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Low load resistance training with blood flow restriction: Adaptations and mechanisms in young and old people

Stephen Patterson

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

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Abstract

Low load resistance training (LLRT) with blood flow restriction (BFR) is a novel form of exercise that has been demonstrated to increase muscle mass and strength. Combined with the fact that as individuals age they lose both of these parameters, LLRT with BFR has been put forward as a method to help reverse/prevent the associated sarcopenia of ageing. This research investigated the effect the effect of LLRT with BFR on muscle strength firstly in younger people and then an older population group following 4 weeks of training. Muscle function measurements of young and old people included dynamic strength, identified as one repetition maximum (1 RM), isometric strength and isokinetic torque at a range of velocities (0.52 – 2.09 rad.s\(^{-1}\)). Vascular adaptations were also measured using venous occlusion plethysmography to assess rest blood flow (R\(_{\text{bd}}\)) and post occlusive reactive hyperemia (PO\(_{\text{obf}}\)). The mechanisms behind any adaptations were measured following acute responses of plasma hormones and growth factors (cortisol, growth hormone (GH), insulin like growth factor 1 (IGF-1), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF)) as well as local skeletal muscle gene expression (IGF-1Ea and MGF mRNA) to LLRT with BFR. LLRT with BFR increased (P < 0.05) all measurements of muscle strength by 13 – 30% in both young and older people. PO\(_{\text{obf}}\) was also increased (P < 0.05) following 4 weeks of LLRT with BFR in both population groups. Acute responses to LLRT with BFR identified an increase (P < 0.05) in GH and VEGF in older people. These are similar response to those seen in the young. Finally local gene expression of MGF mRNA was elevated (P < 0.05) 24 hours post LLRT with BFR in both young and older people. Any changes in muscle and blood flow adaptations may be as a result of increased hormones and growth factors at a circulation and local level.

Key words: Blood flow restriction, blood flow, muscle strength, growth hormone, IGF-1
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Publications and Conference Presentations

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Chapter 1

Introduction
1.1. GENERAL INTRODUCTION

The demographics of the world’s population are ever-changing. Improved survival rates at older ages and a low birth-rate have resulted in European countries having the oldest populations in the world (Ferrucci et al. 2008). This trend will continue into the future with one in four Europeans projected to be aged 65 years and older by the year 2030 (Fig 1.1).

![Figure 1.1 Population ageing throughout the world (Ferrucci et al. 2008)](image)

Due to this increased life expectancy there becomes a greater reliance on the taxpayer to help pay for the associated increase in costs for the aged population. With ageing there is a decrease in the functional ability of individuals which may lead to increased medical costs. A loss of muscle mass and the resulting decrease in muscle strength leads to a decrease in function (Janssen et al. 2002; Buchman et al. 2007) in daily tasks such as stair walking, raising from a chair and postural control (Tinetti et al. 1988; Skelton et al. 1994; Izquierdo et al. 1999; Foldvari et al. 2000). There are a few studies that have investigated the effects of strength training protocols on selected functional abilities in older people (Fiatarone et al. 1994; Suetta et al. 2004a; Caserotti et al. 2008). Fiatarone et al. (1994) demonstrated, in a large study consisting of 100 mixed sex individuals aged 72-98, that ten weeks of heavy resistance training (80% 1-RM) increased knee extensor muscle strength (↑ 113%). This was seen
alongside an improvement (12%) in the individuals' habitual gait velocity. Others have shown, alongside an increase in strength, that resistance training improves other functional tasks such as the ability to raise from a chair (Taaffe et al. 1999), maximum walking velocity (Hakkinen et al. 2000) and stair climbing (Rooks et al. 1997). Therefore strategies that can have positive benefits on muscle strength and daily function should be considered and investigated in order to improve daily living for the ageing population.

Low load resistance training (LLRT) with blood flow restriction (BFR) is a new and novel way to increase both the strength and size of skeletal muscle (Takarada et al. 2000b; 2002). For individuals who lose both strength and muscle mass with ageing i.e. sarcopenia, this format of training may be very useful as it involves the use of light workloads but offers the benefits of heavy conventional resistance training (Takarada et al. 2000b). Specifically ageing is associated with a decrease in both the number (Frontera et al. 2000; Andersen 2003) and size (Lexell and Taylor 1991; Coggan et al. 1992) of type II muscle fibres. This reduction in type II fibres may contribute to a greater decline in muscle power than strength (Lexell 1995), therefore having important negative consequences for power specific tasks. LLRT with BFR is very effective in recruiting these faster type II fibres (Takarada et al. 2000b; Moore et al. 2004) and making them grow in size as evidenced in young individuals (Yasuda et al. 2005).

The mechanisms behind an increase in strength and muscle size following LLRT with BFR are still relatively unknown, but some research has started to identify some of the main causes. For example Kubo et al. (2006) specifically investigated the adaptations to LLRT with BFR when compared to heavy resistance training, over a 12 week period. They found that muscle volume, physiological cross sectional area and pennation angle all improved following LLRT with BFR. In contrast heavy resisted training improved muscle volume, physiological cross sectional area, pennation angle, muscle activation levels, specific tension and tendon stiffness, demonstrating that the adaptations seen are not always through the same mechanisms. It seems that the adaptations in strength following LLRT with BFR are mainly hypertrophy related, thus investigations have focused on the hormonal and molecular signalling pathways that play a role in skeletal muscle size. Circulating hormones such as growth hormone
(GH) and insulin-like growth factor 1 (IGF-1) have been shown to increase following LLRT with BFR (Takarada et al. 2000a; Takano et al. 2005). Furthermore key targets on the mTOR pathway have been demonstrated to be upregulated as has protein synthesis in both young and older men following acute LLRT with BFR (Fujita et al. 2007; Fry et al. 2010), demonstrating that this method of exercise can acutely signal the start of the muscle hypertrophy process.

Alongside the age reduced muscle strength, there is a decrease in resting blood flow and the ability for the vessels to vasodilate (Wahren et al. 1974; Kenny 1997; Dinneno et al. 1999; 2001), which in turn makes it more difficult to meet the energy demands of daily movements or exercise. Ferguson and Brown (2006) demonstrated that athletes who are specifically trained to perform muscle contractions under ischemic conditions, have significantly enhanced vascular capacity, suggesting that LLRT with BFR, which has a high ischemic component, may improve both resting blood flow and the capacity of blood flow in response to vasodilation. Moreover, vascular endothelial growth factor (VEGF) plays a role in angiogenesis (Ferrara et al. 2009), but is also increased following LLRT with BFR in young men (Takano et al. 2005). It is possible that LLRT with BFR may increase the number of capillaries surrounding the muscle and thus maintain or improve blood flow measurements post exercise, but as of yet there has been a lack of research into this area thus adaptations to LLRT with BFR and the mechanisms behind such changes needs to be investigated, specifically in older people.

The aim of this research was to investigate the effect of LLRT with BFR on limb muscle strength (dynamic, isometric and isokinetic) and resting blood flow adaptations and in young and older people and the mechanisms that may explain changes in size, strength and blood flow. The first experiment (Chapter 3) examined the effect of LLRT with BFR on skeletal muscle strength and limb blood flow in younger people. Considering the importance of muscle strength and blood flow to help maintain daily function and prevent disease in the ageing population the 2nd study carried out investigated the LLRT with BFR in an older sample group (Chapter 4). The final chapters in the thesis investigated the acute effects of LLRT with BFR on circulating (Chapter 5) and local (Chapter 6) hormone and growth factor responses to provide information on how and why adaptations may have come about.
Chapter 2

Literature Review
2.1 AGEING AND MUSCLE STRENGTH

Cross sectional studies indicate that skeletal muscle strength peaks at about 30 years of age and is maintained or slightly decreases until about the 50th year of life, with further, more rapid decreases of approximately 12-15% per decade, after 60 years (Larsson et al. 1979; Viitasalo et al. 1985; Narici et al. 1991; Metter et al. 1997; Lindle et al. 1997; Fig 2.1). Strength losses occur in all muscles but lower body muscles are more affected than upper body, which may be related to a reduction in physical activity such as walking and running (Frontera et al. 1991; Lynch et al. 1999; Jansen et al. 2000; Candow and Chilibeck 2005). It has been hypothesised that older individuals supplement weaker lower body movements with upper body movements, for example when rising from a chair, they may use their arms and upper body musculature to help push themselves upwards rather than using their legs, thus maintaining upper body strength whilst lower body measures decline (Macaluso and DeVito 2004).

The knee extensors are a well-researched muscle group, due to their ease of measurement and functional importance. Compared with younger individuals, knee extensor isometric and dynamic (isokinetic) strength of older individuals in their seventh and eighth decades of life are between 20-40% lower than their younger counterparts (Larsson et al. 1979; Murray et al. 1985; Young et al. 1984; 1985) and up to 50% less than in those in their 9th decade and older (Murray et al. 1985). Generally, similar losses of strength are reported in distal muscle such as the plantar- and dorsiflexors (Cunningham et al. 1987; Vandervoort and Hayes 1989). Kubo et al. (2007) investigated isometric calf muscle strength of healthy males, split into across the age span (20, 30, 50 and 70 years). They found that the 70 year age group had lower strength levels than all other groups with strength being 49% lower compared to the 20 year age group. A further cross-sectional study established that plantar-flexor strength did not begin to decline rapidly until the 6th decade of life but then proceeded to decrease at a rate of 1.3% per year (Vandervoort and McComas 1986).
Figure 2.1. Age and gender related differences in concentric (A) and eccentric (B) peak torque of knee extensors (Lindle et al. 1997).

Longitudinal investigations indicate a greater loss of muscle strength than cross sectional studies. For example, in one 12 year study, quadriceps strength of older men was seen to decrease at a rate of 2.5% per year (Frontera et al. 2000). Others have reported decreases of approaching 5% per year (Aniansson et al. 1986; Aniansson et al. 1992; Bassey and Harries 1993), however not all have shown this decrease, with men and women aged 79-89 years of age showing no decline in strength over an eight year period (Greig et al. 1993). Hughes et al. (2001) reported a decline in knee extensor strength of 1.2-1.8% per year for both men and women. Similar findings have been shown for the plantar- and dorsi-flexors with reports of a 30% and 25% decrease of the plantar-flexors and less dramatic 9.5 and 3.3% loss in the dorsi-flexors for men and women respectively (Winegard et al. 1996).
Along with a decrease in strength seen with ageing, explosive power has also been shown to decrease initially after 40 years of age (Metter et al. 1997) and occurs across both genders. Skelton et al. (1994) demonstrated strength declined at a rate of 1-2% per year, whereas leg muscle power declined at a rate of approximately 3.5% per year, with similar decrements across genders. This suggests a greater more rapid decline in power when compared to strength. Metter et al. (1997) demonstrated a similar power decline, at a rate 10% greater than strength losses, from the 4th decade onwards. This loss of power has been shown to have an impact on functional capabilities such as stair climbing, rising from a chair and walking (Bassey et al. 1992; Suzuki et al. 2001) whilst lower levels of strength may be associated with functional limitations in normal daily activities, as well as the risk of falling (Wolfson et al. 1995), hip fractures (Langlois et al. 1998) and maximal and habitual gait velocities (Suzuki et al. 2001).

**2.1.1 Mechanisms for the decline in muscle strength**

There are many factors that may help explain some of these age associated losses in strength including neural changes, a decline in muscle mass and quality (composition) of the muscle tendon unit.

**2.1.1.1 Neural changes**

The ability of a muscle to produce maximal force is dependent on the number of motor units and their firing frequency (Jones et al 2005). Therefore the ability to maximally activate a muscle during a voluntary contraction plays a major role in force production. Alterations in the central nervous command result in an impairment of agonist activation and/ or an increased antagonist coactivation which may affect muscle strength capability in older people (Macaluso et al. 2002; Morse et al. 2004).

Recent evidence suggests that skeletal muscle activation levels may not be complete in young people as demonstrated in numerous muscle groups (Belanger et al. 1981; Bulow et al. 1993; Allen et al. 1996; Herbert et al. 1996; Herbert and Gandevia 1999). Studies that have investigated the level of muscle activation with age have shown no impairment in the ankle dorsi-flexors, plantar-flexors and knee extensors between young (20 – 32 years) and older people (60 – 100 years) (Vandervoot and McComas
1986; Kent-Braun and Ng 1999; Roos et al. 1999). However this is not always the case as a clear deficit in the quadriceps muscle activation (69-93% of full activation) has been reported in very elderly (85 – 97 years) adults (Harridge et al. 1999). Furthermore a lower activation level of the quadriceps (Stevens et al. 2003) and plantar-flexor (Morse et al. 2004) muscle has been demonstrated in old (64-84 years) versus young (18 – 32 years) males and females. Therefore it seems that under certain conditions both young and older individuals are unable to voluntarily activate the full number of motor units available to them.

Surface electromyography (EMG) is a non-invasive method used to assess activation levels of agonist and antagonist muscles (Hakkinen et al. 1998a; Merletti et al. 1992; 2002). As well as a reduced activation of the agonist muscles, greater coactivation or co-contraction of the antagonist muscles can impair force production of the agonist muscle (Crone and Nielsen 1989; Carolan and Cafarelli 1992). As with agonist muscle activation levels, studies investigating ageing and antagonist coactivation have been highly variable. Evidence suggests a higher coactivation occurs in older individuals during maximal contractions in various muscle groups such as the elbow flexors and knee extensors (Hakkinen et al. 1998a; 2000; Izquierdo et al. 1999; Klein et al. 2001; Macaluso et al. 2002). However, this is not always the case as no differences were observed between young and older individuals during contractions of the plantar-flexors (Klass et al. 2005). It is difficult to draw a conclusive answer from the literature as to whether voluntary activation and coactivation are affected by ageing. This is mainly due to the physical fitness of the participants, the type of muscle group measured and the type of contraction performed (Klass et al. 2007).

2.1.1.2 Muscular changes

Age-related declines in strength are typically associated with a loss of skeletal muscle mass, defined as sarcopenia. Using whole body autopsies total muscle mass has been shown to peak about the age of 24 and like strength remains constant or declines slightly (approx 10%) until the age of 50 (Lexell et al. 1988), however the muscles from cadavers does not give direct strength measurements. Between the ages of 50 and 80 there is a further 30% decrease in muscle mass resulting in a total of 40% loss of muscle mass from 24-80 years of age (Lexell et al. 1988).
Cross sectional area (CSA) of different limbs can be assessed using many different methods such as, ultrasound (US), computer tomographic scanning (CT), and magnetic resonance imaging (MRI). The CSA of quadriceps of older individuals (> 70 years) are reported to be 25-35% less than those of younger counterparts, when measurements are taken with US (Young et al. 1984; 1985). This is similar to the results seen for CT scans for the quadriceps and other muscles (Rice et al. 1989; Klitgaard et al. 1990; Overend et al. 1992). Recent techniques such as MRI have allowed for more muscles to be analysed at once and more subjects than previous studies. Studies using these techniques have further emphasised the wasting of contractile muscle occurring in older muscle, in particular recent data of 468 individuals from 18 to 88 years, show a decline after the 5th decade of 1.9 and 1.1 kg/decade in men and women, respectively, with a preferential decrease in the lower body. (Gallagher et al. 1997; Janssen et al. 2000).

Sarcopenia and the loss of muscle as measured at a whole muscle level may be caused by both a reduction in the number of muscle fibres and a reduction in the size of the existing muscle fibres, known as atrophy. Cross sectional studies indicate a loss in the number of muscle fibres with ageing (Lexell et al. 1983; 1986; 1988). A 5% decline in fibre number has been reported between the ages of 24 and 50, which increases to 35% between 52 and 77 years of age (Lexell et al. 1986). At the same time the numbers of excitable motor units are reduced from 60 – 70 years of age (Campbell et al. 1973; Doherty et al. 1993). More recently, McNeil et al. (2005) found a 39% decrease in the number of motor units in the tibialis anterior muscle of old (61-69 years) compared to young individuals (23-32 years). In the very old individuals (80-89 years) there was an even greater decline (61%), however no difference in isometric strength was observed in the young and old but was reduced in the very old. This suggests the loss of motor units does not cause a change in the functional ability of the muscle until a certain critical threshold is reached. An early cross sectional study reports a decrease in the percentage of both type IIa and IIx fibres, therefore increasing the type I/II fibre ratio (Larsson et al. 1978) which may have implications for the contractile ability of the muscle. Most recent studies have not seen this phenomenon showing a lower number of muscle fibres occurring equally between all skeletal muscle fibre types (Aniansson et al. 1986; Lexell et al. 1983; 1988; Kiltgaard
et al. 1990). This is also confirmed in longitudinal studies, with evidence suggesting a decrease in number of both type I and II fibres, with a greater loss of type II fibre number up to the age of late 70’s and after 80 years of age a greater decline in type I fibres occurs (Frontera et al. 2000; Andersen 2003).

A reduction in the size of individual fibres has also been shown to affect the loss of muscle mass (Aniansson et al. 1986; Lexell et al. 1988; Kiltgaard et al. 1990). Lexell et al. (1988) found a slightly lower (26%) CSA of type II fibres, with no difference of type I fibres, in men, across the ages of 15-83 years. This difference in type II fibres has also been found in women they as they exhibited 24% and 30% lower CSA in type IIa and IIx fibres, respectively (Coggan et al. 1992). In a cadaver study Lexell and Taylor (1991) investigated 5 different regions of the whole vastus lateralis, in 20 men aged 19-84 years of age. Older individuals were found to have a significantly smaller CSA of type II fibres when compared to younger people, with no reported differences in type I fibres. This reduction in type II fibres may contribute to a greater decline in muscle power than strength (Lexell 1995), therefore having important negative consequences for power specific functional tasks.

Although there are significant changes in the actual size and number of muscle fibres, this does still not fully explain the changes in strength and power observed with old age. Therefore, factors within the muscle may also be implicated. The maximum isometric force that can be produced by a muscle is partly determined by the muscle CSA (Lieber 2002). Anatomical cross section area (ASCA) is a measure of muscle size that is made perpendicular to the long axis of the muscle, obtained from scanning techniques such as MRI. This measure has been demonstrated to have a moderate to high correlation with maximal voluntary force (Maughan et al. 1984). However the use of ACSA to determine the size of skeletal muscle is limited when comparing groups of muscles with varying architectural features (Morse et al. 2005), specifically overestimating the contractile area of skeletal muscles with a smaller pennation angle (Narici et al. 1992) In pennate muscles, fibres run at an axis of traction of the muscle. This arrangement of fibres can directly influence the amount of force generated through voluntary contraction, however the ASCA does not represent the cross section perpendicular to the fibres in the muscle (Lieber 2002). Therefore a better measure is the physiological cross sectional area (PSCA), which measures
perpendicular to the direction of the muscle fibres. This refers to the maximum number of cross bridges that can be activated in parallel during contraction (Fukunaga 2001). In older people the pennation angle of the actual muscle fibres has been identified to be smaller in the vastus lateralis (Kubo et al. 2003) and medial gastrocnemius (Kubo et al. 2003; Narici et al. 2003; Morse et al. 2004) compared to younger individuals, thus affecting the force generating capacity of older skeletal muscle.

A further determinant of muscle strength is highlighted by observing the relationship between force and CSA, i.e. specific tension (Maganaris et al. 2001). Recent findings in older individuals suggest a greater specific tension in human muscle fibres expressing MHC IIx than fibres expressing MHC I only (Bottinelli et al. 1999; Stienen et al. 1996). Recent evidence has shown a greater proportion of hybrid fibres in aged muscle containing more than 1 MHC isoform (Andersen et al. 1999; Kiltgaard et al. 1990). Furthermore, in studies on human skinned and freeze dried muscle fibres, specific tensions of type I muscle fibres are lower than those of Type II (Larsson et al. 1993; Stinen et al. 1996). At a whole muscle level evidence suggests that specific tension is reduced in older adults, specifically the quadriceps (Larsson et al. 1997; Macalusso et al. 2002; D’Antona et al. 2003; Yu et al. 2007), however, this may be unaffected in the dorsi-flexors (Kent-Braun and Ng 1999). The change in specific tension provides evidence of quantitative changes in contractile properties of human skeletal muscle with ageing, which is likely to play an important part in the age-related impairment of skeletal muscle function by reducing power and speed of movement (Larsson et al. 1997)

Finally, along with a reduced number and size of muscle fibres there may be changes in the number of myonuclei and satellite cells (SC). SC or ‘muscle stem cells’ are the sole source for generation of new myonuclei in vivo in skeletal muscle tissue, therefore deemed essential for repair and hypertrophy of skeletal muscle (Allen et al. 1999; Shefer et al. 2006). There is a discrepancy in the findings of myonuclei number and domain size with ageing, with some finding no change in myonuclei number alongside a reduction in muscle fibre size (Roth et al. 2000; Dreyer et al. 2006; Petrella et al. 2006). In contrast others have reported an increase in the number of myonuclei with increased age (Kadi et al. 2004; Verdijk et al. 2007). At specific fibre
level Verdijk et al. (2007) demonstrated a reduction in SC number in type II muscle fibres in old compared to young people. This supports the idea that SC content is strongly associated with myofiber maintenance and implies that the age-related reduction in type II muscle fiber SC content might represent an important factor in the specific loss and atrophy of type II muscle fibers with ageing. A decreased SC content has been reported in the vastus lateralis (Sajko et al. 2004; Verdijk et al. 2007), biceps brachii (Renault et al. 2002) and the tibialis anterior (Kadi et al. 2004) in aged muscle, however other have found no change (Roth et al. 2000; Dreyer et al. 2006; Petrella et al. 2006). Many variables may account for the differences in the study’s findings, though it appears SC content may be maintained up to the 7th decade of life (Petrella et al. 2006; Roth et al. 2000) but decline thereafter (Renault et al. 2002; Kadi et al. 2004; Verdijk et al. 2007).

2.1.1.3 Hormonal Changes

Age related muscle wasting and sarcopenia may lead to hormonal and gene expression changes both at rest and in response to exercise. Two of the main hormones/genes examined in older people have been growth hormone (GH) (Rudman et al. 1981; 1990; Welle et al 1996) and insulin-like-growth factor (IGF-1) (Butterfield et al. 1997; Hameed et al. 2003; 2004; Roubenoff et al. 2003). GH is a single-chain peptide of 191 amino acids produced and secreted mainly by the somatotroph cells of the anterior hypothalamus (pituitary gland) (Davidson 1987). GH secretion occurs in a pulsatile pattern with a high release at the onset of slow-wave sleep and less observed secretion episodes a few hours after meals (Ho et al. 1988; Hartman et al. 1991), thus circulating GH levels vary greatly depending on the time of day. The pulse rate of GH is controlled by two hypothalamic factors, growth hormone releasing hormone (GHRH), which stimulates GH secretion and somatostatin, which inhibits GH secretion (Giustina et al. 2008). The secretion of GH is maximal at puberty (Moran et al. 2002), with circulating levels of GH progressively declining over the ageing process (Rudman et al. 1981). Between the ages of 20 and 70 it has been reported that GH values are reduced by approximately 50% in both sexes (Veldhuis et al. 1997). Mean pulse amplitude, duration and fraction of GH secreted but not pulse frequency all decrease with ageing (Lamberts et al. 1997). Several studies have demonstrated a significant inverse relationship between peak stimulated
GH levels and body mass index (Bonert et al. 2004; Corneli et al. 2005; Franco et al. 2006).

GH stimulates insulin-like growth factor 1 (IGF-1) synthesis in most tissues (Gostelli-Peter et al. 1994) with the GH/IGF-1 axis being recognised as an important regulator of muscle mass (Goldspink and Harridge 2004). IGF-1, is a 7.6 kDa, 70 amino acid polypeptide, with systemic IGF-1 being synthesized primarily in the liver. GH administration causes rapid upregulation of IGF-1 mRNA and protein in the liver (Matthews et al. 1986) and mice with liver specific IGF-1 deletions show only 10-25% of serum IGF-1 levels compared with controls (Sjogren et al. 1999). IGF-1 is also produced in multiple tissues, where it acts locally as an autocrine/paracrine growth factor under the control of multiple hormones (Stewart & Rotwein, 1996; Laviola et al. 2007). While IGF-1 has very complex functions it is mainly involved in important physiological functions during growth and development. In muscle cell culture studies it has been demonstrated that IGF-1 has an anabolic function as shown by its ability to increase the diameter of myotubes, suppress protein degradation, increase amino acid uptake and stimulate protein synthesis (Florini 1987; Rommel et al. 2001). Extra hepatic tissue production of IGF-1 has been shown to support postnatal growth (Sjogren et al.1999; Straitikopoulos et al. 2008). A role for IGF-1 in muscle hypertrophy is supported by overload experiments in mice, where hypertrophy and increases in IGF-1 at both mRNA and protein levels are observed (Adams and Haddad 1996). IGF-1 upregulation is also observed in muscle after stretch induced hypertrophy (Czerwinsji et al. 1994) and during muscle regeneration following injury (Levi-novitz et al. 1992).

The effects of IGF-1 are mediated mainly by the type I IGF-1 receptor (IGF-R1), which has tyrosine kinase activity and signals through the phosphatidylinositol 3 kinase (P13K)/ AKT pathway. IGF-1 also binds to the insulin receptor (IR) but with much lower affinity than to the IGF-1R (Pandini et al. 2002). There are six IGF-binding proteins (IGFBPs). The IGFBPs were initially isolated from serum and are proteins of about 30kDa able to bind IGF-1 and IGF-II but not insulin (Bach et al. 1993). Most systemic IGF-1 is found in tripartite complex with IGFBP3 and the acid labile subunit (ALS). IGF-1 IGFBP complexes cannot leave the circulation and access tissue unless they are bound to the LS. In serum, they increase the circulating half life
and delivery of IGF-1 to tissues. In tissues they modulate IGF action as they have higher affinity for IGFs than the receptors. IGFs are released by proteolysis of IGFBPs or binding of the IGFBPs to the extracellular matrix (Parker et al. 1998). IGFBPs can also be phosphorylated and this affects their affinity for IGFs (Kajantie et al. 2002). IGF-independent actions have been described for most IGFBPs and can involve intracellular localization (Xu et al. 2004) or integrin binding (Jones et al. 1993).

