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**Flow mediated vasodilation changes in older and younger adult groups
after 4 weeks of low intensity hand grip isometric training with vascular
occlusion**

by

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in partial fulfillment of the requirements for the degree of

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ABSTRACT

Introduction:

Low intensity blood flow restricted exercise (BFRE) has been shown to increase muscle strength similarly to high intensity (HI) exercise in young adults. This may be beneficial in older adults to attenuate sarcopenia but without the negative side effects of HI exercise. The purpose of this study was to compare brachial artery flow mediated dilation (FMD) before and after 4 weeks of low intensity isometric hand grip BFRE. The comparison will be between three cohorts, namely a young adult group with BFRE (YR), an older adult group with BFRE (OR) and an older adult group with HI exercise (OH). My hypothesis is that there will be a significant difference between FMD, brachial artery diameter and peak flow velocity responses to reactive hyperemia in YR and OR as compared to OH after the 4-week training.

Methods:

Eight individuals comprised YR, ten individuals comprised OR and nine individuals comprised OH. YR and OR performed low intensity (20% maximum voluntary contraction (MVC)) isometric handgrip training exercises during 5 minutes of BFRE three times weekly for 4 weeks. OH performed high intensity (75% MVC) isometric handgrip training exercises without blood flow occlusion. Before and after 4 weeks of training, resting brachial artery blood flow velocity

and diameter, and peak brachial artery blood flow velocity and diameter during reactive hyperemia were measured. FMD as well as shear rate (SR) were calculated for all groups.

Results:

No significant differences were found between brachial artery diameter and peak blood flow velocity during reactive hyperemia, nor for FMD and SR, when compared before and after training for individual subjects. Neither were any significant differences found for these parameters between YR, OR, and OH groups.

Conclusion:

Data from this study did not support the hypothesis nor other findings in the literature. The differences in these results may have been related to study design or implementation including variation in training type, intensity and frequency as well as accuracy of data collection. Several limitations of this study, including sonographer experience, small sample size, occult vascular aging changes and inconsistencies in ultrasound measurements due to timing and position, may have affected the results. Future studies controlling for these limitations may have a different outcome.

CHAPTER 1. INTRODUCTION

By 2050, there will be 83.7 million people aged 65 and older, double the 43.1 million people age 65 and older in 2012 (1). Sarcopenia, muscle loss with aging, and endothelial dysfunction are two degenerative changes related to age. Sarcopenia is related to age alone, independent of other major chronic morbidities and diseases (2). Functionally and histologically, the muscle atrophy from sarcopenia is the same as other muscle wasting conditions such as muscle atrophy from muscle disuse and neurologic injury. Exercise decreases but does not eliminate sarcopenia in older adults (3).

Endothelial dysfunction as a result of abnormal nitrous oxide signaling pathways may be a result of oxidative stress, cardiac diseases, atherosclerosis and other inflammatory vascular disease. Older adults have basal endothelial dysfunction and abnormalities of the nitrous oxide (NO) dilator pathways (4). Nitrous oxide signaling pathway dysfunction may be part of age related hemodynamic changes (5).

Guidelines set forth by the American College of Sports Medicine recommend resistance exercise training at least 70% of one-repetition maximum (1-RM) load to increase muscle size and strength (6). Resistance training in older adults has been shown to increase muscle strength and endurance (7). High intensity exercise, however, can exacerbate hypertension and orthopedic problems in older adults. (8). An exercise technique that restricts blood flow to exercising muscle with low intensity exercise is called KAATSU (9). Low intensity blood flow restricted exercise (BFRE) has yielded similar increases in muscle

size and strength as compared to high intensity exercise without blood flow restriction in both young and old adults (10,11,12,13,14). This method may be an alternative to traditional resistance exercise that uses the aforementioned heavy muscle loading (9).

Muscular improvements from BFRE training may benefit those with decreased strength and joint stability such as older adults, post surgical patients, or athletes undergoing rehabilitation (15). Low intensity BFRE create lower joint and ligament stress forces when compared to high intensity exercise (12, 17). Vascular occlusion training may also prove beneficial for astronauts while in zero gravity environment (16). As one example, in a 16-week low intensity handgrip exercise study at 30-50% 1RM with blood flow occlusion in older women, increases in elbow flexor muscle strength and muscle cross sectional area (CSA) were similar to high intensity (HI) exercise (13). This study used 3 sets of dumbbell curl exercise (2 second contraction, 2 second relaxation) with occlusion for 5 minutes with 1-minute rest in between twice per week (13). Plasma lactate levels were found to be significantly increased in the BFRE group as compared to the HI group (13). Blood flow restricted exercise therefore may provide benefits for reducing sarcopenia in older adults. However increased pain scores with low intensity BFRE can lead to a lower number of repetitions or failure to exercise (18).

The effect of BFRE on vascular changes is not fully understood. Some studies have shown beneficial changes such as increased filtration capacity (19), increased brachial artery diameter (20) and increased arterial compliance (21). However other BFRE studies have shown either no change or a decrease in flow mediated dilation (22, 23).

Flow mediated vasodilation (FMD), or vasodilation caused by endothelial release of nitric oxide, can be assessed noninvasively using ultrasound to evaluate diameter and blood flow (BF) velocity in larger blood vessels (24). FMD measurement can be used to assess peripheral artery disease and monitoring endothelial dysfunction in older adults (25). Endothelial dysfunction precedes atherosclerosis such that assessment of endothelium mediated vasodilation has been used to evaluate endothelial function (26). Endothelial nitric oxide synthase (eNOS), an enzyme that produces nitric oxide (NO), helps maintain normal endothelial function in homeostasis (26). However, eNOS can switch through eNOS uncoupling and generate reactive oxygen species (ROS) (26). ROS causes sustained endothelial activation leading to endothelial dysfunction (26). FMD can be used to diagnose subclinical coronary vessel disease before overt atherosclerosis is present (27). In individuals with subclinical coronary artery disease, as confirmed by exercise myocardial perfusion imaging with computed tomography, there has been found a correlation with abnormal brachial artery FMD response (27). This change in brachial artery FMD is found before

atherosclerotic plaques are detected in coronary vessels by angiography studies (27). A decreased FMD response in older adults with peripheral artery disease is predictive of long term cardiovascular events; FMD has been shown to be useful in predicting future cardiac events (28, 29). Patients with atherosclerosis and coronary risk factors causing impaired brachial artery endothelial function independently predicted long term cardiovascular events such as cardiac death, myocardial infarction, and stroke (30).

The changes in FMD with BFRE have shown mixed results in the literature. Studies with forearm BFRE in younger adults have shown increased peak blood flow and brachial artery diameter but no change in FMD (20) while other studies have shown decreased FMD (22). Also with younger adults but with BFRE using leg muscles, FMD decreased (23). No studies with older adults with BFRE measured FMD. However in one study with older adults using resistance exercise, occlusion was applied for 5 minutes after training and FMD was found to increase (31).

Increased FMD secondary to increased NO production may be evident with exercise lasting less than 4 weeks, before increases in arterial size normalize shear rate levels (32). NO signaling pathway dysfunction may be part of age-related hemodynamic changes (33). Older adults have basal endothelial dysfunction and abnormalities of the nitrous oxide (NO) dilator pathways (4). Because these hemodynamic and vascular changes occur with aging, the FMD response following low-intensity blood flow-restricted exercise may be different

compared to younger adults. To my knowledge, no study has compared the effects of low intensity BFRE on FMD between young and older adult groups. In addition, no study has assessed FMD responses with low intensity BFRE in older adults compared to high intensity exercise in older adults.

As the older adult population is increasing so will the number of people affected by degenerative age-related changes namely, sarcopenia and peripheral artery disease. High intensity exercise can attenuate these changes but high load exercise can cause complications in older adults. BFRE may be beneficial in older adults because it uses lower exercise intensity and produces less joint stress. However, there have been few studies in older adults. Also the efficacy of BFRE may be limited as a long term exercise program because it causes pain. Assessment of FMD can be used to monitor vascular response to exercise, as well as to monitor vessel health in disease. Assessment of FMD in BFRE has shown mixed results after 4 weeks of training. These studies have all been completed in younger adults.

