THE EFFECTS OF ACUTE AND CHRONIC EXERCISE ON CARDIOVASCULAR RESPONSES IN FEMALE SMOKERS

by

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(Under the Direction of Rodney K. Dishman)

ABSTRACT

Among women, smoking is the leading cause of preventable death and disease. Endothelial dysfunction, measured by flow mediated dilation (FMD) and hyper-reactivity to neurovascular challenges are two potential mechanisms by which this may occur. It has been suggested that acute and chronic exercise may improve endothelial function and attenuate cardiovascular reactivity to neurovascular stressors. This research was conducted to determine the effect of acute (Study 1) and chronic (Study 2) moderate-intensity cycling exercise on FMD and cardiovascular responses to two neurovascular stressors (Stroop Color-Word conflict test (CWT) and forehead cold) among sedentary female smokers and non-smokers. FMD was determined by brachial arterial diameter, arterial velocity and blood flow measured by Doppler Ultrasonography; beat-to-beat finger blood pressure (BP), heart rate (HR), arterial velocity, arterial diameter, and blood flow were assessed in responses to forehead cold and the Stroop CWT. Acute exercise (Study 1) increased FMD, baseline HR, arterial velocity and blood flow; and reduced baseline systolic and diastolic BP, HR, arterial velocity, and blood flow in both groups. In addition, acute exercise decreased arterial velocity and blood flow responses during the Stroop CWT and forehead cold in both groups. In study 2, FMD was impaired and decreases

in arterial diameter and blood flow during forehead cold were augmented at baseline among female smokers compared to non-smokers. Three weeks of cycle exercise training at a moderate intensity did not improve endothelial function measured by FMD or alter stress reactivity during neurovascular stress in smokers or non-smokers. Overall, acute moderate intensity exercise improves endothelial function and alters some aspects of stress reactivity, but these effects do not extend to 3-weeks of short-term exercise training.

INDEX WORDS: Endothelial function, Exercise, Flow mediated dilation, Physical activity, Stress reactivity

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) is the primary cause for morbidity and mortality among women in the United States (Thom, et al., 2006). CVD is responsible for 1/3 of all women deaths, and there are 38.2 million women in the United States living with the disease (Thom, et al., 2006). Women are more likely than men to die from CVD, for reasons that remain unknown (Mosca, et al., 2007; Thom, et al., 2006). In 2006, the health care cost related to CVD rose to $403 billion dollars, 2 times more than the estimated costs associated with cancer and 13 times more than HIV (Mosca, et al., 2007). The apparent public health burden of CVD in women shows the vital need for effective prevention and treatment methods.

Multiple treatment options are available to both prevent and treat CVD in women. Treatment methods can be categorized into lifestyle, major risk factor, and preventive drug interventions. Drug therapy is effective in the secondary treatment of CVD; however adverse side effects, cost, and pregnancy in women are reasons that may discourage their use. One recommended therapy option that is beneficial in preventing and treating CVD is physical activity and exercise (Mosca, et al., 2007). Overwhelming longitudinal data has shown that exercise and/or physical activity is a lifestyle intervention that can decrease risk for CVD during any stage of prevention and treatment in women (Blumenthal, et al., 2004; M.B. Conroy, Cook, Manson, J.E., & Lee, 2005; Hambrecht, et al., 2004b; Hillsdon, Thorogood, Murphy, & Jones, 2004; Knoops, et al., 2004; Lee, Sesso, Oguma, & Paffenbarger, 2003). A meta-analysis of 30 studies reported that in women, low- and moderate-intensity activity reduces the overall risk of CVD by 82% and 78%,

respectively (T. Y. Li, et al., 2006). In addition, physical activity is beneficial in decreasing the effects of other risk factors related to CVD such as smoking.

Cigarette smoking is the leading risk factor for preventable death in the United States and is related to increased risk for CVD, peripheral arterial disease, stroke, and congestive heart failure ("Cigarette smoking among adults--United States, 2004," 2005). After controlling for other risk factors (i.e., age, diet, physical inactivity, BMI, and other chronic diseases), smoking has been shown to be the most important indicator for cardiovascular mortality (Luoto, Prattala, Uutela, & Puska, 1998). Endothelial dysfunction (Schroeder, et al., 1999) and hyper-reactivity to neurovascular stressors (Treiber, et al., 2003) are two plausible mechanisms that may explain smoking-related morbidity and mortality.

A non-invasive hyperemic technique used to assess endothelial function is commonly referred to as "flow-mediated dilation" (FMD). FMD is a result of increased blood flow that puts a shear stress or frictional force on the endothelium, resulting in vasodilation of the vessel. The primary mechanism responsible for FMD is mediated by nitric oxide (D. Green, 2005) which is important for the maintenance of vascular health and vascular tone (Hutcheson & Griffith, 1991).

Neurovascular stressors, such as the Stroop Color –Word conflict test (CWT) (an autonomic vasodilator) and forehead cold (an autonomic vasoconstrictor), are two commonly used cardiovascular reactivity tests (Treiber, et al., 2003). Physiological responses to active stressors, such as the Stroop CWT, include increased HR, SBP, CO, withdrawal of vagal tone, and β-adrenergic mediated vasodilation (Dietz, et al., 1994; Freyschuss, Hjemdahl, Juhlin-Dannfelt, & Linde, 1988; Halliwill, et al., 1997). On the other hand, passive stress such as forehead cold (response is similar to the mammalian dive reflex), result in increased in vagal tone leading to

decreased HR (Heindl, Struck, Wellhoner, Sayk, & Dodt, 2004; Treiber, et al., 2003). Forehead cold is also associated with increased resistance because of α-adrenergic mediated vasoconstriction (Dishman, Nakamura, Jackson, & Ray, 2003). To date, studies that point to a direct relationship between vascular reactivity and laboratory stressors, however, are inconclusive (Jackson & Dishman, 2006). Reported differences in the studies may be due to inability to standardize the type of stressor, not accounting for pre-test cardiovascular stress responses such as HR and blood pressure, baseline cardiorespiratory fitness levels, and measurement of vascular responses. In addition, underrepresentation of women is a consistent problem.

The exact components within cigarette smoke that is responsible for endothelial dysfunction remains unknown. It is believed that cigarette smoke includes high amounts of free radicals and pro-oxidants that increase oxidative stress and degrade endothelium derived relaxing factors (i.e. nitric oxide, prostacyclin, and endothelium derived hypopolarizing factor) that are important for vessel tone, compliance, and vasodilatory function (Furchgott & Zawadzki, 1980). In healthy young adults FMD is impaired 2-fold in smokers compared to non-smokers. These effects on endothelial function are dose-dependently related to how long individuals have smoked in their lifetime (Celermajer, Sorensen, Georgakopoulos, Thomas, et al., 1993). Nicotine in cigarette smoke is also known to increase heart rate (HR), blood pressure (BP), circulating catecholamines, and peripheral vasoconstriction (Cryer, Haymond, Santiago, & Shah, 1976; Perkins, Epstein, Jennings, & Stiller, 1986). When the effects of smoking are combined with stress, cardiovascular reactivity is increased (MacDougall, Dembroski, Slaats, Herd, & Eliot, 1983). For example, non-smokers compared to chronic smokers (whether or not an individual smokes), show increased BP (Tsuda, Steptoe, West, Fieldman, & Kirschbaum, 1996) and HR

(Perkins, Grobe, Fonte, & Breus, 1992) reactivity to neurovascular stressors, indicative of increased risk for disease.

Effects of Acute Exercise on Endothelial Function and Stress Reactivity

There is longitudinal evidence that increased physical activity and/or exercise reduces the risk of morbidity and mortality associated with CVD in women smokers (M.B. Conroy, et al., 2005; Hambrecht, et al., 2004b; T.Y. Li, et al., 2006; Noda, Iso, Toyoshima, Date, Yamamato, et al., 2005). Specifically, dynamic exercise increases blood flow and shear stress, which leads to an increase in NO synthesis and release (Fisslthaler, Dimmeler, Hermann, Busse, & Fleming, 2000; Malek & Izumo, 1995; Resnick & Gimbrone, 1995), important for endothelial function. To date, few studies have tested the effect of acute exercise on FMD (Padilla, Harris, & Wallace, 2007), and only one focused on women (Harvey, et al., 2005). Harvey et al. (2002) found that after 45 minutes of treadmill exercise at an intensity of 60% VO_2peak, FMD was increased in premenopausal women, though non-significantly. Other studies that measured FMD assessed healthy sedentary men using high intensity exercise (Pullin, et al., 2004; Rundell, Hoffman, Caviston, Bulbulian, & Hollenbach, 2007), combined exercise with diet (Padilla, Harris, Fly, Rink, & Wallace, 2006), or looked at populations with impaired endothelial function (Gresele, Migliacci, Procacci, De Monte, & Bonizzoni, 2007; Harris, Padilla, Hanlon, Rink, & Wallace, 2007; Silvestro, et al., 2006; Silvestro, et al., 2002). These studies found no change, an increase, or a decrease in FMD. Based on these findings, the relationship between FMD and acute exercise remains inconclusive. Inconsistencies may result from the diverse populations studied or the timing of FMD measurements after exercise. It remains unclear the optimal time to measure FMD post-exercise, but studies that measured FMD within 60 minutes observed larger

increases in FMD (Cosio-Lima, et al., 2006; Harvey, et al., 2005; Padilla, et al., 2006) compared to those that waited longer than an hour (Pullin, et al., 2004).

Similar to the effects of acute exercise on FMD, the benefit of acute exercise on cardiovascular reactivity remains unclear. A meta analysis found that in 10 out of 15 studies, acute exercise significantly reduced BP responses during neurovascular stressors (Hamer, Taylor, & Steptoe, 2006). Six of the fifteen studies tested women, 3 studies used the Stroop CWT and only 1 used forehead cold as a stressor. Thirty minutes of dynamic exercise at 50%VO_2peak was the minimum dose necessary to show an exercise-induced reduction in pressor responses during the tasks. None of these studies used a standardized version of the Stroop CWT that controls for task difficulty and social-interaction or measured vascular responses.

We were unable to find any studies of female smokers that measured the effect of an acute bout of exercise on FMD. Benjamin et al. (2004) (Benjamin, et al., 2004) found that chronic smoking decreased FMD and an acute 6-minute walk test increased FMD in a group of men and women, but the findings weren't reported separately for women. In males, exercise has been shown to equally improve FMD in smokers and non-smokers (Clarkson, et al., 1999; Gaenzer, et al., 2001). To date, the benefit of exercise on FMD in woman smokers remains uncertain.

Only a few studies have addressed the relationship between stress response in smokers and the benefit of acute exercise. The earliest study examined 12 male sedentary smokers responses to mental arithmetic after 20 minute of cycling (P.O. Russell, L.H. Epstein, & K.T. Erickson, 1983). Exercise did not reduce stress reactivity as measured by electromyographic activity, skin conductance, and heart rate. Measures of vascular health were not obtained and no control group was used. A more recent study examined the effect an acute bout of 15 minutes of brisk

semi-self paced walking session has on the BP response to three psychosocial stressors (A. Taylor & Katomeri, 2006). Participants performed the Stroop CWT, a speech task, and were exposed to a lit cigarette under a period of temporary abstinence from smoking. Exercise reduced blood pressure after the three stress conditions by 3.8 mmHg compared to non-exercise controls. This study though it used a short bout of exercise, found a small but significant change in blood pressure

Effects of Chronic Exercise on Endothelial Function and Stress Reactivity

If the vascular stress, or shear stress, associated with acute exercise exposure is repeated over time, long term vascular adaptations may occur (Selye, 1951). During chronic exercise training, the endothelium, which is an important regulator of arteriolar tone, is exposed to repeated episodes of increased blood flow. This repeated increase in flow, results in improved function of the endothelium; specifically, increased synthesis of NO (Sun, Huang et al. 1994; Sessa, Pritchard et al. 1993) and an upregulation of NO synthase (Sun, Huang et al. 1994; Sessa, Pritchard et al. 1993). In addition, acute and chronic exercise has anti-hypertensive effects (Whelton, Chin, Xin, & He, 2002), that may account for changes in autonomic nervous system activity. These changes may result in attenuated sympathetic responses to neurovascular challenges (de Geus, van Doornen, & Orlebeke, 1993; Hamer, et al., 2006; Sothmann, et al., 1996). Utilizing both an acute and chronic model of exercise helps determine both the temporary effects and long-term adaptations of exercise on the vascular endothelium.

To date, the results of whole body exercise training on FMD in humans remains inconsistent. Studies have looked at healthy populations (Clarkson, et al., 1999; D. J. Green, et al., 2003; Kingwell, Sherrard, Jennings, & Dart, 1997; Ostergard, et al., 2006), individuals with cardiovascular risk factors (D. J. Green, et al., 2003), heart failure patients (Hambrecht, et al.,

1998; Hornig, Maier, & Drexler, 1996; Linke, et al., 2001; Maiorana, et al., 2000), and patients with a recent myocardial infarction (Vona, et al., 2004). Differences in responses depended on the type of patient, as well as, the length, and intensity of the exercise training protocol. It has been suggested that individuals with impaired endothelial function are more likely to show changes in endothelial vasodilation after a moderate training program, compared to healthy subjects with preserved endothelial function (D. J. Green, et al., 2003). In addition, between 2-4 weeks of exercise training, FMD is significantly increased from baseline but structural remodeling begins to occur (Tinken, Thijssen, Black, Cable, & Green, 2008). After this point, the shear stress stimulus is more likely to return to normal due to increases in arterial size. Based on this evidence, improvements in FMD are most likely noticeable in individuals with impaired endothelial function, such as smokers, after 2-4 weeks of exercise training. Thus, measuring FMD, after exercise training protocols lasting 3 weeks appears optimal for obtaining any training-induced improvements.

The benefit of repeated bouts of exercise may also result in cardiovascular adaptations that reduce stress reactivity. For example, exercise training lasting 2 to 24 weeks has been shown to reduce SBP and DBP by -3.48 and -2.58 mmHg, respectively (Whelton, et al., 2002). It is plausible that these physiological adaptations may occur during exposure to stressful stimuli. For example, Vona et al. (2004) found that 3 months of moderate intensity exercise training, attenuated arterial diameter reactivity to the hand cold pressor, a sympathetic vasoconstrictor. On the other hand, a more recent review found that out of 19 randomized exercise training studies that included a control group, reactivity did not change (Jackson & Dishman, 2006). Few of these studies, focused on women or individuals at risk for cardiovascular disease, such as smokers. In addition, the cardiovascular parameters measured (primarily HR and BP) did not

afford a thorough understanding of the underlying mechanisms whereby exercise training alters stress reactivity.

To date, we were unable to find any training studies that measured FMD in healthy female smokers. Likewise, it remains unknown what effect dynamic exercise training has on reactivity to neurovascular challenge in female smokers. It is plausible that the potential benefits of acute exercise, as well as the chronic adaptations observed in healthy non-smoking individuals, can extend to female smokers. The current research aims to determine the acute effects and chronic adaptations of exercise on FMD and cardiovascular responses during neurovascular stressors in female smokers and non-smokers.

CHAPTER 2

LITERATURE REVIEW

Effects of Smoking on Endothelial Function

Cigarette smoking is one of the primary modifiable predictors of atherosclerosis and vascular disease (Burns, 2003; Henderson, 1991). Endothelial function, an important indicator of atherosclerosis progression and rate of cardiovascular events (Brevetti, Silvestro, Schiano, & Chiariello, 2003; Verma, Buchanan, & Anderson, 2003; Widlansky, Gokce, Keaney, & Vita, 2003), is impaired in smokers (Celermajer, Sorensen, Bull, Robinson, & Deanfield, 1994; Celermajer, Sorensen, Georgakopoulos, Bull, et al., 1993; Esen, et al., 2004; Raij, DeMaster, & Jaimes, 2001). A non-invasive hyperemic technique used to assess endothelial function is commonly referred to as "flow-mediated dilation" (FMD) (Celermajer, et al., 1992). In arteries with a healthy endothelium, reactive hyperemia results in vasodilation (Laurent, et al., 1990; Rubanyi, Romero, & Vanhoutte, 1986). Factors important for vasodilation in response to increases in blood flow include NO (Koller, Sun, & Kaley, 1993), prostacyclin (Koller, et al., 1993), endothelial-derived hyperpolarizing factor and acetylcholine (Martin, Beltran-Del-Rio, Albrecht, Lorenz, & Joyner, 1996). The mechanisms responsible for FMD are primarily mediated by nitric oxide (NO) (Celermajer, et al., 1992; D. Green, 2005; Joannides, et al., 1995).

The mechanisms responsible for endothelial dysfunction in smokers remain unknown. The toxic substances associated with cigarette smoke include high amounts of free radicals and pro-oxidants, augmenting superoxide-anion-mediated degradation of endothelium-derived relaxing factors (Galle, Mulsch, Busse, & Bassenge, 1991). This potentially results in increased

oxidative stress and decreased NO biosynthesis (Barua, et al., 2001, 2002; Barua, Ambrose, Srivastava, DeVoe, & Eales-Reynolds, 2003; Raij, et al., 2001). In smokers, this increase in oxidized free radicals is evidenced by increased oxidized low-density lipoprotein (plasma indicator of lipid peroxidation) (Galle, et al., 1991; Minor, Myers, Guerra, Bates, & Harrison, 1990), which has been shown to inhibit endothelium-dependent vasodilation (Flavahan, 1992). This enhances oxidized LDL stimulated monocyte adhesion, a first step in the process of atherogensis (Weber, Erl, & Weber, 1995). The amount of oxygen-derived free radicals results in direct cellular damage, as well as scavenging of nitric oxide and decreased synthesis of NO via NO synthase (Puranik & Celermajer, 2003). Damage to NO production also impairs NO-mediated vascular tone, an early marker of atherosclerotic vascular changes (Kiowski, et al., 1994).

