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Luis Penailillo

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PhD Thesis

MUSCLE DAMAGE AND METABOLIC PROFILES OF ECCENTRIC CYCLING

Luis PEÑAILILLO
Pt, MSc

Doctor of Philosophy

School of Exercise and Health Sciences
Faculty of Health, Engineering and Science
Edith Cowan University, Australia

Date of submission: 16th December 2013
Esta tesis esta dedicada a mi familia, padres y hermanos, por su incondicional apoyo y cariño.

This thesis is dedicated to my family, parents and siblings for their unconditional support and love.
DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

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ABSTRACT

Eccentric cycling, in which the knee extensor muscles perform eccentric contractions while trying to brake the backward rotational movements of the cranks of a cycle ergometer, has been shown to effectively increase muscle function and volume with a low metabolic cost. However, acute responses to repeated eccentric cycling bouts have not been well documented. Thus, the primary purposes of this PhD project were to investigate muscle damage and metabolic profiles of eccentric cycling in comparison to concentric cycling (Studies 1-3), and muscle-tendon behaviour (Study 4) during eccentric cycling in relation to muscle damage.

Study 1 compared muscle damage and metabolic profiles between a bout of concentric cycling (CONC) and two bouts of eccentric cycling (ECC1, ECC2) performed by 10 healthy men (28 ± 8 y), with a 2-wk interval between bouts. All cycling bouts were performed for 30 min at 60% of CONC maximal power output (PO\text{max}). Heart rate (HR), oxygen consumption, blood lactate (BLa) and rate of perceived exertion were 19-65% lower during ECC1 than CONC, and HR and BLa were 12-35% lower during ECC2 than ECC1. Exercise-induced decreases in knee extensor maximal voluntary contraction (MVC) torque and vertical jump height as well as increases in muscle soreness were significantly greater after ECC1 than CONC and ECC2, and no significant changes in these variables were found one day after CONC and ECC2. It was concluded that eccentric cycling was less metabolically demanding than CONC, and muscle damage was minimal after the second eccentric cycling bout.

Study 2 examined fat and carbohydrate utilisation during and immediately after cycling, and resting energy expenditure before and both 2 and 4 days post-cycling using indirect calorimetry. An oral glucose tolerance test was performed before, and 1 and 3 days post-cycling. Fat utilisation was greater during ECC1 (72%) and ECC2 (85%) than CONC, and was 48% greater during ECC2 than ECC1. Post-exercise energy expenditure and fat utilisation were less after ECC1 than CONC (30% and 52%, respectively), but similar between CONC and ECC2. Glucose uptake increased 3 days...
post-ECC1. These results suggest greater fat utilisation during and after eccentric than concentric cycling without glucose uptake impairment.

Study 3 tested the hypothesis that rate of force development (RFD) would be a more sensitive marker of muscle damage than MVC torque by comparing the changes in MVC torque and RFD after CONC, ECC1 and ECC2. Decreases in MVC torque were significantly greater immediately and 1-2 days after ECC1 than CONC and ECC2. RFD decreased immediately after all cycling bouts, but RFD measured in the interval 100-200 ms ($RFD_{100-200}$) decreased at all time points after ECC1 (24-32%) as well as immediately after ECC2 (23%), but did not change after CONC. The magnitude of decrease in $RFD_{100-200}$ after ECC1 was 7-19% greater than MVC torque. These suggest that $RFD_{100-200}$ is a more specific and sensitive marker of eccentric exercise-induced muscle damage than MVC torque.

To investigate the mechanisms underpinning the repeated bout effect in eccentric cycling, Study 4 examined the hypothesis that vastus lateralis muscle-tendon behaviour would be different between two (i.e. repeated) eccentric cycling bouts. Eleven healthy men (27.1 ± 7.0 y) performed 10 min of eccentric cycling at 65% of CONC $P_{O_{max}}$ twice (ECC1, ECC2) separated by 2 weeks. Greater muscle soreness was developed 1-2 days after ECC1 than ECC2. Electromyogram and crank torque were similar between bouts, but the magnitude of fascicle elongation during ECC2 was 16% smaller than ECC1. These results suggest that smaller elongation of fascicles was associated with less muscle soreness after ECC2, and possibly the repeated bout effect.

These studies revealed the muscle damage profile of eccentric cycling, one of the potential mechanisms of the repeated bout effect, and metabolic characteristics of repeated eccentric cycling bouts. Since muscle damage is minimal and can be abolished by proper prescription, eccentric cycling may be an ideal exercise for elderly and frail individuals with impaired muscle oxidative function (e.g. diabetes and chronic obstructive pulmonary disease). Further studies are warranted in these populations.
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USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
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CHAPTER 1

Introduction

1.1 Background

Human movements are generated by muscle contractions consisting of isometric (static), concentric (shortening) and eccentric (lengthening) actions. During eccentric contractions, muscles are lengthened under tension, as opposed to concentric contractions in which muscles are shortened [1]. Eccentric contractions are performed during daily activities such as walking down stairs, sitting down on a chair, getting into a car, lowering an object or hiking downhill. Typical eccentric exercise models that are used in laboratory to investigate eccentric contractions include isokinetic joint flexions and extensions [2], lowering weights [3], downhill running or walking [4] and stepping exercises [5]. Several studies [6-8] have also investigated eccentric cycling, in which knee extensor muscles perform eccentric contractions while resisting against the backward rotational movements of the cranks generated by an installed electrical motor as shown in Figure 1.1.
Figure 1.1: Eccentric cycling ergometer (Eccentric trainer, Metitur, Finland). The red-circled arrow represents the backward rotational movement of the cranks, and the blue-straight arrow represents the force applied by the subject to resist the crank movement.

Eccentric cycling was first documented by Abbot et al. [9] in 1952, who used two cycles linked to each other in which one person was pedalling forward and the other was resisting the backward movement of the pedals. Abbot and colleagues reported that eccentric cycling required significantly less oxygen than concentric cycling [9]. This early finding was later confirmed using more sophisticated eccentric ergometers, which incorporated an electric motor that rotate the cranks backward at a selected velocity. For instance, Knuttgen et al. [10] and Bigland-Ritchie and Woods [11] reported that eccentric cycling required only 21–29% of the oxygen of concentric cycling. More recently, it has been also reported that eccentric cycling requires only 25–30% of oxygen than that required for concentric cycling at the same workload (330 W) [8], and that heart rate is 34% lower and cardiac output is 39% lower during eccentric cycling than concentric cycling at maximal concentric power output (PO_{max}) [12], and blood lactate did not increase during eccentric cycling [8]. Remarkably, eccentric cycling can produce 4–7 times greater workload compared with concentric cycling at same relative intensity (65% HR_{peak} or 1 L\cdot min^{-1} oxygen consumption: VO_{2}) [6, 7].
Due to these unique characteristics, eccentric cycling can introduce a greater mechanical stimulus to the knee extensor muscles at a low metabolic cost, and thus eccentric cycling may be an ideal exercise modality for muscle mass and strength acquisition. In fact, several studies have reported that eccentric cycling training induced 26–60% greater increases in muscle isometric strength and 52–60% greater increases in muscle fibre cross-sectional area compared with concentric cycling training [6, 7] or resistance training [13] in young and old individuals, respectively.

One possible negative aspect of eccentrically-biased exercise is the induction of muscle damage, represented by prolonged muscle weakness and delayed onset muscle soreness (DOMS) after exercise, especially when it is performed first time or with a long interval between bouts [14, 15]. Muscle damage after eccentric exercise is directly evidenced by histological changes such as disruption of contractile and/or non-contractile proteins and sarcolemma [16, 17]. However, indirect markers of muscle damage are commonly used to assess muscle damage, and these markers include large increases in muscle proteins in the blood, prolonged loss of muscle function, swelling and DOMS [18, 19]. Interestingly, when eccentric exercise is performed for a second time within several weeks after the initial bout, changes in these muscle damage markers are attenuated, and this adaptation is referred to as the “repeated bout effect” [20]. Potential adaptations to explain this effect have been classified as mechanical, neural and cellular [14]. However, the precise underpinning mechanisms are currently not known, and are most likely multifactorial.

Eccentric cycling has also been shown to induce muscle damage after an initial cycling bout. For instance, Friden et al. [17] reported that 30 min of eccentric cycling performed at 80–100% of concentric VO₂max resulted in 13–24% decreases in knee extensor maximal voluntary isometric contraction (MVC) torque for 3 days after exercise, accompanied by myofibrillar Z-band disruption predominantly seen in fast-twitch muscle fibres. Several studies have also shown other markers of muscle damage such as increases in muscle proteins in the blood [21-26] and muscle soreness [22,
25] following eccentric cycling exercise (15–60 min) performed at different range of intensities (50–150% of concentric PO\textsubscript{max}). However, no previous studies have systematically examined muscle damage and repeated bout effect in eccentric cycling.

Although MVC torque has been extensively used, and advocated, as the best indirect marker of muscle damage [27], it has also been documented that its ability to differentiate muscle fatigue from damage is poor when measured immediately after eccentric exercise [28]. Therefore, new more sensitive tools to assess muscle damage, especially soon after the exercise, are needed. It is known that the rate of force development (RFD) could inform about different mechanical properties of the muscle including the muscle stiffness and contractility of the muscle-tendon complex [29]. Thus, it is possible that RFD could be more informative of the muscle function after eccentric exercise than MVC torque, but more research is necessary to determine the effectiveness of RFD as an indirect muscle damage marker.

Several studies [22, 30-32] have demonstrated negative effects of eccentric exercise on glucose metabolism in skeletal muscle. Asp et al. [22] reported a transient decrease in muscle glucose transporter type 4 (GLUT-4) for 3 days after 20 min one-legged eccentric cycling bout. They speculated that the inflammatory responses and sarcolemmal disruption after muscle damage impaired the intra-cellular insulin signal transduction, leading to decreases in the insulin-mediated glucose uptake [22]. However, the negative effects appear to be eliminated when eccentric exercise is repeated. Green et al. [4] reported that increases in insulin resistance assessed by an oral glucose tolerance test (OGTT) performed 2 days after the first bout of downhill running exercise were not observed after a second bout of the same exercise performed 14 days later. Conversely, Nikolaidis et al. [33] have shown that eccentric exercise enhances the blood lipid profile by reporting decreases in total cholesterol (14%), triglycerides (18%) and low-density lipoprotein cholesterol (25%), and an 8% increase in high-density lipoprotein cholesterol for 3 days after 75 maximal isokinetic eccentric hamstrings contractions, but these changes were smaller when the same
exercise was repeated four weeks later. Furthermore, downhill hiking training for 8 weeks improved glucose tolerance by 6.2% [34] and 16 weeks of eccentric cycling training decreased glycosylated haemoglobin by 8% in female type 2 diabetics [35]. However, no previous studies have examined the substrate (i.e. fat and carbohydrates) utilisation during and after eccentric cycling in comparison to concentric cycling, or repeated eccentric cycling bouts, which could increase our understanding of alterations in lipid and glucose profiles in the blood.

It has been suggested that energy expenditure is a determining factor for weight loss management, and that exercise can induce increases of the resting energy expenditure (REE), which, if accompanied with caloric restriction, could lead to weight reduction and improvement in health [36]. It is well known that REE decreases with advancing age (2–3% per decade) and this decrease is mainly attributed to the loss of muscle mass, and could lead to obesity and metabolic diseases [37]. Paschalis et al. [38] reported that maximal isokinetic eccentric exercise (75 maximal contractions) increased REE more than concentric exercise 2 days after exercise, and attributed this to the greater muscle damage and increased muscle protein synthesis rate. The same research group showed larger increases in REE in overweight than lean women after 75 knee extensor isokinetic maximal eccentric contractions [39]. These results suggest that muscle damage induced by eccentric exercise influences the REE; however, REE after eccentric cycling and the effect of repeated eccentric cycling bouts have not been studied. It seems possible that, in addition to increases in muscle mass, increases in muscle oxidative capacity could contribute to an enhanced blood lipid profile and increased REE after eccentric exercise.

B-mode ultrasound imaging techniques have been used to investigate human skeletal muscle in vivo [40]. Several studies have used these techniques to examine the behaviour of muscle fascicles (i.e. fascicle length and angle) and tendinous tissues during movements [40-42]. Some studies have also examined muscle and tendon behaviour during eccentric contractions. For instance, Finni et al. [43] showed greater vastus lateralis (VL) fascicle elongation during fast
(180°⋅s⁻¹) than slow (60°⋅s⁻¹) maximal isokinetic eccentric contractions, and Ishikawa et al. [44] reported that relative tendinous tissue elongation decreased with increasing fascicle lengthening during stretch-shortening contractions (i.e. drop jump) as intensity increased. However, no previous studies have investigated muscle-tendon behaviour during repeated bouts of eccentric exercise. It is of great interest to examine muscle fascicle and tendon behaviour during repeated eccentric cycling bouts, which might shed light on the mechanisms underlying the repeated bout effect and muscle damage; in particular, whether rapid adaptations occur that reduce overall fascicle lengthening and thus subsequent muscle damage and soreness.

1.2 Purposes

The overall main purposes of this thesis project were to examine muscle damage and metabolic profiles of eccentric cycling, and to investigate a possible mechanism underpinning the protective effect conferred by a single eccentric cycling bout. This thesis includes four studies, and the specific purposes of each study were: 1) to compare the metabolic demand of both initial and repeated eccentric cycling bouts to concentric cycling, and the changes in muscle damage markers following two eccentric cycling bouts and one concentric cycling bout (Study 1); 2) to compare the substrate utilisation during and immediately after exercise, and the changes in REE and glucose uptake between two bouts of eccentric cycling and a bout of concentric cycling (Study 2); 3) to examine the changes in the RFD of the knee extensors following two bouts of eccentric cycling and a bout of concentric cycling, to test they hypothesis that RFD could be a better (more sensitive and discriminatory) marker of muscle damage (Study 3); and 4) to quantify vastus lateralis muscle fascicle and tendon behaviour during two (i.e. repeated) eccentric cycling bouts, and to explore whether changes in fascicle and/or tendinous tissue behaviour during eccentric cycling are related to the repeated bout effect (Study 4).
1.3 Significance

It was expected that this research would increase our understanding of eccentric cycling, which appears to be an ideal exercise for individuals with limited exercise tolerance who could perform eccentric cycling due to its lower metabolic cost and potential for increasing muscle mass and strength. However, little is known about the acute responses and mechanisms underpinning the physiological changes elicited by eccentric cycling exercise, and it is possible that metabolic and muscle damage profiles are different between an initial and repeated eccentric cycling bouts. Thus, it is important to improve our understanding of the acute physiological responses to eccentric cycling exercise prior to using this exercise modality in fragile individuals or clinical patients. Furthermore, changes in the muscle oxidative capacity after eccentric exercise have been poorly investigated, and it is possible that eccentric cycling could also induce some muscle oxidative adaptations due its submaximal eccentric contractions and long exercise duration (30 min) characteristics. This could stimulate significant health benefits, especially in patients with metabolic disorders. This research will contribute to the development of more effective and scientifically based eccentric cycling training protocols. The findings of this research will also contribute to our fundamental understanding of the underpinning mechanism of the repeated bout effect, and thus will benefit clinicians, exercise professionals and researchers who wish to safely implement eccentric cycling, optimise its prescription, understand its adaptations and possibly extend its health or clinical uses.
CHAPTER 2

Review of Literature

2.1 Introduction

Eccentric muscle contractions are performed when active muscles are lengthened against an external load [1]. Eccentric contractions of the lower limbs are performed during daily activities such as walking down stairs or sitting down on a chair, where the knee extensor muscles exert a braking force (or absorb energy) during knee flexion movements. Eccentric contractions of the knee extensor muscles have been mainly studied during isotonic, isokinetic and eccentric-based exercise modalities [45-47]. Isotonic eccentric exercises are performed against gravity by using body’s weight and/or additional load such as the downward phase of the squat [48] and descending dumbbells or weights [49]. Isokinetic eccentric contractions are performed in an isokinetic dynamometer, which permits controlled velocity and range of motion of the contraction [50]. Eccentric-based exercises include downhill walking or running, as well as eccentric cycling (Figure 2.1A) and stepping (Figure 2.1B), which have received attention recently due to their effectiveness as exercise interventions in frail and clinical populations [46, 51-53].

It is known that eccentric contractions are associated with the production of greater muscle force than concentric or isometric contractions [54-56]. For example, Kellis et al. [54] reported that maximum eccentric knee extensor torque exceeded the concentric torque by 23% at 30°·s⁻¹ and 107% at 150°·s⁻¹. This has also been confirmed by other studies showing 28% and 40% greater muscle force in eccentric than concentric contractions of the knee extensors [57] and elbow flexors [58], respectively. Griffin [59] reported that maximum eccentric isokinetic elbow flexor torque (at
30°·s⁻¹ and 120°·s⁻¹) was 7-9% greater than maximum isometric torque. This greater force production likely results from multiple events, including molecular events involved in the cross-bridge cycle [60], the spring-like properties of structural proteins of the sarcomeres and tendinous tissue [61, 62], and specific neural control strategies [63]. Several studies showed that the energy cost of performing eccentric work is less than concentric work [64, 65]. In an animal study, 10 maximal lengthening contractions of the medial gastrocnemius of rats showed 70% lower energy cost (i.e. high-energy phosphate consumption) than concentric contractions [66]. Ryschon et al. [67] explored the ATP-utilisation using ³¹P-nuclear magnetic resonance in the human tibialis anterior muscle, and estimated that the biochemical efficiency (i.e. ATP production rate/work produced) was 20% greater during submaximal eccentric than concentric contractions. Therefore, eccentric exercise has a possibility to exert a high level mechanical stimulus that could promote mechanically-dependent signalling for muscle growth and strength development with relatively low energy cost [47]. Roig et al. [47] reviewed the effects of eccentric and concentric resistance training on muscle strength and mass and concluded that eccentric exercise induces greater improvements in muscle strength and mass gains following training, when compared to concentric training. The authors speculated that the superiority of eccentric training is possibly mediated by the higher forces developed during the exercise [47].

Eccentric exercise training has been also demonstrated to produce greater health benefits than concentric exercise. Specifically, a greater reduction in cholesterol and triglyceride concentrations and a parallel increase in high-density lipoprotein concentration was observed after 8 weeks of eccentric–biased resistance training of the knee flexors, compared with concentric–based training [38]. Additionally, it was reported that glucose tolerance was improved and reductions in C-reactive protein in blood were evident following 8 weeks of downhill hiking, compared to uphill hiking [34]. These finding supports the potential benefits of eccentric training for health, and suggest that eccentric exercise may also enhance the muscle oxidative capacity via
improvements in blood lipids, blood glucose levels, and inflammatory indices, as previously shown [34, 38].

Isner-Horobeti et al. [46] and LaStayo et al. [45] reviewed eccentric exercise training for improving skeletal muscle function, and concluded that the mechanical, metabolic and cardiocirculatory characteristics of eccentric exercise could be widely used to improve muscle function in various populations including elite athletes and aging individuals [46]. However, both review articles stated the necessity of better understanding of the physiology of eccentric exercise, and to develop new parameters for optimising the intensity, duration, and modes of exercise, aiding athletes, patients, coaches and clinicians to take full advantage of the benefits of eccentric exercise training [45, 46]. Both LaStayo et al. [45] and Isner-Horobeti et al. [46] focused on eccentric cycling and stepping training, and LaStayo et al. [45] stated that in order to implement eccentric-based exercises, external assistance would be required to move the load concentrically prior to resisting the load eccentrically. However, currently motorised ergometers (i.e. cycle and stepper) deliver the eccentric load only, which make eccentric exercise easier and safer within a clinical setting. Furthermore, eccentric cycling and stepping exercises appear more practical when compared with isokinetic eccentric exercises or downhill running, since the recumbent position is easier and more secure to adopt, and workload can be adjusted and monitored easily.

Eccentric cycling was first reported by Abbot et al. [9] in 1952, who connected two stationary cycle ergometers back-to-back with a single chain such that one person pedalled forward and the other resisted this motion by braking the backward-moving pedals, therefore producing eccentric contractions of the quadriceps muscle group. Later technology permitted the integration of an electric motor that rotates the cranks backwards at a constant velocity (40–60 revolutions per minute: rpm), and eccentric stepping has recently also been used (15–20 rpm) as an eccentric-based exercise modality. Eccentric cycling (Figure 2.1A) and stepping (Figure 2.1B) are similar eccentric exercise modalities in which the knee extensor muscles perform eccentric contractions by resisting
the backward movement (i.e. toward the individual) of a cycle ergometer crank or footstep, respectively.

![Figure 2.1: Eccentric cycling (A) and eccentric stepping (B). Modified from [13, 46]](image)

This review focuses on studies that have used only eccentric cycling or stepping exercises, as different from the previous reviews [45, 46] in which all eccentric-based exercises were included. Furthermore, this review examines our understanding of glucose metabolism during eccentric cycling exercise, which has not been systematically covered in previous review papers. This review primarily focuses our current understanding of the characteristics of a bout of eccentric cycling exercise in comparison to concentric cycling, and the adaptations conferred by eccentric cycling and stepping exercise training.

### 2.2 Characteristics of Eccentric Cycling

Nineteen studies were found to describe the characteristics of eccentric cycling (Table 1), but no study has reported acute responses of eccentric stepping. The characteristics of eccentric cycling reported in the previous studies were categorised as: 1) metabolic responses, 2) muscle damage, and 3) measurements related to health or clinical applications, as shown below. Table 1 shows the measurements taken during eccentric cycling and for several days (i.e. 1–9 days) after exercise. If eccentric cycling was compared with concentric cycling, the results are displayed as a percentage difference between the two.
2.2.1 Metabolic Responses

One of the most interesting and attractive characteristics of eccentric cycling is the lower oxygen required to perform the same mechanical work (i.e. power output/workload) as concentric cycling [7-11, 51, 68, 69]. Abbott and colleagues [9] reported 41–67% lower oxygen consumption (VO$_2$) during eccentric cycling when compared to concentric cycling. This finding was later confirmed by Asmussen [68] and Knuttgen et al. [10, 69], who demonstrated a 60–73% lower VO$_2$ and 40% lower heart rate (HR) during eccentric cycling [69]. In addition, Bigland-Ritchie et al. [11] also reported a 79% lower VO$_2$, and expanded on previous finding by attributing the lower VO$_2$ to a 50% lower muscle activity of the knee extensor muscles during eccentric cycling. They speculated that fewer muscle fibres were recruited to perform the same amount of work during eccentric cycling, in comparison to concentric cycling [11]. Although a different eccentric exercise model was used (i.e. knee extensor isokinetic eccentric contractions at 60°·s$^{-1}$), McHugh et al. [57] reported a higher electromyogram mean frequency during eccentric contractions as compared to concentric contractions at different exercise intensities, and speculated that a greater proportion of fast-twitch motor units were active during submaximal eccentric contractions. However, some controversy exists as to whether fewer and selected (more fast-twitch muscle fibres) muscle fibres are recruited during eccentric contractions, since the lower muscle activity during eccentric exercise may depend on the joint angle and the pre-activation mode [70]. Therefore, the lower muscle activity of eccentric contractions, when compared to concentric contractions, is not fully confirmed and further research is needed. It is important to note that earlier studies reporting lower levels of VO$_2$ during eccentric cycling used individuals who were accustomed to the exercise [9-11, 68, 69, 71], so it is not known how individuals respond to eccentric cycling when they are initially exposed to it, or how the responses differ when they are exposed to the same exercise for a second time.

Smaller increases in body temperature have also been shown during eccentric than concentric cycling [23, 72]. For instance, body temperature (i.e. oesophageal) was 0.7°C lower after 40 min of eccentric cycling when compared to concentric cycling at same metabolic intensity.
(ECC=381 W and CONC= 102 W for 30-40 min cycling) [72]. Another study reported that rectal temperature was not different between 30 min of concentric cycling performed at 65% of maximal power output (PO\text{max}) and eccentric cycling performed at 100–150\% PO\text{max} [23]. Nadel et al. [72] showed that skin and intramuscular temperature of the quadriceps muscle were 1–2°C higher during eccentric than concentric cycling; Elmer et al. [73] found that knee extensor muscles accounted for 54\% of the work produced during eccentric cycling, with the hip extensors and ankle plantar flexors contributing 34\% and 11\%, respectively. Therefore, peripheral (i.e. muscle–skin) increases in temperature may be explained by the greater muscular work (i.e. force) performed by the quadriceps during eccentric actions. Smaller increases in body temperature during and after eccentric cycling compared with concentric cycling for the same work are considered to be clinical advantages of eccentric cycling exercise for elderly individuals, since they are less tolerable for heat because of impaired thermoregulation [74].

More recent studies have confirmed the lower metabolic demand and higher power output (for the same level of effort: VO\text{2}, HR and RPE) of eccentric cycling. Perrey et al. [8] demonstrated that the metabolic response to high-intensity eccentric cycling (330 W) was similar to low-intensity concentric cycling (70 W), as indicated by comparable O\text{2} consumptions (~1.2 L\cdot\text{min}^{-1}), blood lactate concentrations (<1 mmol/l) and rates of perceived exertion (RPE) (~5 of 10). Dufour et al. [12] reported that eccentric cycling induced less cardiocirculatory stress than concentric cycling, as shown by a 39\% lower cardiac output (Q), a 40\% lower arteriovenous oxygen difference ((A-v)O\text{2}), and a 34\% lower HR response, when compared to concentric cycling at the same given workload (~300 W).

Although the lower energetic cost of eccentric contractions was first reported by A.V. Hill in 1960, who showed a decreased energy liberation in a muscle that was stretched during contraction [65], relatively little further investigation has been conducted to understand the underpinning mechanisms. Nevertheless, it has been proposed that the lower energy cost of eccentric cycling might be due to: 1) the non-adenosine triphosphate (ATP)-dependent mechanical detachment of the
actin-myosin cross-bridges during eccentric contractions [46, 75]; 2) the spring-like property of the muscle and tendinous tissue, allowing the store and recoil of potential energy during eccentric contractions [62]; and 3) the lower muscle fibre recruitment at a given level of muscle force, which, in conjunction with the spring-like property of muscle and tendinous tissue, may contribute to the lower energy used during eccentric contractions [8, 11, 76]. However, the exact mechanism of the lower metabolic demand of eccentric contractions is yet to be agreed. Therefore, further research is necessary to fully understand the underpinning mechanisms of the lower metabolic cost during exercises such as eccentric cycling.

### 2.2.2 Muscle Damage

A common consequence of performing eccentric contractions, especially for the first time or when a significant time (e.g. > 24 weeks) has elapsed since the last exercise bout, is muscle damage, which has been evidenced by direct (e.g. muscle fibres disruption) and indirect (e.g. strength loss, muscle soreness and muscle proteins in blood) markers [15]. Friden et al. [17] reported that muscle damage was induced after a 30-min bout of eccentric cycling, which was associated with Z–band disruption predominantly seen in fast–twitch muscle fibres, and a 12-24% decrease in isometric and 12-31% decrease in isokinetic strength 1–3 days and 1–6 days after exercise, respectively [17]. Four other studies have reported decreases in muscle function (i.e. isometric strength, squat jump and countermovement jump) [25, 71] and performance (i.e. decrease in maximal concentric cycling, knee extensor power, power-pedalling rate) [77, 78], which were recovered 1-6 days after eccentric cycling. However, the heterogeneity (i.e. duration, rest, intensity) of eccentric cycling protocols makes comparisons difficult (Table 1). Several studies have also reported increases in muscle soreness and blood creatine kinase concentrations 1–4 days [22, 25, 73, 78] and 1–9 days [21-26] after eccentric cycling, respectively. Similarly, increases in other muscle proteins in blood (i.e. myoglobin [Mb], aspartate aminotransferase and alanine aminotransferase) have also been observed 1-7 days after eccentric cycling [21-23]. Other biochemical markers of muscle damage and inflammation reported after eccentric cycling include
increases in white-cell infiltration [23], interleukin-1 and interleukin-6 (IL-1 and IL-6) concentrations [21] and growth factors [24, 26]. Greater increases in inflammatory and muscle damage markers in blood were observed in young rather than older individuals, which was associated with the greater absolute workload performed by younger subjects [23, 24].

These negative, muscle damage-related consequences of eccentric cycling are probably not desirable when applying eccentric cycling in fragile individuals. However, it is well documented that the magnitude of muscle damage is smaller when eccentric exercise is repeated within several weeks after an initial bout, which is known as the repeated bout effect [15]. Nonetheless, the effects of repeated eccentric cycling bouts have not yet been systematically examined. Thus, further research is necessary to examine muscle damage responses to repeated eccentric cycling bouts.