In this study, brachial artery flow mediated dilation (FMD) before and after 4 weeks of low intensity isometric hand grip BFRE will be compared. The comparison will be between three cohorts, namely a young adult group with BFRE (YR), an older adult group with BFRE (OR) and an older adult group with HI exercise (OH). The purposes of this study were to 1) compare flow mediated dilation in two populations (YR and OR) before and after 4 weeks of low-intensity hand grip isometric training with vascular occlusion, and 2) compare flow

mediated dilation in two older populations (OR and OH). My hypothesis is that there will be a significant difference between FMD, brachial artery diameter and peak flow velocity responses associated with reactive hyperemia after release of vascular occlusion in YR and OR as compared to OH after the 4-week training period

CHAPTER 2. LITERATURE REVIEW

Aging hemodynamics

Sarcopenia is muscle degeneration due to age. Functionally and histologically, the muscle atrophy from sarcopenia is the same as other muscle wasting conditions such as muscle atrophy from muscle disuse and neurologic injury. The rate of muscle loss with age appears to be relatively consistent, approximately 1%–2% per year after the age of 50 years (3) but it accelerates after the age of 65 (2). The mechanisms of sarcopenia are not fully understood; however, with age there is a loss of skeletal muscle fiber number as well as decrease in cross sectional area of the remaining fibers (3). There is an inverse association of grip strength and age (2). This muscle loss has been shown to be related to age alone, independent of other major chronic morbidities and diseases (2). Therefore, sarcopenia occurs in older adults who are otherwise apparently healthy (2). Exercise decreases but does not eliminate sarcopenia in older adults (3). Advanced sarcopenia has been independently associated with an increased likelihood of functional impairment and disability in older adults (33).

Blood flow changes may be related to sarcopenia. In healthy older men, basal whole limb blood flow and vascular conductance decreased progressively with advancing age (31). Capillary density is also reduced with advancing age (34). There was an associated lack of expression of transforming growth factor-1 (TGF-1) and vascular endothelial growth factor (VEGF) in this study (34). Older adults can develop abnormalities in basal endothelial function as well as

abnormalities of the prostaglandin (PG) and NO dilator pathways (4). In a study comparing younger men and women to older men and women, a diminished endothelium-dependent responsiveness resulted from a decrease in NO-mediated vasodilation (5). Therefore, abnormalities in the NO signaling pathway are part of the age-related hemodynamic changes (5).

Flow mediated vasodilation

Reactive hyperemia is a transient increase in blood flow caused by brief ischemia (35). Reactive hyperemia is a form of intrinsic control of blood flow, matching the local needs of the tissue via changing blood flow. During this ischemia, tissue hypoxia occurs and vasodilatory metabolites including lactic acid, adenosine, hydrogen ions, and potassium ions increase, as well as NO, causing dilation of arterioles which in turn decrease peripheral vascular resistance (36). After the occlusion to blood flow is released, there is an increase in blood flow in the conduit arteries such as the brachial artery (35). This increase in blood flow increases shear stress in the conduit artery distal to the occlusion, which stimulates the endothelial release of NO, causing vasodilation (36). The ability of the endothelium to dilate in response to increased blood flow and shear stress is a unique ability dependent on endothelial receptors and cascades with the end release of NO.

The shear stress is sensed by the endothelium through changes on the cell membranes such as the primary cilia or mechanosensitive ion channels. One

such channel is the calcium activated potassium channel activation which causes hyperpolarization of the endothelial cell which increases the driving force for calcium entry into the endothelial cell (37). Increased calcium increases a G protein that increases phosphokinase A (38). This activates endothelial nitric oxide synthase (eNos) activity, which then converts L-arginine to generate NO (38). Nitric oxide then acts in a paracrine fashion on smooth muscle cells by activating soluble guanylate cyclase which converts GTP to cGMP through a decrease in calcium in the cytosol, leading to vasorelaxation (39). cGMP activates protein kinase G causing a reuptake of calcium into the sarcoplasmic reticulum leading to opening of calcium activated potassium channels (40). Decreased calcium in the cytosol inhibits myosin light chain kinase which leads to decreased phosphorylation of the myosin molecule. This stops cross bridge cycling and leads to smooth muscle relaxation (40). The release of NO is central to flow mediated vasodilation.

Increased shear stress from increased blood flow leads to increased vasodilation termed endothelial dependent flow mediated dilation. Flow mediated dilation can be induced locally by intermittent occlusion to blood flow to the peripheral circulation during reactive hyperemia or it can be induced globally by various parenterally administered drugs that induce NO release. One of the first non-invasive methods used to measure flow mediated dilation was using a dual crystal pulsed Doppler system to calculate width of the moving blood column to infer vascular diameter (41). More recently, other noninvasive methods have

been used including the FMD method (42), pulse wave analysis (PWA) (41), pulse contour analysis (PCA) (41) and photoplethysmographic pulse amplitude (PPG) (43). Each method has different pros and cons that make them suitable to different clinical or research applications. The FMD method, which uses 2-D B-mode and pulse wave Doppler, is accurate and reproducible but operator dependent, expensive and technically demanding requiring considerable expertise (42). PWA is inexpensive and portable but has more variability and lower reproducibility (44). PCA and PPG require little training and are operator independent but are more variable (44, 43). Although the FMD method has a higher learning curve to use, it is the most commonly used method for FMD studies (45).

Various protocols have been used with the FMD method and different study design factors can influence the outcome. These factors include occlusion pressure, occlusion duration, cuff location, ultrasound machine capabilities and post-processing FMD analysis. For consistency and subject comfort, cuff occlusion pressure to blood flow should be 25-50 mmHg above resting systolic blood pressure (45). Cuff duration should be 5 minutes (46). No greater maximal arterial dilation is observed after 5 minutes (46). Also arterial dilation with cuff duration lasting more than 5 minutes is more influenced by non-NO mediated vasodilation (46). There is no consensus on cuff location, either distal or proximal to the measurement of the blood vessel; however, a proximal location has greater peak hyperemic response and FMD measurement (45). Ultrasound

machine capability should include Duplex mode that allows simultaneous acquisition of B-mode and Doppler for vessel diameter and velocity (45). Duplex mode allows measuring the cumulative exposure to shear rate experience by the artery that contributes to FMD by calculating the shear rate under the curve area (47). Post-processing FMD analysis should include edge detection software (48). Edge detection software allows a more robust and sensitive assessment of FMD and removes operator error (48). Peak blood flow arterial velocity occurs in the first 15 seconds after blood flow occlusion is released during reactive hyperemia and peak artery vasodilation occurs 45-80 seconds after occlusion is released (49). Although Duplex mode and edge detection software is better to measure shear rate integral over time to more accurately reflect FMD measurement, acceptable and reproducible FMD measurements can be made by capturing peak hyperemic during the first 15 seconds after blood flow occlusion release and then switching to 2-D B mode to measure maximal brachial artery over the subsequent 2 minutes (49).

Flow mediated dilation with aging

Arterial stiffness increases with age in sedentary healthy women and men (50, 51). Aging is associated with loss of elasticity in arterial conduits and causes increased systolic pressure (52). In older adults, it has been shown that muscle blood flow is decreased and vascular resistance is increased in the legs (53). Increasing age is associated with decreasing endothelium-dependent vasodilation (54). A decline in flow mediated dilation of brachial arteries is seen

in men as young as 40 years of age (24). This decline is delayed in women which may be due to a protective effect of estrogen on the arterial wall, because menopause is associated with a decrease in FMD (24).

Increased age is associated with decreased exercise hyperemia which may be caused by decreased NO production from the endothelium (55). In a study with older adults using dynamic handgrip exercise, the contribution of NO to exercise hyperemia was reduced 45% compared to younger adults (55). N-nitro-L-arginine-methyl-ester (L-NAME) was used to inhibit NO synthase and ketorolac was used to inhibit PG, and the effect of this inhibition on vasodilation was compared between the younger and older adult groups (56). In older adults, NO and PG inhibition have less effect on exercise hyperemia due to decreased availability of NO and PG mediated vasodilation seen with age (55). The role of PG in vasodilation is lost with aging in skeletal muscle (55). Pharmacological inhibition of NO in older adults using low intensity 20% 1-RM hypoxic exercise did not decrease the vasodilation response, suggesting a blunted role of NO in reactive hyperemia in older adults (56). In older adults performing handgrip exercise, endothelium dependent vasodilation was improved when ascorbic acid was administered (57). Ascorbic acid, acting as an antioxidant, improves vasodilation in these older adults by increasing NO bioavailability (57).

The mechanisms to explain the changes in age associated decrease in endothelium-dependent vasodilation are not fully understood and likely multiple

mechanisms are involved. One possible mechanism is cumulative damage over time to vessel function by reactive oxygen species. Increased oxidative stress with age may decrease prostaglandin bioavailability and thereby decreasing reactive hyperemia response (58). To investigate this possibility further, researchers used a rat model and found no difference between young and old rat groups when comparing resting arteriolar diameter and blood flow (58). However, with muscle contraction, arteriolar diameter and blood flow was greater in the young rat group compared to the older rat group (58). Indomethacin was used to inhibit cyclooxygenase activity but didn't change the hyperemic response to contracting muscles in either the younger or older rat group; therefore, decreased prostaglandin bioavailability is an unlikely mechanism to explain the decreased hyperemic response in older rats with exercise (58).

