Angiotensin II Receptor Blockade and Insulin Sensitivity in Overweight and Obese Adults with Elevated Blood Pressure

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ABSTRACT

Currently, it is reported that ~65% and 34% of the U.S. population is overweight and obese, respectively. Obesity is a major risk factor for cardiovascular disease. Overweight and obese individuals are also at an increased risk of developing hypertension. Whole-body insulin sensitivity is reduced in obesity, resulting in insulin resistance and increased risk of type 2 diabetes. One possible mechanism contributing to insulin resistance in obesity hypertension is renin-angiotensin system (RAS) overactivation. The RAS exhibits vasocontricting and sodium-retaining properties, yet *in vivo* and *in vitro* animal experiments suggest impairment of whole-body insulin sensitivity with increased angiotensin II (Ang II) exposure. Furthermore, evidence from clinical studies indicates Ang II receptor blockers (ARBs) may reduce the incidence of new-onset diabetes compared to other antihypertensive agents in at-risk hypertensive patients. However, it is unclear if whole-body insulin sensitivity is improved with Ang II receptor blockade in humans. Thus, we tested the hypothesis that 8-week Ang II receptor blockade with olmesartan would improve whole-body insulin sensitivity in overweight and obese individuals with elevated blood pressure (BP). Olmesartan was selected for the present study because it is devoid of partial PPARγ agonist activity. To test our hypothesis, intravenous glucose tolerance tests were performed to measure insulin sensitivity before and after control and ARB treatment in a randomized crossover manner. Because skeletal muscle tissue accounts for ~75-90% of insulin-stimulated glucose uptake, a secondary exploratory aim was to examine skeletal muscle

inflammatory and collagen response in relation to insulin sensitivity during ARB treatment. No baseline differences were observed between treatments (P>0.05). Both systolic (-11.7 mmHg; P=0.008) and diastolic (-12.1 mmHg; P=0.000) BP were reduced with ARB treatment. Insulin sensitivity was not different between treatments (P>0.05). No correlates of insulin sensitivity were identified. In addition, skeletal muscle inflammatory and collagen gene expression did not change from pre- to post-ARB treatment (P>0.05). Our findings suggest that short-term RAS blockade in overweight and obese adults with elevated BP does not improve whole-body insulin sensitivity, despite a significant BP reduction. Further studies are needed to clarify the role of individual RAS blockers on insulin sensitivity during RAS inhibition in obesity hypertension.

DEDICATION

My husband, Andrew, deserves recognition for his patience, motivational speeches, and endless support and love during my graduate training. He understood my desire to earn an advanced degree, moved to Blacksburg with me, and never let me forget or forgo my career goals. Therefore, I dedicate my doctoral degree to Andrew, as I would not have done it without him.

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CHAPTER I

Introduction

Obesity rates have climbed at unprecedented rates over the past 3 decades. Overweight and obese individuals (BMI> 25 kg/m^2) currently account for 68% of the U.S. adult population.[1] Obesity is now a worldwide problem, with rising prevalence observed in other developed and underdeveloped countries. Obesity and its comorbidities greatly increase the risk of cardiovascular disease (CVD), type 2 diabetes, and cancers, among other chronic conditions.[1] Obesity and the corresponding rise in cardiovascular morbidity and mortality have placed an immense burden on the global public health system and economy. Obesity-related health care costs continue to rise[2], while effective prevention strategies have not been generally established. Thus, it is necessary to further develop treatment strategies to relieve the health and economic consequences of obesity and its associated complications.

Hypertension is a cardiovascular risk factor strongly associated with obesity.[3, 4] Overweight and obesity contribute to ~60-70% of hypertensive cases.[5] The rising prevalence of essential hypertension echoes the surge in obesity rates.[6] A proposed mechanism by which obesity leads to hypertension is activation of the renin-angiotensin system (RAS). The classic role of the RAS is maintenance of total peripheral resistance via vasoconstriction and sodium excretion. However, chronic elevation in angiotensin II (Ang II) elicits detrimental effects on systemic and local environments. Endogenous and infused Ang II regulates glucose metabolism, insulin and endothelial secretion, cell proliferation and apoptosis, and fibrosis via activation of Ang II type 1 receptors.[7-14] Moreover, Ang II appears to augment inflammatory and oxidative stress response in adipose and skeletal muscle tissue, resulting in impaired insulin sensitivity and signaling *in vivo* and *in intro*.[7, 12, 15-20] Overall, chronic overactivation of the RAS may play a role

in the etiology of pathophysiological conditions, including type 2 diabetes and CVD. Therefore, the RAS is an important target of antihypertensive therapy in obese hypertensives to impede disease progression.

RAS inhibitors, such as angiotensin converting enzyme inhibitors and Ang II receptor blockers (ARBs), interrupt RAS activity and are commonly used to lower blood pressure. However, recent studies suggest that the benefit of RAS inhibition may extend beyond its blood pressure-lowering abilities. Over the last decade, multiple large-scale clinical trials indicate a reduction in CVD morbidity and mortality with long-term use of RAS antagonists compared to other antihypertensive medications or placebo in at-risk hypertensive patients.[21-25] Post-hoc analysis of the data also reveals significant reductions in new-onset diabetes in the non-diabetic populations.[26-29] Recent prospective studies in patients with hypertension and/or CVD risk factors provide additional support for reduced incidence of new-onset diabetes during RAS blockade.[30, 31] However, it is unknown if the reduction in new-onset diabetes is related to direct beneficial actions of RAS inhibition or detrimental effects of comparator drugs.[29, 32] Accordingly, the primary mechanisms responsible for the additional benefits of RAS blockade have not been clarified in humans and further investigation is required.

As such, we tested the hypothesis that RAS antagonism improves whole-body insulin sensitivity in humans. We selected olmesartan as the ARB for the present study because it is devoid of peroxisome proliferator-activated receptor gamma (PPARγ) agonist activity[33] and PPARγ agonist increased insulin-stimulated glucose disposal.[34] Our overweight and obese subjects had elevated blood pressure and were treated with olmesartan or no drug for 8 weeks each in a randomized crossover fashion. Changes in insulin sensitivity were assessed by an intravenous glucose tolerance test before and after each treatment period. In addition, insulin

resistance is associated with alterations in the extracellular matrix and inflammatory milieu in skeletal muscle. Therefore, we also sought to determine if changes in skeletal muscle inflammation and collagen were related to changes in insulin sensitivity in our subjects.

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CHAPTER II

Review of Literature

Obesity: An Expanding Public Health Problem.

Obesity is the leading health risk factor for cardiovascular disease (CVD), type 2 diabetes and some cancers, among other chronic conditions.[1] These disease states dramatically increase the risk of cardiovascular morbidity and mortality, individually and combined.[2] Within the last decade, obesity has reached epidemic proportions in industrialized as well as developing countries.[1-3] As recently reported, 68% of U.S. adults are overweight (25.0 $kg/m^2 \leq BMI < 30.0$ kg/m^2) and 33.8% of adults are obese ($BMI \geq 30.0$ kg/m^2).[1]

Obesity has placed a significant burden on the U.S. economy and health care system. Obesity accounted for 9.1% of total U.S. medical expenses in 1998[4] and the medical costs of obesity have been estimated to be as much as $147 billion per year.[5] There seems to be no relief in sight as trends predict that by year 2030, ~90% of the U.S. population and more than 3 billion people worldwide will be overweight or obese[6, 7] and related health care costs will surge over 860 billion dollars.[7]

Obesity-related conditions contribute to productivity loss with an average of 9.2-11.4 lost work days per year and obese workers miss more long-term consecutive workdays (>2-week period) than normal weight coworkers.[8] The financial consequence of obesity cannot be ignored, although the impact on public health status may be a greater priority. Obese individuals have a reduced life expectancy and quality of life[9, 10] as well as a greater risk of diverse chronic diseases, particularly CVD, which is currently one of the leading causes of death in the U.S.[11, 12] Determining appropriate treatment for overweight and obesity, along with their corresponding

conditions, is of utmost importance to slow and potentially reverse the incidence of overweight and obesity and their dire economic and health-related consequences.

