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Effects of 4 weeks of low intensity hand grip isometric training with vascular occlusion in older adults

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**Effects of 4 weeks of low intensity hand grip isometric training with
vascular occlusion in older adults**

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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ABSTRACT

Introduction: By the middle of this century, the number of people of age 65 and over will more than double to 80 million (US census bureau, 1993). As more and more older people are getting added to the population, it becomes vital to understand the mechanisms and processes which can play a crucial role in their overall well being. The goal of the present study was to study the benefits of a novel method of training which can be an alternative to high intensity training and also be equally beneficial without the risks of heavy training in older adults. It was hypothesized that low intensity hand grip isometric training with vascular occlusion leads to increased peak forearm blood flow (FBF) and forearm vascular conductance (FVC) as compared to high intensity training in older adults.

Methods: Older participants (60 years or more) were recruited from the Ames community (Training group, n=9; control group, n=10). They were non smokers, did not participate in any structured hand grip exercise and did not have any diagnosed cardiovascular disease, PVD or diabetes. Intervention group did low intensity hand grip isometric exercise with vascular occlusion [20% Maximum voluntary contraction (MVC), 130% resting SBP] while the control group did high intensity isometric exercise (75% MVC) for 4 weeks. Resting FBF, peak FBF, resting FVC and peak FVC were calculated before and after the training in each group.

Results: No statistically significant changes were seen in either FBF or FVC in any of the groups. Also, strength and size changes did not reach significance.

Conclusion: Previous literature convincingly proves that conventional resistance training leads to strength and size gains. However, there were no changes in either group in the current study. The study also had a few limitations. The 4 weeks study period might not have been long enough to evoke a convincing and significant outcome. Some gender based differences in FBF response were seen as well. Further research is warranted before any conclusions can be drawn regarding the applicability of low intensity exercise with vascular occlusion.

Chapter 1. INTRODUCTION

By the middle of this century, the number of people age 65 and over will more than double to 80 million (US Census Bureau, 1993). As more and more older people are getting added to the population, it becomes vital to understand the mechanisms and processes which can play a crucial role in their overall well being. Exercise training helps to offset some of the common physiological, metabolic, psychological and functional disorders in elderly. Regular exercise decreases myocardial oxygen demand, increases serum HDLs (Williams, 1996), positively alters body fat distribution (Schwartz et al., 1991), increases insulin sensitivity (Rosenthal et al., 1983), prevents and mitigates osteoporosis (Sinaki et al., 2010) and improves muscle health (Laforest et al., 1990).

Resistance training has been identified as an efficacious method for developing musculoskeletal strength and is frequently prescribed for prevention and rehabilitation of orthopedic injuries (AACPR, 1991; ACSM, 1998; Fleck and Kraemer, 1997; Pollock, et al., 1994). Resistance training also improves physical functional capacity and quality of life, especially in the elderly or weak and less fit individuals (Fiatarone et al., 1994). The natural process of aging leads to physiological declines which lead to sarcopenia. Consequently, older adults are more prone to fatigue and orthopedic injuries and cardiovascular complications. These factors must be taken into consideration when prescribing resistance training programs for this population. (ACSM , 1998; Fletcher et al., 1995 ; Pollock, et al., 1991; Pollock et al, 1994).

In older adults, there is a dose-response relationship between the intensity of resistance training and strength gains and functional improvements (Seynnes et al, 2004). High-

intensity resistance training has also been shown to have a positive impact on the perception of pain in older adults (Knutzen et al, 2007). However, there have been reports which have cautioned against the risks associated with high load training as documented by the review of literature. An exercise program with similar benefits without the risks would be ideal for the elderly population. Low intensity exercise done in the presence of restricted blood flow has been linked with muscular hypertrophy and increased strength (Shinohara et al., 1998; Takarada et al., 2000; Takarada et al., 2002; Takarada et al., 2004; Moore et al., 2004; Abe et al., 2005; Yasuda et al., 2005; Madarame et al., 2008). Various physiological adaptations like increase in growth hormone concentration, endothelium-based vasodilation and angiogenesis form the basis of such a response [Takarada et al. (2000a), Esbjornsson et al. (1993), Roca et al., 1998].

The goal of the present study was to identify an alternative to high intensity training which can be equally beneficial without the risks of heavy training in older adults. In young adults, vascular occlusion combined with low intensity exercise provides a similar milieu as high intensity exercise. Since the vasculature in the older population differs vastly than in younger adults, the current study assessed the hyperemic response of the forearm microvasculature after handgrip training combined with occlusion in older adults. It was hypothesized that low intensity training with vascular occlusion leads to increased maximal forearm blood flow and vascular conductance as compared to high intensity training in older adults.

Chapter 2. REVIEW OF LITERATURE

2.1 Changes in hemodynamics due to aging

Old age is accompanied by a decrease in the blood flow (BF) capacity of the skeletal muscle (Wahren et al., 1974, Proctor et al., Lawrenson et al., 2003, Donato et al., 2006). In old rats, the BF to kidneys, spleen, stomach, pancreas, small and large intestine was seen to decrease during submaximal exercise. Even though the amount of BF was similar in the hindlimbs of young and old rats during exercise, there was a profound redistribution of blood from highly oxidative to highly glycolytic muscle. Consequently, there is an altered distributed of blood between different muscles and also within a muscle (Musch et al., 2004).

An aberration of endothelium-dependent vasodilation is likely one of the mechanisms underlying the altered muscle blood flow in old age (DeSouza et al., 2000; Muller-Delp et al., 2002; Woodman et al., 2002). Muller-Delp et al. (2002) established that this dysfunction originated in the nitric oxide (NO) signaling pathway. NO bioavailability is critical in matching oxygen supply and demand in skeletal muscle during the rest to exercise transition (Ferreira et al., 2006). The contribution of NO to exercise hyperemia is reduced in older subjects (Schrage et al., 2007); however, the presence of NO is not mandatory to increase muscle blood flow during exercise in young subjects (Frandsenn et al., 2001; Radegran and Saltin, 1999).