Reversing the age-associated loss in endothelium dependent vasodilation through regular aerobic exercise lowers cardiovascular disease risk in older men (59). Exercise may have this beneficial effect on endothelium dependent vasodilation because of increased eNOS expression, increased PG release and decreased sympathetic vasoconstrictor tone (59). Endothelial dysfunction precedes atherosclerosis and assessment of endothelium mediated vasodilation has been used to evaluate endothelial function (26). Measurement of the brachial artery FMD has become the gold standard for assessing conduit artery endothelial health (26). The response of the endothelium as measured by FMD can show the transition to unstable disease states such as atherosclerosis (26).

Decreased arterial compliance can lead to increased cardiovascular

diseases such as coronary heart disease, stroke and atherosclerosis (60). A decreased FMD response in older adults with peripheral artery disease is predictive of long term cardiovascular events (30). Patients with atherosclerosis and coronary risk factors causing impaired brachial artery endothelial function independently predicted long term cardiovascular events such as cardiac death, myocardial infarction, and stroke (30).

With exercise, the increase in blood flow and flow mediated vasodilation is decreased in older adults compared to younger adults. This endothelial dysfunction can be assessed by measuring FMD.

Flow mediated dilation with exercise

Guidelines set forth by the American College of Sports Medicine recommend exercising at least at 70% one-repetition maximum (1-RM) load to induce changes in muscle size and strength (6). Resistance training in older adults has been shown to increase muscle strength and endurance (13). A robust dose response relationship in older adults between intensity of resistance training and strength gains has been noted (61). This gain in strength from resistance training in older adults leads to functional improvements (61). In a study in older adults assessing knee extensor strength endurance, the low intensity group, training at 40% 1-RM, revealed insufficient strength increase compared to the high intensity group training at 80% 1-RM (61).

Higher load exercises however can cause complications in older adults. In one study, older women exhibited higher levels of muscle damage after chronic high intensity exercise as compared to young women (62). High intensity exercise can cause hypertension and orthopedic problems in older adults. (8). In another study, older men had a lower heart rate but higher blood pressure in response to isometric exercise compared to the young men (8). Systolic pressure increased progressively with exercise and showed a greater change in older subjects (63). In contrast, total peripheral resistance at rest increased with age as well (63). Regular aerobic exercise decreased blood pressure in older adults while increasing vasorelaxation in older adults with hypertension (64). This decrease in blood pressure in older adults with regular aerobic exercise was noted in another study as well using healthy participants (65).

Chronic regular exercise may improve endothelium dependent function (66). Exercise-induced changes in shear stress provide the principle stimulus to flow mediated endothelial function and vascular remodeling. (67). Brachial artery peak reactive hyperemia increased in healthy men following 8 weeks of handgrip training (67). High intensity exercise in lean adults showed increased FMD as compared to obese adults (68). And in another study, FMD increased with moderate intensity moderate duration and high intensity short duration aerobic exercise in healthy young men (69). Even in active overweight men acute exercise resulted in increased FMD compared to inactive overweight men (70). And also in some cardiovascular disease conditions FMD may show improvement with exercise. In a study with people with coronary artery disease

brachial artery FMD increased after a single acute session of exercise (71). In conclusion, FMD improves with exercise in healthy or overweight young adults with aerobic or handgrip exercise, and even in people with cardiovascular disease.

However, in a study with young men completing resistance exercise over 12 weeks five days a week with 2-3 sets of 10-12 repetitions of various upper and lower body exercises, it was found that FMD did not change (72). In this study, brachial artery diameter increased and post occlusion blood flow increased (72). FMD may not have changed since the endothelium may have been maximally functional before training or the shear stress may have been too short and transient to cause changes in conduit artery endothelial function (72). Although FMD did not change, this study showed peripheral artery remodeling does occur (72). Increased FMD due to increase NO may be evident in the short term (less than 4 weeks) before increases in arterial size normalize shear rate levels (32). In a study with 8 week treadmill and cycle exercise with young healthy males, FMD was increased at 2 and 4 weeks but normalized at 8 weeks (32).

Moderate exercise may lead to attenuated FMD response as was the case in a study of healthy older women undergoing moderate intensity leg cycling (73). As compared to the younger adult women cohort in this study, the blunted FMD response may have been the result of age associated reduction in cardiac output

and muscle mass and impaired local vasodilation (73). In a different study older healthy sedentary men underwent chronic aerobic walking exercise and acetylcholine-mediated vasodilation increased 30%, similar to levels of middle aged endurance trained men (59). Acetylcholine-mediated FMD increases NO through activation of eNOS. In a study with older individuals with heart failure completing daily handgrip exercise (70% MVC) with no occlusion acetylcholine mediated FMD increased (74). FMD response therefore can be affected by the type of exercise, the intensity and duration of exercise and by arterial or cardiovascular disease.

In a study using young men, regular handgrip exercise with no occlusion for 4 weeks produced a 30% increase in reactive hyperemic blood flow (75). This was measured with a strain gauge plethysmograph using a venous occlusion technique after 10 minutes of arterial occlusion (75). Overall resistance decreased and localized skeletal muscle forearm work was associated with increased localized vasodilation (75). Isometric handgrip training lowered resting arterial blood pressure while FMD was unchanged in one study with normotensive individuals (76). In a study of older individuals with hypertension, FMD only improved in the trained arm but blood pressure did decrease (76). Reactive hyperemic blood flow and FMD may be a local effect but even with isolated isometric exercise overall blood pressure and resistance may be decreased.

In conclusion high-intensity (HI) exercise, or exercise greater than 70% 1-RM, has been shown to increase muscle mass and strength in older adults but

can cause complications such as hypertension. Regular aerobic and isometric exercise can improve vasodilation. FMD improves with exercise especially with acute high intensity exercise. Even if FMD and reactive hyperemic blood flow occur locally, overall blood pressure can decrease. However, FMD is affected by the type of exercise, the intensity and duration of exercise and by arterial or cardiovascular disease.

Arteriogenesis, shear stress, and exercise

Larger arteries such as the brachial artery are termed conduit arteries. These supply blood flow to smaller resistance vessels such as arterioles. Arteriogenesis describes an increase in diameter of conduit arteries that is not caused by either increased pressure or wall compliance. Rather, it reflects actual remodeling of the cell types that make up the vessel wall, namely endothelial cells, smooth muscle cells and fibroblasts (77). Arteriogenesis involves remodeling arterioles into collateral arteries (77). The main driving force of arteriogenesis is shear stress (77). The increased blood flow leads to vessel enlargement (78). In one experiment, using rabbits an arteriovenous fistula was created between the left common carotid artery and external jugular vein. This chronic increase in blood flow led to increased endothelial NO production and increased artery diameter by 75% compared to vessels where NO was inhibited

by N-nitro-L-arginine-methyl-ester (L-NAME) (78). In another study, increased NO from increased shear stress led to vascular remodeling by growth factor induction, and matrix metalloproteinase (MMP) activation (79). The vessel wall remodeling increased vessel diameter (79). Monocytes, after adhering to the vessel wall and becoming macrophages, are important for arteriogenesis through the release of cytokines and growth factors (80). These include MCP-1, which attracts more macrophages, and TNF2-alpha which creates an inflammatory environment (80). These act with MMPs to remodel 'old' arterial structures (80).

There is a robust association between resting wall shear stress and FMD (81). The luminal diameter of blood vessels, as shown in healthy men, changes to maintain a constant level of shear stress as chronic changes in blood flow occur. This is described by the relationship: Wall shear stress = Blood viscosity \times Blood flow velocity/Internal diameter (81). For example, increased shear stress from a 400% increase in blood flow led to increased artery lumen by 33% by day 7. In addition there was a 37% and 18% increase in size in the medial and intimal layers, respectively, by the second day (81). Also cell densities increased 26% and 44% in the intimal and adventitial layers by the second day (82). Gene expression of endothelial nitric oxide synthase (eNOS) was also increased by the second day (82). VEGFR2, an endothelial cell receptor for VEGF, acts as a shear stress sensor and transduces the signal of a change in blood flow (83). Conduit artery modification occurs in response to localized short term resistance exercise training secondary to altered shear stress (67).

The term arteriogenesis describes an increase in blood vessel diameter from remodeling as a result of increased NO that is induced by shear stress from increased blood flow. Increased blood flow leads to increased internal pressure causing increased radial wall stress on the endothelial surface which increases wall mass according to the Laplace principle. This increases shear rate as the blood flow velocity is slower at the vessel wall, increasing friction between the blood and vessel wall. The increased shear rate increases shear stress. The shear stress is sensed by VEGFR2 and leads to increased NO and eNOS. Exercise causes increased blood flow and shear stress leading to blood vessel changes.

