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The Effect of Statins on Resting Blood Pressure and the Peak Blood Pressure Response to a Graded Exercise Stress Test

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The Effect of Statins on Resting Blood Pressure and the Peak Blood Pressure Response to a Graded Exercise Stress Test

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Chapter 1 - Introduction

Background and Significance

Hypertension (HTN) is defined as a systolic blood pressure (SBP) ≥140mmHg and/or diastolic pressure (DBP) ≥90mmHg, and/or taking antihypertensive medication (1) (see Table 1). The Seventh Joint Committee Blood Pressure Classifications (JNC7) added a new category of blood pressure classification, prehypertension, due to the increased risk of cardiovascular complications associated with above normal blood pressure levels (2). Approximately 30% of adults in the United States that are ≥20 years of age have prehypertension (an untreated SBP of 120-139 mmHg and/or DBP of 80-89 mmHg) and are twice as likely to develop HTN (3). HTN affects one in three adults in the United States (1) and is prevalent in one billion individuals worldwide (2). HTN is the most commonly diagnosed cardiovascular condition (1) and is a major risk factor for heart disease, stroke, congestive heart failure, and kidney disease (3). However, approximately one in five individuals who have HTN are unaware and go undiagnosed. (3).

Table 1

The Seventh Joint National Committee Blood Pressure Classifications (JNC7) (2)

JNC7 Category	**SBP (mmHg)**		**DBP (mmHg)**
Normal	< 120	**AND**	<80
Prehypertension	120-139	**OR**	80-89
Stage 1 Hypertension	140-159	**OR**	90-99
Stage 2 Hypertension	≥ 160	**OR**	≥ 100

SBP; systolic blood pressure, DBP; diastolic blood pressure

JNC7 recommends lifestyle intervention as the first line of therapy to prevent, treat, and control HTN, including eating a healthy diet, maintaining a healthy body

weight and being physically active (2). The American College of Sports Medicine (ACSM) recommendations for the frequency, intensity, time and type, or the FITT principle, for exercise and HTN is displayed in Table 2 (9).

Table 2. ACSM Exercise Recommendations (9)

*F*requency	On most but preferably all day s of the week
*I*ntensity	Moderate intensity (40-60% VO_2R)
*T*ime	≥30 minutes of continuous or accumulated physical activity per day (i.e. 3 bouts of 10 minutes per day)
*T*ype	Primary focus should be focused on aerobic endurance physical activity, supplemented by resistance exercise.

VO_2R; maximal oxygen uptake reserve

Exercise lowers blood pressure 5-7mmHg among those with HTN (9) and 2-3 mmHg among those with prehypertension (10). Although increasing physical activity is an important recommendation for reducing HTN, less than 50% of Americans engage in the amount recommended to lower blood pressure (3). By not meeting these minimal recommendations, physical activity is under utilized as an antihypertensive therapy.

In addition to lifestyle modifications, antihypertensive medications are prescribed to control HTN (4). Approximately 70% of individuals with HTN use antihypertensive medication to control blood pressure (5). Thiazide-type diuretics have been the basis of antihypertensive therapy to lower blood pressure compared to placebo, followed by angiotensin inhibitors, aldosterone receptor blockers, beta blockers and calcium channel blockers (2). These antihypertensive medications have successfully reduced elevated blood pressure levels, as well as reduce the complications of HTN (2). However, less than half (46.6%) of the individuals who use antihypertensive medications have controlled blood pressure levels (3).

Not typically prescribed for treating HTN, statins are the most commonly prescribed medications for reducing low-density lipoprotein (LDL) cholesterol and are the most potent monotherapy for lowering LDL by 18-55% of initial levels (11). The reductions are dose dependent: the greater the daily medication dose amount, the greater the reduction in LDL (11). Atorvastatin is the most widely prescribed statin and is the second most potent, next to Rosuvastatin (11). Atorvastatin also reduces trigylcerides by 7-30% of initial levels and increases low high-density lipoprotein (HDL) by 5-15% of initial levels (11). Again, the greater the daily medication dose amount, the greater the reduction (11). The greatest reductions usually occur in individuals who have elevated initial triglyceride levels, and the greatest increases in HDL usually occur in individuals with low initial HDL levels (11). In addition to lowering LDL cholesterol levels, there is evidence that suggests statin therapy also reduces resting systolic blood pressure by 5-8mmHg and diastolic blood pressure by 3-5mmHg (6). However, these pleiotropic blood pressure lowering effects of statins are controversial because some researchers have seen no effect of statins on blood pressure (6, 12).

Milinois et al. (6) meta-analyzed the literature examining 17 different studies that involved statins and examined their effect on blood pressure. Among those 17 studies, 12 studies were found to reduce resting blood pressure, while the remaining 5 studies reported no change. Although a majority of studies reported a reduction in blood pressure, there was a large range of variability in the statin induced blood pressure reductions of 4.0 mmHg to 13 mmHg. The subject population of the 17 studies included both individuals with normal blood pressure and HTN, and normal and elevated cholesterol levels. However, the meta-analysis but did not report any additional

descriptive characteristics (age, body mass index, etc.) of the study subjects that were included in the analysis. With such heterogeneity of response, they concluded it is inconclusive whether statins truly reduce resting blood pressure or not (6).

Similarly, Sarafidis et al. (12) meta-analyzed 25 studies examining the effect of statins on blood pressure and found 20 of these studies reported reductions in resting blood pressure. The range of statin induced blood pressure reductions in this meta-analysis was 2mmHg to 37mmHg (12), which were even larger than that reported by Milinois et al. (6). It is apparent that the literature on the blood pressure lowering effects of statins is mixed and additional research is required in order to accurately determine if statins do possess blood pressure lowering effects.

Having HTN increases an individual's risk for developing cardiovascular disease. Experiencing an exaggerated SBP response to a graded exercise stress test (GEST) can also put individuals at risk for developing HTN and cardiovascular disease (13). An exaggerated blood pressure response to a GEST is defined as an exercising systolic blood pressure of >220 mmHg for men, >190mmHg for women. It can also be defined as an increase of >10 mmHg in diastolic blood pressure or diastolic blood pressure >90mmHg for both men and woman (8). Therefore, it is possible that if statins lower blood pressure at rest, they may lower the peak blood pressure response to a GEST and reduce the risk of developing hypertension or further cardiovascular disease. Among current literature, there have been no studies that have examined the effect of statins on exercising blood pressure or the peak blood pressure response to a GEST.

Problem Statement

HTN is recognized as a major risk factor for cardiovascular disease and an exaggerated blood pressure or hypertensive response to a GEST is also considered a risk factor for the development of HTN. Statins have been inconclusively found to reduce resting blood pressure. Their efficacy to lower the peak blood pressure response to a GEST has not been investigated. Therefore, the purpose of this study is to examine the effect of statins on resting blood pressure and the peak blood pressure response to a GEST.

Specific Aims

The first specific aim is to examine the influence of statins on resting blood pressure before and after 6 months of atorvastatin or placebo drug therapy. We hypothesize resting blood pressure will be reduced in subjects assigned to atorvastatin compared to placebo.

The second specific aim is to examine the influence of statins on the peak systolic blood pressure response to a GEST before and after 6 months of atorvastatin or placebo drug therapy. We hypothesize the peak blood pressure response to a GEST will be reduced in the subjects assigned to atorvastatin compared to placebo.

Clinical Significance

It is common for individuals to have dyslipidemia and HTN simultaneously (1). As mentioned previously, statins are the most effective pharmacological treatment for reducing low-density lipoprotein levels. If statins are found to effectively reduce elevated blood pressure levels, as well, physicians will be able to prescribe one medication to

combat both dyslipidemia and hypertension, two major cardiovascular disease risk factors simultaneously.

Chapter 2 - Review of Literature

Introduction

The lipid lowering effects of statins have been well established and several clinical trials have reported additional beneficial effects of statins (35). Law et al. (15) meta-analyzed 164 short-term, randomized controlled trials and their effect on lowering LDL cholesterol, reducing ischemic heart disease and reducing stroke events. These researchers examined the six classifications of statins (Atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) and dose amounts of 5, 10, 20, 40 and 80 mg (15). All the classes of statins and doses resulted in a reduction of LDL cholesterol (15). As the dose amount increases, the percentage of reduction in LDL cholesterol also increased (15). Ischemic heart disease events were decreased by 61% and stroke events by 17% (15). The results of this meta-analysis clearly display the additional beneficial effects that statins can have beyond improving the lipoprotein profile.

Brugts et al. (16) also examined the effects of statins on reducing cardiovascular disease risk. The objective of this meta-analysis was to investigate whether statins reduce all cause mortality and major coronary and cerebrovascular events in individuals without established cardiovascular disease but who have cardiovascular disease risk factors (16). The researchers examined 10 trials that included a placebo or control group with at least a 1 year follow-up. The studies also had to report that 80% or more of the participants were without established cardiovascular disease and included outcome data on mortality and major cardiovascular disease events. Of the 70, 388 subjects, 34% were women, 23% had diabetes mellitus and the mean follow-up was 4.1 years. There were no significant

differences between drug treatments (type of statin, dosage, etc.) used in each study or effect of treatments among the studies examined. Brugts et al. (16) found that statin therapy significantly reduced the risk of all cause mortality (odds ratio 0.88), major coronary events (0.81), and major cerebrovascular events (0.81). For patients without established cardiovascular disease but with risk factors, statin therapy was associated with significantly better survival and large reductions in major cardiovascular disease events compared to control or placebo (16). Statin therapy was associated with a 30% decrease of major coronary events, 12% decrease in all cause mortality, and a 19% decrease in cerebrovasclular events associated with the use of statins. The meta-analysis only reported the lipid lipoprotein profile in relation to statins among the cardiovascular disease risks, but with a major decrease in major coronary events associated with statin use, statins ensure better survival among those with cardiovascular disease (16).

Statin Mechanism

Statin medications work by directly acting to reduce cholesterol biosynthesis, mainly in the liver (Figure 1) (17). Cholesterol is produced by mevalonate, the product of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase reaction. When HMG-CoA is converted to mevalonate by the HMG Co-A reductase enzyme, mevalonate is then coverted into squalene, which in turns becomes lanosterol, and thus becomes cholesterol. Statins work by inhibiting and alleviating activation of the HMG Co-A reductase enzyme, therefore limiting the amount of HMG Co-A which becomes mevolnate, thus reducing the amount of cholesterol that is produced (17).