The endothelium dependent vasodilating response decreases with advancing age in the forearm as well as with primary hypertension (Taddei et al., 1995). Aging is characterized by

a derangement of endothelial cells which results in a fall in NO production (Mayhan et al., 1990; Stewart et al., 1987). It appears that there is a generalized abnormality of basal endothelial function in older adults, with an impairment of NO dilator pathway in human forearm (Singh et al., 2002). These are crucial observations as endothelial cells markedly affect local vascular reactivity.

Vascular conductance is defined as flow divided by mean arterial pressure. As age is associated with increases in blood pressure (Dinenno et al., 2001), conductance decreases with increasing age (Dinenno et al., 1999). Total peripheral resistance increases with age (Julius et al., 1967). Almost 85% of the cardiac output to the maximally exercising muscles is distributed by the peripheral resistance vasculature (Mellander and Johansson, 1968). Maximum blood flow recovery to resting values after exercise (Half time to recovery) increases in older people (Olive et al., 2002). Half time to recovery was determined as the time where blood flow dropped to one half the magnitudes between maximum flow and resting flow values. This can be attributed to a reduced ability to wash out the metabolites and has been associated with a loss of endothelium dependent vasodilation (DeSouza et al., 2000) and decreased NO production (Singh et al., 2002). Post exercise, the arteriolar diameter and reactive hyperemic response was found to be much higher in younger rats as compared to old rats , even though resting blood flow in the skeletal muscle was not affected by aging (Hammer and Boegehold, 2005). In addition to an altered vascular response, capillary density is reduced with advancing age (Shimada et al., 2004; Sadoun and Reed, 2003;). Gastrocnemius muscle capillarization was found to be about 25% lower in the older rats (Coggan et al., 1992).

2.2 Angiogenesis

Angiogenesis is defined as the formation of new blood vessels by way of sprouting of preexisting mature endothelial cells (Folkman, 1995). There is considerable evidence supporting delayed and impaired neovascularization response with aging (Shimada et al., 2004; Kitlinska et al., 2002 ; Sadoun and Reed, 2003; Rivard et al., 1999; Reed et al., 1998). The tissues of aged rats showed less newly deposited collagen and a lack of Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor (TGF) expression (Sadoun and Reed, 2003). A major factor for the diminished angiogenesis is the decreased level of VEGF in aging (Rivard et al., 2000). The magnitude of the effect of hypoxia on VEGF expression and the absolute VEGF protein level decline significantly during aging. All this leads to a significant decline in the new capillary formation in old age. Thus, aging leads to impaired oxygen diffusion, inadequate hyperemic response to exercise, inefficient vasodilation and a blunted angiogenic response.

2.3 Role of shear stress, VEGF and nitric oxide in angiogenesis

Shear stress

Acute muscle contraction significantly increases capillary red blood cell velocity (Tym and Cheng, 1995; Anderson et al., 1997). Such an increase leads to an elevation in the mechanical force faced by the endothelial walls of the blood vessels, thereby increasing the shear stress (Berg et al, 1997). Long term increases in muscle blood flow, stimulated either by vasodilator treatment or stimulation, is also associated with greater shear stress (Egginton

et al, 1998). Prolonged administration of the vasodilator dipyridamole led to an increased capillarity in skeletal muscle of rats (Tornling et al, 1980) and long term prazosin, an alpha-receptor blocker caused a threefold rise in muscle blood flow (Ziada et al, 1989).

VEGF

Vascular endothelial growth factor, or VEGF, is a family of growth factors which includes VEGF-A, B, C, D and placenta growth factor (PlGF). VEGF-A (or simply VEGF) is a very potent growth factor that has been shown to be linked to angiogenesis both in vivo and in vitro (Ferrara, 2001). Goto et al. (1993) cultured bovine capillary endothelial cells within collagen gel and studied the combined activity of VEGF and basic fibroblast growth factor (bFGF). They found that VEGF activity lasts twice as long as bFGF and their combined action resulted in the formation of tube-like structures in the medium. VEGF also acts as a chemoattractant (Matsumoto and Claesson-Welsh L, 2001), which seems to help in endothelial cell migration. All types of VEGF signal by binding to tyrosine kinase receptors on the endothelial cell surface, primarily VEGFR1 and VEGFR2. The latter is responsible for facilitating almost all of the VEGF-generated endothelial cell proliferation and migration (Bernatchez et al., 1999; Gille et al., 1997; Waltenberger et al., 1994; Wu et al., 2000). In a study by Milkiewicz et al. (2003), it was seen that capillary growth is heavily dependent on receptor-2 presence, even if muscle VEGF levels are high.

Abumiya et al (2002) studied shear stress in human umbilical vein endothelial cells and found that it augments the function of VEGF by upregulating the expression of VEGF receptor 2. They suggested that when blood flow increases, the consequent increase in shear

stress promotes the endothelial cells to express receptor 2, and thereby stimulate angiogenesis. On VEGF-deletion inducing intramuscular injection in mice, there was 67% decrease in capillary-to-fiber ratio and a 69% decrease in capillary density within 8 weeks (Tang et al., 2004). Thus, VEGF also has a substantial role in sustaining muscle capillarity.

Nitric oxide

Nitric oxide (NO) was originally discovered as an endothelium-derived relaxing factor and is known to be a strong vasodilator (Ignarro et al., 1999 and Furchgott & Zawadzki, 1980). It is also another crucial factor in vascular remodeling, released by shear stress and acting as a mediator in the functioning effects of VEGF. There seems to be an interrelationship, since VEGF-2 activation leads to NO release via mobilization of calcium stores (Cunningham et al., 1999) while NO can also activate VEGF expression (Tsurumi et al., 2001). Hudlicka et al. (2006) examined the role of NO in capillary growth in rats. Ankle flexors were chronically stimulated for 8 hr/day for 2 or 7 days with or without N-omega-nitro-L-arginine (L-NNA, a nitric oxide synthase inhibitor). It was observed that shear stress doubled after 2 days of muscle stimulation (8hr/day) but normalized after 7 days in L-NNA treated rats. This is explained by the fact that L-NNA restricts the increase in capillary shear stress after two days by inhibiting NO production. This confirmed the important role of NO in the early elevation of shear stress that promotes angiogenesis.