### 2.2.3 Health and Clinical Aspects

There are few data describing changes in health-related parameters after a single bout of eccentric cycling exercise. Impaired glycogen replenishment associated with decreased glucose uptake resulting from muscle damage has been demonstrated to be a negative consequence of eccentric cycling. O’Reilly et al. [79] showed a 43% lower muscle glycogen content immediately after 45 min eccentric cycling, which remained 39% lower for 10 days after exercise and was also accompanied by Z-band disruption and muscle fibre oedema. Additionally, Asp et al. [22] reported an increase in catecholamine hormone concentrations during eccentric cycling and a decrease in muscle glycogen and glucose transporter type 4 (GLUT-4) protein concentration 1-2 days post-eccentric cycling. These authors speculated that eccentric cycling caused a transient decrease in muscle GLUT-4 protein concentration, leading to an impaired glycogen re-synthesis. On this basis, muscle membrane damage is thought to be involved in the development of insulin resistance following eccentric cycling [22]. This could be of particular significance when eccentric cycling is considered as a therapeutic/training modality for insulin-resistant patients such as those with metabolic syndrome or type 2 diabetes. Nevertheless, more research is necessary to investigate
whether these findings are due to exercise intensity or can be attenuated/abolished with repeated bouts of eccentric cycling.

2.3 Adaptations to Eccentric Cycling and Stepping Training

Twenty-seven eccentric cycling and stepping training studies (i.e. 5-16 weeks in duration) were found. Due to the potential for eccentric cycling to promote greater gains in muscle strength and size at a lower metabolic cost than concentric cycling, eccentric cycling, and more recently eccentric stepping, have been used as training modalities for individuals with limited cardiorespiratory function as well as in elderly individuals and athletes [7, 53, 80-82]. Eccentric cycling and stepping are particularly beneficial for individuals with limited exercise tolerance in order to maintain or increase their muscle function, ultimately promoting independence and quality of life. The main results of these training studies are summarised in Table 2, with variables showing significantly greater improvements through eccentric cycling training in comparison to other training modalities being highlighted (e.g. concentric cycling, resistance training and standard rehabilitation protocols).
Table 1: Characteristics of eccentric cycling

<table>
<thead>
<tr>
<th>Author</th>
<th>Intensity</th>
<th>Time</th>
<th>N</th>
<th>Subjects characteristics</th>
<th>Main Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbot et al. (1952)</td>
<td>[9]</td>
<td>25-35 &amp; 52 rpm</td>
<td>45-250 W</td>
<td>13' Male, 20 yr old</td>
<td>NE 41-67% VO₂ than CONC</td>
</tr>
<tr>
<td>Asmussen (1953)</td>
<td>[68]</td>
<td>45, 68, 85, 92 &amp; 102 rpm</td>
<td>7' Male, 20 yr old</td>
<td>60% VO₂@163 W than CONC</td>
<td>NE mean muscle lengthening</td>
</tr>
<tr>
<td>Knuttgen et al. (1971)</td>
<td>[10]</td>
<td>15-130 W</td>
<td>20-100 rpm</td>
<td>7' Healthy males @ 2 weeks</td>
<td>NE 73% VO₂ than CONC, 40% HR than CONC @ 130 W @60 rpm</td>
</tr>
<tr>
<td>Nadel et al. (1972)</td>
<td>[72]</td>
<td>115-440 W</td>
<td>NE</td>
<td>40' Male, 20 yr old</td>
<td>NE 0.7 °C oesophageal temp, 1-2 °C higher skin temp, 1 °C higher intra-muscular temp</td>
</tr>
<tr>
<td>Bigland-Ritchie et al.</td>
<td>[11]</td>
<td>2.5-15 kg resistance</td>
<td>50 rpm</td>
<td>≤75% HR max</td>
<td>NE 79% VO₂, 50% iEMG</td>
</tr>
<tr>
<td>Knuttgen (1982)</td>
<td>[69]</td>
<td>PO Peak</td>
<td>30''</td>
<td>NE</td>
<td>NE 70-75% VO₂ than CONC</td>
</tr>
<tr>
<td>Friden et al. (1983)</td>
<td>[17]</td>
<td>80-100% of CONC workload @ VO₂max</td>
<td>60 rpm</td>
<td>30' Males</td>
<td>NE 12% 1-24% ISOM strength @ 1-3 days, 12-31% lower ISOK strength @ 1-6 days, Z-band disruption predominantly in type 2 fibres</td>
</tr>
<tr>
<td>Evans et al. (1986)</td>
<td>[21]</td>
<td>250 W</td>
<td>NE</td>
<td>45' 5 untrained, 4 highly endurance trained</td>
<td>NE 33-30 fold CK activity in untrained group, 2.3-5 fold CK activity for 1 days in trained group</td>
</tr>
<tr>
<td>O'Reilly et al. (1987)</td>
<td>[79]</td>
<td>ECC cycling @ 90, 80 and 70% of CONC PO max</td>
<td>3 x 15'</td>
<td>5 Untrained healthy males</td>
<td>NE During exercise: 41-48% VO₂max, Immediately after: NE 43% muscle glycogen levels, 10 days after: 43% muscle glycogen levels, Frank muscle necrosis</td>
</tr>
<tr>
<td>Asp et al. (1995)</td>
<td>[22]</td>
<td>4 x 5 min cycling</td>
<td>2 min rest</td>
<td>63 W average power output (single leg)</td>
<td>Average HR: 129 beats min^-1, Average VO₂: 34% of CONC VO₂max</td>
</tr>
<tr>
<td>Bruunsgaard et al. (1997)</td>
<td>[23]</td>
<td>ECC: 20 min @ 150% of CONC PO max + 10 min @ 100% of CONC PO max</td>
<td>CON: 30 min @ 65% of CONC PO max</td>
<td>20' Young active males</td>
<td>NE During exercise: Adrenaline and noradrenaline = Rectal temp, 450% IL-6 @ 2 h after ECC, 3000-5000 UI/L CK activity @ 2-4 days, NK @ 20 min after ECC, During recovery: 9-13 fold ASAT &amp; 4-5 fold ALAT @ 4-7 days after ECC</td>
</tr>
<tr>
<td>Kinetics</td>
<td>Value</td>
<td>Value</td>
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<td>----------------------------------------------</td>
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<tr>
<td>Mean CO2 production during exercise (%)</td>
<td>1.5</td>
<td>2.0</td>
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<td></td>
<td></td>
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<tr>
<td>Fatigue (%)</td>
<td>20</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Resting Heart Rate (%)</td>
<td>70</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Heart Rate (%)</td>
<td>160</td>
<td>170</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Lactate Level (%)</td>
<td>4.2</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal Oxygen Uptake (%)</td>
<td>40</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Output (%)</td>
<td>60</td>
<td>65</td>
<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:**
- CONC: concentric cycling
- ECC: eccentric cycling
- PO: power output
- VO2: oxygen consumption
- HR: heart rate
- temp: temperature
- iEMG: integrated amplitude electromyogram
- MPF: mean power frequency
- RPE: rated perceived exertion
- ISOM: isometric strength
- ISOK: isokinetic strength
- SOR: muscle soreness
- CK: creatine kinase
- Mb: myoglobin
- IL-1 and IL-6: interleukin 1 and 6
- GLUT-4: glucose transporter 4
- NK: natural killer cells
- ASAT: aspartate aminotransferase
- ALAT: alanine aminotransferase
- sTNF-R1: soluble Tumor Necrosis Factor Receptor 1
- MGF: mechanical growth factor
- Q: cardiac output
- (A-v)O2: arteriovenous oxygen difference
- CMJ: counter movement vertical jump

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**Data Source:**
- Perrey et al. (2001)
- Toft et al. (2002)
- Dufour et al. (2004)
- Klossner et al. (2007)
- Hameed et al. (2008)
- Elmer et al. (2010)

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2.3.1 Muscle Function and Size

Several articles have reported increases in isometric [6, 7, 52, 81, 83-86] and isokinetic concentric knee extensor strength [83] following eccentric cycling or stepping training. These strength gains have been reported across various populations, including healthy young [7, 87] and elderly individuals [13, 85], young skiers [82], coronary artery disease patients [83], Parkinson’s disease patients [81, 84], cancer survivors [88-90], obese and glucose intolerant individuals [52], and individuals who had anterior cruciate ligament (ACL) reconstruction [91] and total knee arthroplasty [80, 92]. However, only four of these studies have reported statistical differences when compared to another training modality (i.e. concentric cycling, resistance training and standard rehabilitation protocols) [6, 13, 83, 89].

Improvements in sprint performance [87], vertical jump power [93] and leg spring stiffness [93] have also been reported in healthy untrained individuals after eccentric cycling training. However, Gross et al. [82] reported no change in vertical jump performance (i.e. squat jump: SJ and countermovement jump: CMJ) following eccentric cycling training in young elite skiers, accompanied by a modest 10% increase in isometric strength, although this was not significantly different from the 12% strength improvement in the resistance training group. The authors speculated that the eccentric cycling training load was lower than their normal training load among the elite skiers. In addition, 12 weeks of eccentric stepping training did not result in significant improvements in strength and was not as effective as standard exercise in patients with multiple sclerosis. However, it may be that the effects of eccentric cycling on muscle function are reduced in this population since multiple sclerosis is a progressive neurodegenerative disease where motor control is impaired and may lead to poor coordination [94].

Overall, previous studies have shown the greater potential of eccentric cycling and stepping training for muscle function improvements. The greater increases in muscle function after eccentric training have been attributed to the greater force production of the eccentric contractions [47], but also the unique motor unit recruitment pattern that may be selective for fast-twitch muscle fibres,
thereby inducing an enhanced motor drive after eccentric training [63, 95]. However, selective fast-twitch muscle fibre recruitment during eccentric exercise is still not fully elucidated and warrants further research.

Eccentric cycling training has also been shown to increase muscle fibre [6, 13] and whole thigh [52] cross-sectional area (CSA), while eccentric stepping training has been shown to increase quadriceps [91, 96] and gluteus maximus muscle volumes [91]. Additionally, increases in quadriceps and gluteus maximus volumes were sustained for one year post-training, when compared to a convectional anterior cruciate ligament (ACL) surgery rehabilitation protocol [91]. Eccentric cycling has also been demonstrated to increase leg lean muscle mass after training, however no statistical difference was found between eccentric cycling training and a combined concentric cycling plus rehabilitation training program [52, 83]. LaStayo et al. [89] reported that eccentric stepping produced a greater increase in average quadriceps (lean tissue) CSA in cancer survivors when compared to usual care (i.e. general recommendations, non-exercise group). In addition, Hansen et al. [90] reported a 3% increase in quadriceps CSA in non-androgen deficient prostate cancer survivors. Interestingly, it has been documented that muscle growth (6% increase in thigh muscle mass) occurred in the absence of anabolic signalling, muscle inflammation or damage, shown at least by no increases in CK activity, insulin-like growth factor 1 (IGF-1) or tumour necrosis factor alpha (TNF-α), during and after training [97].

Only four studies have shown statistically greater improvements in muscle size when compared to another training group. Specifically, two studies have demonstrated a greater increase in muscle fibre CSA after eccentric than concentric cycling training [6] and resistance training [13]. Two studies showed significantly greater increases in muscle volume after eccentric cycling and stepping when compared to standard care and resistance training [80, 81]. Changes in muscle fibre composition have provided more equivocal results, with no change in muscle fibre type composition observed in healthy young participants [87], yet increases in type IIa muscle fibres in cardiac patients [98] and decreases in the type IIx/type II muscle fibre ratio in elderly subjects [85].
Interestingly, the only study to investigate eccentric cycling training in an athletic population found a modest increase in isometric strength and thigh lean muscle mass in young skiers [82]. Therefore, it appears that eccentric cycling and stepping promote muscle strength and mass gains to a greater extent, at least in individuals with decreased muscle function (e.g. due to aging or chronic disease), than concentric training, most likely due to the greater mechanical stimuli to the muscle during eccentric contractions. However, more research is necessary to examine whether such muscle strength and mass gains are greater when compared to other training modalities (i.e. resistance training) while performed at a similar workload, and whether eccentric cycling training can benefit athletic populations.

2.3.2 Functionality and Quality of Life (QoL)

Functionality is the ability of an individual to produce whole-body complex movements and includes both cognitive and physical aspects, which ultimately determines an individuals level of independence [99]. Eccentric cycling training has been shown to increase the functionality of elderly subjects [13] and those who suffer from cardiac [83], chronic obstructive pulmonary disease (COPD) [53] and Parkinson’s disease [81], those with type 2 diabetes and impaired glucose tolerance [52], cancer survivors [88-90], and those who have had ACL reconstruction surgery [91] or total knee arthroplasty [80, 92]. More specifically, eccentric cycling and stepping training were effective in improving the lower extremity function scale score [92], stair ascent [81, 92] and descent times [13, 89], the time-up-and-go [13, 85, 88] 200-m fast walk [86] and six-minute walk test [52, 81, 86, 89, 92] times, gait speed [92], the number of steps taken per day [52], hopping performance [100], total range of motion [92] and coordination [82, 85]. Nonetheless, no change in the six-minute walk test was reported after eccentric cycling training in COPD [53] and type 2 diabetes patients [35] or stair ascent and descent times in multiple sclerosis patients [94], so under some conditions the training might not be effective.

Four studies showed significant improvements in functionality when compared to another training modality (i.e. standard rehabilitation, resistance training) [13, 80, 81, 89]. QoL has not been
well examined, and although no substantially greater benefit in QoL was noted in post-ACL reconstruction surgery and COPD patients after eccentric stepping training when compared to other training modalities [53, 91, 100], these studies were complicated due to both exercise interventions inducing a significant increase in QoL after training. However, eccentric stepping training improved the physical component summary of the Short Form-36 in total knee arthroplasty patients (59% improvements) [92]. Therefore, eccentric cycling and stepping training have shown potential to induce improvements in functionality and QoL, which were most likely attributed to the increases in lower limb muscle strength that would have increased mobility and the overall independence of individuals with restricted functionality. However, examination of these benefits compared to other training modalities is necessary and assessments of QoL should be considered in future eccentric training interventions.

2.3.3 Balance

Balance refers to the ability to maintain the vertical position with minimal postural sway, and depends on sensory (i.e. proprioceptive receptors in the joints and vestibular system) and motor (i.e. muscle) systems to detect and correct the displacement of the body [101]. To the best of our knowledge, only two studies have reported an improvement in postural balance after eccentric cycling training. LaStayo et al. [13] reported a 7% increase in the balance berg test after eccentric cycling training when compared to resistance training in older individuals (range: 70–93 years). Conversely, Hayes et al. [94] noted only a 4% increase in balance after eccentric stepping training in comparison to the 15% increase reported after a standard exercise training (i.e. aerobic and balance exercises) in multiple sclerosis patients. However, it is possible that the smaller balance improvements in multiple sclerosis patients result from the neurogenic weakness present in this condition ultimately limiting the peripheral adaptations to eccentric training. Therefore, more research is necessary to test the hypothesis that muscle strength and mass gains are transferrable to improvements in postural balance after eccentric training in individuals with neurologic stability.
2.3.4 Adaptations for Clinical Applications

The metabolic adaptations to eccentric cycling and stepping training have not been extensively investigated. However, it was shown that eccentric cycling training increased 6-minute walk test performance in patients with COPD (from baseline) without inducing dyspnoea or reducing haemoglobin saturation during exercise [53]. In addition, similar ventilation frequencies [53] and diastolic pressure [51] were reported during eccentric and concentric cycling training, although eccentric cycling induced greater increases in cardiac function (i.e. mean left ventricular fraction ejection) in coronary artery disease patients after training [51]. These characteristics suggest eccentric cycling to be an ideal and safe exercise for individuals with cardio-pulmonary impairments. Furthermore, eccentric cycling training has been also shown to decrease body mass index (BMI) and abdominal fat in type 2 diabetic women and elderly individuals, respectively [35, 85]. Additionally, although a significant (8%) reduction in glycosylated haemoglobin (HbA1c) was reported after 16 weeks of eccentric cycling training combined with aerobic training, it was not statistically greater than the 5% reduction in HbA1c induced by aerobic training alone [35]. However, eccentric cycling training did not modify the whole body insulin resistance in women with impaired glucose tolerance [52]. Thus, these results regarding eccentric cycling and stepping training are promising for individuals who are unable to perform demanding cardio-pulmonary exercise (given their disease state), yet require exercise as a therapeutic modality to improve functionality, body composition and, perhaps, insulin sensitivity. However, these aspects need to be investigated further.

2.3.5 Potential Mechanisms

The positive adaptations to eccentric exercise training shown above most likely result from the greater mechanical stimulus imposed on the muscle when compared to concentric cycling or standard rehabilitation protocols [70]. However, since greater muscle strength was also found after eccentric cycling training than resistance exercise training, it is possible that eccentric contractions induce specific adaptations within the muscle and tendon tissues that are different to a conventional
resistance training, although more research is needed to investigate this possibility. It has also been speculated that eccentric exercise induces greater tendinous tissue remodelling, which could enhance the muscle force transmission systems and consequently increase muscle strength [102]. Furthermore, eccentric exercise may produce a unique motor pattern, improving muscle fibre recruitment after eccentric training [63, 95]. Therefore, it seems possible that eccentric exercise induces specific/different adaptations within the skeletal muscle compared to those after concentric exercise. However, more research is needed to gain a greater understanding of the underpinning mechanisms of eccentric cycling and stepping in order to explain the muscle strength and mass gains induced by eccentric training.
<table>
<thead>
<tr>
<th>Author</th>
<th>Intensity</th>
<th>Training period</th>
<th>&amp; frequency</th>
<th>Subjects</th>
<th>Training modality</th>
<th>Results</th>
<th>Authors</th>
<th>Groups</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Friden et al. (1983)</td>
<td>~100-300 W</td>
<td>8 wk</td>
<td>2-3 x wk</td>
<td>15</td>
<td>ECC cycling</td>
<td>375% PO</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LaStayo et al. (1999)</td>
<td>50-60 rpm</td>
<td>6 wk</td>
<td>2-5 x wk</td>
<td>9</td>
<td>ECC: 4 CONC: 5</td>
<td>300% PO</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LaStayo et al. (2000)</td>
<td>54-65% HRmax</td>
<td>15' - 30'</td>
<td>2-4 x wk</td>
<td>13</td>
<td>ECC: 7 CONC: 6</td>
<td>400% PO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LaStayo et al. (2003)</td>
<td>65-70 rpm</td>
<td>15' - 20'</td>
<td>3 x wk</td>
<td>21</td>
<td>ECC: 7 CONC: 6</td>
<td>500% PO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer et al. (2003)</td>
<td>175-200 bpm</td>
<td>30'</td>
<td>3 x wk</td>
<td>13</td>
<td>ECC: 7 CONC: 6</td>
<td>62% mean left ventricular fraction</td>
<td>4</td>
<td>10%</td>
<td>60% CSA muscle fibre</td>
</tr>
<tr>
<td>Time: 5 wk</td>
<td>Time: 12 wk</td>
<td>Time: 16 wk</td>
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<td>ECC=10 x wk</td>
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<td>ECC=10 x wk</td>
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<td>Average 3%</td>
<td>Average 20%</td>
<td>Average 20%</td>
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<tr>
<td>Strength</td>
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<tr>
<td>KE ISOM 9%</td>
<td>KE ISOM 32%</td>
<td>KE ISOM 32%</td>
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<tr>
<td>IGF-1 mRNA</td>
<td>COX-1 mRNA</td>
<td>COX-1 mRNA</td>
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<td>78%</td>
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<tr>
<td>Time: 6 MWT</td>
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<td>6%</td>
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<tr>
<td>Time: 11 wk</td>
<td>Time: 11 wk</td>
<td>Time: 11 wk</td>
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<tr>
<td>ECC=10 x wk</td>
<td>ECC=10 x wk</td>
<td>ECC=10 x wk</td>
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<td>Gerber et al. (2009)</td>
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<td>6 wk</td>
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<td>60 rpm</td>
<td>Muscle strength, knee laxity, daily living activities</td>
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<td>CONC + cycling + standard rehabilitation</td>
<td>3 x wk</td>
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<td>60 rpm</td>
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<td>23% quadriceps femoral muscle volume</td>
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<td>21% gluteus maximus muscle volume</td>
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<td>33% KE ISOK CONC (60° - 1 s) strength</td>
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<td>50% Hop test</td>
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<td>22% type IIX/II ratio</td>
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<td>7.5% KE ISOM strength</td>
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<td>43% muscle coordination</td>
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<td>Mueller et al. (2009)</td>
<td>ECC cycling training</td>
<td>12 wk</td>
<td>ECC</td>
<td>5 min</td>
<td>Body composition, muscle mass, daily living activities</td>
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<td>Resistance training (RT) + Cognitive training (CT)</td>
<td>2 x wk</td>
<td>RT</td>
<td>7.5 min</td>
<td>Muscle mass, daily living activities</td>
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<td>5% whole body fat</td>
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<td>7% thigh fat</td>
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<td>2.5% thigh muscle mass</td>
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<td>LaStayo et al. (2009)</td>
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<td>12 wk</td>
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<td>3 x wk</td>
<td>RT</td>
<td>60 - 80 rpm</td>
<td>Muscle mass, daily living activities</td>
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<td>9% quadriceps volume</td>
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<td>15% KE ISOM</td>
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<td>29% TUG</td>
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<td>32% stair ascent</td>
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<td>31% stair descent</td>
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<td>Hansen et al. (2009)</td>
<td>ECC stepper</td>
<td>12 wk</td>
<td>ECC</td>
<td>7 rpm</td>
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<td>Non-ADT</td>
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<td>7.5 rpm</td>
<td>Muscle mass, daily living activities</td>
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<td>3% quadriceps volume</td>
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<td>4% FACT - P physical subscale</td>
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<td>Gremeaux et al. (2010)</td>
<td>CONC cycling training</td>
<td>5 wk</td>
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<td>5 min</td>
<td>Body composition, muscle mass, daily living activities</td>
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<td>ECC cycling training</td>
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<td>17% VO2peak</td>
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<td>17% ankle flexor ISOM strength</td>
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<td>7% ankle flexor ISOM strength</td>
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<td>Gross et al. (2010)</td>
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<td>60 - 80 rpm</td>
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<td>2% leg soft tissue lean mass</td>
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<td>10% KE ISOM strength</td>
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<td>6% KF ISOM strength</td>
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<td>4.3% SJ</td>
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<td>6.5% CMJ</td>
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<td>50% precision during ECC cycling</td>
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<td>Treatment Outcome Measures</td>
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<tr>
<td>LaStayo et al. (2010)</td>
<td>ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Old cancer survivors (&gt;60 y)</td>
<td>300% PO = SOR</td>
<td>11% KE ISOM = 14% TUG (1.2 s)</td>
<td>ECC vs Usual care</td>
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<tr>
<td>Rocha et al. (2011)</td>
<td>ECC cycling training</td>
<td>60% CONC VO$_{2\text{peak}}$</td>
<td>Time: 20'</td>
<td>5 wk, 3 x wk</td>
<td>Severe COPD males</td>
<td>VE = 60% CONC VO$_{2\text{peak}}$, TUG = 1.2 s</td>
<td>CK &lt;140 UL/L, SaO$_2$ &gt; 90%</td>
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<td>Marcus et al. (2011)</td>
<td>ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Total knee arthroplasty patients (40-70 y)</td>
<td>59% SF - 36pcs</td>
<td>47% 6MWT, 55% LEFS, 30% gait speed, 29% stair climbing leg power, 12% total ROM, 107% KE ISOM strength, 93% maximal voluntary power output</td>
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<tr>
<td>Flann et al. (2011)</td>
<td>ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Healthy university students</td>
<td>59% CK activity * = SOR * = 7.5% thigh volume, 26% KE ISOM, 55% IGF-1Ea mRNA</td>
<td>7.5% thigh volume, 26% KE ISOM, 55% IGF-1Ea mRNA</td>
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<td>LaStayo et al. (2011)</td>
<td>ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Usual care = 20 Males=8, Females=12</td>
<td>4% quadriceps average CSA of lean tissue * = 11% KE ISOM strength = 29% stair climbing leg power * = 12% 6MWT * = 21% stair descent time *</td>
<td>1% quadriceps lean tissue average CSA * = 1% KE ISOM * = 8% stair climbing leg power * = 2% 6MWT * = 5% stair descent time</td>
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<tr>
<td>Hayes et al. (2011)</td>
<td>Standard exercise + ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Multiple sclerosis patients</td>
<td>22% KF (left) ISOM strength = 20% KE (left) ISOM strength</td>
<td>8% HF (left) ISOM strength = Fatigue (FSS) = TUG = 6MWT</td>
<td>8% HF (left) ISOM strength = Fatigue (FSS) = TUG = 6MWT</td>
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<td>Flann et al. (2011)</td>
<td>ECC stepper</td>
<td>7-13 RPE</td>
<td>Progressively increased</td>
<td>12-18 rpm, 5-20'</td>
<td>Healthy university students</td>
<td>59% CK activity * = SOR * = 7.5% thigh volume, 26% KE ISOM, 55% IGF-1Ea mRNA</td>
<td>7.5% thigh volume, 26% KE ISOM, 55% IGF-1Ea mRNA</td>
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<td>Marcus et al. (2011)</td>
<td>ECC stepper</td>
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<td>12-18 rpm, 5-20'</td>
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<td>4% quadriceps average CSA of lean tissue * = 11% KE ISOM strength = 29% stair climbing leg power * = 12% 6MWT * = 21% stair descent time *</td>
<td>1% quadriceps lean tissue average CSA * = 1% KE ISOM * = 8% stair climbing leg power * = 2% 6MWT * = 5% stair descent time</td>
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*Note: ECC = Eccentric, CONC = Concentric, PO = Power Output, RPE = Rate of Perceived Exertion, VE = Ventilation Efficiency, SaO$_2$ = Oxygen Saturation, CK = Creatine Kinase, SOR = Systolic Oxygen Reserve, TUG = Timed Up and Go Test, 6MWT = 6-Minute Walk Test, LEFS = Lower Extremity Functional Scale, COPD = Chronic Obstructive Pulmonary Disease, CON = Control, HF = Hamstring Force, FSS = Fatigue Severity Scale, CSA = Cross-Sectional Area.
ECC cycling training and CONC cycling training resulted in 54-66% HRmax and exercise time: 10-30' respectively. CONC cycling was designed to perform the same total work in the shortest amount of time.

Healthy men and women completed 7 weeks of training, 3x per week, and were able to maintain an RPE between 6 and 8.1%

Abbreviations:
- CONC: concentric cycling
- ECC: eccentric cycling
- SOR: muscle soreness
- VO2: oxygen consumption
- VO2max: maximal oxygen consumption
- VE: pulmonary ventilation
- PO: power output
- KE: knee extensors
- KF: knee flexors
- HF: hip flexors
- ISOM: isometric strength
- ISOK: isokinetic strength
- RPE: rated perceived exertion
- CSA: cross-sectional area
- BBT: Berg Balance Test
- TUG: Timed Up-and-Go Test
- 6MWT: Six Minute Walk Test
- LEFS: Lower Extremity Function Scale
- QoL: quality of life
- SF-36pcs: Physical component summary of the Short Form-36
- HbA1c: glycosylated haemoglobin
- BMI: body mass index
- CNIC: cardiovascular and cerebrovascular disease
- CAD: cardiac artery disease
- ADT: androgen deprived therapy
- COX-4: cytochrome c oxidase subunit IV
- IGF-1: insulin-like growth factor I
- LHFALC: glucose-mediated hemoglobin

*P<0.05 between training modalities.
2.4 Conclusion

A number of studies have examined the effects of acute and chronic eccentric cycling and stepping training, however little is known about the underpinning mechanisms of the adaptations induced by such exercise. It has been shown that eccentric cycling and stepping are attractive training alternatives for individuals who cannot tolerate high (or moderate) exercise intensities because they are associated with a lower metabolic cost (and hence cardiovascular response) concomitant with a greater mechanical workload for the lower limbs. However, muscle damage and soreness can be negative consequences of eccentric cycling and stepping, particularly in those individuals with decreased muscle function. Thus, it is important to determine whether repeated eccentric cycling bouts can attenuate muscle damage symptoms after exercise. Further investigation of eccentric cycling and stepping exercises in comparison to concentric cycling and resistance exercise training is required. Specifically, future research should focus on understanding whether muscle strength and mass gains are due to the higher workload performed during eccentric cycling and stepping or because of specific adaptations to the eccentric contractions characteristics. Furthermore, since few oxidative improvements have been shown after eccentric exercise training, a systematic exploration of oxidative adaptations induced by eccentric cycling and stepping may reveal novel effects, and these training modalities could be extended to other populations, such as those with metabolic conditions (e.g. metabolic syndrome and diabetes).
CHAPTER 3

Study 1: Metabolic and Muscle Damage Profiles of Concentric Versus Repeated Eccentric Cycling

3.1 Introduction

Eccentric contractions are often performed during activities of daily living such as walking down stairs or sitting down on a chair, as well as in exercises such as downhill running or walking [104], stepping exercise [5] and a variety of resistance exercises [2, 3]. Eccentric cycling is also an exercise modality in which eccentric contractions predominate, as the knee extensor muscles perform eccentric contractions when resisting against the backward rotational movements of the cranks. Eccentric cycling was first introduced by Abbott et al. [9] in 1952, where two inter-linked bicycles were used with one person pedalling forward (i.e. concentric) and the other resisting the backward movements (i.e. eccentric) imposed on their bicycle. In the classical study, Abbott et al. [9] reported that oxygen consumption ($\text{VO}_2$) was 41%, 49% and 66% lower during eccentric cycling performed at 25, 35 and 52 revolutions per minute (rpm), respectively, when compared with concentric cycling at intensities ranging between 24 to 245 W. These findings were later confirmed by Asmussen [68], Knuttgen et al. [10] and Bigland-Ritchie et al [11], and Bigland-Ritchie et al [11] also showed that muscle activation was lower during eccentric than concentric cycling. More recently, electric motors have been used to drive the backward rotations of the cranks, against which the person works. Researchers have shown that eccentric cycling requires only 25-30% of the oxygen required for concentric cycling at the same workload [8, 69], and that a 4-7 times greater
workload can be produced in eccentric cycling compared with concentric cycling at an intensity of 65% HR_{peak} [6] or at the same (i.e. 1 L-min^{-1}) VO$_2$ [7]. Additionally, several studies have shown that eccentric cycling training produces greater increases in muscle strength and size compared with concentric cycling training [6, 7, 13]. Therefore, it has been advocated that eccentric cycling might be an ideal exercise to induce muscle mass and strength gains in the elderly, and for use by patients with pulmonary or coronary disease where cardiorespiratory fitness is reduced but that increases in muscle mass and strength are required [103].