The damaging effects of smoking on the endothelium appear to be immediate and increase the longer an individual smokes. For example, acute exposure to 2 tobacco cigarettes in non-smokers results in twice the number of nuclear-damaged endothelial cells in circulating blood compared to non-tobacco containing cigarettes (M. C. Davis & Matthews, 1990). In addition, in 10 healthy smokers, FMD was reduced immediately (-66%), 30 minutes (-64%), and 60 minutes (-54%) after smoking a cigarette compared to baseline values (Lekakis, et al., 1998). Likewise, long-term smoking, results in a decrease or absence of endothelium function, measured by FMD (Celermajer, Sorensen, Georgakopoulos, Bull, et al., 1993; Celermajer, et al., 1992; Gaenzer, et al., 2001). The length an individual smokes (measured by life-time dose) appears to be dose dependently related to decreased endothelium function (Celermajer, Sorensen, Georgakopoulos, Bull, et al., 1993; Gaenzer, et al., 2001). Celermejer et al. (1993) found that in healthy current smokers, FMD is inversely related to the life-time dose smoked (6.6±4% very

light, 4.0±3.1 light, 3.2± 3.2% moderate, and 2.6±1.2% heavy smokers). Life-time dose ranged from 1 pack years (defined as having smoked at least 20 cigarettes per day for 1 year) to 20 and endothelial dysfunction had a smoking dose threshold of 20 pack years. This relationship between reduced FMD and smoking was independent of vessel diameter, baseline flow, and degree of reactive hyperemia. This dose-dependent effect implies that smoking may have a causal relationship with endothelial dysfunction, which is the earliest detectable event before structural damage occurs (i.e., increased intramedial thickness) (Faggiotto, Ross, & Harker, 1984; Poredos, Orehek, & Tratnik, 1999; Ross, 1986). These damaging effects of smoking on vascular function are partially reversible by smoking cessation (Celermajer, Sorensen, Georgakopoulos, Bull, et al., 1993), however, women smokers are more likely to relapse (Borrelli, Papandonatos, Spring, Hitsman, & Niaura, 2004) and less likely to successfully quit smoking (Sherman, Fu, Joseph, Lanto, & Yano, 2005). Thus, there is a need to identify other lifestyle interventions that may also improve vascular health and potentially decrease cardiovascular risk in smokers.

Effects of Acute and Chronic Exercise on Endothelial Function

Improvements in endothelial function, or FMD, have been previously (Celermajer, et al., 1994; Maiorana, O'Driscoll, Cheetham, et al., 2001; Maiorana, et al., 2000; Neunteufl, et al., 2000; Silvestro, et al., 2002) used in intervention studies to test for changes in CVD risk . There is longitudinal evidence that increased physical activity and/or exercise reduces the risk of morbidity and mortality associated with CVD in women smokers (M. B. Conroy, Cook, Manson, Buring, & Lee, 2005; Hambrecht, et al., 2004a; T. Y. Li, et al., 2006; Noda, Iso, Toyoshima, Date, Yamamoto, et al., 2005). To date, the effects of acute and chronic exercise training on

FMD remain unclear. Utilizing both an acute and chronic model of exercise helps determine both the temporary effects and long-term adaptations of exercise on the vascular endothelium.

A study by Harvey et al. (2002) (Harvey, et al., 2005) assessed the effects of a single bout of treadmill exercise (60% VO2peak) on FMD in pre- and post-menopausal women. Compared to baseline, post-exercise FMD was higher after exercise in both groups; however, this increase was only significant in post-menopausal women. Premenopausal women (12.1 ± 1.5) did have significantly higher FMD compared to postmenopausal women (5.3 ± 1.3), implying a "ceiling effect". This in turn could potentially limit the ability to detect a significant difference due to exercise. Another study, aimed at testing FMD in renal patients (Cosio-Lima, et al., 2006), found that FMD in the healthy (men and women) counterparts of renal patients improved after 30 minutes of treadmill walking (RPE = 13). This effect of acute exercise, which has not been observed in other healthy patients after a single bout of exercise (Harvey, et al., 2005; Pullin, et al., 2004), may be due to the older population (D. J. Green, et al., 2003; Thijssen, de Groot, Smits, & Hopman, 2007) studied (aged 30-65). There are other instances where endothelial function does not change (Pullin, et al., 2004; Rundell, et al., 2007) or decreases (Gresele, et al., 2007; Silvestro, et al., 2002) after an acute bout of exercise. Inconsistencies may be due to the range of populations studied, timing of FMD measurement after exercise, and only making comparisons of post-exercise FMD. It remains unclear, the most optimal time to measure FMD post-exercise, but it is believed that immediately following exercise FMD is maximal (Padilla, et al., 2007).

If the vascular stress, or shear stress, associated with acute exercise (Fisslthaler, et al., 2000; Malek & Izumo, 1995; Resnick & Gimbrone, 1995) exposure is repeated over time, long term vascular adaptations may occur (Selye, 1951). During chronic exercise training, the

endothelium is exposed to repeated episodes of increased blood flow leading to improved endothelium function, increased synthesis of NO (Kingwell, et al., 1997; Sessa, Pritchard, Seyedi, Wang, & Hintze, 1994; Sun, Huang, Koller, & Kaley, 1994) and up-regulation of NO synthase (Sessa, et al., 1994; Sun, et al., 1994). During chronic exercise training, the endothelium, which is an important regulator of arteriolar tone, is exposed to repeated episodes of increased blood flow. This repeated increase in flow, results in improved function of the endothelium; specifically, increased synthesis of NO (Sessa, et al., 1994; Sun, et al., 1994) and an upregulation of NO synthase (Sessa, et al., 1994; Sun, et al., 1994). Other adaptations associated with enhanced exercise-induced endothelium dependent vasodilation may include genetic (endothelin, growth factors, regulators of fibrinolysis) or structural alterations (cytoskeletal redistribution, cell shape change) (Huonker, Halle, & Keul, 1996).

In animals, exercise training has been shown to improve vascular dilation in response to various chemical stimuli (Johnson, Rush, Turk, Price, & Laughlin, 2001; Koller, Huang, Sun, & Kaley, 1995; Sessa, et al., 1994; Wang, Wolin, & Hintze, 1993), independent of oxidative capacity (Lash and Bohlen 2001). One of the early studies (Delp, McAllister, & Laughlin, 1993) on exercise training in rats found increased endothelium vasodilation in response to acetylcholine (Ach, endothelium dependent dilator) after 12 weeks of training in rats. These training-induced adaptations appear to be specific to the endothelium and increased NO production, because endothelium independent dilation was not improved.

In humans, endothelial function has been assessed after handgrip training in healthy individuals (Bank, Shammas, Mullen, & Chuang, 1998; Franke, Stephens, & Schmid, 1998; D. J. Green, Cable, Fox, Rankin, & Taylor, 1994), in whole body dynamic exercise training in healthy individuals (Bergholm, et al., 1999; Clarkson, et al., 1999; DeSouza, et al., 2000; Goto, et al.,

2003; Higashi, et al., 1999; Kingwell, et al., 1997; Maiorana, O'Driscoll, Dembo, et al., 2001), and in whole body training in diseased populations (i.e., congestive heart failure, arterial disease, hypertension, etc.) (D. J. Green, Maiorana, O'Driscoll, & Taylor, 2004). In these studies, measurement of endothelial function included responses to Ach (endothelium-dependent NO vasodilator) and FMD. In healthy participants, whole body training resulted in no change, decreased, or increased endothelial function. In human subjects with existing cardiovascular disease or risk factors (i.e., hyperlipidemia, heart disease, and diabetes), 8 weeks of supervised circuit training (aerobic and resistance) improved endothelial function independent of changes in lipid profile, BMI, blood glucose, and blood pressure (D. J. Green, et al., 2004). It has been suggested (Maiorana, O'Driscoll, Dembo, et al., 2001), that individuals with impaired endothelial function are more likely to show changes in endothelial vasodilation, compared to healthy subjects with preserved endothelial function, after a moderate training program. This point is supported by the improvement in cardiovascular risk factors, associated with endothelial dysfunction, after exercise training (Maiorana, et al., 2000). Conversely, in healthy subjects, the effects of exercise training on endothelial function are mixed. For example, Clarkson et al. found that 10 weeks of aerobic and anaerobic exercise training improved brachial artery FMD by 77% in male military recruits (Clarkson, et al., 1999). On the other hand, in healthy subjects undergoing 8 weeks of supervised exercise training (Maiorana, O'Driscoll, Dembo, et al., 2001), Ach stimulated FBF did not improve, even with increases in fitness. Similarly, 10-weeks of high intensity endurance training (70% VO2peak) did not change FMD in type 2 diabetic offspring or their healthy controls (Ostergard, et al., 2006).

There are several potential explanations for the incongruent findings in healthy subjects. The first explanation has to deal with the structural adaptations that occur with extended exercise

training. Tinken et al. (2008) found that exercise-induced increases in brachial artery FMD occur between 1-2 weeks. Between two to four weeks FMD begins to decline but remains significantly different from pre-training FMD. Aerobic training after 4 weeks, results in a consistent decline in FMD. This reduction in FMD is inversely related to increases in arterial remodeling (Tinken, et al., 2008), measured by conduit artery dilator capacity or peak vasodilator capacity (Naylor, Weisbrod, O'Driscoll, & Green, 2005). Based on this evidence and previous work conducted by Laughlin and colleagues in animals (Laughlin, 1995; Laughlin, et al., 2003), functional adaptations, such as FMD occur prior to structural adaptations or remodeling. Thus, the longer the training program, the more likely the shear stress will return to normal due to increases in arterial size and structural remodeling. Based on this evidence the most appropriate length for an exercise training intervention, should be between 2-4 weeks. This will increase the likelihood a change in FMD is observed.

Aside from one study in male military recruits of average fitness (Clarkson, et al., 1999), we were unable to find any studies using a healthy population, <55 years of age, that found an exercise training-induced increase in FMD. Further evaluation of this study revealed that Clarkson et al. (1999) employed a training protocol that include both aerobic and anaerobic resistance training of the lower and upper limbs. The upper limb anaerobic resistance training resulted in improved upper limb anaerobic fitness. On the other hand, a similar study (Maiorana, O'Driscoll, Dembo, et al., 2001) that used both aerobic and resistance training only included select torso and upper body exercises. This protocol did not include any handgrip or forearm exertion and did not alter muscular strength (measured by maximal isotonic voluntary contractile strength). Thus, it is likely that by employing a training program that includes exercises utilizing forearm muscles, such as Clarkson et al. (1999), localized adaptations may occur. This in turn,

can lead to improved endothelial function of the trained extremity (Allen, Geaghan, Greenway, & Welsch, 2003; Hornig, et al., 1996).

Overall, it remains unclear whether acute or chronic exercise can improve endothelial function measured by FMD. Since improvements in FMD appear to be more noticeable in certain cases where endothelial function is attenuated (i.e., high fat diet or post-menopause), it is likely that improvements in FMD should be more noticeable in instances where endothelium function is impaired, such as smoking (Barua, et al., 2001; Celermajer, Sorensen, Georgakopoulos, Bull, et al., 1993; Gaenzer, et al., 2001; Lekakis, et al., 1998; Poredos, et al., 1999; Stoner, Sabatier, Edge, & McCully, 2004).

Smoking, Exercise, and Endothelial Function

To our knowledge, the information available on the effect of exercise on endothelial function, measured by FMD, in smokers is limited to dynamic whole body exercise, primarily in men (Clarkson, et al., 1999). To date, no study has directly addressed the effect of exercise on endothelial function in female smokers. Clarkson et al. (1999) found that exercise improved endothelial function in healthy smoking and nonsmoking military recruits. Although smokers had slightly lower FMD, the pre to post change in FMD after exercise was not different between smokers and non-smokers. Gaenzer et al. (2001) utilized two methods of endothelial vasodilation, exercise-induced flow-mediated dilation and hyperemia (post occlusion) induced FMD. They found among smokers, baseline FMD was impaired following ischemia and during exercise in the femoral artery. The forearm ischemia-induced vasodilatory response of the endothelium was highly correlated with the ability of the femoral artery to dilate during whole body exercise (r=.88) (Gaenzer, et al., 2001). It is imperative that researchers address the benefit of exercise on endothelial function in female smokers, an underreported population. It is

additionally important to identify other predictors of cardiovascular risk in women that may be improved by exercise, such as stress reactivity.

Stress Reactivity and Laboratory Stressors

It has been proposed, that cardiovascular reactivity tests are a useful tool for predicting risk for cardiovascular disease (Manuck, Kaplan, & Clarkson, 1983; Manuck, Olsson, Hjemdahl, & Rehnqvist, 1992; Menkes, et al., 1989; J. Turner, 1994). This offers the means to study risk factors for vascular disease and the impact of interventions. Hyper-reactivity to these tasks is associated with the development of hypertension (Carroll, Ring, Hunt, Ford, & Macintyre, 2003; Carroll, et al., 2001; Matthews, et al., 2004) and atherogenesis (Barnett, Spence, Manuck, & Jennings, 1997; Jennings, et al., 2004; Kamarck, et al., 1997). Responses to these tasks involve multiple physiological and behavioral reactions activated by the, autonomic nervous system, sympathoadrenal system, hypothalamic-pituitary-adrenocortical axis (HPA), renin-angiotensin system, and systems involving vasopressin and endogenous opiods (Chorus & Gold, 1992). Autonomic and endocrine responses cause the release of catecholamines (norepinephrine and epinephrine) from the hypothalamus and adrenal medulla, and cortisol from the adrenal cortex.

Psychophysiological stressors differ in their characteristic features (active vs. passive coping) and physiological responses (i.e. heart rate, blood pressure, stroke volume, cardiac output, and peripheral resistance) (Buckworth & Dishman, 2002). Common tasks include active coping stressors, which result from the organism either avoiding or overcoming stressful stimuli. Examples of this task include mental arithmetic, psychomotor reaction time, and the Stroop CWT. Passive stressors cannot be overcome and are met with little to no resistance. Forehead cold and cold pressor are two examples of a passive stressor. Key physiological responses to active stressors, such as the Stroop CWT, include increased HR, SBP, CO, withdrawal of vagal

tone, and β-adrenergic mediated vasodilation (Dietz, et al., 1994; Freyschuss, et al., 1988; Halliwill, et al., 1997). On the other hand, passive stress such as forehead cold (response is similar to the mammalian dive reflex), result in increased in vagal tone leading to decreased HR (Heindl, et al., 2004; Treiber, et al., 2003). Forehead cold is also associated with increased resistance because of α-adrenergic mediated vasoconstriction (Dishman, et al., 2003). Although there are common cardiovascular responses to stress, individual differences are present. These differences are not simply a function of task difficulty or how engaged participants were by the task (J. R. Turner & Carroll, 1985). Instead, factors such as gender play a role in how an individual will respond to stress (Dishman, et al., 2003; Saab, 1989; Schmidt, 1983). A large portion of the literature on stress reactivity has been conducted in males with less consideration for females (Jackson & Dishman, 2006). Since the physiological responses to stress are different (Stoney, Davis, & Matthews, 1987), results shown in males cannot be generalized to women. Thus more studies need to be conducted that assess stress reactivity in women and factors that may augment this response.

Stress Reactivity and Cigarette Smoking

It has been suggested, that cigarette smoking and stress interrelate; thereby, increasing lifetime risk for disease (Epstein & Jennings, 1986). Epstein and Jennings (1986) proposed 3 primary hypotheses whereby smoking and stress may directly or indirectly increase risk of CHD: a) under stressful conditions, smokers are prone to smoke more as a means of coping (Pomerleau & Pomerleau, 1987; Rose, Ananda, & Jarvik, 1983; Schachter, 1978). For example, puff rate and smoking intensity was increased during impromptu videotaped monologue or concentration tasks compared to quiet rest. The higher the dose smoked is dose dependently related to risk of CHD. b) Smokers are more inclined to report lower feelings of subjective anxiety when smoking

("nicotine paradox") (Jarvik, Caskey, Rose, Herskovic, & Sadeghpour, 1989). This decrease in subjective feelings of anxiety are associated with decreased sensitivity to aversive stimuli (Pomerleau & Pomerleau, 1987) which results in longer exposure to the stressful stimulus to the point where coping is no longer advantageous (Epstein & Jennings, 1986). c) Smoking and stress combined play an additive role in cardiovascular arousal (Byrne, 2000; M. C. Davis & Matthews, 1990; MacDougall, Musante, Castillo, & Acevedo, 1988; Tersman, Collins, & Eneroth, 1991; Tsuda, et al., 1996). Similar to stress, the nicotine present in cigarettes smoke is known to increase heart rate (HR), blood pressure (BP), circulating catecholamines, and peripheral vasoconstriction (Cryer, et al., 1976; Perkins, et al., 1986). Thus, in stressed smokers, cardiovascular reactivity is heightened compared to stress alone and to smoking alone (MacDougall, et al., 1983), and even more exaggerated during sham smoking and relaxation (Byrne, 2000). The latter hypothesis allows the determination of the psychophysiological mechanisms whereby smoking and stress can potentially increase risk for cardiovascular disease.

The additive effects of smoking on the vasculature have been consistently shown using multiple psychophysiological stressors for heart rate, blood pressure, cortisol, and catecholamines (J. W. Davis, Shelton, Eigenberg, Hignite, & Watanabe, 1985; MacDougall, et al., 1988; Perkins, et al., 1992; Pomerleau & Pomerleau, 1987; Robinson & Cinciripini, 2006). Unfortunately, the measurement of cardiovascular responses has been limited to BP and HR, thus restricting discussion on potential mechanisms. In addition, few studies (MacDougall, et al., 1988) have addressed the relationship between smoking and stress in current women smokers. There are multiple mechanisms proposed that heightened reactivity during stress in smokers may increase risk for disease. It is likely that altered peripheral vascular responses in target tissue interact with endocrine and autonomic signaling from the central nervous system to accelerate

disease progression (Lovallo & Gerin, 2003). Thus, in this population, it is important to develop lifestyle interventions that can prevent or slow the progression of disease.

Effects of Acute and Chronic Exercise on Cardiovascular Reactivity

Similar to exercise and FMD, it is plausible that physical activity or exercise can reduce risk for disease in women smokers (M. B. Conroy, et al., 2005; Hambrecht, et al., 2004a; T. Y. Li, et al., 2006; Noda, Iso, Toyoshima, Date, Yamamoto, et al., 2005), by decreasing cardiovascular arousal and stress reactivity. Acute exercise has also been shown to reduce post-exercise blood pressure (Kenney & Seals, 1993; MacDonald, 2002; Pescatello & Kulikowich, 2001). In a comprehensive meta-analysis (Petruzzello, Landers, Hatfield, Kubitz, & Salazar, 1991), dynamic exercise lasting approximately 40 minutes in length results in a small but significant reduction in systolic (-3.2mmHg) and diastolic (-1.6mmHg) blood pressure. These effects were found in both sedentary men and women, and were a function of their baseline blood pressures.