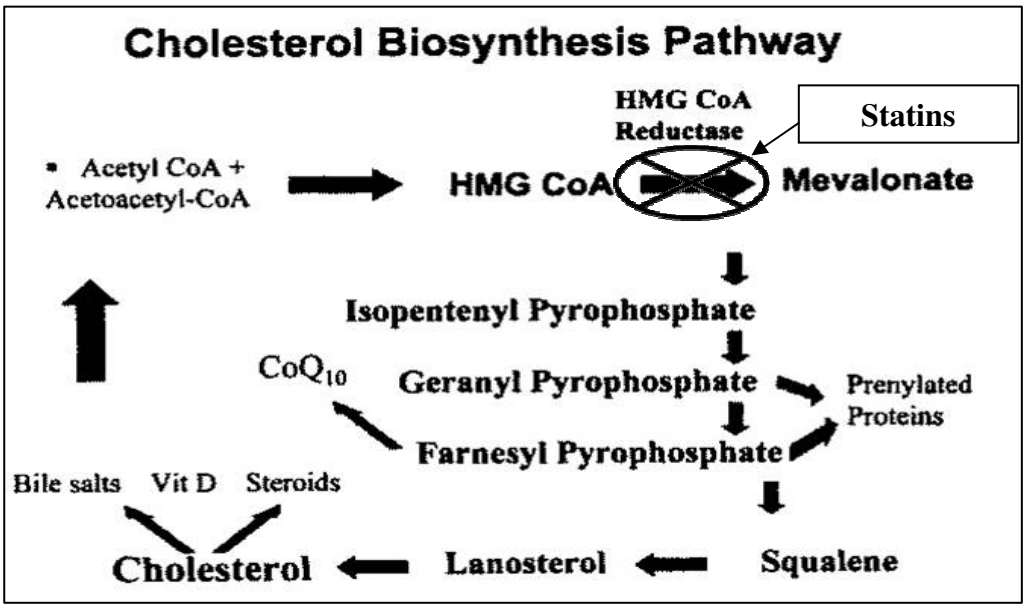

Figure 1. Statin mechanism of action (27).

Statins and Reducing Blood Pressure

Statins not only effectively improve the lipoprotein lipid profile and improve cardiovascular disease event risk, but may have a positive effect on BP as well. In addition to lowering LDL cholesterol levels, there is evidence that suggests statin therapy also reduces resting systolic blood pressure (SBP) by 5-8mmHg and diastolic blood pressure (DBP) by 3-5mmHg (6). However, these pleiotropic BP lowering effects of statins are controversial because some researchers have seen no effect of statins on BP (6, 12).

Milinois et al. (6) meta-analyzed 17 different studies that included statin use and that considered the relationships between hypertension and lipid abnormalities. Milinois et al. (6) wanted to examine the clinical and experimental data regarding statins on BP. The subjects included in the analysis had normal, pre-hypertension, and Stage 1 and Stage 2 hypertension BP levels. The subjects also had normal and high cholesterol levels. The subjects included may also have had diabetes mellitus or a combination of these cardiovascular disease risk factors. Among those 17 studies, 12 studies were found to

reduce resting BP, while the remaining 5 studies reported no change. Although a majority of studies reported a reduction in BP, there was a large range of reductions from 4.0 mmHg to 13 mmHg. Milinois et al. (6) reported statin treatment is associated with an average decrease of 5-8 mmHg in SBP and a 3-5 mmHg decrease in DBP. However, the heterogeneity of the reported BP effects led to an inconclusive conclusion of whether statins truly reduce resting BP or not. The researchers also attributed the heterogeneity of responses to the different groups of patients (e.g. men versus women, subjects with normal BP and HTN). It is important to note the studies that were examined did not include statin treatment on BP as part of the study design. The beneficial effect on BP among individuals with hypertension could have been attenuated by the large number of subjects with normal BP, in which no effect may have occurred and antihypertensive drugs may have masked any beneficial effects of statins (6).

Similarly, Sarafidis et al. (12) meta-analyzed 25 studies to examine the current knowledge regarding the effect of statins of BP and found 20 of these studies reported favorable reductions in resting BP. This review included multiple studies that were also used in the Milinois et al. (6) meta-analysis. Again, subjects included in this analysis ad normal BP, prehypertension, Stage 1 and 2 hypertension BP, along with normal to high cholesterol levels. One noticeable difference between this meta-analysis than Milinois et al. (6) was the presence of studies that used statins in addition to a BP lowering medication. The range of these reductions reported by Sarafidis et al. (12) were from 2 mmHg to 37 mmHg, which were much larger than reported by Milinois et al. (6). The inclusion of studies that used combination therapy (BP lowering medication with a statin treatment) may have resulted in the large range of differences reported by Sarafidis et al.

(12). Sarafidis et al. (12) concluded that their meta-analysis supported the notion that statins do have a favorable effect on BP but the studies are limited by several factors. These factors include small sample sizes, inadequate design, and not having changes in BP as a primary end-point. Sarafidis et. al. (12) suggested that long-term studies should be conducted with BP change as the primary variable.

Included in the meta-analysis by Sarafidis et al. (12) were studies that used combination therapy. An example of how why this would affect BP in regards to statins is explained by Danaogolu et al. (18). Danaogolu et al. (18) evaluated the effect of the angiotensin converting enzyme (ACE) inhibitor lisinopril alone and in combination with simvastatin on BP in 56 patients with hypertension and normal lipoprotein levels. The subjects assigned to lisinopril alone experienced a large reduction in BP (32 mmHg SBP / 22 mmHg DBP). The combination of the lisinopril and simvastatin treatment resulted in similar reductions in BP (38 mmHg SBP / 23 mmHg DBP), with a slightly greater SBP reduction (18). Sarafidis et al. (12) did not perform any between-group comparisons to examine a possible difference but it appeared that although the reductions were similar, those taking the combination of the ACE inhibitor and statin experienced a slightly greater reduction in BP (18). The lack of the between-group analysis within this meta-analysis may be because studies included in the analysis may not have performed between-group analysis and therefore, had nothing to report.

It should be also be noted that many of the studies included in the meta-analyses by Milinois et al. (6) and Sarafidis et al. (12) included studies that were conducted as early as 1990-2005. It is apparent that the literature on the BP lowering effects of statins is mixed and more recent research is required in order to accurately determine if statins

do possess BP lowering effects. In additional to the heterogeneous results regarding statins and BP, the mechanisms by which statins affect BP still remain a question. Previous studies report a reduction in BP due to statins, but fail to explore the effect. Although a single mechanism has not been established, many researchers seem to agree that the answer resides in the renin-angiotensin system.

Renin-Angiotensin System and Blood Pressure

During times of extreme stress, norepinephrine acts on alpha-adrenergic receptors on vascular smooth muscle and strongly constricts afferent arterioles that inhibit filtrate formation. This action indirectly triggers the renin-angiotensin mechanism by stimulating prorenin to form renin in the juxtaglomerular cells of the kidneys (19, 29). The kidneys' macula densa cells are then activated and release renin (19, 29).

When the hormone renin is released it acts enzymatically on angiotensinogen (Figure 2). Angiotensinogen is a hormone that causes vasoconstriction and stimulates the release of aldosterone, which causes sodium retension within the kidneys (19). These actions of angiotensinogen cause BP to increase. When renin acts on angiotensinogen, it is converted into angiotensin I. Angiotensin I is converted into angiotensin II by the angiotensin converting enzyme (ACE). The ACE enzyme is associated with the capillary endothelium in various body tissues, particularly the lungs. Angiotensin I can also be converted into angiotensin II by non-ACE ezymes such as chymase (19). Angiotensin II is the predominant physiological regulator of BP (29). Angiotensin II also triggers the release of aldosterone from the adrenal cortex (29). When aldosterone binds to the mineralocorticoid receptor (MR), it contributes to electrolyte and water balance in the

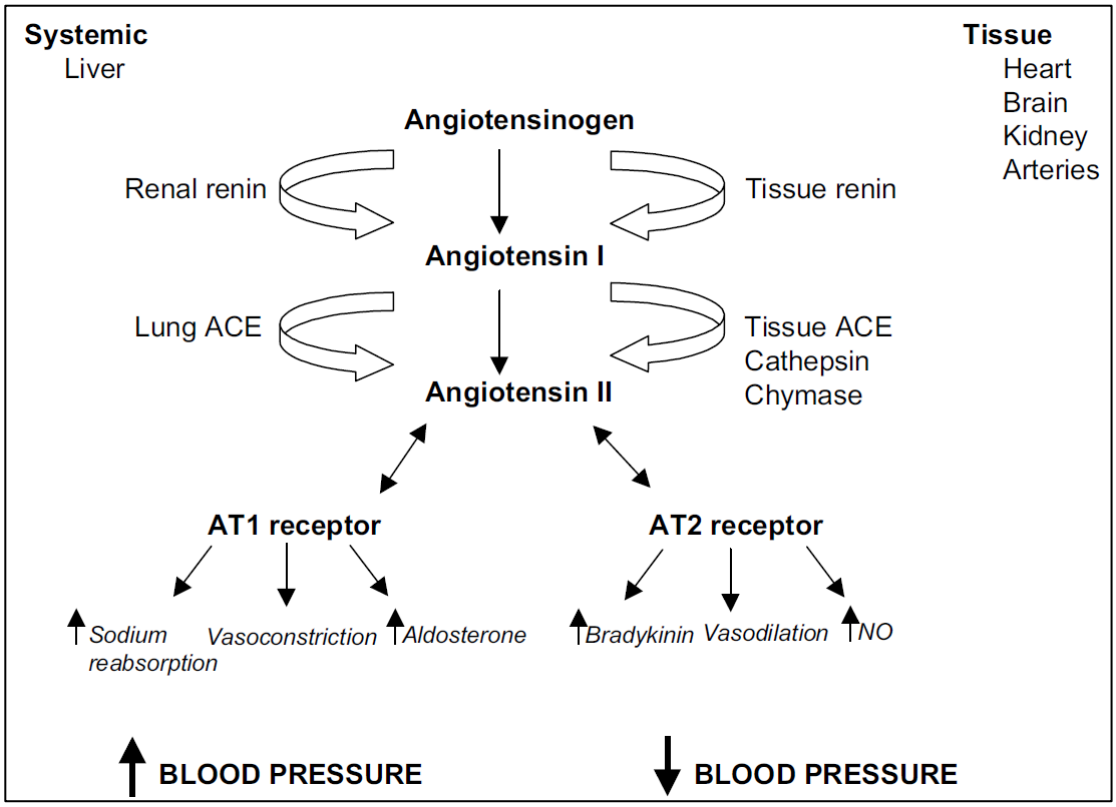

Figure 2. Representation of the overall role of systemic and tissue renin-angiotensin-aldosterone system (RAAS). ACE, angiotensin-converting enzyme; AT1, angiotensin II type 1; AT2, angiotensin type II type 2; NO, nitric oxide (24).

body (29). Angiotensin II is activated through two different receptors: Angiotensin 1 (AT1) receptor or the Angiotensin 2 (AT2) receptor (19, 29).

The AT1 receptor acts in ways that can affect BP and extracellular fluid volume. Angiotensin II activates the AT1 receptor which results in vasoconstriction and activates smooth muscle of arterioles throughout the body and raises BP. Angiotensin II stimulates reabsorption of sodium through directly acting on the renal tubules and indirectly triggering the release of aldosterone from the adrenal cortex. Water follows sodium osmotically, therefore this results in an increase in blood volume and BP. The hypothalamus can be directly stimulated by angiotensin II causing the thirst center to activate, which leads to an increase in blood volume and BP. Angiotensin II also increases fluid reabosrption by decreasing peritubular capillary hydrostatic pressure; this

occurs when the efferent arterioles constrict and more fluid moves back into the peritubular capillary bed. Lastly, angiotensin II targets the glomerular mesangial cell, causing them to contract and reduce the glomerular filtration rate. This is caused by a reduction in the total surface area of glomerular capillaries available for filtration, and thus the kidneys retain more water, increasing fluid volume and BP (19).