One possible negative aspect of eccentric-dominant exercise is the risk of muscle damage, which is characterized by muscle weakness and delayed-onset muscle soreness (DOMS) after exercise. This is especially prevalent when it is performed first time or with a long interval from the previous exercise bout [14]. Muscle damage after eccentric exercise is directly evidenced by histological changes such as disruption of contractile and/or non-contractile proteins and plasma membrane [16, 17]. However, more common markers of muscle damage are increases in muscle proteins in the blood (e.g. creatine kinase: CK), prolonged loss of muscle function, swelling and DOMS [19]. When eccentric exercise is repeated within several weeks of the initial bout, changes in muscle damage markers are attenuated and recovery is enhanced, and this adaptation is referred to as the repeated bout effect [15].

Several studies have reported muscle damage induced by eccentric cycling exercise. Friden et al. [17] reported that 30 min of eccentric cycling performed at 80-100% of VO$_{2\text{max}}$ resulted in a 13 – 24% decrease in maximal isometric knee extensor strength (MVC) for three days after exercise, accompanied by myofibrillar Z-band disruption predominantly seen in type 2 muscle fibres. Klossner et al. [25] reported a 6% decrease in countermovement jump performance one day after 15 min of eccentric cycling at 50% of the concentric maximal power output (PO$_{\text{max}}$). Additionally, several studies have shown increases in muscle proteins in the blood [21-26] and muscle soreness [22, 25] following eccentric cycling exercise (15 – 60 min) at different intensities ranging from 50 to 150% of concentric PO$_{\text{max}}$. In order to safely apply eccentric cycling training in
elderly and/or clinical populations, it is necessary to understand the characteristics of eccentric cycling, including metabolic and muscle damage responses to the initial and secondary eccentric cycling bouts. Friden et al. [87] showed a decreased magnitude of muscle architectural disruption after 8 weeks of eccentric cycling training evidenced by well-preserved muscle fibres and non-affected Z-band widths after the last session of eccentric cycling training, when compared to that after the first eccentric cycling session. These data indicated that prolonged exposures to eccentric cycling might allow for a protective effect to accumulate. However, no previous studies have systematically examined muscle damage profile of eccentric cycling when it is repeated after an initial bout.

The purposes of this study, therefore, were to compare the metabolic costs of concentric cycling to both initial and secondary eccentric cycling bouts, and to subsequently compare changes in muscle damage markers following the three cycling bouts. We hypothesized that eccentric cycling would be less metabolically demanding than concentric cycling. Regarding muscle damage, we hypothesized that concentric cycling would not induce muscle damage, and also that severe muscle damage would be present after the first eccentric cycling bout but only minimal muscle damage would be present after the second bout.

### 3.2 Methods

#### 3.2.1 Participants

Ten healthy men who had not performed lower limb resistance training regularly in the past six months, and who reported no history of neurological disorders or orthopaedic lower limb injuries, completed an informed written consent form and a medical questionnaire before participating in the study. Ethical approval from the Institutional Human Research Ethics Committee was sought prior to the study. The participants’ mean (± SD) age, height, body mass, body mass index and peak oxygen consumption were 28.4 ± 8.3 yr, 179.0 ± 4.6 cm, 81.6 ± 13.1 kg,
25.5 ± 3.8 kg·m$^{-2}$, and 3.1 ± 0.5 L·min$^{-1}$, respectively. The sample size was estimated using the data from a previous study [105] in which changes in MVC strength of the knee extensors following isokinetic eccentric exercise (50 maximal isokinetic eccentric contractions of the knee extensors) were compared between the first and second bouts. Based on an $\alpha$ level of 0.05 and a power (1-$\beta$) of 0.8, with a potential 8% difference in the isometric strength between bouts at 1 day post-exercise, it was found that 10 subjects would be sufficient.

### 3.2.2 Study Design

Participants reported to the laboratory on three occasions each separated by two weeks, in which they performed one bout of 30 min of concentric cycling (CONC; visit 1) followed by two 30-min eccentric cycling bouts (ECC1 & ECC2; visits 2 & 3). In order to minimize possible effects of eccentric cycling on concentric cycling (we considered the effects of concentric cycling on eccentric cycling to be minimal), concentric cycling was performed first by all subjects. A familiarisation session for maximal voluntary isometric knee extensor strength and vertical jumps was performed 2-4 days before testing. Metabolic variables, including heart rate (HR), oxygen consumption (VO$_2$), blood lactate (BLa), tympanic temperature and rate of perceived exertion (RPE) data, were obtained during the 30-min cycling bouts. Also, surface electromyogram (EMG) data were recorded from vastus lateralis (VL) during cycling. In addition, maximal voluntary isometric knee extensor strength (MVC) and squat (SJ) and countermovement jump (CMJ) height were measured before, immediately after and 1-4 days after each eccentric cycling bout, and 1-2 days after concentric cycling. Plasma CK activity and muscle soreness ratings were measured before and 1-4 days after each eccentric cycling, and 1-2 days after concentric cycling. The shorter follow-up after eccentric cycling (i.e., 2 days) compared with eccentric cycling (i.e., 4 days) was due to the lack of significant changes in MVC, SJ and CMJ following concentric cycling found in a pilot study.
3.2.3 Cycling Exercise

Both the concentric and eccentric cycling bouts were performed at 60 rpm for 30 min at 60% of maximal concentric power output (PO\textsubscript{max}) based on the VO\textsubscript{2peak} test. Our pilot studies showed that 60% of the concentric PO\textsubscript{max} was close to the highest concentric power output that could be maintained for 30 min by our subjects. The VO\textsubscript{2peak} test was performed at least 96 h before the concentric cycling, and consisted of an incremental test using an electromagnetically braked recumbent ergometer (Tunturi F30R, Australia). The test started at 50 watts (W) for 4 min followed by 25-W increments every minute until volitional exhaustion. Cadence was kept at 60 rpm and participants received verbal encouragement during the test. Concentric cycling was performed on the same ergometer as that used for the VO\textsubscript{2peak} test, and eccentric cycling was performed on a recumbent ergometer with a motor that moved the cranks of the ergometer backwards at a selected cadence (Eccentric Trainer, Metitur, Finland). Participants were instructed to resist the backward movements of the cranks and maintain a steady level of power output displayed on a screen, in which a line was drawn at the target power output. This required eccentric contractions of mainly the knee extensor muscles. A familiarization period was performed immediately before the first eccentric cycling bout, which consisted of 5 min of cycling at ~50 W.

3.2.4 Metabolic and Physiological Parameters

Metabolic and physiological parameters included VO\textsubscript{2} measured using a metabolic cart (TrueOne 2400, Parvo Medics, USA), HR recorded by a Polar heart rate monitor (Polar RS800sd, Finland), BLa obtained from finger prick and measured by a Lactate Pro analyser (Arkay KDK, Japan) and RPE measured using Borg’s 6 – 20 scale. VO\textsubscript{2} and HR were recorded throughout the 30-min cycling bouts, and BLa and RPE measurements were taken at 10, 20 and 29 min of exercise. These time points were chosen because a pilot study showed that steady state was reached within 5 min, and no further changes in the metabolic parameters were found during cycling for the remaining 25 min. The average of these three time points was used for analyses. Tympanic temperature was measured before and immediately after cycling by a digital thermometer (First
Temp, Genius, USA), and the magnitude of change in the temperature from pre- to post-exercise was used for further analysis. Tympanic temperature was measured as an indicator of internal body temperature, since tympanic temperature has been shown to change similarly to rectal temperature during exercise [106].

### 3.2.5 Surface Electromyography

The surface electromyogram was recorded from VL during cycling using a Bagnoli-8 desktop EMG system (Delsys, USA) with a bipolar electrode configuration (DE-2.1 SEMG sensor, Delsys, USA) with 10-mm inter-electrode distance. Skin was shaved and cleansed with alcohol, and the electrodes were placed at the 2/3 of the distance from the anterior superior iliac spine to the patella according to SENIAM guidelines. The sampling frequency was set at 2000 Hz and an off-line digital filter was applied with a band pass of 10-450 Hz. EMG was recorded throughout the 30-min cycling bouts, but analysis was made for selected time points since a pilot study data did not show significant changes in root-mean-square (RMS) sEMG amplitude after 1 min of cycling. Thus, EMG analyses were made for the data at 1-2 min, 15-16 min and 29-30 min of each cycling. The 1-min time point was included since it would provide data in a non-fatigued state. RMS analysis was performed over 10 complete revolutions, and the average of the 10 revolutions was calculated and used as representation of each time point. EMG epochs were determined when EMG amplitudes increased more than two standard deviations above the baseline amplitude. The median frequency (MDF) of the power spectrum was obtained by a fast Fourier transform of 1024 points using a Hanning window with 50% overlap using the same epochs as for the RMS analyses [107].

### 3.2.6 MVC Strength

MVC strength of the knee extensors of the dominant leg (i.e. kicking leg) was measured at 70° of knee flexion [108], due to 70° being shown optimum for isometric torque production [109]. It was measured in a custom-made rigid chair with a load cell (Xtran S1W, Australia). After the participants performed a warm-up of 5-min cycling on an ergometer (Monark 828E, Sweden) at
9.81 N and 60 rpm, they were seated in the chair and performed three submaximal contractions (i.e. 50%, 50% and 80% of perceived maximum for 3 s each and 30 s of rest between contractions). The participants performed three 3-s maximal isometric contractions with a 1-min rest between contractions, and the maximum value was used for further analysis. The participants were instructed to contract as fast and hard as possible, and visual feedback was provided in real time on a computer screen.

3.2.7 **Vertical Jump**

After performing the MVC strength test, the participants were assessed for maximal squat jump (SJ) and counter movement jump (CMJ) height, in this order. For SJ height measurement, participants positioned themselves in a squat position (90° of knee flexion) and were instructed to jump from the position without any counter movement. CMJ height was measured in a jump where they started from a stand position and used countermovement prior to the upward (concentric) phase to jump as high as they could. Jump height was measured by a jump mat (Jump – MD, TKK 5106, Japan). The highest of three jumps was used for further analysis.

3.2.8 **Muscle Soreness**

Thigh muscle soreness was quantified using a 100-mm visual analogue scale (VAS) in which 0 indicates no pain and 100 represents the worst pain imaginable [15]. The participants were asked to mark the level of perceived pain of the quadriceps femoris muscle on the VAS whilst sitting on and standing from a 42-cm chair three times [110]. Pressure pain threshold (PPT) was also assessed at three sites using a digital algometer (Somedic AB, Sweden), including vastus medialis (VM) at 80% of the distance between anterior superior iliac spine (ASIS) and the patella, VL at 50% of the distance between ASIS and the patella, and rectus femoris at 50% of the distance between ASIS and the patella. The probe of the PPT algometer (1 cm² stimulation area) was placed perpendicular to the site and the investigator gradually applied force at a rate of 50 kPa·s⁻¹ until the
participants reported a pain from each muscle. The average of three measurements of muscle soreness (VAS) and PPT was used for further analysis [110].

3.2.9 Plasma CK Activity

A 35 μL blood sample was taken by a finger prick, and plasma CK activity was measured (without replication) by a spectrophotometer (Reflotron, Roche Diagnosis, Germany) using standard procedures.

3.2.10 Statistical Analysis

A one-way analysis of variance (ANOVA) was used to compare the average power output performed during each cycling bout. Average HR, VO₂, RPE and BLa during cycling were compared amongst the three cycling bouts by a one-way ANOVA. When significant differences were found, a Fisher’s LSD post hoc test was used to determine the location of the change. A two-way repeated measures ANOVA (bout x time) was used to compare changes in MVC, CMJ and SJ, VAS and PPT over time between CONC and ECC1, and CONC and ECC2, and between ECC1 and ECC2. A two-way repeated measures ANOVA was also used to compare changes in RMS and MDF during the 30-min of cycling in the same way to the muscle damage parameters. If a significant bout, time or interaction effect was found, a Bonferroni post hoc test was used for pairwise comparisons. The significance level was set at P<0.05. All statistical analyses were performed with PASW Statistics 19 software for Mac (SPSS inc, IBM company, USA). Data are presented as mean ± standard error of mean (SEM).

3.3 Results

3.3.1 Metabolic and Physiological Parameters

Average cycling power output was 158.5 ± 9.2 W, 169.9 ± 26.7 W and 179.3 ± 6.1 W for CONC, ECC1 and ECC2, respectively, with no significant difference being found between bouts.
As shown in Figure 3.1, HR was 19% lower (126.7 ± 8.5 vs. 156.1 ± 5.9 beats min$^{-1}$, $P=0.79$), BLa was 65% lower (2.7 ± 0.5 vs. 7.6 ± 0.8 mmol L$^{-1}$, $P<0.001$), VO$_2$ was 50% lower (1.2 ± 0.1 vs. 2.3 ± 0.1 L min$^{-1}$, $P<0.001$), and RPE was 22% lower (10.6 ± 2.9 vs. 13.6 ± 2.4, $P=0.001$) in ECC1 when compared to CONC. HR, BLa, VO$_2$ and RPE during ECC2 were also lower than those during CONC (29%, 77%, 51% and 23%, respectively, $P<0.05$). In addition, a 12% lower HR (111.4 ± 8.5 vs. 126.7 ± 8.5 beats min$^{-1}$, $P=0.003$) and 35% lower BLa (1.7 ± 0.4 vs. 2.7 ± 0.5 mmol L$^{-1}$, $P=0.002$) were seen in response to ECC2 when compared to ECC1. A significant increase in tympanic temperature from pre- to post-exercise (0.45 ± 0.16°C) was found only after CONC.

**Figure 3.1:** Comparison of the average oxygen consumption (A), heart rates (B), rates of perceived exertion (C) and blood lactate values (D) between concentric (CONC) and the first (ECC1) and second eccentric (ECC2) cycling bouts during 30 minutes of cycling. #: significantly ($P<0.05$) different from CONC, †: significantly ($P<0.05$) different from ECC1.
3.3.2 Surface Electromyography

A significant main effect for bout was found for RMS EMG amplitude between CONC and ECC1 (P=0.01), CONC and ECC2 (P=0.00), and ECC1 and ECC2 (P=0.016). Although there was no interaction effect, pairwise comparisons revealed that RMS EMG amplitude was greater (P=0.01) during CONC than ECC1 and ECC2 at all time points (1, 15 and 29 minutes), and EMG amplitude was lower (P=0.02) during ECC2 than ECC1 at 15 min (Figure 3.2A). Significant interaction effects were found when MDF was compared between CONC and ECC1 (P=0.002), and CONC and ECC2 (P=0.012) without a main effect for bout. Pairwise comparisons revealed that MDF was greater (P=0.04) during ECC1 compared to CONC only at 1 min (Figure 3.2B), and significant increases in MDF over time were observed only for CONC.
**Figure 3.2:** Root mean square sEMG amplitude (A) and sEMG mean frequency (B) at 1, 15 and 29 min during concentric (CONC) and the first (ECC1) and second eccentric (ECC2) cycling bouts. *: significant (P<0.05) bout-by-time interaction effect, #: significantly (P<0.05) different from CONC, †: significantly (P<0.05) different from ECC1.

### 3.3.3 MVC Strength

No significant difference in the baseline MVC strength was evident between CONC (282.3 ± 14.7 Nm), ECC1 (268.4 ± 15.5 Nm) and ECC2 (282.1 ± 13.1 Nm). Nonetheless, significant bout-by-time interaction effects were found between CONC and ECC1 (P=0.04), and between ECC1 and ECC2 (P=0.044). Also, significant main effects for bout were found between CONC and ECC1 (P=0.045), and between ECC1 and ECC2 (P=0.001) for the changes in MVC strength (Figure 3.3A). MVC strength was lower (P=0.005) immediately after and 1-2 days after ECC1 compared with CONC and ECC2, and was lower (P=0.001) at 1-4 days following ECC1 than ECC2. MVC strength returned to baseline by 1 day after CONC and ECC2, but remained below the baseline until 4 days after ECC1. An increase in MVC strength (6.5 ± 2.7%; P=0.04) was seen at 4 days post-ECC2.

### 3.3.4 Vertical Jump

No significant differences in SJ (average: 41.3 ± 5.2 cm) or CMJ height (45.4 ± 4.4 cm) were evident before exercise between CONC, ECC1 and ECC2. However, significant bout effects were found between CONC and ECC1 (P=0.045), and ECC1 and ECC2 (P=0.037), with an interaction effect between ECC1 and ECC2 (P=0.015). Significant decreases in CMJ height (7–12%) from baseline were seen at 1-3 days following ECC1 only (Figure 3.3B). CMJ height was lower (P=0.045) 1-2 days post-ECC1 compared with CONC, and it was lower (P=0.037) than ECC2 at 2-3 days. Similarly, significant bout effects were found between CONC and ECC1 (P=0.046), and ECC1 and ECC2 (P=0.045), with significant interaction effects between CONC and
ECC1 (P=0.025), and ECC1 and ECC2 (P=0.0016) for SJ. Pairwise comparison revealed that SJ height decreased significantly only after ECC1, and was 17-22% lower (P=0.046) immediately and 1 day post-exercise for ECC1 than CONC (Figure 3.3C). When comparing between ECC1 and ECC2, SJ height was 12-14% lower (P=0.045) at 1-2 days after ECC1.
Figure 3.3: Normalized changes in maximal voluntary isometric knee extensor strength (A), squat jump height (B) and counter movement jump height (C) from baseline (100%) immediately after (Post) and 1-4 days following concentric (CONC) and the first (ECC1) and second eccentric (ECC2) cycling bouts. *: significant (P<0.05) bout-by-time interaction effect, #: significantly (P<0.05) different from CONC, †: significantly (P<0.05) different from ECC1.

3.3.5 Muscle Soreness

Significant bout and interaction effects were evident between CONC and ECC1 (P=0.001 and P=0.001, respectively), and ECC1 and ECC2 (P=0.003 and P<0.001, respectively) for muscle soreness assessed by VAS. There was significant muscle soreness 1-2 days after ECC1 compared to CONC, and 1-4 days when compared to ECC2, but no increase in muscle soreness was found after CONC (Figure 3.4A). PPT results were similar for VM, VL and RF, and Figure 3.4B shows data for VM. The baseline PPT was 839.7 ± 82.5 kPa, and no significant difference was seen between CONC, ECC1 and ECC2. Significant main bout and interaction effects (P=0.006 and P=0.005, respectively) were found when CONC and ECC1 were compared, and an interaction effect was found between CONC and ECC2 (P=0.05). Significant decreases in PPT were observed 1-3 days after ECC1, 1-2 days after ECC2 and 1 day after CON.

3.3.6 Plasma CK Activity

Small but significant increases in plasma CK activity were evident 1 day after CONC (219.5 ± 40.3 IU·L⁻¹) and ECC1 (246.5 ± 33.0 IU·L⁻¹), but not after ECC2 (173.1 ± 16.0 IU·L⁻¹) from the baseline (average: 147.4 ± 21.1 IU·L⁻¹). No significant difference in the change was seen between the bouts.
Figure 3.4: Changes in muscle soreness responses assessed by a visual analogue scale (0-100 mm) (A) and normalized changes in pressure pain threshold of vastus medialis (B) from baseline (100%) before (Pre) and 1-4 days following concentric (CONC) and the first (ECC1) and second eccentric (ECC2) cycling bouts. *: significant (P<0.05) bout-by-time interaction effect, #: significantly (P<0.05) different from CONC, †: significantly (P<0.05) different from ECC1.

3.4 Discussion

Oxygen consumption during eccentric cycling was approximately 50% of that during concentric cycling for the same workload (~165 W), confirming the findings of previous studies and highlighting the fact that eccentric cycling is less cardiopulmonary demanding than concentric cycling [8, 69]. A new finding of the present study, however, was that heart rate and blood lactate
responses were further reduced by 12-35%, when eccentric cycling was repeated. This is suggestive of a further reduction in metabolic stress during the repeat exercise bout. Regarding muscle damage, eccentric cycling resulted in loss of muscle function (i.e. MVC strength and vertical jump) and noticeable DOMS. However, almost no symptoms of muscle damage were observed when it was performed two weeks later. These results support our hypothesis that muscle damage would be minimal after the second eccentric cycling bout.

3.4.1 Metabolic Profile

Previous researchers have reported that eccentric cycling requires only 25-30% of the oxygen (VO\textsubscript{2}) required for concentric cycling at the same workload [8, 11, 69], that HR during eccentric cycling is about two thirds of that during concentric cycling at maximal intensity [12], and that eccentric cycling does not promote an noticeable increase in BLa [69]. However, as shown in Figure 3.1, VO\textsubscript{2} during eccentric cycling was 50% of that measured during concentric cycling, HR during eccentric cycling was 81% of that in concentric cycling, and a low BLa concentration (2.7 mmol\textperiodcentered L\textsuperscript{-1}) was found after the first eccentric cycling bout. Thus, the metabolic demand of eccentric cycling found in the present study was greater than that reported in previous studies [10, 11]. A possible explanation is that the magnitude of the difference in the metabolic cost between eccentric and concentric cycling becomes greater at higher workloads, and that eccentric cycling is relatively more metabolically efficient at higher loads. The workload was set at 60% of the concentric PO\textsubscript{max} (~165 W) in the present study, and it was close to the limit that could be maintained by participants for the 30-min concentric cycling bout. However, this is much lower than that (330-600 W maintained only for 30-s to 6 min) used in previous studies [8, 69]. It is also important to note that the participants in the present study were unaccustomed to eccentric cycling and were deliberately given no familiarisation, whereas participants in previous studies were accustomed to it. When comparing the first and second eccentric cycling bouts, a 12% lower HR was elicited in the second bout compared with the first, and only a low BLa concentration was detected in the second bout.
Although no significant differences between the first and second eccentric cycling bouts were found for VO$_2$ and RPE (Figure 3.1), it may be that VO$_2$ and RPE during eccentric cycling decrease with repeated exposures and the metabolic differences between eccentric cycling and concentric cycling become greater.

It was found that eccentric cycling did not stimulate an increase in tympanic temperature during either the first or second bouts, while a significant increase in temperature was evident after concentric cycling. Nadel et al. [72] found that internal (i.e. oesophageal) temperature was consistently lower (~0.7°C) during eccentric cycling (~37.6°C) compared with concentric cycling (~38.3°C), but quadriceps intramuscular and skin temperatures were higher during eccentric cycling (40°C and 34°C, respectively) compared with concentric cycling (38.8°C and 31°C, respectively) at same metabolic intensity (ECC=381 W and CONC= 102 W for 30-40 min cycling). It is interesting that the substantial muscle work performed during eccentric cycling did not increase internal temperature in the present study. The lack of increase in the internal temperature during eccentric cycling could be due to total work being done by the muscles during eccentric cycling, which therefore consumed significantly less oxygen than concentric cycling. This could induce less cardiopulmonary distress and also less activation of the respiratory muscles during eccentric cycling, inducing a smaller increase in internal temperature. However, the effect of elevated muscle temperature on muscle function in healthy and fragile individuals has been poorly investigated. Importantly, it is possible the lack of response could be beneficial for cardiac and respiratory patients, or elderly people with impaired thermoregulatory capacity. Eccentric cycling thus appears to be a low-risk exercise from a temperature regulation perspective, when compared to concentric cycling.

The lower metabolic cost of eccentric cycling is likely attributable to the lesser muscle activity when compared with concentric cycling. As shown in Figure 3.2A, EMG amplitude was consistently lower for eccentric cycling compared with concentric cycling. Kellis and Baltzopoulos [54] reported that VL EMG amplitudes during maximal eccentric isokinetic knee extensor
contractions at different velocities were 11-52% lower than during concentric contractions, which may be attributed to a smaller portion of the motoneuron pool being recruited during eccentric contractions [63]. It is possible, therefore, that the 38% (ECC1) and 53% (ECC2) lower EMG amplitudes obtained during eccentric cycling compared to concentric cycling are also reflective of a reduced motoneuron activation. The lesser muscle activity during ECC2 compared with ECC1 could represent an enhanced cycling efficiency (i.e. maintenance of power output with a lesser muscle activity), which is evidenced by the decreased HR and BLa in ECC2. LaStayo et al. [111] observed a decreased EMG amplitude during eccentric cycling in subjects after 8 weeks of eccentric cycling training compared to a group of subjects unaccustomed to eccentric cycling, and speculated that the decrease was related to a lower level of motor neurone activation or to an activation of only a subset of the entire motor unit population within the muscle, or both [111].

Interestingly, the EMG median frequency (MDF) was 13% greater during ECC1 than CONC at the beginning of exercise as shown in Figure 3.2B (i.e. 1 min), which could be taken to indicate that a greater proportion of fast-twitch motor units were activated or that motor units were fired at a higher rate during eccentric cycling [112]. McHugh et al. [57] found a greater MDF and lesser VL EMG amplitude during maximal eccentric compared to concentric isokinetic (60°·s⁻¹) contractions of the knee extensors, and speculated that a greater proportion of fast-twitch motor units were active during the eccentric contractions [57, 113]. However, controversy exists as to whether the greater MDF is indicative of a preferential recruitment of fast-twitch motor units during eccentric contractions, and a faster motor unit firing rate has been proposed to explain the difference in mean or median frequency between eccentric and concentric contractions [112]. Indeed, it has been recently proposed that changes in EMG amplitude and frequency are also affected by adaptations in the muscle fibres themselves [114]. Dimitrov et al. [114] suggested that the shift in the EMG frequency content towards lower frequencies and decreases in EMG amplitude during the second bout of eccentric contractions could be attributed also to changes in the shape of the intracellular action potential (i.e. increase in intracellular action potential duration and in the negative after
potentials). They speculated that these were due to the long lasting elevated resting cytoplasmic Ca$^{2+}$ caused by an increase in membrane permeability after eccentric exercise, and due to motor unit synchronisation after muscle damage induced by eccentric contractions. Therefore, it could be that peripheral (i.e. intracellular action potential shape), in addition to central (i.e. motor unit recruitment) adaptations affect the amplitude and frequency changes observed when eccentric exercise was performed repeated.

### 3.4.2 Muscle Damage Profile

Several researchers have reported significant muscle damage being induced by eccentric cycling [17, 21-25]. However, for the first time, the magnitude of muscle damage between two consecutive eccentric cycling bouts and a concentric cycling bout were compared in the present study. The results revealed 10–25% decreases in muscle function after the first eccentric cycling bout, which lasted for 2 - 3 days (Figure 3.3), and that moderate DOMS was developed after the cycling (Figure 3.4). However, the increase in plasma CK activity in the present study was small after both the first and second eccentric cycling bouts, and was not different from concentric cycling. If a large increase in plasma CK activity indicates muscle fibre necrosis [115], then the small increases in CK activity detected in the present study suggest that myocellular disruption was minimal. One might speculate that the decreased muscle function and DOMS after eccentric cycling resulted from damage and inflammation to the muscle extracellular matrix occurring without significant myocellular disruption. Unfortunately, markers of connective tissue damage were not measured in the present study, however markers such as matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, plasmatic fragments of collagens I - III should be examined in the future.