Several other studies highlight the importance of NO in VEGF-induced vascular permeability as well as angiogenesis (Parenti et al., 1998; Ziche et al., 1999 and Morbidelli et al., 1996). There are three isoforms of NO synthase (NOS): eNOS, nNOS and iNOS. eNOS

mediates VEGF-induced angiogenesis and VEGF-induced permeability of microvessels to circulating macromolecules. NO also mediates blood flow in VEGF-induced angiogenesis vessels. It dilates the vessels, inducing vessel sprouting and maintaining blood flow in angiogenic vessels (Fukumura et al., 1997 and Carmeleit, 2000).

2.4 Effect of hypoxia on angiogenesis

Hypoxia is one of the foremost stimuli responsible for angiogenesis. Long-term disparities between the perfusion dynamics of the vascular system and the metabolic demands of the body tissues leads to compensatory vascular adjustments. VEGF production is stimulated by hypoxia in a variety of pathophysiological conditions (Dor et al., 2001; Semenza, 2003). An attestation to the physiologically-driven stimulation of VEGF due to hypoxia is provided by a study which shows growth of retinal vasculature (Stone et al., 1995). The hypoxia induced as a result of neuronal activity is detected by glial cells which secrete VEGF in response. Knighton et al. (1983) studied the macrophage angiogenesis expression in healing hypoxic wounds. Macrophages were exposed to various O₂ concentrations for 24 hours and they showed an oxygen dependent change in morphology. Angiogenesis accompanied the healing of hypoxic wounds and there was a compensatory development of collateral vascular supply in otherwise dormant ischemic tissues. Evidence for a role of VEGF in pathological conditions comes from an investigation on a specimen of malignant human brain neoplasm which revealed that VEGF mRNA levels vary depending upon the oxygen tension in the specimen (Shweiki et al., 1992). Decreasing the oxygen supply led to marked proliferation and migration of endothelial cells (i.e., tumor

neovascularization) while bringing the supply back to normal levels reduced the VEGF mRNA expression to its low constitutive levels and attenuated endothelial proliferation. It was also noted that capillary bundles were seen to align preferentially along the VEGF-expressing cells. Hypoxia induces transcription of the VEGF gene via a transcription factor, hypoxia-inducible factor (HIF-1 α) (Semenza , 2002). Inhibition of HIF-1 α binding sequence leads to termination of a major percentage, though not all, of HIF-1 α -inducible VEGF (Carmeliet et al., 1998; Forsythe et al., 1996 and Wood et al., 1996).

Milkiewicz et al. (2006) studied the VEGF mRNA and protein expression during ischemia in an iliac artery ligation rat model. Even though their levels were elevated, VEGF mRNA and protein expression level did not peak at the same time as ischemia. VEGF mRNA registered more than 7-fold increase during first 3 days, fell to normal levels in 7 days and increased about 1.5-2 times again after 14 days. VEGF protein expression followed an almost similar trend but it was lower than the control when capillary growth was reported. So, it can be concluded that hypoxia is not the only stimulus for capillary growth. The restoration of blood supply in ischemic muscles seems to be a crucial key in facilitating angiogenesis as demonstrated by Fulgenzi et al. (1998). The administration of prazosin improved the capillary to fiber ratio, blood flow and muscle performance in chronically ischemic muscles. Muscle performance, which was measured by a fatigue index, peaked after 2 weeks of ischemia. Breen et al. (1996) also found that hypoxia at rest doubled VEGF mRNA levels and exercise in hypoxia further raised its concentration, as was noted during the first 4 hour of post exercise period.

2.5 Effect of exercise on angiogenesis

It is well established now that skeletal muscle adapts to exercise stimulus by increasing capillary density and/or capillary-to-fiber ratio, in order to match better to the increased metabolic requirements of the involved tissues (Brodal et al., 1977; Hang et al., 1995 and Zumstein et al., 1983). Richardson et al. (1995) reported wide differences in PO₂ between blood and intracellular tissue in intact human skeletal muscle throughout an incremental exercise protocol in both normoxic as well as hypoxic conditions. It suggests a considerable drop in the local muscle oxygen tension during exercise. This is a possible explanation for exercise-induced capillary growth via upregulation of growth factors.

Muscle contractions induced by means of electrical stimulation are associated with an upregulation of VEGF mRNA. Amaral et al. (2001) electrically stimulated rat muscle for 8 hours per day for 7 days. It led to significant increases in vessel density and serum VEGF protein level. Similarly, VEGF protein level and capillary density was found to be increased after chronic motor nerve stimulation in the glycolytic skeletal muscle which otherwise has a lower capillary density than an oxidative muscle (Annex et al., 1998). Hang et al. (1995) showed similar increases in VEGF mRNA levels. On chronic electrical stimulation of rat peroneal motor nerve for 21 days, VEGF mRNA had a sixfold increase within 4 days of stimulation and then decreased over the next several days, but still remained much higher than the control.

Breen et al. (1996) demonstrated the upregulation of VEGF, bFGF and another growth factor (TGF-beta 1) after a bout of acute exercise in rats. Maximum increases of two to four fold were seen in the group which exercised at 20 m/min as compared with 15 m/min. This increase peaked in the first 4 hours after exercise and returned to normal levels by 8-24 hours. Hypoxic exercise augmented the rise in VEGF mRNA levels. There is evidence that suggests a similar phenomenon in human skeletal muscle as well. Gustafsson et al. (1999) examined the effect of one-legged dynamic constant-load knee-extension exercise on mRNA expression of VEGF and FGF-2 in human skeletal muscle. They reported 178% increase in the VEGF mRNA expression and concluded that it was proportional to the changes in HIF-1 α and HIF-1 β mRNA. We now have enough literature to reason that the upregulation of VEGF is an important consequence of exercise training and it plays a crucial role in the exercise-induced angiogenesis. Acute resistance training has also been found to have similar angiogenic effects as aerobic exercise with respect to timing and magnitude (Gavin et al., 2007). High intensity training with 60-80% of 1-RM increased skeletal muscle VEGF, VEGF receptor and angiopoietin receptor expression.