Angiogenesis, hypoxia and exercise

Angiogenesis is the creation of new blood vessels from pre-existing vessels (77). The main driving force for angiogenesis is hypoxia (77). Vascular endothelial growth factor (VEGF) is an important mediator in the initiation of hypoxia induced angiogenesis (77). With endurance training, the number of capillaries increase (84). Increased capillary growth found with endurance training has been noted to be similar in men, women and in the older population (85). Exercise increases capillary density in skeletal muscle as a response to

increased metabolic demand (86). With training the number of capillaries per muscle fiber is higher in endurance trained women than non-trained women (86).

Hypoxia-induced angiogenesis is largely influenced by VEGF (87).

Hypoxia leads to increased transcription of factor HIF-1, increasing genes for NOS and VEGF (88). NOS and VEGF activate endothelial cells which leads to vessel remodeling and growth (88). VEGF then acts through endothelial surface tyrosine kinase receptors, VEGFR1 and VEGFR2, which activates endothelial cells for proliferation and migration (89). Increased shear stress leads to NO activation, increasing VEGFR2 and VEGF expression and then to angiogenesis (90). Arteriogenesis is also initiated by increased NO from shear stress and activation of VEGFR2 receptors. Ets-1, a transcription protein activated by VEGF, regulates expression of proteases, such as MMP-1, and allows migration of endothelial cells into the interstitial space to initiate angiogenesis (91).

Hypoxia increases VEGF and mediates hypoxia-initiated angiogenesis (92). Hypoxic exercise increases VEGF mRNA levels (93). In older adults, diminished angiogenesis is correlated with decreased VEGF levels (94). However, in a study using hypoxic exercise training in rats, gene expression of VEGF and its receptors was not found although an increase in skeletal muscle capillarity was (95). Therefore VEGF is a main factor in hypoxia initiated angiogenesis but likely is not the only factor. In a study using acute low intensity ischemic leg extension exercise in men, both growth hormone (GH) and VEGF were increased (96). In another study, GH also increased with low intensity ischemic exercise when compared to moderate exercise without occlusion (97).

With ischemia only, GH does not increase, but with blood flow restricted exercise, circulating GH concentration does increase (98). In another study with low intensity ischemic exercise, the metabolic stress, as measured by decreased intramuscular phosphocreatine and pH, needed for fast twitch fiber recruitment was greater than low intensity exercise without ischemia (99). However, the metabolic stress in low intensity ischemic exercise was less than high intensity exercise without ischemia (99).

In summary, exercise and hypoxia lead to an increase in capillary density. Hypoxia is the main driving force for angiogenesis. Hypoxia increases VEGF which activates endothelial cells to create new blood vessels. With exercise, VEGF increases. VEGF levels decrease with age. However, other factors than hypoxia, such as GH, contribute to angiogenesis.

Blood flow restricted exercise

Yushiaki Sato from Japan patented an exercise technique of vascular occlusion moderator training known as KAATSU or blood flow restricted exercise (BFRE) (9). This technique restricts blood flow to exercising muscles and increases muscle mass and strength even with low intensity exercise (9). Muscle hypertrophy by this method is an alternative to traditional resistance exercise that uses heavy muscle loading (9). In injured athletes, post surgical patients, individuals with neurological issues or older individuals where high intensity

exercising may be contraindicated, BFRE may provide benefits in preventing nonuse muscle atrophy and sarcopenia. For example, in patients after anterior cruciate ligament repair surgery, 16 weeks of BFRE produced increased cross sectional area of the extensor knee muscles (100). In this study, a pressure band with 180mmHg pressure was applied across the proximal thigh; straight leg raising and hip joint abducting exercises were completed in 2 sets (20 times for each set) daily six times per week.

Low intensity BFRE has resulted in similar increases in muscle size and strength compared to high intensity exercise without blood flow restriction in both young and old adults. In young men, low intensity BFRE increased maximum isometric strength by 8-10% and muscle cross sectional area (CSA) by 4-7% (101). Training was twice a day while walking with proximal leg occlusion for 6 days a week for 3 weeks (101). Training was for 2 minutes on a treadmill at 50 meter per minute for 5 repetitions, with 1-minute rest intervals in-between (101). In young untrained men, low intensity exercise, using isometric knee extension at 40% MVC for 3 minutes was done 3 days per week for 4 weeks, comparing one leg with occlusion and one leg without occlusion (12). Increased muscle strength was measured in the occluded leg (12). It was concluded that even at a low intensity, exercise with occlusion was beneficial (12). In another study, maximal voluntary contraction increased by 8.3% in untrained young men following moderate intensity at 50% 1RM isometric elbow flexion with occlusion training for 8 weeks 3 times per week using a defined variable amount of repetitions in each set (102).

Athletes, who may already have maximal endothelial vasodilatory function from training, were shown to still benefit from BFRE. In a study with rugby players, moderate intensity exercise at 50% MVC with occlusion results in increased muscle strength and cross sectional area of knee extensor muscles (17). Knee extensor training was four sets of repetitions until failure with 30 second rests in between (17). The training lasted about 10 minutes with occlusion and was for twice a week for 8 weeks (17). In another study, the effects of low to moderate intensity exercise at 30-50% 1-RM with occlusion in older women was compared to high intensity at 80%1-RM exercise without occlusion (13). After 16 weeks of training, it was found both groups had similar increase in muscle strength and CSA (13). Training used dumbbell curls using 3 sets of repetition until failure with one minute rests (13). Training with occlusion lasted 5 minutes and was twice a week for 16 weeks (13).

Increased pain scores with low intensity BFRE can lead to a lower number of repetitions or failure to exercise (18). In a study with young men and women completing extensor knee exercise with blood flow occlusion, many participants were unable to complete as many sets of exercise when using wider and less elastic cuffs as compared to narrower and more elastic cuffs because of high levels of pain (18). Besides cuff size, the number of exercise sets, duration and rest periods can also increase pain perception reporting with BFRE compared to

low intensity exercise with no occlusion (103). The efficacy of BFRE may be limited as a long term exercise program because of this associated pain.

Although direct comparisons between these studies are difficult because of differences in the variables of age, gender, exercise type, exercise intensity and exercise duration, some general trends are observed. These studies demonstrate that similar or improved gains in muscle strength and size can be accomplished using low to moderate intensity BFRE compared to traditional high intensity exercise in both young and older adult populations.

Blood flow restricted exercise and metabolic changes

Changes in metabolic factors during ischemic exercise may explain mechanisms of arteriogenesis and angiogenesis in blood flow restricted exercise. In a comparison of 30% 1-RM BFRE to high intensity exercise at 70% 1-RM without occlusion, it was found that growth hormone concentrations were higher in the exercise group with occlusion (97). This study involved young adults completing bicep curls and leg calf presses, performing 3 sets to failure with one minute rest periods in between. Each participant performed the exercises, at both low intensity with occlusion and with high intensity without occlusion, but on separate days (97). In another study with young men and low intensity knee extension exercise at 10-20% 1-RM with approximately 10-minute occlusion, CSA increased by 10.3% ($\pm 1.6\%$) (104). Growth hormone also increased in the low intensity exercise group with blood flow occlusion compared to either low intensity exercise with no occlusion or vascular occlusion with no exercise (104).

In this study five sets were completed to failure with one-minute rest periods twice a week for 8 weeks.

In a 3-week study in young men, blood flow occlusion of the thigh muscles while walking on a treadmill at 50m/minute showed significant increase in muscle strength, CSA and growth hormone (11). Training was twice a day for six days for 3 weeks consisting of 5 sets of 2 minute sessions on the treadmill with 1-minute rest periods. In a 16 week study with older women, growth hormone was increased with low to moderate intensity blood flow restricted exercise at 30-50% 1-RM (13). Training used dumbbell curls using 3 sets of repetition until failure with one minute rests lasting 5 minutes, twice a week for 16 weeks, with blood flow occlusion. These studies demonstrate an increase in growth hormone concentration does not have to be through high intensity traditional exercise, but can be increased with low intensity exercise as well when using blood flow occlusion. Growth hormone plays a role in increasing muscle strength, muscle mass, angiogenesis and arteriogenesis.