The IGF-1 gene comprises of six exons, two of which contain multiple transcription initiation sequences which through alternative splicing can result in a large number of IGF-1 mRNAs (Rotwein et al. 1986; Hameed et al. 2002). IGF-1 exists in two isoform, as a result of alternative splicing if the IGF-1 gene. Yang et al (1996) were the first to clone and sequence the cDNA of the two isoforms of the IGF-1 gene expressed in the tibilias anterior and extensor digitorum longus muscle of rabbits which had been challenged to grow using a stretch and immobilization model. The first isoform discovered is often referred to as the liver-type or systemic IGF-1 isoform (termed IGF-1Ea). The second differed by the inclusion of a 52 base pair insert (49 base pairs in human) in exon 5 of the e domain of the IGF-1 gene (IGF-1Ec). In contrast to the overloaded muscle, only the Ea isoform type was expressed in resting muscle, leading to the idea that the IGF-1Ec isoform was sensitive to mechanical signals or the micro damage caused and prompted this isoform to be termed ‘mechano growth factor’ or MGF (Yang et al. 1996).

Circulating levels of IGF-1 have been shown to decrease with an increase in age (Harris et al. 1997; Roubenoff et al. 1998). This decrease in circulating IGF-1 has been associated with increased mortality in older men and women (Roubenoff et al. 2003; Brugts et al. 2008). Recently, in an 8-year study, Brugts et al. (2008) have shown that higher circulating IGF-1 bioactivity was associated with better overall survival in older adult men, since individuals in the lowest quartile of IGF-1 bioactivity had a 1.8-fold increased mortality risk compared with individuals in the highest quartile. Ageing is also reported to lower levels of IGF-1 mRNA at rest (Welle et al. 2002; Dennis et al 2008; Leger et al. 2008) however, these studies do not specify which splice variant so therefore may be both IGF-1Ea and MGF. Ageing is associated with decreases in IGF-1R (receptor) content and IGF-1R phosphorylation
in muscle (Li et al. 2003). Furthermore, IGF-1 is known to accelerate SC proliferation and differentiation, also mice over expressing IGF-1 have significantly more muscle mass than controls (Hayashi et al. 2004; Shavlakadze et al. 2005). Therefore GH and IGF-1 at both a circulating and local level may play a role in sarcopenia in older people.

Along with changes in circulating hormones and muscle specific growth factors, pro and anti inflammatory cytokines may play a role in muscle mass loss with old age. One such cytokine investigated in the literature is Interleukin-6 (IL-6), which is produced by a variety of cell types such as monocytes, fibroblasts vascular endothelial cells and skeletal muscle fibres (Akira et al. 1993; Jonsdottir et al. 2000). Chronically elevated levels of IL-6 have been associated with conditions such as cachexia and insulin resistance (Kern et al. 2001; Baltgalvis et al. 2008). IL-6 may also play a causal role in sarcopenia, with high levels positively associated with low walking speed and poor muscle strength (Taaffe et al. 2000b). However muscle contractions acutely increase circulating IL-6 (Febbraio and Pedersen 2002), overload of skeletal muscle increases IL-6 gene expression (Carson et al. 2002) and in the recovery phase of disuse atrophy expression of muscle IL-6 is also increased (Childs et al. 2003). Typically, IL-6 is the first cytokine released into the circulation during exercise. The level of circulating IL-6 increases in an exponential fashion (up to 100-fold) in response to exercise and declines in the post-exercise period (Nieman et al. 1998; Steensberg et al. 2001). The appearance of IL-6 in the circulation is by far the most marked and its appearance precedes that of other cytokines. Moreover, it appears that muscle-derived IL-6 may account for most of the systemic IL-6 response to exercise (Steensberg et al. 2000).

IL-6 has increasingly been shown to have diverse roles, such as inflammation (Cantini et al 1996) and influencing muscle repair by promoting satellite cell proliferation and myogenic differentiation (Cantini et al. 1995; Serrano et al. 2008). IL-6 also plays a role on other major hormones in humans. IL-6 injection increases adrenocorticotropic hormone (ACTH) in rates (Naitoh et al. 1988). The IL-6 receptor is present in the
human pituitary gland (Hanish et al. 2000) and adrenal cortex (Gonzalez-Hernandez et al. 1994), therefore IL-6 may play a role in stimulating cortisol release from humans. A dose-dependent relationship between IL-6 and cortisol in humans has been demonstrated (Tsigos et al. 1997), with a consistent increase of cortisol reported when plasma IL-6 is ~50 pg/ml or higher (Febbraio et al. 2004; Hiscock et al. 2005; Krogh-Madsen et al. 2006; Nemet et al. 2006). IL-6 also mediates intracellular signalling via the Janus kinase/ signal transducers and activators of transcription (JAK/STAT) cascade (Wegenka et al. 1993). This signal pathway is necessary for the regulation of cell growth and phenotype adaptation (Hirano et al. 2000). Several STAT isoforms are expressed within skeletal muscle and upon activation promote myoblast proliferation, fusion and differentiation (Sun et al 2007; Serrano et al. 2008; Wang et al. 2008), with STAT3 critical for satellite cell proliferation and differentiation (Wang et al. 2008; Yang et al. 2009). IL-6 has been shown to exert marked effects of STAT3 during skeletal muscle myogenesis (Serrano et al. 2008). Trenerry et al (2011) demonstrated IL-6 protein expression increased 17.5 fold following acute strength training but only 5 fold following 12 weeks of strength training. Previous work using plasma IL-6 observed elevations following acute exercise but not following long term periods of exercise training (Crossier et al. 1999; Willoughby et al. 2003). Therefore it seems that IL-6 is not influenced by training but is important for muscle repair therefore is responsive following acute exercise irrespective of the training status of the muscle.

2.1.2 Enhancing muscle strength in older people

As previously discussed it has been observed that muscle strength decreases with age which can have a significant impact on functional ability (Young and Skelton 1994). Therefore, any way of increasing muscle strength in older people would have great benefits on the functional capacity of older people and thus their quality of life. Resistance training involving high force contractions appears to be a mode of exercise that may play a role in offsetting this decreased strength. Indeed, resistance training is recognised as an important activity such that it is now included in physical activity guidelines in the United States of America (Nelson et al. 2007).
Early studies from the 1980’s investigating the effect of resistance training on muscle strength showed positive effects for older individuals (Larsson 1982; Frontera et al. 1988). As in younger counterparts, the programmes undertaken are of a similar structure and nature. The duration of the studies vary from as little as four (Sherrington and Lord 1997) to eighty-four weeks (McCartney et al. 1996), with a frequency of 2 – 3 sessions per week (Aniansson et al. 1980; Frontera et al. 1988; Harridge et al. 1999; Hakkinen et al. 2001) and an intensity of 70-80% 1-RM (Frontera et al. 1988; Pyka et al. 1994; Taaffe et al. 1999; Hakkinen et al. 2001)

The actual improvements observed following resistance training programmes in older people vary depending on the variables discussed above, as well as the actual modalities used to measure and perform the strength exercises. A large number of studies have demonstrated gains in maximal muscle strength in older people following resistance training with changes in 1-RM ranging from 23 – 174 % (Aniansson et al. 1980; Frontera et al. 1988; Fiatarone et al. 1990; ; Hakkinen et al. 1998a, b; Harridge et al. 1999; Suetta et al. 2004a; Caserotti et al. 2008) (See table 1).
Table 2.1. Effect of resistance training on muscle strength and size in older individuals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Gender (no of subject)</th>
<th>Age (yrs)</th>
<th>Exercise(s)</th>
<th>Intensity (% 1RM)</th>
<th>Sets</th>
<th>Reps</th>
<th>Frequency (Sessions/week)</th>
<th>Duration (days)</th>
<th>Change in muscle strength (% )</th>
<th>Change in muscle mass (% CSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontera et al. (1988)</td>
<td>M (12)</td>
<td>60-72</td>
<td>KE</td>
<td>80</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>84</td>
<td>+107 1RM +17 IS</td>
<td>+9 (CT) +3 type I fibre area +28 type II fibre area</td>
</tr>
<tr>
<td>Pyka et al. (1994)</td>
<td>M/F (25)</td>
<td>61-78</td>
<td>LP, KE</td>
<td>75</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>365</td>
<td>+53-95 1RM</td>
<td>-</td>
</tr>
<tr>
<td>Lexell et al. (1995)</td>
<td>M/F (23)</td>
<td>70-77</td>
<td>KE</td>
<td>85</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>77</td>
<td>+163 1RM</td>
<td>-</td>
</tr>
<tr>
<td>McCartney et al. (1996)</td>
<td>M/F (113)</td>
<td>60-80</td>
<td>LP</td>
<td>80</td>
<td>3</td>
<td>12</td>
<td>2</td>
<td>588</td>
<td>+32 1RM</td>
<td>+9 CT</td>
</tr>
<tr>
<td>Sherrington and Lord (1997)</td>
<td>M/F (21)</td>
<td>64-94</td>
<td>BW</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>28</td>
<td>+ 22 IS</td>
<td>-</td>
</tr>
<tr>
<td>Hakkinen et al. (1998a)</td>
<td>M (10)</td>
<td>61</td>
<td>KE</td>
<td>-</td>
<td>3-6</td>
<td>3-15</td>
<td>3</td>
<td>70</td>
<td>+17 IS</td>
<td>+9 (MRI)</td>
</tr>
<tr>
<td>Hakkinen et al. (1998b)</td>
<td>M/F (20)</td>
<td>70</td>
<td>KE</td>
<td>50-80</td>
<td>3-6</td>
<td>3-15</td>
<td>2</td>
<td>182</td>
<td>+26 1RM</td>
<td>+6 (US)</td>
</tr>
<tr>
<td>Harridge et al. (1999)</td>
<td>M/F (11)</td>
<td>85-97</td>
<td>KE</td>
<td>80</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>84</td>
<td>+134 1RM +37 IS</td>
<td>+10 (MRI)</td>
</tr>
<tr>
<td>Hunter et al. (1999)</td>
<td>M/F (11)</td>
<td>64-79</td>
<td>KE</td>
<td>-</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>84</td>
<td>+39 1RM</td>
<td>-</td>
</tr>
<tr>
<td>Taaffe et al. (1999)</td>
<td>M/F (46)</td>
<td>65-79</td>
<td>LP, KE</td>
<td>80</td>
<td>3</td>
<td>8</td>
<td>3</td>
<td>168</td>
<td>+28 1RM</td>
<td>-</td>
</tr>
<tr>
<td>Hakkinen et al. (2001)</td>
<td>M/F (21)</td>
<td>71</td>
<td>LP</td>
<td>70-80</td>
<td>3-6</td>
<td>10-18</td>
<td>2</td>
<td>182</td>
<td>+26 1RM +26 IS</td>
<td>+32 type I fibre area +32 type II fibre area</td>
</tr>
<tr>
<td>Scaglioni et al. (2002)</td>
<td>M (14)</td>
<td>65-80</td>
<td>PF</td>
<td>80</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>112</td>
<td>+24 1RM +18 IS</td>
<td>-</td>
</tr>
<tr>
<td>Reeves et al. (2004)</td>
<td>M/F (9)</td>
<td>74</td>
<td>LP, KE</td>
<td>70-75</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>98</td>
<td>+23 1RM +14 1RM</td>
<td>-</td>
</tr>
<tr>
<td>Suetta et al. (2004a)</td>
<td>M/F (138)</td>
<td>60-86</td>
<td>LP, KE</td>
<td>65</td>
<td>3-5</td>
<td>8</td>
<td>3</td>
<td>84</td>
<td>+24 1RM</td>
<td>+12 (CT)</td>
</tr>
<tr>
<td>Kryger and Andersen (2007)</td>
<td>M/F (30)</td>
<td>85-98</td>
<td>KE</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>84</td>
<td>+134 1RM +37 IS</td>
<td>+10 (MRI) +5% ↑ type IIa fibre area</td>
</tr>
<tr>
<td>Morse et al. (2007)</td>
<td>M (11)</td>
<td>73</td>
<td>LP, CR</td>
<td>70</td>
<td>3</td>
<td>8-10</td>
<td>3</td>
<td>365</td>
<td>+39 IS</td>
<td>+29 (MRI)</td>
</tr>
</tbody>
</table>

BW body weight, CR calf raise, CT computerized tomography, F female, IS isometric strength, KE knee extension, LP leg press, M male, MRI magnetic resonance imaging, NS non significant, 1-RM one repetition max, PF plantar-flexion, US ultrasound
The improvement in muscle strength induced by resistance training in older people results in improved ability to perform functional tasks, such as chair rising, stair climbing and walking speed, over a wide age (60 – 90 years) range (Fiatarone et al. 1990; 1994; Judge et al. 1993; Hakinnen et al. 2000; Suetta et al. 2004a, b; Beyer et al. 2007; Caserotti et al. 2008). Fiatarone et al. (1994) reported that following a resistance training program at an intensity of 80% 1-RM, both stair walking speed (28%) and maximal horizontal walking speed (12%) were increased in frail nursing home residents (87.1 yrs). Other studies have shown positive improvements in both stair walking (Suetta et al. 2004b) and horizontal walking speed (Fiatarone et al. 1990; Hakinnen et al. 2000; Judge et al. 2005; Beyer et al. 2007; Caserotti et al. 2008). The chair rise test is another well used test of functional capacity, consisting of asking an individual to rise from a standard chair as quickly as possible with their arms folded (Fiatarone et al. 1990;Earles et al. 2000; Beyer et al. 2007; Caserotti et al. 2008). Improvements of up to 21% in this functional ability following resistance training have been reported (Fiatarone et al. 1990; Beyer et al. 2007; Caserotti et al. 2008). However, other studies have suggested that certain functional ability tasks are not improved with resistance training. For example Earles et al. (2000) did not demonstrate any improvement in chair rising and other tests such as walking speed and a 6 minute walking test following 12 weeks of resistance training (50 – 70 % 1-RM) of the lower body. It is likely that the highly functioning level of the participants and the fact these participants were allowed to practice the tests may have resulted in no improvement following training.

2.1.3 Mechanisms for improvements in muscle strength

It is clear that following a period of resistance training in older people there is improvement in strength and potential improvements in functional capability. However strength may increase through different mechanisms; mainly adaptations occur through both the neural and muscular systems.

2.1.3.1 Neural Changes

There are many different factors that contribute to the changes in strength following resistance training. In the early phases of a resistance training program these factors
may be due to a learning effect, followed rapidly by other adaptations to the neurological system (Sale 1988; Hakkinen et al. 2001).

One such adaptation is an increased motor unit activation level of the trained agonist muscles following resistance training (Hakkinen et al. 1998a; Scaglioni et al. 2002; Morse et al. 2007; Sueta et al. 2007; 2009), with Harridge et al. (1999) reporting a strong positive relationship ($r = 0.86 – 0.92$) between individual changes in central activation levels and isometric strength. Reports of increased EMG magnitude of agonist muscles may be the result of increased number of active motor units and/or an increase in their firing frequency (Kamen and Knight 2004). A further improvement is the amount of antagonist coactivation during muscular contractions. When elevated before training, antagonist coactivation decreases in older people following resistance training (Hakkinen et al. 1998a; 2001), however some suggest that an increased antagonist coactivation may occur (De Boer et al. 2007). It may be that the increased antagonist coactivation in the De Boer et al. (2007) study may be a safety mechanism to stabilize the ankle joint due to the increased agonist force production following training. Finally, fine motor control appears to be improved following resistance training in older people, as demonstrated by improvements in both force steadiness and force accuracy (Hortobagyi et al. 2001; Tracy et al. 2004; Tracy and Enoka et al. 2006).

2.1.3.2 Muscular Changes

Resistance training in older people leads to an increase (5-12%) in both ACSA and PCSA and volume of the muscle, as measured by non-invasive techniques, such as US, CT scan and MRI (Frontera et al. 1988; Hakkinen et al. 1998a,b; Harridge et al. 1999; Sueta et al. 2004a; Reeves et al. 2004). See table 1.

Training induced increases CSA of type I and II muscle fibres have been reported in older people when assessed by muscle biopsy sampling (Frontera et al. 1988; Pyka et al. 1994; Hakinnen et al. 1998b; 2001; Kryger and Andersen 2007; Suetta et al. 2008). However other studies have not observed an increase in muscle fibre size in older males and females (Lexell et al. 1995; Hunter et al. 1999). It has been shown that older people exposed to lifelong resistance training have a larger (18-27%) type II
fibre area when compared to aged matched controls (Korhonen et al. 2006; Aagaard et al. 2007).

Resistance training may also lead to an increase in the pennation angle of the trained muscle (Morse et al. 2007; Reeves et al. 2004; 2009; Suetta et al. 2008). Reeves et al. (2009) found that 14 weeks of concentric resistance training increased the pennation angle of the vastus lateralis from 14.7° to 19.8°. In the same study the investigators identified that pennation angle did not increase following eccentric training, however this type of training did lead to a greater increase in fascicle length compared to concentric resistance training. It was speculated that the stimulus for adding sarcomeres in-series (fascicle length increase) and sarcomeres in-parallel (pennation angle increase) may be different, thus greater increases in pennation angle may be the result of metabolic stress rather than mechanical stress, thus allowing for a greater change in muscle fibre CSA than ACSA (Aagaard et al. 2001; Suetta et al. 2008).

Given the fact that circulating hormones and the local factors such as satellite cell proliferation and local growth factors are important in the control of muscle mass, it is likely that these factors play some role in the modulation of muscle mass with resistance training. In both mice and humans, GH deficiency minimally affect birth size, but lead to reduced growth during childhood and adolescence resulting in diminished body size (Savage et al. 1993; Zhou et al. 1997). Supraphysiological GH in the young leads to pituitary gigantism, whereas adult-onset GH tumours result in a condition known as acromegaly, characterized by an overgrowth of bony tissue, osteoarthritis, carpal tunnel syndrome, headaches, cardiomyopathies, hyperglycaemia, hypertension and diabetes mellitus (Ayuk and Shepperd 2006). In mice an enlargement of both the heart and general organ size are seen following systemic GH overproduction or administration (Kopchick et al. 1999). Individuals who are GH deficient tend to have increased body fat and decreased fat free mass in comparison to control subjects. This is alongside decreased muscle strength and exercise tolerance (Ayuk and Shepperd 2006; Mollitch et al. 2006; Woodhouse et al. 2006). Strength has been shown to increase in GH deficient patients following 6 months GH administration (Cuneo et al. 1991), but changes in myofibre CSA were not observed (Cuneo et al. 1992), indicating GH does not affect muscle mass in adult humans.
In individuals with acromegaly it is evidenced that high levels of circulating GH and IGF-1 may be detrimental to muscle function. Despite their large muscle mass, they have less specific force and often present with histological signs of myopathy (Nagulesparen et al. 1976; Woodhouse et al. 2006). Furthermore, overexpression of GH in transgenic mice has been reported to increase type I fibres (Dudley and Portanova 1987), whilst GGH receptor knockout mice there are fewer type I fibres and more type II fibres relative to the wild type animals (Sotiropoulos et al. 2006). These observations imply a negative impact of long term supraphysiological circulating GH on power output or strength. Due to often other present hormonal abnormalities, acromegaly may not be the best model to examine the effect of GH on strength and muscle mass.

GH response following acute resistance training is highly correlated with type I and II muscle fibre hypertrophy ($r=0.62-0.74$)(McCall et al. 1999). However resistance training does not seem to affect chronic resting levels of GH in men or women of various ages (Kraemer et al. 1999; Hakkinen et al. 2000). This is consistent with the dynamic feedback mechanisms of GH and its role in homeostatic control of variables such as glucose (Kraemer and Ratamess 2005). Rudman et al (1990; 1991) showed that administration of GH resulted in increased lean body mass and decreased fat/muscle ration in older individuals. It seems that this increase in body mass may not be a result of increased muscle mass with Taffe et al. (1994; 1996) showing no change in myofibre CSA and strength gains following resistance training and GH administration. Other studies using both GH and resistance exercise have found no effect over the placebo group for strength, power or hypertrophy gains following 12 weeks (Lange et al. 2002) or 6 months training (Hennessey et al. 2001). Welle et al. (1996) did report differences in strength following 12 weeks GH administration in older men, but did not observe differences between GH and placebo groups for both mean fractional rates of myofibrillar protein breakdown or mean postabsorptive fractional rate of myofibrillar protein synthesis. Exercise results in both increased protein synthesis and breakdown and that it is net protein synthesis which is important outcome for hypertrophy (Rennie et al. 2004). Yarasheski et al. (1993) also demonstrated that there was no effect of 2 weeks GH administration on quadriceps protein synthesis rates or whole body protein breakdown in young weight lifters.
suggesting no beneficial effect of GH administration and resistance exercise for muscle gains.

Despite a lack of evidence for anabolic activity of GH in healthy humans, there is some evidence of anticatabolic activity of GH and IGF-1. Clemmons et al. (1992) demonstrated that infusion of GH and IGF-1 reduced negative nitrogen balance during calorific restriction in humans. In a subsequent and similar study the combination of both IGF-1 and GH resulted in a positive nitrogen balance within 2 days of initiation of treatment (Kupfer et al. 1993). Also a decline in GH and IGF-1 secretion has been correlated with bone mineral density (BMD) loss in postmenopausal women (Zhao et al. 2008). GH stimulate the proliferation of cells of the osteoblastic lineage (Slootweg et al. 1988) suggesting a direct effect of GH on bone formation. In addition IGF-1 treatment has been shown to induce gene expression of osteoblastic markers (Tanaka et al. 1994), and enhance the formation of osteoclasts-like cells in bone marrow cultures (Jonsson et al. 1996). Mechanical loading, as seen during resistance training, also upregulates IGF-1 and IGFBP2 mRNA transcripts in osteocytes as well as collagen production (Reijnders et al. 2007). This suggests that GH ad IGF-1 may prevent muscle loss through anticatabolic properties and improve strength by increasing bone strength and collagen.

Cell culture studies allow for the investigation of signalling pathways in skeletal muscle using cultured muscle cells or myoblasts derived from muscle explants. Treatment of proliferating C2C12 myoblasts with IGF-1 leads to increased proliferation, but once cells have stopped proliferating, treatment leads to increased fusion and hypertrophy of the resulting myotubes (Rommel et al. 2001). Hypertrophy has also been observed in human muscle cells following IGF-1 treatment (Jacquemin et al. 2004; 2007). IGF-1 increases the size of myotubes by activating protein synthesis, inhibiting protein degradation and inducing fusion of reserve cells.

Direct infusion of either GH or IGF-1 into rat muscle results in increased mass providing evidence that it is local autocrine/paracrine rather than systemic endocrine effects that are important for hypertrophy (Adams and McCue 1998). In animal models hypertrophy of an exercised muscle is accompanied by increases in IGF-1 mRNA and IGF-1 peptide production specifically in the exercised muscle, with this
preceding increases in muscle DNA and protein content (Adams and Haddad 1996). Igf-1 upregulation is also observed in muscle after stretch-induced myofibre hypertrophy (Czerwinski et al. 1994) and during muscle regeneration following injury (Levinovitz et al. 1992). Local upregulation of IGF-1 also seems to augment the resistance training response even in animals with normal GH levels (Lee et al. 2004). In this study, rats were directly injected with IGF-1 into the muscle, resulting in increased mass and force production over and above GH or resistance training alone. In the same study, injection of IGF-1 also prevented loss of muscle mass following a period of detraining.

IGF-1 has is involved in numerous hypertrophic signalling processes, including the phosphatidylinositol-3 kinase (PI3K)/Akt/m TOR and the P13K/Akt/Glycogen synthase kinase 3 β (GSK3 β) pathways (Glass 2005; Rennie et al. 2004). Downstream targets of the mammalian target of rapamycin (m TOR) pathway are the translational regulators p70S6 kinase (p70S6K) and eukaryotic initiation factor-4E-binding protein (4E-BP) and downstream of the P13K/Akt/ GSK3 β pathway is the eukaryotic initiation factor 2B (e IF2B) as seen in figure 2.2 (Adams 2002; Glass 2005; Rennie et al. 2004). Activation of these pathways results in an increase in both protein synthesis and cell growth (Bolster et al. 2004; Glass 2005). There is a direct correlation between the increase in ribosomal protein S6 kinase beta-1 (S6K1) seen following acute exercise and the percent change in muscle mass measured 6 weeks after training as measured in rats (Barr and Esser 1999) therefore suggesting this may be effective marker for long term changes in muscle mass. IGF-1Ea and MGF mRNA are both up-regulated following high-intensity resistance exercise in young people (Bamman et al. 2001; Hameed et al. 2003; Petrella et al. 2006), however MGF has been reported not to increase following acute exercise in older individuals (Hameed et al. 2003; Petrella et al. 2006) which is similar to work in animals that show MGF mRNA up-regulation may decline with age (Owino et al. 2001). This is in contrast to more recent evidence that suggests MGF mRNA is up-regulated following both concentric (Roberts et al. 2010) and eccentric exercise (Hameed et al. 2008).
2.2 Ageing and muscle blood flow

The performance of sustained exercise and physical tasks is largely dependent on the adequate supply of blood to the contracting muscles. The increase in blood flow allows the constant supply of nutrients and oxygen to the working muscles. Ageing not only results in a loss of skeletal muscle strength and power but has been found to affect skeletal muscle blood flow.

Venous occlusion plethysmography can be used to measure both resting blood flow and post-occlusive reactive hyperaemia (PO$_{bf}$) (Engelke et al. 1996; Higashi et al. 1999). PO$_{bf}$ is the increased blood flow that follows a period of ischemia, through the dilation of resistance vessels. The classical theory states that both metabolic and myogenic autoregulation contribute to the increased blood flow following ischemia.
(Joyner et al. 2001). This vasodilation has also been attributed to the release of local metabolites such as adenosine (Shinoda et al. 1997), prostaglandins and nitric oxide (Engelke et al. 1996; Dakak et al. 1998). Measuring post occlusive PO$_{bf}$ is also frequently used to establish maximal vasodilator responses in limbs as well as evaluate structural as opposed to vasomotor changes in the circulation (Joyner et al. 2001; Proctor, Le, & Rideout, 2005; Rideout, Parker, & Proctor, 2005).

Basal limb blood flow is reduced in aged individuals compared to their younger counterparts (Dinenno et al. 1999; 2001; Moreau et al. 2002). Dinenno et al. (2001) reported that, in a cross sectional study using 142 men between the ages of 19-79, basal femoral artery blood flow (18-22%) and femoral vascular conductance (20-30%) was lower and vascular resistance (25-38%) was higher in older men than younger counterparts (Figure 2.2). When basal limb blood flow was corrected for fat free mass the measurement was still 12% lower than the younger individuals, with a gradual decline in blood flow with age occurring with no obvious age were decline actually begins.