2.6 Effect of blood flow restricted exercise on angiogenesis

Suga et al. (2009) studied the intramuscular metabolism during a single bout of low intensity resistance exercise with partial vascular occlusion. They concluded that the intramuscular metabolic stress during this protocol significantly increased as compared to low-intensity alone. A higher percentage of phosphocreatine breakdown and a greater drop in muscle pH were reported. Muscle plasma concentration increases significantly during low-

intensity exercise with vascular occlusion of 100mm Hg as compared with high intensity exercise alone (Takarada et al., 2000). Metabolic alterations such as pH change, local metabolite concentration and local hypoxia due to exercise are an important signal in the upregulation of VEGF, thus triggering angiogenesis (Adair et al., 1990; Shweiki et al., 1992; Nomura et al., 1995; Roca et al., 1998).

In the previously mentioned study by Gustafsson et al. (1999), the subjects were divided into two groups. One group performed knee extension under conditions of blood flow restriction (R) of 15-20% and the other exercised under non restricted blood flow (NR). The R group had an increase of 236% and the NR group a rise of 111% in the VEGF mRNA expression after a 45-minute bout of exercise. Thereby suggesting the extent of VEGF expression was dependent upon the metabolic stress during the exercise. Esbjornsson et al. (1993) found a greater capillary to fiber ratio after exercise in blood flow restricted conditions in one leg when compared with training at a similar power output with normal blood flow in the other leg. The authors also stated that ischemic training changes the muscle metabolic profile in order to facilitate aerobic metabolism. Sundberg and Kaijser (1992) performed an experiment to quantify a human ischemic model. They found that the application of 50 mm Hg of pressure on the exercising leg led to reduction in venous oxygen saturation by 12 percentage units. This clearly signifies that oxygen tension dips even further during blood flow restricted exercise.

Another effect of blood flow restricted exercise is an additional recruitment of fast twitch fibers even with moderate intensity exercise. Krstrup et al. (2008) found an alteration in the

normal muscle fiber recruitment (i.e., Size principle, Henneman et al., 1965)), with more fast twitch fibers being recruited during sub maximal knee extensor exercise with thigh occlusion. Takarada et al. (2000) found a dramatic increase in plasma lactate concentration with low intensity exercise at 100mm Hg of occlusion. Increased plasma lactate levels in and around the muscle fibers inhibit the contraction of muscle fibers; this leads to additional motor unit recruitment to maintain a constant force generation (Moritani et al., 1992; Sundberg, 1994). The recruitment of fast twitch muscle fibers provide an augmented stimulus for angiogenesis as it takes a precedence for growth of capillary network over slow twitch fibers (Adair et al, 1990).

Muscular responses to aging

Increasing age is associated with a decreasing capacity to perform different physical activities (Beere et al., 1999; Cartee and Farrar., 1987). The age-associated decline in VO_2 max can be attributed to the loss of muscle mass in old age (Fleg and Lakatta, 1988). The cross sectional areas of the hamstring, gluteus maximus and quadriceps diminish with aging. The quadriceps volume declines to 67% while the quad oxidative capacity reduces to 53% of the adult value (Conley et al., 2000). Other cardiopulmonary variables also show a downward slope with increasing age (Inbar et al., 1994). Behnke et al. (1995) found a temporal and spatial mismatching between oxygen delivery and oxygen demand during the rest to contraction transition. This can be attributed to a compromise in the blood flow dynamics as there is a reduced RBC-to-capillary surface contact time. The harmony between red blood cell velocity peak time and capillary RBC flux peak time is altered in older population. This

increases the O₂ capillary transit time, thus limiting oxygen diffusing capacity (Kindig et al., 2002). With advancing age, the RBC flux does not increase over time with muscular contractions (Copp et al., 2009). This further substantiates the fact that O₂ delivery gets impaired with aging.

These changes can have a critical role in many diseases or conditions associated with old age. Endothelial dysfunction can be a major influence on the myocardial infarction prognosis (Kurotobi et al., 2004). This is because of a reduced ability to develop collaterals as a result of weakened NO expression. Furthermore, there is decreased angiogenic potency in the bone marrow cells of old patients (Li et al., 2010). These marrow cells are used for the treatment of ischemic diseases. Impaired oxidative capacity and impaired blood flow due to aging contribute to decreased functional and exercise performance (Astrand et al., 1973).

2.7 Benefits of exercise training

Exercise training improves vasodilation in skeletal muscle

Treadmill running for 60 min/day, 5 days a week for a period of 12 weeks in rats led to an increase in Acetylcholine-induced dilation in isolated feed arteries (Trott et al., 2009). Post-training, the vasodilator response of the senile exercising rats was found to be similar to that of sedentary and exercising young rats. Spier et al. (2007) found that exercise training restores and enhances flow-induced vasodilation in gastrocnemius muscle arterioles from old and young rats, respectively. The authors attributed these result as being induced through a NOS mechanism.

Exercise training improves hyperemic response and increases muscular blood flow

The maximal muscle blood flow is higher in physically active individuals, irrespective of age. Olive et al. (2002) found 30 % greater blood flow capacity and higher maximal conductance in active subjects as compared with sedentary subjects. Regular exercise also prevents the age associated loss in the endothelium dependent vasodilation (Martin et al., 1991; DeSouza et al., 2000).

2.8 Low intensity resistance exercise with vascular occlusion

Effect on Cross Sectional Area

It is well established now that low intensity exercise with vascular occlusion (LIVO) creates an intramuscular milieu analogous to that of high intensity exercise (HI) (Takarada, Takazawa and Ishii, 2002 ; Takarada et al., 2000). LIVO also results in better or comparable results with respect to muscular strength and hypertrophic gains when compared to HI (Shinohara et al., 1998; Takarada et al., 2000; Takarada et al., 2002; Takarada et al., 2004; Moore et al., 2004; Abe et al., 2005; Yasuda et al., 2005; Madarame et al., 2008). Abe et al. (2005) recorded an increase of 9% in muscle cross-sectional area (CSA) and an increase of 17% in squat strength in a training protocol of 2 sessions per day for a period of 2 week, with a load of 20% 1 Repetition Maximum (RM) along with venous occlusion. Yasuda et al. (2005) used a similar protocol with occlusion pressure of 160-240 mm Hg. In addition to the gains of 7.8% in muscle CSA and 14% in squat strength, they also reported hypertrophy in the muscle fibers; with much higher CSA in type II muscle fibers (27.6%) as compared with

type I muscle fibers (5.9%). This proved that increases in muscle CSA during LIVO is due to an increase in muscle fiber size and is not merely due to extracellular tissue/liquid gain. Takarada et al. (2000) registered significant and comparable gains in strength and CSA of elbow flexors with LIVO (50-30% RM; 110 mmHg) and HI (80-50% 1RM) in sixteen weeks of training with older women. The effects of LIVO are not restricted to the normal, untrained population alone. LIVO resulted in similar benefits in highly trained athletes as well in a training period of 8 weeks with 50% 1RM and occlusion pressure of 200 mmHg (Takarada et al., 2002).