A meta-analysis of 15 studies addressing the benefit of acute exercise on cardiovascular reactivity found that exercise reduced systolic and diastolic blood pressure by a mean effect of 0.38 SD and 0.40 SD, respectively (Hamer, et al., 2006). Six of the fifteen studies tested women, 3 studies used the Stroop CWT and 1 used forehead cold as a stressor. In addition, exercise attenuated the diastolic blood pressure responses by -.55 SD and -.17 SD to the Stroop CWT and forehead cold, respectively. None of these studies used a standardized model of difficulty for the Stroop CWT. Although no studies used the proper dose-response design to determine the relationship between exercise intensity and duration, moderate (W. Rejeski, Gregg, Thompson, & Berry, 1991) and vigorous (West, Brownley, & Light, 1998) activity resulted in reduced blood pressure responses. The use of multiple stressors has presented mixed results with some

reporting response to both task (W. J. Rejeski, Thompson, Brubaker, & Miller, 1992) or only the first task (habituation) (Steptoe, Kearsley, & Walters, 1993).

After moderate intensity exercise, a subsequent reduction in blood pressure occurs (Kenney & Seals, 1993; MacDonald, 2002; Pescatello & Kulikowich, 2001). The additional benefit of repeated bouts of acute exercise may eventually result in cardiovascular adaptations that reduce stress reactivity. Some argue that the benefits of exercise on stress reactivity can only occur within a post-exercise window (Hamer, et al., 2006). Thus, chronic exercise or regular daily activity would increase the likelihood individuals were within this window. It has been suggested that in order to study the impact of acute and chronic exercise, the two interventions must be studied separately (Hamer, et al., 2006). Acute exercise may overtime result in chronic cardiovascular and metabolic adaptations to exercise that extend to non-exercise stressors (Thompson, et al., 2001).

Chronic exercise training has been shown to attenuate stress reactivity but with mixed results. An earlier meta-analytic review found a homogenous effect of exercise of 0.48 of aerobic exercise (both acute and chronic) on psychosocial stress response. This effect equates to approximately 0.50 SD effect of exercise compared to control or baseline values. A major limitation of this meta-analysis was that responses both during stress and recovery were combined to give an overall mean effect. In addition, the authors combined both acute and chronic exercise training which have differing responses on the vasculature. Chronic exercise results in cardiovascular adaptations that may alter cardiovascular responses during stress (i.e., HR, blood pressure, increased SV and VO2peak, decreased/increased sensitivity to adrenergic (β or α) stimulation. A more recent meta-analysis (Jackson & Dishman) assessed study features (i.e., study design, stressor tasks, dependent measures and populations at risk for CVD) that

might moderate the relationship between exercise training and stress reactivity. It was found that out of 19 randomized exercise training studies that included a control group, reactivity did not change (Jackson & Dishman, 2006). The only study feature that modified the relationship between exercise training and reactivity was the dependent variable measured. HR and peripheral resistance resulted in positive effects, where as, negative effects were generally observed with limb blood flow. Because the meta-analysis included multiple studies that utilized several different laboratory stressors, it is uncertain what effects may occur when using the Stroop CWT or forehead cold. Specific studies that have utilized the Stroop CWT or forehead cold also reported mixed results (Bond, et al., 1999; Ray & Hume, 1998; Rogers, Probst, Gruber, Berger, & Boone, 1996; Sothmann, Hart, & Horn, 1992; Stein & Boutcher, 1992; Vona, et al., 2004). The cause for null findings after chronic exercise training may be: 1) an acute reduction in disease risk (PEH) that diminishes before any impact on health is observed 2) larger and more observable effects are present the closer to the exercise session and diminishes as time lapses, and 3) the health impact of acute exercise may not be large enough to observe in smaller clinical trials (Haskell, 1994).

Exercise, Smoking, and Cardiovascular Reactivity

Though the evidence discussed illustrates how physical activity and exercise may be beneficial in improving cardiovascular reactivity and vascular health, less has been studied on its benefits in women smokers, an underrepresented population, at high risk for CVD (Jackson & Dishman, 2006). In addition, we were unable to locate any studies that addressed chronic adaptations to exercise training in female smokers.

A few studies have addressed the relationship between stress response in smokers and the benefit of acute exercise. The earliest study examined 12 male sedentary smokers responses to

mental arithmetic after 20 minute of cycling (P. O. Russell, L. H. Epstein, & K. T. Erickson, 1983). Exercise did not reduce stress reactivity as measured by electromyographic activity, skin conductance, and heart rate. Measures of vascular health were not obtained and no control group was used. A more recent study examined the effect an acute bout of 15 minutes of brisk semi-self paced walking session has on the BP response to three psychosocial stressors (A. H. Taylor, Katomeri, & Ussher, 2005). Participants performed the Stroop CWT, a speech task, and were exposed to a lit cigarette under a period of temporary abstinence from smoking. Exercise reduced blood pressure after the three stress conditions by 3.8 mmHg compared to non-exercise controls. This study though it used a short bout of exercise, found a small but significant change in blood pressure. The attenuated increase in blood pressure responses during the Stroop CWT among smokers (A. H. Taylor, et al., 2005) after exercise were similar to results from previous studies of non-smokers that showed attenuated reactivity during the Stroop CWT (Hobson & Rejeski, 1993) (Probst, Bulbulian, & Knapp, 1997; W. J. Rejeski, et al., 1992) and the cold pressor test (Ebbesen, Prkachin, Mills, & Green, 1992). A primary limitation of both studies includes the absence of a control group of non-smokers.

Purpose

To date, few studies have addressed the relationship between acute and chronic exercise on endothelial function and stress reactivity among female smokers and non-smokers. Based on the available evidence, the effect of exercise on endothelial function and stress reactivity remains inconclusive. The purpose of this study was to compare the effects of acute and chronic moderate intensity cycling exercise on FMD and on cardiovascular reactivity during neurovascular stress in sedentary female smokers versus non-smokers. Novel features of this research are the use of multiple measures of cardiovascular responses in addition to HR and BP,

including arterial diameter, arterial velocity, and blood flow during vasodilatory (i.e., Stroop CWT) and vasoconstrictive (i.e., forehead cold) stress.

Hypothesis

It is expected that:

1. FMD will be impaired and stress reactivity augmented in smokers compared to non-smokers.
2. Acute and chronic exercise will improve FMD and attenuate cardiovascular responses to neurovascular stressors similarly in both groups. We expect that acute exercise will oppose increased blood pressure during stress by decreasing limb blood flow and vascular resistance during the Stroop CWT and by decreasing vascular resistance during forehead cold. It is hypothesized that these mechanisms of attenuated stress reactivity after acute exercise will lead to cardiovascular adaptations with chronic exercise training.

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CHAPTER 3

ACUTE EXERCISE IMPROVES ENDOTHELIAL FUNCTION AND VASCULAR RESPONSES DURING STRESS IN FEMALE SMOKERS AND NON-SMOKERS[1]

[1]Rooks, C.R., McCully, K.K., Dishman, R.K. To be submitted to Medicine & Science in Sports & Exercise

ABSTRACT

The purpose of this study was to determine the effect of an acute bout of moderate-intensity cycling exercise on endothelial function, measured by flow mediated dilation (FMD), and cardiovascular responses during neurovascular stress among sedentary female smokers and non-smokers. FMD was determined by brachial arterial diameter, arterial velocity and blood flow measured by Doppler ultrasonography after forearm occlusion. Those measures and beat-to-beat finger blood pressure (BP) and heart rate (HR) were also assessed in response to 2 min of forehead cold and 4 min of the Stroop Color-Word conflict test (CWT) (separated by 8 min of rest). Individuals participated in two counterbalanced conditions: 30 minutes of passive rest or an acute bout of cycling exercise (~50% VO_2peak; perceived exertion rating of 12-13). Baseline FMD and stress responses were not different between smokers and non-smokers. Compared to passive rest, exercise increased FMD and reduced baseline systolic and diastolic BP, HR, arterial velocity, and blood flow in both groups. In addition, exercise decreased arterial velocity and blood flow responses during the Stroop CWT and forehead cold in both groups. Overall, exercise improved vascular function and led to reduced limb blood flow during both dilatory and constrictive neurovascular stress in the presence of post-exercise hypotension among female smokers and non-smokers.

INTRODUCTION

Vascular endothelial dysfunction (87) and cardiovascular reactivity during neurovascular challenge (98) are regarded as risk factors in the prediction of future development of cardiovascular disease (CVD). It has been suggested that acute exercise may improve endothelial function and augment cardiovascular reactivity to neurovascular stressors, but the supportive evidence is mixed (49, 70). In addition, few studies have addressed the effects of exercise on vascular health and vascular reactivity in women or women who are at high risk for CVD because they smoke (67).

Epidemiological evidence (9) supports a link between smoking and CVD. Among women, smoking is the leading cause of preventable death and disease (1) and 22% of deaths among smokers are associated with CVD (67). Endothelial dysfunction (87) and hyper-reactivity to neurovascular challenges (98) are two plausible mechanisms that may explain smoking-related morbidity and mortality. Endothelial function is commonly assessed by flow-mediated dilation (FMD), which is a non-invasive hyperemic technique (13). FMD occurs in response to shear stress or frictional force on the endothelium resulting from increases in blood flow (74). The mechanisms responsible for FMD are primarily mediated by nitric oxide (40) which is important for the maintenance of vascular health and vascular tone (56). Neurovascular challenges, such as the Stroop Color-Word conflict test (CWT) (an autonomic vasodilator that acts by sympathetic withdrawal or β-adrenoreceptor activation) (24, 47) and forehead cold (an autonomic vasoconstrictor that acts by α1-adrenoreceptor activation) are commonly used cardiovascular reactivity tests (53, 98). A typical limitation of studies that employ these tasks is that only blood pressures and heart rate are measured as cardiovascular responses. This limits

the conclusions that can be drawn about vascular responses and the underlying mechanisms of the autonomic nervous system that regulate limb blood flow.

Chronic smoking results in impaired endothelial function (12, 38) and, when combined with stress, increases cardiovascular reactivity (65). It is believed that cigarette smoke includes high amounts of free radicals and pro-oxidants that increase oxidative stress and degrade endothelium derived relaxing factors (i.e. NO, prostacyclin, and endothelium derived hypopolarizing factor) that are important for vessel tone, compliance, and vasodilatory function (37). In addition, nicotine in cigarettes smoke is known to increase heart rate (HR), blood pressure (BP), circulating catecholamines, and peripheral vasoconstriction (21, 71).

A meta-analysis of 30 studies reported that among women, low- and moderate-intensity physical activity reduces the overall risk of CVD by 82% and 78%, respectively (68). Specifically, a single bout of dynamic exercise increases blood flow and shear stress, which leads to an increase in NO synthesis and release (33, 66, 79), important for endothelial function. Exercise-induced hyperemia occurs in both active and inactive limbs (41, 42), and is observed with a systemic increase in NO production (41). To date, few studies have tested the effect of acute exercise on FMD (70), and only one focused on women (52). Harvey et al. (2002) found that after 45 minutes of treadmill exercise at an intensity of 60% VO_2peak, FMD was increased in premenopausal women, although non-significantly. Other studies that measured FMD after acute exercise among men or mixed groups of men and women found no change (76, 90), an increase (20, 51, 69), or a decrease in FMD (83, 89). Based on these cumulative findings, the effect of acute exercise on FMD is inconclusive.

Inconsistency in the findings of past studies may have resulted from the diverse populations studied or the timing of FMD measurements after exercise. The optimal time to

measure FMD after exercise remains unclear, but studies that measured FMD within 60 minutes observed larger increases in FMD (20, 52, 69) compared to those that waited longer than an hour (76).

Similar to the effects of acute exercise on FMD, the benefit of acute exercise on cardiovascular reactivity remains unclear. A meta analysis found that in 10 of 15 studies, acute exercise significantly reduced BP responses during neurovascular stressors (49). Six of the 15 studies tested women, 3 studies used the Stroop CWT and only 1 used forehead cold as a stressor. Thirty minutes of dynamic exercise at 50%VO_2peak was the minimum dose necessary to show an exercise-induced reduction in pressor responses during the tasks. None of these studies measured vascular responses or used a standardized version of the Stroop CWT that controls for task difficulty and social-interaction.

We were unable to find any studies of female smokers that measured the effect of an acute bout of exercise on FMD. A recent study found that chronic smoking decreased FMD and an acute 6-minute walk test increased FMD in a group of men and women, but the findings weren't reported separately for women (3). In males, exercise has been shown to equally improve FMD in smokers and non-smokers (14, 38). To date, the benefit of exercise on FMD in woman smokers remains uncertain.

Likewise, only a few studies have examined the effects of acute exercise on cardiovascular reactivity during stress among smokers. The earliest study (84) found no effect of cycling on HR responses to mental arithmetic in sedentary, male smokers. A more recent study (96) of male and female smokers found that 15 minutes of brisk, self-paced walking reduced BP after three separate stress conditions (including the Stroop CWT) by 3.8 mmHg compared to non-exercise controls. The attenuated increase in blood pressure responses during the Stroop

CWT among smokers (97) after exercise were similar to results from previous studies of non-smokers that showed attenuated reactivity during the Stroop CWT (55, 75, 78) and the cold pressor test (29). Limitations of both studies (84, 96) were the absence of an exercising control group of non-smokers and no measures of vascular responsiveness.

In addition, post-exercise hypotension moderated reactivity during stress in the Taylor et al. study (97). Therefore, changes in blood pressure responses during stress after exercise may be confounded with a reduction in resting blood pressure. Previous studies have accounted for the potential confounding effects of resting blood pressure by assessing stress responses (i.e., change from baseline) rather than comparing absolute levels during stress to pre-exercise levels (55, 75, 78).

The purpose of this study was to compare the effects of an acute bout of moderate intensity cycling exercise on FMD and on cardiovascular reactivity during neurovascular stress in groups of sedentary female smokers versus non-smokers. In general, reactivity to laboratory stressors has focused on HR and BP responses. Novel features of this study are the use of multiple measures of cardiovascular responses in addition to HR and BP, including arterial diameter, arterial velocity, and blood flow during vasodilatory (i.e., Stroop CWT) and vasoconstrictive (i.e., forehead cold) stress. Neurovascular stress can impair endothelial function (28, 54, 63). Therefore, we measured FMD after recovery from exposure to the two stressor tasks. Two hypotheses were tested: 1) FMD will be impaired and stress reactivity augmented in smokers compared to non-smokers, and 2) exercise will improve FMD and attenuate cardiovascular responses to neurovascular stressors similarly in both groups. Specifically, we expected that acute exercise would oppose increased blood pressure during stress by decreasing

limb blood flow and vascular resistance during the Stroop CWT and by decreasing vascular resistance during forehead cold.

METHODS

Twenty-three participants were recruited, 11 regular cigarette smokers (who reported an average of 10 cigarettes/day for at least 1 year) and 13 non-smokers, from the University of Georgia and local Athens, Georgia community. The mean pack years ± SD reported by smokers was 1.38 ± 0.79. The mean age ± SD was 20.64 ± 2.06 yr for smokers and 20.54 ± 2.33 yr for non-smokers. The study was approved by the Institutional Review Board and all participants gave informed consent prior to testing.

Participants completed 3 successive sessions separated by 48 hours: a maximal exercise test, experimental protocol + passive rest, and experimental protocol + acute exercise. Experimental days that included passive rest or exercise were counterbalanced. The first session included the maximal exercise test. Prior to that test, participants were asked to complete a pre-exercise medical history questionnaire, a historical leisure activity questionnaire (61), and a 7-day physical activity recall interview (PAR) (5). If no contraindications to exercise were present (i.e., untreated diabetes or other endocrinological disorder, renal or peripheral venous disease, uncontrolled hypertension, heart disease, or lung disease, alcohol, dietary supplement, or drug use that would complicate interpretation of the results, or use of psychoactive, anti-inflammatory, or analgesic medications within the previous week), participants then performed an incremental exercise test using a standard procedure to measure peak oxygen consumption (VO_2peak). After instruction about the test procedures, participants then sat on a Lode Excalibur Sport (Groningen, Nederland) electronically braked cycle ergometer while a Polar model S810i (Polar Oy, Kempele Finland) HR monitor, mouthpiece, and nose clip were affixed. VO_2peak

($ml \cdot kg^{-1} \cdot min^{1}$) was determined by open circuit spirometry using a ramped protocol. Participants sat on the cycle ergometer without moving for 30 seconds, followed by a low intensity (50 W) 5-minute warm up. After the warm up, ergometer resistance increased continuously at a rate of 24 watts per minute until volitional exhaustion. Resistance was controlled by computer software via an interference cable connected to the ergometer. HR and ratings of perceived exertion (RPE), using the Borg 6-20 category scale (7) were obtained every minute. Once peak effort was reached, participants underwent a 2-min low intensity (25 W) cool down. Oxygen consumption was measured continuously using a Sensormedics Model 2900 metabolic cart. The metabolic cart was calibrated with standard gases. VO_2peak ($ml \cdot kg^{-1} \cdot min^{1}$) was defined as the highest attained oxygen consumption when at least two of the following criteria were met: a HR within 90% of the age predicted maximal HR, a respiratory exchange ratio above 1.1, and an RPE value greater than 18. VO_2peak was used to standardize a subsequent moderate-intensity (~50%) exercise bout.

Experimental Protocol.

Baseline measurements and stress reactivity were assessed by systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), HR, arterial velocity, arterial diameter, and blood flow before and after exercise or passive rest. In addition, FMD in response to reactive hyperemia was determined before and after exercise. Figure 3-1. illustrates the order of baseline measurements, exposure to stressor tasks, and FMD measurements. Individuals were tested during the first 12 days of their menstrual cycle (i.e. the onset of menstrual flow is day 1), which was determined by oral confirmation (8). Participants were asked to fast 2 hours prior to testing and refrain from alcohol, caffeine, and exercise at least 24 h prior to testing. Smokers were instructed to abstain from smoking at least 2 hours prior to

arriving to the lab. Because abstinence from smoking increases vascular reactivity (99) and decreases FMD (94), smokers were given a low nicotine (~0.5mg nicotine) cigarette to smoke upon arrival to the lab. This also served to standardize smokers last smoking session. Fifteen minutes elapsed between exposure to the cigarette and data collection. Low nicotine cigarettes (<10mg nicotine) have been shown to not alter vascular measurements, such as BP and HR (59). The rest of the protocol was identical for smokers and non-smokers.