Muscle damage markers after the first eccentric cycling bout were similar in magnitude to those reported after other eccentric exercise modalities. For instance, the magnitude of loss in MVC strength was similar to that seen 1-3 days after 45 min of downhill (-10% slope) running [116] and 1-2 days after 75 submaximal (75%) isokinetic eccentric contractions of the knee extensor muscles [49]. However, MVC strength loss following eccentric cycling was smaller when compared with
that (30-40% decrease) measured 1-3 days after 50 maximal isokinetic eccentric contractions [117], and MVC strength in the present study recovered faster than after 30 min of downhill (-15% slope) running [104]. Increases in plasma CK activity after eccentric cycling were smaller compared to those found after 30 min of downhill running on a -15% slope (peak CK activity: 462 IU·L⁻¹), possibly due to the greater muscle mass involved in the exercise [104] and smaller than after 50 knee extensor maximal isokinetic eccentric contractions (2,815 IU·L⁻¹) [117], most likely due to the maximal instead of submaximal eccentric contractions. However, muscle soreness levels appeared to be similar to those observed after other eccentric exercise modalities for the knee extensors [49, 104, 117]. Therefore, the magnitude of knee extensor muscle damage induced by eccentric cycling at 60% of concentric POmax seems similar to other submaximal eccentric exercises.

Following the second eccentric cycling bout, however, changes in MVC strength, jump height and muscle soreness were minimal, and were not different from those after concentric cycling. This represents a typical repeated bout effect that is characterised by smaller changes in, and faster recovery of, muscle damage markers following the second eccentric bout compared with the first, which has been reported previously for numerous other eccentric exercises [3, 14, 15]. The repeated bout effect has been speculated to be associated with neural, mechanical and cellular adaptations [14]. Typically, neural adaptations include a change in the motor unit activation pattern for a given muscle force, increasing activation of slow-twitch fibres, and a decreasing stress on fast-twitch fibres; mechanical adaptations may include changes in tendon and/or muscle connective tissue stiffness, or an improved efficiency of muscle-tendon force transmission during the second bout; and cellular adaptations might include a reduced inflammatory response and requirement for remodelling of muscle fibres and the extracellular matrix [14]. From the present data, it is not possible to determine the mechanisms underpinning the repeated bout effect, however an important finding is that muscles seem to adapt to eccentric cycling rapidly to minimize muscle damage in the subsequent bout of eccentric cycling. Thus, the potential for muscle damage after eccentric cycling training should not be a major factor influencing the decision to use eccentric cycling in the longer
term. Since only young, healthy men were used in the present study, further studies are required to examine the muscle damage profile in response to eccentric cycling in older individuals and clinical populations.

In conclusion, eccentric cycling was less metabolically demanding than concentric cycling, and the metabolic stress of eccentric cycling was further reduced in a second bout performed two weeks later. In addition, less metabolic stress was induced during eccentric cycling shown by a smaller increase in HR and BLa, lower RPE and lack of change in EMG MDF during cycling compared to concentric cycling. Eccentric cycling resulted in moderate muscle damage when it was performed for the first time, but the second bout of eccentric cycling resulted in little or no sign of muscle damage. Thus, the potential for muscle damage and the subsequent muscle soreness should not be a factor influencing the decision to use eccentric cycling, and eccentric cycling could be an effective and well tolerated exercise modality for elderly individuals and clinical populations.
CHAPTER 4

Study 2: Energy Expenditure and Substrate Oxidation During and After Eccentric Cycling

4.1 Introduction

Eccentric cycling requires knee extensor muscles to perform lengthening (eccentric) contractions whilst braking the backward rotation of the cranks. It was introduced in 1952 for the first time in the literature [9], but its health benefits have only been documented in the last 10 years or so. For example, LaStayo et al. [13] showed that eccentric cycling training produced 60% greater increases in muscle strength and muscle fibre cross-sectional area than resistance training in elderly individuals. Since oxygen consumption during eccentric cycling is 40–50% of that of concentric cycling at the same given workload [69, 71], eccentric cycling appears to be an attractive training modality for elderly, cardiac and pulmonary patients who may not tolerate high-intensity exercise.

An inability to oxidise lipids (e.g. decreased activity of lipoprotein lipase and citrate synthase, and carnitine palmitoyl transferase content) appears to be an important factor in the aetiology of obesity, and a reduced level of fat oxidation is associated with a high rate of weight gain [118]. Exercise has been used to improve oxidative function in obese individuals [119]. Although eccentric cycling training has been used in obese and overweight women [52], the substrate utilisation during and after exercise has not been described. It is well documented that carbohydrates (CHO) and free fatty acids are the dominant fuels oxidised by the muscles during exercise, and that the absolute and relative contributions of these fuels are influenced by exercise intensity and duration, as well as training status [120]. Many studies have used indirect calorimetry
to estimate CHO and fat oxidation from the respiratory exchange ratio (i.e. $\text{VCO}_2/\text{VO}_2$; RER) [121]. It has been reported that fat utilisation rate is maximal during concentric cycling at 43% and 50% of the maximal oxygen consumption for untrained and trained individuals, respectively [122]. Shuenke et al. [123] showed an 11% increase in fat utilisation after 31 min of resistance exercise, which remained significantly elevated (19–21%) at 38 h post-exercise. However, despite its use as exercise modality in overweight individuals [52], the substrate utilisation during and after eccentric cycling has not been documented. Before the prescription of eccentric cycling to elderly and diseased individuals, it is important to understand the CHO and fat oxidation characteristics of eccentric cycling in comparison to concentric cycling.

An increase in energy expenditure has been suggested to be an important factor for weight management [36], and resting energy expenditure (REE) accounts for approximately 60–75% of an individual’s daily energy expenditure [124]. Importantly, REE increases after exercise and is thought to significantly impact the regulation of body weight [124]. For example, REE is reported to be increased by 5% at 1–3 days after a resistance exercise bout [125], and Paschalis et al. [39] reported 25% and 9% increases in REE in overweight and lean individuals, respectively, 1–3 days after 5 sets of 15 maximal isokinetic eccentric contractions of the knee extensors. These authors speculated that a higher proportion of fast-twitch muscle fibres in the overweight individuals might account for the greater muscle damage induced. Thus, it is possible that an initial eccentric cycling bout induces a greater perturbation in muscle homeostasis, which requires more energy for restoration, than a second bout because of the well-known repeated bout effect [14, 15]. One might speculate, therefore, that repeated bouts of eccentric cycling would not yield beneficial energy expenditure changes in comparison to concentric cycling. In Study 1 (Chapter 3) it was reported that an initial eccentric cycling bout induced significant muscle damage, but that repeated bouts (performed 2 weeks later) induced little or no muscle damage [15, 71]. Thus, it is possible that resting energy expenditure after eccentric cycling differs between the first and second eccentric cycling bouts.
Given the above, the aims of the present study were to compare a bout of concentric cycling and two bouts of eccentric cycling performed at the same workload for substrate utilisation during and immediately after exercise, and for changes in REE and glucose tolerance. We hypothesized that energy expenditure during exercise would be lesser and fat utilisation greater during eccentric than concentric cycling. We also hypothesized that post-exercise energy expenditure and fat oxidation would be less after both eccentric cycling bouts than concentric cycling, and that resting energy expenditure and the decrease in glucose tolerance would be greater after the first than the second eccentric cycling bout.

4.2 Methods

4.2.1 Participants

Ten healthy men who had not performed lower limb resistance training regularly in the past six months and who had no history of neurological disorders or orthopaedic lower limb injuries, completed an informed written consent form and a medical questionnaire before participating in the study. Ethical approval was sought from the institutional Human Research Ethics Committee prior to the study. The participants’ mean (± SD) age, height, body mass, body mass index (BMI) and peak oxygen consumption during cycling were 28.4 ± 8.3 y, 179.0 ± 4.6 cm, 81.6 ± 13.1 kg, 25.5 ± 3.8 kg⋅m⁻², and 38.6 ± 6.7 ml⋅min⁻¹⋅kg, respectively. As in Study 1, the sample size was estimated using the data from a previous study [105] in which changes in MVC strength of the knee extensors following isokinetic eccentric exercise (50 maximal isokinetic eccentric contractions of the knee extensors). Based on an α level of 0.05 and a power (1-β) of 0.8, with a potential 40% difference oxygen consumption between the concentric and eccentric cycling bout, it was found that 10 participants would be sufficient.
4.2.2 Study Design

Participants reported to the laboratory for four testing blocks consisting of 1–4 days each over 7 weeks. In the first visit a control (non-exercise) baseline measure of resting energy expenditure (REE) was taken, and the first oral glucose tolerance test (OGTT) was performed. At 3 days after the baseline measure, participants performed the first exercise bout of 30 min of concentric cycling (CONC) and recovery from the exercise was followed for 2 days. With two-week intervals, they performed the first 30-min eccentric cycling bout (ECC1) and then a second eccentric cycling (ECC2), and recovery was followed for 4 days after each exercise. As we considered that the effects of concentric cycling on eccentric cycling would be minimal, concentric cycling was performed first by all subjects. Dietary intake was determined before exercise testing and participants were asked not to change their food intake throughout the testing days. The OGTT was repeated 1 day after CONC, ECC1 and ECC2, and 3 days after ECC1 and ECC2. REE was measured at 2 days after CONC, ECC1 and ECC2, and 4 days after ECC1 and ECC2. REE and OGTT measurements were taken on different days to eliminate the effect of diet-induced thermogenesis (i.e. 75 g glucose ingestion), and time constrains. The shorter follow-up after concentric cycling (i.e., 2 days) compared with eccentric cycling (i.e., 4 days) was due to the lack of significant REE changes following concentric cycling found in a pilot study.

4.2.3 Cycling Exercise

Both the concentric and eccentric cycling bouts were performed at 60 rpm for 30 min at 60% of concentric maximal power output (PO_max) based on the peak oxygen consumption (VO_2peak) determined by an incremental exercise test. Our pilot studies showed that 60% of the concentric PO_max was close to the highest concentric power output that could be maintained for 30 min by participants who were similar to those used in the present study. In this study, the concentric and eccentric cycling bouts were equated for PO_max, since it was thought that the work-matched design would be a better comparison than intensity-matched design, because of the pronounced characteristic of eccentric cycling being its less metabolic demand and possible greater work than
The test was performed on an electromagnetically braked recumbent ergometer (Tunturi F30R, Australia) at least 4 days before the concentric cycling. The test started at 50 watts (W) for 4 min followed by a 25-W increment every minute until volitional exhaustion, and the cadence was kept at 60 rpm. The participants received verbal encouragement during the test, and the peak oxygen consumption value obtained during the last incremental stage completed for at least 30 s was regarded as VO$_{2\text{Peak}}$. Oxygen consumption was determined using a ParvoMedics TrueOne 2400 metabolic cart (ParvoMedics, Sandy, USA) that was calibrated according to the specifications of the manufacturer [126]. Concentric cycling (CONC) was performed on the same ergometer as that used for the VO$_{2\text{Peak}}$ test, and two bouts of eccentric cycling (ECC1 & ECC2) were performed on a recumbent ergometer with a motor that moved the cranks of the ergometer backwards at a selected cadence (Eccentric Trainer, Metitur, Finland). Participants were instructed to resist the backward movements of the cranks and maintain a steady level of power output displayed on a screen. A familiarisation period was performed immediately before the first eccentric cycling bout, which consisted of 5 min of cycling at ~50 W. Room temperature during exercise was 25 ± 1°C with 30-40% relative humidity.

### 4.2.4 Resting Energy Expenditure (REE)

REE was measured in the morning (06:30–9:00 am) at 3 days before CONC (control), 2 days after CONC, and 2 and 4 days after ECC1 and ECC2. Participants refrained from food or any liquid except water for 12 h, and were instructed to maintain their normal diet. They reported their diet during the first week of the study, including food names, serving sizes and times of ingestion, and this report was provided to the participants so they could reproduce the diet during the following experimental weeks. After entering the laboratory the participants rested supine for 10 min prior data collection without wearing a mouthpiece [127]. Steady state REE was measured in supine position for 30 min in a semi-darkened, quiet and thermoregulated room (23°C). Subjects were advised to remain awake and not to move or talk during the measurement. REE was
determined using the respiratory exchange ratio (RER) from the data provided by a ParvoMedics TrueOne 2400 metabolic cart (ParvoMedics, Sandy, USA), which was calibrated before each test. RER was collected for 30 min, and REE was estimated using the last 15 min of the data (steady state), and 24-h REE was also estimated using a Weir equation [128] as previous studies (e.g. [38, 121] did.

4.2.5 Substrate Oxidation

Fat and CHO utilisation were estimated from the RER measured by the metabolic cart (ParvoMedics, Sandy, USA). According to the specifications of the manufacturer [126], the oxygen and carbon dioxide analysers were calibrated by nitrogen and two primary standard gases, and the pneumotachometer was calibrated using a 3-L syringe to deliver fixed volumes at variable flow rates. Fat and CHO utilisation were estimated during the 30 min of cycling, and at 10 min after cycling for 30 min in a supine position in a semi-darkened, quiet, thermoregulated room (23°C) [129]. The participants were advised to remain awake and not to move or talk during the measurement. Substrate oxidation was estimated using the stoichiometric equations assuming a non-protein respiratory quotient [121].

\[
\text{Carbohydrate oxidation} = 4.585 \cdot \text{VCO}_2 - 3.226 \cdot \text{VO}_2 \quad [130]
\]

\[
\text{Fat oxidation} = 1.695 \cdot \text{VO}_2 - 1.701 \cdot \text{VCO}_2 \quad [130]
\]

4.2.6 Oral Glucose Tolerance Test (OGTT)

An oral glucose tolerance test (OGTT) with 75 g of glucose was performed after an overnight (8-12 h) fast at 4 days before CONC, 1 day after CONC, and 1 and 3 days after ECC1 and ECC2. Blood samples (~3.5 ml) were taken from the antecubital vein by a standard venipuncture technique using a vacutainer fluoride tube (Vacutainer, BD, Australia) before and 30,
60, 90 and 120 min after the glucose ingestion. The blood samples were centrifuged at 3,000 g for 15 min at 4°C to obtain plasma, which was aliquoted to tubes and stored at -80°C until analyses. Plasma glucose concentration was assessed by a glucose oxidase assay kit (BioAssay Systems, Australia). The peak and area-under-the-curve (AUC) of blood glucose for the 120 min period were calculated using the trapezoidal method [4].

4.2.7 Statistical Analysis

A one-way analysis of variance (ANOVA) was used to compare the average power output performed during cycling between conditions (CONC, ECC1 and ECC2). Total energy expenditure and substrate oxidation during and after exercise were also compared between conditions by a one-way ANOVA. If a significant main effect was found, a Fisher’s least significant difference post-hoc test was used to determine differences between the bouts. A two-way repeated measures ANOVA was used to compare changes in REE and OGTT (peak and AUC) over time (before, and 1 or 2 days post-exercise) between CONC and ECC1 or ECC2, and specific comparisons between ECC1 and ECC2 for the changes in REE and OGTT over time (before, 1 and 3 days for REE, and before, 2 and 4 days for OGTT) were also made using a two-way repeated measures ANOVA. When significant interaction effects were found, a Fisher’s least significant difference post-hoc test was used to compare between bouts for each time point. The significance level was set at P<0.05. All statistical analyses were performed with PASW Statistics 21 software for Mac (SPSS inc, IBM company, USA). Data are presented as mean ± standard deviation (SD).

4.3 Results

4.3.1 Cycling Exercise

All subjects completed the three cycling conditions as instructed. Average cycling power output was 158.5 ± 9.2 W, 169.9 ± 26.7 W and 179.3 ± 6.1 W for CONC, ECC1 and ECC2,
respectively, with no significant difference between the bouts (P=0.79). The cycling intensities in CONC, ECC1 and ECC2 were 76.9 ± 5.1%, 38.6 ± 10.6% and 37.1 ± 22.2% of VO$_{2peak}$, respectively. No significant difference was evident between ECC1 and ECC2 (P=0.54), but the metabolic intensity was significantly lower for ECC1 and ECC2 than CONC (P<0.001).

### 4.3.2 Substrate Oxidation During Exercise

Figure 4.1 shows the total energy expenditure during cycling and the estimated CHO and fat contributions. Total energy expenditure during cycling was not different between ECC1 and ECC2 (P=0.617), but was 36% and 40% smaller during ECC1 and ECC2, respectively, than CONC (P<0.001). CHO utilisation during exercise was 42% and 52% less (P<0.001) during ECC1 and ECC2 than CONC, respectively, but there was no difference between ECC1 and ECC2 (P=0.195). All participants showed a significantly greater fat utilisation during ECC1 (72%; range difference: +9.6 to 154.2 kJ; P=0.004) and ECC2 (85%; range difference: +97.9 to 313.3 kJ; P<0.001) when compared with CONC. Furthermore, fat utilisation during exercise was significantly greater during ECC2 compared with ECC1 for all participants (48%; range difference: +7.9 to 179.1 kJ; P=0.001).

**Figure 4.1:** Carbohydrate (CHO) and fat oxidation-dependent energy expenditure during the 30-min concentric cycling bout (CONC), and the first (ECC1) and second eccentric (ECC2) cycling bouts. There was less total energy expenditure during ECC1 and ECC2 than CONC, but greater fat
was utilized during ECC1 and ECC2 than CONC. 

- **a**: significantly (P<0.05) less total energy expenditure than CONC,
- **b**: significantly (P<0.05) greater total fat oxidation than CONC,
- **c**: significantly (P<0.05) less total CHO oxidation from CONC, and
- **d**: greater (P<0.05) different total fat oxidation from ECC1.

### 4.3.3 Substrate Oxidation After Exercise

Figure 4.2 shows the total post-exercise energy expenditure for the 30 min from 10 to 40 min after cycling, and the substrate (CHO, fat) utilisation. Energy expenditure after exercise was 30% less following ECC1 (P=0.048) when compared with CONC, and there was a tendency (P=0.068) toward a greater (28%) total post-exercise energy expenditure following ECC2 than ECC1 (range difference: -27.9 to 250.7 kJ). CHO utilisation after cycling was not different between exercise bouts (P=0.158). Compared with CONC, fat utilisation was 52% smaller after ECC1 (range difference: -180.7 to 35.9 kJ; P=0.021), but no difference was found between CONC and ECC2 bouts (range difference: -77.8 to 83.0 kJ; P=0.648). There was a tendency for a greater fat utilisation after ECC2 than ECC1 (47%; range difference: -27.9 to 250.7 kJ; P=0.056).

![Figure 4.2: Carbohydrate (CHO) and fat oxidation-dependent energy expenditure after- concentric cycling (CONC), and the first (ECC1) and second eccentric (ECC2) cycling bouts. There was a](image-url)
lesser energy expenditure and fat utilisation after ECC1 compared to CONC, but no statistical difference was found between CONC and ECC2. *a:* significantly (P<0.05) less total energy expenditure than CONC, *b:* significantly (P<0.05) lesser total fat oxidation than CONC.

### 4.3.4 REE

REE was steady during the last 15 min of the expired gas collection as shown by a 5.7 ± 2.7% average coefficient of variation (CV), which was similar to that reported in a previous study [131]. As shown in Figure 4.3A, REE did not change after any cycling condition. Estimated 24-h REE did not change after cycling compared to baseline (7791.9 ± 1040.1 kJ), and no significant difference between bouts was evident as is shown in Figure 4.3B.

![Figure 4.3](image)

**Figure 4.3:** Resting energy expenditure for 15 minutes (A) and 24-h estimation (B) before (control) and 2 days after concentric cycling (CONC), and 2 and 4 days after the first (ECC1) and second eccentric (ECC2) cycling bouts. There were no significant differences between cycling bouts and pre-exercise (control) condition.

### 4.3.5 OGTT

Peak blood glucose concentration during OGTT did not change after any cycling bout from the baseline (9.1 ± 0.9 mmol·L⁻¹). No significant changes in AUC from baseline (786.3 ± 65.2 mmol·L⁻¹·120 min⁻¹) were observed at 1 and 3 days after any cycling bouts.
**Figure 4.4:** Plasma glucose concentration area under the curve (AUC) during the oral glucose tolerance test before (control) and 1 day after concentric (CONC), and 1 and 3 days after the first (ECC1) and second eccentric (ECC2) cycling bouts. Glucose uptake was up-regulated 3 days after ECC1 when compared to control (Pre-exercise) measurements. *: Significantly (P<0.05) different from control.

### 4.4 Discussion

It was hypothesised that 1) energy expenditure during eccentric cycling would be less but fat utilisation would be greater than concentric cycling at the same workload; 2) post-exercise energy expenditure would be less after both eccentric cycling bouts than concentric cycling; and 3) resting energy expenditure would be increased and glucose uptake would be decreased more after the first than second eccentric cycling bout. The results show that fat utilisation was significantly greater during both eccentric cycling bouts compared with concentric cycling at the same given workload, supporting the initial hypothesis (Figure 4.1). In partial support of the second hypothesis, the increase in post-exercise energy expenditure was less after the first eccentric cycling bout compared with concentric cycling, but was not different to concentric cycling after the second bout, and fat
utilisation tended to be greater after the second than the first eccentric cycling bout (Figure 4.2). Although speculative, the unexpected lack of significant difference in post-exercise energy expenditure between the concentric and second eccentric cycling bouts may be indicative of a greater cellular synthesis rate concomitant with improved oxidative capacity after repeated eccentric cycling. The third hypothesis was not supported by the results, because there was no observable change in resting energy expenditure 2-4 days after any cycling bout and glucose uptake did not decrease after any cycling bout. Collectively, the present data strongly indicate that a shift toward a greater oxidative metabolism occurred after repeated eccentric cycling bouts without a reduction in glucose uptake.

Oxygen consumption during concentric cycling (CONC; 77% of VO$_{2peak}$) was significantly greater than that during the first (ECC1; 39% of VO$_{2peak}$) and second (ECC2; 37% of VO$_{2peak}$) eccentric cycling bouts for the same workload (~165 W). It is well known that carbohydrate (CHO) utilisation is greater at higher exercise intensities (i.e. >65% of VO$_{2max}$) [121], so it is not surprising that the majority of the energy used during CONC came from CHO oxidation (98%). Previous studies have reported that CHO is the predominant energy source for oxidation above an exercise intensity of 50% VO$_{2max}$, and that the contribution of fat oxidation to energy supply is negligible when the intensity is above 80% VO$_{2max}$ [129, 132]. It should be noted that ECC1 and ECC2 utilized 70–85% greater fat during cycling when compared with CONC. It would be interesting to know whether the same level of fat utilisation would be found between concentric and eccentric cycling if the concentric cycling intensity was reduced so that energy expenditure, rather than the power output, was identical. Regardless, the present data show that a significant fat oxidation can be achieved during eccentric cycling at relatively high power outputs, which were previously shown to also elicit substantial muscle size and strength increases [6, 13]. Eccentric cycling, therefore, may offer broad health benefits for individuals that cannot tolerate high-intensity exercise.

It has been shown that exercise intensity greatly influences both the magnitude and duration of the post-exercise energy expenditure [133, 134]. In the present study, post-exercise energy
expenditure was 30% greater after CONC than ECC1, which is probably related to the 50% greater oxygen consumption during CONC than ECC1. In concentric cycling, it has been reported that energy expenditure in the 3 h post-exercise is 47% greater after high- (75% of VO_{2max}) than low-intensity (50% of VO_{2max}) cycling exercise [134]. However, post-exercise energy expenditure after ECC2 (389 kJ) in the present study was not different to CONC (399 kJ), even though the oxygen consumption during ECC2 was 52% lower. Furthermore, there was a tendency for the post-exercise energy expenditure to be greater after ECC2 than ECC1 (28%; P=0.068), in spite of a similar oxygen consumption during exercise. To the best of the author’s knowledge, no previous study has compared the post-exercise energy expenditure between repeated eccentric exercise bouts. However, Binzen et al. [133] reported that the energy expenditure remained significantly elevated for at least 1 h above resting level after strenuous resistance exercise, with a preference for fat oxidation. The authors speculated that strenuous resistance exercise relied on the anaerobic metabolism of phosphocreatine and glycogen for energy, which depleted glycogen stores sufficiently for lipid metabolism to predominate during recovery. Interestingly, our results revealed a tendency for post-exercise energy expenditure to be greater after ECC2 than ECC1 (28%, P=0.068), indicating that eccentric cycling may have similar effects on energy expenditure and fat oxidation, and that repeated bouts could increase this effect. Sedlock [135] stated that post-exercise energy expenditure seemed to be a function of an increased mitochondrial respiration, and recovery energy expenditure was suggested to be affected primarily by the magnitude of the homeostatic disturbance.

Rattray et al. (2013) have recently reported that mitochondrial function was significantly depressed due to the substantial increase in mitochondrial Ca^{2+} content after an acute bout of downhill running in rats, but no such decrease was found when animals were subjected to 5 weeks of downhill running training [137]. Therefore, since myoplasmic Ca^{2+} has been shown to be significantly increased during and after a single eccentric exercise bout compared to concentric or isometric contractions [136], one might speculate that it is possible that a loss of Ca^{2+} homeostasis
after the first eccentric cycling induced a smaller post-exercise energy expenditure due to mitochondrial function depression. However, a greater energy expenditure was found after the second bout, where the magnitude of muscle damage has been shown to be minor [71], and presumably enhanced Ca\(^{2+}\) handling was induced after the first eccentric cycling bout. Alternatively, the smaller post-exercise energy expenditure after ECC1 might be explained by the muscle damage and inflammation induced after ECC1, which negatively affected (inhibiting) muscle oxidative metabolism. However, when the eccentric exercise was repeated the increases in Ca\(^{2+}\) occurred without muscle damage or inflammation, therefore, allowing the muscle cells to activate their machinery and increase energy expenditure. Thus, whether muscle metabolism is affected by a loss of Ca\(^{2+}\) homeostasis induced by eccentric contractions warrants further investigation.

As shown in Figure 4.2, post-exercise fat utilisation was 52% greater after CONC than ECC1, but was ~47% greater after ECC2 than ECC1 (P=0.056). It has been documented that fat oxidation is greater after low- than high-intensity cycling [129, 134]. For instance, Phelain et al. [137] reported that total fat utilisation was 25% greater during and for 3 h after low- (50% of VO\(_{2\text{max}}\)) than high-intensity (75% of VO\(_{2\text{max}}\)) cycling. However, this does not explain why fat oxidation was greater after ECC2 than ECC1, since the exercise intensity was similar between bouts. One possibility is that the increased energy requirements after ECC2 could be achieved through lipid metabolism given there would have been little cellular damage to impair lipid transport and affect mitochondrial function. Another possibility is that the initial eccentric exercise bout induced an increase in the capacity for fat oxidation. In fact, our results are in agreement with Hody et al. [138] who have recently shown that glycolytic enzyme expression was down-regulated and oxidative enzyme expression up-regulated after a second maximal eccentric exercise bout performed six weeks after. They speculated that this shift toward oxidative metabolism was triggered by a reduction in the relative contribution of the glycolytic system during eccentric
contractions. It would be interesting to investigate whether greater fat oxidation is still observed if eccentric training was regularly performed.

It has been suggested that energy expenditure is a determining factor for weight loss management, and exercise can increase energy expenditure that, when accompanied by caloric restriction, leads to weight reduction and health improvements [36]. It is well known that REE increases for 2-3 days after exercise [125, 139]. For instance, Jamurtas et al. [139] reported that single bouts of 60-min resistance exercise (70–75% 1-RM) or running (70–75% of VO_{2max}) induced 6% and 8.5% increases in REE at 1 and 2 days after exercise, respectively. Also, Dolezal et al. [140] reported an 11-18% increase in REE 1–2 days after an acute resistance exercise bout with accentuated eccentric loading (i.e. 8 sets of a 6-RM leg press) that increased CK activity (>1100 IU·L^{-1}) and muscle soreness 1–3 days after exercise. They speculated that the acute phase responses after muscle damage, including inflammatory responses and skeletal muscle protein synthesis, were probably involved in the increased REE. It has been shown that eccentric cycling training produces muscle hypertrophy, and it should be noted that protein synthesis requires ATP. Thus, if eccentric cycling stimulates protein synthesis greater than concentric cycling, it is likely that more energy is required after eccentric cycling. However, no significant changes in REE were observed from control measurements after any cycling bouts in the present study, which is in agreement with a previous study [141] that reported no change in REE 48 h after 100 eccentric contractions (100% of 1-RM) for knee extension and chest press. It was reported that only small changes in muscle damage markers are observed after eccentric cycling using the same protocol, as indicated by a lack of significant increase in plasma CK activity and only minor development of muscle soreness [71]. It seems unlikely, therefore, that severe muscle damage was induced by this eccentric cycling, which may explain the lack of increase in REE in the days after exercise.