Age associated alterations in the ability to increase limb blood flow both in response to exercise and PO$_{bf}$ may play important roles for cardiovascular regulation and physical function (Koch et al. 2003; Proctor et al. 2003; 2005). Evidence suggests leg blood flow is reduced during sub-maximal (Proctor et al. 2003) and peak exercise (Proctor et al. 2004). Furthermore reductions in vasodilatation capacity have been seen following PO$_{bf}$ and vascular conductance in older men (Proctor et al. 2005), with an inverse relationship reported between age and peak hyperaemia in women (Lind et al. 1999; Sarabi et al. 1999). Others have reported lower PO$_{bf}$ and vascular conductance for older people compared to younger individuals (Martin et al. 1991; Olive et al. 2002), however not all evidence points towards lower vasodilatation capacity in older versus young people (Rueckert and Hanson 1995). This may be related to the age of the participants as the Rueckert and Hanson (1995) individuals had an average age of 35 and 57 for the young and older groups, respectively.
Figure 2.3. Relationship between femoral artery haemodynamics at rest (Dinenno et al. 2001).
2.2.1 Mechanisms for the decline in blood flow

There are several different mechanisms that may help explain the decrease in resting blood flow with age. Firstly a decrease in resting limb blood flow, with age is associated with corresponding reductions in limb fat-free mass and estimated limb oxygen demand (Dinneno et al. 1999; 2001). This may not be due to a reduced physical activity as when the values were adjusted for activity levels both limb fat-free mass and estimated limb demand both decreased (Dinenno et al. 2001). Age related decline in vascular conductance may be due to an increased muscle sympathetic vasoconstrictor nerve activity (MSNA) in both sedentary and physically active males (Sundolf and Wallin 1978; Ng et al. 1994; Davy et al. 1998). Dinneno et al. (1999) reported an inverse relationship between MSNA and basal limb vascular conductance in young and older men. Tonic release of nitric oxide from the vascular endothelium, a powerful vasodilator, decreases progressively with age (Taddei et al. 2000). This decrease in nitric oxide may play a role in the reduced blood flow seen during exercise in older people as Crecelius et al. (2010) recently demonstrated that the release of nitric oxide plays a role in increasing blood flow during exercise in older adults. Thus this decrease in nitric oxide may play a role in age-associated reductions in basal limb blood flow and vascular conductance.

The smooth muscle of a blood vessels vasoconstricts when the transmural pressure increases and in turn relaxes (vasodilates) when the pressure decreases and is thus known as the myogenic response (Johnson 1980). In-vitro animal studies suggest that myogenic responses in skeletal muscle arterioles are impaired with ageing (Muller-Delp et al. 2002). They demonstrated that arterioles from older animals exhibited less vasoconstriction as transmural pressure was incrementally increased. In a study on older humans Lott et al. (2004) demonstrated an attenuated peak blood velocity to altered transmural pressure and an attenuated vasoconstriction response to an increased transmural pressure. Numerous different mechanisms are proposed for this response in humans. It may be that the enhanced vasoconstriction associated with ageing impairs the endothelium’s release of vasodilators opposing the myogenic stimulus to vasoconstrict, as it is clear that endothelial substances (nitric oxide and endothelial relaxing factors) are important modulators of the myogenic response (de Wit et al. 1998; Schubert and Mulvany 1999; Pohl et al. 2000). Secondly structural
changes associated with ageing may affect the ability to initiate vasodilation with ageing (Vaitkevicius et al. 1993; Marin 1995).

Older adults appear to be limited in their ability to vasodilate to different tasks such as dynamic exercise, energy intake and heat stress (Wahren et al. 1974; Meneilly et al. 1995; Kenny 1997). Two large studies have shown that endothelial function, as seen with a reduced ability to vasodilate, in resistance vessels declines with age in healthy men and women (Taddei et al. 1995; Gerhard et al. 1996). Recent work by Taddei et al. (1996) found that the age effect on endothelium dependent vasodilatation in women was evident only after menopause but was a linear decrease in men from the age of 20. This has been confirmed by Sarabi and Lind (1999) as they found maximal blood flow during PO_{bf} to be reduced with age similar to previous research (Celermajer et al. 1994). Maximal blood flow during PO_{bf} has been used as a measurement of structural changes in resistance vessels (Sarabi and Lind 1999). Humeral factors (such as humeral immunity factors and hormones in the circulation) might also contribute to maximal flow during PO_{bf} and evidence presented that prostacyclin, NO, lactic acid and potassium all might modulate this measurement (Tagawa et al. 1994). However Nugent et al. (1999) found that nitric oxide production does not make a significant contribution to the vasodilatation associated with PO_{bf} in human forearm. Prostaglandins are reported to contribute to peak flow following PO_{bf} (Engelke et al. 1996) but have been found to be reduced in older adults and may play a role in the decreased vasodilatation capacity (Schrage et al. 2007).

### 2.2.2 Enhancing blood flow in older people

Endurance exercise training has been shown to result in a greater skeletal muscle blood flow in response to PO_{bf} in young and older people (Baynard et al. 2003; Witkowski et al. 2010). The mechanisms behind these increases may be a result of increased arterial diameters (Dinenno et al. 2001), capillary density (Borisov et al. 2000; Charles et al. 2006), vascular reactivity (Shoemaker et al. 1998), and endothelial function (Clarkson et al. 1999). Sinoway et al. (1986) investigated the maximal vasodilatation in the dominant and non-dominant forearms of tennis players and control subjects by measuring the maximal reactive hyperaemic blood flow. It was found that the maximal blood flow following 5 minute arterial occlusion coupled with 1 minute of exercise resulted in a 42% greater flow in the dominant (trained)
forearm compared to the non-dominant (untrained) forearm in the tennis players. It was postulated that this was due to an increase in the cross-sectional area of the skeletal muscle capillary bed. Snell et al. (1987) also measured vascular conductance during PO_{bf} produced by ischemic exercise to fatigue, which was performed by sedentary and endurance trained individuals. It was demonstrated that maximal conductance capacity was significantly higher in the trained compared the sedentary subjects again suggesting that endurance training increases the capacity for vasodilatation in the active limbs.

The studies described above highlight the adaptation in vascular capacity in response to endurance type training. There is now emerging evidence to suggest resistance training may also improve limb blood flow. An increase in PO_{bf} and vascular conductance was seen following 4 weeks of hand grip resistance training in young men (Alomari and Welsch 2007). They had their participants perform 20 minutes of hand grip contractions at 60% isometric strength, five days a week for 4 weeks. Other forms of resistance training such as low intensity resistance training with slow movement has also been shown to increase basal limb blood flow (Tanimoto et al. 2009), however the evidence is far from conclusive. For example, high intensity resistance training has been found to decrease PO_{bf} in healthy young males (Bond et al. 1996). Kawano et al. (2009) recently demonstrated no improvement in PO_{bf} following resistance training, however did find an increase when resistance training was combined with endurance training.

Resistance training has been shown to increase both basal limb blood flow and vascular conductance in older people (Anton et al. 2006). Indeed, resistance trained men do not have an age related decrease in limb blood flow as seen in non trained age matched controls (Miyachi et al. 2005). Therefore early research indicates different forms of resistance training may have an important role to play in limb blood flow of older people.

2.2.2.1 Mechanisms for improvements in blood flow

Skeletal muscle blood flow adaptations may occur for a number of different reasons. Specifically the adaptations may occur as a result of changes in either function or
structure. Hambrecht et al. (2003) provided an insight into the mechanisms responsible for exercise-mediated improvements in endothelial function by demonstrating that exercise training improved endothelial function in-vivo by up-regulating nitric oxide synthase protein expression and by increasing phosphorylation of this enzyme. These effects are consistent with a shear-stress mechanism for enhanced nitric oxide bioactivity with training. A further explanation for improved vascular function with training has been indirectly proposed by the results of Goto et al. (2003), who studied the effects of 12 weeks of endurance exercise undertaken at low, moderate and high intensity and observed improvement in endothelial function in the moderate intensity group only. This improvement occurred in the absence of changes of oxidative stress, whereas the high intensity group demonstrated increased oxidative stress and no improvement in endothelial function. This suggests that the impact of exercise training on endothelial function may be dependent upon the intensity of exercise performed and the consequent balance between acute oxidative stress, chronic changes in antioxidant defences and their impact on nitric oxide bioavailability. Reactive oxygen species degrade nitric oxide before it has a vasodilator impact on vascular smooth muscle. However, repeated stimulation of nitric oxide production as a result of exercise training may reduce its degradation by free radicals (Fukai et al. 2000), directly decrease free radical production (Adams et al. 2005), or increase the expression of genes encoding for antioxidant enzymes (Ennezat et al. 2001).

The mechanisms behind structural adaptations have demonstrated a link between changes in flow and arterial remodelling in animals (Langille and O’Donnell 1986). More recent studies confirm that the stimulus to arterial remodelling is dependent on shear stress (Tuttle et al. 2001) and that vessels enlarge to regulate wall shear in nitric oxide-dependent manner (Tronc et al. 1996). Shear stress mediated arterial enlargement, which is at least partly nitric oxide-dependent, acts to mitigate the increases in transmural pressure and wall stress brought about by repeated exercise bouts (Langille et al. 1989; Tronc et al. 1996; Lloyd et al. 2001; Prior et al. 2003). The structural normalization of shear stress may prevent the need for ongoing and acute functional adaptations (Maiorana et al. 2003; Green et al. 2004). One recent study in humans provides some support for this notion, as functional adaptations were superseded by apparent changes in artery size (Tinken et al. 2008). Other structural
adaptations may be an increased angiogenesis. Exercise induced angiogenesis leads to an increase in the diffusion of O2 through an increased capillary surface area and decreases the distance of O2 to the mitochondria (Wagner 2000). Vascular endothelial growth factor (VEGF) is essential for angiogenesis (Carmeliet et al. 1996; Ferrara et al. 2009), maintenance of the capillary supply in normal skeletal muscle and exercise induced increases in skeletal muscle vascular density (Amaral et al. 2001). It is well established that endurance training promotes increases in oxidative enzymes and the number of capillaries surrounding muscle fibres (Andersen and Henriksson 1977), however resistance training can also induce angiogenesis (McCall et al. 1996; Green et al. 1999; Campos et al. 2002). VEGF mRNA is increased 2.9 fold above rest 24 hours post resistance training in both young and old men (Jozsi et al. 2000). VEGF can be transcripted through the PI3K/AKT/mTOR pathway as an over expression of AKT in C2C12 cells increases VEGF transcription independent of hypoxia inducing factor 1 (HIF-1) (Takahashi et al. 2002). Resistance training increases capillaries surrounding a muscle fibre whilst maintaining or decreasing capillary density due to the concomitant increase in muscle fibre size (McCall et al. 1996; Green et al. 1999). Therefore it seems that resistance training may help preserve and reverse the age associated decreases in vascular function seen with normal physiological ageing.

2.3 Resistance training with blood flow restriction

So far in this review it has been highlighted that skeletal muscle in older people is very responsive to hypertrophy and strength gains. Indeed, this is also the case in very old people up to the age of 97 years (Harridge 1999). However, in order to obtain substantial gains in strength it is suggested that the targeted muscles must be subjected to a relatively high workload. The American College of Sports Medicine (Nelson et al. 2007) recommend that, during resistance training, the load should exceed 70% of 1-RM in order to achieve maximum hypertrophy and strength responses. An increasing number of research studies in the past ten years have shown that significant hypertrophy and strength gains can occur following resistance training at relatively low loads (i.e. 20 – 50 % 1-RM) during which the blood flow to the active musculature is either fully or partially restricted (Takarada 2002; Burgomaster et al. 2003; Abe et al. 2005b; Reeves et al. 2006).
The idea for this type of training originated in Japan around 40 years ago and has developed into a commercialized training method (known as “Kaatsu”) that is used in many training centres throughout the country. This type of training is widely used and is not only performed during resistance training but also been used during endurance type exercise i.e. treadmill walking (Abe et al. 2006). It is even used without exercise, in which the application of only the pressure cuff has been shown to prevent muscle atrophy in immobilisation (Kubota et al. 2008).

What is evident however is that throughout the literature there is no clear definition of the exact method used in terms of the combination of cuff occlusion pressure and nature of the sets and repetitions. Occlusion cuff pressure varies (100 – 300 mmHg) between studies which result in either partial or full restriction of blood flow and the number of repetitions performed vary from study to study with some working to a fixed number of repetitions whilst others complete each set to the point of muscular failure (Takarada 2002; Burgomaster et al. 2003; Abe et al. 2005b; Reeves et al. 2006). Recent work has tried to establish a definitive method for resistance training with blood flow restriction (BFR) and concluded that training at 20% 1-RM with continuous partial pressure would lead to the most appropriate way to produce fatigue and in turn the authors suggested this may be the most appropriate method to increase growth (Cook et al. 2007).

2.3.1 Adaptations to exercise with BFR

Generally strength adaptations are assessed by measuring isometric, isokinetic or dynamic muscle strength, which may be the result of neural or morphological adaptations, or both. Conventional resistance training results in significant gains in muscle strength, however a growing body of evidence suggests similar adaptations are seen following resistance training with BFR that are commensurate with the adaptations seen during traditional high load resistance training.

2.3.2 Muscle strength

Numerous studies have shown an increase in dynamic strength (↑ 8-23%) following resistance training with BFR (Burgomaster et al. 2003; Moore et al. 2004; Yasuda et
al. 2005; Abe et al. 2006) as well as isokinetic strength (↑ 9-23%) across a range of velocities (0-180°.sec) (Takarada et al. 2000b; 2002; 2004; Burgomaster et al. 2003; Moore et al. 2004). Resistance training with BFR also causes an increase in isometric strength ranging from 8 to 26% over a four to sixteen week period (Shinohara et al. 1998; Takarada et al. 2000b; Moore et al. 2004; Abe et al. 2005b). Not all research indicates a different strength response between resistance training with BFR and low intensity resistance training (50% 1-RM). Following 8 weeks of unilateral elbow flexion training both isometric and dynamic strength were seen to increase in the BFR group with no changes seen in the control group, however isokinetic strength increased by 10.5% and 9.6% in both groups respectively and dynamic strength by 22 and 23%. It is unclear why both limbs would respond in a similar fashion and contrary to all other published research but it is likely due to the mode of testing being similar to both isokinetic and dynamic strength. For a general review of strength and muscle mass adaptations following resistance training with BFR see Table 2.
Table 2.2. Effect of LLRT with BFR on muscle strength and size

<table>
<thead>
<tr>
<th>Reference</th>
<th>Gender (no of subject)</th>
<th>Age (yrs)</th>
<th>Training methods</th>
<th>Exercise(s)</th>
<th>Intensity (% 1RM)</th>
<th>Sets</th>
<th>Reps</th>
<th>Pressure (mmHg)</th>
<th>Frequency (Sessions/ week)</th>
<th>Duration (days)</th>
<th>Change in muscle strength (%)</th>
<th>Change in muscle mass (% CSA)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shinohara et al. (1998)</td>
<td>M (5)</td>
<td>23 (19-29)</td>
<td>Isometric +BFR</td>
<td>Knee extension (unilateral)</td>
<td>40 of MVC</td>
<td>1</td>
<td>36</td>
<td>Full &gt;250</td>
<td>3</td>
<td>28</td>
<td>+26 IS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Takarada et al. (2000b)</td>
<td>F (11)</td>
<td>58±2</td>
<td>Dyn + BFR</td>
<td>Elbow flexion (unilateral)</td>
<td>53</td>
<td>3</td>
<td>~ 18</td>
<td>110</td>
<td>2</td>
<td>112</td>
<td>+18 ISK</td>
<td>+20 (MRI)</td>
<td>Training to failure</td>
</tr>
<tr>
<td>Takarada et al. (2002)</td>
<td>M(6)</td>
<td>25±1</td>
<td>Dyn + BFR</td>
<td>Knee extension (bilateral)</td>
<td>50</td>
<td>5</td>
<td>~ 16</td>
<td>~196</td>
<td>2</td>
<td>56</td>
<td>+14 ISK</td>
<td>+12 (MRI)</td>
<td>Training to failure</td>
</tr>
<tr>
<td>Burgomaster et al. (2004)</td>
<td>M(8)</td>
<td>20±0</td>
<td>Dyn + BFR</td>
<td>Elbow flexion (unilateral)</td>
<td>50</td>
<td>3-6</td>
<td>~ 5-10</td>
<td>~100</td>
<td>2</td>
<td>56</td>
<td>+11 ISK, +23 1RM, +10 IS</td>
<td>-</td>
<td>5 mins rest between sets 3 and 4.</td>
</tr>
<tr>
<td>Takarada et al. (2004)</td>
<td>M(6)</td>
<td>21±1</td>
<td>Dyn + BFR</td>
<td>Knee extension (bilateral)</td>
<td>16-22</td>
<td>5</td>
<td>15-23</td>
<td>~218</td>
<td>2</td>
<td>56</td>
<td>+9 ISK</td>
<td>+10 (MRI)</td>
<td>Training to failure</td>
</tr>
<tr>
<td>Abe et al. (2005a)</td>
<td>M(9)</td>
<td>24±8</td>
<td>Dyn + BFR</td>
<td>Squat + knee flexion</td>
<td>20</td>
<td>3</td>
<td>15</td>
<td>160-240</td>
<td>12 (6d/wk, 2 x d)</td>
<td>14</td>
<td>+17 and +23 1RM</td>
<td>+8 and +11 (MRI)</td>
<td>-</td>
</tr>
<tr>
<td>Abe et al. (2005b)</td>
<td>M(9)</td>
<td>-</td>
<td>Dyn + BFR</td>
<td>Squat</td>
<td>20</td>
<td>3</td>
<td>15</td>
<td>160-240</td>
<td>14 (7d/wk, 2xd)</td>
<td>8</td>
<td>+10 1RM</td>
<td>+6 (Ultrason d)</td>
<td>-</td>
</tr>
<tr>
<td>Yasuda et al. (2005)</td>
<td>M(3)</td>
<td>20-47</td>
<td>Dyn +BFR</td>
<td>Squat and KF</td>
<td>20</td>
<td>3</td>
<td>15</td>
<td>160-240</td>
<td>12 (6d/wk, 2 x d)</td>
<td>14</td>
<td>14% IRM</td>
<td>+8% (MRI), +28% (Type II fibre area)</td>
<td>-</td>
</tr>
<tr>
<td>Kubo et al. (2006)</td>
<td>M(9)</td>
<td>25±2</td>
<td>Dyn + BFR</td>
<td>Knee extension (unilateral)</td>
<td>20</td>
<td>4</td>
<td>12-25</td>
<td>180-240</td>
<td>3</td>
<td>84</td>
<td>+8 IS</td>
<td>+6 (MRI)</td>
<td>+17% IS in HRST group</td>
</tr>
<tr>
<td>Madarame et al. (2008)</td>
<td>M(8)</td>
<td>22±2</td>
<td>Dyn + BFR</td>
<td>Knee extension (unilateral)</td>
<td>30</td>
<td>3</td>
<td>15-30</td>
<td>160-240</td>
<td>2</td>
<td>70</td>
<td>+20 IS</td>
<td>+12 (MRI)</td>
<td>-</td>
</tr>
<tr>
<td>Laurentino et al. (2008)</td>
<td>M(8)</td>
<td>24±3</td>
<td>Dyn + BFR</td>
<td>Knee extension (unilateral)</td>
<td>80 or 60</td>
<td>3-4</td>
<td>6-12</td>
<td>126</td>
<td>2</td>
<td>56</td>
<td>+35 1RM</td>
<td>+5 (MRI)</td>
<td>Exercise to failure -37% ↑ 1RM in HRST limb</td>
</tr>
<tr>
<td>Clark et al. (2010)</td>
<td>M(15)</td>
<td>24±2</td>
<td>Dyn + BFR</td>
<td>Knee extension (unilateral)</td>
<td>30</td>
<td>3</td>
<td>30-50</td>
<td>130% SBP</td>
<td>3</td>
<td>28</td>
<td>+8 IS</td>
<td>-</td>
<td>Exercised to failure. ~13% ↑ HRST group</td>
</tr>
<tr>
<td>Karabult et al. (2010)</td>
<td>M(37)</td>
<td>56.8±0.6</td>
<td>Dyn + BFR</td>
<td>Leg Press + Knee extension</td>
<td>20</td>
<td>3</td>
<td>15-30</td>
<td>160</td>
<td>3</td>
<td>42</td>
<td>+19 1RM</td>
<td>-</td>
<td>KE ↑ in HRST No ∆ in LLRT</td>
</tr>
<tr>
<td>Evans et al. (2010)</td>
<td>M(9)</td>
<td>20±1</td>
<td>Dyn +BFR</td>
<td>Heel Raise</td>
<td>10% +</td>
<td>4</td>
<td>50</td>
<td>150</td>
<td>3</td>
<td>28</td>
<td>+18 IS</td>
<td>-</td>
<td>Deflation between sets. No ∆ in Control. ↑ FC.</td>
</tr>
</tbody>
</table>

Dyn dynamic, F female, IS isometric strength, ISK isokinetic strength, 1RM one repetition max, M male, MRI magnetic resonance imaging
One possible explanation for the change in strength seen following LLRT with BFR may be related to motor unit recruitment patterns. Two studies investigating BFR and resistance training have shown that EMG values from the working muscles are significantly higher than a control group (Takarada et al. 2000b; Moore et al. 2004). Specifically, Takarada et al. (2000b) showed that the EMG of the biceps brachii during LLRT with BFR (40% 1-RM) was almost equal to that observed during conventional heavy resistance exercise (80% 1-RM). This would imply that during BFR training, motor units that are normally inactive are actually recruited. The elevated activation of the muscle at a low force level may be due to an intramuscular environment, in which more fast twitch, glycolytic (Type II) are activated in order to maintain the same level of force production (Sundberg 1994). Numerous studies (Vollestad et al. 1984; Sahlin et al. 1997; Houtman et al. 2003) have shown recruitment thresholds for motor units decrease during fatiguing exercise at a sub-maximal loads so that type II fibres are recruited as the point of force production failure draws closer. Direct measurement of single fibre activity have been made where it was observed that thigh occlusion (200mmHg) during intense dynamic knee extensor exercise resulted in a greater activity (recruitment) of type II, fast twitch muscle fibres (Krustrup et al. 2009). In order to further investigate the effects of LLRT with BFR on muscle fibre recruitment Suga and colleagues (2009) investigated intramuscular metabolism (using $^{31}$P-magnetic resonance spectroscopy) during LLRT with BFR and found that fast twitch fibre recruitment, as measured by inorganic phosphate splitting, occurred in only 31% of the participants during LLRT with BFR compared to 70% of the participants in the heavy resistance training group. They suggested that LLRT with BFR may not adequately recruit fast twitch muscle fibres in order to increase muscle size or strength. Previous work that has suggested that inorganic phosphate splitting provides a non invasive marker for type II fibre recruitment has been provided by Yoshida and Watari (1993; 1994) who investigated the time course of the inorganic phosphate splitting during progressive exercise to fatigue. They concluded that, because the inorganic phosphate peak appears with increasing exercise intensity, the splitting might be attributable to the delayed recruitment of type II muscle fibres. However, it has been suggested that inorganic phosphate splitting may not be an appropriate measure of type II muscle fibre recruitment patterns as this simple splitting into 2 distinct separate regions may be an oversimplification (Rossiter et al. 2002), and therefore the work by Suga et al. (2009)
must be treated with caution. Most evidence suggests that LLRT with BFR does enhance the recruitment of fast twitch muscle fibres due to an enhanced mechanical load on the muscle fibres in large motor units (Meyer 2006). As a greater number of fast twitch fibres are recruited it is possible that this may play a role in increasing the size of the muscle.

Several studies have reported significant increases in skeletal muscle size and volume following LLRT with BFR (Takarada et al. 2002; 2000b; Abe et al. 2006; 2005b; Yasuda et al. 2005). Moreover, Abe et al. (2005b) demonstrated an increases in muscle volume of the quadriceps, biceps femoris and gluteus maximus after only 2 weeks of LLRT with BFR compared to intensity matched control group. Similar adaptations using heavy conventional resistance training usually take 3 months to achieve (McCall et al. 1999; Goto et al. 2005). Similar results have been found in postmenopausal women (Takarada et al. 2000b), strength trained athletes (Takarada et al. 2002) and following a high frequency training program consisting of walking with BFR (Abe et al. 2006). This walk training with BFR resulted in an increase in muscle volume (4-7%) and maximal strength (8-10%) following 3 weeks of training (Abe et al. 2006). Yasuda et al. (2005) observed a 8% and 2% increase in quadriceps muscle CSA in a BFR and control group, respectively, following 2 weeks of training at 20% 1-RM. They also observed that type I and II muscle fibre CSA increased by 6% and 28%, respectively following LLRT with BFR with no change in the control group. Kubo et al. (2006) also demonstrated an increase in the pennation angle of trained muscle following LLRT with BFR. This, alongside an increase in PSCA and muscle volume, indicates a greater force production.

As well as increasing skeletal muscle hypertrophy, BFR alone has been found to preserve muscle CSA and strength following prolonged periods of immobilization. Ohta et al. (2003) found that following rehabilitation from anterior cruciate ligament surgery the participants who performed rehabilitation without BFR exhibited 8% less quadriceps CSA and 35% less knee extension strength in their injured limb in comparison to their healthy limb, whilst a group performing rehabilitation exercises with BFR exhibited similar CSA and 15% less strength than the injured limb. Kubota et al. (2008) demonstrated that BFR alone offset the skeletal muscle size and strength.
losses associated with immobilization. Both the control group and an isometric exercise group both lost size and strength whereas BFR protected against the loss.

As with conventional heavy resistance training the adaptive mechanisms to skeletal muscle hypertrophy to LLRT with BFR may be similar. Several investigations have demonstrated that GH responses are significantly higher following LLRT with BFR compared to resistance exercise at the same relative intensity, without BFR (Reeves et al. 2006; Takano et al. 2005; Takarada et al. 2000a). For example, Takarada et al. (2000a) found GH to be elevated 290 times above baseline levels following 5 sets of bilateral knee extensions at 20% 1-RM with BFR in a fully occluded state (~ 214 mmHg). Similarly, Takano et al. (2005) reported that partial occlusion (1.3 times greater than systolic pressure) at 20% 1-RM elevated GH levels to 100 times baseline measures. Both these studies found that exercise at the same intensity without BFR failed to result in similar GH responses. These observations highlight that LLRT with BFR elicit similar GH responses to those reported during conventional heavy load resistance exercise (> 75% 1-RM) without occlusion (Kraemer et al. 1990). Reeves et al. (2006) observed that light resistance training at 30% 1-RM with partial BFR resulted in GH increasing forty-one times resting levels (Fig 2.4). This was somewhat lower than the levels seen in previous studies using BFR, however this may have been due to the fact that the biceps brachii and gastrocnemius and/or soleus muscle are smaller in CSA than the quadriceps group studied by both Takarada et al. (2000a) and Takano et al. (2005), therefore the metabolic demand placed on the muscles by BFR would be lower. Secondly, afferent feedback from the relatively low mechanical stress provides the stimulus for hormonal response, which would be less in the Reeves et al. (2006) study when compared to studies using larger active muscle groups (Takarada et al. 2000a; Takano et al. 2005).
Figure 2.4 The pattern of serum GH (GH) response during low-intensity (LI: 20% maximal isometric strength), LI BFR knee extension exercise, and BFR without exercise. The arrows indicate the beginning and cessation of BFR (Pierce et al. 2006).