Obesity and Hypertension.

The relationship between obesity and hypertension has been repeatedly confirmed in experimental, clinical and epidemiological studies.[13, 14] Collectively, these studies show that blood pressure (BP) rises with weight gain, the development of hypertension is significantly predicted by excess weight gain, and BP reductions occur with weight loss in most hypertensive individuals.[15-20] As with obesity, the prevalence of essential hypertension (≥140/90 mmHg) in adults has also risen steadily over the last decade to 29% of U.S. adults[21], which may be a slight underestimation as ~8% of the population may have undiagnosed hypertension.[12] An examination of the National Health and Nutrition Examination Survey (NHANES) 1999-2004 and NHANES III provided data demonstrating a linear relationship between rising body mass index (BMI) levels and systolic, diastolic, and pulse pressures in American adults.[22] Other studies support this observation, though there appears to be a slight dissociation in the hypertension and obesity relationship when extended to various racial and ethnic groups.[23-25] Nevertheless, most hypertensive individuals are overweight according to the Framingham Heart Study, which reported obesity itself possibly contributed to 70% and 61% of essential hypertension cases in men and women, respectively.[26] Moreover, obesity may not need to be long-term to induce hypertension since children and adolescents have recently mirrored the upward swing in excess weight gain and elevated BP prevalence as seen in adults.[27, 28] Therefore, obesity hypertension should be considered as a significant form of essential hypertension and investigated thoroughly to determine health-related consequences contributing to increased

cardiovascular morbidity and mortality. Importantly, various mechanisms of structural (e.g., renal dysfunction or compression, altered vascular structure and function), hormonal (e.g., hyperinsulinemia, hyperleptinemia), neurochemical (e.g., enhanced sympathetic nervous system (SNS) activity), and neurohormonal (e.g., renin-angiotensin system (RAS) activity) nature have been postulated to contribute to obesity hypertension.[18, 19, 29, 30] The latter will be the focus of this review.

The Renin-Angiotensin System and Obesity Hypertension.

i. The RAS Cascade. The primary role of the RAS is to regulate arterial pressure and sodium excretion. Classic RAS pathway signaling involves intrarenal baroreceptor detection of declining arterial perfusion pressure, alteration in the delivery of sodium chloride to the macula densa cells, and during times of stress or trauma, increased SNS activity on the arterioles of the juxtaglomerular apparatus. These physiological signals trigger the breakdown of prorenin to renin and its subsequent release into the renal and systemic circulations. Renin, a proteolytic enzyme, then converts circulating angiotensinogen into angiotensin I (Ang I), a mild, ineffective vasoconstrictive decapeptide. Within minutes, Ang I is acted upon by angiotensin-converting enzyme (ACE), which primarily lines the endothelial cells of the vasculature in the lungs, to form a smaller octapeptide, angiotensin II (Ang II). This short-lived, powerful pressor peptide largely induces vasoconstriction in renal and peripheral arterioles. This action is mediated by the Ang II type 1 receptor, a seven transmembrane G-protein-coupled protein distributed on a number of target organs including the kidney, heart, smooth muscle vasculature, brain, adrenal gland, and adipose tissue. This action raises total peripheral resistance to acutely increase BP. A secondary, longer-term effect of Ang II action is the reduction of sodium and water excretion by

the kidneys, thereby increasing extracellular fluid and arterial pressure over hours and days. Other systemic effects of Ang II include stimulation of thirst to expand blood volume and promotion of aldosterone secretion from the adrenal gland to increase sodium and fluid reabsorption and further raise BP. Elevated circulating Ang II concentration also inhibits the release of renin, the rate-limiting enzymatic step, via a negative feedback loop. Thus, Ang II acts to restore circulatory homeostasis.

Although these intrinsic adjustments are necessary to maintain appropriate BP, an obese state is associated with RAS activation. Chronic elevations in circulating Ang II concentrations contribute, in part, to a rightward shift in pressure-natriuresis such that a higher BP is required to maintain sodium balance, and thus, the development of hypertension. All RAS components, including plasma renin activity, plasma angiotensinogen, ACE activity and plasma Ang II levels, are increased 2- to 3-fold in obese hypertensive subjects.[31] In addition, chronic Ang II type 1 receptor activation leads to oxidative stress, inflammation, fibrosis, increased endothelial secretion, and overactivation of the SNS. Together, chronic activation of the RAS contributes to the development of cardiovascular and renal disease. Thus, the RAS is a vital target of antihypertensive therapy to interrupt or reverse the progression of cardiovascular and renal disease.

ii. RAS Blockade. Antihypertensive therapy via inhibition of the RAS has been well documented.[32-34] The primary short-term aim of RAS inhibition is to lower BP to <140/90 mmHg in hypertensive-only individuals or <130/80 mmHg in high-risk hypertensive patients, such as those with diabetes.[35] Two common forms of RAS inhibitors are ACE inhibitors (ACEIs) and Ang II receptor blockers (ARBs). Common ACEIs include captopril, ramipril, enalapril and lisinopril. ACEIs effectively inhibit RAS activity by blocking the conversion of Ang I to Ang II.

They have been studied thoroughly for efficacy and safety, demonstrating tolerability in patients. However, the most frequent severe side effects of ACEI treatment, cough and angioedema, may drive patients away from ACEIs to a different blood pressure-lowering medication or to stop treatment altogether.

ARBs, which have only been in use since the late 1990's, include losartan, valsartan, irbesartan, candesartan, telmisartan and olmesartan. ARBs induce RAS inhibition by direct blockade of the Ang II type 1 receptor. This is beneficial because all known clinical effects of Ang II are mediated by the Ang II type 1 receptor (Ang II type 1a in murine models) and ARBs competitively bind to the type 1 receptor with high affinity and selectivity and have slow dissociation.[36] ARBs further inhibit Ang II binding produced from ACE-independent pathways at these receptors. Like ACEIs, ARBs have remarkable efficacy and likely a better tolerability profile as there is an absence of cough and the side effects are reported to be no different than placebo. Consequently, ARBs may elicit more complete blockade of the RAS with minimal side effects, making them sufficient alternatives to ACEI therapy.

Other RAS inhibitors, aliskiren and remikiren, have been recently developed as direct inhibitors of the catalytic activity of renin. However, their role in hypertension, heart failure and kidney disease treatment is currently under investigation.

iii. Clinical Studies. Multiple large-scale, multi-center clinical studies have been performed to examine the impact of treatment with ACEIs and/or ARBs and other classes of antihypertensive drugs (e.g., β-blockers, calcium channel blockers, diuretics) on cardiovascular morbidity and mortality events as well as other secondary outcomes.[37-42] Most of these studies included patient populations who were middle-aged or older adults with hypertension with or without type 2 diabetes, left ventricular hypertrophy (LVH), microalbuminuria or other cardiac

conditions.[43] The majority of the clinical studies demonstrated reduced cardiovascular morbidity and mortality in the ACEI, ARB or combination treatment compared to other antihypertensive or placebo groups though all groups displayed comparable BP reductions.[43-45] Unfortunately, the studies had mean BMI measures of <30 kg/m^2.[43] Although some obese individuals may have participated, the results may not be applicable to the generalized public in regards to BP management and cardiovascular outcomes. Additional studies are needed to examine the same comparisons of RAS inhibitors to other antihypertensives or placebo in obese hypertensives.