Other factors besides growth hormone have been found to be elevated in BFRE. In a study with young men using a KAATSU belt that applied 1.3 times resting systolic blood pressure and doing leg extensions, growth hormone as well as VEGF and insulin like growth factor (IGF-1) were significantly increased (96). Compared to resting baseline levels, GH increased from 0.11 ± 0.03 to 8.6 ± 1.1 nanograms/ml ($p < 0.01$), VEGF increased from 41 ± 13 to 103 ± 38 picograms/m

($p < 0.05$) and IGF increased from 201 ± 40 to 236 ± 56 nanograms/ml ($p < 0.01$) (96). Training was for 4 sets until failure, with 20 second rest periods. However, in another study, there were no significant differences between VEGF and VEGF mRNA expression after acute exercise with leg occlusion using a band at 50mmHg above atmospheric pressure and exercise without occlusion (105). In this study, young men performed leg extensions for 45 minutes at 60 rpm. In a study with young men with low intensity squat and leg curl exercises at 20% 1RM with leg blood flow occlusion, muscle CSA increased as well as IGF-1 (11). Training was for about 10 minutes twice per day 6 days per week for 2 weeks for 3 sets of 15 repetitions with 30 second rest periods. In a study with young men with low intensity exercise at 20% 1-RM with blood flow occlusion, biopsy revealed type II muscles were increased showing CSA was increased due to muscle fiber size (106). Training was completed performing 3 sets squat and leg curls twice daily for 2 weeks.

In conclusion these studies show metabolic changes with BFRE that may influence angiogenesis and arteriogenesis. In both young and older adult groups with low intensity ischemic exercise, growth hormone increased as compared to high intensity exercise with no occlusion. Insulin like growth factor was also shown to increase in young adults with BFRE. Both GH and IGF may influence the effect of VEGF on vessel changes. Although VEGF change in these studies showed no change to increased changes, the design of the studies may have influenced the results.

Blood flow restricted exercise and flow mediated dilation

Brachial artery blood flow increases with resistance exercise (72), after occlusion and no exercise (108) and with low intensity blood flow restricted exercise (20). In a study of young men completing resistance exercise over 12 weeks five days a week with 2-3 sets of 10-12 repetitions of various upper and lower body exercises, it was found that FMD did not change (72). In this study, however, brachial artery diameter increased and post occlusion blood flow increased (72). FMD may not have changed since the endothelium may have been maximally functional before training or the shear stress may have been too short and transient to cause changes in conduit artery endothelial function (72). Although FMD did not change, this study showed peripheral artery remodeling does occur (72). In a study involving young men, blood flow, as measured by strain gauge plethysmography, was found to be higher with high intensity handgrip exercise at 75% MVC with blood flow occlusion and low intensity handgrip exercise at 25% MVC with blood flow occlusion, compared to blood flow with five minutes of occlusion with no exercise (108). Blood flow increased similarly for both high and low intensity groups. Training consisted of one contraction per 4 seconds for 20 minutes 5 days a week for 4 weeks (108).

The changes in FMD with chronic BFRE have shown mixed results even though blood flow increases. In a study with young men with dynamic handgrip exercise at 40% 1RM with occlusion until fatigue at a rate of 20 contractions per minute for three sets with one minute inter-set rest periods, FMD did not change

but peak blood flow after ischemia and baseline diameter did (20). Training was 3 days per week for 4 weeks and occlusion cuff pressure was 80mmHg (20). Structural enlargement of the brachial artery may account for the decreased FMD as larger arteries have smaller vasodilation responses (20). On the other hand, perhaps the training load in this study was not enough in occlusion pressure and duration to cause a change in FMD measurement as resting brachial artery diameter only changed 3% (20). In a study with young adults using leg occlusion walking on a treadmill at 2 miles/hour, the FMD of the popliteal artery decreased significantly (23). The training was 5 two minute sets on 2 different days separated by a week (23). The decreased FMD may have been due to decreased endothelial function through ischemia-reperfusion injury (23). In this study, since both legs were occluded there was a further decrease in stroke volume return to the heart, increasing heart rate and myocardial oxygen demand as well as conditions for ischemia-reperfusion injury (23). These factors in addition to the ischemia reperfusion injury causing endothelial damage could be a cause of the decreased FMD (23).

In a 4- week study with young adults using using brachial artery occlusion during handgrip exercise at 60% MVC, FMD decreased 30.36% (22). The training was for 15 grips per minute for 20 minutes 3 times per week (22). In this study the opposite arm also completed exercise also at 60% MVC for 15 grips per minute for 20 minutes 3 times per week for 4 weeks but without occlusion and FMD increased 24.19% (22). Muscle strength increased in both arms: muscle strength increased 16.17% exercise with occlusion and muscle strength

increased 8.32% exercise with no occlusion (22). Oxidative stress was a possible explanation for the decreased FMD measurement in the arm with BFRE (22). Another possible explanation was higher pretraining FMD in participants who regularly exercised had less to 'gain' from BFRE on endothelial response to shear stress (22). However, in a study with older men, aged 81 ± 5 years, using isometric handgrip training at 60% MVC, FMD increased (31). Training was one contraction per 4 seconds for 20 minutes four times a week for 4 weeks (31). FMD was measured after 5 minutes of forearm occlusion after training. In this study heart rate and blood pressure did not increase (31). In conclusion, structural changes to blood vessels increasing diameter after BFRE, oxidative stress and ischemia-reperfusion injury causing endothelial damage after BFRE and inadequate exercise load during BFRE in terms of occlusion duration, pressure and number of repetitions could all be possible reasons why FMD decreased or stayed the same in these studies, even when vessel blood flow or diameter increased.

In acute exercise lasting 1-4 weeks, NO bioavailability plays a role in increasing FMD response (20). Exercise lasting greater than 4 weeks, structural changes, such as arteriogenesis, can occur in the blood vessel (20). Increased FMD secondary to increased NO may be evident in exercise lasting less than 4 weeks before increases in arterial size normalize shear rate levels (32). With increased blood vessel diameter, shear stress is normalized (20). This

generalization however does not consider the variation of training type, intensity, frequency and duration, which could help explain the variable changes in FMD reported in the literature. Even though most of the studies discussed above were 4 weeks in duration, the number of times per week and number of sets and repetitions differed. This changes the total volume of exercise completed and could have had an affect on arteriogenesis. Given this evidence above it is likely both the brachial artery diameter and velocity will increase in the present study. FMD may increase but a direct comparison to similar studies found in the literature is difficult since the study design variables are considerably different.

CHAPTER 3. METHODS

General Experimental Design

Younger and older adults completed four weeks of isometric handgrip training three times a week. A young adult group with BFRE (YR) and an older adult group with BFRE (OR) completed low intensity BFRE while an older adult group with HI exercise (OH) trained with the same protocol but without BFRE and at high intensity. Training exercise for YR and OR was at 20% MVC for three sets of isometric handgrip contractions with blood flow occlusion for 5 minutes with one minute rest periods between sets. Training exercise for OH was at 75% MVC for three sets of isometric handgrip contractions without blood flow occlusion for 5 minutes with one minute rest periods between sets.

Prior to and after training, participants sat in a comfortable rested position with the forearm in a supported supinated position using a table. Resting baseline brachial artery diameter and velocity were recorded using 2-D and Pulsed wave Doppler respectively. Isometric handgrip exercises were then completed, using the same protocol used for training, and brachial artery diameter and velocity were again measured after exercise was completed. These measurements were used to calculate shear rate, SR, and flow mediated dilation, FMD.

Subjects

Twenty seven individuals participated in this study. Group YR was comprised of eight individuals (mean 22.2 years old, range 19-25 years old, 3 female, 5 male). Group OR was comprised of ten individuals (mean 63.2 years old, range 60-67 years old, 6 female, 4 male). Group OH was comprised of nine individuals (mean 62.1 years old, range 59-72, 5 female, 4 male). Each participant completed a self-reported medical history. Exclusion criteria included smoking, on antiplatelet, such as Plavix, or anticoagulant, such as Coumadin, medications, on blood pressure medications, diabetes, hypertension, cardiovascular disease, peripheral vascular disease, hand arthritis, orthopedic or musculoskeletal injuries or pain during gripping activities or any other condition where weight lifting exercises were not advisable. Each participant signed an informed consent. The study design was approved by the Iowa State University Institutional Review Board (Appendix).

Instrumentation

Heart rate was measured using an electrocardiogram with three electrodes on the chest (lead II, Life Pak 5, Physio-Control, Redmond, WA). Blood pressure was measured using the oscillometric technique with a cuff placed on the dominant arm and an automated sphygmomanometer (Dinamap-XL cuff, Johnson and Johnson Medical, Inc., Tampa, FL). Maximum voluntary contraction (MVC) was measured using a handheld dynamometer (Jamar-type,

Fabrication Enterprises, Inc., Irvington, NY). The dynamometer was interfaced with a BIOPAC A/D board (BIOPAC Systems, Inc., Santa Barbara, CA). Participants maximally squeezed the hand held dynamometer over three trials and the peak force generated was used as MVC. Brachial artery diameter and peak flow velocity were measured with an ultrasound unit (Siemens Acuson Sequoia C512) using a 15MHz broadband linear array transducer for continuous two dimensional (2D) grayscale imaging and pulse wave (PW) Doppler imaging.