It has previously been reported that glucose uptake is impaired 1–3 days after 20 min of eccentric cycling at similar intensity to that in present study, but performed with a single leg, resulting in greater (395–587 U·L^{-1}) CK activity 1–7 days after exercise [22], and after 30 min
downhill running (-17% slope) [32]. The impaired glucose uptake was presumably due to sarcolemmal damage interrupting the Glut-4-mediated active transport of glucose, which caused transient glucose intolerance [22, 32]. Furthermore, the impairment of the insulin signal transduction by acute eccentric exercise at the level of insulin receptor substrate-1, phosphatidylinositol-3 kinase and protein kinase B (Akt-kinase) [30], may offer another potential mechanism influencing inflammatory responses (increases in Interleukin-6 and Tumor necrosis factor – α) with the decline in insulin sensitivity. However, as shown in Figure 4.4, the OGTT AUC revealed a lack of change in glucose uptake after both eccentric cycling bouts. Nevertheless, these results clearly demonstrate that glucose tolerance was not impaired after eccentric cycling. It is possible that sarcolemmal damage was relatively minor after both eccentric cycling bouts as shown by non-significant increases in plasma CK (246 UI/L) activity, shown in Study 1 [71], in comparison to previous eccentric exercise protocols [22, 32]. Therefore, glucose uptake has not been significantly reduced because glucose transport was not impaired even after ECC1. Thus, the moderate-level muscle damage after eccentric cycling in the present study did not induce glucose uptake impairment and could be safely implemented in subjects with impaired glucose tolerance.

The present data suggest that eccentric cycling could be used as an exercise modality for elderly individuals and those with cardiac and/or respiratory limitations. Eccentric cycling training has been reported to induce greater increases in muscle strength and mass than concentric cycling and resistance training [6, 13], and the present data extend these findings by showing that eccentric cycling can increase post-exercise metabolism and fat utilisation without impairing glucose uptake. The lack of glucose intolerance is important because it is known that elderly and both cardiac and respiratory disease patients have a higher risk of developing insulin resistance and dyslipidemia [142], and muscle damaging exercises may induce or exacerbate transient glucose intolerance. Previous studies have reported the efficacy of eccentric training for metabolic syndrome; for example, downhill hiking training for 8 weeks improved glucose tolerance by 6.2% [34] and 12 weeks of eccentric cycling training decreased abdominal fat by 3.7% for obese glucose intolerant
women [52]. As shown in the present study, eccentric cycling may increase fat oxidation after repeated bouts without glucose uptake impairment, thus could be a useful strategy for individuals with exercise intolerance and with impaired glucose metabolism (i.e. diabetes), in addition to the muscle mass and strength gains shown after training. Nonetheless, further studies are necessary to more completely determine the effects of eccentric cycling training on metabolic and oxidative adaptation within skeletal muscles to elucidate whether concurrent adaptations (e.g. protein synthesis and mitochondrial biogenesis) occur after eccentric cycling exercise.

In conclusion, the results of the present study showed that an acute bout of 30 min eccentric cycling induced greater fat utilisation during exercise compared with concentric cycling at the same workload, and that post-exercise fat utilisation was similar between concentric and the repeated bout of eccentric cycling, regardless of the lower metabolic cost of the exercise. It should be investigated further whether the greater fat utilisation in eccentric cycling is still evident when the exercise intensity is the same between eccentric and concentric cycling. If this is the case, eccentric cycling training may be beneficial for exercise intolerant patients since it could increase fat utilisation without inducing glucose uptake impairment in addition to the gains in muscle mass and strength shown in previous research. Further studies are necessary to investigate this.
CHAPTER 5

Study 3: Rate of Force Development as a Measure of Muscle Damage

5.1 Introduction

Exercise involving eccentric (lengthening) contractions results in muscle damage, especially when it is performed without previous exposure to eccentric contractions of the muscles used in the exercise [143]. Although histological alterations are direct indicators of muscle damage, eccentric exercise-induced muscle damage is also assessed by increases in muscle proteins in the blood (e.g. creatine kinase: CK) and ratings of delayed onset muscle soreness (DOMS), and decreases in both muscle strength and range of motion [15]. Among these, a prolonged decrease in maximum voluntary contraction (MVC) peak force or torque has been considered to be the best indirect measure of eccentric exercise-induced muscle damage [27, 28]. For example, large decreases in MVC peak torque (30–50%) are evident for several days following maximal eccentric exercise of the elbow flexors together with abnormalities shown by ultrasound and/or magnetic resonance imaging [144] and myofibril necrosis [145].

The magnitude of decrease in MVC peak torque after eccentric exercise is smaller for leg muscles when compared with arm muscles [49], and the MVC peak torque of the knee extensors does not decrease to a large extent after running [146] and playing team sports such as soccer [147] and rugby [148]. In Study 1 (Chapter 3) it was reported that MVC peak torque of the knee extensors decreased 25% immediately after 30-min eccentric cycling, and remained below the baseline at 1 (11%), 2 (11%) and 3 (5%) days post-cycling, but returned to the baseline by 4 days post-cycling [71]. However, it is important to note that decreases in knee extensor peak torque were also
observed immediately after concentric cycling, although the magnitude of decrease was smaller (12%) than eccentric cycling [71]. Thus, it is not possible to separate the effects of metabolic (or neuromuscular) fatigue and muscle damage by measuring MVC peak torque immediately after exercise, and the magnitude of damage can only be determined by testing on subsequent days [28, 71]. It would be of substantial clinical and rehabilitative interest to find a non-invasive marker that can be measured during and/or immediately after exercise that is specifically reflective of muscle damage. Then, if muscle damage was being induced, decisions could be made as to whether to continue the exercise and/or implement specific recovery strategies. Such a marker would be particularly beneficial for clinicians, coaches and athletes.

The rate of force development (RFD) is defined as the slope of the force-time curve obtained under isometric contractions, and is a measure of the ability of the neuromuscular system to generate rapid force at the onset of contraction [149]. RFD has been reported to change in relation to the proportion of fast-twitch muscle fibres [150, 151], muscle cross-sectional area [108], stiffness of the muscle-tendon complex [152] and the neural drive such as enhanced initial firing rate and decreased recruitment threshold of motor units [153, 154]. It is possible that muscle damage induced by eccentric exercise results in greater decrease in RFD than MVC peak torque, if greater proportion of fast-twitch than slow-twitch muscle fibres are damaged in eccentric exercise [17]. Several studies [155-160] have examined changes in RFD of elbow flexors [155, 156], knee extensor [157, 160, 161] and knee flexor [158] muscles after a single bout of maximal isokinetic eccentric contractions. For example, Crameri et al. [157] reported 18–24% decrease of the relative RFD (i.e. RFD normalised to MVC peak torque) at 4 h and 1 day after 210 maximal isokinetic eccentric contractions of the knee extensor muscles, and explained that rapid force characteristics were more affected than MVC peak torque, suggesting an impairment of the intra muscular force transmission pathways. Edman et al. [29] have shown in frog muscle that early portions of the force-time curve (e.g. before reaching 50% MVC) is a combination of muscle series of elastic components (SEC) by 40% and muscle activation process (e.g. calcium release-binding) by 60%,
but the force-time curve beyond the 50% MVC is more associate with muscle contractile capacity (i.e. cross-bridged kinetics) than stretching of SEC. Thus, it may be that the effect of muscle damage on RFD is different between the early (0–200 ms) and late (100–200 ms) phases of the force-time curve. However, no previous studies have examined the changes in early and late phases of the RFD after eccentric exercise in relation to muscle damage. It is also possible that RFD is a marker that could distinguish muscle damage from muscle fatigue at immediately after eccentric exercise.

In eccentric cycling, eccentric contractions of the knee extensor muscles are performed while trying to brake the backward rotational movement of the cranks of a cycle ergometer [73]. When eccentric cycling is performed for an extended period of time (e.g. 30 min), many submaximal eccentric contractions (e.g. 60 revolutions per min × 30 min = 1800 eccentric contractions) are performed, inducing greater muscle metabolic fatigue in comparison to maximal eccentric contraction protocols that are often used as models of muscle damage (e.g. 100 maximal isokinetic eccentric contractions). Thus, eccentric cycling appears to be an ideal exercise model to investigate muscle damage and fatigue occurring in sporting activities, and to examine whether RFD can be used to distinguish muscle damage from muscle fatigue at immediately after exercise.

Therefore, the purpose of this study was to examine changes in RFD of the knee extensors before, immediately after and 1-2 days following a bout of concentric cycling and two bouts of eccentric cycling to test the hypothesis that RFD is more sensitive marker of muscle damage than MVC peak torque, differentiating muscle damage from muscle fatigue immediately post-exercise.

5.2 Methods

5.2.1 Participants

As in Study 1, the sample size was estimated using the data from a previous study [105] in which changes in MVC strength of the knee extensors following isokinetic eccentric exercise (50
maximal isokinetic eccentric contractions of the knee extensors). It showed that at least 8 participants were necessary for this study. Ten men who had not performed lower limb resistance training regularly in the past six months and who reported no history of neurological disorders or orthopaedic lower limb injuries completed an informed written consent form and a medical questionnaire before participating in the study. Ethical approval was obtained from the institutional Human Research Ethics Committee prior to the study commencement. The participants’ mean (± SD) age, height, body mass, body mass index and peak oxygen consumption obtained during a recumbent ergometer incremental test were 28.4 ± 8.3 y, 179.0 ± 4.6 cm, 81.6 ± 13.1 kg, 25.5 ± 3.8 kg/m², and 38.6 ± 6.7 ml·min⁻¹·kg, respectively. They were instructed not to perform any exercise other than the included in this study, consume any anti-inflammatory medication and apply any treatment (e.g. massage, cryotherapy).

5.2.2 Study Design

The participants reported to the laboratory on three sessions consisting of 3 days for each session, with a two-week interval between sessions. All participants performed a 30-min bout of concentric cycling (CONC) in the first session, and two 30-min eccentric cycling bouts (ECC1, ECC2) in the second and third sessions, respectively. This order was chosen because it was considered that the carry-over effects of concentric cycling on eccentric cycling would be minimal, but the first eccentric cycling would confer a protective effect against the second eccentric cycling bout (i.e. repeated bout effect). Peak torque, RFD, and root-mean-square (RMS) electromyogram (EMG) amplitude and mean power frequency (MPF) were assessed during maximal voluntary isometric contractions (MVC) of the knee extensors before, immediately after and 1-2 days after each cycling bout.

5.2.3 Cycling Exercise

Both concentric and eccentric cycling exercises were performed at 60 revolutions per minute (rpm) for 30 min at 60% of the maximal concentric power output (PO_max) obtained during a
recumbent ergometer incremental test performed at least 96 h before the concentric cycling bout. The test consisted of incremental cycling starting at 50 watts (W) for 4 min followed by 25-W increment every minute until volitional exhaustion using a recumbent electronic ergometer (Tunturi F30R, Australia), while the cadence was kept at 60 rpm and the subjects received verbal encouragement during the test. The eccentric cycling was performed in a recumbent ergometer conditioned with a motor that moved the cranks of the ergometer backwards at the selected cadence (Metitur, Finland). Eccentric contractions were mainly performed by the knee extensor muscles when the participants resist the backward movement of the cranks [73]. A 5-min familiarization period was performed at a low (~50 W) power output before ECC1 and ECC2. The cadence of 60 rpm was chosen because the same cadence was used in previous studies [6, 13], and our pilot study showed that this was the best cadence to compare between concentric and eccentric cycling, since a slower cadence was difficult to maintain during concentric cycling to reach the target power output, and a faster cadence was difficult to control during eccentric cycling. A line to indicate the 60% concentric power output was shown on a screen of the ergometer, and the subjects were instructed to maintain the power output as close as possible to the target line.

5.2.4 MVC Peak Torque

Before testing, the participants performed a 5-min cycle warm-up on an ergometer (Monark 828E, Sweden) with a load of 9.81 N at 60 rpm. MVC peak torque and RFD of the knee extensors were measured for the dominant leg (i.e. kicking leg) at 70° of knee flexion [108] in a custom made rigid chair equipped with a load cell (Xtran S1W, Australia). After seated in the chair, the participants performed three submaximal contractions at 50%, 50% and 80% of self-perceived maximum intensity for 3 s each, with a 1-min rest between contractions. Participants then performed three 3-s MVCs with a 1-min rest between contractions, and if any countermovement was evident, an additional MVC was measured. The peak value of each trial was obtained for each attempt, and the attempt of the greatest trial was used for analyses. For the MVC measures, the
participants were instructed to contract as fast and hard as possible, and visual feedback was provided as a real-time display of the torque output on a computer screen. Torque data was recorded at a sampling frequency of 1000 Hz and was smoothed using a digital low-pass filter with a cut-off frequency of 14 Hz in subsequent off-line analysis [149].

5.2.5 Rate of Force Development (RFD)

RFD was measured as the slope of the torque-time curve in the time intervals 0–30 ms (RFD_{0-30}), 0–50 ms (RFD_{0-50}), 0–100 ms (RFD_{0-100}), 0–200 ms (RFD_{0-200}) and 100–200 ms (RFD_{100-200}) as shown in Figure 5.1 [108]. RFD_{100-200} is thought to be influenced by different underlying mechanisms than the early force rise (i.e. 0–100 ms) and could therefore provide different information with respect to muscle function. In particular, it was thought to be less influenced by stretch of the series elastic component (SEC) and more reflective of muscle contractile potential, as Edman and Josephson [29] showed that ~40% of the force rise time to 50% of MVC in frog muscle fibres (devoid of external tendon) was attributable to the requirement to stretch the SEC whilst ~60% of the force rise time was attributable to the contractile process. Some evidence for this has also been found for the human quadriceps muscle [162], which has a substantial SEC volume and is attached to its insertion via the long quadriceps tendon. Peak RFD (RFD_{peak}) was also determined using a 20 ms window throughout the torque-time curve as the highest RFD at any time during the contraction [159]. The onset of muscle contraction was defined as the time point at which the torque curve exceeded the baseline by >7.5 Nm [149], and if torque dropped >5 Nm below the baseline at the beginning of a contraction, the contraction was not used for the RFD analysis, since it was considered that a countermovement was performed. Analyses were performed using Matlab version 7.12 software (MathWorks, R2011a). The attempt that showed the greatest MVC among the three MVC measures at each time point was used for RFD analyses.
Figure 5.1: An example of torque-time curve obtained before (Before Cycling) and immediately after concentric (After CONC) and the first eccentric (After ECC) cycling bouts from a participant. Vertical lines indicate time intervals of 50, 100 and 200 ms relative to the onset of contraction.

5.2.6 Surface Electromyography (EMG)

Vastus lateralis (VL) surface EMG was recorded from the dominant (i.e. kicking leg) leg during the MVC measurements. The skin was shaved and cleaned with alcohol swaps and electrodes were placed at 2/3 of the distance from the anterior superior iliac spine and the patella, according to SENIAM guidelines. A Bagnoli-8 desktop EMG system (Delsys, USA) was used to collect EMG data using a bipolar electrode configuration (DE-2.1 SEMG sensor) with a 10-mm inter-electrode distance. Electrode placement was marked on the skin with a semi-permanent marker to re-position the electrodes during subsequent testing days. Sampling frequency was set at 2000 Hz and an off-line digital filter was applied with a band pass of 10–450 Hz. The RMS of the EMG amplitude was calculated from the central 1 s of the MVC. The MPF of the power density spectrum was obtained by fast Fourier transform of 1024 points using a Hanning window with 50%
overlap, using same epochs as for RMS [71, 107]. The highest of three MVC contractions was used for analyses.

5.2.7 Statistical Analysis

A one-way analysis of variance (ANOVA) was used to compare the average power output performed among the three cycling bouts, and to examine changes in MVC peak force, RFD, EMG amplitude and MPF for each cycling bout. A two-way repeated measure ANOVA was used to compare the changes in MVC peak force, RFD, EMG amplitude and MPF over time (before, immediately post- 1-2 days) between CONC and ECC1, CONC and ECC2, and ECC1 and ECC2. Furthermore, to compare the magnitude of change of MVC peak torque and RFD after ECC1 over time, another two-way repeated measures ANOVA was performed. If a significant effect was found, a post-hoc comparison using a Fisher’s Least Significant Difference (LSD) test was followed. A Pearson product-moment correlation coefficient (r) was calculated to determine whether the magnitude of decrease in RFD immediately after eccentric cycling was correlated with the magnitude of decrease in MVC peak torque at 1 day after eccentric cycling that represents the magnitude of muscle damage. For this analysis, the RFD in each time period was analysed separately, and the relationship between the RFD and MVC peak torque was examined for ECC1 and ECC2, separately. All statistical analyses were performed with PASW Statistics 19 software for Mac (SPSS inc, IBM company, USA). The significance level was set at P<0.05. Data are presented as mean ± standard deviation (SD) in text, but figures show mean ± standard error of the mean (SEM).
5.3 Results

5.3.1 Cycling Exercise

Average cycling power outputs during CONC, ECC1 and ECC2 were 158.5 ± 9.2 W, 169.9 ± 26.7 W and 179.3 ± 6.1 W, respectively, and no significant differences were evident between them.

5.3.2 MVC Peak Torque

Baseline MVC peak torque was not different between bouts (CONC= 282.3 ± 14.7, ECC1= 268.4 ± 15.5 and ECC2= 282.1 ± 13.1 Nm). Changes in MVC peak torque were significantly greater after ECC1 compared with CONC and ECC2 (Figure 5.2). MVC peak torque decreased 11–25% from immediately to 2 days after ECC1 (P<0.05), and decreased 12% (P=0.0001) and 19% (P=0.006) from baseline to immediately after CONC and ECC2, respectively.

![Figure 5.2](image)

**Figure 5.2**: Changes in maximal voluntary isometric contraction torque of the knee extensors (MVC Torque) before (Pre), immediately after (Post) and 1-2 days after concentric (CONC), first eccentric (ECC1) and second eccentric (ECC2) cycling bouts. Significantly (P<0.05) different from the pre-exercise value for CONC (†), ECC1 (*) and ECC2 (#).
5.3.3 RFD

Figure 5.1 shows the torque-time curve of one subject before and immediately after CONC and ECC1 cycling bouts. Changes in RFD for all intervals were significantly greater after ECC1 compared with CONC and ECC2, but no significant differences between CONC and ECC2 were found. As shown in Figure 5.3, RFD calculated within all time intervals decreased immediately, and both 1 and 2 days after ECC1 compared to the baseline (P<0.05), but only immediately after ECC2 (P<0.05). RFD measured from contraction onset (i.e. 0-30, 0-50, 0-100 and 0-200 ms) decreased immediately after CONC, but there was no change in RFD_{100-200}. The magnitude of decrease in RFD immediately post-exercise for all time intervals except RFD_{100-200} was similar between CONC, ECC1 and ECC2. RFD_{100-200} showed a 30–32% decrease at immediately, and 1 and 2 days after ECC1, and a 23% decrease immediately after ECC2 from the baseline; however, the magnitude of decrease immediately post-exercise was not different between ECC1 and ECC2 (P=0.13). Furthermore, RFD_{100-200} showed a 7–19% greater decrease immediately, and both 1 and 2 days after ECC1 when compared to the MVC peak torque decreases (P=0.002, P=0.006 and P=0.003, respectively).

5.3.4 Correlation Between MVC Peak Torque and RFD

Figure 5.4 shows the correlations between the magnitude of change in RFD immediately after ECC1 and ECC2, and the magnitude of change in MVC peak torque 1 day after ECC1 and ECC2. Only RFD_{100-200} was significantly correlated (r=0.58, P=0.04) with MVC peak torque after ECC1. For ECC2, RFD_{peak} and RFD_{100-200} were correlated with the MVC peak torque loss (r=0.66, P=0.02 and r=0.61, P=0.03, respectively).
Figure 5.3: Changes in the rate of force development (RFD) for peak (A), and 0-30 ms (B), 0-50 ms (C), 0-100 ms (D), 0-200 ms (E) and 100-200 ms (F) slots before (Pre), immediately after (Post) and 1-2 days after concentric (CONC), first eccentric (ECC1) and second eccentric (ECC2) cycling bouts. Significantly (P<0.05) different from the pre-exercise value for CONC (†), ECC1 (*) and ECC2 (#).
Figure 5.4: Correlation between the magnitude of change in MVC torque from the baseline at 1 day post-exercise (% of pre) and the magnitude of change in rate of force development from the baseline at immediately after exercise (% of pre) for the first eccentric (ECC1) and second eccentric (ECC2) cycling bouts for time intervals of 0-30 ms (A, B) 0-50 ms (C, D), 0-100 ms (E, F), 0-200 ms (G, H), peak RFD ms (I, J) and 100-200 ms (K, L). In each graph, correlation coefficient (r) and P values are included, and a regression line is inserted where a significant correlation (P<0.05) was found.

5.3.5 EMG Amplitude and MPF

Baseline EMG amplitude and MPF during MVC was similar between CONC (amplitude: 0.19 ± 0.09 mV, MPF: 85.8 ± 12.1 Hz), ECC1 (0.17 ± 0.07 mV, 84.6 ± 10.9 Hz) and ECC2 (0.16 ± 0.05 mV, 82.7 ± 11.1 Hz). No significant changes over time were found for EMG amplitude and MPF after any exercise bout (P>0.05).

5.4 Discussion

It was hypothesized that RFD would prove to be a more specific and sensitive marker of muscle damage than MVC peak torque, differentiating muscle damage from muscle fatigue immediately after exercise. A significant decrease in MVC peak torque was found immediately after concentric (CONC) and the first (ECC1) and second (ECC2) eccentric cycling bouts, without a significant difference between bouts. Since metabolic fatigue is likely to be significant and muscle damage negligible after CONC, peak MVC torque could not be considered a useful marker of muscle damage when measured immediately after exercise - its discriminative power was therefore poor under these conditions. The MVC peak torque decrease did, however, remain for 2 days after ECC1 only (Figure 5.2), which was most likely attributed to substantial muscle damage being induced. Also, RFD measured from contraction onset decreased similarly immediately after CONC,
ECC1 and ECC2 (Figure 5.3), indicating that these measures were not able to discriminate muscle damage and fatigue but may be indicative of muscle damage when measured in the days after exercise. These data suggest that MVC peak torque and RFD measured from contraction onset are useful markers of muscle damage only when they are assessed from 1 day post-exercise onward. Nonetheless, RFD_{100-200} did not decrease immediately after CONC, and showed a 7–19% greater decrease than MVC peak torque after ECC1. Furthermore, the magnitude of decrease in MVC peak torque at 1 day post-exercise, which is considered to indicate muscle damage, significantly correlated with the magnitude of decrease in RFD_{100-200} immediately after both ECC1 and ECC2, but not CONC (Figure 5.4). These results suggest that RFD_{100-200} is a more specific and sensitive marker of eccentric exercise-induced muscle damage in the knee extensors than MVC peak torque, and could be assessed during or immediately after exercise sessions as a reasonable indicator of muscle damage.

The magnitude of decrease in MVC peak torque after eccentric cycling reported in the present study was similar to that reported after other exercises such as downhill (-10% slope) running [116], submaximal (75%) isokinetic eccentric contractions of the knee extensor muscles [49], a 30-km run [163] and a 90 min match-simulated soccer [164]. A prolonged (>1 day) decrease in MVC peak torque has been regarded as the best marker of muscle damage [27]. However, in the present study the magnitude of decrease in MVC peak torque immediately after exercise was not significantly different between the three bouts, although it remained below baseline 1–2 days after ECC1 only. This indicates that little or no muscle damage was induced by CONC or ECC2. It is assumed that the peak torque loss induced immediately after CONC was probably due to metabolic muscle fatigue. In contrast, the MVC peak torque loss after ECC1 was likely induced by a combination of muscle fatigue and muscle damage, including damage to force-generating (i.e. actin, myosin) and/or force-transmitting (e.g. aponeurosis) structures, and failure to activate the intact force-generating structures [165]. Warren et al. [165] estimated that most (~75%) of the force loss was attributed to excitation-contraction (E-C) coupling failure, and the remaining (25%) was due to
damage of force-generating and/or force-transmitting structures within the muscle, for the first 2-3 days after eccentric exercise. In the present study, no EMG amplitude or mean power frequency (MPF) changes were observed after any cycling bouts, suggesting that immediate and prolonged MVC peak torque loss after eccentric cycling was not associated with a failure of muscle activation at or distal to the neuromuscular junction, supporting previous research [166, 167].

RFD is indicative of the ability of the neuromuscular system to generate rapid increases in muscle force soon after the initiation of a contraction [108]. It has been shown that RFD, when measured from the contraction onset, is associated with fast-twitch muscle fibre proportion [150], muscle stiffness [152] and efferent neural drive [108]. Changes in RFD after eccentric exercise were reported in several studies, and all studies showed significant decreases in RFD after exercise, although the magnitude of decrease vary among the studies [155-160]. For example, Crameri et al. [157] reported 23–43% decreases in RFD<sub>0-50</sub> at 4 h and 1 day after exercise and 16–34% decreases in RFD<sub>0-100</sub> at 4 h and both 1 and 8 days after eccentric knee extensor exercise. Moreover, greater decreases in RFD relative to MVC peak torque (relative RFD at 1/6 and 1/2 of MVC) were found 4 h and 1 day after the eccentric exercise, showing that RFD was more impaired than MVC peak torque [157]. The authors stated that the greater decrease in rapid force production than MVC peak torque after eccentric exercise suggested a reduced stiffness of serial and/or lateral cellular mechanotransduction pathways, possibly due to changes in cytoskeletal integrity. In the present study, significant decreases were observed in MVC peak torque and RFD, but not in the magnitude of decrease between the RFD at various intervals calculated from the onset of contraction (to 200 ms) and MVC peak torque. This may be due to the difference in the magnitude of muscle damage such that the magnitude of decrease in MVC peak torque in the present study was much smaller than that of the study by Crameri et al. [157]. Nevertheless, it does not appear that RFD assessed from the contraction onset provides any additional information to that which is already provided by MVC peak torque. However, it is important to note that RFD<sub>100-200</sub> showed greater decreases than
MVC peak torque, and it appears to provide different information from MVC peak torque and other
RFD measures as discussed below.

It should be noted that RFD, except when calculated from 100–200 ms, was decreased
immediately after all cycling bouts (Figure 5.3). It has been shown that early phases of the RFD are
greatly (~40%) influenced by the SEC of the muscle whereas the later phases (~60%) may be
related to the activation process, including the release, diffusion and binding of Ca\(^{2+}\) and the rate of
cross-bridge binding to thin filaments [29]. Thus, the decreases in early RFD (i.e. 0–200) may have
been only partly influenced by disruption of the activation process immediately after eccentric
cycling and therefore were not completely reflective of muscle damage. It is important to note that
CONC and ECC2 elicited a decrease in RFD immediately after exercise, although muscle damage
after CONC and ECC2 was considered to be minimal. Therefore, it seems that muscle fatigue also
influenced the decreases in the RFD (especially after CONC), suggesting that RFD calculated from
torque onset cannot exclusively assess muscle damage.

The possibility existed that RFD\(_{100-200}\) would more clearly differentiate muscle damage from
muscle fatigue than MVC peak torque. It seems possible that this later region (i.e. 100–200 ms) of
the torque-time curve could be more reflective of cross-bridge kinetics (binding, translation and
detachment), affecting the muscle contractility, because the SEC will have been elongated
substantially by the early force rise and further increases in force at this point would cause less
stretch in those structures [29]. Additionally, there would be less influence of slow-twitch fibre
activation in the interval RFD\(_{100-200}\) because these are largely activated early in the force rise [168],
and therefore the highly damage-susceptible fast-twitch fibres [17] will have a greater influence in
this region of the force-time curve [168]. The data showed that CONC did not induce any decrease
in RFD\(_{100-200}\) after exercise but that RFD\(_{100-200}\) decreased 7–19% more than MVC peak torque from
immediately after to 2 days after ECC1 (Figure 5.3F). This suggests that RFD\(_{100-200}\) was more
sensitive in detecting the changes in muscle contractibility after eccentric exercise than MVC peak
torque and RFD calculated from other time intervals. Accordingly, one might speculate that
disruption of mainly fast-twitch fibres during eccentric cycling would have influenced RFD<sub>100-200</sub> and that this change would have not been masked by a significant stretch of the SEC, allowing it to be a more specific marker of muscle damage. Interestingly, a decrease in RFD<sub>100-200</sub> was detected immediately after ECC2, which may reflect minor muscle damage that was not associated with a decrease in MVC peak torque and possibly resulted from transient E-C coupling failure in fast-twitch fibres, an increase in muscle compliance and/or slowed cross-bridge kinetics. This finding may further highlight the sensitivity of RFD<sub>100-200</sub>, given that some damage may have been expected after ECC2.

The correlations between the magnitude of decrease in MVC peak torque 1 day after eccentric cycling (which is typically assumed to be a good indicator of muscle damage) and the magnitude of decrease in RFD immediately after eccentric cycling (ECC1, ECC2) were examined to investigate whether the RFD immediately post-exercise could predict the muscle damage observed 1 day post-exercise (Figure 5.4). Interestingly, only changes in RFD<sub>100-200</sub> were correlated with the MVC peak torque loss 1 day after ECC1 (Figure 5.4K), and changes in RFD<sub>peak</sub> and RFD<sub>100-200</sub> were correlated with the MVC peak torque loss 1 day after ECC2 (Figure 5.4J and 5.4L). Thus, RFD<sub>100-200</sub> was the only parameter that was statistically related to the MVC peak torque loss at 1 day after both eccentric cycling bouts. In fact, despite there being a significant correlation between MVC peak torque and RFD<sub>100-200</sub> after ECC2, the change in MVC peak torque did not reach statistical significance, suggesting that only the RFD<sub>100-200</sub> measure was sensitive enough to detect the likely minor muscle damage present after ECC2. Therefore, RFD<sub>100-200</sub> seems to be a differentiating measure between muscle function loss induced after concentric (i.e. metabolic fatigue) and eccentric contractions (i.e. decreased muscle contractility). Nonetheless, additional research may refine assessments to further improve sensitivity in order to predict performance and recovery after exercises that include eccentric contractions.