Nevertheless, one interesting observation noted in the clinical studies was that some hypertensive subjects (non-obese or obese) may experience additional benefits with ACEI or ARB use. There is growing evidence that these antihypertensive therapies may also play a role in preventing metabolic dysfunction that accompanies obesity, such as insulin resistance and type 2 diabetes. In both the Heart Outcomes Prevention Evaluation and Studies of Left Ventricular Dysfunction projects, the relative risk for new-onset diabetes was 0.66 and 0.26, respectively, for treatment with an ACEI compared to placebo during the 3-5 year follow-up period.[40, 46] Similarly, the relative risk for new-onset diabetes was 0.75 and 0.79 in two studies evaluating long-term ARB treatment compared to other antihypertensive medications (β-blocker and calcium channel blocker).[38, 47] These observations suggest that RAS inhibition may have additional benefits beyond BP control in lean and obese hypertensive patients who are at increased risk for developing insulin resistance and type 2 diabetes. Unfortunately, these studies were retrospective in nature. Prospective studies with the development of new-onset diabetes as a primary outcome in patients with hypertension and/or with CVD or risk factors are required.

Recently, initial reports from the prospective DREAM and NAVIGATOR studies have demonstrated that the use of RAS inhibitors improves glucose homeostasis and reduces the

development of diabetes.[48, 49] The DREAM study reported a trend of reduced (~9%) new-onset diabetes incidence with use of an ACEI; additionally, ~42% of patients showed regression to normoglycemia. In contrast, there was a 14% reduction ($P<0.001$) in the likelihood of developing new-onset diabetes in the ARB compared to placebo group in the NAVIGATOR trial. The reported differences in outcomes for the two studies may be primarily due to the patient population and study time course. The NAVIGATOR study followed older patients with increased diagnosis of prediabetes and hypertension for 5 years compared to younger, healthier patients in the shorter (3 years) DREAM study.[48, 49] The significant effects observed in the NAVIGATOR study may be related to the enrollment of more patients with overactive RAS compared with the DREAM study.

It needs to be determined if the reduced rate of new-onset type 2 diabetes is a direct mechanistic effect of RAS inhibition or a reflection of a consistent decrease in insulin sensitivity with long-term use of the comparative classes of antihypertensive therapies (e.g., β-blockers and thiazide diuretics).[43, 50] Additional studies should also determine whether the mechanistic differences between ACEIs and ARBs lead to significant clinical differences in the natural course of diabetes. Moreover, clinical and experimental studies are needed to define the beneficial effects of RAS agents in the prevention of microvascular and macrovascular complications in the development of type 2 diabetes as well as the contribution of RAS blockade to improve cardiovascular endpoints in individuals with prediabetes and/or the presence of CVD risk factors.[51] With type 2 diabetes occurring at high rates in the U.S.[51], the need for pharmacologic (and lifestyle) interventions to reduce hyperglycemia and insulin resistance in hypertension is imperative and RAS inhibitors are highly attractive pharmacological agents.

Effect of the Renin-Angiotensin System on Metabolism.

i. The RAS and the Cardiometabolic Syndrome. The cardiometabolic syndrome encompasses a number of deleterious health characteristics that increase the risk of developing type 2 diabetes and CVD. Two well-known organizations have published guidelines for the recognition of the cardiometabolic syndrome in at-risk individuals. The National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) defines the cardiometabolic syndrome as the inclusion of three of the following conditions: central obesity (waist circumference >102 cm for men and >88 cm for women), elevated triglyceride concentrations (>150 mg/dL), reduced high-density lipoprotein cholesterol concentrations (<40 mg/dL for men and <50 mg/dL for women), elevated BP (>135 mmHg systolic and/or >85 mmHg diastolic BP) or current antihypertensive medication usage, and elevated fasting plasma glucose concentration (>100 mg/dL).[52] The World Health Organization criteria differ slightly, with a required presence of diabetes, impaired glucose tolerance, impaired fasting glucose or insulin resistance, as well as two other factors among hypertension, microalbuminuria, or similar measurements for dyslipidemia and central obesity as in NCEP ATP III.[53] These classic cardiometabolic characteristics are typically associated with insulin resistance and a proinflammatory and prothrombotic milieu. The prevalence of the cardiometabolic syndrome is ~20% in the U.S. population.[54] Therefore, mechanisms controlling the expression of the interrelated characteristics of the cardiometabolic syndrome need to be explored.

The results of numerous studies suggest the RAS directly affects all defined cardiometabolic components.[54] Thus, many investigations exploring the effect of Ang II and RAS inhibition on components of the cardiometabolic syndrome have been developed.

ii. The RAS and Energy Balance. Obesity is a state of extra calorie storage within adipose tissue during periods of excess caloric intake and/or inactivity. A positive association between systemic RAS activity and body weight, specifically body fat, has been observed in obese individuals.[55] Alternatively, there is a reduction in RAS activity and adipose tissue mass with weight loss.[55] These observations suggest RAS activity may influence energy balance. The role of the RAS in the etiology of obesity has been examined with systemic RAS inhibition, genetic deletion models and Ang II administration in rodents. ACEI administration led to a leaner phenotype via decreased caloric intake during ad libitum or high-fat diets in young rats.[56-58] Adipocyte hypotrophy was responsible for the observed fat mass reduction in these rats. On the other hand, deletion of a RAS component (e.g., angiotensinogen, ACE, or Ang II type 1a receptor) did not affect food intake in young mice, yet each modified group weighed less than the control group.[59-61] Significant differences in energy expenditure were present between the genetically-altered and wild-type mice. This suggests that the attenuation of weight gain may also be due to increased locomotive activity or intrinsic energy expenditure.[59-61]

In contrast, central administration of Ang II surprisingly showed similar reductions in energy intake in rats as with systemic ACEI treatment.[62] A possible explanation is the inability of ACEIs to cross the blood-brain-barrier. ACEIs only interrupt the conversion of Ang I to Ang II in the periphery, while the brain continues to produce Ang II. Conversely, rats with reduced central RAS activity exhibit hyperphagia.[63]

Collectively, these observations support a differing neuronal versus peripheral role for Ang II in energy storage and balance. These studies show that the diverse actions of Ang II are dependent on the specific tissue it stimulates, RAS activation level and/or physiological state of the animal.[58] Despite extensive evidence from rodent studies, the regulation of normal body

weight associated with RAS activity in humans is not clear. Further investigations are needed to understand the role of systemic and central RAS on body weight regulation in overweight and obese humans.

iii. The RAS and Glucose Metabolism. Glucose metabolism involves processes in the body to uptake, store and use glucose. Glucose homeostasis is tightly controlled by the organs and tissues requiring glucose for energy production, predominantly the skeletal muscle, pancreas and liver. One proposed key regulator of glucose homeostasis is the RAS. RAS overactivation observed in obesity and hypertension is associated with hyperglycemia, glucose intolerance, and increased hepatic gluconeogenesis.[64] Therefore, RAS inhibition may be an excellent first-line therapy to minimize dysregulation in glucose metabolism.