Vascular Measurements of Stress Reactivity. Individuals were directed to the Vascular Biology Laboratory where they were fitted with a Polar model S810i heart rate monitor (Polar Oy, Kempele, Finland). Participants were instructed to lie on a cushioned table in supine position, and the right arm was placed on an extended table to access the brachial artery. All measurements of arterial diameter and arterial velocity were taken on the right arm for each testing session. A GE 400CL duplex color Doppler imager (GE Medical) was used to assess diameters of the brachial artery, while simultaneously measuring arterial velocity before, during, and after stress and reactive hyperemia. Blood pressure was determined by placing a small finger cuff on the middle finger to assess continuous finger arterial pressure using an Ohmeda 2300 Finapres blood pressure monitor. Data acquisition of blood pressure occurred using the Biopac MP100 physiological data acquisition system (Biopac Systems Inc., Goleta, CA) and AcqKnowledge software (v3.7.3) (Biopac Systems Inc). Participants were given ~10 min during setup for quiet rest prior to testing and exposure to stressors. Four minutes of baseline recordings were taken.

Following baseline assessments, individuals were exposed to forehead cold or the Stroop CWT. Stressor administration was counterbalanced between participants. Forehead cold was administered by placement of a bag of ice water on the forehead for a total of 2 minutes. A

computerized version of the Stroop CWT was administered. The test randomly presented in the middle of the screen a color word (red, blue, green, and yellow) along with four color word options at the bottom of the screen. The purpose of the test is to choose the word at the bottom that describes the color of the word in the middle. A four-button controller was used by the participant to record their response. The test was administered for four minutes. Since task difficulty is associated with neurovascular responses during the Stroop CWT (11, 36), the task was programmed to adjust the speed of word presentation according to the number of correct answers in order to maintain the difficult of the task at a correct percentage of 60% ± 10%. After exposure to each stressor, individuals were given 8 minutes for recovery.

Vascular Measurements of FMD. After 8 minutes of recovery from laboratory stressors, during which blood pressures returned to within 2 or 5 mmHg of baseline diastolic or systolic values, FMD measurements were taken. Baseline measurements of brachial artery diameter were recorded every 30 seconds for 2 minutes, while arterial velocity was continuously measured. A pressure cuff (Hokanson Inc., Bellevue, WA) was previously placed on the right forearm distal to ultrasound measurements upon entry to the lab. After baseline recordings, the cuff was rapidly inflated to ~50 mmHg above SBP for 5 minutes while diameter measurements were continued every 30 seconds. After 5 minutes, (22, 94) pressure was quickly released from the cuff inducing reactive hyperemia. Arterial diameters were then recorded continuously for 3 minutes. Averaged diameter readings (across 3 minutes) at baseline and peak hyperemia (averaged across 2 seconds) were used for analysis. The between-days coefficient of variation for baseline FMD was 32%, which is consistent with values between 28-50% previously reported in the literature (22, 94). FMD (FMD %) was defined as the peak hyperemic diameter-baseline/baseline*100. Area under the curve (AUC) was used to quantify reactive hyperemia for 30 seconds after cuff

deflation. AUC was computed by summing the area of consecutive post-occlusion trapezoids (trapezoidal rule) for 30 sec and was calculated as:

$\sum\{y_i[x_{(i+1)} - x_i] + (1/2)[y_{(i+1)} - y_i][x_{(i+1)} - x_i]\}$. The within-day coefficient of variation for reactive hyperemia was 24% and the between-day coefficient of variation was 30%. The shear stress stimulus, or 30 sec postocclusion hyperemic stimulus, was calculated as: $SS_{AUC} = 4 \cdot AUC/\text{arterial diameter (cm)}$. FMD, normalized to shear stress, was expressed as FMD:SS_{AUC} ratio.

Arterial diameter and velocity. Quantitative Doppler ultrasound imaging (GE 400CL) was used to determine simultaneous measurement of arterial velocity and diameter. A 5-10 MHz linear array ultrasound transducer was used. All measurements were taken on the right arm brachial artery during each testing period. Velocity values were collected across the entire length of the artery and time-averaged maximal velocity (T_{max}) was determined using pulsed Doppler ultrasound with a maximum Doppler angle of 60°. Calculations were conducted using GE's advanced vascular program for the LogiQ 400CL. Integrated GE medical software was used to determine the time averaged maximum velocity for every heart beat and recorded real-time. A consistent section of artery diameter was measured using B-mode imaging and actual diameter was measured using semi-automated edge-detection software (LabView, National Instruments (Austin, TX). A representative section of the arterial wall was identified, and a line of best fit was identified. The within-day reliabilities (ICC-2) for 4 minutes of baseline values for arterial velocity and diameter > .95. The within-day coefficient of variation was ~ 3%. The between-day reliabilities of baseline values for arterial velocity and diameter > .90. Blood flow was computed as ml/min by using arterial velocity and diameter scores (velocity * π * $(\text{diameter}/2)^2$). Vascular resistance (arterial resistance unit (aru)) was estimated by dividing MAP (1/3*pulse pressure + diastolic blood pressure) by arterial blood flow. Vascular

conductance (arterial conductance unit (acu)) was determined by dividing arterial blood flow by MAP.

Exercise and Passive Rest Condition. After vascular measurements were completed, participants walked (~1 minute) to the same room where the maximal exercise test was administered. They then engaged in passive rest or an acute bout of moderate-intensity cycling exercise (50% VO_2peak; perceived exertion rating of 12-13), lasting 30 minutes as recommended in the 2007 Guidelines for Cardiovascular Disease Prevention in Women (67). Exercise intensity was verified by analysis of expired gases using the Sensormedics metabolic cart every five minutes. During passive rest, individuals sat quietly alone for 30 minutes in the same room as the cycle ergometer. The order of acute exercise or passive rest was counterbalanced between participants. Upon completion of exercise or rest conditions, participants were led back to the Vascular Biology Laboratory for posttest measurement of cardiovascular stress reactivity, and FMD. Individuals were given 10 minutes to recover prior to testing. Blood pressure (within 2-5 mmHg of resting values) was used to assess whether cardiovascular parameters returned to normal.

Data Analysis. Statistical analysis was performed using SPSS 16.0 computer software (SPSS, Chicago, IL). The dependent measures were HR, SBP, DBP, MAP, arterial diameter, arterial velocity, and blood flow each averaged before and during stress. In addition, FMD, reactive hyperemia, shear stress, and normalized FMD were dependent variables during FMD testing. Change scores were calculated to analyze stress reactivity and FMD. Average response during the baseline period, before each stressor, was subtracted from the average response during each stressor. Group comparisons on pre-task baseline scores for each dependent variable were tested by a 2 (group) x 2 (time) mixed model ANOVA with time repeated. Linear regression

analysis was used to assess the relations between body weight and vascular responses during stress. In addition, linear regression analysis was used to determine the relations between stress responses and baseline values. Changes in the dependent variables during the baseline stressor tasks were tested for all participants using directional paired samples t-test. Comparisons of tasks before passive rest or exercise were tested by a 2 (group) x 2 (task) x 2 (time) mixed model ANOVA with task and time repeated. Outlying effects for one participant were replaced with the overall group mean for that variable (45).

Hypothesis 1 comparing groups on pre-condition stress responses, FMD, and AUC were tested by the group effect in a 2 (group) x 2 (time) mixed model ANOVA with time repeated. Hypothesis 2 comparing the effects of exercise with passive rest on pre-task baseline scores, responses during stress (change from baseline), FMD, and AUC were tested by the condition effect in a 2 (group) x 2 (condition) ANCOVA with condition repeated. Responses before exercise or passive rest were included as the time-varying covariate. Eta squared was used to represent effect sizes. Twenty-four subjects were needed to provide a statistical power of .80 at an $\alpha<0.05$ (30). Data are presented as means ± SD, except where stated otherwise. Significance tests were two-tailed and set at $p<.05$.

RESULTS

Participant Characteristics

Participant characteristics (M ± SD) are presented in table 3-1. Groups were not significantly different at baseline on age, height, VO_2peak, daily energy expenditure during the previous week, and historical leisure time physical activity. Non-smokers weighed more than smokers ($p=.023$), however, body weight was not related to vascular responses during either stressor (β's < -.10, p's > .24).

Baseline means and standard deviations pre and post conditions for dependent variables are presented in Table 3-2. Averaged across testing days, baseline SBP [t(22)=.786, p=.44], DBP [t(22)=1.10, p=.28], MAP [t(22)=1.10, p=.28], HR [t(22)=.891, p=.38], arterial diameter [t(22)=1.26, p=.22], and arterial BF [t(22)=1.45, p=.17] were not different between groups. Smokers had significantly higher baseline arterial velocities [t (22) =2.50, p=.02].

Stress Responses

Responses to the Stroop CWT and forehead cold pressor before and after exercise are presented as change scores (M±SD) in table 3-3.

Stroop CWT. SBP [t (23) =7.61, p<.001], DBP [t (23) =5.72, p<.001], and MAP [t (23) =4.65, p<.001] increased significantly from baseline during the Stroop CWT. HR [t (23) =1.63, p=.06] increased, but the change did not reach statistical significance. Arterial velocity [t (23) =.64, p=.27] was not changed. There were small, non-significant decreases in arterial diameter [t (23) =1.04, p=.16] and blood flow [t (23) =.89, p=.19].

Forehead Cold Pressor. There were significant increases in SBP [t (23) =5.47, p<.001], DBP [t (23)=5.00, p<.001], and MAP [t (23)=3.77, p<.001]. In addition, there were significant decreases in HR [t (23) =3.04, p=.003], arterial velocity [t (23) =5.50, p<.001], arterial diameter [t (23) =1.81, p=.04], and blood flow [t (23) =4.54, p<.001] during forehead cold.

The Stroop CWT resulted in higher SBP [F (1, 22) =11.89, p=.002, η^2=.35] and DBP [F (1, 22) =12.44, p=.002, η^2=.36] responses compared to forehead cold. Pre-condition responses were lower for forehead cold than the Stroop CWT for arterial velocity [F (1, 22) =52.96, p<.001, η^2=.71], HR [F (1, 22) =20.82, p<.001, η^2=.49], and blood flow [F (1, 22) =29.40, p<.001, η^2=.57].

Hypothesis Testing

Group Differences

Vascular Responses during Stressors. None of the responses during the Stroop CWT [$F(1,22)<2.35$, $p>.14$] and forehead cold [$F(1,22)<2.37$, $p>.14$] differed between smokers and non-smokers.

Endothelial Function. FMD [$F(1, 22) = 0.01$ $p=.93$] and reactive hyperemia [$t(1, 22) =0.47$, $p=.50$] were not different between groups before conditions.

Effects of Acute Exercise

Baseline Vascular Responses. After exercise, compared to passive rest, there was a significant reduction in SBP, [$F(1,21)=7.48$, $p=.013$, $\eta^2=.27$], DBP [$F(1,21)=20.95$, $p<.001$, $\eta^2=.51$], MAP [$F(1,21)=19.01$, $p<.001$, $\eta^2=.49$], and vascular resistance [$F(1,21)=33.69$, $p<.001$, $\eta^2=.63$]. Baseline HR [$F(1,21)=99.57$, $p<.001$, $\eta^2=.83$], arterial velocity [$F(1, 21)=61.64$, $p<.001$, $\eta^2=.75$], blood flow [$F(1,21)=48.30$, $p<.001$, $\eta^2=.70$], and vascular conductance [$F(1,21)=52.91$, $p<.001$, $\eta^2=.73$] increased after exercise. No group x condition interactions [$F(1, 21) <1.78$, $p>.20$] or group differences were observed [$F(1, 21) <0.941$ $p>.345$].

Vascular Responses During Stressors. During the Stroop CWT, increases in HR [$F(1, 21) =4.08$, $p=.056$, $\eta^2=.16$], arterial velocity [$F(1, 21) =30.99$, $p<.001$, $\eta^2=.60$], blood flow [$F(1, 21) =20.94$, $p<.001$, $\eta^2=.50$], and vascular conductance [$F(1, 21) =23.71$, $p<001$, $\eta^2=.54$] responses were all attenuated after exercise compared to passive rest. In addition, vascular resistance was higher after the Stroop CWT compared to passive rest [$F(1, 21) =9.51$, $p = 006$ $\eta^2=.32$]. During forehead cold, reductions in arterial velocity [$F(1, 21) =39.57$, $p<.001$, $\eta^2=.60$], blood flow [$F(1, 20) =30.21$, $p<.001$, $\eta^2=.60$], and vascular conductance [$F(1, 21) =34.48$, $p<.001$, $\eta^2=.63$] were augmented after exercise compared to passive rest. In addition, an attenuated reduction in HR response during forehead cold approached significance [$F(1, 20)$

=3.23=.08, η^2=.13]. No significant differences after exercise compared to passive rest were observed for SBP [F (1, 21) =2.64, p=.12], DBP [F (1, 21) =2.50, p=.13], and MAP [F (1, 21) =1.89, p=.19] responses during the Stroop CWT. In addition, there were no significant differences after exercise compared to passive rest for SBP [F (1, 21) =0.70, p=.41], DBP [F (1, 21) =.01, p=.98], and MAP [F (1, 21) =1.35, p=.26] responses during forehead cold. There were also no group x condition effects for any of the dependent variables during the Stroop CWT or forehead cold [F (1, 21) <1.34, p>.26]. In addition, there were no differences between smokers and non-smokers in their responses to the Stroop CWT or forehead cold [all F (1, 21) <2.07, p>.13].

After exercise, responses during the Stroop CWT and forehead cold, were inversely related to post-exercise baseline values for arterial velocity (β= -.42 and -.70, p < .001) (Figure 3-2) and blood flow (β= -.48 and -.68, each, p < .001) (Figure 3-3), respectively. After passive rest, change in arterial velocity (Figure 3-2) and blood flow (Figure 3-3) were inversely related to post-rest baseline values during forehead cold (β= -.53 and -.61, p < .001) but not during the Stroop CWT (β= .002 and -.147, ps > .16). HR responses were not related to post-condition baseline values for either stressor task (β= -.01 and .09, ps > .51).

Endothelial Function. There was a significant main effect of condition on FMD [F (1, 21) =11.33, p=.003, η^2=.35] (Figure 3-4). FMD was higher in both groups after exercise (13.16 ± 2.40%) compared to passive rest (8.93 ± 2.07%). There was also a main effect of condition on reactive hyperemia [F (1, 21) =41.52, p<.001, η^2=.66] (Figure 3-5) and shear stress [F(1,21) = 30.64, p <.001) (Figure 3-6). Reactive hyperemia was higher after exercise (2051.23 ± 585.57) compared to passive rest (1550.82 ± 424.50). In addition, shear stress was higher after exercise (2527.34 ± 862.86) compared to passive rest (1942.88 ± 624.02) in both groups. The effect of

exercise on FMD was abolished when normalizing FMD to shear stress [F= (1,21)=1.00, p = .33] (Figure 3-7).There was no group x condition effect for FMD [F (1, 21) =.29, p=.60], reactive hyperemia [F (1, 21) =.24, p=.63], or shear stress [F(1,21) = .60, p = .45]. In addition, FMD [F (1, 21) =.04, p=.844], reactive hyperemia [F (1, 21) =1.50, p=.235], and shear stress [F(1,21) = .97, p = .34] were not different between groups.

DISCUSSION

The effects of exercise on endothelial function measured by FMD and hemodynamic responses during neurovascular stress were examined in female smokers and non-smokers. Consistent with our hypothesis, acute exercise increased FMD in both groups. The beneficial effects of exercise in both smokers and non-smokers also extended to some cardiovascular responses during stress. During the Stroop CWT, increases in HR, arterial velocity, and blood flow were attenuated after exercise. Similarly, decreases in arterial velocity, blood flow, and HR were augmented during forehead cold. In addition, resting SBP, DBP, MAP, HR, arterial velocity and arterial BF were reduced after exercise.

Based on our results, it is likely that among female smokers and non-smokers, exercise improved FMD through a NO endothelium dependent pathway as previously shown (58). These changes occurred in the presence of post-exercise hypotension (PEH), which can result from a decrease in resting cardiac output after exercise (64). The hyperemic response and shear stress to forearm occlusion was higher after exercise compared to passive rest in this study. This suggests that increased flow and shear stress stimulus acted on the endothelium, improving FMD by increasing NO bioavailability, production, or vasodilator function (43, 74). When FMD was normalized to shear stress (77), the increase in FMD, compared to passive rest, was no longer

present after exercise. This implies that increased shear stress accounted for the increase in FMD after exercise.

As expected, we observed postexercise hypotension (PEH) at rest, which was accompanied by increases in HR, arterial velocity, and blood flow below pre-exercise levels. There are multiple mechanisms postulated to cause PEH. Reduced cardiac output (34, 46, 82) and decreased peripheral resistance (15, 17, 57) can each explain PEH (MacDonald 2002), but the underlying explanations for either mechanism remain unknown (64). In this study, elevations in HR and blood flow after exercise decrease the likelihood that PEH resulted from a reduction in baseline cardiac output. The most likely explanation is a reduction in vascular resistance which was accompanied by an increase in vascular conductance in this study. Decreases in efferent sympathetic nerve activity, afferent nerve activity (i.e., baroreceptors), or reduced vascular transduction of neurohumoral influences (64) may explain the reduced peripheral resistance. Previous research has shown, however, that PEH can occur with an increase in sympathetic outflow and decreased vagal tone which offset peripheral vasodilation during PEH (73). The increase in HR, arterial velocity, and blood flow support increased sympathetic activity. Although an increase in arterial vasodilation did not occur, forearm vasodilation in the microvasculature could explain the reduction in blood pressure downstream.

The results are not consistent with our hypotheses that exercise would lower blood pressures by reducing limb blood flow and vascular resistance during the Stroop CWT and reducing vascular resistance during forehead cold. Exercise did not affect blood pressure responses during either stressor task despite mitigation of increased arterial velocity and blood flow during the Stroop CWT and augmentation of decreased arterial velocity and blow flow during forehead cold. Hence, although we observed PEH and improved flow-mediated dilation

among smokers and non-smokers, the pattern of cardiovascular responses suggests an increase in vascular resistance or a reduction in conductance during stress in both groups. This is supported by higher vascular resistance and lower arterial conductance during the Stroop CWT after exercise compared to passive rest. In addition, during forehead cold, the reduction in arterial conductance was augmented.

After exercise, there was a significant inverse relationship between post exercise baseline values and arterial velocity and blood flow responses during the Stroop CWT and forehead cold. After passive rest, arterial velocity and blood flow responses during forehead cold were related to post-rest baseline values. Higher baseline arterial velocity and blood flows after exercise appear to account for the augmented stress responses after exercise compared to passive rest. This is inconsistent with a previous review (49) that suggests baseline blood pressure is unlikely to affect stress related blood pressure responses. In the review, that conclusion was based on the limited studies in the literature that report PEH along with an attenuated stress response. However, in the few studies that PEH did occur (6,75,93), an attenuated stress response also occurred. Future studies should consider the importance of baseline values after exercise and their relation to stress responses.