In conclusion, the present data indicate that RFD could be used as an additional indirect marker of muscle damage and that RFD<sub>100-200</sub> could more specifically detect muscle damage in the
knee extensors. It is proposed that RFD\textsubscript{100-200} is a sensitive and specific marker for eccentric exercise under these conditions. These results in the context of monitoring muscle damage during and after an exercise session, match or sports competition since exercise may be terminated prior to significant damage being elicited or recovery strategies (e.g. rest, dietary supplementation or treatment) may be more rapidly implemented. However, the determination of RFD requires specific equipment that may not be easily accessed in practice, thus the practicality of the RFD measures as muscle damage markers should be investigated further. Future research might further refine these RFD measurements, and determine the optimum time intervals in which RFD might be measured in order to reflect muscle damage in other muscles, since force rise times will differ markedly between muscle groups.
CHAPTER 6

Study 4: In Vivo Vastus Lateralis Fascicle Behaviour During Repeated Eccentric Cycling in Relation to Muscle Damage

6.1 Introduction

Exercise incorporating of eccentric (lengthening) contractions induces muscle damage characterized by prolonged loss of muscle function and delayed onset muscle soreness (DOMS), especially when it is performed by muscles that have not been exposed to eccentric contractions regularly [143]. However, when the same eccentric exercise is performed within several weeks, DOMS is less and recovery of muscle function is faster [71, 169]. This adaptation conferred by a single bout of eccentric exercise is referred to as the “repeated bout effect” [169]. McHugh [14] categorized potential adaptations as neural, mechanical and cellular, and concluded that the mechanisms underpinning the repeated bout effect were likely multifactorial.

It is documented that eccentric exercise-induced muscle damage is triggered by muscle strain while the muscle is active, resulting in Ca$^{2+}$-mediated myocellular remodeling, damage to connective tissue structures and over-elongation and disruption of sarcomeres [17, 61, 136, 170]. Muscle strain during eccentric contractions is considered the primary cause of changes in indirect markers of muscle damage such as DOMS and loss of muscle function [171, 172]. It has been speculated that passive and (or) dynamic muscle stiffness increase after the first eccentric exercise bout, which reduces muscle strain and confers a protective effect against subsequent eccentric exercise-induced muscle damage [57, 172]. If reductions in muscle strain occur after the first eccentric exercise bout, it is possible that muscle behavior during the second eccentric exercise bout
is different from the first such that muscle fibers lengthen less and overall muscle strain is reduced. However, to the best of our knowledge, no previous study has compared the muscle behavior between two eccentric exercise bouts to investigate the repeated bout effect.

The B-mode ultrasound technique has been used to examine human skeletal muscle in vivo [40]. In particular, several studies have used this technique to examine the behavior of muscle fascicles (fascicle length and angle) and tendinous tissue (TT; outer tendon + aponeurosis) of a muscle-tendon unit (proximal tendon + muscle + distal tendon; MTU) during movements [40-42]. Some studies have shown that MTU lengthening mainly results from TT elongation with a quasi-isometric behavior of muscle fascicles during eccentric contractions in tibialis anterior [173] or gastrocnemius [174]. Other studies reported that vastus lateralis (VL) fascicles and TT of the muscle were simultaneously lengthened during eccentric contractions of the knee extensors [41, 43]. Ishikawa et al. [44] reported that the relative TT elongation decreased with increasing in VL fascicle length during stretch-shortening contractions (i.e. drop jump) as intensity increased. These studies show that the ultrasound technique could detect a difference in muscle and tendon behavior during eccentric contractions, if any, between bouts.

In Study 1 (Chapter 3) it was reported that an initial bout of eccentric cycling induced greater decreases in muscle function (maximum voluntary isometric contraction strength, countermovement and squat jump height) and increases in muscle soreness 1-3 days after eccentric cycling, when compared with the second eccentric cycling bout performed two weeks later [71]. In eccentric cycling, many submaximal eccentric contractions (e.g. 60 revolutions per min × 10 min = 600 eccentric contractions) are performed, which appears to be an ideal exercise model to investigate muscle damage occurring in sporting/exercise activities. We speculated that the differences in the symptoms of muscle damage between the first and second eccentric cycling bouts might be associated with differences in muscle-tendon behavior. Therefore, the present study investigated the muscle fascicle and tendon behavior of the VL during two eccentric cycling bouts performed two weeks apart to test the hypotheses that muscle fascicle elongation would be less
during the second eccentric cycling bout when compared with the initial bout, and that a smaller
fascicle elongation during the second eccentric cycling bout would be associated with the repeated
bout effect.

6.2 Methods

6.2.1 Participants

Eleven men who had not performed lower limb resistance training regularly in the past six
months and who reported no history of neurological disorders or orthopedic lower limb injuries
completed an informed written consent form and a medical questionnaire before participating in the
study. The sample size was calculated on the basis of an α level of 0.05 and a power (1-β) of 0.8,
with an estimated 20% difference in fascicle length change between the first and second eccentric
cycling bouts using the VL fascicle length data of a previous study [43]. Ethical approval was
obtained from the institutional Human Research Ethics Committee prior to study commencement.
The participants’ age, height, body mass, body mass index and peak oxygen consumption during
concentric cycling exercise (mean ± SD, n=11) were 27.1 ± 7.0 y, 178.4 ± 8.2 cm, 73.6 ± 6.2 kg,
23.1 ± 1.3 kg/m², and 3.3 ± 0.6 L·min⁻¹, respectively. Participants were instructed not to perform
any exercise, take anti-inflammatory medication, or to apply any treatments (e.g. massage,
stretching) from 2 days before to 2 days after each cycling bout.

6.2.2 Study Design

The participants reported to the laboratory for two 3-day blocks (one exercise day followed
by two recovery days) separated by two weeks, since our previous study [71] showed a strong
repeated bout effect of eccentric cycling for this period. The participants performed 10 min of
eccentric cycling at the same intensity for each block (ECC1, ECC2), and maximal voluntary
isometric contraction (MVC) torque of the knee extensors and muscle soreness by visual analogue
scale (VAS) were assessed before and 1-2 days after each cycling bout. During cycling, VL fascicle
behavior and the surface electromyogram (EMG), crank torque and knee joint angle were recorded. Changes in MVC torque and muscle soreness, and the variables measured during cycling were compared between bouts.

6.2.3 Cycling Exercise

An incremental cycling test to determine VO$_{2peak}$ was performed at least one week before ECC1 using a recumbent ergometer (Tunturi F30R, Australia). The test started at a power output of 50 W for 4 min and increased 25 W every 1 min until volitional exhaustion. Cadence was kept at 60 revolutions per minute (rpm) and the participants received verbal encouragement during the test.

Eccentric cycling was performed using a recumbent cycle ergometer conditioned with a motor that moved the cranks of the ergometer backwards at a selected cadence (Metitut, Finland). Eccentric contractions were mainly performed by the knee extensor muscles when the participants tried to resist the backward movement of the cranks. A 5-min familiarization period was performed at a low power output (50 W) before a 10-min cycling (60 rpm) set at 65% of the maximal power output (PO$_{max}$) obtained during the VO$_{2peak}$ test. We have previously shown that 30 min of eccentric cycling at 60% PO$_{max}$ induced moderate muscle damage [71]. Based on this and a pilot study, we assumed that 10 min of eccentric cycling at 65% PO$_{max}$ would be sufficient to induce some symptoms of muscle damage (e.g. DOMS, loss of muscle function).

6.2.4 Crank Torque and Knee Range of Motion

Crank torque was continuously measured by a KIP power meter bicycle crank (Breakaway Innovations, Australia) implemented to the left crank at a sampling frequency of 477 Hz, and the data were stored in a data logger; average power output was instantaneously calculated and displayed on a screen (Rider 50, Bryton, Taiwan). The cycle ergometer was equipped with a switch (crank angle of 270°) that sent a pulse to the ultrasound apparatus and the PowerLab system (ADInstruments, Australia), which allowed us to synchronize the ultrasound, EMG, crank torque and knee joint angle data. After synchronization, ultrasound video clips, EMG and crank torque
data of three revolutions at 1 and 10 min of cycling were analyzed, since muscle fascicle length changes were not significantly different among 10 revolutions, as shown below. Knee joint range of motion was continuously measured by an electronic goniometer (SG, Biometrics, USA). The data collected at different frequencies were resampled at 200 Hz to relate the changes in variables over a revolution and to estimate tendinous tissue length changes in relation to knee joint angle changes. Prior to cycling for both ECC1 and ECC2, maximal voluntary isometric contraction torque was measured at 90° of knee flexion (0° = full extension) with 100° hip flexion on the ergometer using the KIP power meter crank to determine the relative torque level and VL EMG activity exerted during eccentric cycling in relation to maximal knee extension torque. Knee extension torque and EMG were simultaneously measured three times with a 60-s rest between attempts, and the attempt with the highest torque value was used for further analyses.

6.2.5 Electromyography (EMG)

Neural adaptations have been speculated to partially explain the repeated bout effect [14]. A previous study showed that EMG amplitude was lower during the second bout of eccentric cycling than the initial bout [71]. Thus, surface EMG was recorded from VL during cycling. The skin was shaved and cleaned with alcohol swaps and electrodes were placed at 2/3 of the distance from the anterior superior iliac spine to the patella, according to SENIAM guidelines. A Bagnoli-8 desktop EMG system (Delsys, USA) was used to record EMG data using a bipolar electrode configuration (DE-2.1 SEMG sensor) with a 10-mm inter-electrode distance. Sampling frequency was set at 2000 Hz and a 10–450 Hz digital band-pass filter was applied. EMG data were then smoothed using a root mean square (RMS) algorithm with a 50-ms window to produce a linear envelope from which average peak amplitude of 10 consecutive revolutions was used for comparisons according to a previous study [57].
6.2.6 Muscle and Tendon Behaviour

With each subject seated on the ergometer, an ultrasound probe (60 mm, Model UST-5712, 7.5 MHz, B-mode, Aloka SSD α-10, Japan) was attached to the left mid-thigh just above the VL EMG electrode (explained above). The probe was firmly placed on the skin by surgical tape, and a custom-made flexible plastic cast (size: $10 \times 20$ cm; surrounding the leg and secured by Velcro straps) was also used to minimize the movement of the probe. Echo absorptive tape was used to ensure that no probe displacement occurred during the 10-min cycling bouts. The ultrasound video clips were captured at a sampling rate of 47 Hz (i.e. 47 frames per pedal revolution) and analyzed off-line with a video analysis software (Tracker 4.7, free download: http://www.cabrillo.edu/~dbrown/tracker/). The superior and inferior aponeuroses and a clearly-identifiable fascicle (i.e. visible echo from the fascicle interspaces that could be followed across the image) were identified to track the same fascicle throughout the movements, and digitized frame-by-frame assuming a linear continuation of the fascicles (see Figure 6.1). For each subject, the entire length of the VL fascicle (Lf) was estimated using trigonometric method as shown in Figure 6.1 [43, 175], because Lf could not be visualized throughout the pedaling cycle. The error for estimating Lf with this method has been reported to be 2-7% [43, 175]. To estimate the reliability of the fascicle length measures, 10 consecutive revolutions were analyzed for one subject. Average change in fascicle length (i.e. max-min) was calculated using averages of 2 to 10 revolutions. Interclass correlation coefficient was 0.995 (95% Confidence Interval [0.994-0.996], P<0.0001). When comparing the average of 2, 3, 4...10 revolutions for changes in fascicle length, the mean ± standard deviation (SD) values were $4.8 \pm 0.3$ cm, $4.9 \pm 0.5$ cm, $4.9 \pm 0.4$ cm, $4.9 \pm 0.4$ cm, $4.8 \pm 0.4$ cm, $4.7 \pm 0.4$ cm, $4.8 \pm 0.5$ cm, $4.9 \pm 0.5$ cm and $5.0 \pm 0.5$ cm, respectively. Since no significant difference in the change in the fascicle length was evident, and considering the time constrains of manual frame-by-frame fascicle length analysis, the average of 3 consecutive revolutions was used to represent the average length change of 60 revolutions per minute. No significant difference in the fascicle length changes was observed over time (1-10 min), thus the results at 1 and 10 min of
cycling are reported in the results. The fascicle length and angle data were filtered using a smoothing spline method [176]. The resting fascicle length was also estimated using the ultrasound images taken when each subject seated in the ergometer with the knee joint at 90° of flexion. Resting fascicle length was measured three times from three different images per subject and the average of the three measures was used for further analyses.

The length of tendinous tissue (L\textsubscript{TT}) was defined as the sum of the proximal and distal tendinous structures, and aponeurosis [177, 178]. Length change in the tendinous tissue (tendon and aponeurosis; L\textsubscript{TT}) was calculated as:

\[ 7 \quad L_{\text{TT}} = L_{\text{MTU}} - L_{f_{\text{total}}} \times \cos \theta \]

Where \( L_{\text{MTU}} \) is the MTU length and \( \theta \) is the angle between fascicle and deeper aponeurosis [174, 178]. \( L_{\text{MTU}} \) changes were estimated using previously derived models based on the knee joint position (angle) data and limb length of each subject using the Hawkins and Hull model [179].

**Figure 6.1:** Estimation of vastas lateralis fascicle length and fascicle behaviour during eccentric cycling at 20°, 80° and 120° of knee flexion.
7.2.7 MVC Torque

The participants performed a 5-min warm-up on a cycle ergometer (Monark 828E, Sweden) with a load of 9.81 N at 60 rpm before testing. MVC torque of the left knee extensors was measured at 70° of knee flexion [108] on an isokinetic dynamometer (Biodex, System 3, NY, USA). Once seated in the chair, the participants performed three submaximal contractions at 50%, 50% and 80% of perceived MVC for 3 s each with 1 min rest between contractions. Participants then performed three MVCs with a 1-min rest between contractions, and the maximum value was used for further analyses. The participants were instructed to contract as fast and hard as possible, and visual feedback was provided as a real-time display of the torque output on a computer screen.

7.2.8 Muscle Soreness

Thigh muscle soreness was quantified using a 100-mm visual analogue scale (VAS) in which 0 indicates no pain and 100 represents the worst pain imaginable [169]. The participants were asked to mark the level of perceived pain of the quadriceps femoris muscles on the VAS whilst sitting down on and standing up from a 42-cm chair three times [110]

7.2.9 Statistical Analysis

A paired t-test was used to compare the average power output performed during each cycling bout. Since no significant difference in fascicle length changes was shown between 1 and 10 min of cycling by a two-way repeated measures analysis of variance (ANOVA), values from 1 and 10 min were averaged to represent the whole exercise to compare between ECC1 and ECC2. After synchronization of crank torque, EMG amplitude, muscle fascicle length and TT length data, the average pattern of changes (mean ± SD) of the pedal revolution are obtained (see Figure 6.3). Paired t-tests were used to compare the changes in fascicle and tendinous tissue length, average fascicle angle, peak cycling torque and peak EMG amplitude between ECC1 and ECC2. A two-way
repeated measures ANOVA was used to compare changes in MVC torque and VAS for muscle soreness over time (before, 1 and 2 days post-exercise) between ECC1 and ECC2. If a significant main effect was found, a post-hoc Fisher’s Least Significant Difference (LSD) test was performed. A significance level was set at P<0.05. All statistical analyses were performed with PASW Statistics 21 software for Mac (SPSS inc, IBM company, USA). Data are presented as mean ± SD.

6.3 Results

6.3.1 MVC Torque and Muscle Soreness

MVC torque of the knee extensors at baseline was not different between bouts (ECC1=270.3 ± 49.9 and ECC2= 275.3 ± 47.9 Nm; P=0.24). Figure 6.2A shows normalized changes in MVC torque from the baseline at 1 and 2 days post-exercise. No significant difference was evident for the changes in MVC torque between ECC1 and ECC2 (P=0.47). However, muscle soreness was greater after ECC2 than ECC1 at 1 (P=0.001) and 2 (P=0.003) days post-exercise, as shown in Figure 6.2B.

![Figure 6.2](image)

**Figure 6.2:** Normalised changes in maximal voluntary isometric contraction (MVC) torque from baseline (A) and changes in muscle soreness assessed by a visual analogue scale (B) before (Pre) and 1-2 days after the first (ECC1) and second (ECC2) eccentric cycling bouts. *: significant (P<0.05) interaction effect. #: significantly (P<0.05) different from ECC1.
6.3.2 Knee Joint Range of Motion

The range of motion of knee joint during cycling was not different between cycling bouts (P=0.27), and it ranged from 18.8 ± 8.7° to 116.8 ± 4.4° (0° full extension).

6.3.3 Cycling Power Output and Peak Crank Torque

Average cycling power output was not different (P=0.19) between ECC1 (189.7 ± 43.2 W) and ECC2 (194.0 ± 48.5 W). Figure 6.3A shows the average crank torque during a complete revolution cycle for ECC1 and ECC2. Torque increased during the pushing phase of the revolution and reached the peak at approximately 70° knee flexion for ECC1 and ECC2, and decreased in the recovery phase. Peak torque generated during cycling was not different (P=0.17) between ECC1 (73.5 ± 17.3 Nm) and ECC2 (79.5 ± 19.1 Nm) as shown in Figure 6.3B. The peak torque generated during cycling was 29.5 ± 11.3% and 33.1 ± 14.2% of the single leg MVC (248.7 ± 44.0 Nm and 240.4 ± 45.4 Nm) for ECC1 and ECC2, respectively.

6.3.4 EMG

Figure 6.3C shows the average EMG amplitude changes during a complete revolution for ECC1 and ECC2. Muscle activity increased during the revolution with increasing torque, and EMG amplitude peaked at ~70° knee flexion for ECC1 and ECC2, followed by gradual decreases during the recovery phase. As shown in Figure 6.3D, EMG peak amplitude was not significantly different (P=0.42) between ECC1 (0.047 ± 0.02 mV) and ECC2 (0.048 ± 0.02 mV). When pushing and recovery phases (first and second half periods of the revolution, respectively) were analyzed separately, the EMG amplitude during pushing phase was not different between ECC1 and ECC2 (P=0.09), but the EMG amplitude during the recovery phase was 60% smaller (P=0.005) during ECC2 than ECC1 (Figure 6.4). EMG peak amplitude measured during cycling was 29.7 ± 19.7%
and 27.0 ± 8.5% of the MVC peak EMG amplitude prior exercise (0.25 ± 0.23 and 0.19 ± 0.07 mV) for ECC1 and ECC2, respectively.
Figure 6.3: Changes (mean ± SD) in crank torque (A), VL EMG amplitude (C), VL fascicle length (E) and tendinous tissue length (G) in relation to knee joint angle during one pedal revolution, and peak crank torque (B), peak EMG amplitude (D), maximal change in fascicle length (F) and tendinous tissue (H) lengths during the first (ECC1) and second (ECC2) eccentric cycling bouts. The SD of ECC1 and ECC2 are shown by drank and light grey shadows, respectively, in panels A, C, E and G. *: significantly (P<0.05) different from ECC1.

Figure 6.4: EMG amplitude (mean ± SD) during the pushing (A) and recovery (B) phases during the first (ECC1) and the second (ECC2) eccentric cycling bouts. *: significantly (P<0.05) different from ECC1.

6.3.5 Fascicle and Tendinous Tissue Behaviour

Resting fascicle length at 90° of knee flexion was not different (P=0.28) between ECC1 (14.1 ± 3.0 cm) and ECC2 (14.2 ± 3.8 cm). The CV of the six revolutions used for analyses was 5.9 ± 1.8% (range: 2.6 – 8.1%), which was similar to that reported in a previous study [40]. As shown in Figure 6.3E, fascicle length increased as the knee joint angle increased, resulting in maximum fascicle lengths at 115.7 ± 4.6° and 111.8 ± 5.9° of knee flexion during ECC1 and ECC2, respectively (without significant difference between bouts), and returning to the resting length when the knee moved into knee extension. No significant difference between ECC1 and ECC2 was evident for the
knee angle at which maximum fascicle length occurred (P=0.09). Fascicle length changed from 8.8 ± 2.7 cm to 16.8 ± 4.8 cm during ECC1, and from 7.7 ± 2.5 cm to 14.4 ± 5.0 cm during ECC2, while the knee moved similarly from ~20° to 120° flexion in both bouts. The minimum (at ~20° of knee flexion) and maximum (at ~120° of knee flexion) fascicle lengths were significantly greater during ECC1 than ECC2 (P=0.007 and P=0.0008, respectively). Also, the magnitude of the fascicle length change (i.e. the difference between the minimum and maximal fascicle lengths) was greater (P=0.017) during ECC1 (8.0 ± 2.6 cm) compared with ECC2 (6.7 ± 3.2 cm), as shown in Figure 6.3F. The maximum fascicle length was observed at low torque (ECC1: -0.14 ± 8.6 Nm; ECC2: -2.04 ± 9.3 Nm) and muscle activity (ECC1: 0.016 ± 0.00 mV; ECC2: 0.006 ± 0.00 mV) levels for both bouts. Peak torque was achieved at a similar knee joint angle (ECC1: 71.5 ± 10.7°; ECC2: 72.6 ± 10.2°, P=0.5) and at a similar fascicle length (ECC1: 14.7 ± 3.4 cm; ECC2: 13.5 ± 3.1 cm, P=0.09) between bouts. Fascicle angle changes were similar (P=0.37) between bouts, and increased significantly from 8.4 ± 2.7° to 14.2 ± 4.6° during ECC1 (P<0.0001), and from 7.8 ± 3.1° to 13.3 ± 4.6° during ECC2 (P<0.0001).

TT length increased as the knee joint moved toward maximum flexion, as shown in Figure 6.3G. There was a tendency (P=0.055) for the maximum TT length to change at a greater knee angle during ECC1 (114.7 ± 4.3°) when compared with ECC2 (111.3 ± 6.9°). However, the maximum changes in TT length were not significantly different between bouts (P=0.36) as shown in Figure 6.3H.

Individual crank torque changes, and fascicle and TT length changes were averaged for the 11 participants during ECC1 and ECC2 separately, and the averaged changes in fascicle (Figure 6.5A) and TT (Figure 6.5B) lengths are plotted against crank torque for ECC1 (solid line) and ECC2 (point line). The ascending segment of the curve shows that the fascicle elongation was not different (P=0.09) at peak torque production between ECC1 (14.7 ± 3.4 cm) and ECC2 (13.5 ± 3.1 cm), but maximal fascicle length was observed at the descending segment of the curve when little torque was produced. The fascicle length was smaller during ECC2 than ECC1 when both the knee
joint was close to maximal flexion and almost no crank torque was generated, which is shown by the dashed area (Figure 6.5A). The TT length where the knee joint was close to maximal flexion and negative crank torque was developed was greater during ECC2 than ECC1 (Figure 6.5B). Peak crank torque was achieved at smaller TT length during ECC1 than ECC2, and after that the TT length tended to be greater during ECC2 than ECC1 as shown in the dashed area.

**Figure 6.5:** Relationship between fascicle (A) and tendinous tissue (B) length changes and crank torque during a pedal revolution during the first (ECC1: solid line) and second (ECC2: dashed line) eccentric cycling bouts based on the average of all participants. The dashed area highlights the smaller fascicle elongation during ECC2 than ECC1. Arrows represent the direction of the movement.

### 6.4 Discussion

The present study examined the *in vivo* vastus lateralis (VL) muscle fascicle and tendinous tissue behavior during two eccentric cycling bouts (ECC1, ECC2) performed two weeks apart to test the hypothesis that the magnitude of fascicle elongation during ECC2 would be smaller than in ECC1. The results showed that moderate muscle soreness was developed only after ECC1 (Figure 6.2B). The two eccentric cycling bouts were performed similarly without significant differences in the average power output, peak crank torque, EMG peak amplitude and knee range of motion,
however the magnitude of fascicle elongation was 16% smaller during ECC2 than ECC1 (Figure 6.3E and 6.3F). These results support the hypothesis that fascicle elongation would be smaller in a repeated bout of eccentric cycling. It is possible that the smaller muscle strain during ECC2 than ECC1 was associated with the reduction in muscle soreness after ECC2.

The study detailed in Chapter 3, in which 30 min of eccentric cycling was performed at 60% of the concentric \( P_{O_{\text{max}}} \) by young healthy men, revealed an 11-12% decrease in MVC torque 1-2 days after the first bout, but the MVC torque recovery was significantly faster after the second than the first bout. The smaller decrease (~5%) in MVC torque in the present study (Figure 6.2A) was likely due to the shorter exercise duration (10 min). It seems likely that the repeated bout effect was not clearly shown for MVC torque, because of its small decreases after exercise in the present study. However, a typical repeated bout effect was observed for muscle soreness such that moderate DOMS was developed only after ECC1 (Figure 6.2B). It has been documented that DOMS is more associated with damage/inflammation to connective tissue surrounding muscle fibers and fascia [180]. It is possible that the 10-min eccentric cycling still affected the connecting tissue, but its effects on muscle fibers were minimal. The lesser muscle soreness after the second eccentric cycling bout when compared with the initial bout was likely due to the repeated bout effect. As discussed below, it may be that smaller fascicle length changes were associated with the lesser muscle soreness after ECC2 than ECC1.

Changes in crank torque during a cycle revolution were similar between bouts (Figure 6.3A). The crank peak torque recorded during both ECC1 and ECC2 was approximately 30% of MVC torque measured at 90° of knee flexion on the ergometer, and also approximately 30% of the MVC torque measured on the isokinetic dynamometer at 70° of knee flexion (Figure 6.3B). The pattern of change in EMG amplitude (Figure 6.3C) was similar to that of the crank torque, and EMG peak amplitude of VL during eccentric cycling was also approximately 30% of the MVC for both cycling bouts (Figure 6.3D). These indicate that the intensity of eccentric contractions during cycling was low; however, it should be noted that more than 600 submaximal eccentric contractions
were performed during cycling including the warm-up. Interestingly, the EMG amplitude during the recovery phase of the cycle revolution was 60% smaller during ECC2 than ECC1 (Figure 6.4B). This shows that VL was de-activated or relaxed faster during ECC2 than ECC1 at the recovery phase, which may be attributed to more efficient motor pattern during ECC2 than ECC1. This could be associated with the repeated bout effect.

To the best of our knowledge, this was the first study to report muscle fascicle behavior during eccentric cycling. However, some studies have reported VL fascicle length changes during concentric cycling or isotonic/isokinetic eccentric contractions of the knee extensors. For example, Muraoka et al. [181] showed that fascicles operated at lengths between 9.1 ± 0.7 cm and 12.7 ± 0.5 cm during slow (40 rpm) concentric cycling in which the knee joint angle ranged from 39 to 114° of flexion. Guilhem et al. [182] reported that VL fascicle length changed from 9.3 to 12.8 cm during isotonic eccentric contractions (120% 1RM) and from 9.2 to 13.5 cm during maximal isokinetic (30°·s⁻¹) eccentric contractions of the knee extensors from 30° to 90° of knee flexion. Thus, the fascicle length changes during eccentric cycling found in the present study appear slightly greater than those reported during isotonic or isokinetic eccentric contractions in previous studies despite using a similar measurement technique [40, 182]. This greater fascicle elongation observed during eccentric cycling is most likely due to the greater range of motion (120°) than that in the previous studies (~90°). The present results showed that fascicle angle changed from 8.4 ± 2.7° to 14.2 ± 4.6° during ECC1 and from 7.8 ± 3.1° to 13.3 ± 4.6° during ECC2, without a significant difference between bouts. Resting VL fascicle angle has been reported to range between 6° and 27° in healthy subjects [183], which is in line with our results. Therefore, reasonably accurate and valid measurements of muscle fascicle length and angle appear to have been obtained in the present study.

In the present study, fascicle length at ~20° of knee flexion was shorter during ECC2 (7.7 ± 2.5 cm) than ECC1 (8.8 ± 2.7 cm), but no significant difference between bouts was evident for the fascicle length measured at 90° of knee flexion prior to (i.e. resting) eccentric cycling (ECC1: 14.1
The fascicle length kept increasing until the knee joint was moved to the maximal flexion (~120°) where the maximal fascicle length was observed. The maximal fascicle length was more than 2 cm greater during ECC1 (16.8 ± 4.8 cm) than ECC2 (14.4 ± 5.0 cm), as shown in Figure 6.3E. Importantly, the magnitude of fascicle length change (i.e. muscle strain) was significantly greater during ECC1 than ECC2 (Figure 6.3F). It is speculated that the smaller fascicle elongation represents a lesser mechanical strain in muscle fascicles and muscle fibers, resulting in less DOMS.