Glucose metabolism has been shown to improve significantly during RAS inhibition in obese individuals and animals or in RAS genetic ablation murine models.[48, 49, 59, 61, 65] Fasting glucose levels, absolute peak glucose values as well as area under the curve during 2-hour oral glucose tolerance tests (OGTT) were significantly reduced in rats treated with an ARB or ACEI compared to control animals.[58, 65] Similar outcomes for glucose clearance during an OGTT were observed in genetically-modified versus wild-type mice.[59, 60]

Multiple mechanisms have been proposed for the improvement in glucose clearance during RAS inhibition. RAS inhibition prevents Ang II-induced vasoconstriction and may increase glucose delivery to liver and skeletal muscle tissues. In turn, fasting hepatic gluconeogenesis may be reduced, lowering the incidence of hyperglycemia.[51, 65] In muscle cells, ACEIs augment skeletal muscle glucose uptake by activating B2 kinin receptors and upregulating the GLUT transport system.[66, 67] Some ARBs are also purported to increase skeletal muscle GLUT-4 protein and ultimately GLUT-4 translocation to enhance glucose uptake.[65, 68]

While the primary mechanisms in glucose homeostasis remain unclear, RAS inhibition appears to be a key treatment for resisting the rise in fasting glucose, glucose intolerance and hepatic glucose production observed in obese hypertensive individuals.

iv. The RAS and Insulin Sensitivity in Obesity Hypertension. Chronic impairment of whole-body insulin-stimulated glucose disposal gradually results in insulin resistance.[69, 70] This condition is commonly observed in obese individuals. Insulin resistance is a major predictor for the development of various metabolic disorders, such as impaired glucose tolerance, type 2 diabetes and CVD.[71, 72] Insulin resistance is not only detected in obesity and glucose intolerant states, but also in hypertension[71]; about 50% of hypertensive patients are insulin resistant[73] and 60-80% of type 2 diabetics are hypertensive and insulin resistant.[74] In accordance, the risk of developing type 2 diabetes is strongly associated with weight gain and increased BP over time.[75-77] Importantly, the recent rise in overweight and obesity contributes to the concomitant progression of type 2 diabetes observed over the last two decades.

Pathophysiological activity of the RAS appears to be a potential link between obesity and insulin resistance.[78, 79] Ang II is generally thought to decrease whole-body insulin sensitivity.[64] Suggested mechanisms include hemodynamic changes, proinflammatory adipocyte activity, skeletal muscle insulin signaling interference, aldosterone production, and effects on other insulin-sensitive organs, such as the liver and pancreas. Recent clinical studies utilizing RAS inhibitors suggest enhanced insulin sensitivity and reduced onset of diabetes in addition to their BP-lowering effect in hypertensive patients.[37, 38, 43, 47, 49, 80] RAS blockade may improve whole-body insulin sensitivity by decreasing circulating Ang II concentration and/or opposing Ang II-mediated changes.

Effects of Local Renin-Angiotensin System

i. Presence of Local Tissue-derived RAS. Although the RAS has far-reaching endocrine effects as discussed, the idea of a "local" RAS was conceived after RAS substrates were observed in unusual tissues (e.g., renin in the brain).[81] Evidence supports that local Ang II synthesis occurs in select tissues.[82-85] Tissues expressing all or most of the components of a local RAS include the heart, smooth muscle vasculature cells, kidney, brain, adrenal gland, pancreas, placenta, testes and adipose tissue. Overexpressing RAS genes in animals and RAS knockout models have allowed investigators to detail the presence and function of a local RAS in a variety of tissues.[82-84, 86-88] Local RAS primarily exerts autocrine and paracrine actions via Ang II activation of the tissue-specific Ang II type 1 receptor.[89] It also interacts with the endocrine RAS and other peptides on multiple levels.[90, 91] The autocrine/paracrine activity of local RAS is important to explore because its effects mediate metabolism, cell growth, and cell proliferation in pathophysiological states, such as obesity hypertension, diabetes and CVD.

ii. Angiotensin II and Adipocytes. Adipose tissue expresses all significant RAS components (e.g., renin, angiotensinogen, ACE, Ang II type 1 and 2 receptors, Ang II).[92] These components are elevated in obese and hypertensive states.[93] Adipose tissue RAS also contributes greatly to circulating angiotensinogen levels.[94, 95] The synthesis and release of angiotensinogen from adipose tissue is sensitive to food intake, body weight and blood glucose concentration.[96, 97] As such, rodent studies demonstrate adipose tissue angiotensinogen overexpression increases adiposity while removal of any component of the RAS elicits a lean phenotype.[60, 61, 95, 98-100] These observations suggest a functional role for adipose tissue RAS in adipocyte metabolism.

Adipose tissue-derived Ang II is purported to influence adipocyte growth and differentiation.[61, 92, 101] Local Ang II stimulates both the Ang II type 1 and 2 receptor, increasing

the enzymatic activity of two key lipogenic compounds, fatty acid synthase and glycerol-3-phospahate dehydrogenase.[92, 102] In addition, Ang II-induced lipogenesis simultaneously inhibits adipose tissue lipolysis *in vitro* and *in vivo*.[102, 103] Furthermore, triglyceride storage is upregulated while preadipocyte recruitment is inhibited by Ang II, resulting in hypertrophy of 3T3-L1 and human adipocytes.[102] In turn, lipotoxicity occurs when the adipocyte triglyceride potential is reached. Consequently, ectopic storage of increased circulating free fatty acids into liver and skeletal muscle tissue ensues.[104]

Local RAS and Ang II may also reduce adipocyte differentiation; however, this effect is controversial. Human preadipocytes exhibit upregulation of RAS components and Ang II type 1 receptor activity during differentiation.[105, 106] RAS knockout mice models oppose this role, suggesting an inhibitory effect of Ang II in adipogenesis.[61, 99] Consistent with Ang II's trophic effect, increased adipose tissue angiotensinogen levels enhance adipocyte hypertrophy and hypoplasia in mice.[95]

To further clarify the role of adipose tissue-derived Ang II in adipocyte growth and differentiation, various animal and cell models have been treated with RAS inhibitors to reduce Ang II exposure. Overall, studies show that RAS blockade reduces adipocyte size and increases the number of smaller adipocytes in adipose tissue[104, 106-108]; though, these changes may not necessarily impact the absolute size of fat depots. Furthermore, these alterations in adiposity are accompanied by improvements in cellular insulin sensitivity[109] and decreased ectopic lipid deposition.[104, 107] Thus, Ang II-induced inhibition of recruitment and differentiation of preadipocytes may be a primary contributing mechanism for insulin sensitivity impairment in overfeeding and obese states.[64]

iii. Angiotensin II and Tissue Remodeling. Long-term pathological conditions, such as hypertension and chronic heart disease, stimulate compensatory structural processes in efforts to preserve tissue and organ function. The heart, kidneys, liver and pancreas are prone to acute injury, and over time, the affected tissues initiate tissue remodeling processes to delay disease progression. Adaptive remodeling processes include cellular hypertrophy and dysfunction, fibrosis, extracellular matrix (ECM) deposition and alteration of gene expression in functional tissue.[110-112] If left untreated, organ failure eventually occurs.

Potential remodeling mechanisms have been studied in detail in cardiac injury models, predominantly hypertensive heart disease and myocardial infarction. In these conditions, LVH initially occurs to offset increased pressure afterload.[113] However, when LVH is prolonged, there is an increase in pressure-induced local Ang II activity. In turn, cardiac tissue damage is advanced by Ang II-induced humoral factors and cardiac function cannot be preserved.[113] Ang II itself can stimulate fibroblast proliferation and the expression of ECM proteins, such as collagen I and III, fibronectin and laminin, via Ang II type 1 receptor activation.[114, 115] Indirectly, Ang II contributes to fibrosis formation by upregulating mRNA and protein expression of growth factors, particularly local transforming growth factor-beta 1 (TGF-β1)[116-121], also via Ang II type I receptor activation.[122-124] TGF-β1 regulates fibroblast proliferation[113], phenotypic conversion to myofibroblasts[125], apoptosis[121, 126], and synthesis of ECM in the heart.[121] In addition, TGF-β1 regulates metalloproteinase activity during fibroblast conversion to myofibroblasts. Tissue inhibitors of metalloproteinase (TIMP-1) are activated to promote fibrosis via collagen I and III accumulation, whereas matrix metalloproteinase (MMP-1) activation is repressed.[126, 127]

Numerous human[128, 129] and experimental animal[123, 130-133] studies have shown increased TGF-β1 expression during cardiac hypertrophy and fibrosis. The degree of myocardium fibrosis

correlates with the upregulation of ACE and TGF-β1.[134] The RAS-associated, nonhemodynamic mechanisms are similar for fibrosis development in other organs, such as the pancreas, kidneys and liver. However, TGF- β1 targets pancreatic stellate cells[135], glomerular mesangial and tubule cells[136] and hepatic stellate cells[137], respectively, as the main sources of ECM and fibrosis accumulation in pathophysiological conditions.