During the Stroop CWT, the attenuation of limb blood flow and arterial velocity indicate reduction in cardiac output that could be explained by the attenuated HR response during the task. An attenuated increase in HR during the Stroop CWT was reported among normotensive men after 30 min of cycling exercise at 60%VO2max (75) and among normotensive male offspring of hypertensive men after 20 min of moderate intensity cycling (75-85% HRR) (48), but not after 20 min self-paced treadmill walking (96). Downregulation of β1 and β2 receptors might explain the attenuated HR response during stress post-exercise (10). It is likely that in the present study the

reduction in HR alone was sufficient to account for decreased arterial velocity and blood flow and thus attenuate the BP response during the Stroop CWT. A limitation of this study was the absence of a measurement of cardiac output to determine whether this was the case.

The augmented reduction in arterial velocity during forehead cold could also be explained by the greater reduction in HR, which could account for a reduction in cardiac output (75) and decreased flow to the periphery (100). These results presented in this study are inconsistent with reduced vascular resistance after exercise (48, 102). Instead, these results suggest an increase in vascular tone in forearm tissue, resulting in stimulation of α1 and α2 receptors (16) and increased vascular resistance (26). Because no change in blood pressure, measured downstream in the radial artery, was observed during forehead cold, but arterial velocity decreased, measured upstream in the brachial artery, forearm skeletal muscle resistance could account for the incongruence. A measurement of forearm vascular resistance would help determine whether after exercise, responses during stress within the microvasculature oppose those within the macrovasculature. Increased sensitivity of baroreflexes, which occurs after acute exercise to maximal effort, is less likely related to augmented stress response (19, 91). An increase in baroreflex sensitivity acts as a countermeasure to post-exercise hypotension by increasing HR. In this study, HR was increased and blood pressure was lowered after exercise compared to passive rest. On the other hand, the increase in blood pressure during forehead cold occurred with an augmented decrease in HR. Thus, these results do not suggest a role for altered baroreflex sensitivity.

It was expected that FMD would be impaired and stress reactivity would be augmented among smokers when compared to non-smoking controls. The smokers used in this study were chronic smokers, but compared to other studies the smoking history was shorter ~ 3 years.

Previous reports have shown that heavy chronic smoking is not required to produce endothelial dysfunction (59, 62, 94, 95) or increase cardiovascular responses to neurovascular tests (23, 72). However, in those studies individuals were commonly required to smoke a high nicotine cigarette immediately before measurements of FMD and stress reactivity. This may have led to some of the smoking induced reductions in vascular health. This study employed the use of low nicotine cigarettes that typically do not increase cardiovascular responses when comparing them to placebo (60). It also enabled us to test the benefits of exercise on FMD in smokers that were not in a state of complete abstinence.

Although, a general effect of smoking is impaired endothelial function (12), it is not uncommon for studies to report similar FMD responses between smokers and non-smokers. For example, Clarkson et al. (1999) found in male smokers and non-smokers that did not smoke prior to vascular measurements, baseline FMD was not significantly different between groups. In addition, when post-smoking baseline arterial diameter was used to compute FMD in chronic smokers (92) and occasional smokers (94), endothelial function was similar to non-smoking controls. Therefore in this study, the use of post-smoking baseline values to compute FMD, the young age of smokers, and slightly higher fitness level, compared to controls, suggests the deleterious effects of smoking were not as pronounced in this group. This is further supported by higher baseline arterial velocity and blood flow levels, which is generally lower with chronic smoking (4, 81).

There is also evidence cardiovascular reactivity among chronic smokers may not differ from controls. For example, a previous study in chronic smokers that were allowed to smoke ad libitum prior to testing found a non-significant increase in BP and HR responses when compared to smokers asked to abstain 18 hours prior to stress testing (2). Similarly, cardiovascular

responses to problem solving tasks among recent smokers were not significantly different when comparing them to their non-smoking counterparts (99). In these studies, cardiovascular responses to stress did increase, but it was only in the state of abstinence that the responses were exaggerated. In the present study, the smokers and non-smokers were healthy, currently inactive (5), and had lifetime historical leisure physical activity that was similar to the non-smoking controls.

Similar to previous studies (27, 32, 85), the pattern of HR, arterial velocity, blood flow, and arterial diameter responses to forehead cold and the Stroop CWT were different. Specifically forehead cold decreased arterial velocity, blood flow, HR, and the reduction in arterial diameter came close to significance. On the other hand, the Stroop CWT either did not change or slightly increased arterial velocity and HR. Inconsistent with previous studies (24, 47) the Stroop CWT did not increase vasodilation of the brachial artery. This may be due to counterbalancing the Stroop CWT with forehead cold. Thus, the typical α- adrenergic response observed during forehead cold (53, 98) may have altered the β–induced vasodilation observed during the Stroop CWT.

Previous research has shown a transient reduction in FMD after exposure to mental stress and cold pressor (28, 54, 63). Risk factors such as hypertension (44) and increased intima wall thickness (25), which impair FMD, commonly increase sympathetic activity. Inversely, decreases in sympathetic activity (i.e. diurnal variation) have been shown to improve FMD in premenopausal women (80). Therefore, it is likely that FMD in this population of premenopausal women was further impaired by first administering both a mental stressor (39, 86, 88), the Stroop CWT, and forehead cold (54). On the other hand, mental stressors, such as a standard arithmetic challenge (50) and the Stroop CWT (24), have been shown to increase FMD

or NO production. In addition, increases in catecholamine production, such as during stress, can cause the endothelium to counteract α-adrenergic mediated vasoconstriction by augmenting NO release and bioavailability (18, 30, 101). Therefore, it is just as plausible that in this study, administration of the Stroop CWT and forehead cold did not attenuate FMD, but instead increased the potential for vasodilation to occur. Nevertheless, this protocol was used consistently during each experimental testing session, and it did not block the beneficial effects of exercise on FMD.

Limitations. This study has a couple of potential limitations. First, the elapsed time between exercise and vascular measurements was not sufficient for all variables such as HR, velocity, and blood flow to fully return to pre-condition levels. After moderate-intensity exercise, heart rate and cardiac output remain elevated for at least 1 hour (17, 35). The increases in cardiac output have been explained by elevated HR since stroke volume does not change (17). We chose to take measurements on stress reactivity and FMD within the first hour after exercise to increase the likelihood increases in FMD would be observed (70). Secondly, during stress, an augmented reduction in heart rate, arterial velocity, and blood flow was observed with no change in blood pressure. We have argued that in order for blood pressure, measured in the radial artery, to not change, there must be an increase in forearm peripheral resistance. This study did not include a measure of local vascular response, but we acknowledge that future research should include an estimate of forearm resistance.

In conclusion, this study illustrates that acute exercise improves endothelial function, measured by FMD, regardless of their contemporary smoking history. These improvements in endothelial function and changes in cardiovascular reactivity were observed after moderate intensity exercise, with a reduction in baseline SBP, DBP, HR, arterial velocity, and blood flow. Some measures of stress reactivity during the Stroop CWT were attenuated; however, responses

during forehead were augmented. It is generally accepted that the benefits of exercise on the vasculature result from an increase in NO dependent endothelium vasodilation. Decreased sensitivity to β-adrenergic stimulation of the heart could explain decreased HR and blood flow responses during stress. These changes in HR and blood flow to the periphery occurred in the presence of increased vascular resistance and decreased conductance. Future studies should include measures of cardiac output and both total peripheral and local vascular resistance to determine the mechanisms associated with exercise-induced changes in reactivity.

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Table Captions

Figure Captions

Table 3-1.

Variable	Non-smoker (n= 13)	Smoker (n= 11)
Age	20.54 ± 2.33	20.64 ± 2.06
Height	65.08±3.16	64.45 ± 2.65
Weight*	148.54 ± 24.37	130.27 ± 7.67
VO2max (ml/kg/min)	30.48 ± 4.80	34.17 ± 4.98
7-Day PA Recall (kcal/d)	35.79 ± 2.21	36.53 ± 2.98
Historical Leisure Time Physical Activity (h/wk)	3.16 ± 3.23	3.31 ± 2.74
Historical Leisure Time Physical Activity (met-h/wk)	18.69 ± 19.51	20.67 ± 17.24

Table 3-2.

	Rest		Exercise	
Variable	Pre-condition	Post-condition	Pre-condition	Post-condition
Arterial Velocity (cm/s)				
Non-smokers	21.42 (11.65)	10.07 (6.49)	17.78 (6.03)	32.68 (14.33)
Smokers	28.45 (11.95)	14.85 (6.41)	30.70 (12.59)	34.54 (12.38)
HR (beats/min)				
Non-smokers	74 (13)	65 (10)	73 (14)	91 (16)
Smokers	78 (11)	71 (9)	77 (12)	84 (11)
SBP (mmHg)				
Non-smokers	117 (7)	118 (11)	112 (10)	110 (12)
Smokers	118 (10)	120 (10)	115 (11)	108 (9)
DBP (mmHg)				
Non-smokers	72 (8)	76 (11)	70 (8)	70 (8)
Smokers	74 (11)	85 (7)	75 (11)	73 (5)
MAP (mmHg)				
Non-smokers	134 (12)	140 (18)	130 (11)	130 (14)
Smokers	138 (18)	153 (12)	138 (18)	132 (9)
Arterial Diameter (cm)				
Non-smokers	3.39 (0.50)	3.36 (0.51)	3.47 (0.39)	3.50 (0.52)
Smokers	3.24 (0.43)	3.21 (0.45)	3.20 (0.37)	3.32 (0.49)
BF ($ml*min^{-1}$)				
Non-smokers	118.04 (91.54)	56.87 (43.03)	101.58 (42.35)	190.62 (103.15)
Smokers	147.28 (76.05)	73.31 (41.33)	154.56 (86.20)	181.64 (89.49)
Vascular Resistance (aru)				
Non-smokers	1.74 (1.40)	3.90 (2.59)	1.49 (.62)	0.97 (0.83)
Smokers	1.44 (1.37)	2.71 (1.58)	1.19 (0.69)	0.87 (0.36)
Vascular Conductance (acu)				
Non-smokers	0.88 (0.62)	0.39 (0.30)	0.80 (0.35)	1.52 (0.92)
Smokers	1.10 (0.59)	0.49 (0.31)	1.15 (0.69)	1.38 (0.68)

Table 3-3.

Variable	Rest				Exercise			
	Pre-condition		Post-condition		Pre-condition		Post-condition	
	Stroop	Forehead Cold	Stroop	Forehead Cold	Stroop	Forehead Cold	Stroop	Forehead Cold
Arterial Velocity (cm/s)								
Non-smokers	-1.48 (5.13)	-11.37 (9.10)	1.88 (2.44)	-3.29 (3.52)	2.37 (4.78)	-5.78 (4.98)	-8.72 (10.13)	-16.76 (10.05)
Smokers	-1.35 (8.31)	-12.29 (11.01)	1.33 (1.93)	-5.80 (5.43)	-2.83 (10.90)	-15.77 (13.16)	-8.43 (6.50)	-21.01 (12.85)
HR (beats/min)								
Non-smokers	0.91 (3.53)	-2.71 (3.37)	1.49 (2.50)	-2.51 (3.64)	-.37 (2.75)	-4.87 (6.92)	-2.36 (1.39)	-7.09 (6.31)
Smokers	0 .73 (3.05)	-1.25 (7.56)	.75 (3.28)	-1.47 (10.12)	1.91 (4.21)	-4.64 (4.85)	.09 (5.33)	-4.48 (7.01)
SBP (mmHg)								
Non-smokers	15.34 (6.92)	9.18 (7.51)	11.56 (12.75)	5.44 (4.45)	6.51 (4.55)	3.02 (4.27)	13.28 (6.44)	6.32 (5.62)
Smokers	7.36 (7.42)	8.47 (7.98)	9.02 (7.14)	9.02 (3.92)	11.39 (9.47)	4.35 (2.53)	12.09 (8.50)	4.51 (5.12)
DBP (mmHg)								
Non-smokers	7.25 (5.86)	3.18 (6.23)	6.22 (4.66)	4.29 (3.68)	3.76 (2.05)	3.29 (3.16)	7.10 (6.10)	3.99 (2.64)
Smokers	4.70 (4.27)	5.10 (4.39)	2.99 (4.57)	3.36 (2.64)	5.33 (4.55)	2.50 (4.41)	5.48 (5.05)	2.91 (3.27)
MAP (mmHg)								
Non-smokers	12.22 (12.57)	8.39 (14.16)	14.69 (14.66)	8.02 (7.92)	8.44 (5.35)	10.31 (7.42)	17.41 (11.28)	7.40 (4.79)
Smokers	12.11 (13.73)	9.58 (8.27)	6.96 (8.17)	7.92 (5.19)	10.84 (8.81)	12.12 (12.10)	11.28 (9.02)	5.35 (5.24)
Diameter (cm)								
Non-smokers	-0.02 (.08)	-0.02 (0.09)	0.01 (0.11)	-0.02 (0.12)	0.02 (0.19)	-0.03 (0.06)	-0.07 (0.13)	-0.11 (0.11)

Smokers	-0.06	-0.07	0.02	-0.03	-0.06	0.02	-0.03	-0.08
	(.11)	(.08)	(0.01)	(0.10)	(0.18)	(0.13)	(0.22)	(0.12)
BF (ml*min^{-1})								
Non-smokers	-12.87	-62.26	26.31	-19.49	20.82	-21.63	-58.12	-100.30
	(41.45)	(67.41)	(49.31)	(24.34)	(35.68)	(51.94)	(69.29)	(64.96)
Smokers	-18.28	-72.54	9.81	-31.31	-22.94	-79.14	-45.85	-114.20
	(47.47)	(68.19)	(17.55)	(35.70)	(66.16)	(84.33)	(43.50)	(86.11)
Vascular Resistance (aru)								
Non-smokers	0.74	1.90	-0.65	1.77	-0.10	0.94	0.35	1.42
	(0.63)	(2.01)	(1.66)	(1.63)	(0.54)	(0.77)	(0.31)	(1.45)
Smokers	-0.01	1.36	-0.26	1.97	0.31	1.85	0.49	1.64
	(0.65)	(1.20)	(0.55)	(1.93)	(0.76)	(1.66)	(0.51)	(1.09)
Vascular Conductance (acu)								
Non-smokers	-0.09	-0.44	0.16	-0.16	0.09	-0.32	-0.59	-0.85
	(0.27)	(0.47)	(0.38)	(0.17)	(0.27)	(0.26)	(0.72)	(0.64)
Smokers	-0.21	-0.59	0.04	-0.23	-0.25	-0.65	-0.41	-0.89
	(0.40)	(0.55)	(0.12)	(0.27)	(0.52)	(0.64)	(0.31)	(0.65)

Figure 3-1.