Due to the large increase in GH seen following LLRT with BFR it has been suggested that this increase may be important for the hypertrophy response observed. However as discussed earlier, the role of GH in muscle hypertrophy per se is somewhat limited. A number of studies have shown correlations between acute increases in GH and type I and II fibre area and CSA following a period of resistance training (McCall et al. 1999; Goto et al. 2005), but they do not prove cause and effect, therefore increased levels of acute GH may imply a greater level of effort, which may lead to increased growth. Taken in combination with evidence that exogenous GH in combination with resistance training does not have any effect of skeletal muscle growth (Rennie 2003), this therefore implies that other mechanisms are responsible.
As discussed previously GH mediates the release of circulating IGF-1, however the response of circulating IGF-1 to LLRT with BFR is equivocal. Abe et al. (2005b) found that LLRT with BFR produced a 24% increase in circulating IGF-1, with this response being similar in magnitude to high intensity resistance training (Borst et al. 2001). In contrast plasma IGF-1 did not increase following LLRT with BFR (Fujita et al. 2007) and walking with BFR (Abe et al. 2006). Others have also found positive increase in IGF-1 using a LLRT with no relaxation between repetitions and short rest periods (Popov et al. 2006). In this study the increased levels of IGF-1 was correlated well with the increase in GH. It is believed though that the increase in IGF-1 may have been caused by plasma volume changes resulting in hemoconcentration. Nevertheless this hemoconcentration may have important physiological consequence as an increased concentration of growth factors and hormones in the blood may result in a greater probability of interactions with receptors (Kraemer and Ratamess 2005). Finally it is possible that acute increases in GH may not cause an immediate increase in IGF-1, as peak values may not be reached until 16-28 hours post GH release (Kraemer and Ratamess 2005). One of the strongest suggestions that the mechanism of LLRT with BFR is endocrine induced comes from the study by Madarame et al. (2008). They found that 10 weeks LLRT with BFR of the knee extensors accompanied by heavy elbow flexion resistance training increased muscular size and strength of the elbow flexors in the BFR group only when compared to a control group performing LLRT on the knee extensors and heavy elbow flexion resistance exercise. This suggests a ‘cross transfer’ effect of circulating growth factors and their ability to stimulate growth in other skeletal muscles.

Although the GH/IGF-1 axis is regarded as an important regulator of muscle mass, as discussed earlier, some evidence suggests that exercise induced increases in GH and IGF-1 do not enhance the acute synthesis of myofibrillar proteins (West et al. 2009). Furthermore, exercise induced increases in these hormones did not enhance strength or hypertrophy following resistance training (West et al. 2009), suggesting local factors may be more important. There is limited evidence on the effect of local gene expression following LLRT with BFR. A recent investigation found that p21, Myo D (satellite cell activity) and MuRF1 (protein turnover) were up-regulated whilst myostatin was down-regulated following LLRT with BFR (Drummond et al. 2008a). However these changes were also seen following LLRT with normal blood flow, thus
the mechanisms behind the changes associated with BFR are still unknown. Along with the above genes both MGF and IGF-1R are not up-regulated 3 hours post LLRT with BFR thus indicating that IGF-1 may not be important for growth (Drummond et al. 2008a). A more recent study has tried to distinguish between the myogenic and proteolytic gene expression in response to LLRT with BFR, 8 hours post exercise (Manini et al. 2011). They found no change in myogenic gene expression (IGF-1, MyoD, myogenin and myostatin) but did find a down-regulation in proteolytic pathway genes (FOXO3A, atrogin-1 and Murf-1). All these studies have taken skeletal muscle biopsies at 3 and 8 hours post exercise thus it may be the timing of biopsy samples may play a role in when genes are up-regulated. In response to acute resistance exercise IGF-1 splice variants are known to be up-regulated between a few hours and a number of days (Adams and Haddad 1996; Hameed et al. 2003; Petrella et al. 2006). One recent study in rats found that when hypertrophy occurred due to restriction of blood flow and resistance training, it was not due to an increase in muscular IGF-1 (Kawada et al. 2005). They believe that IGF-1 may not be essential for hypertrophy if other factors such as myostatin, HSP-72 and NOS-1 would change in favour of muscular growth. Using this study to describe what happens in humans is difficult because exercise and restriction only occur for 5-10 minutes compared in humans compared to 14 days in rats. However research in this area is lacking thus understanding of the GH/IGF-1 axis and the role it plays in muscular hypertrophy following LLRT with BFR is needed.

One recent study has actually looked at a possible cellular mechanism that may explain an enhanced protein synthesis during LLRT with BFR (Fujita et al. 2007). They found that S6K1, a key downstream effector of the mTOR signalling pathway, became phosphorylated and muscle protein synthesis was stimulated following an acute bout of LLRT with BFR. The authors failed to establish the reason behind the activation of S6K1 and believe it may be due to either changes in hormonal response, metabolic stress and/or mechanotransduction signalling. The mTOR signalling pathway plays a significant role in stimulating translation initiation and muscle protein synthesis (Wang and Proud 2006). Recent studies have shown that the activation of mTOR signalling pathway is gradually activated during the recovery phase of resistance exercise (Bolster et al. 2003; Dreyer et al. 2006; Koopman et al. 2006). Furthermore, the increase in the phosphorylation of S6K1 associated with LLRT with
BFR suggests an up-regulation of translational efficiency allowing for the synthesis of specific mRNAs essential for the muscle growth. There is a direct correlation between the increase in S6K1 measured at 6 h after an acute exercise bout and the percent change in muscle mass measured after 6 wk of training (Barr and Esser 1999), suggesting the phosphorylation of S6K1 could be a marker for the long-term increase in muscle mass. The same groups have recently shown that mTOR is up-regulated and protein synthesis increase following LLRT with BFR in older men (Fry et al. 2010). For a general review of acute responses of LLRT with BFR (see Fig 2.5).
Figure 2.5. Conceptual Model of physiological responses following LLRT with BFR (Manini and Clark 2009).
GH indicates growth hormone; BP, blood pressure; O2, oxygen; IGF-1, insulin-like growth factor-1; p21, cyclin-dependent kinase inhibitor 1A; mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase; HSP, heat shock protein; ROS, reactive oxygen species; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase
2.3.3 Blood flow

Although much of the work to date investigating the beneficial effects of LLRT with BFR have focussed on muscle strength parameters it is conceivable that adaptations may occur within the skeletal muscle vasculature. Indirect evidence for this can be seen by the observation that LLRT with BFR leads to greater muscular endurance than low-intensity resistance training alone (Sumide et al. 2009; Takarada et al. 2000b; 2002). Furthermore, Takarada et al. (2002) observed that LLRT with BFR increased in muscular endurance despite an increase in muscle CSA. The authors believe that this increase in endurance performance was caused by an increase in oxidative energy metabolism and acid buffering capacity rather than an increase in the resistance to fatigue. This is because the exercise training did not cause any change in the EMG signal in either the initial or last ten of the fifty contractions. However, improved endurance in a trained muscle could be related to an enhanced blood supply, which would facilitate a greater oxygen delivery.

Indirect evidence has demonstrated that ischemic exercise like that seen during LLRT with BFR can improve vascular capacity. Ferguson and Brown (1997) demonstrated that rock climbers have a higher PO_{bfs} than non trained individuals suggesting that adaptations may occur due to high ischemic component of their training. Similar findings have also been shown high ischemic component with Sinoway et al. (1986) demonstrating that the forearms of tennis players that held the tennis racket had a 42% greater PO_{bfs} than the non dominant forearm. The improvements seen following ischemic exercise could be due to the adaptations discussed earlier such as a change in arterial structure, a change in the availability of nitric oxide or an increased capillarity. There is currently limited data on these adaptations during LLRT with BFR in both young and old people. Very recent evidence has shown that LLRT with BFR increases microvascular filtration capacity following 4 weeks training (Evans et al. 2010). Calf filtration capacity is determined by Starling forces – that is, the surface area available for filtration and permeability per unit of surface area (Brown et al. 2001). There is a significant correlation between increases in calf filtration capacity and the length of contact between capillaries and muscle fibres in older men following endurance training (Charles et al. 2006). This strong relationship between capillary surface area and calf filtration capacity therefore allows the latter to be used as an
indirect measure of capillarization (Gamble et al. 2000; Brown et al. 2001). VEGF is associated with both an increase in capillarization and angiogenesis (Amaral et al. 2001) and therefore suggests that this type of training may play a role in changes in vascular function following a period of sustained training. Indeed, Takana et al. (2005) found that plasma VEGF was significantly elevated following an acute bout of LLRT with BFR. Coupled with the increase in calf filtration capacity following LLRT with BFR suggests it may increase angiogenesis (Evans et al. 2010). Furthermore, there may be a link between the GH response seen following LLRT with BFR and vascular function. Acute administration of exogenous GH has been shown to lower peripheral vascular resistance and stimulate endothelial function (Napoli et al. 2003).

2.4 Aims and Objectives

This review has discussed an indentified that as individuals become older and age, there is a loss of skeletal muscle strength and mass, as well as a decrease in vascular function. A new and novel form of training known as LLRT with BFR has been identified to help improve some of the main consequences of ageing, namely improving muscle mass and strength. Moreover, it has been suggested that exercise with a high ischemic component may also help improve vascular function. Therefore the main aim of this thesis was to investigate the effects of LLRT with BFR and its effects on both young and older individuals. Firstly it was hoped to establish whether LLRT with BFR could improve strength and blood flow parameters in both young and older people. Furthermore, we hope to gain insight into the mechanism behind which these alterations may occur by investigating both circulating and local growth factor responses to LLRT with BFR.

The specific hypotheses were therefore:

In chapter 3

Strength parameters would increase by a greater extent following LLRT with BFR at 50% 1 RM when compared to training at 25% 1 RM.
PO_{bf}, would be increased by a greater extent following LLRT with BFR compared to LLRT alone in young people.
In chapter 4
Strength parameters will also increase by a greater extent following LLRT with BFR when compared to LLRT alone in a population of older people. PO$_{bf}$ would be increased by a greater extent following LLRT with BFR compared to LLRT alone in older people.

In chapter 5
LLRT with BFR would provide a sufficient stimulus to increase anabolic (GH and IGF-1), inflammatory (IL-6) and vascular (VEGF) hormones and cytokines in response to an acute bout of exercise in older people.

In chapter 6
IGF-1 isoforms associated with muscle growth will be up-regulated 24 hours following a bout of LLRT with BFR and to a greater extent than LLRT alone. The expression of IGF-1 isoforms will be greater in the young compared to the older individuals.
Chapter 3

Increase in calf post-occlusive blood flow and strength following short term resistance exercise training with BFR in young women.
3.1 Introduction

It is well known that heavy resistance exercise has a potent effect to increase the size and strength of skeletal muscle. The load required to achieve these responses should generally exceed 70% 1 RM (ACSM 2002). It is therefore interesting that several studies have demonstrated that resistance training at relatively low levels of force (20-50% 1 RM) when combined with BFR results in gains in strength and hypertrophy that are commensurate with traditional high load resistance training (Takarada et al. 2002; Burgomaster et al. 2003). For example, Takarada et al. (2002) demonstrated a 14% increase in isokinetic knee extensor torque of young subjects when LLRT at an intensity of 50% 1 RM was combined with BFR compared with no change in strength with resistance training alone. In another study, Takarada et al. (2000b) demonstrated in the elbow flexors of older women that LLRT combined with BFR resulted in a similar increase in strength as that resulting from high load training (80% 1 RM) without occlusion. Several other studies (Takarada et al. 2002; Burgomaster et al. 2003) show similar increases in muscle size and strength during LLRT with BFR compared to conventional heavy resistance training alone using a variety of exercise modalities including the so-called Kaatsu walking (Abe et al. 2006). Indeed, some observations of increases in strength as a result of muscle hypertrophy, following LLRT with BFR have been made within short timescales such as 2 weeks (Abe et al. 2005b).

Along with increases in strength it is also of interest that recent work has demonstrated that resistance training exercise has also been shown to enhance peak PO$_{bf}$ (Rakobowchuk et al. 2005). Alomari and Welsch (2007) have also shown that 4 weeks handgrip resistance training increased peak PO$_{bf}$ in the trained arm. These findings combined with the observation that athletes who are specifically trained to perform ischemic contractions have significantly enhanced vascular capacity (Ferguson & Brown 1997) suggests that LLRT with BFR may also increase PO$_{bf}$ to a greater extent compared to LLRT alone.

Although research in the area of LLRT with BFR has been increasing in interest, the studies thus far have used varying protocols making it difficult to establish an optimum training regime. This is especially true for the workloads used during
exercise, with investigators using loads ranging between 20-80% 1 RM (Burgomaster et al. 2003; Moore et al. 2004; Reeves et al. 2006; Laurentino et al. 2008). Work by Cook et al. (2007) investigated the effect of varying protocols for exercise load, occlusion pressure and occlusion duration. They suggested that the most potent stimulator for muscle growth was one that resulted in the greatest fatigue and thus concluded that training at a load equating 20% maximum with partial but continuous restriction of blood flow was the most fatiguing, therefore resulting in the most growth. This study, however, only investigated the acute effects of resistance training with BFR, with longer term changes unknown for lighter workloads. Until recently it was not clear if training at higher workloads with BFR further enhanced strength and hypertrophy gains, however Laurentino et al. (2008) investigated the effect of heavy (60 – 80% 1 RM) resistance training with and without BFR and did not observe any difference in the changes in size or strength between groups.

Therefore we currently know that: (i) resistance training can increase PO_{bf}, (ii) BFR does not enhance strength gains if high load training is used, (iii) LLRT without BFR does not enhance strength, and (iv) LLRT with BFR evokes a similar increase in the strength and size of a muscle when compared to high load training without restriction. However it is currently unknown if low (25% 1 RM) and moderate (50% 1 RM) load resistance training with BFR may improve PO_{bf} and secondly what the load needs to be to induce a gain in strength when combined with BFR. Therefore, we hypothesised that PO_{bf} would be increased by a greater extent following LLRT with BFR compared to LLRT alone. We also hypothesised that strength parameters will also increase by a greater extent following resistance training with BFR at 50% 1 RM when compared to training at 25% 1 RM. We have used the calf muscle as this provides a suitable model for the study of both strength and blood flow parameters using well defined techniques.
3.2 Methods

Participants

16 young healthy females volunteered to participate in the study. They were assigned to one of 2 groups that were matched for maximal dynamic plantar flexion strength determined from a 1-RM. These were 25% 1 RM (n = 8; age 23 ± 3 yr, height 168.6 ± 6.8 cm, body mass 62.6 ± 8.5 kg) or 50% 1 RM (n = 8; age 22 ± 3 yr, height 162.6 ± 5.8 cm, mass 61.3 ± 6.2 kg). All participants were habitually physically active but none specifically performed resistance exercise training. The participants were fully informed of the purposes, risks and discomfort associated with the experiment before providing written, informed consent. This study conformed to current local guidelines and the Declaration of Helsinki and was approved by Loughborough University Ethics Committee.

Overview of experimental procedures

Participants initially performed a familiarisation trial before the experimental protocol in order to become accustomed to all testing procedures and training devices. Participants were instructed in proper use of the resistance exercise equipment and also performed several plantar-flexions using a light load (<25% 1 RM) in order to mimic the type of actions performed during the training.

The experimental protocol consisted of (i) baseline measurement of resting limb blood flow (Rbf) and blood flow following 5 minutes occlusion (PObf) as well as baseline measurements of plantar-flexor strength, (ii) a 4 week plantar-flexor resistance training program with and without BFR, and (iii) post-training blood flow and strength measurements that were conducted in an identical manner to the baseline measurements. All pre- and post-testing and training procedures were performed on both limbs. In the RBF condition all training was performed with an occlusion cuff, whereas the contralateral limb training was performed without restriction. It has been recently demonstrated that changes in vascular capacity are limited to the trained region following resistance training (Alomari and Welsch 2007) and there are no cardiovascular effects on the contra-lateral limb (Mourtzakis et al. 2004; Saltin et al. 1976). All pre-training tests were performed 3-5 days prior (baseline) to the
commencement of the resistance training program and post-training measurements performed 3-5 days after the final training session.

**Limb blood flow**

Calf blood flow measurements were carried out in a supine position using venous occlusion strain-gauge plethysmography, using mercury-in-rubber strain gauges (Hokanson, Bellvue, WA, USA). The basis of this technique is that a “pressure cuff” is inflated around the lower limb to a pressure less than diastolic so arterial inflow may continue whereas venous outflow is obstructed. Under this condition the limb “swells” and the volume increases. Mercury strain gauges were placed on the widest circumference of the calf. Inflatable pressure cuffs (CC17RB and SC10RB, Hokanson, Bellvue, WA, USA) were positioned 2-3 cm above the knee and around the ankle. Strain gauges were placed at the widest girth of the calf and attached to a dual channel plethysmograph (EC6 Plethysmograph, Hokanson, Bellvue, WA, USA) with blood flow traces being sampled on line at 100 Hz (Powerlab, AD Instruments, NSW, Australia), for offline analysis. The diameter of the strain gauge was 1 or 2 cm lower than the widest girth of the calf. Venous drainage was facilitated by placing a 15 cm foam block under the ankle and a 7 cm foam block under the knee and ensuring the limb was positioned in line with the heart. This method has been previously used to prevent contamination due to venous congestion (Tschakovsky et al. 1995). Rapid inflation of the cuffs occurred by connecting the thigh cuff to a pneumatic air source (E20 Rapid cuff inflator and AG101 Cuff Inflator Air Source, Hokanson, WA, USA). The BF was measured at rest and post- 5 min occlusion. The release of thigh cuff after ischemia leads to a large vasodilation, which is known as reactive hyperemia (PObf). Both Rbf and PObf were measured in each limb in a counterbalanced order. The coefficient of variation (CV) over repeated test occasions for Rbf and PObf was 10-11% and 7-10%, respectively. These correspond with values obtained from previous studies (Thijssen et al. 2005).
Resting limb blood flow ($R_{bf}$)

Following instrumentation participants rested for 20 min in a supine position. Thirty seconds prior to the measurement of blood flow, arterial blood flow to the foot was occluded by inflating the ankle cuff to 200 mmHg. The measurement was performed by inflating the thigh cuff to a venous occlusion pressure of 50 mmHg for 7 seconds after which the cuff was deflated. This process was repeated 3 times, with approximately 30 seconds between each measurement, and the average taken. The ankle cuff was deflated immediately after the final blood flow measurement was obtained.

$PO_{bf}$

After $R_{bf}$, the measurement of $PO_{bf}$ was performed while participants remained in the supine position by inflating a thigh blood pressure cuff to 200 mmHg to induce arterial occlusion for five minutes. With 30 seconds left of arterial occlusion an ankle cuff was inflated to 200 mmHg. Following rapid deflation of the thigh cuff blood flow measurements were obtained within 15 seconds following arterial occlusion and every 15 seconds thereafter for two minutes. $PO_{bf}$ was taken as the highest value obtained after occlusion. Total blood flow following 5 mins occlusion was expressed in absolute terms as area under the time-flow curve (AUC) calculated by the trapezoid method (Meeking et al. 2000).

Data Analysis

The plethysmographic signal was transmitted to a computer for off-line analysis, with blood flow being calculated from the slope of the volume change over the first cardiac cycle, using Chart version 5 software (ADInstruments, NSW, Australia). The plethysmographic chart speed was set at 5mm/sec to record the arterial inflow. The sensitivity of the chart speed represents that for each centimetre deflection on the chart is the equivalent of a 0.2% change in volume of the limb. Blood flow values were obtained by applying the classic triangular method. The tangent slope increases vertically from the baseline to the top of the recording paper. Values were calculated with the formula, which is 60 seconds multiplied by the full chart range and divided by the longitudinal distance (mm), which reflects the slope between the baselines to
the top of the recording paper (see Fig 3.1. This results in a % volume change per minute. All vascular values are presented in mL. 100mL tissue⁻¹.min⁻¹ and is equivalent to percentage change per minute (Whitney 1953).

**Muscular strength**

Plantar-flexion torque was recorded on both limbs unilaterally with participants lying prone in an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY), with the knee at full extension and the lateral malleolus aligned with the axis of rotation identified on the dynamometer. The foot of each participant was firmly secured to the foot adapter of the dynamometer and participants were restrained at the waist, shoulders and the distal part of the thigh. Before measurements of isometric strength subjects performed 5 submaximal isometric plantar-flexion contractions as a warm up. Three maximal isometric contractions were performed at a joint angle of 0° (the sole of the foot at 90° with respect to the tibial axis, as measured using a goniometer). The participants were asked to gradually but quickly attain peak isometric strength and hold for ~2-3 seconds during which constant verbal encouragement and feedback was provided by the investigator. The value for isometric strength during each contraction was determined as the highest value obtained, with the highest of the 3 values taken as isometric strength.

Isokinetic torque was assessed by measuring maximal plantar-flexion torque during three, single maximal repetitions. Prior to the maximal repetitions, five warm up contractions were performed to accustom the participant to the required velocity. Torque production was assessed during the concentric phase of the movement only, at 3 (identified by pilot work) different contraction velocities (0.52, 1.05 and 2.09 rad.s⁻¹). The highest torque value recorded during any of the three repetitions was taken as peak torque. The performance of each velocity was randomised and 1 minute rest was given between each maximal effort.

After 15 min rest, dynamic plantar-flexor 1-RM of each limb was assessed with a straight leg in a supine position on a leg press machine (Ortus Fitness, Valencia, Spain). In order to bring the leg-press plate into position for straight leg plantar-
flexion exercise the weight was initially pushed using knee extension with the help from an investigator. Once in a straight leg position the plantar-flexion exercise could commence. After warming up, the load was set at 80% of the predicted 1-RM. Following each successful lift the load was increased by ~5% until the subject failed to lift the load through the entire range of motion. A test was only considered valid when the participant used proper form and completed the entire lift in a controlled, unassisted manner. Approximately 2-3 mins of rest was allowed between each attempt to ensure recovery. After it was considered 1-RM had been achieved, following a sufficient rest period each participant had the load increased one last time to ensure that they could not lift any more weight. On average each participant needed 5 attempts to reach 1-RM. Following the 4 week training period 1-RM was reassessed. On this occasion, following a warm up, the load was set close to the previous 1-RM to ensure a maximal effort was achieved before fatigue occurred. The CV for MVF, isokinetic torque at 0.52, 1.05, 2.09 rad.s\(^{-1}\) and 1-RM was 2.5, 3.9, 4.3, 5 and 3.5% respectively.

Training protocol
The 4 week training program consisted of 3 sessions per week of supervised resistance training. Training consisted of unilateral plantar-flexion resistance exercise using one of the two respective loads (25 or 50% 1 RM). Following a warm up involving 5 minutes cycling at 50 W, participants performed single leg plantar-flexion exercise in a supine position using the same device employed for the dynamic 1-RM strength test. Within each group participants were counterbalanced with 4 participants performing LLRT with BFR on their dominant limb whilst 4 performed LLRT with BFR with their non dominant limb. For all training sets the restricted blood flow limb was trained first in which participants completed 3 sets of exercise to the point of failure, with 1 min rest interval between sets. This allowed for the repetitions to be matched for the control leg. BFR was maintained at an occlusion pressure of 110 mmHg, just above the knee, as this has been shown to compress underlying arteries and veins causing a pooling of blood (Takarada et al. 2000b). This pressure was maintained for the entire 3 sets (including rest periods) which resulted in a duration of ~ 5-8 minutes. Once the LLRT with BFR was completed the participants then performed 3 sets of LLRT, with 1 min rest between sets. The number of repetitions
performed by the normal blood flow leg was matched to that completed by the restricted condition. 1 RM of each limb was reassessed after 2 weeks of training and loads were adjusted to maintain the required training intensity.

**Statistical analysis**

Results are expressed as means ± standard deviation (SD) for all variables. There were no significant differences between resting values for any of the variables measured. Changes in $R_{bf}$, $PO_{bf}$, 1 RM, isometric strength and isokinetic strength at each velocity were examined using a two-way (load (25 vs. 50% 1-RM) x condition (Restricted vs. normal)) mixed ANOVA with repeated measures design. The absolute response for $R_{bf}$, $PO_{bf}$, blood flow AUC were analysed using a three-way (time (Pre vs. Post) x load (25 vs. 50%) x condition (Restricted vs. Normal)) mixed ANOVA with repeated measures design. $R_{bf}$ was not normally distributed, therefore statistical analysis was performed on the logarithmic transformation of the data. Where significant interactions between load and condition used have been found they are reported. If the interaction between load and condition was not significant but a significant main effect for load or main effect for condition was found, the main effects are only reported. Statistical significance was accepted at $P < 0.05$. 
3.3 Results

All the participants in both training groups were able to successfully complete all training sessions with 100% compliance and free of injury. No differences were found in any baseline variables between the restricted blood flow condition and normal blood flow condition. There were no significant interactions between the load used and the condition used for training for any of the variables measured.

Changes in limb blood flow

\( R_{bf} \) did not change with resistance training either with or without BFR (Table 3.1). Regardless of training load, following LLRT with BFR peak PO\(_{bf}\) increased (P < 0.05) by a greater extent when compared to LLRT with normal blood flow (Table 3.1, Figure 3.2). Similarly, the AUC increased (P < 0.05) to a greater extent following LLRT with BFR compared to with normal blood flow (Table 3.1).