Importantly, fibrosis in injured tissues may not be permanent. RAS ablation or pharmacological RAS inhibition may prevent or reverse the profibrotic effects of Ang II.[138-143] In particular, Nagashio et al.[144] observed an attenuation in pancreatic fibrosis in Ang II type 1a receptor-deficient (AT1a -/-) mice compared to wild-type mice after repeated acute tissue injury. In similar experimental conditions, tissue fibrosis was also reduced in the heart[145], kidneys[146] and liver[147] of AT1a -/- mice compared to their wild-type counterparts. Furthermore, ACEIs and ARBs equally reduced fibrosis progression in these same tissues *in vivo*[137, 148] and *in vitro*.[137, 149] Moreover, chronic losartan treatment in adult rats reversed cardiac hypertrophy and fibrosis via attenuation of multiple Ang II-associated mediators, including reduced Ang II concentration, downregulation of collagen I and TGF- β1 mRNA expression, stimulation of MMP-1 activity and concomitant inhibition of TIMP-1 expression.[127, 150]

Overall, local Ang II and TGF-β1 are currently recognized as critical mediators of Ang II-induced tissue fibrosis; however, other mediators may include hyperaldosteronism[151], oxidative stress[152-154], and other proinflammatory cytokines (e.g., C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1)).[155, 156] Thus, further investigation into mechanisms and pathways contributing to tissue remodeling during states of injury and pathophysiological conditions is needed to determine the best practice to delay, reverse or prevent the loss of tissue structure, function and, ultimately, organ failure.

The Renin-Angiotensin System, Inflammation and Obesity.

i. Origin of Chronic Inflammation in Obesity. Adipose tissue is no longer considered an inert storage depot, but an active endocrine organ and metabolic tissue that contributes to the low-grade systemic inflammation characterized in obesity. Just over a decade ago, overexpression of tumor necrosis factor-alpha (TNF-α) in white adipose tissue was first discovered in obese animals[157], suggesting a role for inflammation in obesity. Since then, the synthesis and secretion of many other adipokines and chemokines, such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and PAI-1, from adipose tissue has been documented.[158] Although the biological actions of some adipokines are still unknown, studies have revealed consistent patterns in adipokine production and release. Most adipokines exert local paracrine and/or systemic proinflammatory actions, rise with fat mass gain and fall with fat mass loss.[158, 159] Conversely, the adipokine adiponectin induces anti-inflammatory action, responds to weight gain and weight loss in an opposing manner as above, and promotes whole-body insulin sensitivity.[160-162]

Transcriptomics has amassed panels of inflammatory genes observed in adipose tissue of obese animals and humans.[163, 164] Stromal vascular fraction (SVF) cells (i.e., macrophages, T cells, preadipocytes, endothelial cell, and fibroblasts) in adipose tissue produce and secrete the majority of the proinflammatory adipokines (i.e., TNF-α, IL-6, IL-1β, MCP-1, and PAI-1)[158, 165, 166], while adipocytes and other cells contribute little to the inflammatory milieu.[165, 167-169] In particular, MCP-1 is an important inflammation promoter in obese states as it recruits additional macrophages to adipose tissue, demonstrated by the enhanced expression of CD68+ cells and increased crown-like structures in adipose tissue.[165, 169-171] Consequently, release of macrophage-secreted factors remains elevated and seems partially dependent on nuclear factor-κB (NF-κB)

pathway activity for the additional generation of proinflammatory response in adipose tissue.[172, 173] Despite evidence for adipose tissue macrophage infiltration in obesity, the cellular and molecular mechanisms responsible remain largely unknown.

ii. Obesity, Insulin Resistance, and Inflammation. A chronic low-grade inflammatory state is observed in obesity marked by abnormal cytokine production, increased acute phase proteins and activation of proinflammatory signaling pathways.[174-179] Higher circulating concentrations and adipocyte protein expression of proinflammatory markers, such as CRP, TNF-α, IL-6 and PAI-1, are typically observed in obese compared to lean individuals.[180, 181] Total and regional adiposity measurements also correlate with the rise in the plasma concentrations of these proinflammatory markers.[174-178] The immune environment shifts from a defensive anti-inflammatory to an enhanced proinflammatory state during the progression of many chronic diseases, characterized by the elevated proinflammatory cytokine and acute phase protein concentrations as well as immune cell infiltration in tissues and organs.[182-184] Inflammation also inhibits postreceptor signal transduction, particularly at the insulin receptor substrate-1 (IRS-1) and -2 of the insulin signaling pathway.[185]

Currently, it is postulated that activation of the innate immune system and the associated rise in inflammatory markers in obesity is involved in the pathogenesis of insulin resistance and type 2 diabetes as well as a predictor for atherosclerosis.[186, 187] More so, the significant observations between inflammatory markers and type 2 diabetes are often attenuated after statistical adjustments with covariates related to adiposity.[188, 189] Furthermore, an inflammatory milieu is frequently observed before insulin resistance is detected. Elevated systemic levels of MCP-1, TNF-α, IL-6, IL-12 and IFN-γ impair whole body insulin sensitivity.[185] However, insulin sensitivity is improved with salicylate treatment via inhibition of the NF-κB pathway.[185]

Taken together, type 2 diabetes may be partially caused by a chronic inflammatory response, mediated by an obese state.

iii. Angiotensin II and Adipose Tissue Inflammation in Obesity. In addition to BP regulation and electrolyte homeostasis, the RAS appears to contribute to the chronic inflammatory state in obese individuals. Local Ang II release, in part, regulates the stimulation and recruitment of proinflammatory cells to sites of injury in various tissues (e.g., cardiac, vascular, renal), accelerating disease processes.[190-193] Of interest, Ang II also indirectly stimulates several transcription factors, notably NF-κB, that control gene expression of numerous adipokines, chemokines and adhesion molecules.[191] Furthermore, Ang II enhances immune cell recruitment to injured areas via an increase in MCP-1 expression.[194]

Accumulating evidence suggests RAS activation may affect insulin action and type 2 diabetes development in obesity.[54, 99, 195, 196] Several reports have linked adipose tissue inflammation to Ang II activity, which may play a role in insulin resistance in obesity. As discussed earlier, supporting evidence includes enhanced activity of various components of the RAS in adipose tissue, especially the expression of Ang II type 1 and 2 receptors.[60, 61, 95, 102, 197, 198] Also, the local expression of angiotensinogen and RAS activity in adipose tissue correlates to human obesity.[197, 199] Furthermore, MCP-1 positively correlates with abdominal fat as increased MCP-1 gene expression in rat preadipocytes *in vitro* and adipose tissue *in vivo*[200, 201] has been reported. Additional reports demonstrate direct effects of Ang II on MCP-1 expression in preadipocytes[200], the SVF[201], and adipocytes *in vitro.*[202] The increased expression appears to occur via Ang II type 1 receptor-mediated and NF-κB-dependent pathways.[202]

Moreover, multiple investigations have observed significant reductions in adipose tissue gene expression of proinflammatory cytokines and macrophages as well as elevations in

adiponectin levels after ARB administration in obese rodents.[101, 170, 201] These inflammatory alterations were associated with an improvement in whole-body insulin sensitivity.[203] Overall, RAS activity may play a key role in adipose tissue inflammation and insulin resistance in obesity. However, it is unknown if adipose tissue inflammation in human obesity is partly stimulated by Ang II activity. Thus, further investigations are needed to determine the effect of Ang II blockade on adipokine expression and release and macrophage infiltration in obese individuals.