Effect on Growth Hormone

Skeletal muscle growth is contingent upon various endocrine hormones for its growth and proper functioning. Growth hormone (GH) is one such hormone and it is known to regulate protein synthesis in the muscles (Healy et al., 2003; Liu et al., 2003). Exercise, in general, is a very potent stimulator of GH (Godfrey et al., 2003). There are multiple factors which contribute to the GH elevation due to exercise, such as neural stimulation, metabolite accumulation and NO (Godfrey et al., 2003). Accumulation of metabolites such as lactic acid during exercise is one of the triggering factors for the production of GH (Kraemer et al., 1991). Takarada et al. (2000a) showed a dramatic increase in both lactic acid and GH concentration; with the latter showing a rise of 290 times the resting value when leg extension at 20% 1RM was performed with occlusion pressure of ~214 mmHg. It remained almost unchanged after the same volume of exercise without occlusion. In another study, a 100 fold rise in GH was reported after 20% 1 RM knee extension exercise with occlusion

pressure of 130% of systolic blood pressure (Takano et al., 2005). So, occlusion combined with low intensity training leads to an increase in GH concentration.

However, occlusion alone is not enough to cause such a rise (Pierce et al., 2006). It was suggested that there is a relationship between fatigue (decrement in muscle force) and GH levels. The subjects who presented the greatest drop in force production had the highest plasma GH concentration. This means the most fatiguing training protocol may be the most effective design for bringing maximum strength and hypertrophy. This can be beneficial in patients suffering from muscle atrophy as low load exercise can bring about similar increases in GH concentration, thus facilitating protein synthesis for muscle mass production.

Various training protocols have been used by different researchers to elicit the above mentioned results. Variables such as exercise intensity (% 1RM for low and high resistance training), occlusion pressure, muscle group, exercise volume and age/gender/occupation of participants are frequently manipulated. However, there is a lack of consensus in literature for the values of the variables manipulated. For instance, Takarada et al. (2000) used 50% and 80% of 1 RM as low and high intensities; Reeves et al. (2006) used 30% 1 RM as low and 70% 1RM as high intensity. Takarada et al. (2000a), Takarada et al. (2004), Abe et al. (2005), Takano et al., (2005), Pierce et al. (2006) and Suga et al. (2009), used ACSM's recommendation of 20% 1RM as low and 65% 1RM as high intensity. Similarly, the optimum occlusion pressure also lacks consensus, with some studies using pressures as low as 110 mm Hg (Takarada et al., 2000) to as high as 300 mmHg (complete occlusion) (Cook et al., 2007). Based on the fact that the most effective treatment protocol would have the greatest fatigue

as suggested above, Cook et al. (2007) determined that 20% 1 RM with continuous partial occlusion of 160 mm Hg evokes the highest degree of fatigue, which was significantly greater than high intensity exercise (80% 1RM).

2.9 Benefits of LIVO in older adults compared with high intensity

DeLorme (1945) proposed the widely accepted and traditional view on resistance training. He suggested that a low load-high repetition protocol induced muscular endurance while high load-low repetition facilitate strength and power development. A high mechanical load of greater than 65% of one repetition maximum is optimum to increase muscle strength and size while training with intensities lower than this are unable to evoke muscular hypertrophy and strength gains (McDonagh and Davies, 1984). Recent literature also suggests that training volumes using a moderate to high number of repetitions at 67-85% of 1 RM have been associated with increased skeletal muscle size and strength (Baechle and Earle, 2000).

Geriatric populations also retain the potential for increases in muscle strength and hypertrophy in response to resistance training (Brown et al., 1990). Frontera et al. (1988) found significant strength and size gains in older men, in addition to increased myofibrillar protein turnover with resistance training at 80% of 1 RM. Hakkinen et al. (2002) documented improvements in neuromuscular profile while reporting no cardiovascular or orthopedic issues even with heavy resistance training in the elderly. But there have been reports of damage caused by strength training. For instance, three cases of sub arachnoid hemorrhage

were reported with biceps curls and leg press (Haykowsky et al., 1996). They suggested that the valsalva maneuver involved with repetitive upper and lower extremity weight training, raised the pressure in the cerebral vasculature and triggered the rupture of a benign aneurysm. In a study by Pollock et al. (1991), 8.7% of the elderly participants got injured during a 26 week training period. They performed 10-12 reps each of 10 resistance exercises to volitional fatigue. Acute blood pressure responses to heavy weight lifting have been observed, even in young healthy subjects (McDougall et al. 1985). The mean value for peak blood pressure was noted to be 320/250 mm Hg during a single biceps curl concentric contraction. It was concluded that mechanical compression of blood vessels during heavy weight lifting, the pressor response and the valsalva maneuver combine together to cause an extreme rise in blood pressure.

Resistance training is a very potent tool for combating various musculoskeletal and cardiovascular compromises in the elderly. On the other hand, high intensity training has been linked with moderate to severe complications. It would be very beneficial for the older adults, if similar benefits can be achieved by a low intensity training which involves far less risk and is more comfortable. We can expect more compliance and adherence to the training protocol if the subject is more confident of its safety. Therefore, we intended to study low intensity resistance exercise with vascular occlusion in the elderly.