Changes in muscle strength

Regardless of the training condition (normal or restricted blood flow) training at 50% 1 RM resulted in a greater (P < 0.05) increase in 1 RM compared to 25% 1 RM (Fig 3.3). Furthermore, when LLRT was performed with BFR 1-RM increased (P < 0.05) by a greater extent (30 ± 11%) when compared to LLRT with normal blood flow (23 ± 12%) (Fig 3.3). Likewise, isometric strength increased by a greater extent following resistance training with BFR (13 ± 12%) compared to LLRT with normal blood flow (4 ± 8%) (Fig 3.4), as did isokinetic torque at 0.52 rad.s\(^{-1}\) and 1.05 rad.s\(^{-1}\) (Fig 3.5a, b). However, there was no effect of either condition or load on isokinetic torque at 2.09rad.s\(^{-1}\)(Fig.5c).
Table 3.1. Resting ($R_{bf}$), post occlusion PObf blood flow (PObf) and area under the blood flow curve (AUC) before and following 4 weeks resistance training with and without BFR at 25% (n=8) and 50% (n=8) 1 RM. Values of blood flow and AUC are in ml.min$^{-1}$.100ml$^{-1}$ and ml.100ml$^{-1}$, respectively. * Significant (P < 0.05) time x condition interaction for restricted vs normal (i.e. data for training groups are collapsed and combined). Following LLRT BFR values were significantly greater than following training with normal blood flow.

<table>
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<th>25% 1 RM</th>
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<td>Pre</td>
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<tr>
<td>$R_{bf}$</td>
<td>2.3 ± 1.1</td>
<td>1.8 ± 0.5</td>
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<tr>
<td>PObf</td>
<td>20.3 ± 4.5</td>
<td>21.4 ± 5.3</td>
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<td>AUC</td>
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Figure 3.1. Strain gauge calf volume profile during occlusion cuff inflation to 50 mmHg. Vertical solid lines indicate calculated blood flow. Horizontal arrow indicate cuff inflation and deflation. Change in tracing observed prior to cuff infaltion was due to resetting the strain gauge prior to measurement.
Figure 3.2. Change in peak PO_{bf} following 4 weeks resistance training with and without BFR. Values are means ± SD. (25% 1-RM, n = 8; 50% 1-RM, n = 8). * Significant (P < 0.05) main effect for restricted vs. normal (i.e. data for training groups are combined).
Figure 3.3. Change in 1 RM following 4 weeks resistance training with and without BFR. Values are means ± SD. (25% 1 RM, n = 8; 50% 1 RM, n = 8). * Significant (P < 0.05) main effect for restricted vs normal (i.e. data for training groups are combined). † Significant (P < 0.05) main effect for 25% 1 RM vs 50% 1 RM (i.e. data for restricted and normal are collapsed and combined).
Figure 3.4. Change in Isometric strength following 4 weeks resistance training with and without BFR. Values are means ± SD. (25% 1 RM, n = 8; 50% 1 RM, n = 8). * Significant (P < 0.05) main effect for restricted vs normal (i.e. data for training groups are collapsed and combined).
**Figure 3.5.** Change in isokinetic torque at (a) 0.52, (b) 1.05 and (c) 2.09 rad.s\(^{-1}\) following 4 weeks resistance training with and without BFR. Values are means ± SD. (25% 1 RM, n = 8; 50% 1 RM, n = 8). * Significant (P < 0.05) main effect for restricted vs normal (i.e. data for training groups are collapsed and combined).
3.4. Discussion

The main finding of the present study was that 4 weeks calf resistance training with partial BFR enhanced the increase in calf PO_{bf} compared to resistance training alone. Furthermore, plantar-flexor 1 RM, isometric strength and isokinetic torque at 0.52 and 1.05 rad.s^{-1} were also enhanced when training was performed with BFR when compared to resistance training alone.

This is the first study to show an enhanced increase in PO_{bf} following LLRT with BFR. Of course the major limitation within this study is that venous occlusion strain-gauge plethysmography does not allow us to elucidate the exact mechanisms behind changes within the vasculature. Indeed, the exact mechanisms that determine peak PO_{bf} are currently unknown. Infusion studies have demonstrated that NO-dependent endothelial function is only likely to have a modest, if any, effect (Tagawa et al. 1994; Engelke et al. 1996) on PO_{bf} and it is more likely a myogenic and metabolic phenomenon (Carlsson et al. 1987). These observations are consistent with the concept that PO_{bf} is mediated by myogenic mechanism, which reduces vascular tone in vessels that are mechanically unloaded during the ischemia (Koller and Kaley 1990). Therefore, it is possible that the enhanced metabolite accumulation created by the BFR during training (Takarada et al. 2000a; Suga et al. 2009), including vasoactive metabolites such as adenosine, contributed to the enhanced PO_{bf}. It is also possible that BFR resulted in an increased capillarity. The potential contribution of angiogenesis to an increased blood flow with exercise training remains equivocal. The influence of muscle fibre recruitment may play an important role since it has been observed that a preferential capillarisation occurs amongst FT fibres (Adair et al. 1990). Low intensity exercise tends to recruit mainly slow twitch (ST) oxidative fibres whereas more intense exercise requires more fast twitch (FT) glycolytic fibre recruitment (Vollestad & Blom 1985). However, the addition of BFR favours additional recruitment of FT fibres even at relatively low workloads (Krustrup et al. 2009) and would therefore provide an additional stimulus for angiogenesis. However the evidence for this increased recruitment is equivocal, with some investigators showing a recruitment pattern during LLRT with BFR that is similar to heavy load resistance training (Takarada et al. 2000b) whilst others show that although higher than LLRT alone, the recruitment is less than heavy load resistance training (Manini
and Clark. 2009). The additional fibre recruitment and associated metabolic consequences of this would also support the role of the important regulator of angiogenesis, VEGF whereby local metabolic changes occurring in response to exercise seem to be an important signal for VEGF up-regulation (Roca et al. 1998) possibly initiated through the VEGF/NO cascade (Milkiewicz et al. 2005). Indeed, serum VEGF has previously been shown to significantly increase in response to resistance training with BFR (Takano et al. 2005).

One other possible suggestion for the change in peak PO\textsubscript{bf} following LLRT with BFR is an increased venous compliance such that the impact of venous congestion on impeding arterial inflow is reduced which could therefore explain the small differences in the increase of peak PO\textsubscript{bf}. Although not measured in the current study it is possible that pooling of blood flow associated with this type of training may result in increased compliance (Convertino et al. 1988). This is a similar response to that seen following endurance training where venous congestion is increased following training with no decrement on tolerance to orthostatic stress (Hernandez and Franke 2005). What is clear is that further research is needed to investigate the exact mechanisms behind the increase in peak PO\textsubscript{bf} found following LLRT with BFR.

Like previous studies we have observed greater increases in strength when LLRT was performed with BFR compared to resistance training alone. For example, Abe et al (2005b) and Kubo et al (2006) reported greater gains in strength of the quadriceps muscle at loads < 50% 1 RM when blood flow was restricted. Although in the present study there were no significant interactions between the load used and the use of BFR on any of the strength or blood flow measurements it is perhaps not surprising that there was a significant main effect for 1 RM in the 50% compared to the 25% group irrespective of the condition used to train. It is well known that intensities of 50% 1 RM can result in a sufficient stimulus for improved strength, most likely due to the specificity of this measure in relation to the training manoeuvre. Indeed, Moore et al. (2004) found that resistance training at an intensity of 50% 1 RM was effective in improving 1 RM independent of the application of BFR.

Regardless of the workload, resistance training in combination with BFR was also effective in increasing strength in non training specific manoeuvres, as shown by the
greater increases in isometric strength and isokinetic strength at 0.52 and 1.05 rad.s\(^{-1}\). The greater changes in strength with BFR were probably due both to neural adaptations, and possibly muscle hypertrophy. As described previously, the addition of BFR results in additional recruitment of FT fibres (Krstrup et al. 2009) which would facilitate an enhanced recruitment of fast motor units as a result of training (Takarada et al. 2000b). Although we have not measured muscle size or neural activation in the present study hypertrophy is not typically thought to occur so early in a traditional resistance training programme. However, numerous studies of LLRT with BFR have clearly shown significant hypertrophy to occur, even after 14 days (Takarada et al. 2000b; Abe et al. 2005b; Madarame et al. 2008). An important regulator of skeletal muscle hypertrophy is the IGF-1 axis. Increases in IGF-1/MGF have been observed following acute bouts of high-load resistance training (Hameed et al. 2004; Petrella et al. 2006), however the effect of resistance training with BFR on local growth factor responses is not known. Serum levels of IGF-1 and GH (GH) are known to significantly increase following resistance training with BFR compared to exercise with normal blood flow (Takarada et al. 2000a; Abe et al. 2005b; Reeves et al. 2006). One recent study (Fujita et al. 2007) observed that S6K1, a key downstream effector of the mTOR signalling pathway (one of the two signalling pathways thought to be activated by IGF-1), became phosphorylated and muscle protein synthesis was stimulated following an acute bout of resistance training with BFR. Although we cannot confidently suggest that 50% 1 RM provides a greater stimulus for increasing strength and blood flow than 25% 1 RM, what is clear is that loads as low as 25% 1 RM when combined with BFR do provide sufficient stimulus for these improvements. Moreover, the increase in blood flow may enhance the endurance capacity of the muscle trained thus improving the exercise tolerance of the muscle by helping reduce fatigue. For example, Takarada et al. (2002) have shown that the endurance capacity of the knee extensor muscles can increase following 8 weeks resistance training with BFR. The evidence therefore suggests that this type of training may be a useful exercise to improve both muscular strength and endurance for individuals who are unable to lift such heavy loads such as those recovering from an injury or older people.
One limitation of the current study is that hormonal status was not measured in our female participants. However, evidence suggests that strength is not influenced by the menstrual cycle (de Jonge 2003; Kubo et al. 2009; Montgomery and Shultz 2010). Recent evidence also suggests that menstrual cycle phase has no effect on hemodynamics. For example, Cooper et al. (2006) found that suppression of ovarian hormones associated with the menstrual cycle does not affect resting calf blood flow. Finally, although hormonal status was not tracked, all females reported normal cycles (28-30 days). Taking this into account and the fact that the training intervention lasted 4 weeks, it is likely that all participants were at the same phase of their menstrual cycle during the pre and post training measurements.

In conclusion, this study is the first to demonstrate enhancements in the blood flow capacity following resistance training with blood flow restriction compared to resistance training alone. Resistance training with BFR also increased plantar-flexor 1 RM, isometric strength, and isokinetic strength to a greater extent than following resistance training alone.
Chapter 4

Resistance training with BFR enhances the increase in strength and peak post occlusive calf blood flow in older people.
4.1. Introduction

Decreased strength (Skelton et al. 1994) and vascular function (Dinneno et al. 1999) are two important changes associated with advancing age in humans. Lower levels of strength in older people are associated with a decreased functional ability to perform daily tasks (Suzuki et al. 2001), an increased rate in the number of falls (Skelton et al. 2002) and physiological changes such as loss of bone mineral density (Blain et al. 2001). Reductions in limb blood flow at rest (Dinenno et al. 2001), during exercise (Proctor and Parker 2006) and following PObf (Sarabi et al. 1999) are also seen with advancing age and are associated with metabolic syndrome (Lind and Lithell 1993) and the impairment of the clearance of atherogenic lipids that contribute to dyslipidemia (Baron et al. 1990). Interventions that will limit or improve these changes and thus improve both the functional ability as well as these risk factors for disease could have profound benefits for older people.

One such intervention that has been recently shown to improve both strength and vascular function is LLRT with BFR (Chapter 3). It was demonstrated in young females that using workloads as low as 25% 1-RM with BFR was sufficient to improve dynamic, isokinetic and isometric plantar-flexor strength by as much as 30% following a 4 week training period. This is similar to the responses seen in other training studies using LLRT with BFR in young (Burgomaster et al. 2003) and post menopausal women (Takarada et al. 2000b). As well as the increases in strength we also demonstrated an increase in blood flow capacity (PObf; Chapter 3).

Although the effectiveness of this type of training is well established for increasing strength in healthy young populations, it has yet to be confirmed whether older people, over the age of 60 years, can counteract the age related declines in strength and vascular function using such an intervention. Chapter 3 was the first to demonstrate the effectiveness of LLRT with BFR to increase strength in young women, with others demonstrating similar adaptations in young men (Abe et al. 2005b; Kubo et al. 2006). Therefore the aim of this study was to determine if LLRT at 25% 1-RM with BFR can improve calf muscle strength and limb blood flow in older people. It was hypothesised that the gains in both muscle strength and limb blood flow will be greater in LLRT with BFR compared to LLRT alone. The calf muscle
group was chosen as it is considered to be functionally important during posture and locomotory tasks. For example, Reeves et al. (2008) demonstrated that the ankle joint plantar-flexors of older people operate close to their maximal joint moment limits during stair descent and are therefore of critical importance during this type of activity. The calf muscle group also permits the non invasive measurement of blood flow using venous occlusion plethysmography which has to be performed on the distal limb to allow attachment of the mercury strain gauge and proximal occlusion cuff. We chose 25% 1-RM based on our previous study (Chapter 3) which demonstrated changes in strength and blood flow following resistance training with occlusion in young women. Moreover, in the same study we showed similar changes in strength and blood flow between 25% and 50% 1-RM. Other studies have used intensities as low as 20% 1-RM which also shown beneficial effects (Abe et al. 2005b).
4.2. METHODS

Participants
10 (2 female, 8 male) older people (age 67 ± 3 yr, height 170.3 ± 6.7 cm, body mass 77.9 ± 7.8 kg; [mean ± SD]) volunteered to participate in the investigation and were selected according to the exclusion criteria used to define ‘medically stable’ older participants for exercise studies, as proposed by Greig et al. (1994). All participants were habitually physically active, (measured using a physical activity questionnaire) i.e. performed regular physical activity such as walking, jogging, gardening (2-3 times per week, 30 mins at a time) but none specifically performed resistance exercise training. The participants were fully informed of the purposes, risks and discomfort associated with the experiment before providing written, informed consent. This study conformed to current local guidelines and the Declaration of Helsinki and was approved by Loughborough University Ethics Committee.

Overview of experimental procedures
Participants initially performed a familiarisation trial before the experimental protocol in order to become accustomed to all blood flow and strength testing procedures and training devices. All measurements and training procedures were conducted on the calf muscle group. Participants were instructed in proper use of the resistance exercise equipment and also performed several plantar-flexion contractions using a light load (<25% 1-RM) in order to mimic the type of actions performed during the training.

The experimental protocol consisted of; (i) baseline measurement of plantar-flexor strength, resting limb blood flow ($R_{bt}$), blood flow following 5 minutes circulatory occlusion ($PO_{bt}$), blood pressure and corrected calf girth (CCG); (ii) a 4 week plantar-flexor resistance training program with and without BFR, and (iii) post-training strength and blood flow measurements that were conducted in an identical manner to the baseline measurements. All pre- and post-testing and training procedures were performed on both limbs. Participants had their legs assigned to be trained with BFR in a counterbalanced manner with 5 participants training their dominant leg and 5 training their non dominant leg, based on dominance from 1-RM strength, with their opposite leg serving as the control leg. All pre-training tests were performed 3-5 days
prior (baseline) to the commencement of the resistance training program and post-training measurements were performed 3-5 days after the final training session.

**Muscular strength**

Plantar-flexion torque was recorded on both limbs unilaterally with participants lying prone in an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY), with the knee at full extension and the lateral malleolus aligned with the axis of rotation identified on the dynamometer. The foot of each participant was firmly secured to the foot adapter of the dynamometer and participants were restrained at the waist, shoulders and the distal part of the thigh. Before measurements of isometric strength (maximal voluntary force MVF) subjects performed 5 submaximal isometric plantar-flexion contractions as a warm up. Three maximal isometric contractions were performed at a joint angle of 0° (the sole of the foot at 90° with respect to the tibial axis, as measured using a goniometer). The participants were asked to gradually but quickly attain peak isometric strength and hold for ~2-3 seconds during which constant verbal encouragement and feedback was provided by the investigator. The value for isometric strength during each contraction was determined as the highest value obtained, with the highest of the 3 values taken as isometric strength.

Isokinetic torque was assessed by measuring maximal plantar-flexion torque during three, single maximal repetitions. Prior to the maximal repetitions, five warm up contractions were performed to accustom the participant to the required velocity. Torque production was assessed during the concentric phase of the movement only, at 3 (identified by pilot work) different contraction velocities (0.52, 1.05 and 2.09 rad.s⁻¹). The highest torque value recorded during any of the three repetitions was taken as peak torque. The performance of each velocity was randomised and 1 minute rest was given between each maximal effort.

After 15 min rest, dynamic plantar-flexor 1-RM of each limb was assessed with a straight leg in a supine position on a leg press machine (Ortus Fitness, Valencia, Spain). In order to bring the leg-press plate into position for straight leg plantar-flexion exercise the weight was initially pushed using knee extension with the help from an investigator. Once in a straight leg position the plantar-flexion exercise could commence. After warming up, the load was set at 80% of the predicted 1-RM.
Following each successful lift the load was increased by ~5% until the subject failed to lift the load through the entire range of motion. A test was only considered valid when the participant used proper form and completed the entire lift in a controlled, unassisted manner. Approximately 2-3 mins of rest was allowed between each attempt to ensure recovery. After 1-RM had been achieved, following a sufficient rest period each participant had the load increased one last time to ensure that they could not lift any more weight. On average each participant needed 5 attempts to reach 1-RM. Following the 4 week training period 1-RM was reassessed. On this occasion, following a warm up, the load was set close to the previous 1-RM to ensure a maximal effort was achieved before fatigue occurred. The CV for MVF, isokinetic torque at 0.52, 1.05, 2.09 rad.s$^{-1}$ and 1-RM was 2.5, 3.9, 4.3, 5 and 3.5% respectively.

**Limb blood flow**

Calf blood flow measurements were carried out in a supine position using venous occlusion strain-gauge plethysmography, using mercury-in-rubber strain gauges (Hokanson, Bellvue, WA, USA). The basis of this technique is that a “pressure cuff” is inflated around the lower limb to a pressure less than diastolic so arterial inflow may continue whereas venous outflow is obstructed. Under this condition the limb “swells” and the volume increases. Mercury strain gauges were placed on the widest circumference of the calf. Inflatable pressure cuffs (CC17RB and SC10RB, Hokanson, Bellvue, WA, USA) were positioned 2-3 cm above the knee and around the ankle. Strain gauges were placed at the widest girth of the calf and attached to a dual channel plethysmograph (EC6 Plethysmograph, Hokanson, Bellvue, WA, USA) with blood flow traces being sampled on line at 100 Hz (Powerlab, AD Instruments, NSW, Australia), for offline analysis. The diameter of the strain gauge was 1 or 2 cm lower than the widest girth of the calf. Venous drainage was facilitated by placing a 15 cm foam block under the ankle and a 7 cm foam block under the knee and ensuring the limb was positioned in line with the heart. This method has been previously used to prevent contamination due to venous congestion (Tschakovsky et al. 1995). Rapid inflation of the cuffs occurred by connecting the thigh cuff to a pneumatic air source (E20 Rapid cuff inflator and AG101 Cuff Inflator Air Source, Hokanson, WA, USA). The BF was measured at rest and post- 5 min occlusion. The release of thigh pressure cuff after ischemia leads to a large vasodilation, which is known as reactive
hyperemia (PO_{bf}). Both Rbf and PO_{bf} were measured in each limb in a counterbalanced order. The coefficient of variation (CV) over repeated test occasions for Rbf and PObf was 10-11% and 7-10%, respectively. These correspond with values obtained from previous studies (Thijssen et al. 2005).

R_{bf}
Following instrumentation participants rested for 20 min in a supine position. Resting blood pressure was obtained from the right arm of each participant (Omron M5-I, Omron Healthcare, Kruisweg, Netherlands) and repeated three times, with the average taken. Thirty seconds prior to the measurement of blood flow, arterial blood flow to the foot was occluded by inflating the ankle cuff to 200 mmHg. The measurement of blood flow was performed by inflating the thigh cuff to a venous occlusion pressure of 50 mmHg for 7 seconds after which the cuff was deflated. This process was repeated 3 times, with approximately 30 seconds between each measurement, and the average taken. The ankle cuff was deflated immediately after the final blood flow measurement was obtained.

PO_{bf}
After R_{bf}, the measurement of PO_{bf} was performed while participants remained in the supine position by inflating the thigh cuff to 200 mmHg to induce arterial occlusion for five minutes. With 30 seconds left of arterial occlusion an ankle cuff was inflated to 200 mmHg. Following rapid deflation of the thigh cuff blood flow measurements were obtained within 15 seconds following arterial occlusion and every 15 seconds thereafter for two minutes. Peak PO_{bf} was taken as the highest value obtained after occlusion. Total blood flow following 5 mins occlusion was expressed in absolute terms as area under the time-flow curve (AUC) calculated by the trapezoid method (Meeking et al. 2000).

Data Analysis
The plethysmographic signal was transmitted to a computer for off-line analysis, with blood flow being calculated from the slope of the volume change over the first cardiac
cycle, using Chart version 5 software (ADInstruments, NSW, Australia). The plethysmographic chart speed was set at 5mm/sec to record the arterial inflow. The sensitivity of the chart speed represents that for each centimetre deflection on the chart is the equivalent of a 0.2% change in volume of the limb. Blood flow values were obtained by applying the classic triangular method. The tangent slope increases vertically from the baseline to the top of the recording paper. Values were calculated with the formula, which is 60 seconds multiplied by the full chart range and divided by the longitudinal distance (mm), which reflects the slope between the baselines to the top of the recording paper (see Fig 3.1. This results in a % volume change per minute. All vascular values are presented in mL. 100mL tissue⁻¹.min⁻¹ and is equivalent to percentage change per minute (Whitney 1953).

**Anthropometry**

Participants’ height was measured with a wall mounted stadiometer (Holtain Ltd, Crymych, UK) and body mass with a beam balance scale (Avery Ltd, Fairmount, MN, USA) whilst participants wore shorts and t-shirt. Calf circumference was measured using a standard anthropometric tape and medial calf skinfold was recorded by means of a vertical fold at the widest section of the muscle using Harpenden skinfold calipers (British Indicators Ltd, Wolverhampton, UK). The measures were taken on both limbs and repeated three times, in order to calculate the average value. From the values obtained CCG was calculated by subtracting π multiplied by the skinfold (in centimeters) from the total calf circumference measurement, previously described by Martin et al. (1990).

**Training protocol**

The 4 week training program consisted of 3 sessions per week of supervised resistance training. Training consisted of plantar-flexion resistance exercise at 25% 1-RM, as used in the previous study (Chapter 3). Following a warm up involving 2 sets of 10 contractions of dynamic planter-flexion at <20% 1-RM, participants performed single leg plantar-flexion exercise using the same leg-press device employed for the dynamic 1-RM strength test. For all training sets the restricted blood flow limb was trained first in which participants completed 3 sets of exercise to the point of failure, with 1 min rest interval between sets. This allowed for the repetitions to be matched
for the control leg. BFR was maintained at an occlusion pressure of 110 mmHg, just above the knee, as this has been shown to compress underlying arteries and veins causing a pooling of blood (Takarada et al. 2000b). This pressure was maintained for the entire 3 sets (including rest periods) which resulted in a duration of ~ 5-8 minutes. Once the LLRT with BFR was completed the participants then performed 3 sets of LLRT, with 1 min rest between sets. The number of repetitions performed by the normal blood flow leg was matched to that completed by the restricted condition. 1 RM of each limb was reassessed after 2 weeks of training and loads were adjusted to maintain the required training intensity.

**Statistical analysis**

Results are expressed as means ± standard deviation (SD) for all variables. Absolute changes for Rbf, peak PObf, blood flow area under the curve (AUC), 1-RM, maximal isometric strength, isokinetic strength and CCG were examined using a two-way (Time x Limb) ANOVA with repeated measures design. Systolic and diastolic blood pressure was examined using a paired sample T-test. Statistical significance was accepted at p<0.05.
4.3. RESULTS

All the participants were able to successfully complete all training sessions with 100% compliance and free of injury or complications. No differences were found in any baseline variables between the restricted blood flow condition and normal blood flow condition.

CCG increased (main effect of time, p<0.01) in the BFR group (34.1 ± 1.7 to 34.5 ± 1.6 cm) and the normal blood flow group (34.0 ± 1.8 to 34.4 ± 1.7 cm). However, there were no differences observed between groups (no main effect for limb, p>0.05, or interaction of limb x time, p>0.05).

Systolic blood pressure did not change (p>0.05) following resistance training (132 ± 22 mmHg and 131 ± 18 mmHg, pre and post training). Diastolic blood pressure was also unchanged (p>0.05) following resistance training (79 ± 16 and 79 ± 15 mmHg, pre and post training).

**Changes in muscle strength**

Values of plantar-flexor 1-RM, isometric strength and isokinetic torque are shown in Table 4.1. 1-RM was similar between conditions at baseline (150 ± 25 vs. 148 ± 25 kg, for the normal and restricted groups, respectively). The increase in 1-RM was greater when LLRT was performed with BFR compared with normal blood flow (20 vs. 5kg, respectively; main effect for limb, p < 0.05; main effect of time, p < 0.001; interaction of limb x time, p< 0.001).

Isometric strength increased by a greater extent following LLRT with BFR compared to LLRT with normal blood flow (15 vs. 3 N.m, respectively; no main effect for limb, p>0.05; main effect of time, p < 0.01; interaction of limb x time, p < 0.01.).

Isokinetic torque at 0.52 rad.s⁻¹ increased by 13 N.m in the restricted condition compared to no change in the normal blood flow condition (no main effect for limb, p>0.05; main effect for time, p=0.05; interaction of limb x time, p<0.01).
Isokinetic torque at 1.05 rad.s$^{-1}$ increased (main effect of time, p<0.01) by 7 and 3 N.m for restricted and normal blood flow conditions, respectively. However, there were no differences observed between groups (no main effect for limb, p>0.05; no interaction of limb x time, p>0.05).

Isokinetic torque at 2.09 rad.s$^{-1}$ increased (main effect of time, p=<0.05) by 4 and 1 N.m, for restricted and normal blood flow conditions, respectively. However, there were no differences observed between groups (no main effect for limb, p>0.05; no interaction of limb x time, p>0.05).

**Changes in limb blood flow**

$R_{bf}$ was similar between conditions at baseline and remained unchanged following LLRT with (2.1 ± 0.5 to 2.4 ± 0.5 ml.min$^{-1}$.100ml$^{-1}$) and without (2.2 ± 0.6 to 2.5 ± 0.8 ml.min$^{-1}$.100m$^{-1}$) BFR (no main effect for limb, p>0.05; time, p>0.05; or interaction of limb x time p>0.05).

$PO_{bf}$ was similar between conditions at baseline. $PO_{bf}$ increased by a greater extent following LLRT with BFR compared to LLRT with normal blood flow (Fig 4.1; no main effect for limb, >0.05; main effect of time, p<0.01; interaction of limb x time, p < 0.01).