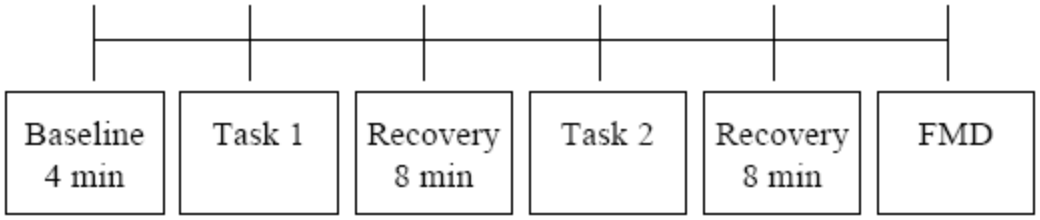


Figure 3-2

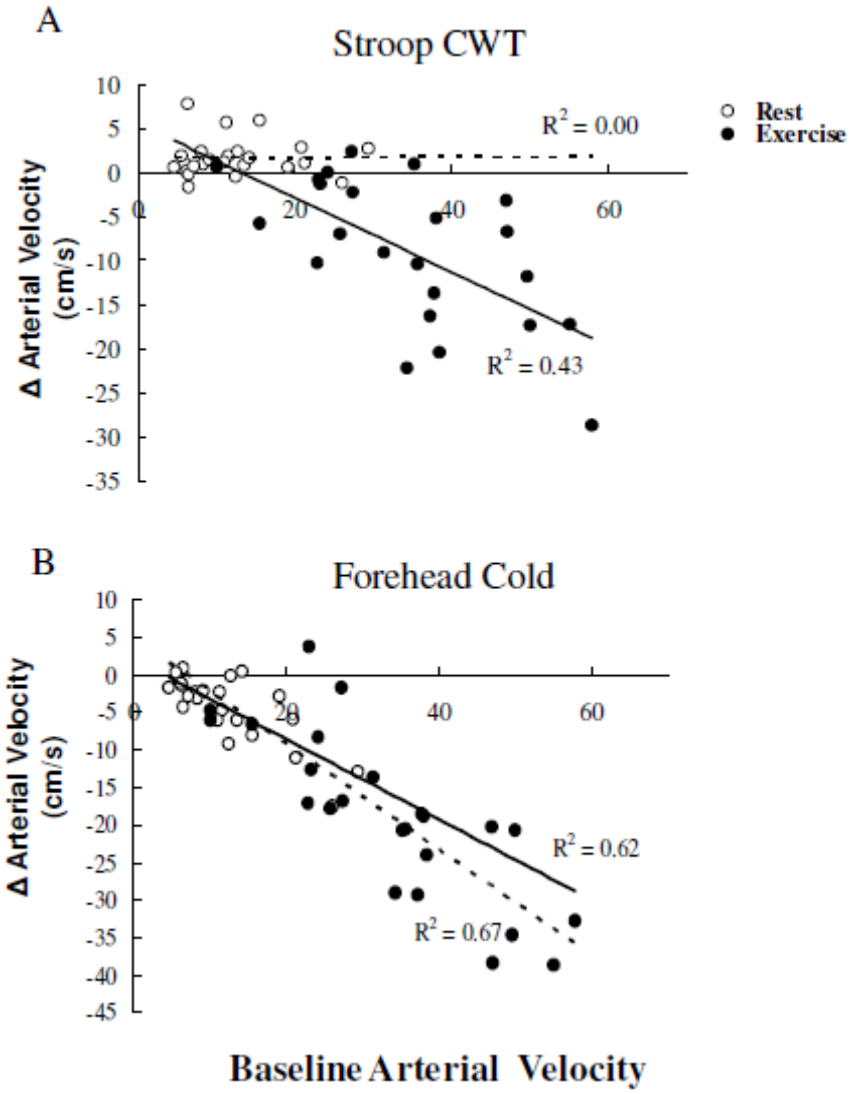


Figure 3-3

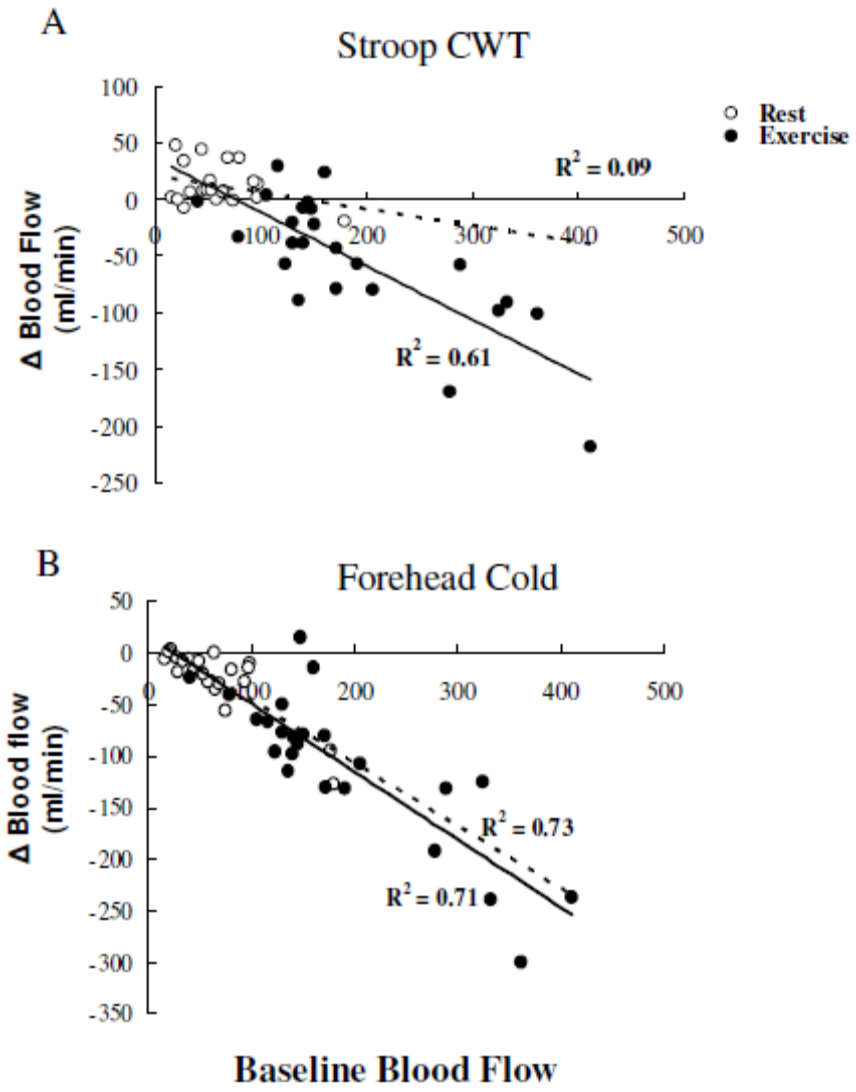

A
Stroop CWT
Rest
Exercise
$R^2 = 0.09$
$R^2 = 0.61$
Δ Blood Flow (ml/min)
100
50
0
-50
-100
-150
-200
-250
0
100
200
300
400
500
B
Forehead Cold
$R^2 = 0.73$
$R^2 = 0.71$
Δ Blood flow (ml/min)
50
0
-50
-100
-150
-200
-250
-300
-350
Baseline Blood Flow

Figure 3-4.

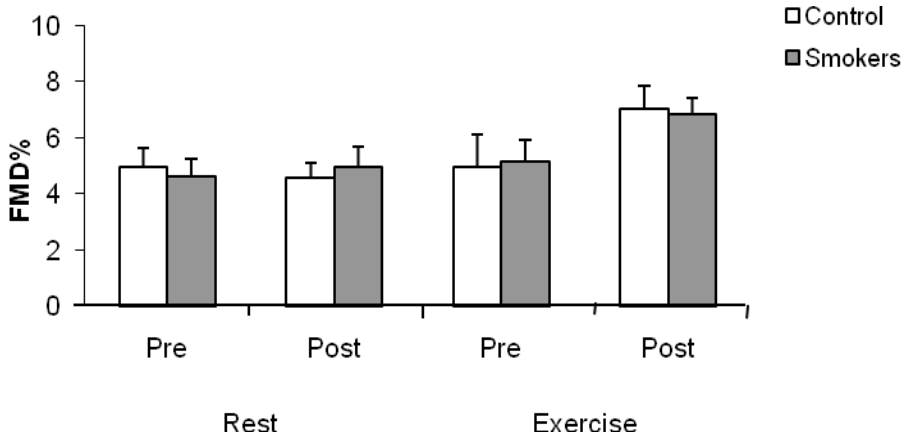


Figure 3-5.

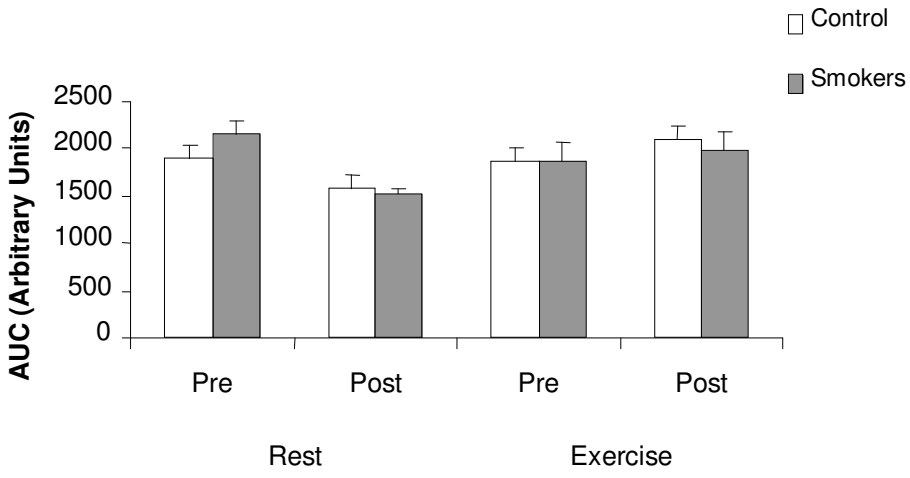


Figure 3-6.

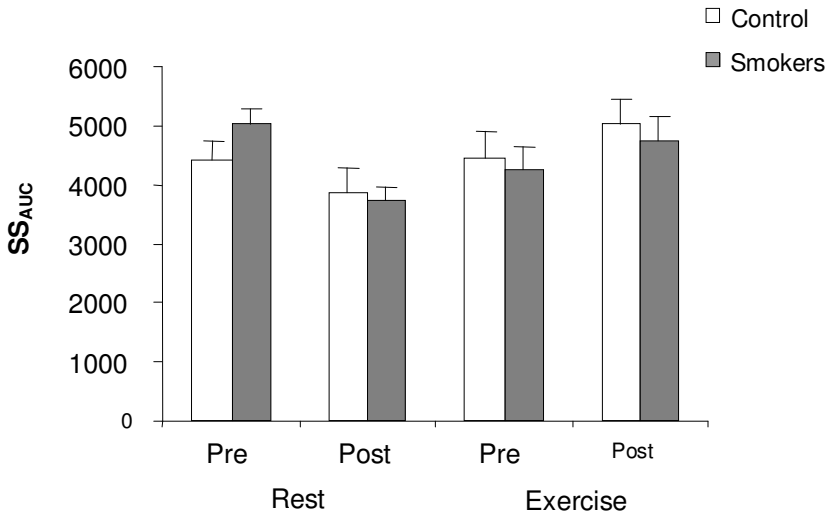
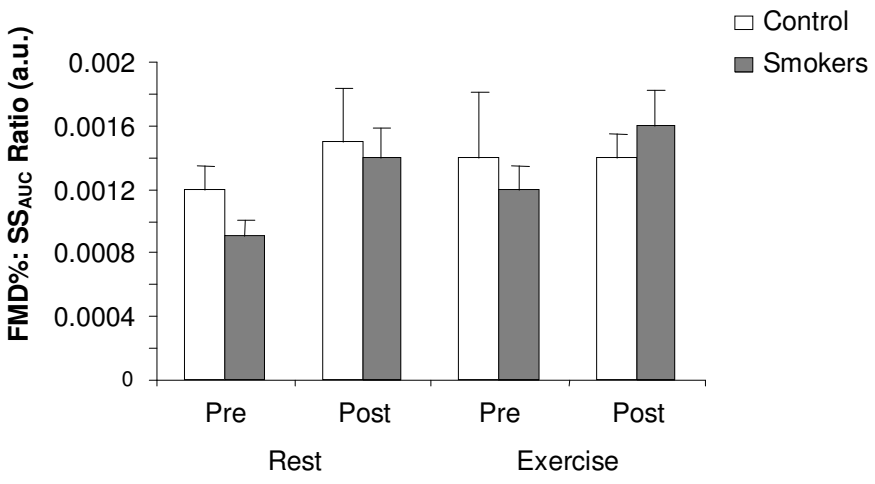


Figure 3-7.



CHAPTER 4

EFFECTS OF CHRONIC EXERCISE TRAINING ON ENDOTHELIAL FUNCTION AND VASCULAR RESPONSES DURING NEUROVASCULAR STRESS IN FEMALE SMOKERS AND NON-SMOKERS[2]

[2]Rooks, C.R., McCully, K.K., Dishman, R.K. To be submitted to Medicine & Science in Sports & Exercise

ABSTRACT

The purpose of this study was to determine the benefits of 3 weeks of aerobic exercise training on endothelial function, measured by flow-mediated dilation (FMD), and cardiovascular responses to neurovascular stress among sedentary female smokers and non-smokers. FMD and stress responses were measured before and after a 3 week moderate intensity (~50%VO_2max) aerobic exercise training program. FMD was determined by brachial arterial velocity, arterial diameter, and blood flow measured by Doppler Ultrasonography. Those measures and beat-to-beat finger blood pressure (BP), and heart rate (HR) were assessed in responses to 2 min of forehead cold and 4 min of the Stroop Color-Word conflict test (CWT) (separated by 8 min of rest) at baseline and three weeks later. Compared to non-smokers, FMD was impaired and decreases in arterial diameter and blood flow responses during forehead cold were augmented at baseline among female smokers. Three weeks of cycle exercise training at a moderate intensity did not improve endothelial function measured by FMD or alter stress reactivity during neurovascular stress in smokers or non-smokers. Based on these results, the impact of 3 weeks of moderate intensity exercise training was insufficient to observe a measurable change in endothelial function or cardiovascular responses during stress.

INTRODUCTION

Cigarette smoking is one of the primary modifiable predictors of atherosclerosis and vascular disease (16, 32). Dysfunctions in vascular health and hyper-reactivity to neurovascular stressors can lead to the development and progression of atherosclerosis and changes in vascular endothelial function, indicative of future CVD risk (37, 42, 72). Chronic exercise has been shown to improve vascular health and vascular responses to neurovascular stressors (10, 75) but to date, this relationship has not been shown in female smokers, a high risk population for cardiovascular disease (CVD) (36).

Cigarette smoking is considered a risk factor for CVD (64) due to the damaging effects it has on the vascular endothelium. The endothelium is important for the maintenance of vascular health and regulating vessel tone (see Ref. (58). The exact mechanism responsible for the effects of cigarette smoke on endothelial dysfunction remains unknown, but it has been suggested that cigarette smoke produces free radicals and pro-oxidants that impair nitric oxide (NO) (endothelium derived vasodilator) biosynthesis (1-3, 20). In addition, compared to non-smoking controls, smoking status (whether or not an individual smokes), is related to increased BP (73) and HR (54) reactivity to neurovascular challenges, also increasing risk for disease.

Flow mediated dilation (FMD) is a nitric oxide mediated, non-invasive hyperemic technique used to assess endothelial function (9). FMD is a result of increased blood flow that puts a shear stress or frictional force on the endothelium, resulting in vasodilation of the vessel (55). Neurovascular stressors, such as the Stroop Color-Word conflict test (CWT) (autonomic vasodilator) and forehead cold pressor (autonomic vasoconstrictor) are commonly used to assess vascular reactivity. The Stroop CWT increases BP while increasing blood flow, whereas, the forehead cold pressor increases BP, while decreasing blood flow. Both FMD and neurovascular

stressors are tools to potentially assess risk for CVD (9, 72), thus making them effective methods for testing interventions known to reduce risk for disease.

There is longitudinal evidence that increased physical activity and/or exercise reduces the risk of morbidity and mortality associated with CVD in women smokers (11, 29, 43, 52). Thus it appears that the effects of smoking are partially reversible. It is plausible that the mechanism responsible for the reduced risk is improved vascular health and hyper-reactivity to a neurovascular stressor. If the vascular stress, or shear stress, associated with acute exercise (18, 49, 61) exposure is repeated over time, long term vascular adaptations may occur (65). During chronic exercise training, the endothelium is exposed to repeated episodes of increased blood flow leading to improved endothelium function, increased synthesis of NO (38, 66, 69) and up-regulation of NO synthase (66, 69). Despite these findings, the results of whole body exercise training in humans remains inconsistent. Studies have looked at healthy populations (10, 25, 38, 53), individuals with cardiovascular risk factors (25), heart failure patients (28, 34, 45, 47), and patients with a recent myocardial infarction (74). Differences in responses depended on the type of patient, as well as, the length, and intensity of the exercise training protocol. It has been suggested that individuals with impaired endothelial function are more likely to show changes in endothelial vasodilation after a moderate training program, compared to healthy subjects with preserved endothelial function (25). In addition, between 2-4 weeks of moderate intensity exercise training, FMD is significantly increased from baseline but structural remodeling begins to occur (71). After this point, the shear stress stimulus is more likely to return to normal due to increases in arterial size. Based on this evidence, improvements in FMD are most likely noticeable in individuals with impaired endothelial function, such as smokers, after 2-4 weeks of

exercise training. Thus, when measuring FMD, exercise training protocols lasting 3 weeks appear optimal for observing any training induced improvements.

The benefit of repeated bouts of exercise may also result in cardiovascular adaptations that reduce stress reactivity. For example, exercise training lasting 2 to 24 weeks has been shown to reduce SBP and DBP by -3.48 and -2.58 mmHg, respectively (75). It is plausible that these physiological adaptations may occur during exposure to stressful stimuli. For example, Vona et al. (2004) found that 3 months of moderate intensity exercise training, attenuated arterial diameter reactivity to the hand cold pressor, a sympathetic vasoconstrictor. On the other hand, a more recent review found that out of 19 randomized exercise training studies that included a control group, reactivity did not change (36). Few of these studies, focused on women or individuals at risk for cardiovascular disease, such as smokers. In addition, the cardiovascular parameters measured (primarily HR and BP) did not afford a thorough understanding of the underlying mechanisms whereby exercise training alters stress reactivity.

The aim of this study was to determine the effects of 3 weeks of moderate intensity cycling exercise on endothelial function, measured by FMD, and on cardiovascular responses to neurovascular challenge in female smokers and nonsmokers, compared to a non-smoking control group that did not exercise. To determine FMD, we measured arterial velocity, arterial diameter, and blood flow. In addition, we measured BP, heart rate, arterial velocity, arterial diameter, and blood flow during forearm hyperemia and during the Stroop CWT and forehead cold. We hypothesized that 1) smokers would have impaired FMD and exaggerated reactivity to neurovascular stressors when compared to non-smokers, 2) FMD would be improved and the magnitude of reactivity to the stressor tasks would be attenuated in both smokers and nonsmokers after 3 weeks of exercise training.

METHODS

The CONSORT diagram in Fig. 4-1 illustrates participant recruitment and group assignment during the study. Thirty-five participants were recruited, 11 regular smokers (who reported an average of 15 cigarettes/day for at least 1 year) and 24 non-smokers, from the University of Georgia and local Athens, GA community. The mean pack years ± SD reported by smokers was 4.70 ± 3.87. All smokers underwent the exercise training protocol. Non-smokers were randomly assigned, by random number generator, into the exercise training (n=12) or to the passive rest group (n=12). Each participant was required to give informed consent prior to beginning testing. This study was approved by the University Institutional Review Board. The mean age was 18.92 ± 1.31 yr for the non-smoking control group, 22.42 ± 6.60 for the non-smoking exercise training group, and 25.73 ± 7.43 yr for the smoking group.

Participants completed 2 separate days of experimental testing before and after the 3 week training protocol. Participants were asked to fast 2 hours prior to testing and refrain from alcohol, caffeine, and exercise at least 24 h prior to testing. On day 1, the FMD test was completed followed by the maximal exercise test. On Day 2 day, cardiovascular responses to two neurovascular challenges (Stroop CWT and forehead cold) were assessed. Because mental stress has been shown to impair endothelial function (15, 33, 44), vascular measurements of stress reactivity were taken on a subsequent day. Distance between testing sessions was no more than 48 hours. Smokers were instructed to abstain from smoking at least 2 hours prior to arriving to the lab. The remaining protocol for smokers and non-smokers were identical. Prior to administering the FMD test, participants were asked to complete a pre-exercise medical history questionnaire, a historical leisure activity questionnaire (40), and a 7-day physical activity recall interview (PAR) (4).

Vascular Measurements of FMD. Upon arrival, individuals were directed to the Vascular Biology Laboratory where they were instructed to lie on a table in supine position, and the right arm was placed on an extended table to access the brachial artery. All measurements of arterial diameter and arterial velocity were taken on the right arm for each testing session. A GE 400CL duplex color Doppler imager (GE Medical) was used to assess diameters of the brachial artery, while simultaneously measuring arterial velocity. Baseline measurements of brachial artery diameter were recorded every 30 seconds for 2 minutes, while arterial velocity was continuously measured. A pressure cuff (Hokanson Inc., Bellevue, WA) was previously placed on the right forearm distal to ultrasound measurements upon entry to the lab. After baseline recordings, the cuff was rapidly inflated to ~50 mmHg above SBP for 5 minutes while diameter measurements were continued every 30 seconds. After 5 minutes, pressure was quickly released from the cuff inducing reactive hyperemia. Arterial diameters were then recorded continuously for 3 minutes. Averaged diameter readings at baseline and peak hyperemia (averaged across 2 seconds) were used for analysis. In our lab, the day-to-day variability of FMD readings approximates 30%. FMD (%ΔFMD) was defined as the peak hyperemic diameter-baseline)/baseline*100. Area under the curve (AUC) was used to quantify reactive hyperemia for 30 seconds after cuff deflation. AUC was computed by summing the area of consecutive postocclusion trapezoids (trapezoidal rule) for 30 sec and was calculated as: $\sum\{y_i[x_{(i+1)} - x_i] + (1/2)[y_{(i+1)} - y_i][x_{(i+1)} - x_i]\}$. The between-day coefficient of variation in the control group was 28%. The shear stress stimulus, or 30 sec postocclusion hyperemic stimulus, was calculated as: $SS_{AUC} = 4 \cdot AUC/\text{arterial diameter (cm)}$. FMD, normalized to shear stress, was expressed as FMD:SS_{AUC} ratio.