Possible risks with LIVO

Even though low intensity training with blood flow restriction is not linked to serious side effects, some concerns have been raised regarding its potential thrombogenic effect (Manini

and Clark, 2009). Complete or severe blood flow occlusion can cause tissue necrosis, blood coagulation and reduced endothelial function (Margovsky et al., 1997). However, a study demonstrated that moderate blood flow restriction has no effect on blood clotting (Fujita et al., 2008). There were no signs of changes in the levels of markers of intravascular clot formation, such as D-dimer and markers of thrombin generation such as plasma thrombin-antithrombin complex and prothrombin fragment. Moreover, no obvious signs of muscle damage were reported as the blood levels of creatine kinase, myoglobin and IL-6 remained unchanged as well. A recent study by Madarame et al. (2010) corroborated these findings. They concluded that low-intensity exercise with blood flow restriction does not activate thrombin generating part of coagulation system. Additionally, a survey was conducted to establish the safety of such exercise, which included approximately 13000 people, including adults over the age of 70 years (Nakajima et al., 2006). It was determined through this study that blood flow restricted exercise poses no grave complications and dangers to the health of elderly.

2.10 Summary

The detrimental results of senescence adversely affect the quality of life in the elderly. The reduced ability to grow new capillaries leading to decreased muscle mass, decreased muscle blood flow, blunted vasodilation response due to endothelial dysfunction and resultant inadequate hyperemic response contribute to their impaired musculoskeletal and cardiovascular health. High intensity resistance training has been shown to be effective in delimiting these factors and improving overall well being in the elderly. But it can be risky

for the older population to be engaged in heavy training, as they often suffer from a host of medical illnesses. Not only is it risky, but the discomfort associated with heavy training may make adherence and compliance with exercise, a challenge. A safer, agreeable and a more reliable alternative may be low intensity training with partial vascular occlusion. No serious side effects and dangers have been reported, even in the elderly. It has been shown to be beneficial in increasing muscular strength and size. LIVO also triggers the angiogenic response by upregulating the VEGF and NO expression. No study has attempted to study the effects of LIVO on the hyperemic response and vascular conductance in the elderly. We aimed to determine the efficacy of LIVO in producing the maximal hyperemic response and vascular conductance in the forearm microvasculature after low intensity isometric handgrip training with partial vascular occlusion in the older adults.

Chapter 3. METHODS

3.1 Subjects

Nineteen relatively healthy older adults above 60 years of age were recruited from the Ames community to participate in the study. They completed a medical health history form. Exclusion criteria were the presence of any diagnosed cardiovascular disease, peripheral vascular disease, hypertension, diabetes, smoking, pain during gripping activities, blood anticoagulant medications, and/or any medical condition that precludes a program of weight lifting. The participants were not involved in any sort of exercise that could possibly affect forearm muscle strength. They were randomly divided into an intervention (n=9) and a control group (n=10). They reported three days per week for the training protocol for a period of four weeks.

The protocol was approved by the Institutional Review Board at the Iowa State University. Informed consent was obtained from each participant prior to the program.

3.2 Experimental Design

Pre test measures

Age, height and weight were recorded for all the participants at the beginning. Maximum voluntary contraction (MVC) was measured by asking the participants to maximally squeeze the hand held dynamometer, with the forearm supported. Training MVC, which was used to train the participant, was defined as the peak force generated during the three trials by the non dominant arm. Resting blood pressure was also measured after the participants had been sitting quietly for ten minutes.

3.3 Instrumentation

MVC was measured using a Jamar-type handgrip dynamometer (Fabrication Enterprises, Inc., Irvington, NY). It was interfaced with a Biopac A/D board (BIOPAC systems, Inc., Santa Barbara, CA). The forearm blood flow was measured by mercury in silastic strain gauge plethysmographic method (EC-5R Plethysmograph, D.E. Hokanson, Inc., Washington). Minute by minute blood pressure was recorded using an automated sphygmomanometer (Dinamap-XL, Johnson and Johnson Medical, Inc., Tampa, FL). Heart rate was monitored and recorded using an electrocardiogram (Life Pak 5, Physio-Control, Redmond, WA).

3.4 Data collection

These following measurements were done for all the participants in both the groups before and after four weeks of exercise training:

Forearm blood flow (FBF): The participant sat with non dominant arm supported at the elbow and at the wrist, and slightly above the heart level. The mercury in silastic strain gauge was placed at the upper one-third of the distance between the olecranon process and the ulnar styloid. To occlude the blood flow, a blood pressure cuff was placed on the upper arm and another cuff on the wrist such that wrist was in the middle of the cuff. After making these arrangements, the participant rested supine quietly for ten minutes. At the end of this time period, resting FBF was taken for the next 5 minutes with the upper cuff inflated to about 45mmHg. In the next phase, it was inflated to a constant pressure of 130% of resting SBP and the wrist cuff to 200mm Hg. After five minutes of this, the wrist cuff pressure was released to zero and upper cuff pressure quickly decreased to 45mmHg as FBF was recorded for another 5 minutes.

Another blood pressure cuff was placed on the contralateral arm to automatically record blood pressure every minute. Any abnormal beats or arrhythmia and heart rate were also measured by using an electrocardiogram throughout the measurement of FBF.

3.5 Training protocol

The control group exercised at 75% of MVC without vascular occlusion and the intervention group at 20% of MVC with a vascular occlusion pressure of 130% resting systolic blood pressure (SBP). Each training session consisted of three sets of handgrip dynamometer isometric contractions to fatigue by the non dominant arm. Volitional stopping or force production of less than 50% of desired MVC during isometric contraction were considered fatigue points. A rest period of 1 min was allowed in between sets (Reeves et al., 2006). If they did not tire, they kept on exercising. The forearm was supported by a table/plinth throughout the training with the shoulder abducted to 90°. The contraction and relaxation time was set at 2 seconds each and participants were prompted by a metronome. MVC was reassessed on the first day of the third week and every participant was trained according to this new set MVC.