Although $PO_{bf}$ increased by a greater extent with BFR, the increase in AUC (main effect of time, p < 0.01) was the same between both the restricted (393 ± 125 to 587 ± 116 ml.100ml$^{-1}$) and normal blood flow (395 ± 196 to 591 ± 269 ml.100ml$^{-1}$) conditions (no main effect for limb, p>0.05; or interaction of limb x time, p>0.05).
Table 4.1. Changes in plantar-flexor strength parameters (1-RM, isometric strength and isokinetic torque) following 4 weeks resistance training with (Restricted) and without (Normal) BFR. Values are mean ± SD. * significant (p<0.05) interaction between normal and restricted.

| Strength Parameter | Normal | | Restricted | | |
|--------------------|--------| | Pre | Post | Pre | Post |
| 1-RM (kg)          | 150 ± 25 | 155 ± 25 | 148 ± 25 | 168 ± 25 | * |
| Isometric strength (N.m) | 92 ± 26 | 95 ± 25 | 85 ± 20 | 100 ± 26 | * |
| 0.52 rad.s⁻¹ (N.m) | 92 ± 25 | 92 ± 26 | 83 ± 27 | 96 ± 21 | * |
| 1.05 rad.s⁻¹ (N.m) | 66 ± 19 | 69 ± 20 | 62 ± 21 | 69 ± 18 | |
| 2.09 rad.s⁻¹ (N.m) | 43 ± 15 | 44 ± 15 | 41 ± 16 | 45 ± 16 | |
Figure 4.1. Change in PO$_{2f}$ following 4 weeks resistance training with (open bars) and without (shaded bars) BFR. Values are mean ± SD. * Significant (p<0.05) main effect for restricted vs normal from pre to post. a Significant (p<0.05) main effect for time, pre to post.
4.4. DISCUSSION

The major finding of the present study was that in older individuals LLRT with BFR resulted in greater improvements in maximal strength (1-RM, isometric strength, isokinetic torque at 0.52 rad.sec\(^{-1}\)) and peak PO\(_{bf}\) when compared to LLRT with normal blood flow.

Previous research in younger individuals has consistently shown increases in strength ranging from 10-22% following 4-16 weeks resistance training of various muscle groups (e.g. biceps and quadriceps) with BFR (Burgomaster et al. 2003; Shinohara et al. 1998; Takarada et al. 2000b). The increases in dynamic plantar-flexion strength observed in the older participants in the present study are consistent with those observed in younger people (Chapter 3) and are of a similar magnitude observed during conventional high-load (> 70% 1-RM) resistance training in older individuals (Harridge et al. 1999; Hakkinen et al. 2001). Although we were not able to directly assess the degree of strength gains compared to conventional high-load resistance training, LLRT with BFR may prove useful for individuals such as those recovering from injury or those suffering from complications that may not allow them to use heavy loads.

One reason is that it is plausible that the increases in strength during the initial stages of this type of training are greater than conventional high-load resistance training, since the average increase in CSA of the muscle is as high as 0.57% per day during LLRT with BFR (Abe et al. 2005b) compared to the reported 0.03-0.26% per day gains seen during high-load resistance training (Wernbom et al. 2007). Secondly, we have demonstrated the feasibility of this type of training in older people given the fact that our healthy participants completed the study with no complications or complaints which may indicate that it is tolerable and safe. A previous report on safety issues of this type of training was by Nakajima et al. (2006) who surveyed over 105 Japanese facilities and their users (30,000 exercise sessions) whom use LLRT with BFR as a regular mode of exercise. They found that the most frequent safety issues were bruising at the location of the cuff (13.1%) and numbness (1.6%), which although on the high side was not reported in the current study. There were reported cases of venous thrombosis (0.06%), but this was below the average number (0.2% - 0.26%)
of cases seen in the Asian population (Klatsky et al. 2000). Further evidence for the safety of this type of training has been demonstrated from the work by Clark et al. (2010). They provided evidence that 4 weeks of LLRT with BFR resulted in no adverse effects on vascular or nerve function, markers of coagulation, fibrinolysis or inflammation.

The changes observed in strength after 4 weeks training may be primarily due to neural adaptations such as increased motor unit recruitment which typically occur during the early stages of resistance training (Folland and Williams, 2007). Addition of BFR during low intensity exercise results in additional recruitment of FT fibres (Krustrup et al. 2009). In fact the level of muscle activation during acute bouts of resistance exercise with BFR is similar to that seen during resistance exercise with heavier loads (Takarada et al. 2000b; Yasuda et al. 2009). However, following LLRT with BFR in young people, both Moore et al. (2004) and Kubo et al. (2006) did not show an increased in activation level, normally associated with resistance training with heavy loads. In these studies the activation levels were measured in younger individuals and were already at a high level, up to 98%, therefore further changes would seem unlikely. However in men over the age of 50 it has been demonstrated that there is a significantly reduced activation level of the plantar-flexors when compared to younger counterparts (Kubo et al. 2007) suggesting increased activation may play a role. As well as changes in neural activation, a change in pennation angle of the muscle may also explain the changes in strength. Muscles with large pennation angles allow more fibres to be arranged in parallel within a given cross-sectional area, thereby increasing a muscle’s force generating potential (Manal et al. 2008). Kubo et al. (2006) demonstrated that LLRT with BFR resulted in a change in the pennation angle of the muscle which has been shown to occur in as little as 20 days (Seynnes et al. 2007). There may also be an increase in the size of muscle as it has been previously shown that changes in muscle size following LLRT with BFR have been seen to occur as early as two weeks (Abe et al. 2005b). In the current study there was a slight increase in CCG, however this was the same between conditions and it is acknowledged that CCG is not a direct measure of muscle size which would be better performed by MRI. Studies using MRI as a measure of muscle mass have demonstrated that CSA can increase by 8.5% following 2-3 weeks of LLRT with BFR (Abe et al. 2005a; 2005b), thus indicating significant hypertrophy occurs within a
short time period. However it is unlikely that hypertrophy played a major role in the current study due to the increase in CCG in both LLRT with and without BFR.

An important finding from the current study is that peak P\textsubscript{bf} was elevated following resistance training with BFR when compared to LLRT with normal blood flow. This is similar to our recent findings in young people (Chapter 3) with the changes in blood flow being of a similar magnitude. The exact mechanisms are unknown and are difficult to interpret using venous occlusion strain-gauge plethysmography but may be due to an enhanced myogenic or metabolic response (Carlsson et al. 1987) or increased capillarity (Esbjörnsson et al. 1993). An increase in muscle capillarity may be important in enhancing the blood-tissue exchange properties, as in turn this increased capillary network may lead to an increased surface area for diffusion, a shortening of the average diffusion path length within the muscle and possibly an increase in the length of time for diffusive exchange between blood and tissue (Prior et al. 2004). This improvement in the blood-tissue exchange may improve functional tasks requiring sustained activity. Moreover, the decrease in peripheral blood flow with ageing (Rideout et al. 2005; Dinenno et al. 1999), including older individuals who are overweight (Acree et al. 2007), is associated with metabolic syndrome, a major precursor to atherosclerotic disease in humans that includes hyperinsulinemia, dyslipidemia, and hypertension (Lind and Lithell 1993). Furthermore, PObf has recently been indicated in being a marker for cardiovascular disease (Addor et al. 2008). Therefore strategies that can counteract this and increase limb blood flow, as we have demonstrated, may be important for reducing disease risk.

One limitation of the current study is the mixed leg design. Cross education is the contralateral effect of chronic motor activity in one limb (Enoka 1988). Resistance training of a single limb has been shown to increase the strength of the non-trained limb, with a recent meta analysis indicating this cross education accounts for an approximate 8% increase in strength (Carroll et al. 2006). Indeed, a cross transfer in strength and hypertrophy has been reported following LLRT with BFR (Madarame et al. 2008) and may be responsible for the increased strength in the control leg in the current study. This cross education therefore may have resulted in the similar changes in torque at 1.05 rad.s\textsuperscript{-1} and 2.09 rad.s\textsuperscript{-1} in the two conditions. However, this may have also been due to a lack of specificity in the testing procedure. This cross education
effect has also been shown to occur in the vascular system (DeSouza et al. 2000) and may be due to the release of vasodilator substances into the circulation following exercise. However not all evidence points to such an effect with other studies not showing any change in the vascular bed of non trained limbs (Gokce et al. 2002; Mourtzakis et al. 2004).

In conclusion, it was demonstrated that both strength and blood flow parameters in older people can be increased to a greater extent following a 4 week training intervention using LLRT with BFR compared to LLRT alone. Future work should aim to establish the mechanisms behind these changes to aid the understanding of reduced muscle mass and blood flow in older people.
Chapter 5

Circulating hormone response to LLRT with BFR in older men
5.1. Introduction

The ageing process is associated with lower levels of strength, muscle mass and vascular function (Skelton et al. 1994; Dinneno et al. 1999; 2001; Lindle et al. 1997; Proctor and Parker 2006). Numerous mechanisms have been used to explain these losses including loss of muscle mass or ‘sarcopenia’ (Kent-Braun et al. 2000), reduced muscle excitability and contractility (Narici et al. 1999) and all of this may be a result of a decreased level of physical activity employed by the ageing population (Brooks and Faulkner 1994). One other proposed mechanism for these losses is an alteration in the circulating concentrations of specific hormones and growth factors specifically GH and IGF-1 (Lamberts et al. 1997).

GH is an endogenous anabolic hormone released from the pituitary gland and has been shown to decline during the ageing process with some reports showing a decline of 50% (Veldhuis et al. 1997). GH mediates its effects on muscle by regulating the synthesis of IGF-1 (Le Roith, 2001). Circulating levels of IGF-1 have been shown to decrease (Harris et al. 1997; Roubenoff et al. 1998) and have been associated with increased mortality in older men and women (Roubenoff et al. 2003). LLRT with BFR is a novel method shown to increase GH (Takarada et al. 2000a; Takano et al. 2005; Reeves et al. 2006) and IGF-1 (Abe et al. 2005b; Takano et al. 2005) following an acute bout of exercise. For example, Takarada et al (2000a) demonstrated GH was elevated 290 times above resting levels following 5 sets of bilateral knee extensions at 20% 1-RM with BFR. These observations elicit a similar, if not greater, acute GH response to heavy load (> 75% 1-RM) resistance exercise without occlusion (Kraemer et al. 1990). It is believed that these acute increases in hormone concentrations may play a role in long term changes in muscle size and strength associated with LLRT and BFR (Takarada et al. 2000b; Abe et al. 2005b).

Work from Chapter 2 and 3 suggests that LLRT with BFR can increase peak vasodilator capacity in young and older individuals. The mechanisms behind these changes are unclear, however an increased capillarity of the muscle bed cannot be ruled out. VEGF has been shown to be a stimulating factor for angiogenesis (Neufeld et al. 1999; Risau 1997). It has been shown to increase in the muscle and circulation following acute endurance exercise (Gavin et al. 2004), resistance training (Gavin et
Reduced oxygen tension has been shown to be a major stimulus to angiogenesis and in turn has been shown to increase VEGF expression (Breen et al. 1996). LLRT with BFR has been shown to decrease tissue oxygenation by as much as 50% during elbow flexor exercise (Manini and Clark 2009), therefore may be a useful method in up-regulating VEGF in older people.

Interleukin-6 (IL-6) has been shown to increase following many different exercise modalities (Takarada et al. 2000a; Izquierdo et al. 2009; Chatzinikou et al. 2010; Leggate et al. 2010). It has been classified as having both pro- and anti-inflammatory properties (Jones et al. 2001), and thought to play a role in tissue remodelling (Pedersen et al. 2003; Steensberg, 2000). IL-6 may also play a causal role in sarcopenia, with high levels positively associated with low walking speed and poor muscle strength (Taaffe et al. 2000b). Further evidence suggests IL-6 release following exercise may be responsible for the increased cortisol response seen following exercise (Steensberg, 2003).

Therefore the aim of the current study was to examine the effect of an acute bout of LLRT with and without BFR on plasma concentrations of cortisol, GH, IGF-1, IL-6 and VEGF in older men, before and 2 hours post exercise. Older men were chosen in this chapter due to the difficulty in obtaining older women who matched the health criteria. The 2 hour time point was chosen as this represents the optimal time for acute changes in plasma hormone concentrations following heavy resistance training and LLRT with BFR (Pierce et al. 2006; Reeves et al. 2006; Takano et al. 2005). The exercise model used in chapter 5 and 6 used the quadriceps rather than the plantarflexors as seen in chapter 3 and 4. The quadriceps was chosen as it provided a bigger muscle, thus a greater stimulus for the hormonal and local growth factor responses (Pierce et al. 2006). It was also the only muscle that the individual trained to take the muscle biopsy (chapter 6) had experience with. We hypothesised that LLRT with BFR would provide a sufficient stimulus to increase anabolic (GH and IGF-1), inflammatory (IL-6) and vascular (VEGF) hormones and cytokines in response to an acute bout of exercise.
5.2. METHODS

Participants
Seven older males volunteered to participate in the study (age 71.0 ± 6.5 yr, height 1.77 ± 0.05 m, body mass 80.0 ± 7.5 kg; BMI 25.6 ± 3.0 kg/ m²; means ± SD) and were selected according to the criteria of Greig et al. (1994) for “healthy” older participants. This criteria ensured individuals were not on any medication throughout the study all participants were habitually physically active but none were specifically trained with resistance exercise. The participants were fully informed of the purposes, risks and discomfort associated with the experiment before providing written, informed consent. This study conformed to current local guidelines and the Declaration of Helsinki and was approved by Loughborough University Ethics Committee.

Overview of experimental procedures
Participants initially performed a familiarisation trial (described below) trial, before the experimental protocol in order to become accustomed to the strength testing procedures and training devices. All measurements and training procedures were conducted on the quadriceps muscle group. Participants were instructed in proper use of the resistance exercise equipment and performed the exact experimental protocol consisting of knee extension contractions using 20% 1-RM.

The experimental protocol consisted of (i) baseline measurement of unilateral knee extension dynamic muscle strength of both limbs; (ii) a pre exercise blood sample from the antecubital vein, followed by 5 sets of unilateral LLRT knee extensions at 20% 1-RM with or without BFR on both limbs (iii) a further blood sample 30, 60 and 120 minutes post exercise and (iv) seven days post 1st experimental protocol, this was repeated on the opposite limb with the other exercise condition. All pre- and post-testing procedures were performed on both limbs.

1-RM Testing & Familiarisation
Maximum unilateral strength 1-RM of both the knee extensors was assessed on a leg extension machine (Ortus Fitness, Valencia, Spain) at least 7 days prior to conducting
Participants began with a warm up of two sets of 10 repetitions at 5kg, each separated by 2 min. Thereafter, the load was set at 80% of the predicted 1-RM. Following each successful lift the load was increased by ~5% until the subject failed to lift the load through the entire range of motion. A test was only considered valid when the participant used proper form and completed the entire lift in a controlled, unassisted manner. Approximately 2-3 mins of rest was allowed between each attempt to ensure recovery. Participants were then familiarised to the nature of LLRT with BFR which consisted of performing a resistance exercise training session of 5 sets of knee extension to failure at 20% 1-RM with blood flow partially occluded (110mmHg), which was repeated on both limbs. This also provided an indication of the number of repetitions performed in the blood flow restricted condition which would be replicated in the non-restricted condition and allow a counterbalanced order in the main experimental protocol ultimately allowing the work done during the experimental and control trials to be matched.

**Experimental Protocol**

Participants completed 2 trials in a counterbalanced order with a minimum of 7 days between trials. Participants arrived at the laboratory in the morning (7 – 9 am) following an overnight fast and having abstained from caffeine, alcohol or strenuous exercise for the 24 h period prior to testing. With the participant resting in a semi-supine position a cannula (21G) for blood sampling was inserted into the antecubital vein. The cannula was kept patent throughout the trial by regular flushing with 0.9% NaCl saline solution. Following 30 minutes rest a blood sample was taken. Participants then performed 5 sets of unilateral knee extension exercise, for the required number of repetitions, at 20% 1-RM with 30 seconds rest between each set. In the experimental condition blood flow was partially occluded with an inflatable cuff (SC12D - 13 cm width, Hokanson Inc, Belvue, WA ) which was placed around the most proximal potion of the thigh. The cuff pressure maintained throughout the entire duration of exercise (8-10 mins). Immediately after the 5th set, the pressure cuff was released and the participants began the same routine on the opposite leg. The number of reps was pre-determined from the habituation session described above. The participant then returned to a seated position and further blood samples were taken 30, 60 and 120 minutes post exercise. These times were chosen due to obtain peak values
for the hormones and growth factors chosen (Reeves et al. 2006; Takano et al. 2005)
In the control condition participants performed the identical exercise protocol
(number of sets and reps) as during the occlusion trial, except the cuff was not inflated
and no pressure was applied to the legs. Blood samples were obtained at the same
time points.

**Blood sampling and analysis**
All blood samples were drawn into 2 x 10 ml pre-treated vacutainers containing
K\(^+\)EDTA (BD Biosciences, San Diego, USA). Hematocrit was determined
immediately from whole-blood in triplicate, using the microcapillary technique.
Hemoglobin concentration was measured in duplicate using a commercially available
kit (Randox, Co Antrim, UK). Plasma volume changes were estimated using the
method described by Dill and Costil (1974) and presented data are corrected for any
changes in plasma volume. For plasma, one vacutainer was centrifuged immediately
at 4000 g for 10 min at 4°C and the plasma aliquoted, frozen and stored at −80°C
until analysis.

Plasma IGF-1 and GH concentrations were measured in duplicate by immunoassay
(Quantikine Immunoassay; R & D Systems, Inc., Minneapolis, MN, USA) using
Eschericia coli-expressed recombinant human IGF-1 and GH to generate the standard
curve. The mean intra- and inter-assay coefficient of variation was 3.0 and 8.0% for
IGF-1 and 2.4% and 6.9% for GH, respectively. Plasma IL-6 was determined via
sandwich enzyme-linked immunosorbant assay (ELISA) as previously described by
Leggate et al. (2010). Briefly, plates were coated with anti-human IL-6 monoclonal
capture antibody (OptEIA, BD Biosciences, Oxford, UK) diluted 1:250 in 0.1 M
sodium carbonate. The following day, the plates were washed and blocked with 5%
bovine serum albumin (BSA; Probumin, Millipore, Illinois, USA) in Trisbuffered
saline (TBS). The plates were incubated for 1 h at room temperature. After 1 h, plates
were washed and samples or standards were added to the wells. Samples were diluted
1:5 in TBS with 10% fetal calf serum (FCS). After 2 h, plates were washed and 100
μL IL-6 detection antibody (OptEIA, BD Biosciences, Oxford, UK) diluted 1:250 in
TBS-T with 1% BSA was added per well. Plates were incubated for a further 1 h
before being washed. The enzyme streptavidin alkaline phosphatase was diluted
1:2,000 in TBS with 1% BSA and 100 μL was added per well. Plates were then incubated for 45 min. After washing, an ELISA amplification system was used (Invitrogen, Paisley, UK). The reaction was stopped with 50 μL of 10% sulphuric acid (stop solution), and the absorbance of the wells was read at 490 nm with a correction of 690 nm (Varioskan Flash, Thermo Scientific, Vantaa, Finland). Samples were analyzed in duplicate with an inter-assay coefficient of 7.4%. This assay measures total IL-6 content and does not distinguish between the soluble and receptor-bound IL-6. Plasma VEGF was determined in duplicate via ELISA using a specific anti-VEGF antibody according to the manufactures instructions (Human VEGF-A ELISA; Bendermedsystems GmbH, Vienna, Austria). The mean intra- and inter-assay coefficient of variation was 6.2% and 4.3%, respectively. Plasma cortisol was measured in duplicate via ELISA using a specific anti-cortisol antibody according to the manufacturer’s instructions (DRG Instruments GmbH, Germany). The mean intra – and inter – assay coefficient of variation was 5.6% and 6.6%, respectively.

**Statistical analysis**

Statistical analysis was carried out using SPSS software (SPSS 15). Data were analyzed by a repeated measures two-way (Trial (2) x Time (4)) ANOVA with post hoc Bonferroni tests. Plasma IL-6 and VEGF data were log-transformed prior to the ANOVA as they failed to meet the assumptions of normal distribution. Statistical significance was accepted as $P < 0.05$. Data are presented as mean ± SD.
5.3. RESULTS

The experimental and control trials were matched for the number of repetitions performed which were: 30 ± 10 in the first set, 9 ± 3 in the second set, 6 ± 3 in the third set, 5 ± 2 in the fourth set and 4 ± 2 in the final set. BFR continued for a duration of 564 ± 55 seconds. There were no differences in plasma volume for the two trials. Plasma volume changes were 1.1 ± 5.6 and -0.4 ± 4.4% from pre-exercise to 30 minutes post-exercise, -3.1 ± 3.9 and -2.4 ± 5.3% from post 30 minutes to 60 min post-exercise, and -1.5 ± 2.1 and -0.1 ± 4.1% from post 60 to 120 min post-exercise in LLRT with and without BFR, respectively.

Plasma GH concentration (Fig 5.1) demonstrated a significant time effect (P < 0.001), trial effect (P < 0.01) and time x trial interaction (P < 0.01). Following LLRT with BFR GH increased (P<0.05) from rest to 30 minutes post exercise and was greater (P <0.05) when compared to LLRT without BFR. GH concentration following LLRT with BFR returned to baseline levels after 120 min.

There was no time effect (P >0.05), trial effect (P>0.05) or time x trial interaction (P>0.05) for plasma IGF-1 concentration (Fig 5.2) following LLRT with and without BFR.

Plasma VEGF concentrations (Fig 5.3) demonstrated a significant time effect (P < 0.01), trial effect (P < 0.05) and time x trial interaction (P < 0.01). Following LLRT with BFR VEGF increased (P < 0.05) from rest to 120 mins post exercise. At this time point VEGF was significantly higher than 30 (P<0.05) and 60 mins (P<0.05) post exercise. Plasma VEGF was higher at 60 (P<0.05) and 120 (P<0.05) minutes following LLRT with BFR compared to LLRT without BFR.

Plasma IL-6 concentrations (Fig 5.4) demonstrated a significant time effect (P < 0.01, Fig 5.4) but no trial (P>0.05) or group x trial interaction (P>0.05) following LLRT with and without BFR. Following LLRT with and without BFR IL-6 levels increased from 30 to 60 (P<0.05) minutes and remained elevated at 120 minutes post exercise.
Plasma cortisol concentrations (Fig 5.5) demonstrated no significant time effect (P>0.05). There was a significant trial effect (P <0.05) and time x trial interaction (P<0.05) however Bonferroni corrected post-hoc tests did not locate any differences.
Figure 5.1. Plasma GH concentration following LLRT with and without BFR. * Significantly (P<0.05) different from LLRT without BFR. # Significantly (P<0.05) different from rest.
Figure 5.2. Plasma IGF-1 concentration following LLRT with and without BFR.
Figure 5.3. Plasma VEGF concentration following LLRT with and without BFR. * Significantly (P<0.05) different from LLRT without BFR.
Figure 5.4. Plasma IL-6 concentration following LLRT with and without BFR. # Significantly (P<0.05) different from 30 min.
Figure 5.5. Plasma cortisol concentration following LLRT with and without BFR
5.4. DISCUSSION

The major finding of this current study was that LLRT with BFR resulted in an increase in plasma concentrations of GH and VEGF when compared to LLRT without BFR. Specifically, GH increased 3.3 fold 30 minutes following LLRT with BFR and did not change following LLRT without BFR. There were no changes in plasma IGF-1 concentration. Plasma VEGF values were highest 120 minutes following LLRT with BFR with no changes occurring in the control condition.

There are numerous studies reporting that an acute bout of LLRT with BFR can increase circulating GH in young adults (Takarada et al. 2000a, Pierce et al. 2006; Reeves et al. 2006; Takano et al. 2005). Takarada et al. (2000a) reported a 290 fold increase in GH following 5 sets of bilateral knee extension with BFR at 20% 1-RM. This is much higher than that seen in the work by Pierce et al. (2006) who reported a 9 fold increase in serum GH following knee extensions with BFR with an occlusion pressure of 280mmHg. Both these studies in younger individuals show greater responses than seen in the current study on older men, which represented only a 3.3-fold increase. The differences may be due to the lower secretion of GH in older men following acute exercise, when compared to younger individuals (Kraemer et al. 1998). It is believed that the lower GH response to acute exercise in older individuals may be due to a blunted pituitary response to resistance exercise (Pyka et al. 1994; Kern et al. 1996). Another reason for the different responses may be due to the occlusion pressure applied to the exercising limb. In the current study BFR was maintained using a pressure of 110mmHg compared to 214 and 280 mmHg in the Takarada et al. (2000b) and Pierce et al. (2006) studies respectively. It may be that the greater pressure leads to a greater stimulation of sensory nerves such as type III and IV afferents. It is postulated that the greater pressure may lead to an increased acidic environment within the muscle thus causing a greater GH response. An increased acidic environment as identified by an increase of lactate in the blood may be one cause of GH release as there is a strong correlation between lactate response and GH (Wahl et al. 2010). An increase in metabolites such as adenosine, K+ and H+ are thought to drive the pressor reflex which in turn leads to an increase in both heart rate and blood pressure, all of which may increase GH (Pierce et al. 2006).
GH is secreted from the anterior pituitary gland in a pulsatile manner, however the physiological role of exercise-induced GH is unknown. One proposed mechanism of its main actions is to stimulate the release of insulin like growth factor 1 (IGF-1) from skeletal muscle and the liver (Harridge et al. 2003) which is involved in numerous important functions including having an anabolic effect by activating SC and stimulating protein synthesis (Hawke and Garry, 2001). In the present study plasma IGF-1 did not change in older men following an acute bout of LLRT with BFR. This is in agreement with other studies following acute bouts of LLRT with BFR (Abe et al. 2006; Fujita et al. 2007). However, there is contradictory evidence to suggest that LLRT with BFR may have an influence on circulating IGF-1 as two studies have demonstrated significant increases in circulating IGF-1 following exercise in young people (Abe et al. 2005b; Takano et al. 2005). It is possible that the acute increases in GH seen in the current study may have not had an immediate impact on circulating IGF-1 because the response of IGF-1 to GH pulses is delayed by 3 to 9 hours and peak values of IGF-1 may not be reached until 16-28 hrs post exercise (Kraemer and Ratamess, 2005), implying that the measurement times for IGF-1 may have been taken too early. Regardless, it is clear that systemic factors may not necessarily have a direct role on skeletal muscle hypertrophy and the locally derived factors are of greater importance for muscle adaptation (Adams 2002; Goldspink et al. 2006). Indeed, it has been demonstrated that circulating hormones do not enhance the hypertrophic response to resistance exercise (West et al. 2009). Only one study has specifically investigated the response of local genes, such as mechano growth factor (MGF), to LLRT with BFR and it was found that there was no increase 3 hrs post exercise (Drummond et al. 2008a). Moreover, recent work suggests that LLRT with BFR increases muscle protein synthesis in older men, which is stimulated through the mammalian target of rapamycin (mTOR) pathway (Fry et al. 2010).