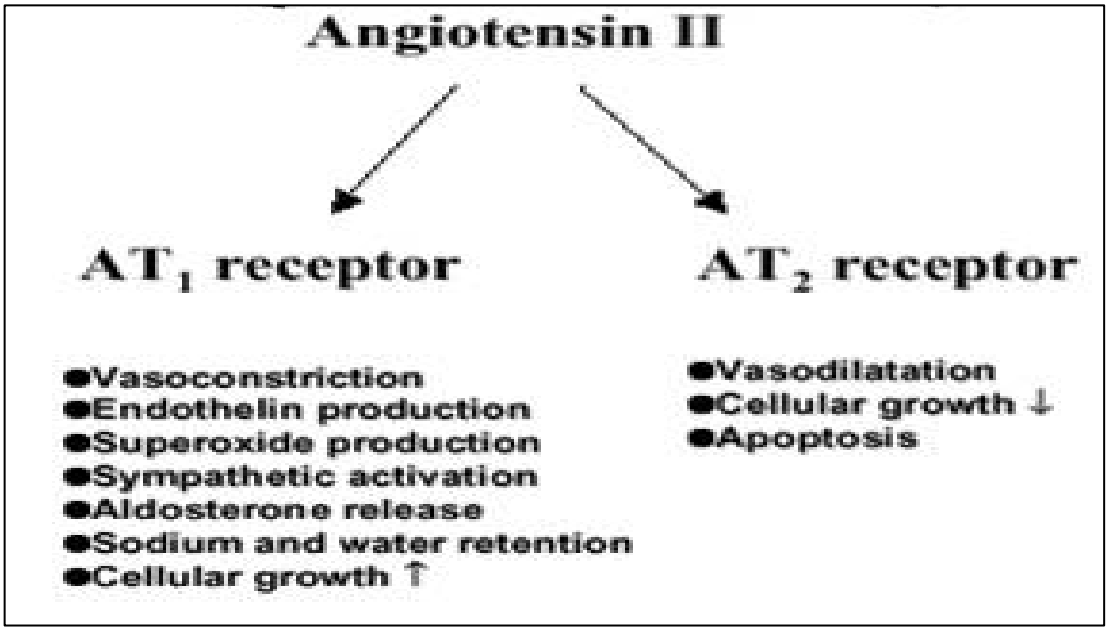

Figure 3. Different effects of the AT1 receptor compared to the AT2 receptor (28). AT1, type I angiotensin receptor; AT2, type II angiotensin receptor.

Angiotensin II activating the AT2 receptor affects BP in a different way than that of the AT1 receptor. When angiotensin II activates the AT2 receptor, angiotensin II acts as a vasodilator among the arterioles and reduces the amount of cellular growth. These actions do not result in an increase in fluid or blood volume, thus resulting in no increase in BP. Angiotensin II via the AT2 receptor also promotes apoptosis, or programmed cell death (19). The different actions of activating the AT1 and AT2 receptor are displayed in figure 3 (28).

Statins and Proposed Mechanisms for Reducing Blood Pressure

The exact mechanism by which statins reduce BP has yet to be determined, but researchers have proposed multiple possibilities. Most of the proposed mechanisms involve statins acting on the renin-angiotensin system. However, it has been suggested that the mechanisms by which statins lower BP are different from the mechanisms by which statins lower lipids (6). Milinois et al. (6) found no correlations between the reductions found in LDL cholesterol and reductions in BP, suggesting that a "non-lipid lowering mechanism" must be present for antihypertensive actions to occur, including decreased expression of the AT1 receptor, restoration of endothelial dysfunction, increased nitric oxide (NO) synthesis, and other potential mechanisms such as urinary sodium by regulating the amount of sodium retained or released (6).

Statins and the Sympathetic Nervous System

The sympathetic nervous system (SNS) and its neural renal controls serve as the needs of the body as a whole to maintain homeostasis (19). When the body responds to stress, it is the SNS that responds and carries out the necessary tasks of the body (19). Deo et al. (39) tested the effectiveness of statins to decrease resting muscle sympathetic nerve activity in heart failure patients and whether those reductions were associated with reductions in reactive oxygen species (ROS, oxygen ions used in cell signaling and homeostasis) (39). Deo et al. (39) tested seven statin-naïve heart failure patients and treated them with 40 mg of Simvastatin for one month. Muscle sympathetic nerve activity was tested by using pulse-synchronous burst pattern in response to the valsalva maneuver measuring burst frequency (burst/minute) and burst incidence (bursts/100 heart beats) (39). Deo et al. (39) found Simvastatin significantly reduced muscle sympathetic nerve

activity in burst frequency and incidence among all patients. ROS were also reduced when patients took Simvastatin. Deo et al. (39) concluded that although findings were novel, it suggests that statins may be beneficial at reducing sympathetic nerve over-activity among individuals with heart failure. Deo et al. (39) demonstrated an additional beneficial use of statins; however, heart rate and SBP remained unaffected by Simvastatin throughout the study. This suggests that the mechanism involved in lowering BP may lie elsewhere within the renin-angiotensin system.

Statins and the Angiotensin Receptors Mechanism

Hypercholesterolemia is associated with an over-expression of the AT1 receptor, which leads to increased angiotensin II induced BP elevation (6). The effects of over-expression of the AT1 receptor activation can cause increased vasoconstriction, endothelin production, superoxide production, sympathetic activation, aldosterone release, sodium and water retention, and cellular growth. All of these factors can lead to elevated BP. However, there is evidence to suggest that statins down-regulate AT1 receptor expression and shunt those negative effects on BP (20). Nickenig et al. (20) measured the effects of the AT1 receptor activation by assessing BP increases after infusion of angiotensin II in 39 men with normal and hypercholesterolemic levels. AT1 receptor expression was assessed on isolated platelets. Eight of the subjects with hypercholesterolemia had their BP and cholesterol assessed before and after receiving statin therapy; however it was not explained why not all subjects were measured. Nickenig et al. (20) found that the subjects with hypercholesterolemia experienced a significant increase in angiotensin II-induced BP elevation. This resulted in enhanced AT1 receptor activity compared to the subjects with normal cholesterol levels. However,

Nickenig et al. (20) also found that subjects treated with statin therapy had reversed the elevated BP response to the angiotensin II infusion and downregulated AT1 receptor density (20). Subjects treated with the statin did not experience elevated BP when angiotensin II was used as stimulation. Subjects not treated with statins experienced an elevated BP response when angiotensin II was used as stimulation.

Nickering et al. (20) found that statins caused a downregulation of the AT1 receptor among men (20). Similarly, Patel et al. (21) tested the effects of the downregulation of the AT1 receptor in mice. Patel et al. (21) hypothesized that mice that receive a pressure overload, in order to cause angiotensin converting enzyme 2 (ACE-2) high BP (ACE2-null mice), can avoided by switching off the AT1 receptor and/or switching on the angiotensin 1-7 Mas receptor axis (21). Angiotensin 1-7 is part of the pathway that includes ACE2 and angiontensin 1-7 receptor (Mas receptor) (21). The actions of the angiotensin 1-7 Mas receptor (Figure 2) counteract the detrimental effects of the AT1 receptor. Patel et al. (21) first used a pressure-overload to elicit hypertrophic cardiac responses (increased left ventricular wall thickness and end-diastolic dimensions). After 2 weeks of pressure overload, Patel et al. (21) used a blockade of the AT1 receptor and supplemented with angiotensin 1-7. At both AT1 receptor blockade and angiotensin 1-7 supplementation resulted in lower SBP, less cardiac hypertrophy and improved heart function (21). The blockade and supplementation also were determined to have equal cardioproective effects. Nickenig et al. (20) found that statins downregulate the AT1 receptor. Patel et al. (21) found that downregulation of the AT1 receptor can result in increased activation of angiotensin 1-7/Mas pathway. The combination of these

mechanisms may be a probable explanation for how statins lower BP; however, it has yet to be truly verified.

Statins and Nitric Oxide

Nitric oxide (NO) is an endothelium-derived relaxing factor that improves endothelial function (6) and positively influences BP. Milinois et al. (6) postulated that it may be the key mediator of the BP lowering effects of statins (6). Statins appear to restore and even improve endothelial function by increasing the bioavailability of NO. Caveolin-1 is a structural protein that is present in endothelial cells when LDL cholesterol is abundant (22). It is related to the amount of extracellular LDL cholesterol and can influence how much cholesterol uptake occurs within the endothelial cells (22). When high levels of LDL are present, caveolin-1 is abundant and decreases the bioavailability of endothelial NO synthase (eNOS) and leads to a decrease in NO production (22). Feron et al. (22) tested if a reduction in circulating LDL, or inhibiting cholesterol synthesis in endothelial cells by using statins would affect caveolin-1 abundance and promote NO production and release (22). They also examined whether statins could reverse endothelial dysfunction by reducing the caveolin-1 expression and promote NO production and release (22).

Feron et al. (22) found was statins inhibition led to a dramatic reduction in caveolin expression even with the lowest dose of statin used (0.01umol/L). Statin inhibition also led to a reduction in the inhibitory caveolin/eNOS interaction in endothelial cells and promoted NO production. The statin, Atorvastatin, reduced the amounts of eNOS that were bound to caveolin within the endothelial cells and significantly increased NO production. Statins also promoted eNOS and the heat shock

protein 90 (Hsp90) interaction. Hsp90 is a proposed molecular chaperone that facilitates long-term activation of eNOS.

Feron et al. (22) concluded that cholesterol abundance leads to an abundance of caveolin-1 within endothelial cells and inhibited eNOS activation. Statins work to restore eNOS activation through downregulation of caveolin-1 expression, even at a low dosage. Inhibition of cholesterol synthesis, via the mevalonate pathway, through statin use may be a postulated mechanism for NO release (22). This study provides a direct relationship to the mechanism of statins inhibiting cholesterol synthesis and promoting NO activation to positively influence BP. NO production can occur through activation of the AT2 receptor, promoting vasodilation (Figure 3) (28). Patel et al. (21) found that downregulating or inhibiting the AT1 receptor, activates the AT2 receptor, and therefore, with the use of statins, NO production may be likely to increase.

Although Milinois et al. (6) reported that NO may be the key mediator for the antihypertensive affect of statins on BP, it is possible that other mechanisms, such as the hormone estrogen, could be a factor.

Statins and Estrogen

Estrogen is a hormone that can readily interact with the renin-angiotensin system to influence BP. Although angiotensinogen is up-regulated by oral administration of estrogen, estrogen is known to downregulate the angiotensin-converting enzyme (ACE) and the AT1 receptor (Figure 4) (23). Despite stimulating a hormone (angiotensinogen) that causes unfavorable affects on BP, estrogen promotes favorable effects on BP (i.e. vasodilation, increased peripheral resistance) (23). In relation to statin therapy, as mentioned previously, it has been proposed that statins can block the AT1 receptor (21).

Because estrogen is a downregulating hormone for the AT1 receptor, when used in addition to statin therapy, the BP lowering effects have the potential to be more prominent in women than men. However, to this author's knowledge, no studies have explored these gender differences with the use of statins and their influence on BP.