3.6 Data Analysis

Maximum FBF was the highest one measured during the first 3 minutes after release of occlusion and maximum FVC was the one corresponding to max FBF. Mean arterial pressure (MAP) was calculated as $[(\text{pulse pressure}/3) + \text{diastolic blood pressure}]$. Forearm vascular conductance was then calculated as FBF/MAP . Resting FBF and FVC were averaged over the first 5 minutes. The variables (FBF, FVC, girth, MVC, HR and MAP) were assessed

using a group (2) X time (2) repeated measure ANOVA and paired t-tests. Statistical significance was set at $p < 0.05$. Data are expressed as mean \pm standard error.

Chapter 4. RESULTS

No significant changes were found in forearm blood flow (FBF) and forearm vascular conductance (FVC) after the training in both the intervention and control groups. Also, no significant changes were seen between the groups after training. Results are shown in the tables below:

Table1. Physical characteristics of subjects (\pm standard error).

	Training group		Control group	
	Pre training	Post training	Pre training	Post training
Age	63.1 \pm 0.8		62.5 \pm 1.1	
Height (m)	1.7 \pm 0.04		1.7 \pm .03	
Weight (kg)	82.3 \pm 6.5		81.7 \pm 4.5	
Forearm Girth (cm)	26.3 \pm 1.2	26.7 \pm 1.9	25.9 \pm 0.8	26.1 \pm 0.8
MVC peak (kg)	33.4 \pm 4.2	36.3 \pm 4.8	34 \pm 4.6	39.8 \pm 5.1
HR	59.4 \pm 2.3	57.3 \pm 2.9	63.3 \pm 3.9	62.9 \pm 3.2
MAP (mmHg)	87.7 \pm 3.2	84.5 \pm 2.8	84.3 \pm 1.9	83.3 \pm 3.0

Table2. Change in study variables pre and post 4 weeks of training (\pm standard error).

	Training group		Control group	
	Pre training	Post training	Pre training	Post training
Maximum FBF (ml/100ml/min)	16.4 \pm 1.6	18.7 \pm 4.2	16.3 \pm 2.4	18.5 \pm 2.8
Resting FBF (ml/100ml/min)	3.9 \pm 0.6	3.4 \pm 0.5	4.6 \pm 0.8	4.2 \pm 0.6
Maximum FVC	0.19 \pm 0.02	0.18 \pm 0.03	0.19 \pm 0.03	0.22 \pm 0.03
Resting FVC	0.05 \pm 0.009	0.04 \pm 0.006	0.04 \pm 0.004	0.05 \pm 0.007

Chapter 5. DISCUSSION

The objective of this research was to test the efficiency of a novel method of resistance training: low intensity hand grip isometric exercise with partial vascular occlusion. The control group did conventional hand grip resistance exercise at a high intensity. It was hypothesized that 4 weeks of training would result in increases in maximum forearm blood flow and vascular conductance as compared to the conventional training. It was found that FBF did not increase significantly in either of the intervention or the control group. Additionally, no statistically conclusive difference was found between the groups.

Most of the previous studies to investigate the effects of low intensity training with vascular occlusion (LIVO) focused on changes in strength and size. To the best of our knowledge, the present study is the first to investigate the role of LIVO on small muscle blood flow in the elderly. In a recent study, Patterson and Ferguson (2011) also studied strength and blood flow in older people with blood flow restriction exercise. Their protocol consisted of 4 week of dynamic plantarflexion with low load training (25% 1 RM) with occlusion pressure of 200mmHg. Both strength and calf blood flow were found to be significantly higher post training. One of the reasons for diminished rise in our study could be that there is a variable pattern between the age-based decline in blood flow (and vascular conductance) in forearm and calf musculature. In addition, it has been seen that gender also plays a role in determining this decline. Ridout et al. (2005) demonstrated that the relative decline in vascular conductance with age is greater in the calf, as compared to forearm, in females. They found a contrasting result in men where the decline was higher in forearm muscle as compared to the calf (Proctor et al., 2004). Our study had 11 female and 8 male

participants. Comparing the pre-training maximum FBF between male and female participants after collapsing the two groups together, the difference was found to be statistically significant ($p < 0.05$). However, post-training difference between the genders' FBF was not significant but it did show a trend ($p = 0.06$). Another crucial factor to consider is the difference in forearm volume in men and women. Thompson and colleagues (2007) demonstrated that absolute FBF in women was significantly lower than men after fatiguing isometric contractions at 20% MVC. In our study as well, absolute max FBF (pre training) in women was found to be significantly lower than men. Since females have lower muscle mass, they generate lower force for a task of same intensity. As a result, they develop much lesser intramuscular force and hence, less vascular occlusion (Heyward and McCreary, 1978). These gender based differences could have contributed to our findings. Moreover, since the muscle volume is related to the amount of vascular occlusion, the wide difference between the sizes of calf and forearm can also help to explain the diminished increase in absolute blood flow in the current study.

Most studies investigating LIVO, including the one by Patterson and Ferguson (2011), have used isotonic training methods. Takarada et al. (2000) found gains in strength and size of elbow flexors with low intensity (50-30%) vascular occlusion (110mmHg). The subjects performed single-arm dumbbell curl with occlusion until failure. Moore et al. (2004) also used a similar protocol with elbow flexion with occlusion for 8 weeks. During dynamic exercise, the blood flow is relatively maintained due to the lengthening and shortening of the muscles. For similar occlusion pressure and exercise intensity, isometric training provides an environment of higher vascular occlusion as compared to dynamic training. So, the results

reported in studies with dynamic exercise may not have been truly due to ‘occlusive exercise’.

The response to LIVO also depends upon the dominant muscle fiber type in the muscle. As was previously mentioned, the increase in cross sectional area with resistance training was higher in type II (27.6%) versus type I muscle fibers (5.9%) (Yasuda et al., 2005). Their protocol consisted of squat training with occlusion pressures of 160-240mmHg. The current study involved the forearm musculature, which has predominantly type I fibers (McIntosh, et al., 1985), so the extent of size gain is expected to be small. We found no increase in girth in either of the groups.