The increase in plasma VEGF following LLRT with BFR in older men is in agreement with the findings of others who demonstrated elevated levels of VEGF following similar exercise in young individuals (Takano et al. 2005). Given the crucial role of VEGF in the generation of new capillaries this would support the recent findings of peak vasodilator capacity following 4 weeks of LLRT with BFR in both young and older people (Chapter 3 and 4). Moreover, calf filtration capacity has been shown to increase following 4 weeks of ischaemic exercise training in young
men (Evans et al. 2010). Calf filtration capacity has a strong correlation with the length of contact between capillaries and muscle fibres (Charles et al. 2006) and thus has been used as an indirect measure of capillarisation (Brown et al. 2001; Gamble et al. 2000). An increased filtration capacity may allow for an increase in microvascular exchange surface area that may be attributable not only to greater anatomical surface area but also to enhanced perfusion of individual exchange vessels (Brown et al. 2001) which would offset the decline in blood flow observed with ageing (Dinenno et al. 2001; Proctor and Parker 2006).

To our knowledge, this is the first study to show an elevation in IL-6 in older people following LLRT with and without BFR. Previous research by Takarada et al. (2000a) found an increase in IL-6 in young men, following LLRT with BFR but did not see an increase in LLRT alone. The mechanisms behind the increase following LLRT without BFR are unclear however exercise at low workloads (knee extensor exercise at 40% peak power output), albeit for long duration (5 hours), have been shown to increase IL-6 (Steensberg et al. 2000). Evidence from this thesis suggests that LLRT at 25% 1-RM can slightly improve strength levels of both young and older people (Chapter 3; Chapter 4), therefore the mechanical stress placed upon the muscle is likely to induce a small inflammatory response. IL-6 has both a pro- and anti-inflammatory role (Pedersen, 2007). Long term IL-6 exposure can lead to catabolic effects on skeletal muscle (Haddad et al. 2005) however acute effects can lead to promotion of angiogenesis (Cohen et al. 1996), and satellite cell proliferation (Cantini et al. 1995). It may also interact with the IGF-1 axis through the suppressors of cytokine signallng (SOCS) family. Increased levels of IL-6 can increase levels of SOCS3 mRNA which in turn provides feedback on IGF-1 signalling, enacting a negative feedback function, resulting in a down-regulation of mTOR (Bodell et al. 2009). Following LLRT with BFR mTOR is up-regulated and IL-6 mRNA has been found not to increase (Fry et al. 2010) therefore suggesting that the acute increase in plasma IL-6 is not catabolic.

Evidence also suggests IL-6 may play a role in enhancing plasma cortisol levels in humans (Steensberg et al. 2003). In the current study no change in cortisol levels were seen between exercise groups, despite a slight increase in IL-6. This is in contrast to previous work showing elevations in cortisol in older men following LLRT with BFR.
(Fry et al. 2010). As with GH response to LLRT with BFR, a lack of stress response in the current study compared to the work by Fry and colleagues (2010) may be related to partial versus full occlusion pressures used in both studies.

In conclusion, a single bout of LLRT with BFR significantly stimulates the exercise induced GH and VEGF responses to exercise in older men. No increase was seen in plasma IGF-1 following LLRT with BFR, however IL-6 was significantly elevated post exercise irrespective of the trial. It is possible that the acute responses observed in the current study may play a role in the hypertrophy and vascular adaptations seen following longer term LLRT with BFR.
Chapter 6

IGF-1 gene response to LLRT with and without BFR in young and older people.
6.1. Introduction

LLRT with BFR has become a novel way to increase muscle size and strength in young people (Takarada et al. 2000b, 2002; Burgomaster et al. 2003; Chapter 3; Abe et al. 2006). As demonstrated in chapter 4 of this thesis it is also effective in increasing strength in older people. Increased CSA is one of the main mechanisms for increased strength following resistance training but as of yet the mechanisms behind the change in the size of skeletal muscle using this technique are not fully understood.

Local growth factors have been hypothesised to play a role in the acute and chronic response to resistance training. One such important growth factor has been IGF-1, which is a peptide hormone that plays a role in development, muscle mass maintenance, adaptation and repair (Hameed et al. 2004). Two isoforms of IGF-1 have been identified and shown to be expressed in human muscle. IGF-1Ea, which is similar to the hepatic endocrine type of IGF-1, and IGF-1Ec, which is mechanosensitive and is therefore termed mechano-growth factor (MGF) (Hameed et al. 2003). Increases in IGF-1Ea and MGF mRNA have been observed following acute bouts and longer term high-load resistance training (Bamman et al. 2001; Hameed et al. 2004; Greig et al. 2006; Petrella et al. 2006), with a significant relationship identified between resting levels of MGF mRNA and whole muscle CSA as measured by MRI (Hameed et al. 2004).

Although it is well established that skeletal muscle in older people is responsive to resistance training by increasing strength and size (Harridge et al. 1999) it has been shown that older muscle responds differently to that of younger muscle with respect to IGF-1 isoform expression. For example, MGF expression is markedly increased in young but not older men following acute heavy resistance training (Hameed et al. 2003). In older men, acute MGF mRNA expression is restored following prolonged periods of resistance training (Hameed et al. 2004). Along with the inability to up-regulate MGF older individuals have lower levels of circulating GH, with levels of approximately one third of those values seen in teenagers (Goldspink 2005). However, recent evidence suggests when resistance exercise is performed along with GH therapy MGF is markedly increased whereas GH therapy alone had no effect (Hameed et al. 2004). More recently, upregulation of MGF has been shown to be
greatest in responders to a 16 week resistance exercise protocol in terms of myofiber hypertrophy, increase in satellite cell number and myonuclear addition (Bamman et al. 2007; Petrella et al. 2008). LLRT with BFR has been shown to increase GH levels up to 290 times resting levels (Takarada et al. 2000a). Research on the effects of supraphysiologic dosing of GH with traditional resistance training in humans is limited. While this research has not indicated increased hypertrophy, it does appear to indicate that GH administration elevates both the liver isoform IGF-1Ea in muscle as well as MGF (Hameed et al. 2004). Indeed in chapter 4 we found that older individuals responded to LLRT with BFR with increased strength levels and again in Chapter 5 that older men significantly increased GH levels following acute LLRT with BFR, and thus may play a role in increasing both IGF-1 isoforms following LLRT with BFR.

The effect of LLRT with BFR on local gene expression is limited. A recent study in rats found that hypertrophy following blood flow occlusion and resistance training, was not due to an increase in local muscle IGF-1 expression, when myostatin was down-regulated (Kawada and Ishii 2005). However this study involved chronic occlusion (14 days) whereas human studies use intermittent occlusion (5-10 mins) and thus did not represent the true nature of LLRT with BFR. Only two studies have investigated the effects of LLRT with BFR on acute muscle gene expression and both were conducted in younger individuals only (Drummond et al. 2008a; Manini et al. 2011). Drummond et al. 2008a found an increase in mRNA expression of hypoxia-inducible factor-1 alpha (HIF -1α), p21, MyoD, and muscle RING finger 1 (MuRF1) as well as a decrease in REDD1 and myostatin in both groups, 3 hours post exercise following LLRT with BFR and LLRT alone. This study also measured MGF and insulin-like growth factor 1 receptor (IGF-1R), however no changes were seen in either group. Manini et al. (2011) performed a similar study but investigated the response of myogenic and proteolytic gene expression 8 hours post LLRT with BFR. They found no change in myogenic genes such as IGF-1 and myostatin, but did see a down regulation in proteolytic gene expression (FOXO3A, atrogin-1 AND Murf-1). With the large increase of GH seen in young individuals following LLRT with BFR (Takarada et al. 2000a), it is likely that up-regulation of the IGF-1 gene would occur following LLRT with BFR. The previous studies (Drummond et al. 2008a; Manini et al. 2011) have taken muscle biopsies samples early (3-8 hours post) in the response to
acute LLRT with BFR, however others, using heavy resistance training have demonstrated that MGF and IGF-1Ea mRNA actually increases between 3 and 72 hours post exercise (Hameed et al. 2003; Greig et al. 2006; Kim et al. 2005a; 2005b). Alongside this, both IGF-1Ea and MGF have not been measured together in the same study to demonstrate if they respond differently to LLRT with BFR and no studies have been conducted in older individuals or females.

The aim of this study, therefore, was to investigate the effect of resistance training with blood flow occlusion on IGF-1 gene expression before and 24 hours following LLRT with BFR in both young and older individuals. It was hypothesised that IGF-1 isoforms associated with muscle growth will be up-regulated following a bout of LLRT with BFR and to a greater extent than LLRT alone.
6.2. Methods

Participants
14 healthy females volunteered to participate in the study. 7 young (age 21.7 ± 3.1 yr, height 166.6 ± 8.1 cm, mass 66.1 ± 10.4 kg; means ± SD) and 7 older participants (age 70.1 ± 4.6 yr, height 163.6 ± 2.5 cm, mass 70.5 ± 6.6 kg; mean ± SD) who were selected according to the criteria of Greig et al. (1994) for “healthy” older participants. All participants were habitually physically active but none had any previous experience of resistance exercise. The participants were fully informed of the purposes, risks and discomfort associated with the experiment before providing written, informed consent. This study conformed to current local guidelines and the Declaration of Helsinki and was approved by Loughborough University Ethics Advisory Committee.

Overview of experimental procedures
Participants initially performed a familiarisation trial (described below) trial, before the experimental protocol in order to become accustomed to the strength testing procedures and training devices. All measurements and training procedures were conducted on the quadriceps muscle group. Participants were instructed in proper use of the resistance exercise equipment and performed the exact experimental protocol consisting of knee extension contractions using 20% 1-RM.

The experimental protocol consisted of (i) baseline measurement of unilateral knee extension dynamic muscle strength of both limbs; (ii) a pre exercise muscle biopsy from the vastus lateralis of the trained limb, followed by 5 sets of LLRT knee extensions at 20% 1-RM with or without BFR; (iii) a post 24hr muscle biopsy from the vastus lateralis of the exercised limb, and; (iv) seven days post 1st experimental protocol, this was repeated on the opposite limb with the other exercise condition. All pre- and post-testing procedures were performed on both limbs. Participants had their limbs assigned to be trained with BFR in a counterbalanced manner with 7 participants exercising their dominant limb with BFR and 7 exercising their non-dominant limb with BFR.
**Familiarisation & 1-RM Testing**

Maximum dynamic strength (1-RM) of the knee extensor muscles was assessed on a knee extension machine (Ortus Fitness, Valencia, Spain) at least 7 days prior to conducting the study. Participants began with a warm up consisting of two sets of 10 repetition at 5 kg, each separated by 2 mins. Following this, the load was set at approximately 80% of the predicted 1-RM. Following each successful lift the load was increased by ~5% until the participant failed to lift the load through the entire range of motion. A test was only considered valid when the participant used proper form and completed the entire lift in a controlled, unassisted manner. Approximately 2-3 mins of rest was allowed between each attempt to ensure recovery. Participants were then fully familiarised to the nature of LLRT with BFR which consisted of performing a resistance exercise test of 5 sets of knee extension to failure at 20% 1-RM with blood flow partially occluded (110mmHg), with 30 sec rest between each set. This familiarisation trial also provided an indication of the number of repetitions performed in the blood flow restricted condition. The number of repetitions would be replicated in the non-restricted condition and in the main experimental protocol which ultimately allowed the work done during the experimental and control trials to be matched.

**Experimental Protocol**

Participants attended the laboratory in the morning (7-9am) after an overnight fast and having refrained from exercise/physical activity for 48hr. Participants lay in the supine position for 20 min before a resting muscle biopsy was obtained. Resistance training was then performed under either control (no BFR) conditions or with BFR. This consisted of performing 5 sets of unilateral knee extension to fatigue at 20% 1-RM with 30 seconds rest between each set. In the BFR condition blood flow was partially restricted with an inflatable cuff (SC12D - 13 cm width, Hokanson Inc, Belvue, WA ) placed around the most proximal potion of the thigh and inflated to 110 mmHg. The cuff pressure was maintained throughout the entire duration of exercise (4-6 mins). Immediately after the 5th set, the pressure cuff was released. The number of repetitions performed was pre-determined from the familiarisation session. In the control condition participants performed the identical exercise protocol (number of sets and reps) as during the occlusion trial, except the cuff was not inflated and no
pressure was applied to the legs. Participants returned to the laboratory 24hrs after exercise and a further muscle biopsy was obtained, 2 cm proximal to the previous biopsy. The control and occluded conditions were repeated for the opposite limb, performed in a randomised order and separated by at least 7 days.

**Muscle sampling and analysis**

Muscle biopsies were obtained from the vastus lateralis immediately prior to the 1st exercise session and 24 hours post exercise. Under local anesthesia (1% lidocaine) samples (~ 20 mg wet wt) were obtained using the needle micro-biopsy technique (Pro-Mag 2.2, Medical Products, IL, USA). Samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

**mRNA analysis**

Muscle samples were analysed for MGF and IGF-1Ea according to the procedures described by Hameed et al. (2003).

**Total RNA isolation**

Frozen muscle samples were weighed. Samples were then homogenised in 0.5 mL Tri Reagent (1 mL/ 50-100 mg tissue) using a hand held homogeniser for 20-50 seconds. Total RNA was extracted by centrifuging at 12,000 x g for 10 mins at 4°C . The supernatant, which contained RNA and protein, was then transferred to a fresh tube. To ensure complete dissociation of nucleoprotein complexes the samples were left to stand for 5 mins at room temperature. 100 µL of chloroform was added to the tube (0.2 mL/ 1 mL Tri Reagent). The samples were then shaken vigorously for 15 seconds allowed to stand for 2-15 mins at room temperature. The resulting mixture was centrifuged at 12,000 x g for 15 min at 4°C. After centrifugation the mixture separates into 3 phases: a red organic phase (containing protein), an interphase (containing cDNA) and a colourless aqueous phase (containing RNA). The aqueous phase was transferred into a fresh tube and mixed with 250 mL of isoproponol. The sample was allowed to stand at room temperature for 5-10 minutes, after which it was centrifuged at 12,000 x g for 10 minutes at 4°C. The RNA precipitate formed a pellet on the side and the bottom of the tube. The extracted RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water and stored at -70°C until required. Total RNA was then treated with 10 units of RQ1 RNase-free DNase (Promega, Madison, WI,USA) at 37°C for
30 minutes followed by incubation at 65°C for 10 minutes in order to avoid amplification of contaminating genomic DNA. The quality of RNA was assessed by visual inspection of ethidium bromide-stained 18s and 28s r RNA under ultraviolet light.

First strand cDNA synthesis
Following RNA extraction total RNA was then reverse transcribed using Omniscript reverse transcriptase (Qiagen, Crawley, UK). 0.5 µg of total RNA was mixed with DEPC treated water in a total volume of 10 µL and heated to 65°C for 5 minutes before transfer to ice. The samples were then mixed with 2 µL first strand buffer (10x), 2 µL dNTP s (5 mM each), 15 pmol of sequence specific primers (MGF, IGF-1Ea), 1 µL RNase inhibitor (10 units/µl) and 1 µL of Omniscript Reverse transcriptase (4 units/µL). The reaction volume was made up to 20 µL using DEPC treated water. The samples were then incubated at 37°C for 1 hour followed by 5 min at 93°C to inactivate reverse transcriptase.

Real-time quantitative PCR
Quantification of mRNA was performed using Light Cycle technology (Roche Diagnostics, UK). Reactions were performed in a volume of 20 µL per capillary for the lightCycler format. The 20 µL reaction mix contained 10 µL of SYBR green master mix (QuantiTect, Qiagen), 5-10 pmol of each forward and reverse PCR primers (Table 6.1), 2 µL cDNA (made from 0.5 µg RNA) and nuclease free water to make up the final reaction volume. SYBR green I was used as the method of detection.
### Table 6.1. DNA oligonucleotide primers used for real time PCR

<table>
<thead>
<tr>
<th>Primer Description</th>
<th>Concentration (pmol/reaction)</th>
<th>Sequence (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1Ea forward</td>
<td>10</td>
<td>GCCTGCTCACCTTCACCAGC</td>
</tr>
<tr>
<td>IGF-1Ea reverse</td>
<td>10</td>
<td>TCAAATGTACTTCTTCTGGGTCTTG</td>
</tr>
<tr>
<td>MGF forward</td>
<td>10</td>
<td>CGAAGTCTCAGAGAAGGAAAGG</td>
</tr>
<tr>
<td>MGF reverse</td>
<td>10</td>
<td>ACAGGTAACTCGTGAGAGC</td>
</tr>
</tbody>
</table>

Serial dilutions of standard DNA (which contained the target sequence of interest) of ‘known’ concentration were included in each run from which a standard curve was created as previously described (Hameed et al. 2003; Fig 6.1). It was relative to this standard curve that samples of unknown concentration were quantified. On completion of PCR, all PCR products formed were melted to attain a melting curve profile, which enabled the specificity of the reaction to be determined (Fig 6.2). Target specificity was further confirmed by running samples on an agarose gel. All target sequences were initially sequenced to ensure specificity of the primers. Runs were performed in duplicate and mean values were subsequently used for analysis. Blinding the identities of the samples during the runs ensured unbiased real time PCR analysis.
**Figure 6.1.** Example of standard curve for MGF mRNA.
Figure 6.2. Example of melting curve.

*Gel Electrophoresis*

PCR products were recovered from the glass capillaries by removing the plastic caps and inverting them into a 1.5 ml epindorf tube, which were then spun down in a bench top centrifuge at no higher than 3000 rpm. 20 µL of these products were mixed with 2 µL of 6 x loading buffer (10.78 g Tris, 5.5 g Boric acid and 0.744 g EDTA made up to 1 L with distilled water) (Fermentas, UK). The 2 % agarose gel was prepared by adding 2 g agarose (Sigma-Aldrich, Dorset, UK) to 100 mL tris/acetate buffer (TAE) and heated in the microwave for approximately 2 minutes to dissolve the agarose. After cooling slightly, ethidium bromide (10 mg/mL) was added to set. The gel was
left at room temperature to solidify before the combs were removed. The PCR products and loading buffer were then fully loaded into the wells as was a size marker (DNA ladder, Fermentas, UK). The gel was then left to run on the gel at 120 V for approximately 45 minutes until good separation of the dye fronts had been achieved. Target specificity was further confirmed by visualising the product bands under ultraviolet (UV) light (Fig 6.3).

![Image of agarose gel]

**Figure 6.3.** PCR products for each target gene were verified by agarose gel electrophoresis (expected PCR product lengths of each PCR product are stated)

**Statistics**

All values are expressed as means ± SD. All data was checked for normal distribution. In the cases of MGF mRNA and IGF-1Ea mRNA expressions they were not normally distributed therefore were Log transformed. Comparisons were performed on the mRNA expressions using a 2 x 2 x 2 mixed measures ANOVA with condition (restricted vs. normal) and time (Pre vs. post) within subjects, and age (young vs. old) between subjects. Fold changes were analysed using a 2 x 2 mixed measures ANOVA with condition (restricted vs. normal) within subjects and age (old vs. young) between subjects.
6.3. Results

1-RM was higher (P<0.05) in the young (52.1 ± 8.1 kg), compared to the older participants (25.7 ± 1.9 kg). The number or repetitions performed by the young and older participants, respectively, during each set was: 1st set, 36 ± 8 vs. 41 ± 15; 2nd set, 8 ± 3 vs. 17 ± 4; 3rd set, 8 ± 3 vs. 11 ± 8; 4th set, 7 ± 6 vs. 7 ± 5; 5th set, 7 ± 7 vs. 7 ± 5.

At rest, MGF mRNA content was higher (P < 0.05) in the young (4.1 x 10⁻⁸ ± 1.6 x 10⁻⁸ ng) compared to the older participants (1.5 x 10⁻⁸ ± 9.3 x 10⁻⁹ ng). There was no time (P > 0.05), or condition effect (P > 0.05) for MGF mRNA expression. There was also no time x age (P > 0.05), condition x age (P > 0.05) or time x condition x age interaction (P > 0.05) (Fig 6.4). However, when both age groups were combined there was a time x condition interaction (P < 0.05) indicating a greater increase in MGF mRNA expression following resistance training with BFR, (Fig 6.5).

At rest no differences were observed for levels of IGF1Ea mRNA between the young (1.4 x 10⁻⁷ ± 6.3 x 10⁻⁸ ng) and older (1.9 x 10⁻⁷ ± 1.2 x 10⁻⁸ ng) individuals. There was no time (P > 0.05), or condition effect (P > 0.05) for IGF-1Ea mRNA expression. Neither was there a time x condition x age interaction (P > 0.05) (Fig 6.6), time x age (P > 0.05), condition x age (P > 0.05) or a time x condition (P > 0.05) (Fig 6.7).
Figure 6.4. MGF mRNA expression before and after (24 hours) LLRT with and without BFR in young and older females. Results represent a fold change and are means ± SD.
Figure 6.5. MGF mRNA expression before and after (24 hours) LLRT with and without BFR. Data for young and old age groups are combined. Results represent a fold change and are means ± SD. * Significant condition x time interaction (P< 0.05)
Figure 6.6. IGF-1Ea mRNA expression before and after (24 hours) LLRT with and without BFR in young and older females. Results represent a fold change and are means ± SD.
Figure 6.7. IGF1-Ea mRNA expression before and after (24 hours) LLRT with and without BFR. Data for young and old age groups are combined. Results represent a fold change and are means ± SD.
6.4. Discussion

The main finding of this study was that MGF mRNA was significantly increased 24 hours following LLRT with BFR, when both groups were combined. There was no effect of LLRT with or without BFR on IGF-1Ea mRNA expression in either age group.

Both MGF and IGF-1Ea mRNA were expressed in skeletal muscle tissue, however MGF levels were lower than that of IGF-1Ea. Baseline IGF-1Ea mRNA expression were of a similar order of magnitude (1 x 10^-7 ng) to those seen in healthy young males (Aperghis et al. 2009). However, although identical protocols for RNA extraction and qRT-PCR were used (Hameed et al. 2003) a lower baseline expression of IGF-1Ea mRNA was observed in both younger and older men. Baseline levels of the MGF mRNA expression were the same magnitude previously reported in young and older men (Hameed et al. 2003) but higher than that seen in middle aged women (Greig et al. 2006). Resting levels of MGF mRNA were lower in older women than their younger counterparts in the present study. Others have reported lower levels of IGF-1 mRNA at rest but do not specify which splice variant so therefore may be both IGF-1Ea and MGF (Leger et al. 2008; Welle et al. 2002).

The most interesting finding of the current study was the fact that MGF mRNA increased following acute LLRT with BFR. Previous evidence suggests that both IGF-1Ea and MGF mRNA increase following high-intensity resistance exercise in young individuals (Bamman et al. 2001; Greig et al. 2006; Hameed et al. 2003; Kim et al. 2005a; 2005b; Petrella et al. 2006). Older individuals respond to acute heavy resistance training by increasing IGF-1Ea mRNA but some evidence suggests they cannot up-regulate MGF mRNA (Hameed et al. 2003; Kim et al. 2005b; Petrella et al. 2006). This observation is similar to studies in animals that show MGF mRNA up-regulation may decline with age (Owino et al. 2001). This inability to up-regulate MGF mRNA is reversed following a period of heavy resistance training (Hameed et al. 2004). However there is some evidence to suggest that acute heavy resistance training will up-regulate MGF mRNA in the older population. Roberts et al. (2010) recently demonstrated increased levels 48 hours post heavy resistance exercise. Indeed, other forms of exercise, such as eccentric cycling, have also demonstrated an
increased up-regulation of MGF mRNA in old people (Hameed et al. 2008). Roberts et al. (2010) suggested that MGF mRNA up-regulation may be delayed following heavy resistance training and that the timing of biopsy samples may explain the differences between the Hammed et al. (2003) study and their own. Of course, the work by Hameed et al. (2008) demonstrated that MGF mRNA can be up-regulated early but this depends on the mode of exercise. They found that MGF mRNA was up-regulated following eccentric cycling, indicating that MGF is involved in the repair process following damage exercise. Previously research has indicated that acute LLRT with BFR does not increase MGF mRNA 3 hours post exercise in young individuals (Drummond et al. 2008a). Interestingly a new case study, involving a 65 year old man has forwarded the idea that 12 weeks of LLRT with BFR can counteract the muscle atrophy associated with the muscle wasting disease inclusion body myositis, specifically by increasing the expression of MGF mRNA by nearly 4 fold (Gulano et al. 2010).

Reports suggest that both IGF-1Ea and MGF mRNA respond differently to a resistance exercise stimulus, with IGF-1Ea being similar to the isoform expressed from the liver and MGF expressed in a mechanosensitive manner (Hameed et al. 2003). In the current study IGF-1Ea mRNA was not up-regulated following LRT with BFR at the 24 hour biopsy time point. Recently evidence suggests that IGF1 mRNA was unchanged at 8 hours post LLRT with BFR in young men and women (Manini et al. (2011). The gene measured in this study is likely to include both MGF and IGF-1Ea as no specific isoform was determined. There is conflicting evidence on the response to IGF-1Ea to heavy resistance training. Some research suggests IGF-1Ea mRNA decreases early (1-6 hours) in response to acute resistance exercise (Psilander et al. 2003), others have found no change (2.5 hours) (Greig et al. 2006; Hameed et al. 2003), whilst some even suggest an increase (2 hours) (Wilborn et al. 2009). Biopsy samples taken at later time points have been a little more conclusive with most pointing towards an increase in IGF-1Ea mRNA at 50 hours (Greig et al. 2006) and 72 hours post acute resistance exercise (McKay et al. 2008), however recent evidence has shown no increase at 48 hours post exercise in both young and older individuals (Roberts et al. 2010). The different response within many of the studies for both of the IGF-1 isoform opens up the suggestion that there may be responders versus non-responders, however the studies in question all have a small
sample size. This is evident from the participants in the current study who have responses ranging from a decrease of 49% to an increase of 190% for MGF mRNA expression 24 hours post LLRT with BFR. These responses are similar to the average 204% increase in MGF mRNA seen 2.5 hours post isometric resistance exercise (Greig et al. 2006), but much lower than the reported 2–864% increase 2.5 hours following heavy resistance exercise (Hameed et al. 2003), which suggests a high degree of biological variability between individuals. Indeed, it has recently been confirmed that individuals reported as non-responders are able to up-regulate IGF-1Ea, but not MGF mRNA following acute heavy resistance exercise when compared to extreme and moderate responders (Bamman et al. 2007). The research from this study and Roberts et al. (2010) indicates that an inability to up-regulate MGF following resistance exercise may be the result of a responders and non-responders theory, rather than age per se.