Among the current literature, an important point to consider is the previously

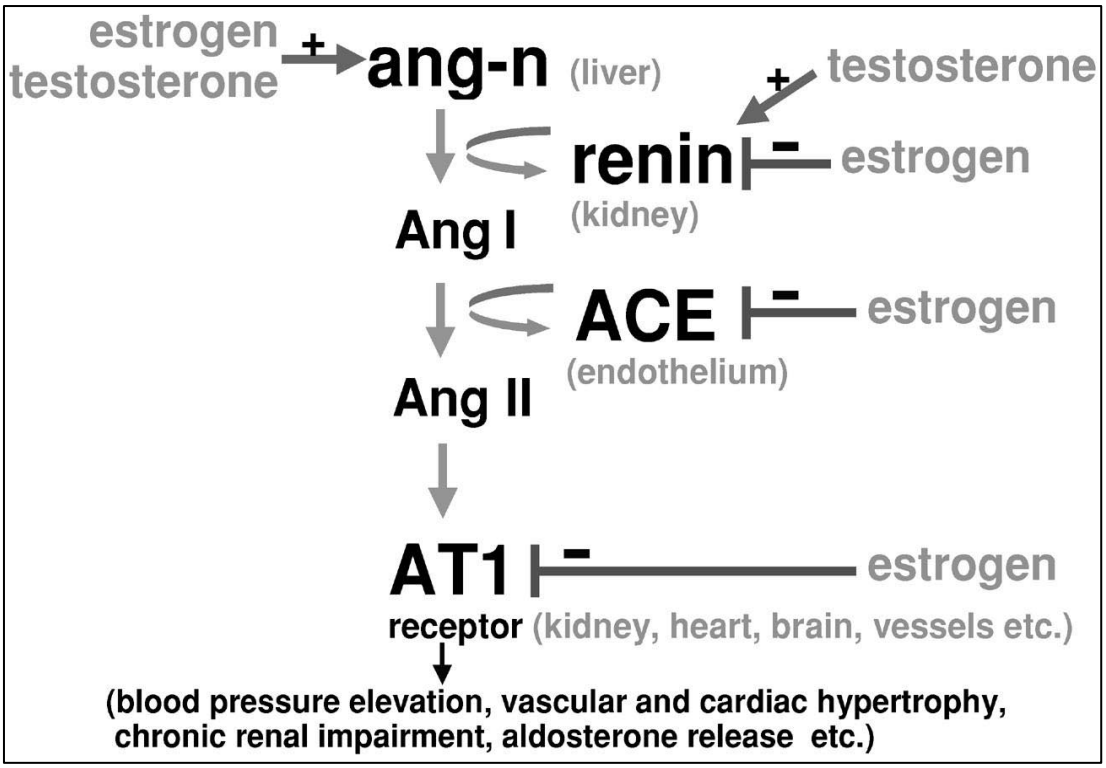

Figure 4. Angiotensin II formation and the influence of estrogen (23). Ang-n, angiotensinogen; Ang I, angiotensin I; Ang II, angiotensin II; ACE, angiotensin converting enzyme; AT1, type 1 angiotensin receptor.

mentioned meta-analyses of statins and BP have only explored the effects on resting BP, and not during exercise. It is also important to explore the effects statins could have on an exaggerated BP response to exercise.

Exaggerated Blood Pressure Response to Exercise

An exaggerated BP response to a graded exercise stress test (GEST) is defined as an exercising SBP of >220 mmHg for men, >190mmHg for women, or an increase of

>10 mmHg DBP or DBP >90mmHg for both men and women (8). An exaggerated systolic BP response to a GEST is a predictor for the development of hypertension and can increase cardiovascular disease risk (24). Patients with exaggerated systolic BP responses to peak exercise also have an increased risk for cardiovascular disease and cardiovascular disease morbidity (31). Additionally, individuals with an exaggerated systolic BP response to a GEST may be at greater risk for cardiovascular complications during vigorous intensity exercise (31).

Balanos et al. (25) found that individuals that have mildly elevated resting BP experienced a greater cardiac response, or increase in BP, to submaximal-exercise compared to control (25). Eleven young men with moderately elevated resting BP (SBP >130 mmHg) and 11 men with normal BP (SBP <120 mmHg) completed a graded submaximal cycling exercise test (25). Heart rate, BP, and oxygen consumption (VO_2) were measured before, during and after the test (25). The researchers found exaggerated heart rate and BP response were greater in the group that had the initial elevated BP levels compared to those with normal BP group (25). The subjects with the greater initial resting BP experienced higher BP levels during submaximal-exercise compared to those with normal BP (25).

It is important, however, to examine whether individuals with elevated resting BP will experience an elevated BP response to a maximal GEST.

Chang et al. (31) sought to explore the mechanisms involved with an exaggerated BP response during a treadmill test (31). Using ultrasound to measure endothelial function, Chang et al. (31) evaluated endothelial function in patients who experienced exaggerated BP response during a treadmill test. The control group included 25 subjects

who experienced normal BP responses to exercise. The experimental group consisted of 25 gender-matched subjects with documented normal BP at rest and an exaggerated BP response during exercise (systolic BP >210 mmHg for men and >190 mmHg for women). All patients completed a Bruce GEST, in which BP, heart rate and a 12-lead echocardiogram were recorded prior to exercise, during the last minute of each 3 minute stage and during the 5 minute recovery (31).

Chang et al. (31) found, as predicted, that individuals categorized into the experimental group experienced a significantly higher peak SBP response to the treadmill protocol than the control group (31). They also found a significant difference in endothelium-dependent vasodilation between the two groups; the experimental group experienced less vasodilatation than the control group, however it did not reach statistical significance (31). However, because the experimental group had lower vasodilation than the control, Chang et al. (31) concluded that the endothelium-dependent vasodilation is impaired in patients with an exaggerated BP response to exercise. The exaggerated increase in SBP during exercise negatively correlated with vasodilation (31). They concluded impaired vasodilatory capacity may be responsible for the exaggerated increase in BP during exercise (31). Exaggerated BP responses to exercise can negatively affect endothelium-dependent vasodilation, which may be caused by eNOS. This relationship observed by Chang et al. (31) could possibly influence the way that statins may act on BP during exercise. However, the influence of statins on exercising BP has not previously been examined.

Although the literature remains inconclusive, it is suggestive that statins aid in reducing resting BP. Therefore, if statins lower BP at rest, they may lower the peak BP

response to a GEST and reduce the risk of developing hypertension or further cardiovascular disease. Glorioso et al. (26) was one of the first controlled trials that clearly demonstrated that HGM-CoA could reduce resting BP and stress-induced BP in patients with essential HTN. In a 32-week double blinded trial, 30 participants received either a placebo or pravastatin (20-40mg/d) (26). There were 25 participants who completed the 32-week trial. Pravastatin decreased BP (8 mmHg SBP and 5 mmHg DBP), as well as blunted the BP increase caused by a cold pressor test (4-9mmHg) when compared to the placebo (26). The results obtained were supported by previous studies that had seen a decrease in mean arterial pressure with the use of statins (26). Because of statins ability to shunt the BP response to an external stimulus designed to increase BP, like the cold-pressor test, it is possible that statins will have the same shunting affects on the BP response to a GEST, especially if it is exaggerated.

Statins and Proposed Mechanisms for Reducing Blood Pressure Response to Exercise

Much like resting BP, no mechanisms for whether or not statins affect exercising BP have been determined or even explored. Among the current literature, to this author's knowledge, there have been no studies to examine the effect of statins on exercising BP or the peak BP response to a GEST. Chang et al. (31) proposed that a possible mechanism for exaggerated BP response to exercise is because of impaired endothelium vasodilation. This proposed mechanism relates directly to Milinois et al. (6) stating that NO may be the key factor involved in the BP lowering effects of statins because of its vasodilator effects. This theory was later discussed through the work of Feron et al. (22), who found that decreasing caveolin abundance using statins increased NO availability (22). NO is known to improve endothelial function. Therefore, the mechanism of action

for the BP lowering effect of statins may be the same during exercise, as it is at rest, involving increasing the bioavailability of NO. Additionally, if the mechanisms are the same, the work of Patel et al. (21) and Nickenig et al.(20) proposed that a blockade of the AT1 receptor and activation of the AT2 receptor would also increase NO availability. However, the proposed mechanisms of the influence of statins on resting and BP response to exercise are purely speculative at the current time.

Conclusion

The mechanism of statins lowering cholesterol is well defined and understood. However, the BP lowering effects of statins and the mechanisms that are involved are not well defined or understood. Despite the literature available, the BP lowering effect of statins still remains inconclusive and studies that examine this effect during exercise are still yet to be seen. Although multiple researchers have proposed plausible mechanisms of action that statins act when affecting BP, no one mechanism has been chosen. Mechanisms involving NO, blockade of AT1 receptor, increasing Angiontensin 1-7 and in the influence of estrogen acting on the AT1 receptor are all possible mechanisms that may influence statins acting on BP. However, it is possible that the BP lowering effects of statins may not entail just one mechanism, but a combination. It is equally as interesting to note the influence estrogen on the lowering effects of statins.

Chapter 3 - Methods

Study Overview

This sub-study is part of a larger study entitled "*The Effect of Statins on Skeletal Muscle Function and Performance* (STOMP)". The STOMP study was funded by the National Institutes of Health (NIH R01HL081893-01A2) and was conducted at three testing sites: Hartford Hospital, Hartford, CT; the University of Connecticut, Storrs, CT; and the University of Massachusetts, Amherst, MA. The purposes of the STOMP study were to determine the incidence of statin-induced muscle complains, defined as myalgia, and the effect of statins on skeletal muscle strength, endurance and performance (35).

In the STOMP study (Figure 1), participants completed a series of six laboratory visits during a 6 month time period. During visit one subjects had a venous blood draw to test for serum lipid alanine aminotransferase (ALT), serum creatinine, thyroid-stimulating hormone (TSH), creatinine kinase (CK), and vitamin D. Following the blood draw, subjects underwent anthropometric measures of height, weight, and waist circumference. In addition, resting blood pressure and heart rate were taken following a seated rest period of 10-15 minutes. Several questionnaires regarding physical activity, pain and psychological well-being were completed with study personnel during the seated rest period. Subjects completed several strength tests beginning with a handgrip test using a handgrip dynamometer. The remaining strength tests were conducted using a Biodex System 3 Isokinetic Dynamometer (Biodex Medical, Shirley, NY) and included elbow extensor and knee flexor isometric and isokinetic tests. Lastly, subjects completed a modified Bruce protocol GEST to test for abnormal responses to a peak exercise test.