Maximal Exercise Test. If no contraindications to exercise were present (i.e., untreated diabetes or other endocrinological disorder, renal or peripheral venous disease, uncontrolled

hypertension, heart disease, or lung disease, alcohol, dietary supplement, or drug use that would complicate interpretation of the results, or use of psychoactive, anti-inflammatory, or analgesic medications within the previous week), participants then performed an incremental exercise test using a standard procedure to measure peak oxygen consumption (VO_2peak. Individual's were instructed on the test procedure and fitted with a Polar HR monitor model S810i (Kempele, Finland). Participants then sat on a cycle ergometer, and a mouthpiece and nose clip were put on with the help of the researcher. VO_2peak ($ml \cdot kg^{-1} \cdot min^{1}$) was determined using open circuit spirometry on a Lode Excalibur Sport (Groningen, Nederland) electronically regulated cycle ergometer using a ramped protocol. Participants then sat on the cycle ergometer without moving for 30 seconds, followed by a low intensity (50 W) 5-minute warm up. After the warm up, ergometer resistance increased continuously at a rate of 24 watts per minute until volitional exhaustion. Resistance was controlled by computer software via an interference cable connected to the ergometer. HR and ratings of perceived exertion (RPE), using the Borg 6-20 category scale (6) were obtained every minute. Once peak effort was reached, participants underwent a 2 min low intensity (25 W) cool down. Oxygen consumption was measured continuously using a Sensormedics Model 2900 metabolic cart. The metabolic cart was calibrated with standard gases. VO_2peak ($ml \cdot kg^{-1} \cdot min^{1}$) was defined as the highest attained oxygen consumption when at least two of the following criteria were met: a HR within 90% of the age predicted maximal HR, a respiratory exchange ratio above 1.1, and an RPE value greater than 18. VO_2peak was used to standardize a subsequent moderate intensity (~50%) exercise bout.

Stress Reactivity Protocol

On Day 2, participants were returned to the Exercise Vascular Biology Laboratory. Baseline measurements and stress reactivity were assessed by BP, HR, arterial velocity, arterial

diameter, and blood flow. Blood pressure was measured by continuous beat-to-beat finger arterial pressure photoplethysmography using an Ohmeda 2300 Finapres BP monitor. Data acquisition of BP occurred using the Biopac MP100 physiological data acquisition system (Biopac Systems Inc., Goleta, CA) and AcqKnowledge software (v3.7.3) (Biopac Systems Inc). Mean arterial pressure (MAP) was computed as 1/3 pulse pressure + DBP. The within-day reliabilities (ICC-2) of baseline values for arterial velocity and diameter > .98. Individuals were tested during the first 12 days of their self-reported menstrual cycle (i.e. the onset of menstrual flow is day 1).

Participants were fitted with a Polar heart rate monitor and instructed to lie on a table in supine position. The right arm was placed on an extended table to access the brachial artery. All measurements of arterial diameter and velocity were taken on the right arm pre and post-training. A GE 400CL duplex color Doppler imager (GE Medical) was used to assess diameters of the brachial artery, while simultaneously measuring arterial velocity. A small finger cuff was placed on the middle finger to assess continuous finger arterial pressure. Participants were given ~10 min during setup for quiet rest prior to testing and exposure to stressors. Eight minutes of baseline recordings were taken. Following baseline measurements, individuals were exposed to forehead cold or the Stroop CWT in an order that was counterbalanced between participants.

Forehead cold was administered by placement of a plastic bag of ice water (4° C) on the forehead for a total of 2 minutes. A computerized version of the Stroop CWT was administered. The test randomly presented in the middle of the screen a color word (red, blue, green, and yellow) along with four color word options at the bottom of the screen. The purpose of the test is to choose the word at the bottom that describes the color of the word in the middle. A four-button controller was used by the participant to record their response. The test was administered

for four minutes. Neurovascular responses during the Stroop CWT differ according to perceived task difficulty (7, 19), so the task was programmed to adjust the speed of word presentation according to the number of correct answers in order to maintain the difficult of the task at a correct percentage of 60% ± 10%. After exposure to the first stressor, individuals were given 8 minutes for recovery while SBP and DP returned to within 5 and 2 mmHG, respectively, of baseline values.

Arterial diameter and velocity. Quantitative Doppler ultrasound imaging (GE 400CL Medical) was used to determine simultaneous measurement of arterial velocity and diameter. A 5-10 MHz linear array ultrasound transducer was used. All measurements were taken on the right arm brachial artery during each testing period. Velocity values were collected across the entire length of the artery and time-averaged maximal velocity (T_{max}) was determined using pulsed Doppler ultrasound with a maximum Doppler angle of 60°. T_{max} calculations were conducted using GE's advanced vascular program for the LogiQ 400CL. Integrated GE medical software was used to determine the time averaged maximum velocity for every heart beat and recorded real-time. A consistent section of artery diameter was measured using B-mode imaging and actual diameter was measured using semi-automated edge-detection software (LabView, National Instruments (Austin, TX). A representative section of the arterial wall was identified, and a line of best fit was identified. Arterial velocity and diameters were recorded and saved for ~10 sec every 30 sec during the first 2 minutes of rest, ice, and continuously during the Stroop CWT. Reliabilities (ICC-2) of baseline values for arterial velocity and diameter > .90. Blood flow was computed as ml/s by using arterial velocity and diameter scores (velocity * π * $(\text{diameter}/2)^2$).

Exercise Training Protocol. The following day, after vascular measurements were completed, individuals were guided to the same room the maximal exercise test was conducted. The exercise training protocol consisted of supervised moderate-intensity exercise (50% VO_2peak; perceived exertion rating of 12-13), lasting 30 minutes as recommended in the 2007 Guidelines for Cardiovascular Disease Prevention in Women (50). Exercise sessions were repeated 4-5 times per week for 3 weeks. Exercise intensity was verified by analysis of expired gases using the Sensor medics metabolic cart every five minutes. During passive rest, individuals sat for 30 minutes in the same room as the cycle ergometer. Average adherence was 94% of all sessions, and all participants attended at least 12 of the 15 scheduled training sessions.

Data Analysis. Statistical analysis was performed using SPSS 16.0 computer software (SPSS, Chicago, IL). The dependent measures were SBP, DBP, MAP, HR, arterial velocity, arterial diameter, and blood flow each averaged before and during stress. In addition, FMD reactive hyperemia, shear stress, and normalized FMD were dependent variables during FMD testing. Change scores were calculated to analyze stress reactivity and FMD. Averages during the baseline period, before each stressor, was subtracted from the average response during each stressor. Group comparisons on pre-training baseline scores for each dependent variable were tested by a one-way ANOVA. Bonferroni post hoc analyses were used to decompose significant effects. Changes in the dependent variables during the baseline stressor tasks were tested for all participants using paired samples t-test.

Study hypotheses were tested using a 3 (group) x 2 (time: pre- vs. post-training) repeated measures ANOVA with time repeated. Hypothesis 1 was tested by the main effect of group, contrasting smokers vs. non-smokers. Hypothesis 2 was tested by the group x time effect on

FMD, reactive hyperemia, shear stress, normalized FMD, and stress reactivity (change from baseline), contrasting the exercise training groups vs. the non-smoking control group. Thirty-six subjects were needed to produce a power of .80 and an $\alpha<0.05$ (17). Eta squared was used to represent effect sizes for tests between groups and stressor tasks. Data are presented as means ± SD, except where stated. Significance tests were two-tailed and set at $p<.05$.

RESULTS

Participant Characteristics

Participant characteristics (M ± SD) are presented in table 4-1. Age [$F(2, 34) = 4.06$, $p=.03$, $\eta^2=.20$], weight [$F(2, 34) = 4.04$, $p=.03$, $\eta^2=.20$], and VO_2peak [$F(2, 34) = 3.52$, $p=.04$, $\eta^2=.18$] were different between groups prior to training. Compared to the non-smoking control group, smokers were older (p=.023), heavier (p=.024), and had a lower VO2peak (p=.05) than the non-smoking control group, but there were no differences between the two exercise training groups.

Participant initial baseline values, before and after 3 weeks of moderate intensity cycling training, on each dependent variables (M ± SD) are presented in table 4-2. There were group differences on pre-training baseline (resting) DBP [$F(2, 34) = 6.49$, $p=.005$, $\eta^2=.29$], MAP [$F(2, 34) = 5.19$, $p=.01$, $\eta^2=.24$], arterial velocity [$F(2, 34) = 3.73$, $p=.04$, $\eta^2=.18$], arterial diameter [$F(2, 34) = 3.94$, $p=.03$, $\eta^2=.20$], and blood flow [$F(2, 34) = 6.28$, $p=.005$, $\eta^2=.28$]. The non-smoking control group had lower DBP than the smoking (p=.007) and the non-smoking exercise training group (p=.03) which did not differ from each other. Smokers had significantly higher MAP (p=.013) and arterial velocity compared to the non-smoking control group ($p = .03$). The smokers had a larger arterial diameter and higher blood flow compared to the non-smoking exercise training group ($p < .03$).

Stress Responses Before Exercise Training

Responses to the Stroop CWT and forehead cold before and after the exercise training period are presented for each group as change scores from baseline in Table 4-3.

Stroop CWT. There were increases (mean ± SD) from baseline in SBP [117.34 ± 11.31 vs. 131.85 ± 13.57; t (34) =10.94, p<.001], DBP [66.72 ± 9.39 vs. 75.18 ± 9.51;t (34) =10.07, p<.001], MAP [127.46 ± 14.82 vs. 143.50 ± 15.41; t (34) =10.07, p<.001], and HR [71.27 ± 8.86 vs. 76.09 ± 9.74; t (34) =4.96, p<.001]. Arterial velocity [t (34) =0.45, p=.66], arterial diameter [t (34) = 0.72, p=.48], and blood flow [t (34) = 0.42, p=.68] were not changed during the task.

Forehead Cold Pressor. There were increases from baseline in SBP [117.34 ± 11.31 vs. 132.07 ± 17.07; t (34) =5.84, p<.001] and DBP [66.72 ± 9.39 vs. 76.82 ± 9.53; t (34) =7.08, p<.001] and a reduction in HR [71.27 ± 8.86 vs. 66.59 ± 6.92; t (34) =4.85, p<.001]. In addition, arterial velocity [20.53 ± 10.02 vs. 10.74 ± 5.29; t (34) =6.73, p<.001], arterial diameter [3.29 ± 0.38 vs. 3.22 ± 0.37; t (34) =2.87, p=.007], and blood flow [107.86 ± 61.49 vs. 54.18 ± 30.26; t (34) =6.79, p<.001] were reduced during the task.

Pre-exercise training responses were lower in response to forehead cold than the Stroop CWT for HR [F (1, 32) =63.30, p<.001, η^2=.66], arterial velocity [F (1, 32) =59.67, p<.001, η^2=.651], and arterial BF [F (1, 32) =50.71, p<.001, η^2=.61]. In response to forehead cold, arterial diameter was lower compared to the Stroop CWT, but the difference did not reach significance [F (1, 32) =3.23, p=.08, η^2=.09].

Hypothesis testing

Group Differences (Hypothesis 1).

Endothelial Function. There was an effect of group on FMD [F (2, 32) =3.74, p=.04] (Fig 4-2). FMD was lower in the smoking group compared to the non-smoking control (p=.02)

and the non-smoking exercise training group (p=.041). However, when baseline arterial diameter was taken into account, there was no group effect on FMD [F (1,32) = 2.99, p = .09]. There was no main effect of group on reactive hyperemia [F (1, 33) =.29, p=.76] (Fig 4-3) or shear stress [F (1, 33) =3.01, p=.09] (Fig. 4-4). When normalized to shear stress, FMD was still significantly different between groups [F (1, 33) =4.10, p=.05] (Fig. 4-5). Smokers normalized FMD was lower (0.0016 ± 0.0012) than non-smokers (0.0023 ± 0.0008).

Vascular Responses during Stressors. There was an effect of group on arterial diameter [F (2, 32) =4.92, p=.03, η^2=.13] and blood flow [F (2, 32) =3.91, p=.03, η^2=.20] responses during forehead cold. The smoking group had greater reductions in arterial diameter (-0.10 ± 0.08 vs. -0.04 ± 0.07; p=.03) and blood flow (-81.69 ± 49.51 vs. -45.12 ± 27.46; p=.05) compared to the non-smoking exercise training group.

Effects of Exercise Training (Hypothesis 2).

Endothelial Function. There were no group x time effects for FMD [F (2, 32) =0.73, p=.49] (Fig 2) reactive hyperemia [F (2, 32) =1.09, p=.35] (Fig 3), shear stress [F(2, 33) =0.76, p=.48 (Fig. 4), or FMD normalized to shear stress [F(1, 33) =.42, p=.66] (Fig. 5). The effect of time was also not significant for FMD [F (2, 32) =.11, p=.74], reactive hyperemia [F (2, 32) =1.99, p=.17], shear stress [F(1, 33) =0.35, p=.56], or FMD normalized to shear stress [F(1, 33) =0.31, p=.58].

Vascular Responses during Stressors. There were no group x time effects on any of the dependent variables during the Stroop CWT or forehead cold [Fs (2, 32) <2.19, p-values >.15]. There were time effects for SBP [F (2, 32) =10.37, p=.003], DBP [F (2, 32) =6.81, p=.01,], and MAP [F (2, 32) =9.54, p=.004] responses during the Stroop CWT.

DISCUSSION

The purpose of this study was to determine the effect that 3 weeks of moderate intensity cycling exercise has on FMD and vascular reactivity to neurovascular challenge in female smokers versus non-smokers. Consistent with previous studies (8, 9), FMD was reduced by ~40% in female smokers compared to non-smokers. Prior to exercise training, smokers also had greater arterial diameter at rest and a bigger reduction in arterial velocity and blood flow during forehead cold than did the non-smokers. Moderate intensity exercise training did not improve endothelial function or stress reactivity during either the Stroop CWT or forehead cold in either smokers or nonsmokers.

FMD was significantly reduced in female chronic smokers compared to non-smoking controls, indicative of endothelial dysfunction (8). The FMD test used is mediated by NO. Cigarette smoking causes the bioavailability of NO to decrease due to increased superoxide and free radical production (21). These results suggest that cigarette smoking impairs endothelial function by inhibiting endothelium derived NO and the capacity for arterial relaxation. Smokers in the present study had larger arterial diameter than non-smokers which has been shown to cause lower FMD (9). However, shear stress, which is the stimulus responsible for vasodilation of the vessel, was similar across all groups. In addition, FMD normalized to shear stress was lower in smokers compared to non-smokers. Hence, smokers had lower FMD that was not changed when normalized to the stimulus that causes vasodilation, shear stress.

At baseline, cigarette smokers also had significantly higher DBP and arterial diameter. Common cardiovascular features of cigarette smoking include increased SBP, DBP, arterial diameter, and increased wall thickness (59). The higher arterial velocity and arterial blood flow are inconsistent with previous literature that reports smoking decreases arterial velocity and blood

flow (41, 56). It remains unclear how this is the case. Future studies should utilize tools such as carbon monoxide analyzer to definitely determine whether smokers had abstained prior to testing (35), to ensure recent nicotine exposure does not confound results.

Cigarette smokers also had greater arterial reactivity (i.e., greater reductions in arterial velocity and blood flow) to the forehead cold pressor compared to their non-smoking counterparts that also underwent exercise training. Cigarette smoking is associated with enhanced production and vascular responsiveness to vasoconstrictors, such as endothelin-1 (23, 26, 39). In addition, cigarette smoking leads to the progression of atherosclerosis by inhibiting vasodilation, increasing vasoconstriction, stabilizing thrombus, initiating inflammation, and modifying lipid profiles (58). Previous research has also shown that endothelial dysfunction, which was observed in smokers in this study, increases vasoconstrictor responses to cold pressor testing (1, 22, 63). Thus, the increased reactivity and decreased endothelial function are indicative of increased risk of cardiovascular disease in this group of female smokers.

Results from studies investigating the effect of exercise training on endothelial function have produced mixed results. Animal studies indicate short-term exercise training (2-4 weeks) increases NO synthase and NO bioavailability (12, 13, 66, 69). On the other hand, vasomotor responsiveness to endothelium dependent dilators in rat soleus did not change after 10 weeks of exercise training. In humans, an 8 week exercise training protocol in type 2 diabetics (46) and CHF (47) patients resulted in improved endothelium function. On the other hand, in healthy subjects, the identical training protocol did not change endothelial function, despite increases in fitness (48). Furthermore, lack of an exercise induced improvement in the NO vasodilator system was observed in healthy tennis players (24). Inconsistencies in these results may be due to arterial remodeling or an increase in dilator capacity (71). This normalizes the shear stress on the vessel

resulting in NO mediated function returning to baseline levels. These changes in arterial structure can occur as early as 2 weeks with no change in arterial diameter or reactive hyperemia (71). In this study, exercise training did not change FMD, arterial diameter, or reactive hyperemia. It is likely that the 3 week training protocol did expose individual's vasculature to increases in shear stress that may have altered NO production or bioavailability. This in turn, may have concealed improvements in FMD by changing arterial capacity. Future studies should include a surrogate measure of conduit artery structure and capacity (51) to determine whether this actually occurred.

Few studies have examined the effect of exercise training on stress responses during the Stroop CWT or cold pressor. Previous studies reported no change (60, 67, 68) or attenuated responses (5, 62, 74) during stress. Inconsistencies in previous studies may have resulted from the length of the training protocol or the comparison of absolute levels during stress to pre-training values (62). Vona et al. (2004) used a 3 month long training protocol that decreased vasoconstriction during hand cold pressor in patients that recently underwent an acute myocardial infarction. Attenuated stress reactivity occurred with improved NO-mediated FMD. It is likely in that study, the attenuated vasoconstriction was related to improved biomarkers of cardiovascular risk factors. It is also likely that increased NO production, which interacted with norepinphrine and sympathetic function during the stressor accounted for the improvements, as they suggested (70). In this study, no change in NO-mediated FMD occurred, which could explain the absence of a training induced reduction in reactivity to forehead cold. Rogers et al. (1996) found a reduction in HR response to the Stroop CWT, but the attenuated response was due to a decrease in baseline HR.