Experimental Procedure

Participants were seated in a comfortable resting position with the forearm in a supported supinated position using a table. Acoustic coupling gel was used to maximize image quality. Resting baseline brachial artery diameter and blood flow velocity were recorded prior to and after training. The brachial artery at ~10mm depth was imaged in longitudinal orientation using 2-D (B mode) ultrasound with the beam angle 90° to the vessel walls. The insonation-angle, the angle between ultrasound and blood flow direction, was held initially perpendicular to the vessel and the image optimized to find the greatest diameter to maximize near and far vessel wall interfaces. Using angle correction, the insonation angle was reduced from 90° to less than 60° to measure maximum brachial artery velocity. The Doppler sample volume, with 1mm gate size, was positioned in the center of the artery and the ultrasound beam aligned with direction of the flow. A blood pressure cuff was placed above the antecubital

fossa and upper arm blood flow occlusion was achieved by inflating the cuff to a constant pressure at 130% of the resting systolic blood pressure. After five minutes, the cuff was rapidly deflated and brachial artery blood flow velocity was recorded approximately every 2 seconds over for the first 14 seconds. A short time lapse occurred between brachial artery velocity (PW Doppler) recordings and diameter (2D grayscale) imaging as these cannot be obtained simultaneously because of differing ultrasound beam orientations and machine limitations. This time lapse ranged from 25 seconds to 68 seconds after cuff release. However, maximal brachial artery diameter usually occurs about 60 seconds after occlusion release (24). In another study, the time to reach peak brachial artery diameter after 5 minutes of ischemia was 61 seconds. (107)

Training Protocol

The intervention groups, YR and OR, trained by performing three sets of isometric handgrip contractions with the upper arm cuff inflated to 130% of systolic blood pressure, during the weekly training sessions. Training exercise was at 20% MVC using subject's nondominant arm to fatigue with blood flow occlusion for 5 minutes. One minute rest periods were allowed between sets, however, the occluding cuff remained inflated during this time. The occluding cuff remained inflated for a total of 5 minutes for each participant regardless if he or she completed 3 sets of exercise to fatigue. If the subject did not fatigue, then exercise continued for the 5 minute duration, with the occluding cuff inflated for

the 5 minute duration. The control group, OH, trained by performing three sets of isometric handgrip contractions, with one minute rest periods between sets, at 75% MVC using their non dominant arm to fatigue without blood flow occlusion. Exercise sessions for group OH also lasted 5 minutes. Each set was 15 repetitions, or until fatigue generating 40% or less of MVC or voluntarily stopping, and lasted approximately one minute with each relaxation-contraction cycle lasting 4 seconds.

The YR and OR groups trained with cuff occlusion while the OH group trained without cuff occlusion. For the intervention groups, fatigue was defined as achieving a force generation of 50% below the target MVC, or the participant voluntarily stopping. For the control group, fatigue was defined as either 15 repetitions, generating 40% MVC, or voluntarily stopping. A metronome was used to prompt the participants to follow a 2 second contraction 2 second relaxation cycle. Occlusion with the cuff remained regardless of either rest or fatigue for 5 minutes. After the first two weeks, MVC was reassessed and this new MVC was used for the remaining study duration. Participants maximally squeezed the hand held dynamometer and the peak force generated was used as the new MVC.

Data Collection

The training protocol was four weeks in duration. Individuals trained three times per week. Before training began, the following information was obtained

and recorded: participant's name and unique identification number, age (years), weight (pounds), height (inches), resting heart rate (beats per minute), resting blood pressure (mmHg, after a 10 minute period of quiet rest), brachial artery diameter (cm, after a 10 minute period of quiet rest), brachial artery blood flow velocity (m/s, after a 10 minute period of quiet rest), corrected insonation-angle (degrees). Also, before training began, three maximum voluntary contraction (MVC) trials using a handheld dynamometer with the non dominant hand to determine maximum hand grip strength were completed. The peak force generated was used to determine MVC for each individual.

Following a 10 minute rest period, 2D ultrasound images of the brachial artery (2D B-mode) were obtained and recorded as 10 second clips. An additional 10 seconds was required to process and store each clip before another recording could be made. Off-line analysis of brachial artery diameter and peak flow velocity were completed using computer software (Siemens' Syngo Dynamics, version 6.0). Resting arterial diameter was measured at end diastole, at the onset of the R-wave as displayed by the ultrasound monitor lead, from the anterior to the posterior walls at the blood vessel interface using inner edge to inner edge on the B mode image. Measurements of resting brachial artery diameter were done for five sequential cardiac cycles and averaged after a ten minute rest, prior to training and after training. Maximal brachial artery diameter, measured during end diastole, was identified after occlusion release. Peak dilation was defined as the largest diameter after releasing the occluding

cuff. FMD was calculated as the peak luminal diameter minus the mean baseline diameter divided by the mean baseline diameter multiplied by 100.

Five sequential cardiac cycle measurements were averaged for resting brachial artery peak flow velocity after a ten minute rest period, prior to training and after training. Doppler blood flow velocity was recorded during the first 15 seconds of reactive hyperemia after occlusion released. Maximal Doppler brachial artery flow velocity was identified after occlusion release as the highest value across a single cardiac cycle. Shear stress, the stimulus for FMD, was calculated as shear stress = viscosity x (velocity/diameter) (109). Shear rate (velocity/diameter) can be used as a surrogate measure for shear stress if viscosity is assumed to be similar among participants (109). Shear rate was calculated by dividing the maximum velocity during reactive hyperemia by the mean resting baseline diameter. Shear rate created by reactive hyperemia is inversely related to baseline diameter (109). Brachial artery baseline diameter variability among subjects leads to shear rate variability, as more narrow diameters increase shear rate, and then also leads to FMD measurement variability (110). Insonation-angle was 60 degrees or less based on the Doppler equation cosine angle. This equation states the Doppler shift frequency (kHz) is equal to $(2FV\cos(\theta))/C$ where F is the ultrasound transmission frequency (MHz), V is the blood cell velocity (cm/sec), $\cos(\theta)$ is the cosine of the angle between the ultrasound beam and flow blood direction, and C is the speed of sound in soft tissue (1540m/sec). Flow velocity increasingly underestimated insonation angle

increases such that an angle of 45° causes a 9% error, 60° causes a 16% error and 70° causes a 26% underestimation error (70). Therefore, variation in angle of incidence among and within subjects can introduce error in data collected.

Statistical analysis

The data analysis was completed with Statistical Analysis System, (SAS Institute, Cary, North Carolina) with two steps. The first step used mixed procedures. The second step used univariate procedure. The mixed procedure is a program in SAS using a variety of mixed linear models for Gaussian data. The mixed procedure, using F-test, was used to test if either group, training or their interaction had significant effects on the difference in pre-training velocity or diameter to post training velocity or diameter. The F-test was also used to test if group had any significant difference between pre- and post-training for FMD and SR. The univariate procedure, t-test, was used for FMD and SR to test within each of the three groups if there was any significant difference between pre-training and post training. A $p < 0.05$ was consider statistically significant.

CHAPTER 4. RESULTS

No significant differences were found in brachial artery diameter, peak brachial artery flow velocity during reactive hyperemia, FMD and SR after training within individuals and between the intervention and control groups.

When comparing the groups YR and OR during pre-training for brachial artery diameter and peak blood flow velocity during rest and following reactive hyperemia, the data were not significant. When comparing the groups YR and OR after training for brachial artery and peak blood flow velocity during rest and reactive hyperemia, the data were not significant.