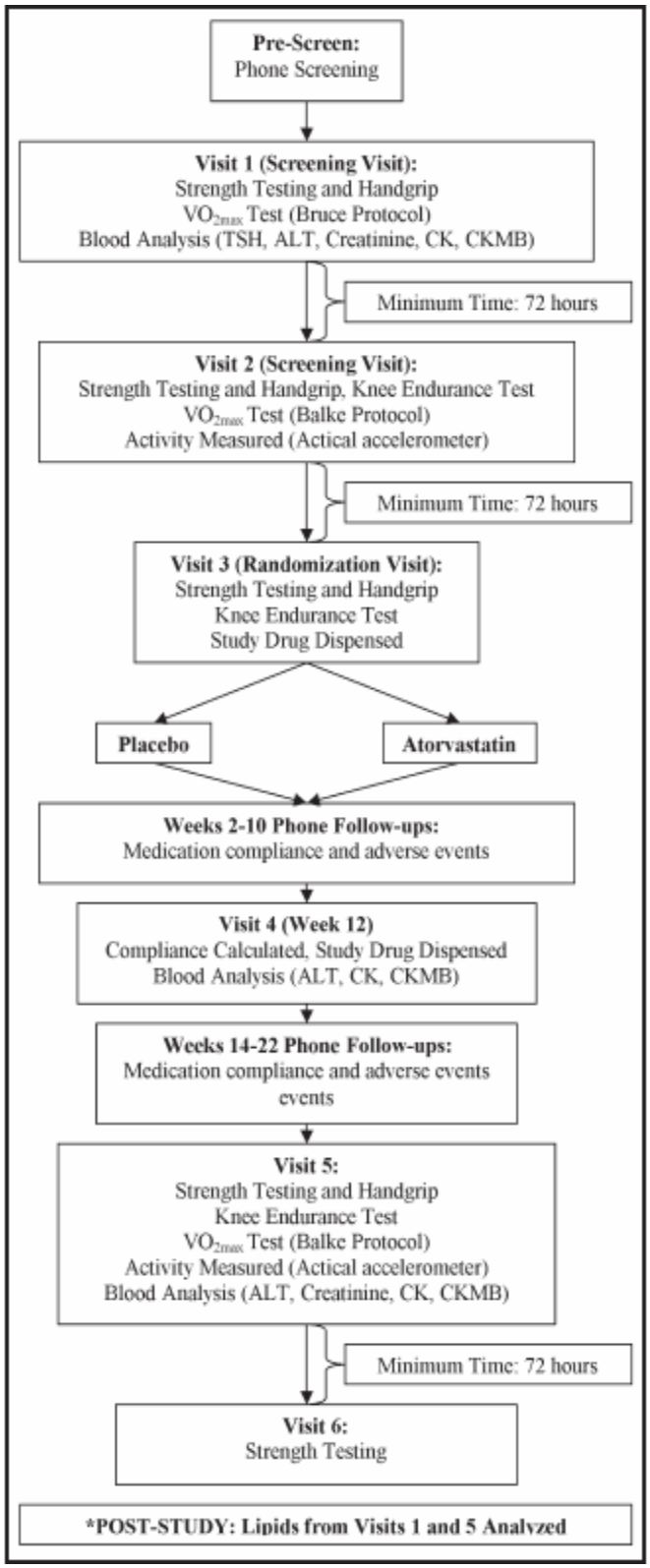

Figure 1. Overview of the STOMP study (35)

Following a minimum of 72 hours between visit one and visit two, subjects returned to the laboratory. During visit two, subjects completed the same series of handgrip and Biodex strength tests that were completed during visit one, as well as an additional knee endurance test using the Biodex system. Subjects also completed a maximal GEST done on a treadmill with the Balke protocol at a speed that could be sustained for the duration of the test (approximately 10-15 minutes). Following the strength and aerobic testing, subjects were given an ACTICAL accelerometer (Mini Meter, a Respironics Inc., Bend, OR) to measure physical activity and asked to wear it for 96 consecutive hours (four days), that included two week days and two

weekend days.

Following a minimum of 72 hours, subjects returned to the laboratory for visit three. During visit three, subjects returned the ACTICAL accelerometer (after wearing it for the 96 hr period) and completed the same strength and Biodex tests that were completed during visit one and two. Prior to subjects leaving the laboratory, they were double-blinded and randomized into receiving a 3 month supply of 80 mg of Atorvastatin or a placebo drug. Each subject was instructed to take one pill capsule at night daily during the next three months. To monitor each subject, research personnel called subjects every other week inquiring about changes in medication use, medication adherence to the study drug, and any symptoms of myalgia.

The fourth visit occurred three months after visit three. During visit four, a blood draw was conducted to test for ALT, serum creatinine and CK. The subjects met with a physician to assess any symptoms of statin-induced myalgia. Subjects also returned their study drug dispenser so that medication adherence could be determined. Prior to leaving the laboratory, subjects were given another three months of supply of 80 mg Atorvastatin or placebo. Study personnel continued to call subjects every other week to monitor changes in medication, medication adherence to the study drug and symptoms of myalgia during the next three months.

The fifth visit occurred three months after visit four and this was approximately six months after study enrollment began. Visit five consisted of the identical testing procedures as the initial visit at the beginning of the study. Subjects underwent a blood draw to test ALT, serum creatinine, TSH, CK, and Vitamin D. Subjects then completed the same handgrip and Biodex strength testing, which included elbow extensor, knee

flexion isometric and isokinetic tests and the knee endurance test. Subjects also completed a maximal GEST done on a treadmill with the Balke protocol. At the conclusion of visit five, an ACTICAL accelerometer was given to the subject to wear for a 96 hour period (four days) that included two week days and two weekend days.

During visit six, subjects returned to the laboratory for the final visit in the STOMP study. Subjects returned the ACTICAL accelerometer to study personnel after wearing it for a 96 hour time period and underwent a final cycle of isometric and isokinetic strength testing on the Biodex. Subjects also returned any remaining study medication to assess medication adherence.

Sub-Study Overview

This sub-study of STOMP used the baseline subject characteristics that were collected during visit one, including the ACTICAL physical activity data collected between visits two and three. Additionally, blood pressure measures from the maximal GEST done on a treadmill with the Balke protocol from visit two and five were used to compare blood pressure changes over six months for this sub-study.

Study Subjects. STOMP enrolled 468 subjects across the three testing sites, including equal numbers of men and women between the ages of 20-39, 40-54, and 55+ yr. The STOMP subjects were recruited by newspaper advertisement, local flyers, posters, and classroom announcements at each of the three testing sites. Prior to participating, subjects were determined eligible via an over-the-phone screening process and were required to complete an informed consent waiver. Subjects were excluded from the study if they had a history of cancer within the previous 5 years, abnormal thyroid stimulating hormone level, and history of cardiovascular disease or diabetes mellitus.

Subjects who had baseline ALT >2 times the upper normal limit (UNL) and creatinine >2 mg/L UNL were also excluded. In addition, subjects were excluded if they had previous or present use of lipid-lowering medication, medications that would alter statin metabolism, or medications known to affect skeletal muscle. If subjects had any muscle complaints or weakness, physical disabilities that would prohibit the strength or aerobic performance testing protocols of the study or had ischemic appearing on an echocardiogram ECG of the GEST done on a treadmill with the Bruce protocol at visit one they were also excluded. Finally, if a subject was currently taking antihypertensive medications to control their blood pressure levels (<140/90 mmHg), they were included. STOMP subjects received monetary compensation up to $720 for their time, travel and number of completed visits in the study.

Baseline Characteristics. At visit one; a blood draw was performed from the antecubital vein using a 21 gauge butterfly needle with a 4.0 mL lithium heparin tube for analysis of the lipoprotein lipid profile (Total, LDL, HDL and Triglycerides cholesterol). The drawn samples were then spun in a centrifuge (VanGuard V6500, Hamilton Bell Co., Inc., Montvale, NJ, USA) at 3400 RPM for 15 minutes. The plasma was then aliquated into 1 mL criovials and frozen and stored at -80°. The frozen samples were then shipped to Clinical Lab Partners (Hartford, CT) for analysis of the lipoprotein profile (Total, HDL, Triglycerides) and LDL were calculated using the Friedwald equation (36). Subjects were asked to list current BP medication use.

Anthropometric Measures. Anthropometric measurements were obtained by study personnel, which included height, weight and waist circumference. Height was measured by a wall-mounted measuring tape in inches which was later converted to

centimeters. Weight was measured using a calibrated balance beam scale and recorded in pounds which were later converted to kilograms. The height and weight measurements were used to calculate the body mass index (BMI) ($kg \cdot m^{-2}$). Waist circumference was measured with a non-distensible tape measure at the narrowest point of the subjects' torso, while they stood in an erect position with their arms relaxed at their sides. Measurements were recorded to the nearest tenth of a centimeter.

Cardiorespiratory Fitness. Peak oxygen consumption [VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)] was determined using the maximal GEST done on a treadmill with the Balke protocol during visit two (Appendix 1). The subjects sat for 5 minutes to establish gas equilibration, at which time a pre-exercise, resting blood pressure was measured via auscultation in the right arm. The Parvomedics True One 2400 Metabolic Cart (ParvoMedics Corp, Sandy, UT) was used to measure and conduct breath-by-breath analysis of expired oxygen and carbon dioxide at rest to establish the subjects' baseline and during exercise to determine VO_{2peak}.

Subjects began the maximal GEST done on a treadmill with the Balke protocol by walking at a 2 mph on a 0% incline for 2 minutes. Following this warm-up, research personnel increased the treadmill speed to a pace that the subject could maintain to complete the test. This speed was subject specific and varied from subject to subject. Once a speed was determined by the research personnel, the incline of the treadmill increased 1% every minute until the conclusion of the test. Heart rate, blood pressure and rating of perceived exertion (RPE) (15) were recorded every third minute of the protocol. Heart rate was recorded with a heart rate monitor, blood pressure was taking manually by auscultation in the right arm, and RPE on the Borg RPE scale 6-20 was recorded (37).

The subject continued to exercise until they reached volitional fatigue or if research personnel had to stop the test for any reason, physical inability to continue or any other reason that the subject felt they could not continue.

Upon cessation of the test, subjects completed an active cool down, walking at a pace of 2.5 mph for 3 minutes then 2.0 mph for 3 minutes, in which gas collection was still conducted. Heart rate, blood pressure and RPE were also recorded during the active and seated cool down. The active recovery was followed by a seated recovery for 3 minutes. This protocol was repeated during visit five to determine the subjects' post-intervention VO_{2peak}.

Resting Blood Pressure and Heart Rate. Resting blood pressure was measured following the 5 minute seated rest prior to maximal GEST done on a treadmill with the Balke protocol during visit two. Systolic (SBP) and diastolic (DBP) blood pressure (mmHg) were measured using auscultation by a trained research associate. Subjects were seated, with both feet flat on the floor, legs uncrossed, and backs supported. Blood pressure was measured in the right arm and the arm supported at heart level by research personnel. A heart rate monitor (Polar Vantage NV™ HR Monitor, Polar Electro Inc., Port Washington, NY, USA) was used to monitor subjects' resting heart rate.

Peak Systolic Blood Pressure. Peak SBP during maximal GEST done on a treadmill with the Balke protocol was established as the greatest BP recorded 1 min prior to the conclusion of the GEST or immediately post-exercise (within 30 second of ceasing exercise) if peak SBP could not be attained at 1 min prior to the conclusion of the GEST. SBP (mmHg) was measured in the right arm by auscultation by trained study personnel. If two BP measurements were recorded at 1 min prior and immediately post-exercise, the

latter BP was used as the peak SBP. Using the peak SBP criteria, only 49 subjects of that total sample had their BP taken to meet all of the criteria limits. In order to properly compare, a subject had to have an accepted recorded peak SBP at visit two and visit five.

Statistical Analysis

Descriptive statistics were conducted on all study variables to confirm normal distributions. All data was reported as mean ± standard error of the mean (SEM).

Independent samples t-tests were used to compare baseline characteristics between men and women by Atorvastatin and placebo groups.

Hypothesis 1. Subjects' resting SBP, DBP and mean arterial pressure will be reduced in those assigned to Atorvastatin compared to placebo after six months. Repeated measures analysis of covariance (RM ANCOVA) was used to test for significant changes in resting SBP, DBP and mean arterial blood pressure measured during the maximal GEST done on a treadmill with the Balke protocol performed at visit two (Pre) compared to visit five (Post) . ANCOVA was used to test differences in the changes in resting SBP, DBP and mean arterial blood pressure between men and women by Atorvastatin and placebo drug groups before and after six months of drug treatment. A Bonferroni correction analysis was used to test for gender by drug effects. For significant drug*gender interactions, ANCOVA was used to test the interactions. The fixed factors were gender, drug group (Atorvastatin vs. placebo), BP medication use, menopausal status and age category and covariates were age, BMI, VO_{2peak} and baseline resting BP.