Forehead cold is an autonomic vasoconstrictor that mainly decreases HR by vagal withdrawal, and increases vascular resistance mainly by α-adrenergic mediated vasoconstriction

(31, 72). On the other hand the Stroop CWT increases HR and blood flow by withdrawal of sympathetic tone or by beta-adrenoreceptor activation (30) and flow mediated mechanisms (74). We did not observe vasodilation during the Stroop CWT as previously found (14, 27). These studies did not measure vasodilation using Doppler ultrasound in the brachial artery. The increases in blood flow observed during the Stroop CWT may not be sufficient to obtain a measureable change in arterial diameter. We did, however, observe a measurable reduction in arterial diameter during forehead cold. This reduction in arterial diameter was accompanied with reductions in HR, blood flow and arterial velocity; and an increase in BP.

Overall, these results suggest that 3 weeks of moderate intensity leg cycling does not change FMD or vascular responses during the Stroop CWT or forehead cold. To our knowledge, this is the first study to determine the effects of exercise training on vascular responses among female smokers, a high risk group for CVD. Arterial remodeling, or peak vasodilator capacity, which begins to occur between 2-4 weeks and results in a decline in FMD, might explain the absence of a training-induced change in FMD (57, 71). To address this issue, future exercise training studies should not only measure FMD once the exercise protocol has ceased, but also between 1-2 weeks when FMD is believed to be optimal (71). Based on these results, the impact of 3 weeks of moderate intensity exercise training was insufficient to observe a measurable change in endothelial function or cardiovascular responses during stress.

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Table Captions

Table 4-1. Baseline characteristics of the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11).

Table 4-2. Initial baseline values (M ± SD), before and after 3 weeks of moderate intensity cycling training, on each dependent variable for the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11).

Table 4-3. Stress responses (change from baseline) (M ± SD), before and after 3 weeks of moderate intensity cycling training, on each dependent variable for the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11).

Figure Captions

Figure 4-1. CONSORT diagram of participant recruitment, assignment to conditions, and flow during the study.

Figure 4-2. FMD response in the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11), before and after 3 weeks of moderate intensity cycling training (50% VO_2peak).

Figure 4-3. Reactive Hyperemia response in the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11), before and after 3 weeks of moderate intensity cyclying training (50% VO_2peak).

Figure 4-4. Shear stress response in the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11), before and after 3 weeks of moderate intensity cyclying training (50% VO_2peak).

Figure 4-5. FMD response normalized to shear stress in the non-smoking control group (NON/Con, n=12), non-smoking exercise training group (NON/ExTr, n=12), and the smoking exercise training group (SMO/ExTr, n=11), before and after 3 weeks of moderate intensity cyclying training (50% VO_2peak).

Table 4-1.

Variable	Non/Control (n= 12)	Non/ExTr (n= 12)	Smoker/ExTr (n= 11)
Age	18.92 ± 1.31	22.42 ± 6.60	25.73 ± 7.43
Height	65.38 ± 2.96	64.67 ± 2.99	64.05 ± 1.93
Weight*	131.92 ± 16.75	152.58 ± 40.12	169.44 ± 33.86
VO_2peak (ml/kg/min)	33.15 ± 2.60	31.83 ± 7.56	26.93 ± 6.38
7-Day PA Recall (kcal/d)	37.88 ± 4.36	35.85 ± 3.72	37.84 ± 4.78
Historical Leisure Time Physical Activity (h/wk)	5.25 ± 5.57	3.62 ± 4.58	8.55 ± 7.33
Historical Leisure Time Physical Activity (met-h/wk)	34.03 ± 37.08	23.06 ± 28.14	55.84 ± 48.91

Table 4-2.

Variable	Pre-Training	Post-Training
Arterial Velocity (cm/s)		
NON/Con	20.57 (8.57)	17.02 (10.29)
NON/ExTr	15.44 (6.94)	22.58 (9.58)
SMO/ExTr	26.04 (11.96)	28.82 (12.26)
HR (beats/min)		
NON/Con	73 (12)	76 (8)
NON/ExTr	72 (7)	73 (12)
SMO/ExTr	69 (7)	68 (9)
SBP (mmHg)		
NON/Con	115 (13)	119 (14)
NON/ExTr	117 (10)	119 (8)
SMO/ExTr	121 (11)	123 (12)
DBP (mmHg)		
NON/Con	60 (10)	64 (7)
NON/ExTr	69 (6)	67 (10)
SMO/ExTr	71 (8)	69 (9)
MAP (mmHg)		
NON/Con	118 (15)	124 (12)
NON/ExTr	131 (11)	128 (16)
SMO/ExTr	135 (13)	132 (15)
Diameter (cm)		
NON/Con	3.29 (0.40)	3.18 (0.44)
NON/ExTr	3.09 (0.17)	3.17 (0.22)
SMO/ExTr	3.50 (0.42)	3.56 (0.42)
Blood flow ($ml*min^{-1}$)		
NON/Con	106.85 (48.01)	84.32 (64.88)
NON/ExTr	70.38 (33.65)	105.21 (42.08)
SMO/ExTr	149.84 (73.82)	171.79 (76.79)

Table 4-3.

	Pre-Training		Post-Training	
Variable	Stroop	Forehead Cold	Stroop	Forehead Cold
Arterial Velocity (cm/s)				
NON/Con	-0.18 (12.62)	-10.17 (9.06)	1.57 (8.35)	-7.77 (6.87)
NON/ExTr	-1.16 (5.80)	-6.57 (7.88)	1.18 (9.66)	-12.27 (7.25)
SMO/ExTr	-3.39 (9.79)	-12.88 (8.39)	-4.03 (8.04)	-13.32 (9.15)
HR (beats/min)				
NON/Con	6.23 (4.80)	-4.52 (7.90)	3.76 (6.38)	-3.09 (5.18)
NON/ExTr	3.03 (5.89)	-5.62 (3.73)	3.83 (4.76)	-4.59 (6.98)
SMO/ExTr	5.26 (6.53)	-3.82 (5.00)	4.01 (4.34)	-2.58 (6.29)
SBP (mmHg)				
NON/Con	14.23 (9.78)	16.97 (14.01)	11.30 (10.27)	14.84 (6.04)
NON/ExTr	13.30 (6.43)	12.18 (19.52)	5.53 (10.17)	13.50 (15.60)
SMO/ExTr	16.12 (7.33)	15.05 (10.34)	8.51 (9.74)	14.28 (7.80)
DBP (mmHg)				
NON/Con	9.24 (5.96)	14.74 (9.01)	6.70 (6.47)	12.07 (5.32)
NON/ExTr	7.02 (3.51)	7.04 (8.98)	3.81 (4.53)	7.61 (7.62)
SMO/ExTr	9.19 (5.24)	8.40 (4.89)	5.26 (3.49)	5.57 (3.44)
MAP (mmHg)				
NON/Con	16.98 (10.09)	25.20 (16.10)	12.64 (11.49)	20.95 (8.04)
NON/ExTr	13.72 (6.38)	13.38 (14.88)	6.89 (9.09)	14.56 (14.39)
SMO/ExTr	17.54 (8.66)	16.13 (8.32)	9.81 (7.65)	12.13 (6.57)
Diameter (cm)				
NON/Con	0.01 (0.09)	-0.05 (0.09)	0.02 (0.13)	-0.08 (0.10)
NON/ExTr	-0.03 (0.09)	-0.03 (0.13)	0.04 (0.08)	0.01 (0.10)
SMO/ExTr	-0.02 (4.27)	-0.11 (0.16)	-0.04 (0.11)	-0.09 (0.10)
Blood flow (ml*min^{-1})				
NON/Con	4.55 (67.21)	-53.61 (41.38)	6.20 (58.21)	-40.89 (43.78)
NON/ExTr	3.61 (25.88)	-30.95 (37.28)	13.29 (53.50)	-55.04 (30.94)
SMO/ExTr	-21.43 (65.81)	-78.55 (52.13)	-26.44 (51.22)	-84.82 (59.81)

Figure 4-1.

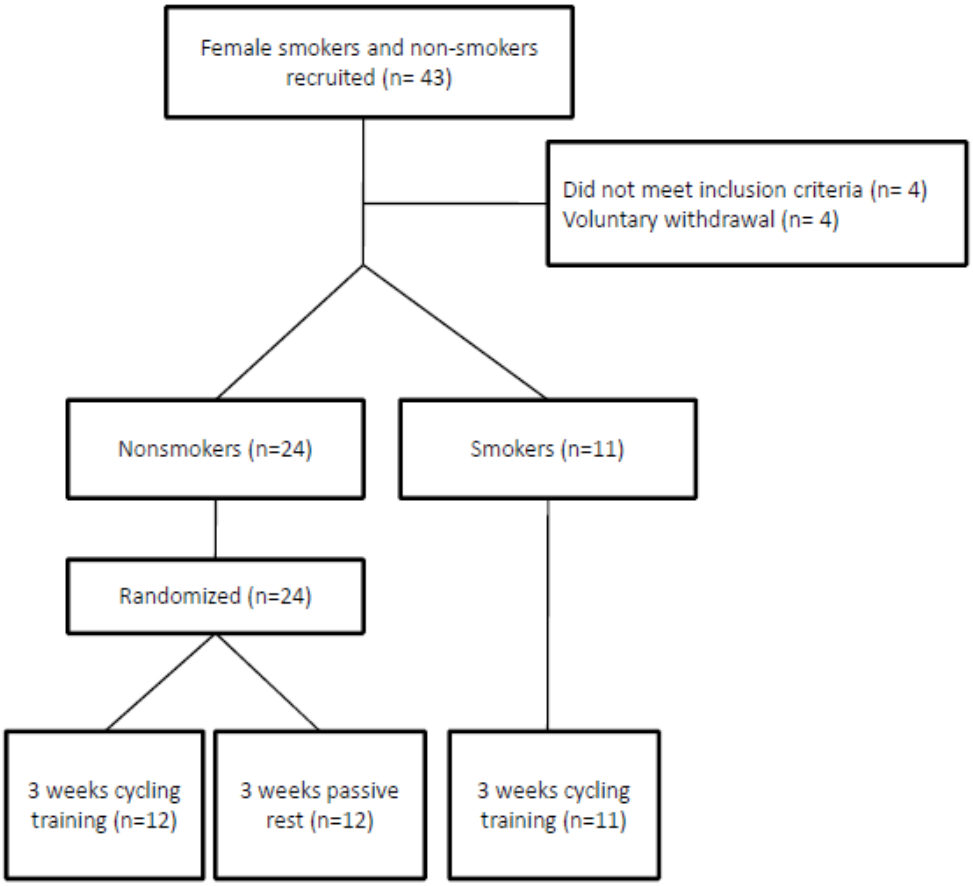

Female smokers and non-smokers recruited (n= 43)
Did not meet inclusion criteria (n= 4)
Voluntary withdrawal (n= 4)
Nonsmokers (n=24)
Smokers (n=11)
Randomized (n=24)
3 weeks cycling training (n=12)
3 weeks passive rest (n=12)
3 weeks cycling training (n=11)

Figure 4-2.

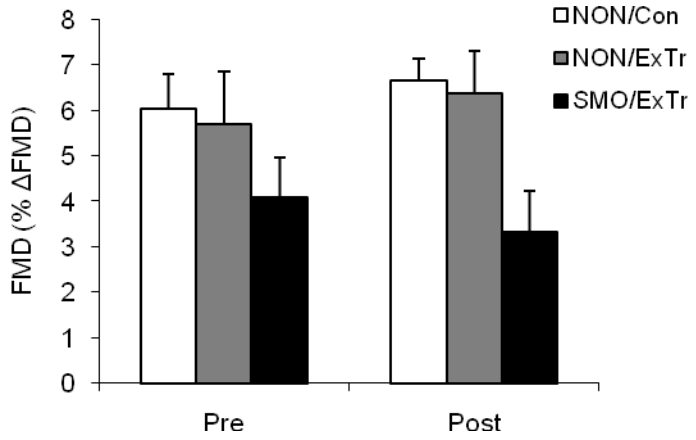


Figure 4-3.

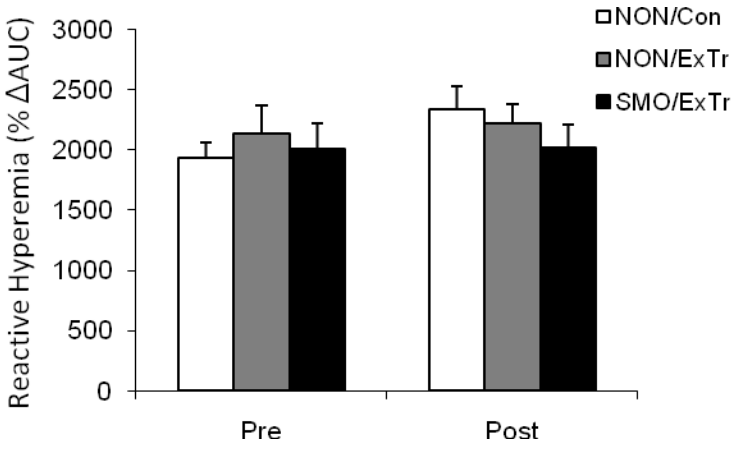


Figure 4-4.

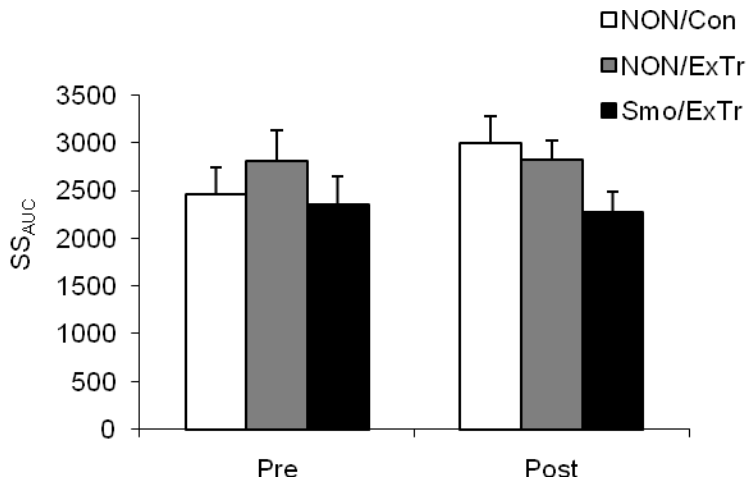
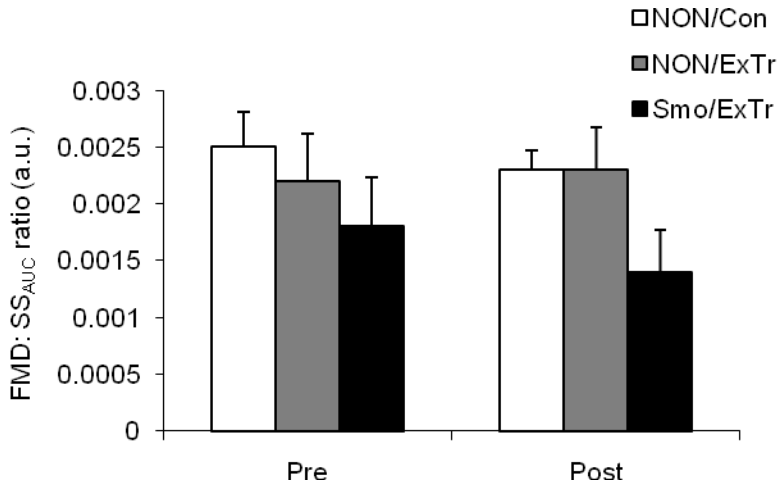


Figure 4-5



SUMMARY AND CONCLUSIONS

The purpose of this research was to examine the effect of acute and chronic exercise on endothelial function, measured by FMD, and vascular responses during two neurovascular stressors (the Stroop CWT and forehead cold pressor). Based on our results, acute exercise improves FMD regardless of smoking status. In addition, resting SBP, DBP, MAP, HR, arterial velocity and arterial BF were attenuated at pre-condition baseline after exercise. The improvement in endothelial function occurred even after exposure to stressful stimuli. When, FMD was normalized to shear stress, the effect of exercise was no longer present. This, along with an increase in reactive hyperemia, suggests that the increase in NO-dependent endothelial function after acute exercise was due to an increase in shear stress stimulus after exercise.

The effects of acute exercise extended to changes in stress reactivity. Specifically, increases in HR and arterial velocity were attenuated during the Stroop CWT. In addition, reductions in arterial velocity and HR during forehead cold were augmented. These results were inconsistent with our hypothesis that acute exercise would attenuate stress reactivity across both tasks. Instead, the pattern of cardiovascular responses suggests an increase in vascular resistance during stress. This increase in resistance during stress occurred with post-exercise hypotension and improved flow-mediated dilation. Future studies should include a measure of peripheral resistance and/or cardiac output to determine the mechanisms whereby exercise attenuated responses during the Stroop CWT and augmented responses during forehead cold.

To our knowledge, this was the first study to examine the effect of aerobic exercise training on FMD and cardiovascular responses during neurovascular stressors among female

smokers. As expected, smokers had impaired endothelial function and an augmented response to forehead cold. The effect of smoking on impairing endothelial depedent FMD remained after normalizing to the shear stress stimulus, even though smokers had a larger arterial diameter. The increased reactivity to forehead cold suggests that smoking and stress combined have additive effects. Exercise training did not improve FMD or attenuate responses to the Stroop CWT or forehead cold in female smokers or non-smokers. Arterial remodeling, or peak vasodilator capacity, which begins to occur between 2-4 weeks and results in a decline in FMD, might explain the absence of a training-induced change in FMD. Future research should measure FMD as early as 1 week. This will decrease the likelihood arterial remodeling will occur.

Overall, acute exercise improved endothelial function and reduced resting blood pressure in sedentary female smokers and non-smokers, implying reduced disease risk. The attenuated responses during the Stroop CWT and augmented responses during forehead cold, after exercise compared to passive rest, suggest that acute exercise alters vascular responsiveness during neurovascular stressors. The effects of acute exercise did not extend to chronic exercise training. A common feature of studies assessing endothelial function includes the normalization of FMD to either the shear stress stimulus or peak hyperemia. In the studies presented, normalization of FMD to shear stress clarified the potential mechanism acute exercise increases FMD. It also illustrated the likely cause for lower FMD in smokers, reduced responsiveness to the same shear stress stimulus. This is most likely caused by lower NO production in smokers. It is likely that the improvements in FMD and changes in vascular responses during stress observed after acute exercise may have diminished, before any chronic adaptations occurred with training.