When comparing the groups OR and OH during pre-training for brachial artery diameter and peak blood flow velocity during rest and reactive hyperemia, the data were not significant according to the following p-values: Resting diameter ($p=0.681$), hyperemic diameter ($p=0.717$), resting velocity ($p=0.467$), hyperemic diameter ($p=0.900$). When comparing the groups OR and OH after training for brachial artery and peak blood flow velocity during rest and reactive hyperemia, the data was not significant according to the following p-values: Resting diameter ($p=0.431$), hyperemic diameter ($p=0.538$), resting velocity ($p=0.279$), and hyperemic velocity ($p=0.827$)

Results are shown in the tables below:

Table 1. Anthropometric characteristics of participants (\pm standard error)

	YR	OR	OH
Age	22.3 \pm 0.65	63.2 \pm 0.76	62.1 \pm 1.32
Height (m)	1.72 \pm 0.04	1.71 \pm 0.037	1.69 \pm 0.034
Weight (kg)	73.5 \pm 11.1	81.5 \pm 12.56	83.5 \pm 11.2
Female	3	6	5
Male	5	5	4

Table 2. Change in measured study variables pre- and post-4 weeks of training (\pm standard error)

	Brachial Artery Diameter (cm)	Brachial Artery Velocity (m/s)
YR		
Pre-training		
Resting	0.32 \pm 0.025	0.70 \pm 0.039
Hyperemia	0.34 \pm 0.022	1.45 \pm 0.079
Post-training		
Resting	0.34 \pm 0.022	0.73 \pm 0.051
Hyperemia	0.36 \pm 0.018	1.18 \pm 0.085
OR		
Pre-training		
Resting	0.36 \pm 0.020	0.71 \pm 0.058
Hyperemia	0.37 \pm 0.021	1.08 \pm 0.085
Post-training		
Resting	0.39 \pm 0.031	0.73 \pm 0.063
Hyperemia	0.43 \pm 0.054	1.12 \pm 0.110
OH		
Pre-training		
Resting	0.37 \pm 0.027	0.72 \pm 0.052
Hyperemia	0.37 \pm 0.029	1.07 \pm 0.094
Post-training		
Resting	0.37 \pm 0.022	0.71 \pm 0.065
Hyperemia	0.38 \pm 0.021	1.12 \pm 0.090

Table 3. Change in calculated study variables pre- and post-4 weeks of training (\pm standard error)

	Flow Mediated Vasodilation (%)	Stress Rate (s ⁻¹)
YR		
Pre-training	12.45 \pm 3.83	655 \pm 95.35
Post-training	12.67 \pm 5.30	457.25 \pm 40.78
OR		
Pre-training	8.59 \pm 3.78	393.2 \pm 27.18
Post-training	9.42 \pm 1.95	381.5 \pm 50.55
OH		
Pre-training	4.63 \pm 2.51	379.7 \pm 47.86
Post-training	8.44 \pm 1.68	389.78 \pm 39.87

CHAPTER 5. DISCUSSION

To my knowledge, no study has compared the effects of low intensity BFRE on FMD, as assessed by changes in peak brachial artery flow velocity and diameter, between younger and older adult groups after 4 weeks of isometric handgrip training. Similarly, no study has assessed the FMD response with low-intensity BFRE in older adults compared to high-intensity exercise in older adults. High-intensity exercise has been shown to improve muscular strength and vascular health in older adults. However, high intensity exercise may be contraindicated in some older adults due to high loading conditions and concurrent medical problems. A low-intensity BFRE exercise program could therefore be beneficial to older adults if similar improvements in muscular strength and vascular health could be achieved with high intensity exercise. One measure of vascular health is the FMD response to changes in blood flow. BFRE in younger adults has been shown to improve muscle strength and blood flow. However, due to hemodynamic and vascular changes associated with aging, the FMD response in older adults after low intensity blood flow restricted exercise may be different compared to younger adults. This study was designed to compare the effect of age on the FMD response by using BFRE training in a younger and an older group. In addition, this study was designed to compare the effect of the type of exercise, namely BFRE vs HI, on the FMD responses in older

There was no significant difference found between the variables measured, namely brachial artery diameter and peak brachial artery velocity, during reactive hyperemia before and after training within individuals and among groups. In addition, there was no significant difference found between calculated variables, namely FMD and SR, before and after training within individuals and among groups. Thus the data from this study did not support the hypotheses and the null hypotheses can not be rejected. There was, however, improvements in forearm strength and girth consequent to the 4 weeks of training in both YR and OR groups. The increase in forearm strength and girth in YR was significant ($p < 0.05$) while the increase in forearm strength in OR was borderline significant ($p = 0.058$) and forearm girth change was not significant. These findings demonstrate the efficacy of the training stimulus to increase strength especially in the YR group ($p = 0.21$). This demonstrates this training protocol was sufficient to induce changes in the forearm muscles. Although muscle size and strength increased, vascular changes, including brachial artery diameter and velocity, did not increase significantly for any of the groups. The reason for this may have been due to technical issues as outlined below, and not due to the training stimulus.

The changes in FMD with BFRE have shown mixed results in the literature. Studies with forearm BFRE in younger adults have shown increased peak blood flow and brachial artery diameter but no change in FMD (20) while other studies have shown decreased FMD (22). Also with younger adults but with BFRE using

leg muscles, FMD decreased (23). No studies with older adults with BFRE measured FMD. However in one study with older adults using resistance exercise, occlusion was applied for 5 minutes after training and FMD was found to increase (31).

Comparing these similar studies, all of which used 4 weeks BFRE training, including the present study, there is quite a bit of variability in the study designs that could affect differences in outcomes. In addition to age, gender, and exclusion criteria as variables that can affect data, other factors include type, intensity and frequency of training. Examples of differences in the studies include: training 3 times per week vs 4 times per week, training at 40% 1RM vs 60% 1RM, occlusion cuff at 80mmHg vs 200mmHg, 1 contraction every 4 seconds vs every 3 seconds and location (leg vs forearm). Different total volumes of training under different conditions can affect muscle and vessel response and this lack of standardization makes direct comparisons between studies difficult. Even cuff position relative to the vessel can have an impact on FMD (111).

The initial size of the artery and variation among individuals could have an effect on FMD calculation. Baseline arterial diameter strongly affects the FMD calculation based on the FMD equation. Smaller vessels have increased shear rate but smaller flow as compared to larger vessels (109). FMD is increased due to the increased shear rate (109). As shown in a previous study, older adults have increased resting baseline arterial diameters (112). Although not statistically significant, our study showed a similar trend. Pre and post training

resting brachial artery diameters for YR, 0.32 ± 0.025 cm and 0.34 ± 0.022 cm respectively, were less than the resting brachial artery diameters before and after training for OR, 0.36 ± 0.020 cm and 0.39 ± 0.031 , and OH, 0.37 ± 0.027 cm and 0.37 ± 0.022 .

The total duration of training can also affect outcome. In acute exercise lasting less than 4 weeks, NO bioavailability plays a role in the increased FMD response. While with chronic exercise of more than 4 weeks, structural changes occur in the blood vessel (20). With increased blood vessel diameter, FMD is normalized since baseline diameter is part of the FMD equation (20). A blunted hyperemic flow has been noted in older individuals with increased aortic stiffness after 5 minutes of resting occlusion ischemia (113). This suggests change in FMD is not reversible due to vascular tone changes but rather due to structural remodeling (113). In a study, this was seen in a blunted response to brachial artery FMD in older people after 5 minute forearm occlusion comparing a younger group (28 years of age ± 8) and an older group (85 years of age ± 8) (114). The brachial artery FMD in the older group was only 2.3% while the younger group FMD changed by 7.7%. In another study using 5-minute occlusion to the forearm, FMD after release of occlusion was decreased with increasing age (115).

Other factors besides shear stress may be involved with FMD and could affect the response. A NO synthase inhibitor, N-monomethyl-L-arginine (L-NMMA) caused decreases in FMD after a 5 minute occlusion (116). However when

occlusion time was increased to 15 minutes, L-NMMA did not affect FMD (116). Other vasodilatory factors, such as prostaglandins, endothelin, and acetylcholine, may be involved with occlusion times over 5 minutes through a “sequential recruitment” mechanism (109). Although shear stress initiates the increase in NO, and thus FMD in reactive hyperemia, there is a “non-NO mediated” component to FMD (117). In our study, because occlusion to blood flow lasted five minutes, non-NO mediated FMD factors were less likely to be involved. This could have had a greater impact on the older groups because NO-mediated hyperemic flow is diminished in older adults. Unfortunately, no significant differences were found between older and younger groups in estimates of FMD before and after training, so it is not possible to compare the influence of NO and non-NO mediated factors in this study.

There were several limitations to this study that could have affected the results. These limitations included sonographer inexperience, participant movement during data collection, lack of edge detection software for measuring vascular diameter, and possible occult vascular, or cardiac disease. It is recommended, by the Intersocietal Commission for the Accreditation of Vascular Laboratories, that at least 100 supervised scans and measurements be performed before independent scanning and reading is attempted (118). Although the scans were performed by the same sonographer, previous experience in human vascular scanning was limited prior to the study. Because ultrasound measurements, especially for Doppler peak velocity, can be

profoundly affected by slight variations in angle of the ultrasound probe, this lack of expertise could have impacted the quality and consistency of ultrasound images collected and subsequent data measurements.