Hypothesis 2. Subjects' peak SBP will be reduced in those assigned to Atorvastatin compared to placebo drug group after six months. RM ANCOVA was used to test for the difference in the absolute change in peak SBP collected during the maximal

GEST done on a treadmill with the Balke protocol performed at visit two (Pre) compared to visit five (Post). ANCOVA was used to test for the difference in the absolute change in peak SBP between men and women by Atorvastatin and placebo groups after six months. A Bonferroni correction analysis was used to test for gender by drug effects. For significant drug*gender interactions, ANCOVA was used to test these interactions. The fixed factors were gender, drug group (Atorvastatin vs. placebo), BP medication use, menopausal status and age category and covariates were age, BMI, VO_{2peak} and baseline resting BP.

Bivariate correlations were run to test for significant relationships between changes in LDL cholesterol and changes in resting and peak SBP.

All statistical analyses for this sub-study were performed using the Statistical package for the Social Science (SPSS) 14.0 program for Windows (SPSS Inc, Chicago, IL). Statistical significant was determined using $p<0.05$.

Chapter 4 - Results

Descriptive Characteristics

Subject Characteristics

The sample for this sub-study (n=419) included healthy men (n=203) and women (n=216) that were middle aged, overweight, had optimal resting BP, above optimal LDL cholesterol, desirable total cholesterol, and normal triglycerides and HDL cholesterol levels (11) (Tables 1 and 2). Resting SBP (p=0.01), DBP (p=0.04), and MAP (p=0.01) were higher among men compared to women (Table 2). Men achieved a higher initial peak SBP response on a GEST compared to women (p=0.001) (Table 3). Among the total sample, men (n=10) and women (n=9) were prescribed antihypertensive medications.

At baseline, men assigned to the Atorvastatin group had a greater BMI (p=0.001), triglyceride levels (p=0.000), VO_{2max} (p=0.000), and energy expenditure (p=0.000) and lower HDL cholesterol levels (p=0.000) than women assigned to the Atorvastatin group (Table 1). Additionally, at baseline, men assigned to the Atorvastatin group had a higher resting SBP (p=0.01) and MAP (p=0.01) (Table 2), and peak SBP response on a GEST (p=0.04) (Table 3) than women assigned to Atorvastatin group.

At baseline, men assigned to the placebo group had a greater BMI (p=0.01), triglyceride levels (p=0.000), VO_{2max} (p=0.000), and energy expenditure (p=0.000) and lower HDL cholesterol levels (p=0.000) than women assigned to the placebo group (Table 1). Additionally, at baseline, men assigned to the placebo group had a higher resting SBP (p=0.02) and DBP (p=0.04) (Table 2), and peak SBP response on a GEST (p=0.000) (Table 3) than women assigned to the placebo group.

At baseline, women assigned to the Atorvastatin group had a significantly higher resting DBP (p=0.04) than women assigned to the placebo group (Table 2). However, there were no other significant differences in baseline descriptive characteristics (Table 1), resting BP (Table 2), or the peak SBP response on a GEST (Table 3) between drug groups or between drug groups when stratified by gender (p>0.05).

Blood Pressure Changes

Resting Blood Pressure

Over 6 months of drug treatment, resting SBP (p=0.60), DBP (p=0.96), and MAP (p=0.74) were not different regardless of drug treatment group among the total sample (Table 2). However, there were significant gender*drug interaction effects for resting SBP (p=0.02) and DBP (p=0.02). Over 6 months of drug treatment, women on Atorvastatin reduced resting SBP (p=0.01) and DBP (p=0.02) more than men on Atorvastatin. However, these gender dependent resting BP effects among the Atorvastatin group were not different than the placebo group for SBP (p=0.20) and DBP (p=0.60).

Peak Systolic Blood Pressure

Over 6 months of drug treatment, the peak SBP response on a GEST was not different regardless of drug group among the total sample (p=0.99) (Table 3). However, there were significant gender*drug interaction effects (p=0.02). Over 6 months of drug treatment, the peak SBP response on a GEST (p=0.02) was lower among women on Atorvastatin, but not placebo (p=0.40). There were no significant drug group differences in the peak SBP response on a GEST among men (p>0.05). Over 6 months of drug treatment, women on Atorvastatin reduced the peak SBP response on a GEST more than men on Atorvastatin (-6.4 ± 3.3 mmHg, p=0.04) (Table 3). However, these gender

dependent differences in the peak SBP response on a GEST among the Atorvastatin group were not different than the placebo group (p=0.66) (Table 3). In contrast, over 6 months of drug treatment, there were no significant differences in the peak SBP response on a GEST among men on Atorvastatin (p=0.31) or placebo (p=0.30) (Table 3).

Resting Blood Pressure and Peak Systolic Blood Pressure Correlations

Over 6 months of drug treatment, there were no significant correlations among the change in resting SBP, DBP, or MAP and peak SBP response to a GEST among the total sample or by drug treatment group (p>0.05) (Table 4).

Lipoprotein Profile Changes

Lipid Lipoprotein Profile

Over 6 months of drug treatment, LDL cholesterol, total cholesterol, and triglyceride levels decreased among the total sample and the Atorvastatin (p=0.000) group but not the placebo group (p>0.05) (Table 5). Over 6 months of drug treatment, triglyceride levels decreased more among men than women among the total sample (p=0.002) (Table 5), Atorvastatin group (p=0.03), and placebo group (p=0.03) (Table 5). Over 6 months of drug treatment, LDL cholesterol, total cholesterol, and triglyceride levels decreased more among the Atorvastatin than placebo group among men (p<0.001) (Table 5). Over 6 months of drug treatment, LDL cholesterol, total cholesterol, and triglyceride levels decreased more among the Atorvastatin than placebo among women (p<0.001) (Table 5).

Lipid Lipoprotein Profile and Blood Pressure Correlations

Over 6 months of drug treatment, there were no significant correlations between the change in LDL cholesterol, total cholesterol, HDL cholesterol over 6 months and

changes in resting SBP, DBP, MAP, and the peak SBP response to a GEST over 6 months among the total sample or by drug group ($p>0.05$) (Table 6). Over 6 months of drug treatment, there was a weak, but significant correlation between the change in resting DBP and triglycerides ($r=0.154$, $p=0.03$) among the Atorvastatin group (Table 6). Over 6 months of drug treatment, there was a weak, but significant correlation between the change in resting MAP and triglycerides ($r=-0.145$, $p=0.03$) among the placebo group (Table 6).

Table 1. Subjects characteristics (Mean±SEM) among the total sample and by Atorvastatin and Placebo groups by gender

Variable	*Total Sample (N=419)*	*Atorvastatin (N=202)*		*Placebo (N=217)*	
		Men (n=99)	*Women (n=103)*	*Men (n=104)*	*Women (n=113)*
Age (yr)	44.1 ± 0.8	43.5 ± 1.6	43.3 ± 1.6	43.6 ± 1.5	45.6 ± 1.6
BMI ($kg \cdot m^{-2}$)	26.4 ± 0.2	27.4 ± 0.4	25.2 ± 0.5**	27.4 ± 0.4	25.6 ± 0.5*
LDL ($mg \cdot dL^{-1}$)	117.4 ± 1.6	121.3 ± 3.1	116.6 ± 3.9	118.6 ± 2.8	113.5 ± 3.2
Total Cholesterol ($mg \cdot dL^{-1}$)	196.6 ± 1.9	196.5 ± 3.3	200.7 ± 4.4	191.6 ± 3.2	197.7 ± 3.8
HDL ($mg \cdot dL^{-1}$)	58.1 ± 0.8	49.9 ± 1.4	65.0 ± 1.6***	50.6 ± 1.1	66.0 ± 1.7***
Triglycerides ($mg \cdot dL^{-1}$)	106.6 ± 2.7	126.0 ± 6.3	95.6 ± 4.1***	112.3 ± 6.2	94.6 ± 4.3*
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	33.9 ± 0.5	38.6 ± 0.9	30.9 ± 0.9***	37.8 ± 0.9	29.0 ± 0.8***
Energy Expenditure ($kcal \cdot day^{-1}$)	624.9 ± 13.7 *(n=404)*	689.1 ± 25.6 *(n=96)*	541.2 ± 21.4*** *(n=99)*	754.5 ± 33.3 *(n=101)*	523.4 ± 20.5*** *(n=108)*
Blood Pressure Medication Use	*n=19*	*n=3*	*n=5*	*n=7*	*n=4*

BMI, Body Mass Index; HDL, High Density Lipoprotein; LDL, Low-Density Lipoprotein; HR, Heart Rate; VO_{2max}, Maximal Oxygen Consumption; TEE, Total Energy Expenditure
*p<0.05, **p<0.001, ***p<0.001 Men vs. Women

Table 2. Average adjusted resting blood pressure change (Mean±SEM) after 6 months of 80mg Atorvastatin or Placebo among total sample and by drug and gender.

Resting Blood Pressure (mmHg)		Total Sample		Atorvastatin		Placebo	
		Pre	Δ	Pre	Δ	Pre	Δ
Total		*n=419*		*n=202*		*n=217*	
	SBP	118.9 ± 0.6	0.5 ± 0.5	115.3 ± 0.9	0.8 ± 0.7	114.7 ± 0.8	0.3 ± 0.6
	DBP	75.3 ± 0.5	0.3 ± 0.5	75.5 ± 0.6	0.4 ± 0.7	75.2 ± 0.6	0.5 ± 0.6
	MAP	87.7 ± 0.7	0.2 ± 0.8	88.5 ± 0.8	0.0 ± 0.8	86.8 ± 1.1	0.5 ± 1.3
Men		*n=203*		*n=99*		*n=104*	
	SBP	117.2 ± 0.8	1.2 ± 0.7	117.7 ± 1.2	2.7 ± 1.0	116.6 ± 1.1	-0.2 ± 1.0 *Φ*
	DBP	76.5 ± 0.6	1.0 ± 0.7	75.0 ± 0.9	2.0 ± 1.0	77.0 ± 0.9	0.2 ± 0.9
	MAP	89.3 ± 1.0	1.2 ± 1.0	90.1 ± 0.9	0.9 ± 1.2	87.7 ± 1.6	1.7 ± 1.8
Women		*n=216*		*n=102*		*n=113*	
	SBP	113.0 ± 0.8 ††	-0.1 ± 0.7	113.0 ± 1.2 †	-1.0 ± 1.1 †	113.0 ± 1.2 †	0.8 ± 0.8
	DBP	74.3 ± 0.6 †	-0.4 ± 0.7	76.0 ± 0.9	-1.2 ± 0.9 †	73.4 ± 0.9 ††, *Φ*	0.8 ± 0.9
	MAP	86.1 ± 0.9 †	-0.7 ± 1.2	86.9 ± 0.9 †	-0.8 ± 1.2	85.9 ± 1.5	-0.7 ± 1.8

Adjusted for age, baseline BMI, baseline VO_{2max} and gender.
Pre: baseline, Δ: Post 6 months, SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MAP, Mean Arterial Pressure
† p<0.05, †† p<0.001 Men vs. Women,
Φ p<0.05, Atorvastatin vs. Placebo

Table 3. Average adjusted peak systolic blood pressure (Mean±SEM) to a GEST after 6 months of 80mg Atorvastatin or Placebo among total sample and by drug and gender.