The Renin-Angiotensin System and Skeletal Muscle Insulin Signaling.

i. Obesity and Skeletal Muscle Insulin Signaling. Skeletal muscle tissue is responsible for ~75-90% of insulin-stimulated glucose disposal post-prandial to maintain normoglycemia.[204] Even so, insulin signaling deficiencies lead to the development of insulin resistance and the cause for these functional disturbances in insulin signaling has not been fully elucidated in obese individuals. However, alterations in the insulin receptor (IR) and signaling cascade have been reported in skeletal muscle from obese subjects.[205] Protein expression of the IR is moderately, but significantly, reduced in obese versus lean skeletal muscle.[205] Moreover, reductions in insulin-stimulated tyrosine phosphorylation of the IR and IRS-1 as well as subsequent diminished phosphatidylinositol 3-kinase (PI 3-kinase) have been demonstrated in obese compared to lean skeletal muscle.[205] Consequently, GLUT-4 release and translocation to the sarcolemmal membrane is essentially reduced with insulin signaling inhibition, even though GLUT-4 protein levels are not affected in obese subjects.[206] A proposed negative regulator of IR tyrosine phosphorylation is serine/threonine phosphorylation activity. Serine/threonine phosphorylation of IRS-1 reduces the ability of the IR proteins to activate PI 3-kinase and

downstream events.[207] Serine/threonine phosphorylation also degrades IRS-1 proteins, minimizing the effect of insulin binding at the IR.[208] Furthermore, protein phosphatases are additional mediators of post-IR signaling and insulin-stimulated GLUT-4 translocation. Specifically, protein tyrosine phosphatase 1B activity inhibits tyrosine phosphorylation at the IR and IRS-1, impairing muscle glucose uptake and whole-body insulin sensitivity.[209]

ii. The RAS and Skeletal Muscle Insulin Resistance. Current epidemiological evidence suggests a potential relationship between RAS blockade and new-onset type 2 diabetes.[38, 43] Moreover, animal experiments and other clinical studies indicate elevated RAS activity in obesity may contribute to the development of insulin resistance and type 2 diabetes.[54, 99, 210] This hypothesis is supported by accumulating evidence that Ang II inhibits insulin action. For example, experimental animals infused with Ang II during a hyperinsulinemic euglycemic clamp become insulin resistant.[211] Crosstalk at the insulin receptor is implicated as Ang II and insulin share the PI 3-kinase signal transduction pathway. Ang II-mediated activation of the skeletal muscle Ang II type 1 receptor stimulates serine phosphorylation on IRS-1 and impedes PI 3-kinase activity and downstream insulin signaling.[212-214] Moreover, acute and chronic blockade of Ang II type I receptors improves glucose disposal via increased insulin-stimulated glucose transport in skeletal muscle *in vivo* and *in vitro*.[65] Of note, skeletal muscle from recent animal and cell culture studies treated with Ang II show increased levels of reactive oxygen species (ROS), via upregulated NADPH oxidase activity.[215] Generation of oxidative stress augments multiple transcription factors[216], primarily NF-κB pathway activity. In turn, these transcription factors diminish insulin-stimulated IRS-1/PI 3-kinase pathway activity and GLUT-4 translocation in these tissues and cells.[215, 217] Overall, the reported outcomes of Ang II treatment *in vivo* and *in vitro* suggest a role for Ang II in insulin resistance in obese individuals and

translate to a viable treatment with specific Ang II receptor blockade to improve whole-body insulin sensitivity and skeletal muscle insulin signaling.

Despite promising evidence from animal and cell experiments[214, 218-220], few randomized controlled studies have examined the effect of Ang II receptor blockade on insulin sensitivity in obese, hypertensive humans. Results from available studies have been contradictory.[221-225] Although the cause for the inconsistent findings is unknown, multiple study flaws may have contributed. These include inadequate study design and methods, such as a small sample size, lack of randomization and use of inadequate measurements for insulin sensitivity (e.g., using HOMA-IR instead of glucose tolerance tests or clamp techniques). Misuse of study medications may also have affected the outcomes as one team of investigators inappropriately combined olmesartan and hydrochlorothiazide, a proposed insulin-sensitizing agent and insulin resistance mediator, respectively, effectively canceling out the individual actions of each substance on insulin sensitivity.[222] Also, the ARBs telmisartan and irbesartan are partial peroxisome proliferator-activated receptor-γ activators, which mimics the beneficial action on insulin sensitivity of antidiabetic medications (i.e., thiazolidinediones), and makes interpretation of study results difficult to compare to other ARB treatments.[226, 227] In general, the effect of Ang II receptor blockade on insulin sensitivity is unclear in obese humans. Moreover, no human studies have explored the role Ang II blockade plays in insulin signaling in skeletal muscle, the main contributor of whole-body insulin sensitivity. Future investigations of molecular events in skeletal muscle insulin signaling with Ang II blockade are needed to establish therapies to improve skeletal muscle glucose uptake and reduce the risk of insulin resistance and type 2 diabetes in obese hypertensives.

The Renin-Angiotensin System and Pancreatic β-Cell Function.

i. Physiologic Role of Local Pancreatic RAS. As discussed earlier, many large clinical trials have shown a reduced incidence of new-onset diabetes after RAS blockade in high-risk populations[43], though the mediating mechanisms have not been resolved. Reduced peripheral insulin sensitivity and impaired skeletal muscle insulin signaling pathway are two primary culprits in insulin resistance and type 2 diabetes. However, pancreatic islet β-cell dysfunction and loss of β-cell mass have recently been recognized as contributors to disease progression.[228]

Pancreatic β-cells synthesize and release insulin in response to a glucose challenge in order to maintain glucose homeostasis. When β-cell function is compromised, insufficient insulin secretion ensues while hyperglycemia is sustained, eventually leading to the development of type 2 diabetes. Recent discovery of an intrinsic RAS within the endocrine pancreas suggests a critical role in islet physiology and pathophysiology.[228]

All constituents of the RAS are present in pancreatic cells, including angiotensinogen, ACE, Ang II, and Ang II type 1 and 2 receptors.[229, 230] Local activation of the pancreatic RAS has been observed during periods of chronic hyperglycemia and hyperlipidemia[231] and in obese, inflammatory[232] and hypertensive[231] states. Importantly, Ang II type 1 receptors are present on islet β-cells.[229, 231, 233, 234] Elevated islet Ang II type 1 receptor expression and activation has been observed in diabetic animal models.[235, 236] Endogenous or infused Ang II upregulates Ang II type 1 receptors in the pancreas of experimental rats and mice. Consequently, islet blood perfusion and (pro)insulin biosynthesis are reduced.[229, 236, 237] Similar outcomes are observed in the islet cell of rodents during states of hyperglycemia or oral glucose challenge, thus inhibiting glucose-stimulated insulin release and delivery.[229, 236, 237] As a result, glucose sensitivity of the islet β-

cells is reduced and glucose intolerance emerges. This novel role of the pancreatic RAS emphasizes the importance of β-cell performance for the maintenance of glucose homeostasis.[238]

The pancreatic RAS also plays a variety of additional autocrine and paracrine roles as previously described, notably regulation of cell proliferation and apoptosis, fibrosis, and oxidative stress.[239] An imbalance between cell proliferation and apoptosis impairs islet functionality due to reductions in β-cell mass. Diabetic murine models and tissue from human donors with impaired glucose tolerance or diabetes exhibit a 2-3 fold increase in apoptotic islet cells as well as concurrent islet cell atrophy.[235, 240-243] Pancreatic connective tissue fibrosis is simultaneously evident in the animal diabetes models as well.[235, 240, 244] However, the main contributor(s) to islet cell damage in the progression to type 2 diabetes has not been determined.