Other technical complications of ultrasound imaging in this study likely had an adverse effect on image accuracy. When the arm cuff was deflated it would sometimes cause the forearm to move slightly, or the participant may have moved his or her forearm slightly during cuff inflation. Consequently, the position and angulation of the original US probe placement had to be changed quickly from resting position in order to visualize the vessel. This movement could have introduced additional error in the results because important data may have been lost during the period of reactive hyperemia while the vessel was relocated and because insonation angle for Doppler data may have changed.

Sonographer inexperience and technical complications of ultrasound imaging adversely effected data collection as compared to data reported in similar studies. In a study using forearm blood flow restricted handgrip exercise in young adults three times weekly for 4 weeks, resting pre-training brachial artery diameter was 0.431 ± 0.28 cm and resting post-training brachial artery was 0.444 ± 0.37 cm (20). In my study resting pre-training brachial artery diameter was 0.32 ± 0.025 cm and resting post-training brachial artery diameter was 0.34 ± 0.022 cm in the young adult group YR. Differences in methods between my study and the later study is the latter study used 200mmHg during training and testing during cuff occlusion and exercise was completed at 40% 1RM (20).

Also the latter study used a distal location on the forearm for cuff position as compared to proximal location in my study (20). This study also used an ultrasound with Duplex capability (20). In another similar study using forearm blood flow restricted handgrip exercise in young adults three times weekly for 4 weeks, the post-training brachial artery diameter after cuff release following 5-minutes occlusion was 0.403 ± 0.68 cm (22). This is in comparison to my study where post-training brachial artery diameter after cuff release following 5 minutes of occlusion was 0.36 ± 0.018 cm in the young adult group YR. Differences in methods between my study and the latter study is the latter study used 200mmHg during cuff occlusion during testing, and 80mmHg during training, and exercise completed at 60% MVC (22). Lastly in a study with older men using isometric handgrip training at 60% MVC, resting brachial artery diameter was 0.447 ± 0.16 cm, post training resting brachial artery diameter was 0.448 ± 0.16 cm, and post-training brachial artery diameter after cuff release following 5-minutes occlusion was 0.467 ± 0.11 (31). Training was one contraction per 4 seconds for 20 minutes four times a week for 4 weeks (31). This is in comparison to my study in the OH group resting brachial artery diameter was 0.37 ± 0.027 cm, post training resting brachial artery diameter was 0.37 ± 0.022 cm, and post-training brachial artery diameter after cuff release following 5 minutes occlusion was 0.38 ± 0.021 . Differences in methods between my study and the latter study is the latter study used 200mmHg during cuff occlusion during testing and used a distal location for the cuff position on the forearm (31).

Cuff occlusion caused discomfort in several participants. Three individuals from YR and three from OR discontinued training due to pain. About 40% of the participants had a twitching arm movement during or after cuff release, likely related to pain. This also would have affected the ultrasound images and measurements because of inconsistent and submaximal alignment with vessel orientation and flow. Measurement accuracy, as well as the practical reliability of participants continuing this type of exercise, was likely limited by pain. Another consequence of pain associated with blood flow occlusion was variability in the number of contractions per training session that each participant could complete. Although cuff occlusion was to remain on for 5 minutes regardless of fatigue as defined in Methods, for some individuals the cuff was removed before five minutes because of pain. This means that the total number of contractions completed by participants and therefore the total amount of training was inconsistent. Participants who completed fewer contractions because of fatigue from pain likely did not have as much gain in muscle strength and size, as well as expected changes in vasculature. This also could have affected the results of this study.

Although baseline diameter, peak diameter and calculated FMD can be measured by experienced sonographers with low variability, edge detection software provides a more sensitive assessment of FMD by removing subjective error in vessel diameter measurement (70). Edge detection software was not available for this study.

Even though individuals were screened to exclude those with a history of smoking, blood pressure medications, diabetes, hypertension, cardiovascular disease, and peripheral vascular disease, apparently healthy older individuals may have endothelial dysfunction without overt signs of cardiovascular disease (119). With this in mind, the small sample sizes for each group was another limitation of this study, as participants were screened only by a self reported questionnaire only and not by other cardiac or vascular screening tests. If individuals with occult cardiovascular disease were present in the study, this may have impacted the results.

Group size variation occurred after recruitment because some individuals left the study for personal reasons, time conflicts or occlusion associated pain. This caused different group sizes namely YR with 8 individuals, OR with 9 individuals and OH with 10 individuals. Age variation also occurred. There was a greater range of ages in the older population compared to the younger population which likely was related to the demographics of the community. Because older adults were selected from the Ames community, and the recruitment age criterion was listed as '60 years of age or older to participate', we expected to have a wider range of ages in this group, compared to the younger group which was recruited from ISU students '30 years of age or under', because the college student age range is smaller.

CHAPTER 6. CONCLUSION

The purpose of this study was to 1) compare flow mediated dilation in the brachial artery in two populations (old and young groups) before and after 4 weeks of low intensity hand grip isometric training with vascular occlusion and to 2) compare flow mediated dilation responses in two groups of older people: the previously described BFRE group and one high intensity exercise group without occlusion. There was no significant difference found between variables measured, namely brachial artery diameter and peak brachial artery velocity, during reactive hyperemia before and after training within individuals and among groups. In addition, there was no significant difference found between calculated variables, namely FMD and SR, before and after training within individuals and among groups. Thus the data from this study did not support the hypotheses and the null hypothesis can not be rejected.

These findings are not consistent with other findings in the literature. Previous studies have shown that blood flow increases with resistance exercise (72), and with blood vessel occlusion and no exercise (108). No studies with older adults with BFRE measured FMD. However, in one study with older adults using resistance exercise, occlusion was applied for 5 minutes after training and FMD was found to increase (31). However, studies of FMD using BFRE have shown mixed results in young participants. For example, FMD appeared decreased (22) or unchanged (20) in young adults with occlusive forearm exercise lasting 4 weeks. However, in another study with young adults using

occlusive leg exercise for 4 weeks of training, FMD was increased (19). The differences in these findings may have been related to study design including differences in training type, intensity and frequency. Several limitations of our study including sonographer inexperience, small sample size, and overt and occult aging changes in the vasculature likely affected results. Additionally, inaccuracies in ultrasound measurements related to timing and position as a result of participant pain from occlusion likely also affected results. Future studies controlling for these limitations may have a different outcome.

If a modification to the BFRE occlusion protocol could be developed to improve participant comfort, then participants become more willing to complete more exercise in the form of sets and contractions, which might lead to measurable differences in results. With modifications to the study design to address the outlined limitations then it might be possible to detect whether improved FMD occurs with blood flow restricted exercise. If so, this would suggest that BFRE could improve endothelial function.

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APPENDIX. INSTITUTIONAL REVIEW BOARD APPROVAL

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Institutional Review Board
Office for Responsible Research
Vice President for Research
1138 Pearson Hall
Ames, Iowa 50011-2207
515 294-4566
FAX 515 294-4267

Date: 12/22/2010

To: Dr. Warren Franke
247 Foraker Bldg

From: Office for Responsible Research

Title: Effects of Blood Flow Restricted Exercise on Vascular Function

IRB Num: 10-300

Approval Date: 12/21/2010 **Continuing Review Date:** 7/5/2011

Submission Type: Modification **Review Type:** Full Committee

The project referenced above has received approval from the Institutional Review Board (IRB) at Iowa State University. Please refer to the IRB ID number shown above in all correspondence regarding this study.

Your study has been approved according to the dates shown above. To ensure compliance with federal regulations (45 CFR 46 & 21 CFR 56), please be sure to:

- **Use only the approved study materials** in your research, including the recruitment materials and informed consent documents that have the IRB approval stamp.
- **Obtain IRB approval prior to implementing any changes** to the study by submitting the "Continuing Review and/or Modification" form.
- **Immediately inform the IRB of (1) all serious and/or unexpected adverse experiences** involving risks to subjects or others; and (2) **any other unanticipated problems** involving risks to subjects or others.
- **Stop all research activity if IRB approval lapses**, unless continuation is necessary to prevent harm to research participants. Research activity can resume once IRB approval is reestablished.
- **Complete a new continuing review form** at least three to four weeks prior to the **date for continuing review** as noted above to provide sufficient time for the IRB to review and approve continuation of the study. We will send a courtesy reminder as this date approaches.

Research investigators are expected to comply with the principles of the Belmont Report, and state and federal regulations regarding the involvement of humans in research. These documents are located on the Office for Responsible Research website <http://www.compliance.iastate.edu/irb/forms/> or available by calling (515) 294-4566.

Upon completion of the project, please submit a Project Closure Form to the Office for Responsible Research, 1138 Pearson Hall, to officially close the project.