True Max Peak SBP (mmHg)	Total Sample		Atorvastatin		Placebo	
	Pre	Δ	Pre	Δ	Pre	Δ
	n=196		*n=98*		*n=98*	
Total	169.6 ± 1.1	0.2 ± 1.1	169.0 ± 1.6	-0.7 ± 1.6	169.9 ± 1.7	0.6 ± 1.4
	n=105		*n=49*		*n=56*	
Men	174.9 ± 1.9	0.7 ± 1.5	175.4 ± 2.6	2.5 ± 2.4	177.7 ± 2.3	-0.9 ± 1.8
	n=91		*n=49*		*n=42*	
Women	164.5 ± 2.0 †	-0.9 ± 1.6	162.6 ±2.6 †	-3.9 ± 2.2* †	162.1 ± 2.8 ††	2.6 ± 2.2 *Φ*

Adjusted for age, baseline BMI, baseline VO_{2max} and gender.
Pre: baseline, Δ: Post 6 months, SBP, Systolic Blood Pressure; GEST, Graded Exercise Test
*p<0.05, Δ Pre to Post
† p<0.05, †† p<0.001, Men vs. Women
Φ p<0.05, Atorvastatin vs. Placebo

Table 4. Pearson correlations among the absolute change (Δ) in resting blood pressure and absolute change (Δ) in the peak systolic blood pressure response to a GEST after 6 months of Atorvastatin or placebo.

Blood pressure (mmHg)	Total Sample (n=192)		Atorvastatin (n=94)		Placebo (n=98)	
	r	p	*r*	p	*r*	p
ΔResting SBP (mmHg)						
ΔTrue Max Peak SBP (mmHg)	-0.087	0.23	-0.036	0.73	-0.143	0.16
ΔResting DBP (mmHg)						
ΔTrue Max Peak SBP (mmHg)	-0.034	0.64	-0.070	0.50	0.023	0.82
ΔResting MAP (mmHg)						
ΔTrue Max Peak SBP (mmHg)	0.094	0.19	0.156	0.12	0.032	0.76

SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean artieral pressure.
*p<0.05 Pearson Correlation

Table 5. Average adjusted lipid lipoprotein profile change (Mean±SEM) after 6 months of 80mg Atorvastatin or placebo among total sample and by drug and gender.

Lipoprotein Measure ($mg \cdot dL^{-1}$)	Total Sample		Atorvastatin		Placebo	
	Pre	Δ	Pre	Δ	Pre	Δ
Total	*n=419*		*n=202*		*n=217*	
LDL	117.7 ± 1.5	-27.9 ± 2.0**	118.9 ± 2.3	-58.9 ± 2.0**	116.0 ± 1.9	0.9 ± 1.4 *Φ*
TC	196.7 ± 1.7	-30.1 ± 2.1**	198.6 ± 2.5	-65.3 ± 2.2**	194.8 ± 2.2	2.7 ± 1.5 *Φ*
HDL	57.9 ± 0.7	-0.21 ± 0.4	57.6 ± 1.1	-0.8 ± 0.6	58.6 ± 1.1	0.3 ± 0.6
Trigs	107.3 ± 2.5	-11.8 ± 2.4**	110.5 ± 3.7	-28.3 ± 3.1**	103.1 ± 3.6	3.5 ± 3.4 *Φ*
Men	*n=203*		*n=99*		*n=104*	
LDL	120.0 ± 2.0	-29.0 ± 1.7**	121.3 ± 3.0	-59.7 ± 2.8**	118.6 ± 2.6	1.7 ± 1.8 *Φ*
TC	194.0 ± 2.1	-32.2 ± 1.9**	196.5 ± 3.1	-66.7 ± 3.1**	191.6 ± 3.0	2.3 ± 2.0 *Φ*
HDL	50.3 ± 0.8	0.44 ± 0.6	49.9 ± 1.3	0.5 ± 0.8	50.6 ± 1.0	0.8 ± -0.3
Trigs	119.1 ± 4.2	-20.3 ± 3.4**	126.0 ± 6.0	-35.0 ± 5.3**	112.3 ± 5.9 Φ	-5.6 ± 4.2 *Φ*
Women	*n=216*		*n=102*		*n=113*	
LDL	115.1 ± 2.2	-29.0 ± 1.7**	116.6 ± 3.6	-58.2 ± 2.8**	113.5 ± 2.7 †	0.2 ± 2.0 *Φ*
TC	199.2 ± 2.6 †	-30.5 ± 1.9**	200.7 ± 4.0	-64.0 ± 3.0**	197.7 ± 3.3 †	3.0 ± 2.1 *Φ*
HDL	65.5 ± 1.1 ††	-0.9 ± 0.6	65.0 ± 1.4	-1.6 ± 1.0	66.0 ± 1.7 ††	-0.9 ± 0.8
Trigs	95.2 ± 2.9 ††	-5.0 ± 3.1 ††	95.6 ± 4.0 †	-21.8 ± 3.2**, †	94.6 ± 4.1	11.8 ± 5.0*, *Φ*, †

Adjusted for age, baseline BMI, baseline VO_{2max} and gender.
Pre: baseline, Δ: Post 6 months, LDL, Low-Density Lipoprotein; TC, total cholesterol HDL, High Density Lipoprotein; Trigs, Trigylcerides
*p<0.001 Pre vs. Post
† p<0.05, †† p<0.001 Men vs. Women,
Φ p<0.001 Atorvastatin vs. Placebo

Table 6. Pearson correlations among the absolute change (Δ) in the lipid-lipoprotein profile and resting blood pressure and the peak systolic blood pressure response to a GEST after 6 months of Atorvastatin or placebo.

Variable	Total Sample (n=419)		Atorvastatin (n=202)		Placebo (n=217)	
	r	p	*r*	p	*r*	p
ΔLDL Cholesterol (mg·dL^{-1})						
ΔResting SBP (mmHg)	-0.029	0.56	0.004	0.95	-0.051	0.45
ΔResting DBP (mmHg)	0.001	0.99	0.094	0.19	-0.086	0.21
ΔResting MAP (mmHg)	0.035	0.47	-0.008	0.91	0.088	0.19
ΔTrue Max Peak SBP (mmHg)	0.007	0.92	-0.131	0.20	0.136	0.18
ΔTotal Cholesterol (mg·dL^{-1})						
ΔResting SBP (mmHg)	-0.024	0.62	0.016	0.83	-0.051	0.45
ΔResting DBP (mmHg)	0.010	0.83	0.116	0.11	-0.083	0.22
ΔResting MAP (mmHg)	0.011	0.83	0.020	0.78	-0.018	0.79
ΔTrue Max Peak SBP (mmHg)	0.027	0.71	-0.111	0.28	0.183	0.07
ΔHDL Cholesterol (mg·dL^{-1})						
ΔResting SBP (mmHg)	0.056	0.26	0.077	0.23	0.038	0.58
ΔResting DBP (mmHg)	0.018	0.71	-0.026	0.72	0.074	0.27
ΔResting MAP (mmHg)	-0.022	0.65	0.057	0.42	-0.076	0.27
ΔTrue Max Peak SBP (mmHg)	0.065	0.36	0.125	0.22	-0.021	0.84
ΔTriglycerides Cholesterol (mg·dL^{-1})						
ΔResting SBP (mmHg)	-0.011	0.82	-0.033	0.65	0.022	0.75
ΔResting DBP (mmHg)	0.038	0.44	**0.154**	**0.03***	-0.054	0.43
ΔResting MAP (mmHg)	-0.065	0.18	0.060	0.40	**-0.145**	**0.03***
ΔTrue Max Peak SBP (mmHg)	0.043	0.55	-0.070	0.49	-0.140	0.17

LDL; low-density lipoprotein, HDL; high-density lipoprotein, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean artieral pressure.
*p<0.05 Pearson Correlation

Discussion

Sub-Study Overview

The main purposes of this sub-study were to examine the influence of statins on resting BP and the peak SBP response to a GEST before and after 6 months of Atorvastatin or placebo drug therapy. We found no significant changes in resting SBP, DBP, or MAP, regardless of drug group or gender among the total sample. Among the total sample there were 19 individuals who were taking antihypertensive medications during the study, however, BP medication use was a nonsignificant covariate in the analysis, by gender and by drug group ($p>0.05$). However, there was a SBP difference of 3.7 ± 1.5 mmHg and DBP difference of 3.2 ± 1.3 mmHg between men and women taking Atorvastatin that favored women. We found no significant changes in the peak SBP response to a GEST, regardless of drug group among the total sample or men. However, women on Atorvastatin reduced the peak SBP response to a GEST after 6 months 6.5 ± 3.1 mmHg more than women on placebo. Also, there was a peak SBP response to a GEST over 6 month difference of 6.4 ± 3.3 mmHg between men and women taking Atorvastatin that favored women. In summary, women experienced greatere reductions in resting SBP (~4 mmHg), DBP (~3 mmHg), and the peak SBP response to a GEST (~7 mmHg) on Atorvastatin compared to men on Atorvastatin. These findings reveal the effects of Atorvastatin on BP were gender specific and affected women more favorably than men.

Statins and Gender Effects

There is limited literature that has examined the gender effects of the BP lowering effects of statins. However, the influence of gender of the effects of statins on

cardiovascular event outcomes has been studied. Petretta et al. (32) examined eight randomized controlled trials of patients with unknown cardiovascular disease. Each study included the effect of lipid-lowering drug therapy for at least 1 clinical outcome (i.e, total mortality, coronary heart disease mortality, nonfatal myocardial infarction, coronary heart disease events) and included women (32). There were ~20,000 women compared to ~30,000 men who were treated with statins for 2-5 years. The participants had mild or moderate hyperlipidemia and were treated with Lovastatin, Pravastatin, Atorvastatin or Simvastatin compared to placebo (32). Dose amount of statin was not reported. Statin therapy reduced the risk of coronary heart disease in men ($r=0.59$, $p<0.001$) but not in women. Women treated with statins free of cardiovascular disease were found to have a reduction in coronary heart disease events but the reduction did not reach statistical significance (0.86, $p=0.0557$).

These results refute the findings of the sub-study because men on Atorvastatin did not experience favorable affects of statins (reductions in BP) compared to women on Atorvastatin. Petretta et al. (32) did report that the length of follow-up and treatment may not have been long enough for results to manifest themselves in women and that the lower number of women included may have affected the results. Another influencing factor may have been the use of statins as primary treatment vs. non-primary treatment. During the analysis of women and coronary heart disease events, two of the six studies were not considered "primary treatment" studies of statins. When these two studies were excluded from the analysis, the statistical significant protection of statins disappeared, and even more so when excluding only one study. The summary RR was strongly influenced by statins being used as primary treatment (32).