Since muscle fascicle elongation was smaller during ECC2 than ECC1, it was expected that TT elongation would be greater during ECC2 than ECC1 for the same total knee range of motion (i.e. same MTU length changes). However, TT elongation was not statistically different between bouts (Figure 6.3G). This could be due to the assumptions associated with the calculation of MTU and TT lengths [178, 179], which might not be sensitive enough to detect small differences between the conditions. However, it is noteworthy that TT continued to be stretched when fascicles were already at maximum length during both ECC1 and ECC2 (Figures 6.3E & 6.3G), but that the maximal TT elongation was reached earlier during ECC2 (111.3 ± 7.0°) compared with ECC1 (114.7 ± 4.3°), as shown in Figure 6.3G. These data suggest that TT tended to be elongated earlier and possibly to a greater extent during ECC2 than ECC1. Thus, it is possible that TT elongated relatively more during ECC2 to “compensate” for the smaller fascicle elongation during ECC2 than ECC1. This could be investigated further when methodological advances allow for more accurate monitoring of tendon length changes.

As shown in Figure 6.5, fascicle elongation did not occur during the torque generation phase (i.e. pushing phase) of the cycle revolution, and the maximal fascicle elongation occurred close to the maximal knee flexion position (i.e. long muscle length). Interestingly, this maximal fascicle elongation occurred when little torque was generated, as shown by the solid line in Figure 6.5A, but no further elongation occurred after the peak crank torque was achieved during ECC2 (dotted line). The dashed area of Figure 6.5 highlights the fascicle and TT length differences in relation to torque.
production between the first and second eccentric cycling bouts throughout a complete cycle revolution. As mentioned above, the greatest fascicle elongation was observed at a low EMG activity for both cycling bouts, but the EMG activity was significantly lower for the relaxation phase during ECC2 than ECC1 (Figure 6.4B). This suggests that muscles were still activated when the fascicles were elongated during ECC1, but muscles were de-activated or relaxed for this phase of the revolution during ECC2, possibly due to an improved motor pattern. It is possible that greater fascicle elongation of activated muscles was responsible for the greater muscle soreness induced after ECC1 than ECC2.

Although the present results do not provide definitive proof of the factors contributing to the 16% smaller fascicle elongation during ECC2 compared with ECC1 (Figure 6.3F), some speculations can be made. A change in the muscle’s dynamic stiffness could have contributed to the difference in the magnitude of fascicle elongation. Animal [184] and human [185] studies have shown that both passive and dynamic muscle stiffness were increased after a bout of downhill running and after six weeks of isotonic eccentric training of the elbow flexors, respectively. McHugh [14] has speculated that increased passive and dynamic muscle stiffness is one of the mechanisms underpinning the repeated bout effect. It might be that muscle extracellular matrix remodeling was induced after the first eccentric exercise, which increased passive and dynamic stiffness of the muscle. Increased muscle structural protein accretion 2-240 h after 30 stimulated eccentric contractions of the tibialis anterior has been reported in experimental animals [186]. The authors [186] hypothesized that reinforcement of the desmin cytoskeleton secondary to transcriptional up-regulation would provide mechanical protection from injury induced by eccentric contractions. Barash et al. [187] reported a three-fold increase in muscle desmin protein content in the tibialis anterior of rats 7 days after a single bout of 30 eccentric contractions. In humans, increases in VL desmin protein content were found 14 days after 30 min of downhill running [170]. Furthermore, increases in of dystrophin, desmin and titin mRNA levels in quadriceps femoris were found after the first downhill running bout in rats, but not after the second bout, and the authors
speculated that sarcomere cytoskeletal remodelling was induced after the first bout [188]. Lemos et al. [189] proposed, using a mathematical and structural modeling of the changes in muscle-tendon length, that the aponeurosis is the major contributor to reduce the fascicle length changes when a muscle is elongated, and speculated that one of the major functional roles of the aponeurosis was the protection of fascicles from excessive lengthening. Therefore, it is possible that remodeling of muscle extracellular matrix was induced after the first eccentric cycling bout, conferring a protective effect against the second eccentric cycling bout by increasing muscle stiffness. Further studies are required to elucidate the molecular mechanisms of the muscle-tendon complex adaptations after a bout of eccentric exercise including eccentric cycling.

In conclusion, the present study showed that VL muscle fascicles were elongated 16% less during the second than the first eccentric cycling bout performed 2 weeks earlier. It seems possible that this was associated with less muscle soreness after ECC2 than ECC1, and changes in muscle-tendon behavior play a major role in the repeated bout effect. It may be that the strain of muscle fascicles during ECC2 was less when compared to ECC1, because of the smaller fascicle length changes. Further studies are necessary to determine why muscle fascicles lengthened less during ECC2 than ECC1, and how tendinous tissue (including the aponeurosis) behave in relation to the smaller fascicle elongation during secondary eccentric cycling bout. It is also necessary to investigate muscle-tendon behavior differences between bouts for other eccentric exercise modalities, such as isokinetic or isotonic eccentric contractions of the elbow flexors and knee extensors, which have been shown to induce a strong repeated bout effect.
CHAPTER 7

Overall Discussion, Future Research Direction and Conclusions

7.1 Overall Discussion

It has been documented that eccentric cycling exercise can achieve greater power output with less energy cost when compared with concentric cycling [8, 9, 11]. This is an advantage as an exercise modality, especially for individuals who are less fit such as elderly and frail. In fact, some beneficial effects of eccentric cycling in elderly individuals [13], cancer survivors [89] and cardiovascular [51] and neurological disease patients [81, 94] have been advocated. These studies showed that eccentric cycling training induced greater increases in muscle function and skeletal muscle volume than standard medical care, and resistance or concentric cycling training [6, 7, 13, 52, 81, 83, 85, 87, 100]. However, a limited number of studies have investigated the acute physiological responses to eccentric cycling, and no previous study has compared between initial and secondary eccentric cycling bouts for metabolic and muscle damage profiles. To better prescribe eccentric cycling, its characteristics should be investigated further, especially potentially-negative muscle damage aspects. Thus, the main purposes of this PhD thesis project were to delineate the metabolic and muscle damage profiles of eccentric cycling, including the mechanism(s) underpinning muscle damage and adaptation conferred by repeated eccentric cycling bouts.

This thesis project consisted of four original studies. The first study compared the metabolic demand of both initial and secondary eccentric cycling bouts with concentric cycling, and the changes in muscle damage markers following two eccentric cycling bouts and one concentric cycling bout. The second study compared the substrate utilisation during and immediately after
exercise, and changes in resting energy expenditure and glucose uptake between two bouts of eccentric cycling and a bout of concentric cycling. The third study examined the changes in knee extensor rate of force development (RFD) and MVC peak torque following two bouts of eccentric cycling and a bout of concentric cycling. The last study quantified vastus lateralis muscle fascicle and tendon behaviour during two eccentric cycling bouts. This chapter aims to highlight the significant findings of each study, integrate the findings, and propose future research directions.

Study 1 (Chapter 3) showed that eccentric cycling was metabolically less demanding than concentric cycling as shown by a 50% lower oxygen consumption, 19% lower heart rate, 65% lower blood lactate and 22% lower rate of perceived exertion during the first eccentric cycling bout (ECC1) than concentric cycling (CONC); and the second eccentric cycling bout performed two weeks later (ECC2), showed a 12% lower heart rate and 35% lower blood lactate concentration than ECC1. These results confirmed previous findings of lower metabolic demand of eccentric cycling compared with concentric cycling [8, 9, 11, 21], and showed for the first time a further reduction in the metabolic demand during a secondary eccentric cycling bout. These results support that eccentric cycling may be an ideal exercise modality for individuals with limited cardiovascular fitness. In Study 1 (Chapter 3), changes in surface electromyogram (EMG) amplitude and frequency during cycling were examined, showing a 38–53% lower EMG amplitude during ECC1 than CONC and ECC2, and a 13% higher EMG median frequency during ECC1 than CONC. These results indicate a lesser muscle activation during eccentric than concentric cycling to produce the same power output, which explains the lower metabolic cost of eccentric cycling. The muscle damage profile of repeated eccentric cycling bouts showed that moderate loss of muscle function and delayed onset muscle soreness were induced for 3 days after ECC1, but muscle function was recovered by 1 day and no muscle soreness was developed after ECC2. Thus, muscle damage should not limit the use of eccentric cycling, if the first exposure is carefully planned and eccentric cycling is prescribed in accordance with the training theory.
Although the lower metabolic cost of eccentric cycling was initially reported in the early 1950s [9, 68] before being confirmed by several other studies [8, 11, 12, 21], the source of the energy utilised has not been documented. Thus, Study 2 (Chapter 4) examined substrate utilisation during and after two eccentric cycling bouts (ECC1, ECC2) and a concentric cycling bout (CONC), as well as the effects of both eccentric cycling bouts and concentric cycling on the resting energy expenditure and glucose uptake in recovery days (1-4 days after exercise). The results showed 72 and 85% greater fat oxidation rates during ECC1 and ECC2, than CONC, respectively. Interestingly, the post-exercise energy expenditure increased more after ECC2 than ECC1, which was not different from that after CONC, despite of the smaller metabolic demand during eccentric than concentric cycling. The greater fat utilisation during eccentric than concentric cycling was likely due to the lower oxygen consumption during cycling found in Study 1 (Chapter 3), but further increases in fat utilisation and greater post-exercise energy expenditure during and after ECC2 were unexpected. These results suggest that eccentric cycling could induce improvements in the muscle oxidative capacity after ECC1 that increased the oxidation of fat as fuel source during the second eccentric cycling bout. Importantly, no glucose uptake impairment was found after eccentric cycling, suggesting that this exercise can be performed by patients with decreased insulin sensitivity without risk.

Study 3 aimed to examine the use of RFD as a better marker of muscle damage, and compared the changes in RFD and MVC peak torque after eccentric and concentric cycling. It was thought that eccentric cycling could be an ideal model to investigate muscle fatigue and damage, because its effects may be similar to that of team sport activities or repetitive daily or occupational tasks. It was assumed that the ability to exert rapid increases in torque (i.e. RFD) would be more impaired than MVC peak torque, which is considered the best indirect marker of muscle damage but is unable to differentiate muscle damage from fatigue immediately after exercise [27, 28]. The results revealed that RFD calculated from the torque raise onset (i.e. 0–200 ms) showed similar decreases to that of MVC peak torque after eccentric cycling. However, RFD calculated later in the
torque-time curve (i.e. 100–200 ms: RFD\textsubscript{100-200}) showed 7–19% greater decreases than MVC peak torque immediately after, and 1 and 2 days after the first eccentric cycling bout, and RFD\textsubscript{100-200} did not decrease after concentric cycling. These results indicate that RFD\textsubscript{100-200} is a more specific and sensitive indirect marker of eccentric exercise-induced muscle damage. It is suggested that RFD\textsubscript{100-200} could be useful for coaches and clinicians to detect muscle damage soon after the exercise, and adopt quicker a rehabilitative/recovery strategy after training or match, speeding up the recovery.

It is well documented that a single bout of eccentric exercise confers a protective effect against muscle damage in subsequent bouts of the same or similar eccentric exercise [15, 190], and as shown in Studies 1 and 3 (Chapters 3 and 5), this was also the case for eccentric cycling. However, the mechanisms underpinning this protective adaptation are not clearly understood. It was thought that mechanical strain would not be the same between the initial and secondary eccentric cycling bouts, which would be associated with different muscle-tendon behaviour between bouts. Thus, the last study of this PhD project (Study 4, Chapter 6) aimed to investigate the repeated bout effect by assessing vastus lateralis muscle and tendon behaviour in two eccentric cycling bouts that were performed two weeks apart. The results showed that elongation of the muscle fascicles was 16\% smaller during the second than first eccentric cycling bout, while crank torque production, muscle activity and knee range of motion were similar between bouts. Greater muscle soreness was developed 1-2 days after the first than second eccentric cycling bout. These results suggest that smaller muscle fascicle elongation is associated with the protective effect against muscle damage observed during repeated eccentric exercise. This finding sheds light on the possible mechanisms underpinning the repeated bout effect.

These findings contribute to the body of knowledge of eccentric cycling and muscle damage, and provide better understanding of muscle damage induced by eccentric exercise. The importance of exercise is increasing in order to cope with progressing health issues (e.g. cardiovascular disease, diabetes, obesity). The world’s population is aging, and it is projected that individuals older than 85 years old will increase 350\% by the year 2050 when compared with year
Two of the main physiological consequences of aging are sarcopenia and decreased aerobic capacity [192], which decrease mobility and increase cardiovascular risk that ultimately decrease quality of life [192, 193]. Furthermore, the number of individuals with limited exercise tolerance due to clinical conditions such as cancer, pulmonary and cardiac diseases has also increased [194]. As such the investigation of new benefits from “exercise as medicine” for those individuals with limited exercise tolerance is crucial. In this context, eccentric cycling has been advocated to be ideal for populations with limited fitness level, mainly due to its potential to increase muscle function and muscle mass with low metabolic cost and cardiovascular stress [45, 46]. This research project has supported the notion that eccentric cycling can be an ideal exercise for all individuals.

### 7.2 Future Directions

There are still numerous unanswered questions, but the present results clarify several areas of future research. One of the limitations of the present project was that all studies used young healthy male volunteers. This was because investigation of young healthy individuals should precede testing elderly and/or frail individuals. Therefore, the findings of the present project may not be necessarily applicable to elderly and/or frail individuals. Although previous studies have already shown that elderly, frail or diseased individuals can perform eccentric cycling with several positive outcomes [13, 35, 51, 53, 81], further studies are necessary to examine whether these populations show similar metabolic and muscle damage profiles of eccentric cycling found to those shown by young healthy men used in the present project.

The present project focused on acute responses to two eccentric cycling bouts, however it should be repeatedly performed as training when it is used as an exercise modality to improve health and fitness. As mentioned previously, eccentric cycling may be an ideal exercise for health promotion and wellness of patients. Future studies should investigate the effects of eccentric
cycling training on health and disease, especially for individuals with impaired metabolic functions such as diabetes, metabolic syndrome and chronic obstructive pulmonary disease patients.

Decreased metabolic demand and increased fat utilisation during and after eccentric cycling were shown in Studies 1 and 2 (Chapters 3 and 4), but it should be noted that the intensity of exercise was different between eccentric and concentric exercise bouts, although the total work was matched between them. Thus, it is of interest to compare fat and CHO utilisation at the same exercise intensity (i.e. oxygen consumption) between concentric and eccentric cycling to examine whether eccentric contractions themselves utilise a greater proportion of fat as an energy source. It is also important to investigate the molecular mechanisms underlying how eccentric cycling training might increase fat utilisation, and whether eccentric cycling induces changes in muscle oxidative capacity after cycling.

The results of Study 1 (Chapter 3) clearly show the repeated bout effect subsequent to an initial bout of eccentric cycling, such that the magnitude of muscle damage after the second eccentric cycling bout was minimal. However, the first eccentric cycling bout induced symptoms of muscle damage lasting for 3 days. It has been shown that low-intensity eccentric contractions confer a potent protective effect against muscle damage induced by maximal eccentric contractions [195]. Although the intensity of eccentric contractions during eccentric cycling was relatively low (e.g. 30% of MVC), it may be better to introduce eccentric cycling from much lower intensities and shorter duration in clinical practice; future research might investigate how to prescribe eccentric cycling training with little or no muscle damage. This is important for elderly and frail individuals, since muscle damage could negatively affect their performance in activities of daily living or likelihood of continuing with the exercise training.

It was found that RFD could be a sensitive and specific marker of muscle damage using eccentric cycling as an exercise model. This needs to be confirmed in a more practical setting (e.g. long distance running, field sports). The present project also showed that muscle-tendon behaviour was different between the first and second eccentric cycling bouts, but the exercise duration was
short, and the repeated bout effect for muscle damage markers was not strong. Therefore, it is
necessary to repeat the study with a longer cycling time (e.g. 30 min) and greater number of
participants, including elderly individuals to investigate the relationship between muscle fascicle
length changes during eccentric contractions and the magnitude of muscle damage. The smaller
fascicle elongation during the second eccentric cycling bout provided evidence to support the
mechanical theory of the repeated bout effect, which was hypothesised to be associated with
structural changes in the muscle fibres, fascicles, fascicular connective tissues, and tendons. Future
studies should investigate changes in cytoskeleton proteins, and extracellular matrix, endomysium
and perimysium histology after a single bout of eccentric exercise.

Eccentric cycling is performed in a specifically-designed cycle ergometer using an electrical
motor to rotate the cranks backwards, thus the ergometer is larger and more costly than a concentric
cycling ergometer. It is therefore not currently accessible for many, and is rarely seen in public
gymnasiums or community health centres. However, the cost of an eccentric ergometer could be
decreased if eccentric ergometers are custom-built using a recumbent ergometer frame and seat, a
motor and speed controller, and a modest amount of custom fabrication as described in a literature
[196]. It is necessary to make eccentric cycling ergometers available to community by decreasing
the cost. It may be that current cycling ergometers in gyms and fitness centres are replaced by
eccentric ergometers in the future.

Like other exercise prescriptions, eccentric cycling also requires closer supervision and
attention, especially during some initial attempts to ensure the correct positioning and coordination
of the individuals to properly perform the exercise and avoid undue stress on joints. To promote this
exercise modality, more studies are necessary to establish the training protocols.

7.3 Conclusions

The present project has provided some novel insights into muscle damage induced by
eccentric contractions, and physiological characteristics of eccentric cycling as described above.
Since muscle damage is moderate and can be attenuated or abolished in the repeated eccentric cycling bouts, muscle damage should not limit the use of eccentric cycling as exercise as “medicine.” Eccentric cycling can be an ideal exercise modality for individuals including patients with impaired muscle oxidative function such as diabetes and chronic obstructive pulmonary disease, because of the less metabolic demand but potent stimulus for muscle growth and strength development. Further studies are warranted to investigate the effects of eccentric cycling training on these populations. Although muscle damage can be minimised by proper training prescription in exercises including eccentric cycling, the mechanisms underpinning eccentric exercise-induced muscle damage and adaptations are not fully understood. Eccentric cycling appears to be a good model to study eccentric exercise-induced muscle damage. More studies are required to better understand eccentric exercise-induced muscle damage and the repeated bout effect.
REFERENCES


APPENDICES

Appendix 1: Ethics Approval Letter

From: Research Ethics  Sent: Monday, 28 February 2011 11:46 AM  To: Luis PENAILILLO  Cc: Ken NOSAKA; Daniel GALVAO; Anthony BLAZEVICH; Research Assessments

Subject: 5228 PENAILILLO ethics approval

Dear Luis

Project Number: 5228 PENAILILLO
Project Name: Characteristics of Eccentric Cycling and Its Health Benefits

Student Number: 10166842

The ECU Human Research Ethics Committee (HREC) has reviewed your application and has granted ethics approval for your research project. In granting approval, the HREC has determined that the research project meets the requirements of the National Statement on Ethical Conduct in Human Research.

The approval period is from 28 February 2011 to 31 December 2012.

The Research Assessments Team has been informed and they will issue formal notification of approval. Please note that the submission and approval of your research proposal is a separate process to obtaining ethics approval and that no recruitment of participants and/or data collection can commence until formal notification of both ethics approval and approval of your research proposal has been received.

All research projects are approved subject to general conditions of approval. Please see the attached document for details of these conditions, which include monitoring requirements, changes to the project and extension of ethics approval.

Please feel free to contact me if you require any further information.

Regards
Kim

Kim Gifkins, Research Ethics Officer, Office of Research & Innovation, Edith Cowan University, 270 Joondalup Drive, Joondalup, WA 6027 research.ethics@ecu.edu.au Tel: +61 08 6304 2170 | Mobile: 0428 035 397 | Fax: +61 08 6304 5044 | CRICOS IPC 00279B
Appendix 2: Information Letter & Inform Consent Studies 1-3

Information Letter to Participants

Thank you very much for indicating your interest in participating in this study. The purpose of this document is to explain the study that you are going to participate. Please read carefully and understand the information below, and do not hesitate to ask any questions.

Project Title
Effects of repeated bouts of eccentric cycling compared to one concentric cycling bout for metabolic demand, resting energy expenditure, insulin sensitivity and muscle damage

Researchers
This research project is being undertaken as part of the requirements of a PhD by Research (Sports Science) at Edith Cowan University (ECU).

PhD Candidate: Luis Penailillo (l.penailillo@ecu.edu.au) 6304 5156
Supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655
Co-supervisor: Assoc. Prof. Antony Blazevich (a.blazevich@ecu.edu.au) 6304 5472
Co-supervisor: Assoc. Prof. Daniel Galvao (d.galvao@ecu.edu.au) 6304 3420

Further details on supervisors and School of Exercise, Biomedical and Health Sciences are available at http://www.sebhs.ecu.edu.au.

Background
Eccentric contractions are performed when muscles are lengthened under tension (i.e. descending a dumbbell very slow), as opposed to concentric contractions in which muscles are shortened (i.e. lifting the dumbbell). Eccentric contractions are performed during daily activities such as walking down stairs or sitting down on a chair. Lowering weights and downhill running or walking have been used as models to study eccentric exercise. Eccentric cycling is a new method of performing eccentric exercise, and it is getting popular. During eccentric cycling your thigh muscles (knee extensor muscles) perform force when resisting against backward rotational movements of the cranks of the bike, this produces the lengthening of your muscles whilst they are contracting, which is eccentric exercise.

One particular consequence of eccentric exercise is muscle damage and muscle soreness. However, when eccentric exercise is performed for the second time within several weeks after initial bout, muscle damage and soreness are attenuated. On the other hand, eccentric cycling training has shown to be beneficial for glycemic control (sugar in blood) and increases whole body metabolism (energy consumed by the body). However, acute
effects after eccentric exercise such as muscle damage have shown produce impairments in the action of insulin hormone (hormone in charge of stimulating the glucose uptake from blood to tissues and organs, specially muscle). Furthermore, the increase in whole body metabolism may be related to the recovery process after muscle damage induced by eccentric exercise. However, no study has investigated both the muscle damage profile after repeated bouts of eccentric cycling exercise and how muscle damage is related to insulin hormone action and whole body metabolism.

**Purpose of the Study**

The purpose of this study is to investigate the effects of two bouts of eccentric cycling on muscle damage profile and its influence over the insulin hormone action and resting metabolism when it is compared with the response after a conventional concentric cycling bout. Therefore, this study will investigate:

1. The muscle damage profile following two bouts of eccentric cycling exercise, and compare it with the response after concentric cycling.
2. The response of insulin hormone and glucose to repeated bouts of eccentric cycling, and compare it with the response after concentric cycling.
3. The response of the whole body metabolism and carbohydrate and fat utilisation during and after repeated bouts of eccentric cycling, and compare it with the response after concentric cycling.

**Eligibility**

You will be eligible to participate in this study if your age is between 18 and 35 years old, if you have no musculoskeletal injuries of the lower extremities and if you have not performed lower limb resistance training within the last 6 months. You will be screened with a generic medical questionnaire consisting of several questions about your health and physical conditions. Once you are found to be eligible for the study, you will be invited to participate in a familiarisation session.

**Requirements**

You will be asked to come to the Exercise Physiology Laboratory (JO 19.150) for one familiarisation session, and three baseline measures sessions one week before the first cycling bout (concentric cycling). During your participation in this Study you will be asked to come to the lab for three blocks of five days, where you will perform 30-min cycling and follow-up measurements for 4 days after each cycling bout, the concentric cycling bout, and the two eccentric cycling bouts. Each cycling bout session as is shown in the Figure 1 (Day 0 - cycling day) will last 2 hours, including a cycling bout of 30-min and immediately after measurements. The follow-up measures (Day 1 to 4) after exercise will take 1 to 2 hour each. Covered shoes and shorts are required for all sessions.
Procedures

The familiarisation session, where procedures and purposes of present study will be explained to you, if your result eligible, you will be scheduled for three days of baseline measurements.

Day 1 baseline, one week before the first cycling bout (concentric cycling), you will report to the laboratory in the morning after an overnight fast for blood sample (oral glucose tolerance test: OGTT, five blood samples within 120 minutes will be taken)

Day 2 baseline you will report to the laboratory in the morning after an overnight fast to assess your resting energy expenditure (REE) using gases analysis during 40 minutes of resting, whilst lying down on a massage table.

Day 3 baseline, the day after you will perform a physiological test until extenuation to determine your maximal aerobic capacity on a stationary bike.

The day of the cycling trial, other parameters such as muscle soreness, presence of protein in blood, and strength will be measured before each cycling bout (see Figure 1).

This study has three experimental blocks. In the first block you will perform 30-minutes of concentric
cycling (conventional cycling) at moderate-low intensity (65%) followed by one-hour of measures immediately after exercise (strength and energy expenditure). Then, you will be asked to come to the lab for the following 4 days to measure your strength, muscle soreness, and muscle damage by images and blood samples from finger prick. You will be asked to come to the lab after an overnight fast (8 – 10 hrs) the 5 days of testing it will be to take blood samples to test the levels of glucose and insulin hormone in your blood (OGTT) and your resting energy expenditure (REE). You will have one week off (you don’t need to go to the lab) after every testing week.

After this one week off, you will be asked to come to the lab for two more experimental blocks, consisting of two eccentric cycling bouts (cycling backwards) with a follow-up period of 4 days each (Tuesday to Friday) 2 weeks apart from first and second ECC cycling. The exercise bouts will consist on 30-minutes eccentric cycling at a low-moderate intensity (65%) followed by one-hour of measures immediately after exercise (muscle strength and energy expenditure), and 4 days of follow-up period after each bout of eccentric cycling (same after concentric cycling), where you will be asked to come to the lab for measures of strength, muscle soreness and muscle damage parameters. At day 1 to 4 you will be asked to come to the lab after an overnight fast for blood samples (OGTT) and resting energy expenditure (REE) assessment.

Measurements

1. **MVC**: The strength measures of your knee muscles will be performed by an isokinetic dynamometer. You will be verbally encouraged to perform two maximal static contractions at the knee joint angle of 60 degrees by holding each contraction for 5 seconds with 1 minute of rest between each effort.

2. **Muscle soreness**: Using a 100-mm visual scale where 0 mm indicates no pain at all and 100 mm is an indication of “unbearable” pain, you will be instructed to place a mark on a 100-mm line while rising from and sitting in a chair two times throughout 90° knee joint range of motion and stretching two times your thigh muscles.

3. **Pressure pain threshold**: Muscle tenderness in your thigh will be assessed by an algometer (a device to apply and measure pressure). You will be asked to report the moment when the pain is perceived. Two measurements will be taken from each site with a 30-second interval between measurements.

4. **Echo Intensity**: Images of your muscle will be obtained from five points of your thigh muscles using an ultrasound machine (i.e. similar to the one used for look at babies during pregnancy). The investigator will place the ultrasound probe on the marked sites on your thigh to obtain both cross-sectional and transverse images.
5. **Resting energy expenditure (REE) and substrate utilisation**: It is measure of how much energy your body expend during resting, furthermore from which kind of energy sources you are taking that energy (fat, carbohydrates or proteins). The REE and substrate utilisation will be estimated during 40 minutes of lie down on a massage table. A **mouthpiece will be placed in your mouth** to collect the gases produced and consumed during the 30 minutes after the cycling exercise. Data will be collected using an oxygen analyser device.

6. **Insulin sensitivity**: It will tell us how your body is using glucose and how long it takes to remove it from blood. It will be assessed by the oral glucose tolerance test (OGTT) that will collect blood samples from your antecubital vein (forearm) **before** glucose (sugar) ingestion (75 grams of glucose diluted in water), and at **30, 60, 90 and 120 min after** glucose ingestion (5 blood samples) that will be analysed for insulin hormone and glucose. For the OGTT you will come to the lab after an overnight fast between 07.00 and 10.00am. 5 blood samples will be collected from your arms veins alternating them.

7. **Creatine kinase (CK) activity**: It is blood marker of muscle damage and a 30 µL blood sample will be extracted from your finger by a capillary tube, and presence of CK protein activity will be measured in your blood.

8. **Blood lactate**: it is a blood marker of muscle metabolism. It will be collected from your earlobe by a prick in order to collect 25µL of blood and instantaneously measure your blood lactate during the 3 cycling modalities.

**Testing Considerations**

Prior to all testing and exercise days you are required to refrain from exercise for at least 48 hours. Participants are also required to abstain from taking any stimulants or depressants (including caffeine or alcohol) for at least 12 hours prior testing. Finally, participants are also encouraged to maintain a normal diet and drink plenty water the day of and the day before testing.