Oxidative stress, and its resultant ROS, is the leading candidate for RAS-induced islet dysfunction. Islet β-cells have low antioxidant activity[245] and are highly susceptible to the deleterious effect of ROS scavengers. Increased expression of NADPH oxidase components and oxidative stress markers in animal models of type 2 diabetes indicate that NADPH oxidase activity is a source of islet β-cell oxidative stress.[246] It has been confirmed that Ang II exposure promotes pancreatic ROS production via both direct NADPH oxidase or protein kinase C (PKC) -dependent activation.[246, 247] ROS are also thought to be key players in β-cell apoptosis. *In vitro* cell and rat studies showed a decrease in insulin expression and an increase in apoptotic β-cells induced by experimental oxidative stress.[245] Local ROS production also shifts the cellular balance by accumulating fibrotic and apoptotic islet cells while limiting pancreatic cell proliferation.[234] Ultimately, the culmination of direct deleterious effects of Ang II in the pancreas results in the loss of β-cell mass and function. Activation of the pancreatic RAS represents a

novel independent mechanism for islet cell damage in the progression of type 2 diabetes development.[231]

ii. RAS Blockade on Pancreatic Function. Recent results from animal experiments show RAS-induced changes in the pancreas are attenuated with RAS antagonism. Numerous murine models demonstrate significant improvements in islet β-cell structure, perfusion and survival during pre- or prolonged treatment with RAS inhibitors.[235-237, 240, 242, 244, 247, 248] First phase glucose-stimulated insulin release and glucose tolerance assessed by oral glucose tolerance tests also improved significantly after ACEI or ARB treatment in many animal studies.[235, 236, 244, 247] In addition, RAS blockade reduced oxidative stress markers in murine pancreatic β-cells.[235, 242, 246, 247] Cell culture and mouse and rat cell studies suggest ARBs indirectly reduced ROS accumulation, PKC activity and NADPH oxidase activity[249, 250] via inhibition of Ang II type 1 receptor activation. Although β-cell function is seemingly preserved and oxidative stress activity reduced in cells and diabetic animal models treated with RAS antagonists, additional studies are needed to define the precise mechanism(s) responsible for the improvements.

Conversely, few studies have assessed changes in pancreatic β-cell function during RAS inhibition in humans. The recently reported outcomes are inconsistent. Bokhari et al.[251] showed no changes while van der Zijl et al.[252] did see improvements in β-cell function during a hyperglycemic clamp with valsartan treatment in their subjects. On the other hand, Suzuki et al.[253] and van der Zijl et al.[252] also reported increased first-phase insulin secretion during an OGTT after treatment with candesartan and valsartan, respectively. The outcome discrepancies may be related to the differences in study population, type and exposure time of the RAS antagonist, and methodology used to estimate insulin secretion and β-cell performance. Thus,

continual investigations are needed to delineate the mechanistic pathways regulating β-cell dysfunction and the subsequent development of type 2 diabetes in humans.

Conclusions.

Obesity and hypertension are major risk factors for the development of type 2 diabetes. Weight loss is effective in improving insulin resistance, but many individuals do not initiate weight loss or are unsuccessful at long-term weight loss. Thus, it is imperative to recognize alternative strategies that uncouple obesity from its health risks. One promising candidate is Ang II receptor blockade. Although ARBs have been developed to control hypertension in individuals at risk for CVD, their beneficial effects seem to extend beyond their blood pressure-lowering capabilities.[54, 254, 255] As presented above, multiple large randomized controlled studies have indicated a reduction in cardiovascular events as well as new-onset type 2 diabetes with RAS inhibition in hypertensive individuals at risk for CVD.[43, 50] However, the specific mechanisms involved have not been completely identified. Although, animal experiments suggest that whole-body insulin sensitivity, skeletal muscle insulin signaling and pancreatic β-cell function is improved with Ang II receptor blockade.[65] However, it is unknown if RAS inhibition via Ang II receptor blockade improves these actions in humans. Therefore, additional studies are needed to directly address the hypothesis that Ang II receptor blockade improves insulin action in obese hypertensives. As such, the results may lead to improved clinical recognition of insulin resistance in obese hypertensives and, in turn, advanced therapy and better health outcomes for these individuals.

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CHAPTER III

Angiotensin II Receptor Blockade and Insulin Sensitivity in Overweight and Obese Adults with Elevated Blood Pressure

ABSTRACT

The renin-angiotensin system (RAS) is chronically activated in obesity hypertension. Reduced incidence of new-onset diabetes has been suggested by multiple large-scale clinical trials in at-risk hypertensive patients during RAS inhibition treatment. We tested the hypothesis that angiotensin II receptor blockade (ARB) would improve whole-body insulin sensitivity in overweight and obese individuals with elevated blood pressure (BP). Olmesartan was selected as the study ARB because it does not activate PPARγ agonist insulin-stimulated glucose disposal. A secondary aim was to determine whether skeletal muscle inflammatory and collagen response was related to insulin sensitivity. Sixteen individuals (8 females, 8 males; age=49.5±2.9 y; BMI=33.0±1.7 kg/m^2) were randomly assigned in a crossover manner to control and ARB interventions. Insulin sensitivity was determined from intravenous glucose tolerances tests before and after each 8-week intervention. Blood pressure, body weight, body fat, lipoproteins and insulin sensitivity were similar at baseline for both treatments (all $P>0.05$). Diastolic BP and triglycerides were higher ($P=0.007$ and $P=0.042$, respectively) at baseline for ARB compared to control treatment. Systolic (-11.7 mmHg; $P=0.008$) and diastolic (-12.1 mmHg; $P=0.000$) BP decreased in ARB treatment. Insulin sensitivity was not significantly different between treatments. No correlates of insulin sensitivity were identified ($P>0.05$). Skeletal muscle inflammatory and collagen gene expression did not change significantly during ARB treatment. In summary, our findings indicate that short-term ARB treatment did not affect whole-body

insulin sensitivity in overweight or obese individuals with elevated BP. Further studies are needed to clarify the effect of individual RAS blockers on insulin sensitivity during RAS inhibition in obesity hypertension.