In conclusion, this study demonstrated that LLRT with BFR increases MGF mRNA expression 24 hours following LLRT with BFR. At the same time point there was no significant increase in the levels of IGF-1Ea mRNA, indicating the splice variants of IGF-1 respond differently to LLRT with BFR. Importantly, there was no effect of age in the current findings indicating that MGF mRNA is up-regulated in response to LLRT with BFR.
Chapter 7

General Discussion
The main aim of this thesis was to investigate the effect of LLRT with BFR on muscle strength and blood flow in young and older individuals. A secondary aim was to elucidate some of the mechanisms that may be responsible for any changes observed. Both knee extensor and plantar-flexor exercise resistance models were used in acute and short-term training studies.

The first study (Chapter 3) demonstrated that LLRT with BFR increased isometric and isokinetic plantar-flexor strength in young people by a greater extent than LLRT alone. The novel finding was that calf PO_{bf} increased following 4 weeks LLRT with BFR, indicating an adaptation to the peripheral vasculature. In order to demonstrate the most appropriate load used during future studies the effect of different loads (25% and 50% 1-RM) were assessed. As no differences were observed between 25 or 50% 1-RM, indicating that 25% 1-RM was equally effective of inducing adaptations, all further studies were carried out in the 20-25% 1-RM range.

Chapter 4 followed a similar design to Chapter 3 but with the intention of investigating the effects of LLRT with BFR on an older population. As with the earlier study in the young, isometric and isokinetic plantar-flexor strength as well as calf PO_{bf} increased to a greater extent following 4 weeks LLRT with BFR training at 25% 1-RM than LLRT alone. As well as demonstrating the beneficial effects of LLRT with BFR on strength and blood flow, the study also highlighted the fact that this training mode is a novel and safe method of training in the health older population.

Chapters 5 and 6 investigated the acute effects of LLRT with BFR, in order to gain an understanding of the mechanisms of adaptation to this type of training in older people. Chapter 5 demonstrated, using knee extension resistance exercise, that LLRT with BFR acutely increased both circulating GH and VEGF in older people. Further evidence of the importance of the local growth factors was demonstrated in Chapter 6 where an increase in the expression of MGF mRNA in skeletal muscle was observed following an acute bout of LLRT with BFR when both young and older individual groups were combined.
Taken together these studies have highlighted novel adaptations and a further understanding of the mechanisms of adaptations that may improve our understanding of the benefit of LLRT with BFR.

**Effects of LLRT with BFR on muscle strength**

In chapter 3 it was demonstrated that plantar-flexor 1 RM, isometric strength and isokinetic torque were increased by 13 – 30% following 4 weeks of LLRT with BFR in young women. These improvements in strength were of a similar magnitude to those seen in previous training studies lasting between 2 and 16 weeks (Abe et al. 2006; Burgomaster et al. 2003; Moore et al. 2004; Shinohara et al. 1998; Takarada et al. 2000b; 2002; 2004; Yasuda et al. 2005). Takarada et al. (2002) demonstrated a 12% increase in isokinetic torque following 8 weeks of LLRT with BFR at 50% 1-RM on the knee extensors. Similarly the same authors found that 16 weeks of twice weekly LLRT with BFR at 30-50% 1-RM of the biceps increased isokinetic torque by 18%, as well as an 18-20% increase in muscle CSA (Takarada et al. 2000b). Shorter training studies lasting 2 weeks, with twice daily sessions have also found an increase in strength (14%) and CSA (8%) following LLRT with BFR (Yasuda et al. 2005). The novel aspect of this thesis is that Chapter 4 followed on from Chapter 3 and demonstrated plantar-flexor 1 RM, isometric strength and isokinetic torque were increased by 14 - 21%, using a similar exercise protocol, in older people. At the time of carrying out this research no other group had investigated this response. However, others have now also demonstrated an increased strength following LLRT with BFR in older men (Karabulut et al. 2010). They found a 19% increase in leg press and knee extensor strength following 6 weeks of LLRT with BFR, 3 times per week, which is very similar to the adaptations seen in Chapter 4.

The decline in strength associated with ageing (Frontera et al. 1991; 2000) was not seen between both groups of individuals in chapter 3 and 4 for any of the strength measurements (P > 0.05). This may have been due to the mixed sex subject group used in Chapter 4 compared to the young females used in Chapter 3. When compared across same sex groups Chapter 5 demonstrated a difference in strength between young and old females., with a higher 1-RM demonstrated in the young (52.1 ± 8.1 kg), compared to the older participants (25.7 ± 1.9 kg). Interestingly although both
Chapters 3 and 4 found significant increases in different forms of strength measurements (e.g. 1-RM, isometric strength, isokinetic torque at 0.52 rad.s\(^{-1}\) (30°.sec\(^{-1}\))), only the young were able to demonstrate an increase in isokinetic strength at 1.05 rad.s\(^{-1}\) with neither seeing an increase at 2.09 rad.s\(^{-1}\). The inability of both groups to increase their strength at 2.09 rad.s\(^{-1}\) may be solely due to the speed of movement carried out during the training protocol being somewhat slower than the testing procedure.

The exact mechanisms behind the strength adaptations seen following LLRT with BFR, although not measured, may be speculated. Although the focus of the studies in this thesis is on peripheral adaptations such as hypertrophy, it is possible that neural adaptations may have played an important role in the increases in strength seen in Chapters 3 and 4. LLRT with BFR is has been shown to increase the recruitment of fast twitch muscle fibres (Takarada et al. 2000b; Moore et al. 2004; Krstrup et al. 2009). Relatively few studies have investigated the neural adaptations of LLRT with BFR. Following heavy resistance training one adaptation may be an increased voluntary activation level of the muscle, however two groups have failed to show the same adaptation occurring in young individuals following LLRT with BFR (Moore et al. 2004; Kubo et al. 2006). In both studies the activation levels measured were already at a high level (>90%), therefore further changes would seem unlikely, however Kubo et al. (2006) compared LLRT with BFR and heavy resistance training and found that although activation levels were greater than 90%, heavy resistance training increased voluntary activation levels, whilst LLRT with BFR did not. It is possible that the greater increase in strength LLRT with BFR was due to an increased level of activation in Chapter 4, as reduced voluntary activation of the plantar-flexors of older men has been reported when compared to younger counterparts (Kubo et al. 2007), but this still does not account for the strength improvements in Chapter 3. Therefore it is possible that other factors may play an important role, such as coactivation levels. A recent cross sectional study indicates that there does not appear to be any differences observed between young and older individuals during contractions of the plantar-flexors (Klass et al. 2005). This combined with the evidence that coactivation levels do not change following LLRT with BFR in young men (Kubo et al. 2006) imply that this may not be the reason for any improvements seen in Chapters 3 and 4. Of course nothing can be ruled either in or out in the current
investigations as they were not measured, however considerable evidence suggests that some neural adaptations do take place.

In Chapter 4 there was a slight increase in CCG indicating an increase in muscle hypertrophy, however this increase was similar between LLRT with and without restriction. A limitation of Chapter 3 and 4 is that a true measurement of skeletal muscle hypertrophy was not carried out, however based on research by others it is likely some form of hypertrophy did occur in as little as 4 weeks. A case study on one individual reported that 1 week of LLRT with BFR at 20% 1-RM resulted in a 3% increase in CSA as measured by MRI (Abe et al. 2005a). Similar short term training studies lasting 2-3 weeks report increases in CSA (8.5%) as measured by MRI (Abe et al. 2005b) and type I (6%) and II (28%) muscle fibre CSA (Yasuda et al. 2005). Kubo et al. (2006) recently demonstrated that LLRT with BFR resulted in an increase in the pennation angle (14.8→ 15.6%) of the vastus lateralis, which has been shown to occur in as little as 20 days (Seynnes et al. 2007). This increase in pennation angle may lead to an increased physiological CSA and therefore a greater force generating capacity (Aagaard et al. 2001)

As discussed above it has been widely demonstrated that skeletal muscle hypertrophy occurs following LLRT with BFR (Abe et al. 2005a;b; Takarada et al. 2000b; 2002; Yasuda et al. 2005), but less is known about the actual causes of this increase. One suggestion for the adaptations seen following LLRT with BFR relates to the idea that an increase in anabolic hormones such as GH may be necessary for muscle growth (Hakkinen et al. 1988), however evidence for this is very limited (West et al. 2009; 2010). This is an interesting hypothesis, indeed LLRT with BFR has been shown to increase GH up to 290 times pre exercise levels following an acute bout of exercise (Takarada et al. 2000a). Takano et al. (2005) demonstrated LLRT with partial BFR resulted in an increased GH concentration, 100 times resting levels, whilst Reeves et al. (2006) demonstrated an increase of 41 times resting levels and Pierce et al. (2006) found a 9 fold increase in GH. Although not as pronounced we also found an increased GH response (3.3. fold) to LLRT with BFR (chapter 5) in older men as have Fry et al. (2010). The differences may be due to the lower secretion of GH in older men following acute exercise or the differences in active muscle mass between older and younger individuals (Hakkinen et al. 1998b; Kraemer et al. 1998). The specific
reason for a GH increase following LLRT with BFR is still unknown but may be due to the an increased stimulation of peripheral afferent nerves (Glustina and Veldhuis 1998; Godfrey et al. 2003), specifically afferents in fast twitch muscle fibres (Gosselink et al. 1998) which are preferentially recruited during LLRT with BFR (Yasuda et al. 2005).

One of the main actions of GH is to stimulate the release of IGF-1 from the liver and IGF-1Ea and MGF mRNA from skeletal muscle (Harridge et al. 2003). Circulating IGF-1 is released directly from the liver, with acute responses to heavy resistance exercise being equivocal (Brahm et al. 1997; Kraemer et al. 1990; 1992; 1995). Like the heavy resistance exercise model, LLRT with BFR has produced different circulating IGF-1 responses. Takano et al. (2005) demonstrated circulating IGF-1 was acutely elevated immediately, 10 and 30 minutes post LLRT with BFR in young men. Using a similar exercise model it has been demonstrated that this is not always the case, with Fujita and colleagues (2007) not finding any increase in circulating IGF-1 in the first 40 minutes post exercise. In Chapter 5 it was demonstrated that circulating IGF-1 was not increased following LLRT with BFR immediately, 30, 60 or 120 minutes post exercise in older men. This is the first study to investigate the response of circulating IGF-1 to acute LLRT with BFR in older people. The ageing process results in decreased GH and IGF-1 (Rudman et al. 1981; Corpas et al. 1993; Lamberts et al. 1997). It was believed that the increase in GH seen following LLRT may play a role in increasing circulating IGF-1 in an older population, however this was not the case in Chapter 5. Although demonstrating an increase in circulating IGF-1, the GH time course responses were quite different (Takano et al. 2005). It has been postulated that acute increases in GH may not cause an immediate increase in IGF-1, as peak values may not be reached until 16-28 hours post GH release (Kraemer and Ratamess 2005) and thus the timing of the circulating IGF-1 sample in Chapter 5 may have been too early to see peak values. However due to the early increase of IGF-1 seen in young individuals following LLRT with BFR BY Takano et al. (2005), it was hypothesised that a similar early rise would e demonstrated inn older individuals also. There is some evidence to suggest that circulating IGF-1 increases following 2 weeks of LLRT with BFR, this is seen alongside an 8.5% increase in CSA (Abe et al. 2005b). What does this increase, specifically GH, mean for hypertrophy following resistance training? Clearly GH does increase following this type of exercise but no
evidence is available to suggest that this increase in GH actually causes an increase in protein synthesis, with recent evidence providing proof that anabolic hormones are not essential for muscle growth (West et al. 2009).

This therefore suggests that the adaptations may take place at a local level within the specific muscle trained. In Chapter 6 we examined the effect of LLRT with BFR at the local level, specifically at splice variants of the IGF-1 gene. IGF-1 has been demonstrated to be involved in numerous hypertrophic signalling processes, including the phosphatidylinositol-3 kinase (PI3K)/Akt/mTOR and the PI3K/Akt/Glycogen synthase kinase 3 β (GSK3 β) pathways (Glass 2005; Rennie et al. 2004). GH treatment has been shown to up-regulate MGF mRNA when combined with resistance exercise (Hameed et al. 2004). It is unknown if the high GH response seen following LLRT with BFR would help up-regulate IGF-1 splice variants. Evidence suggests heavy resistance training is effective in increasing IGF-1Ea and MGF mRNA following acute (Greig et al. 2006; Hameed et al. 2003) and long term training (Liu et al. 2008). On the other hand evidence is lacking for LLRT with BFR. Previous findings have found that MGF or IGF-1R mRNA is not up-regulated 3 hours post LLRT with BFR (Drummond et al. 2008a) and Manini et al. (2011) have recently demonstrated that the IGF-1 mRNA is not changed 8 hours post exercise. It is possible that the time point of 3 hrs is not the optimum time point for changes in MGF and IGF-1Ea mRNA, with Kim et al. (2005a; 2005b) demonstrating that both MGF and IGF-1Ea are up-regulated 24 hours post heavy resistance training and McKay et al. (2008) demonstrating MGF and IGF-1 mRNA are up-regulated at different time points, 24 and 72 hours respectively, following acute eccentric resistance exercise. Indeed in chapter 6 we found an increase in MGF mRNA, 24 hours post exercise, following LLRT with BFR when both the old and young groups were combined. There was no change in IGF-1Ea mRNA in either group 24 hours post exercise.

This increase in MGF mRNA expression is of specific interest as previous research has indicated that older skeletal muscle has an inability to up-regulate MGF mRNA following acute resistance training (Hameed et al. 2003). This acute mRNA response does seem to be reversed when resistance training is performed over a 12 week period (Hameed et al. 2004). More recent research indicates MGF mRNA can be up-regulated in older individuals following acute resistance training (Roberts et al. 2010),
with the differences between the studies may a result of responders and non responders. This increase in MGF mRNA expression is important in helping understand the mechanisms behind LLRT with BFR at a local level.

It has been proposed that the MGF mRNA may play a role in the early activation of SC (Hill and Goldspink 2003) which are required for hypertrophied fibres to maintain their DNA to protein ratios (Kadi and Thornell 2000). Kadi et al. (2004) demonstrated older people had a lower (40%) number of SC relative to the total number of nuclei, therefore the increased MGF mRNA may help activate greater numbers of SC. As a result of resistance training, SC have been shown to contribute to the generation of new fibres, replicate to increase the pool of available stem cells, or serve as nuclear donors to existing fibres (Allen et al. 1999). In humans, satellite cell frequency can be elevated as early as 24 hours after a single bout of exercise and can remain elevated above baseline after several weeks of training (Kadi et al. 2005). A single bout of eccentric exercise resulted in dramatic increases in satellite cell numbers of untrained young men between 24 hours and 4 days post exercise (Cramer et al. 2004, Dreyer et al. 2006). Numerous resistance-training studies, ranging from 9 to 16 weeks in duration and some including both young and old men and women, have reported a significant elevation in SC from pre- to post-training (Kadi and Thornell 2000; Roth et al. 2001; Kadi et al. 2004; Olsen et al. 2006). Activated SC express myogenic factors such as Myo D, myogenin and myostatin (Cornelison et al. 1997; Yablonka-Reuveni and Rivera 1994), however these did not change following LLRT with BFR 8 hours post exercise (Manini et al. 2011). It could be implied that there is a delayed reaction of myogenic genes such as the ones described above due to the loading levels of LLRT with BFR as evidenced by the increase in MGF at 24 (Chapter 5) and not 8 hours (Manini et al. 2011) as both studies have used similar loading stratagies. On the other hand McKay et al. (2008) demonstrated that IGF-1 protein was expressed in the satellite cell compartment of skeletal muscle at 24 hours post resistance exercise, coinciding with peak MGF mRNA expression. At 72 and 120 hours post exercise IGF-1 protein levels were seen to increase in both the satellite cell compartment and myofibre suggesting IGF-1 may be responsible for preparing and proliferating SC into terminal differentiation (Hill and Goldspink 2003; Ates et al. 2007). Crameri et al. (2004) attempted to link satellite cell activation with high intensity exercise and found that they were activated 2 days after exercise in young people. In the same study they
found that there was an absence of myogenin-positive SC following 8 days and they therefore concluded that these SC may need at least another bout of exercise for terminal differentiation.

In order for muscle hypertrophy to occur there must be an increase in muscle protein synthesis (MPS). Acute bouts of heavy resistance training have been shown to increase MPS within the first few hours of exercise and remain elevated for 48 hours in young and older people (Drummond et al. 2008b; Phillips et al. 1997; Rasmussen et al. 2000; Yarasheski et al. 1993). The mTOR pathway plays a significant role in stimulating MPS following heavy resistance exercise (Wang and Proud 2006). This occurs specifically at its downstream target S6K1 which is responsible for mRNA translation initiation and increasing MPS and muscle hypertrophy (Baar and Esser 1999). It is clear that LLRT with BFR causes an increase in MPS through the mTOR pathway in both young (46 %↑) (Fujita et al. 2007) and older people (56% ↑) (Fry et al. 2010). This increase in the mTOR pathway is specifically through increased phosphorylation of S6K1 whose acute response to resistance training has been positively correlated to the percentage change in muscle mass (Terzis et al. 2008). Others have reported a reduced response (Kumar et al. 2009) whilst equivocal timing responses indicating both, a delay (Drummond et al. 2008b) no delay (Kumar et al. 2009) of MPS to acute resistance exercise. Neither has been demonstrated with LLRT with BFR, however studies have only investigated these effects at 3 hours post exercise and not compared between old and younger individuals. Like chapter 5, Fujita et al. (2007) and Fry et al. (2010) demonstrated a significant increase in GH following acute LLRT with BFR. They observed this increase in GH alongside an increase in MPS, however it is unknown if this played any role in signalling the mTOR pathway.

Effects of LLRT with BFR on blood flow

Alongside a decrease in skeletal muscle strength, with ageing, it has also been demonstrated that there is a negative change in the local vasculature at rest (Dinenno et a 1999; 2001), during exercise (Proctor et al. 2003; 2004) and in response to vasodilatation (Lind et al. 1999; Proctor et al. 2005; Sarabi et al. 1999). Therefore interventions that can improve these negative adaptations to ageing will have a
positive impact. Chapters 3 and 4 both demonstrated that LLRT with BFR had no impact on resting limb blood flow. This is stark contrast to the research showing both heavy resistance training and low intensity resistance training with slow movement speed improving basal femoral blood flow in young individuals (Tanimoto et al. 2009) and heavy resistance training increasing rest blood flow in an older population (Anton et al. 2006; Miyachi et al. 2005). One of the resistance training protocols used by Tanimoto et al. (2009) involved working at a low intensity (50-60% 1-RM) but used a slow contraction speed, 3 seconds for concentric and 3 seconds for eccentric contractions with no relaxation phase. This type of training may be similar to LLRT with BFR in that continuous force generation at >40% isometric strength can suppress both blood inflow to and outflow from the muscle due to an increase in intramuscular pressure (Bonde-Petersen et al. 1975). Interestingly, although no change was seen for $R_{bf}$ in the young participants in Chapter 3, there was a tendency for an increase following LLRT with 50% 1-RM. In both studies in this thesis interventions lasted 4 weeks, however in the work by Anton et al. (2006) and Tanimoto et al. (2009) the intervention periods were 13 weeks. Therefore it is possible the intervention was not long enough to see any proposed adaptations that may occur with a longer intervention period. It was hypothesized that we would have seen a positive adaptation in the older participants (Chapter 4) as they were likely to have a reduced resting blood flow, however as discussed above there was no improvement following the 4 week intervention. The old individuals (Chapter 4) in this thesis did not have significantly lower $R_{bf}$ compared to young participants (Chapter 3) (2.2 vs. 2.4 ml.min$^{-1}$.100m$^{-1}$, for old and young respectively) prior to the start of the exercise intervention period. It may be that the older individuals recruited had a greater activity level than many of the other studies showing a decrease in $R_{bf}$.

LLRT with BFR has been shown to improve $PO_{bf}$ in young (chapter 3) and older (Chapter 4) participants. This may be important as some evidence suggests $PO_{bf}$ may be lower in older people when compared to younger people (Sarabi et al. 1999). Of interest heavy resistance training alone has not been previously found to improve $PO_{bf}$ with some showing a decrease (Bond et al. 1996) and some remaining the same (Kawano et al. 2009). On the other hand 4 weeks of hand grip isometric resistance training has been shown to increase $PO_{bf}$ in young men (Alomari and Welsch 2007). Previous evidence suggests that like the studies in the current thesis, exercise with a
high ischemic component may help improve $\text{PO}_{\text{bf}}$. Sinoway et al. (1986) first demonstrated in tennis players that the forearm that held the tennis racket had a 42% greater $\text{PO}_{\text{bf}}$ than the non dominant forearm. Couple that with research demonstrating that rock climbers have a higher $\text{PO}_{\text{bf}}$ than non trained individuals suggests that adaptations may occur due to ischemia (Ferguson and Brown 1997). To our knowledge chapters 3 and 4 were the first to investigate LLRT with BFR, however a few studies have since been carried out in this area. Credeur et al. (2010) recently demonstrated that hand grip resistance training at 60% isometric strength with BFR actually decreased brachial artery flow mediated dilation (FMD). This type of training along with BFR resulted in complete restriction of the blood vessels for at least 20 minutes, compared to a maximum of 10 minutes in the protocols using LLRT with BFR, and thus resulted in a decreased endothelial function. Oxidative stress and inflammation have been shown to play a role in impaired endothelial function (Fisher 2008). Previous isometric exercise protocols have demonstrated an increase in oxidative stress (Alessio et al. 2000) as have studies investigating ischemia (Korthuis et al. 1985; Tsutsumi et al. 2007). This increase in oxidative stress may result in an increased vascular permeability (Korthuis et al. 1985). On the other hand LLRT with partial BFR as used in the protocols in the current thesis demonstrates an attenuated response to oxidative stress when compared to heavy resistance training (Goldfarb et al. 2008), however chapter 5 demonstrated a slight increase in circulating IL-6 following LLRT with BFR. Acute circulating IL-6 responses have been demonstrated to play an important role in inflammation (Cantini et al 1996), but it is unlikely to have any negative effect on limb blood flow as LLRT without BFR also seen a slight increase. In contrast 10 weeks of walk training with BFR actually increases carotid arterial compliance in older adults (Ozaki et al. 2010), thus suggesting the time period of BFR seen in the current thesis does not negatively effect oxidative stress and thus endothelial function, whereas a longer time period such as that seen in the Credeur et al. (2010) study does.

So what may be responsible for these improvements in blood flow capacity? Evans et al. (2010) recently demonstrated an increased filtration capacity following LLRT with BFR, which has been used as an indirect measure of capillarization (Brown et al. 2001; Gamble et al. 2000). Angiogenesis is the formation of new capillaries from an already established capillary network (Carmeliet 2000; Risau 1997). It has been
demonstrated that lowered tissue oxygenation or related metabolic alterations as seen in models of reduced blood flow during exercise can increase angiogenesis in animals (Roberts et al. 1997; Yang et al. 1994) and humans (Esbjornsson et al. 1993). Numerous studies have shown that VEGF is plays an important role in angiogenesis and the development of the cardiovascular system (Risau 1997). Chapter 5 demonstrated that plasma VEGF was increased following acute LLRT with BFR in older adults. This is similar to the increased circulating VEGF seen in young people following a similar protocol (Takano et al. 2005) thus suggesting a role of VEGF and improved PO2bf following LLRT with BFR.

**Limitations**

Due to the design of chapters 3 and 4 it is likely that there was a cross-over effect for strength adaptations following the 4 week training period. Resistance training of one limb has been shown to increase the strength of the contralateral muscle group in both upper and lower body muscles (Cannon and Cafarelli 1987; Hortobagyi et al. 1997), however some studies have not found this to occur (Garfunkel and Cafarelli 1992; Housh et al. 1992). A recent meta analysis demonstrated that this cross-over effect accounted for on average a 7.8% improvement in the contralateral limb trained. This may have caused the improved strength of the control leg that was trained with LLRT alone seen in some measurements in chapter 3 and 4. However in most cases the strength improvements seen following LLRT with BFR were greater than those seen with LLRT alone. The cross over effect has also been demonstrated in the vascular system (DeSouza et al. 2000), however most evidence suggests this not to be the case (Gokce et al. 2002; Mourtzakis et al. 2004) and in chapters 3 and 4 this was not the case.

One of the major limitations of LLRT with BFR is that it may not be needed in healthy individuals. Although the results of the current thesis are novel and interesting they are similar to what would be expected following heavy resistance training. It may be that in individual cases were rapid hypertrophy is needed to return normal function (i.e. following bed rest, injury etc) then LLRT with BFR may be a useful tool. Evidence suggests that LLRT with BFR can be used with a high frequency and return rapid hypertrophy due to the low mechanical demand placed on the trained muscle (Abe et al. 2005a; b). Issues also arise regarding the type of exercise that can be
performed with BFR, as it seems only limbs can be exercised safely, leaving it difficult to train the chest, back and shoulder muscles.

**Future directions**

- It has become apparent that plasma VEGF increases following acute LLRT with BFR therefore investigations are needed to establish the effect of LLRT with BFR on VEGF at a local level.
- Protein synthesis increases 3 hours post exercise however a time course study investigating myogenic growth factor responses to LLRT with BFR would give a better understanding on the adaptive response.
- MGF mRNA is up-regulated 24 hours post exercise and is known to have an effect on satellite cell proliferation. A time course study investigating the effect of LLRT with BFR on satellite cell adaptations is needed.
- Chapters 3 and 4 demonstrate an increase $P_{O_{bf}}$ following LLRT with BFR, however some evidence suggests this is not always the case, therefore investigations focusing on the cuff pressure and adaptations are needed.
- Resting blood flow increases following long term heavy resistance training but not following short 4 week LLRT with BFR. Longer training studies are required to investigate if similar adaptations occur.
- Walking with BFR has been shown to increase muscle strength and size. An investigation into the mechanisms behind these changes would greatly add to the literature.
- Longer term training studies are essential to allay safety risks and concerns.
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