A recent study by Fahs et al. (2011) compared acute vascular responses of high intensity (HI), low intensity (LI) and low intensity exercise with blood flow restriction (LI-BFR). It was seen that the increase in calf vascular conductance was highest in HI, slightly elevated in LI and unaffected during LI-BFR. In addition to this, calf blood flow was also not found to be increased after LI-BFR. Karabulut et al. (2010) studied the effect of high intensity (80% 1 RM) and low intensity with occlusion (20% 1 RM; mean occlusion pressure 205.4 ± 4.3 mmHg) on leg extension strength for a training period of 6 weeks in older men. Higher intensity exercise was found to have significantly greater strength gains than low intensity with occlusion. The presence of contrasting literature warrants further research in this area before any conclusion can be drawn regarding the applicability of LIVO.

The current study’s most significant limitation was the small sample size. A statistically more powerful trend might have been seen in a bigger group. This is supported by a very small Cohen’s *d* for both maximum FBF (0-.012) and peak MVC (-0.23), indicating no

difference between the groups. A sample size of 16000 was calculated as required to demonstrate differences between the groups. Another limitation in the research design could be the fact that BP was not measured throughout the exercise period. Blood pressure rises with isometric exercise and may have exceeded the occlusion pressure at 130% of resting SBP. So, effectively, the occlusion pressure may have been less than 130% SBP. Additionally, in order to avoid any errors in the training method and to habituate the participants to their respective protocol and devices, no familiarization trials were conducted. This may have led to ineffectiveness in the initial few sessions.

Additionally, the 4 week study period might not have been sufficient to evoke a convincing and significant outcome. Most of the studies done to study the chronic effects of LIVO have used 8 to 16 weeks of time to study the effect. For instance, Moore et al. (2004) trained young men for 8 weeks, Takarada et al. (2002) used 8 weeks for young athletes, Takarada et al. (2000) exercised old women for 16 weeks while Madarame et al. (2008) trained men for 10 weeks. Since, venous compliance of the elderly is compromised; a longer duration of the study would have led to a clearer interpretation.

Table 3. Protocols used in the training groups by various studies (\pm standard deviation).

Study of reference	Participant	Length of study	Type of exercise	Intensity	Occlusion pressure	Outcome
Abe et al. (2005)	Men (23.6 \pm 6.5 yrs)	2 weeks (12 sessions)	Isotonic (squat; leg curl)	20% 1RM	160-240mmHg	Significant increase in CSA and strength
Cook et al. (2007)	Men and women (27.7 \pm 4.9 yrs)	5 sessions	Isotonic knee extension	20-40% MVC	160-300mmHg	20% continuous occlusion of 160mmHg resulted in greater decrement than high intensity
Karabulut et al. (2010)	Men (56.8 \pm 0.6 yrs)	6 weeks (18 sessions)	3 upper body; 2 lower body	Upper body: 80% 1RM Lower body: 20% 1RM	205.4 \pm 4.3m mHg	Significant increase in leg extension strength but significantly lower than high intensity exercise
Madarama et al. (2008)	Men (21.6 \pm 2.4 yrs)	10 weeks (20 sessions)	Dumbbell curl, knee extension, knee flexion	50% 1 RM (arm); 30% 1 RM (leg)	160mmHg, (+20mmHg every week)	Significant increases in isometric torque, CSA & 1 RM
Moore et al. (2004)	Men (19.5 \pm 0.4 yr)	8 weeks (24 sessions)	Elbow curls	50% 1 RM	100mmHg	Both dynamic and isometric strength increased significantly

Table 3. continued.

Patterson & Ferguson (2011)	2 women & 8 men (67±3 yrs)	4 weeks (12 sessions)	Dynamic plantar flexion	25% 1RM	110mmHg	Peak blood flow increased significantly
Shinohara et al. (1998)	Men (23.2 yrs)	4 weeks (12 sessions)	Isometric knee extension	40% MVC	>250 mmHg	Maximal rate of torque generation and MVC were significantly high
Takarada et al. (2000)	Women (58.2±6.6 yrs)	16 weeks (32 sessions)	Dumbbell curls	50%-30% 1 RM	110±7.1 mmHg	Significant increases in CSA and isokinetic strength
Takarada et al. (2002)	Men (25.0±0.6 yrs)	8 weeks (16 sessions)	Isotonic knee extension	50% 1RM	200mmHg	Significant increases in isokinetic knee extension torque and knee CSA.
Takarada et al. (2004)	Men (21.3±0.6 yrs)	8 weeks (16 sessions)	Isotonic knee extension	20% 1 RM	218±8.1 mmHg	Significant increases in isokinetic & isometric strengths and also in knee CSA
Yasuda et al. (2005)	Men (20-47 yrs)	2 weeks (2/day)	Dynamic lower body exercises	20% 1RM	160-240mmHg (+10mmHg/day)	Squat strength, quad CSA and type II muscle fiber CSA increased significantly

One important point to be considered was the discomfort reported by the participants. Though none of them dropped out because of discomfort or had any major health issue, there were consistent complaints of very unpleasant feelings during the occlusion. As a result, there were training sessions when they could not tolerate vascular occlusion for the full 5 minutes. One participant in the training group experienced petechiae during a training session. Because of this, she had to take a break for a couple of sessions. It led to inconsistencies during training sessions leading to different exercise volumes between the intervention and control group. This limitation might have prevented true training effects.

The uneasiness experienced by the subjects calls for an alteration in the protocol to make it more comfortable. The practical implications of the current protocol are dependent on its acceptability by the target group. The primary factors which can be manipulated are the occlusion pressure and the time. An occlusion pressure of 110% SBP may be more tolerable while the total time period can be extended more than 5 minutes.

Chapter 6. CONCLUSION

The result from the study showed that neither blood flow nor vascular conductance changed after 4 weeks of training in any of the groups. This outcome does not support our hypothesis. It should be noted that even the conventional high resistance training group did not report any significant change, although it has been well established that the geriatric population benefits from resistance training (Fiatarone et al., 1994), as has been seen in studies with a long term intervention program (e.g., 10 weeks used by Seynnes et al, 2004, 26 week used by Pollock et al., 1991, 12 months used by Pyka et al., 1994).

This study is the first to measure FBF and FVC after hand grip training with occlusion in the elderly. The true effects could have been obscured by the limitations of the study. A better research design may have provided a better conclusion. Further research is needed to demonstrate the vascular benefits of occlusive training in the elderly.

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