Similar to Petretta et al. (32), Dale et al. (33) meta-analyzed the influence of gender on statin efficacy in reducing cardiovascular events (33). The 14 included studies compared statin treatment to a control or placebo group. The analysis included ~62,166 men compared to ~18,000 women. The statins were: Lovastatin (20-80mg), Simvastatin (20-40mg), Pravastatin (40mg), Atorvastatin (10-80mg), or Fluvastatin (80mg). To be included each study had a follow up period of ≥48 weeks, ≥100 enrolled patients, and gender specific data on death or incidence of cardiovascular events, myocardial infarction and stroke (33). Cardiovascular events were reduced in individuals on statin therapy among men (RR 0.76) and women (RR 0.79) compared to the control groups. The results of this study support the findings of the sub-study because women did have a reduction in cardiovascular events when using statins. In the sub-study, women on Atorvastatin experienced greater reductions in resting BP and the peak SBP response to a GEST compared to their male counterparts on Atorvastatin. Despite seeing a significant risk reduction among women taking statins, only 25% of the evaluable data and cardiovascular events were accounted for by women. The reduction in cardiovascular events among men taking statins appeared to be driven by reduction in myocardial infarction, death, and stroke. Women taking statins did not have a reduction in death or stroke, but had reductions in unstable angina and need for revascularization (33). Even with an 11% reduction in myocardial infarction, the lack of female population to evaluate this outcome may explain the lack of statistical significant among women (33).

Petretta et al. (32) and Dale et al. (33) both demonstrate that statins do reduce cardiovascular events, especially among men. However, these results do not support the findings of the current study. Both studies reported that women were poorly represented

among the study populations and this may have affected results. In the sub-study, women were equally represented in the study population compared to men. Dale et al. (33) included two studies that examined only men, leaving no data available of women to be examined. Unlike the meta-analyses, women were able to be equally analyzed compared to men, both overall and by drug group. Women on Atorvastatin experienced a favorable reduction in BP compared to men taking Atorvastatin (~4 mmHg). Although, the sub-study did not examine cardiovascular disease outcomes, it did focus on one major cardiovascular component: BP.

Reduction of BP leads to reduction in cardiovascular complications that are related to elevated BP levels, including the development of HTN (3). This affect was more favorable among women taking Atorvastatin than men taking Atorvastatin, which differs from the two meta-analyses (32, 33). It also should be noted that the meta-analyses examined outcome data and did not explore the physiological effects that may have influenced the results of their studies. However, there is limited literature of gender effects of statins and reductions in BP. Studies that have displayed favorable reductions in cardiovascular events when statins are used demonstrate that statins can improve cardiovascular health. No literature has examined the effect of statins on the peak SBP response to a GEST or the gender effects of statins on the peak SBP response to a GEST.

Proposed Mechanisms for Influence of Statins on Resting Blood Pressure

This sub-study has refuted previous research finding men benefited more from statin use than women in regards of reducing cardiovascular events. Among previous literature in which men showed more favorable affects, the physiological mechanisms that would promote those findings were not discussed. In addition, the physiological

mechanisms involved in the pleiotropic effects of statins have yet to be determined. Milinois et al. (6) meta-analyzed 17 studies in which statins were used and of those 17 included studies, 12 reported favorable BP reductions among resting BP measures. In additional to examining the BP effects of statins, Milinois et al. (6) also explored possible mechanistic pathways. In the 12 studies that reported a favorable reduction in BP, all reported no correlation between the LDL cholesterol change and reduction (change) in BP. Milinois et al. (6) concluded a non-lipid lowering mechanism is responsible for the reductions in BP and may explain the cardioprotective properties of statins (6). In this sub-study, no significant correlations were observed between the change in LDL-cholesterol, resting SBP, DBP and MAP, or the peak SBP response to a GEST over 6 months of Atorvastatin treatment (Table 6) suggesting the mechanisms for reducing BP are non-lipid lowering.

Currently, there are no established mechanisms to explain the effect of statins and reducing BP. However, several mechanisms have been proposed, mostly involving the renin-angiotensin system. Researchers have proposed that statins blockades the AT1 receptor as one possible mechanism (20, 21). Blockade of the AT1 receptor would result in more favorable affects on BP, such as increasing vasodilation and increases nitric oxide (NO) to decrease BP (20,21). Another proposed mechanism is statins increase bioavailability of NO due to inhibition of cholesterol synthesis (22, 30). Increased bioavailability of NO can lead to improved endothelial function, which can result in reductions in BP (22, 30). In order to explain the gender effects of statins, it has been proposed that the presence of estrogen enhances the effect of statin (21, 23).

Estrogen stimulates angiotensinogen production that causes unfavorable effects on BP, such as vasoconstriction which can increase BP. Estrogen is the primary sex hormone among women but can also be found in men (19). However, higher levels of estrogen produce more angiotensinogen (23). This explains the higher circulating levels of angiotensinogen in women as compared to men as it relates to higher levels of estrogen in women (23). However, estrogen can also promote favorable effects on BP (i.e. vasodilation) by blocking the AT1 receptor (23). These favorable effects are enhanced when statins are used (23). When estrogen is present and statins are used to block the AT1 receptor, NO availability increases by activation of the AT2 receptor (20, 21). When NO availability is increased, it influences endothelial function which may result in favorable reductions in BP (22). This mechanism is supported by Feron et al. (22), who found that Atorvastatin reduced the amounts of eNOS that were available to bind to caveolin, which resulted in a significant increase in NO production. In the sub-study, women experienced more favorable effects on BP when taking Atorvastatin compared to men. Although speculative, assuming estrogen levels were higher among women than men, the combination of estrogen and taking Atorvastatin may be an explanation for why the gender effects of the BP reductions favored women.

In summary, in agreement with Milinois et al (6), NO does appear to be a possible key component in the influence of statins on BP. However, estrogen may be a key mediator in the gender differences found when examining the influence of statins on BP. Estrogen and statins are both considered downregulaters of the AT1 receptor, which in turn promote activation of the AT2 receptor. By activating the AT2 receptor, NO

availability can increase endothelial function, allowing for better relaxation of the endothelial and elicit favorable BP effects (22).

Proposed Mechanisms for Influence of Statins on the Blood Pressure Response to Exercise

Previous literature has only examined the influence of statins on resting BP but not the peak SBP response to a GEST. Individuals with a higher resting BP are likely to have a greater BP response to exercise than those with a normal resting BP (25). In the sub- study, resting BP was in the normal range (2) and the average peak SBP achieved was below levels of a hypertensive response to exercise among the total sample (169.6 ± 1.1 mmHg) (4). There were no significant correlations found between change in resting BP and the change in peak SBP response to a GEST, suggesting that the mechanisms that cause reductions in resting BP are not the same that cause reductions in the peak SBP response to a GEST (Table 4). However, exercise may affect the endothelium by increasing the amount of NO produced that results (38) but may impair shear stress-induced NO release causing decreased vasodilation (38). The production of NO may be greater in women because of the affect that statins in the presence of estrogen have on NO availability (23). If statins and estrogen block the AT1 receptor and promote NO availability at rest before exercise, and exercise increases NO availability, it is possible that these mechanisms could result in a lower peak SBP response to a GEST. However, this mechanism is purely speculative and needs to be explored further.

Limitations and Strengths

There are several limitations to the sub- study. Firstly, it was a sub-study of the larger STOMP study (4). The original objectives of the STOMP study did not include a

resting BP or the peak SBP response to a GEST as major outcomes. The data for this sub study were collected at multiple test sites which may have resulted in interpretation bias during data collection and entry. Lastly, the subjects for this sub-study were selected for the STOMP study and had to pass a very stringent inclusion/exclusion criteria. Therefore, the sample used in the sub-study was a self-selected sample. The analysis of this sub-study could not be analyzed with intention to treat because subjects that reported myalgia to research personnel did not have fully complete data to be included.

Despite the limitations of this sub-study, there were also multiple strengths. The subjects recruited for the STOMP study had an equal number of healthy men and women, with no disease or major medical conditions. The research personnel were all highly trained individuals from the field of cardiovascular exercise physiology. The STOMP study conducted weekly meetings to ensure all documentation, database usage, equipment and study protocols were uniform among all sites to ensure testing and data entry integrity.

Conclusion

In conclusion, there were no significant differences of resting BP or the peak SBP response to a GEST among the total sample, regardless of drug group. However, there were gender dependent effects. Women assigned to Atorvastatin experienced a reduction in resting SBP (~4 mmHg), DBP (~3 mmHg) and the peak SBP (~7 mmHg) response to a GEST compared to men assigned to Atorvastatin over 6 months. Although speculative at this time, there is evidence to suggest that estrogen may have been the key mediating factor for the gender effects in reducing BP among women taking Atorvastatin. This speculative mechanism not only involves estrogen and Atorvastatin but also components

of the renin angiotensin system. The ability of Atorvastatin to block the AT1 receptor, resulting in activation of the AT2 receptor and promoting favorable affects of BP. An emphasis on Atorvastati n and estrogen increasing NO production and bioavailability are important contributors to the mechanistic pathway that resulted in more favorable affects on resting BP and the peak SBP response to a GEST among women taking Atorvastatin. Further research should be considered to confirm this mechanistic pathway.

Future Research

Future research should include the BP response to statin use as a primary outcome. Future studies should include a double-blind randomized controlled trial with equal representation of men and women. Blood pressure measurements should be meticulously measured both at resting and during a peak GEST. The markers for determining the mechanistic pathway should be measured and are, but not limited to, estrogen, NO or eNOS, angiontensinogen, and endothelial function. All treatment (statin or placebo) should be monitored for any symptoms related to the drug therapy.

Clinical Significance

Clinically, statins have already been established as the most effective lipid-lowering medications. It has been suggested that statins may reduce BP and the results of this sub-study suggest statins may possess certain gender dependent affects on reducing BP. Further investigation is needed with a randomized controlled trial intentionally designed to determine the effectiveness of statins to lower BP among men and women with hypertension to confirm the gender dependent effects we observed.

Appendix A

Subject Code: __ __ - __ __ __ Subject Initials: ___ ___ ___

MAXIMAL CARDIORESPIRATORY FITNESS TEST (BALKE PROTOCOL)

Time of Last Meal: ___ ___ : ___ ___ (24 hr clock) # HRS FASTING: _____ (must be 8-12 hours)

Weight: _____.___ ÷ 2.2 = _____.___ lbs kg

Age-predicted HR_{max} _____ bpm 85% HR_{max} _____ bpm

Stage	Speed (mph)	Grade (%)	Stage Duration (min)	TM Clock Time	HR (bpm)	BP (mmHg)	RPE	Comments
SEATED REST (GAS COLLECTION)			5					
0	STANDING							
Warm-Up	2.5	0	2	0-2				
1		0	1	2-3				
2		2.0	1	3-4				
3		3.0	1	4-5				
4		4.0	1	5-6				
5		5.0	1	6-7				
6		6.0	1	7-8				
7		7.0	1	8-9				
8		8.0	1	9-10				
9		9.0	1	10-11				
10		10.0	1	11-12				
11		11.0	1	12-13				
12		12.0	1	13-14				
13		13.0	1	14-15				
14		14.0	1	15-16				
15		15.0	1	16-17				
16		15.0	1	17-18				
Cool Down	2.5	0	3					ACTIVE RECOVERY
Cool Down	2.0	0	3					(GAS COLLECTION)
Cool Down	SEATED		3					

Final Treadmill Time ___ ___ : ___ ___ minutes HR_{max} _____ bpm $\%HR_{max}$ _____ %

VO_{2max} _____ ml/kg/min VO_{2max} _____ L/min

Staff Initials: ___ ___ ___

Date: __ __ / __ __ __ / __ __ __ __ (DD MMM YYYY)

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