**Risks**

- You will expect muscle soreness for few days after performing eccentric cycling.
- During the experiments venous blood will be collected from the forearm using needle puncture. In total 38 venous punctions will be performed within 7 weeks, (5 per each OGTT). Bruising to the area where the blood will be collected may occur. There is also a small risk of infection, but this will be minimised using aseptic procedures. This is a standard procedure with no reported adverse reactions or outcomes. A trained researcher, accredited with the procedure, will conduct the procedures.
• To place the electromyography electrodes, the skin must be shaved and cleaned. However, some skin irritation would be produced after shaving.
• During baseline measures for VO$_{2\text{max}}$ and maximal strength you may feel fatigue and soreness for a few days.
• The mouthpiece utilized to collect the air and gases production/consumption may be uncomfortable to maintain for a long period of time.

Benefits
• You will be able to know your maximal aerobic capacity measured by modern equipment.
• You will be able to experience three 30-minutes training bouts performing conventional concentric cycling training and a novel cycling training (eccentric cycling).
• You will be tested for insulin sensitivity, which is one of the main parameter for diabetes mellitus diagnosis. Furthermore, you will be able to look at the influence of exercise on this parameter.
• You will be able to find out about the metabolic cost of concentric and eccentric cycling exercise.

Confidentiality of Information
Your anonymity is ensured as much as is possible during the investigation and, by the assigning of number codes to subjects by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the Chief Researcher during the period of the study. The information and data gathered from you during the study will be used to answer the research question of this study. People who will have access to the raw information for this study are only limited to the researcher and the Supervisors. Data collected will be stored in a password-protected computer and is only available to the researchers. Hard copy data (paper etc) will only be kept in the researcher’s office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

Results of the Research Study
The results of this study are intended for completion of a PhD by Research thesis and may be presented in conferences/seminars and published in peer-reviewed journal(s), as magazine articles, as an online article or part of a book section and reports. Published results will not contain information that can be used to identify participants unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.
Voluntary Participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose not to participate. Your decision if you do not want to participate or continue to participate will not disadvantage you or involve any penalty.

Withdrawing Consent to Participate

You are free to withdraw your consent to further involvement in this research project at any time. You also have the right to withdraw any personal information that has been collected during the research with your withdrawal.

Questions and/or Further Information

If you have any questions or require any further information about the research project, please do not hesitate to contact:
Luis Penailillo (PhD student - Researcher)
Office 19.127, School of Exercise, Biomedical, and Health Sciences, Edith Cowan University
270 Joondahup Drive, Joondalup, WA 6027, Australia.
Tel: (+61 8) 6304 5156
Email: l.penailillo@ecu.edu.au

Independent Contact Person

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:
Kim Gifkins (Research Ethics Officer)
Building 1, Block 'B', Level 3, Room 333, Edith Cowan University, 100 Joondalup Drive, JOONDALUP WA 6027
Phone: (+61 8) 6304 2170
Email: research.ethics@ecu.edu.au
Website: http://www.ecu.edu.au/GPPS/ethics

Approval by the Human Research Ethics Committee:

This research project has been approved by the ECU Human Research Ethics Committee. Attached is the letter of approval for your information.
Informed Consent Form

Project: Effects of repeated bouts of eccentric cycling compared to one concentric cycling bout for resting energy expenditure, insulin sensitivity and muscle damage

I, as a participant in this study
- Have been provided with a copy of the Information Letter, explaining the research study;
- Have read and understood the information provided;
- Have been given the opportunity to ask questions and have had any questions answered to my satisfaction;
- Am aware that if I have any additional questions, I can contact the research team;
- Understand that the participation in the research project will involve all procedures that are listed in the Participants Informed Letter;
- Understand that the information provided will be kept confidential, and that the identity of participants will not be disclosed without consent;
- Understand that the information provided will only be used for the purposes of this research project, and understand how the information is to be used;
- Understand that I am free to withdraw from further participation at any time, without explanation or penalty;
- Freely agree to participate in the study.

Name: ___________________________ Date: ___________________

Signature: ___________________________

Researchers contact:
Luis Penailillo PT., MSc. - PhD Candidate -
Office 19.127, School of Exercise, Biomedical, and Health Sciences, Edith Cowan University
270 Joondalup Drive, Joondalup, WA 6027. Australia.
Tel: (+61 8) 6304 5156
Email: l.penailillo@ecu.edu.au

Professor Ken Nosaka - Assoc. Prof. Anthony Blazevich - Assoc. Prof. Daniel Galvao
Supervisor - Co-supervisor, Co-supervisor
Tel. (61) 8 6304 5655 - Tel. (61) 8 6304 5472 - Tel. (61) 8 6304 3420
Email: k.nosaka@ecu.edu.au - Email: a.blazevich@ecu.edu.au - Email: d.galvao@ecu.edu.au
Appendix 3: Information Letter and Inform Consent Study 4

Information Letter to Participants

Thank you very much for indicating your interest in participating in this study. The purpose of this document is to explain the study that you are going to participate. Please read carefully and understand the information below, and do not hesitate to ask any questions.

Project Title
Comparison of in-vivo muscle-tendon behaviour between two eccentric cycling bouts

Researchers
This research project is being undertaken as part of the requirements of a PhD by Research (Sports Science) at Edith Cowan University (ECU).

- PhD Candidate: Luis Penailillo (l.penailillo@ecu.edu.au) 6304 5156
- Supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655
- Co-supervisor: Assoc. Prof. Anthony Blazevich (a.blazevich@ecu.edu.au) 6304 5472
- Co-supervisor: Assoc. Prof. Daniel Galvao (d.galvao@ecu.edu.au) 6304 3420

Further details on supervisors and School of Exercise and Health Sciences are available at http://www.sebhs.ecu.edu.au.

Background
Eccentric contractions are performed when muscles are lengthened under tension (i.e. descending a dumbbell very slow), as opposed to concentric contractions in which muscles are shortened (i.e. lifting the dumbbell). Eccentric contractions are performed during daily activities such as walking down stairs or sitting down on a chair. Lowering weights and downhill running or walking have been used as models to study eccentric exercise. Eccentric cycling is a new method of performing eccentric exercise, and it is getting popular. During eccentric cycling your thigh muscles (knee extensor muscles) perform force when resisting against backward rotational movements of the cranks of the bike, this produces the lengthening of your muscles whilst they are contracting, which is eccentric exercise.

It has been shown that eccentric cycling consume 70% lesser energy than concentric cycling at the same intensity, and eccentric cycling can produce 4 to 7 times greater force compared with concentric cycling at moderate intensity (65% of the maximum heart rate). However, other characteristics of eccentric cycling have not been fully investigated.
This study will investigate the muscle and tendon behaviour on real time during eccentric and concentric cycling. By using a new technique to imaging what is happening underneath the skin, specifically your muscle an ultrasound machine (i.e. same machine to look at babies during pregnancy) will be used to look at your thigh muscles, which is a non-invasive technique to visualise muscle and tendon movement during exercise, this study has as aim to compare between eccentric cycling and concentric cycling for muscle and tendon behaviour, looking at how really muscles move and change their length, and compare the differences of the energetic cost of these two exercise modalities. These measurements will give us an idea about the relationship about your muscle and tendon behaviour and the energy that is necessary for eccentric and concentric cycling.

**Purpose of the Study**

The purpose of this research is to investigate the relationship between force production, energetic cost and muscle-tendon behaviour during eccentric cycling in comparison with concentric cycling.

**Eligibility**

You will be eligible for this study if your age is between 18 and 35 years old, and you have no musculoskeletal or neurological injuries of the lower extremities and you have not performed lower limb resistance training in the last 6 months. You will be screened with a generic medical questionnaire consisting of several questions about your health and physical condition. Once you are found to be eligible for the study,
you will be invited to participate in a familiarisation session.

**Requirements**

You will be asked to come to the Exercise Physiology Laboratory (JO 19.150) 11 times for:

1) A familiarisation session (30 minutes)
2) Baseline measurements (1h);
3) Concentric cycling trial (90 minutes) plus 2 subsequent days (15 min each);
4) Eccentric cycling trial 1 (2 hours) plus 2 subsequent days (15 min each); and
5) Eccentric cycling trial 2 (90 minutes) plus 2 subsequent days (15 min each).

Firstly, a familiarisation and demonstration of the procedures will be performed. Second, one week before the concentric cycling trial, baseline measurements will be performed; it will be followed by a test to measure your maximal aerobic capacity and another test to test your thigh muscles maximal strength (concentric and eccentric strength). Once you have completed the familiarisation and baseline testing sessions, you will be scheduled for the three experimental trials (concentric, eccentric 1 and eccentric 2 cycling) separated by two weeks. In addition, after each cycling trial day you will be asked to come back to the lab for the 2 subsequent days to test your maximal strength and muscle soreness if any, it will take 15 min each day. Covered shoes and shorts are required for all sessions.

**Measurements**

The familiarisation session will include explanation and demonstration of the equipment that will be used, and also height and weight measures will be taken. In the baseline measures session, you will be tested for maximal concentric and eccentric strength, where you will be verbally encouraged to perform two maximal contractions of your thigh muscles with 1 minute of rest between each effort. Additionally, an incremental maximal cycling test will be performed to assess your maximal aerobic capacity, This test will require you to cycle on a stationary bike at an easy workload (50 watts) for 4 minutes, after which the intensity will get progressively harder (increase by 20 watts) every 2 minutes until your exhaustion. Throughout the duration of this test you will wear a mouthpiece attached to a gas analyser to measure how much oxygen you consume.

For the eccentric and concentric cycling trial, an ultrasound probe will be attached to the mid-belly of the outside of your right thigh (vastus lateralis muscle) and a second probe will be attached to the end of this same muscle (tendon). Electrodes will be placed to the right thigh muscles (vastus lateralis, rectus femoris, vastus medialis and biceps femoris) to record the muscle activity of those muscles during the different cycling modalities. Another two probes will be placed and taped to the left thigh (vastus lateralis and rectus femoris muscles) to measure the oxygen content of your muscles during exercise. A mouthpiece will be placed in your mouth to collect the air during the cycling, and oxygen consumed will be analysed.
The exercise will consist of 10 minutes cycling at 80% of your maximal concentric intensity at a cadence of 60 revolutions per minute. The order of the exercise trials will be always concentric cycling first followed by eccentric cycling trial 1 two weeks later and a second eccentric cycling trial.

**Testing Considerations**
Prior to all baseline and experimental testing days you are required to refrain from exercise for at least 48 hours. Participants are also required to abstain from taking any stimulants or depressants (including caffeine or alcohol) for at least 12 hours prior testing. Participants are also encouraged to maintain a normal diet and drink plenty water the day of and the day before testing.

**Risks**
- Ultrasound scans and muscle blood oxygenation are safe and non-invasive method to study muscle physiology; however some discomfort may be felt by keeping the probes attached to the skin by tape.
- To place the electromyography electrodes, the skin must be shaved and cleaned. However, skin may be irritated after shaving.
- You may also expect some muscle soreness for few days after performing eccentric cycling.

**Benefits**
- You will be able to experience a new method to assess muscle-tendon behaviour in-vivo and understand how muscles move during different type of contractions.
- You will be able to find out about your maximal aerobic capacity and your maximal strength.
- You will be able to experience a 30 minutes eccentric and concentric cycling training.
- You will experienced a different cycling modality.

**Confidentiality of Information**
Your anonymity is ensured as much as is possible during the investigation by the assigning of number codes to subjects by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the Chief Researcher during the period of the study. The information and data gathered from you during the study will be used to answer the research question of this study. People who will have access to the raw information for this study are only limited to the researcher and the Supervisors. Data collected will be stored in a password-protected computer and is only available to the researchers. Hard copy data (paper etc) will only be kept in the researcher’s office and locked in a specific drawer/filing cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

**Results of the Research Study**
The results of this study are intended for completion of a PhD by Research thesis and may be presented in conferences/seminars and published in peer-reviewed journal(s), as magazine articles, as an online article or part of a book section and reports. Published results will not contain information that can be used to identify participants unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.

**Voluntary Participation**

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose not to participate. Your decision if you do not want to participate or continue to participate will not disadvantage you or involve any penalty.

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Email: l.penailillo@ecu.edu.au

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Building 1, Block 'B', Level 3, Room 333, Edith Cowan University, 100 Joondalup Drive, JOONDALUP WA 6027
Phone: (+61 8) 6304 2170
Email: research.ethics@ecu.edu.au

**Approval by the Human Research Ethics Committee**

This research project has been approved by the ECU Human Research Ethics Committee. Attached is the letter of approval for your information.
Informed Consent Form

Project: Comparison between eccentric and concentric cycling

- You do not have any problems participating in the study
- You will report to the laboratory 11 days over 5 weeks time
- You may experience muscle soreness
- Your legs will be taped to place the ultrasound probes, blood oxygenation and EMG electrodes.

I, as a participant in this study
- Have been provided with a copy of the Information Letter, explaining the research study;
- Have read and understood the information provided;
- Have been given the opportunity to ask questions and have had any questions answered to my satisfaction;
- Am aware that if I have any additional questions, I can contact the research team;
- Understand that the participation in the research project will involve all procedures that are listed in the Participants Informed Letter;
- Understand that the information provided will be kept confidential, and that the identity of participants will not be disclosed without consent;
- Understand that the information provided will only be used for the purposes of this research project, and understand how the information is to be used;
- Understand that I am free to withdraw from further participation at any time, without explanation or penalty;
- Freely agree to participate in the study.

Name: ____________________________________ Date: __________________
Signature: _________________________________

Researchers contact:

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270 Joondalup Drive, Joondalup, WA 6027. Australia.
Tel: (+61 8) 6304 5156
Email: l.penailillo@ecu.edu.au

Professor Ken Nosaka                 Assoc. Prof. Anthony Blazevich                 Assoc. Prof. Daniel Galvao
Supervisor                          Co-supervisor.                                Co-supervisor
Tel. (61) 8 6304 5655               Tel. (61) 8 6304 5472                      Tel. (61) 8 6304 3420
Email: k.nosaka@ecu.edu.au          Email: a.blazevich@ecu.edu.au              Email: d.galvao@ecu.edu.au
Appendix 4: Medical Questionnaire for Participants

**PRE-EXERCISE MEDICAL SCREENING QUESTIONNAIRE**

Metabolic and Muscle Damage Profiles of Eccentric Cycling

The following questionnaire is designed to establish a background of your medical history, and identify any injury and/ or illness that may influence your testing and performance. Please answer all questions as accurately as possible, and if you are unsure about anything please ask for clarification. All information provided is strictly confidential.

**Personal Details**

Participant ID: ________________________________

Date of Birth (DD/MM/YYYY): __________________

**PART A**

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Are you a regular smoker or have you quit in the last 6 months?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Did a close family member have heart disease or surgery, or stroke before the age of 60 yrs?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Do you have, or have you ever been told you have blood pressure above 140/90 mmHg, or do you currently take blood pressure medication?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you have, or have you ever been told you have a total cholesterol level above 5.20 mm/L (200 mg/dL)?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Is your BMI (weight/height²) greater than 30 kg/m²?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PART B**

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you ever had a serious asthma attack during exercise?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Do you have asthma that requires medication?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Have you had an epileptic seizure in the last 5 yrs?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Do you have any moderate or severe allergies?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Do you, or could you reasonably, have any infectious disease?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. Do you, or could you reasonably, have an infection or disease that might be aggravate by exercises

**PART C**

1. Are you currently taking any prescribed or non-prescribed medications?
   - YES  
   - NO

2. Have you had, or do you currently have, any of the following? If YES, please provide details
   - · Rheumatic fever
     - YES  
     - NO
   - · Heart abnormalities
     - YES  
     - NO
   - · Diabetes
     - YES  
     - NO
   - · Epilepsy
     - YES  
     - NO
   - · Recurring back pain that would make exercise problematic, or where exercise may aggravate pain?
     - YES  
     - NO
   - · Recurring neck pain that would make exercise problematic, or where exercise may aggravate pain?
     - YES  
     - NO
   - · Any neurological disorders that would make exercise problematic, or where exercise may aggravate the condition?
     - YES  
     - NO
   - · Any neuromuscular disorders that would make exercise problematic, or where exercise may aggravate the condition?
     - YES  
     - NO
   - · Recurring muscle or joint injuries that would make exercise problematic, or where exercise may aggravate the condition?
     - YES  
     - NO
   - · A burning or cramping sensation in your legs when walking for short distances?
     - YES  
     - NO
   - · Chest discomfort, unreasonable breathlessness, dizziness or fainting, or blackouts during exercise?
     - YES  
     - NO
**PART D**

1. Have you had the flu in the last week?  
   YES | NO  
2. Do you currently have an injury that might affect, or be affected by exercise?  
   YES | NO  

**PART E**

1. Have you ever had significant periods of dizziness or disorientation after performing maximal exercise?  
   YES | NO  
2. Do you have any injuries or medical conditions that you believe might be problematic for maximal exercise performance?  
   YES | NO  
3. Are you a diabetic?  
   YES | NO  
4. Have you ever been told by a medical practitioner, or do you think it is likely, that you have a blood clotting disorder?  
   YES | NO  
5. Are you currently taking any supplements (e.g. aspirin, fish oil supplements) or blood thinning drugs that might affect blood clotting?  
   YES | NO  
6. Have you ever had a complication (including fainting or dizziness, uncontrolled bleeding or post-procedure bruising) from a medical blood test?  
   YES | NO  
5. Do you have a heart pace maker?  
   YES | NO  
6. Do you have any metallic implants? (e.g. bone pins)  
   YES | NO  

* Is there any other condition not previously mentioned that may affect your ability to participate in this study?  
   YES | NO  

**Name of the Participant**  
Signature of the Participant  
**Date**  

**Signature of the Investigator**  
**Date**
Declaration (to be signed in the presence of the researcher)

I acknowledge that the information provided on this form, is to the best of my knowledge, a true and accurate indication of my current state of health.

Participant

Name:_________________________ Date (DD/MM/YYYY):______________

Signature:_________________________

Researcher:

Signature:_________________________

Date (DD/MM/YYYY):______________

Parent/ Guardian (only if applicable)

I, ____________________________, as parent / guardian of Mr/ Miss ____________________________, acknowledge that I have checked the answers provided to all questions in the medical questionnaire and verify that they are correct to the best of my knowledge.

Signature:_________________________

Date (DD/MM/YYYY):______________
**Practitioner** (only if applicable)

I, Dr ____________________________ have read the medical questionnaire and information/ consent form provided to my patient Mr/Miss/ Ms______________________________, and clear him/ her medically for involvement in exercise testing.

Signature: ____________________________

Date (DD/MM/YYYY): ____________________
Appendix 5: Study 1 Publication

Metabolic and Muscle Damage Profiles of Concentric versus Repeated Eccentric Cycling

LUIS PENAILILLO1,2, ANTHONY BLAZEVICH1, HIDEO NUMAZAWA1, and KAZUNORI NOSAKA1
1Center for Exercise and Science Research, School of Exercise and Health Sciences, Edith Cowan University, Joondalup, WA, AUSTRALIA; 2School of Kinesiology and Research Center, Faculty of Medicine, Universidad Finis Terrae, Santiago, CHILE; and 3Department of Sports and Wellness, Rikkyo University, Saitama, JAPAN

ABSTRACT

PEÑAILILLO, L., A. BLAZEVICH, H. NUMAZAWA, and K. NOSAKA. Metabolic and Muscle Damage Profiles of Concentric versus Repeated Eccentric Cycling. Med. Sci. Sports Exerc., Vol. 45, No. 9, pp. 1773–1781, 2013. Purpose: Eccentric cycling is an exercise modality that could elicit multiple health benefits with low metabolic cost, but unaccustomed performance results in significant muscle damage. It is not known whether muscle damage is attenuated when eccentric cycling is repeated; thus, this study compared metabolic and muscle damage responses to concentric (CONC) and two consecutive eccentric (ECC1 and ECC2) cycling bouts. Methods: Ten men (28 ± 8 yr) performed each cycling bout for 30 min at 60% of the maximal concentric power output at 60 rpm, with 2 wk between bouts. HR, oxygen consumption (V\text{\textsubscript{\text{O}2}}), blood lactate (BLa), RPE, and muscle activity (EMG) data were collected during cycling. Maximal voluntary isometric knee extensor (MVC) strength, squat (SJ), countermovement jump (CMJ) height, muscle soreness indicators, and plasma creatine kinase (CK) activity were measured before, immediately after, and 1–4 d after exercise. Results: Average HR, V\text{\textsubscript{\text{O}2}}, BLa, and RPE were lower (P < 0.05) during ECC1 than CONC, and EMG amplitude was also lower during ECC1 than CONC. Decreases in MVC, CMJ, and SJ and the increase in muscle soreness were greater (P < 0.05) after ECC1 than CONC. Increases in creatine kinase were minimal after all bouts. When comparing ECC1 and ECC2, HR and BLa were lower (P < 0.05) during ECC2 than ECC1, and decreases in MVC, CMJ, and SJ and the increase in muscle soreness were greater (P < 0.05) after ECC2 than ECC1. After ECC2, MVC, CMJ, and SJ did not change and no muscle soreness was developed. Conclusions: Eccentric cycling was less metabolically demanding than concentric cycling, and HR and BLa were further reduced during ECC2. Muscle damage is minimal after ECC2 and should not influence the choice to undertake eccentric cycling training. Key Words: LENGTHENING CONTRACTIONS, DELAYED ONSET MUSCLE SORENESS, RECUMBENT BICYCLE, OXYGEN CONSUMPTION, REPEATED BOUT EFFECT

Eccentric contractions are often performed during activities of daily living such as walking downstairs or sitting down on a chair, as well as in exercises such as downhill running or walking (11), stepping exercise (36), and a variety of resistance exercises (10,19). Eccentric cycling is also an exercise modality in which eccentric contractions predominate, as the knee extensor muscles perform eccentric contractions when resisting against the backward rotational movements of the cranks. Eccentric cycling was first introduced by Abbott et al. (2) in 1952, where two interlinked bicycles were used with one person pedaling forward (i.e., concentric) and the other resisting the backward movements (i.e., eccentric) imposed on their bicycle. In the classic study, Abbott et al. (2) reported that oxygen consumption (V\text{\textsubscript{\text{O}2}}) was 41%, 49%, and 66% lower during eccentric cycling performed at 25, 35, and 52 rpm, respectively, when compared with concentric cycling at intensities ranging between 24 and 245 W. These findings were later confirmed that eccentric cycling training produces greater increases in muscle strength and size compared with concentric cycling. More recently, electric motors have been used to drive the backward rotations of the cranks, against which the person works. Researchers have shown that eccentric cycling requires only 25%–30% of the oxygen required for concentric cycling at the same workload (23,40), and that a four to seven times greater workload can be produced in eccentric cycling compared with concentric cycling at an intensity of 65%HR\text{\textsubscript{\text{peak}}}(27) or at the same (i.e., 1 L min^{-1}) V\text{\textsubscript{\text{O}2}}(28). In addition, several studies have shown that eccentric cycling training produces greater increases in muscle strength and size compared with concentric cycling training (26–28). Therefore, it has been advocated that eccentric cycling might be an ideal exercise to induce muscle mass and strength gains in the elderly and for use by patients with pulmonary or coronary disease, where cardiorespiratory fitness is reduced but that increases in muscle mass and strength are required (41).

One possible negative aspect of eccentric-dominant exercise is the risk of muscle damage, which is characterized by muscle weakness and delayed-onset muscle soreness (DOMS) after...
Appendix 6: Abstract for Conference (Study 1)

METABOLIC CHARACTERISTICS AND MUSCLE DAMAGE PROFILE OF REPEATED BOUTS OF ECCENTRIC CYCLING IN COMPARISON TO CONCENTRIC CYCLING
Peñailillo, L.1, Blazevich, A.1, Numazawa, H.2, Nosaka, K.1
1Edith Cowan University (Australia), 2Rikkyo University (Japan)

Introduction
Metabolic cost of eccentric (ECC) cycling is lower at a given intensity (1), and ECC cycling training produces greater muscle mass and strength gains compared with concentric (CON) cycling (2). ECC cycling has been reported to result in prolonged strength loss and myofibrillar disruption (3). It is important to know muscle damage profile in ECC cycling to safely implement it in training. Although it is well known that the magnitude of muscle damage is attenuated when the same ECC exercise is repeated, it is unknown whether this is also the case for ECC cycling. Thus, this study compared the first and second ECC cycling bouts and a bout of CON cycling.

Methods
Ten men (28.4 ± 8.2 y) performed a single bout of CON cycling and two bouts of ECC cycling with a two-week interval between bouts. All bouts consisted of 30 min cycling at 60% of the maximal CON power output at 60 rpm (169.2 ± 52.6 W). Heart rate (HR), oxygen consumption (VO\textsubscript{2}), blood lactate (BLa) and rate of perceived exertion (RPE) were measured during cycling, and tympanic temperature (TEMP) was assessed before and immediately after cycling. Maximal voluntary isometric contraction strength of knee extensors (MVC), squat jump (SJ) and counter movement jump height (CMJ), muscle soreness and plasma creatine kinase activity (CK) were measured before, immediately after and 1-4 days after exercise. Changes in these variables over time were compared across the three bouts by a two-way repeated measures ANOVA.

Results
The average HR, VO\textsubscript{2}, BLa and RPE were lower (P<0.05) during the first ECC cycling (ECC1) than CON cycling, and HR and BLa were even lower (P<0.05) during the second ECC cycling (ECC2) compared with ECC1. TEMP increased only after CON cycling by 0.45°C. Decreases in MVC, CMJ and SJ, and increase in muscle soreness were greater (P<0.05) after ECC1 than CON and ECC2. Increases in CK were small after all bouts. Following ECC2, little changes in the variables were found such that MVC, CMJ and SJ did not decrease from baseline and no muscle soreness was developed, which were similar to those seen after CON.
Discussion
The results confirmed that ECC cycling is less metabolically demanding than CON cycling at the same work. The new findings of the present study were that metabolic demand was further reduced during the second than the first ECC cycling bout, and muscle damage was little or minimum after the second ECC cycling. This is in line with the repeated bout effect shown in other ECC exercises. Thus, muscle damage should not limit the use of ECC cycling.

References
Appendix 7: Abstract for Conference (Study 4)

VASTUS LATERALIS FASCICLE BEHAVIOUR DURING ECCENTRIC CYCLING IN RELATION TO MUSCLE DAMAGE

Peñailillo, L., Blazevich, A., Nosaka, K.

Edith Cowan University (Australia)

Introduction
Eccentric exercise induces muscle damage, but confers a protective effect on subsequent bouts of the same or similar exercise (1). The mechanisms underpinning this protective effect are unclear. It is possible that muscle-tendon behaviour during exercise is not the same between the initial and secondary eccentric exercise bouts. B-mode ultrasound technique has been used to assess muscle-tendon behaviour in vivo during exercise (2). The present study used the ultrasound technique to compare between the first and second eccentric (ECC) cycling bouts for vastus lateralis (VL) fascicle behaviour in relation to muscle damage.

Methods
Eleven untrained men (27.1 ± 7.0 y) performed two bouts of ECC cycling (ECC1, ECC2) for 10 min (60 rpm) at 65% of the maximal concentric cycling power output (190.8 ± 44.2 W) separated by 2 weeks. Maximal voluntary isometric contraction strength of the knee extensors (MVC) and visual analogue scale (VAS) for muscle soreness were assessed before and 1-2 days post-exercise. An ultrasound probe was attached to the middle portion of the VL to record muscle movements during ECC cycling. Surface electromyogram (EMG) was recorded from the VL, and cycling torque and knee joint angle were measured during exercise. Three revolutions at 1 and 10 min of cycling were averaged for the fascicle behaviour, and 10 revolutions were averaged for peak EMG amplitude (EMGpeak). Fascicle length (L_f) and angle (θ_fas) were determined using the trigonometric method, and muscle-tendon unit (MTU) length and tendinous tissue (TT) length were estimated (2).
Changes in these variables were compared between ECC1 and ECC2 by two-way repeated measures ANOVAs and paired t-tests.

**Results**

Peak VAS was greater (P=0.000) after ECC1 (32.3 ± 23.5 mm) than ECC2 (8.0 ± 9.0 mm), but decreases in MVC (e.g. 8% at 1 day post-exercise) were not significantly different between bouts. Peak torque was consistent for 10 min, and was not different between bouts. EMG$_{\text{peak}}$ did not change significantly over time and was not different between bouts. The magnitude of fascicle elongation during ECC2 was 19.2 ± 30.1 mm smaller than that of ECC1 at 1 min (P=0.03), but this was not the case at 10 min. $\theta_{fas}$ did not change significantly over time and was not different between bouts. MTU length changes were not different between bouts, but the $L_f$ changes were 3.2% smaller (P=0.036), and TT changes were 6.1% greater (P=0.07) relative to the MTU length changes during ECC2 compared with ECC1 at 1 min.

**Discussion**

These results indicate that fascicles were lengthened less during ECC2 than ECC1, and elongation of TT tended to be greater during ECC2 than ECC1 for the same MTU length changes. It seems that less fascicle strain was imposed during ECC2 than ECC1, which could be related to the less muscle soreness after ECC2 and one of the mechanisms of the protective effect.

**References**