Key words: Insulin sensitivity index, olmesartan, hypertension, renin-angiotensin system

Introduction

Obesity and its related comorbidities are major risk factors for the development of cardiovascular disease (CVD), type 2 diabetes, cancers and other chronic conditions.[1] More than half of overweight and obese individuals develop hypertension.[2] Reduced whole-body insulin sensitivity is also observed in obesity, resulting in insulin resistance and increased risk of type 2 diabetes.[3-5] Overactivation of the renin-angiotensin system (RAS) is recognized as a contributor to insulin resistance and type 2 diabetes development in overweight and obese hypertensives.[6, 7] The primary role of the RAS is to regulate arterial pressure and sodium balance. However, chronic elevation angiotensin II (Ang II) impairs whole-body insulin sensitivity and insulin signaling in skeletal muscle via Ang II type 1 receptor activation.[8-12] Alternatively, multiple recent large-scale clinical trials indicate a reduction in CVD morbidity and mortality in high-risk hypertensive populations receiving long-term RAS inhibition treatment.[13-18] Furthermore, post hoc analysis of these outcomes suggests a decrease in the incidence of new-onset diabetes.[19-23] However, the mechanisms responsible for the additional benefits of RAS blockade accompanying reduced blood pressure remain unclear. It is unknown if RAS antagonism directly attenuates or comparative antihypertensive treatments augment insulin resistance during type 2 diabetes development.[22, 24]

We tested the hypothesis that Ang II receptor blockade with olmesartan would improve insulin sensitivity in middle-aged to older overweight and obese adults with elevated blood pressure. To address this, we conducted a randomized crossover study and assessed insulin sensitivity using the insulin-modified intravenous glucose tolerance test (IVGTT) before and after each treatment period. Olmesartan was selected as the RAS inhibitor to avoid the partial peroxisome proliferator-activated receptor gamma (PPARγ) agonist action associated with

improved whole-body insulin sensitivity observed with use of other Ang II receptor blockers (i.e., telmisartan; ARBs).[25, 26] Moreover, skeletal muscle is the primary tissue responsible for insulin-stimulated glucose disposal and inflammation and alterations in the extracellular matrix of skeletal muscle have been associated with the development of insulin resistance. Therefore, a secondary exploratory aim of the study was to determine whether changes in skeletal muscle inflammatory response predicted changes in insulin sensitivity in our subjects.

Materials and Methods

Subjects. Sixteen sedentary (moderate to hard activity ≤3 d/wk), overweight and obese (BMI ≥25 kg/m^2 or body fat ≥20% for males and ≥25% for females) men (n=8) and women (n=8) aged 18-75 years served as subjects. The subjects were weight stable (±2.0 kg) for the previous 6 months and were not taking any medications known to affect weight or study measures. No post-menopausal females were on hormone replacement therapy. The subjects had elevated blood pressure (BP ≥120/80 mmHg but <160/100 mmHg), but were free of other overt disease (assessed by a Health History Questionnaire). Two female subjects discontinued their current BP medication for the duration of the study with their physician's approval. Their BP was monitored during a 2-week washout period before participation in study testing sessions to ensure that their BP remained within the study BP requirements. One smoker was included in the study. All potential subjects were evaluated and approved by our study Medical Director during a medical examination prior to study participation. All study procedures were approved by the Virginia Polytechnic Institute and State University Institutional Review Board. The nature, purpose, risks, and benefits of the study were explained to potential subjects, all questions were answered, and individuals provided written consent prior to participating in the study.

Intervention. Subjects first completed baseline testing sessions within a 2-week period. They were then randomized to one of two groups: olmesartan medoxomil (Benicar; ARB) or no medication (control) for 8 weeks. The study employed a randomized crossover design. Figure A1 illustrates the study design. When assigned to the olmesartan group, subjects were provided with daily 20 mg pills for the first 2 weeks. For the remainder of the intervention period, they received additional daily doses of 40 mg olmesartan; however, if their BP fell below 110/70 during the first 2 weeks, their dose remained at 20 mg per day. They also continued taking the drug during the 2-week follow-up period. There was no drug intervention during the control period. After the first 8-week intervention, subjects participated in post-testing sessions, followed by a 2-week washout period. Subjects then completed the testing sessions again and participated in the opposite intervention. This was followed by another set of post-testing sessions after 8 weeks. During each intervention period and the washout period, BP was measured weekly. Subjects were asked to maintain their current physical activity level, dietary intake and body weight throughout the study.

Measurements. All testing sessions were performed between 7 a.m. and 1 p.m. after a 12-hour fast. Before each testing session, subjects recorded recent infection and/or illness on an Infection/Inflammation Questionnaire. If an infection/illness was reported, testing was delayed 1-2 weeks for recovery. Subjects refrained from vigorous physical activity for 48 hours before testing. They also abstained from ingesting non-steroidal anti-inflammatory drugs, ibuprofen, or other medication that may have interfered with study measurements for 72 hours prior. Blood pressure measurements were documented at each visit.

Resting Blood Pressure. Blood pressure measurements were performed between 7 and 11 a.m. with serial BP appointments scheduled at the same time for each individual. Automated

sphygmomanometry (Pilot model 9200, Colin Instruments Corp.) measured upright seated BP. Measurements were taken every 3 min after a 5-10 min seated rest and continued until BP stability (±6 mmHg SBP and DBP*)* was reached between 3 consecutive recordings. Baseline BP stability was established after three BP sessions spanning at least one week were completed before any testing sessions were performed . For individuals who discontinued their BP medications, six BP sessions over two weeks were completed to ensure BP stability (<160/100 mmHg).

Body Mass and Composition. Body weight was measured to the nearest ±0.1 kg on a digital scale (Model 5002, Scale-Tronix, Inc.). Height was measured to the nearest ±0.1 cm using a stadiometer. Body composition (total fat and fat-free mass) was analyzed by dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, software version 8.10e).

Dietary and physical activity assessment. Before and after each 8-week intervention period, habitual dietary intake was assessed from self-reported 4-day food records. A Registered Dietitian provided subjects with written and verbal instruction for accurately measuring and recording food intake. The Nutrition Data Systems for Research software (NDS-R 2006, University of Minnesota) was used to estimate energy and macronutrient content. Habitual physical activity level was measured by an accelerometer (Actigraph, MTI) worn for 4 consecutive days before and after each intervention.

Plasma Lipid and Lipoprotein Concentrations. Blood samples were drawn with minimal venous stasis. Plasma total cholesterol, high-density and low-density lipoprotein, and triglyceride concentrations were analyzed by Solstas Lab Partners (formerly Carilion-Spectrum Laboratory) via conventional enzymatic methods.[27]

Circulating Inflammatory Peptides. A Bioplex Pro™ coupled magnetic bead assay (Bio-rad, Hercules, CA) assessed plasma concentrations of inflammatory markers, TNFα, IL-6, IL-10 and MCP-1. Enzyme-linked immunosorbant assays (ELISA) were performed to measure total and high-molecular weight adiponectin (ALPCO Diagnostics, Salem, NH).

Estimated Insulin Sensitivity. Systemic insulin sensitivity was estimated using Bergman's minimal model (MINMOD software, version 6.02) during a modified frequently-sampled IVGTT. [28] An intravenous catheter was inserted in an antecubital vein of each arm for blood collection or glucose and insulin injection. Two baseline plasma blood samples (*(t)* = -10 and -1 min) were drawn. Glucose (0.3 g/kg; 50% solution) was injected at time 0 and insulin (0.025 U/kg) at *(t)* = 20 min. Additional blood samples (6 mL) were collected at *(t)* = 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min during the 3-hr protocol. Glucose concentration (mg/dL) was analyzed immediately using a YSI Glucose Analyzer (Yellow Springs, OH). Insulin (μU/mL) was determined later via ELISA (ALPCO Diagnostics, Salem, NH). Samples were analyzed in duplicate. Thirteen individuals successfully completed all IVGTTs. Reasons for not completing these tests included voluntary withdrawal and inability to find at least one viable antecubital vein for catheter placement. One outlier was removed from statistical analysis.

Skeletal Muscle Biopsy. Skeletal muscle samples were taken by needle aspiration from the vastus lateralis. The skin was cleaned with a povidone-iodine solution and local anesthetic (50:50 2% xylocaine/0.25% bupivacaine; 10 mL total) was used to numb the skin and tissue. A small (~1/4") incision was made with a #10 scalpel. Approximately 500 mg of skeletal muscle tissue was collected using suction applied to a 5 mm Bergstrom needle. The incision was cleaned with saline and closed with sterile bandage strips. Ice and pressure was applied to minimize

discomfort. Tissue samples were immediately washed in 0.9% sterile saline to remove blood and connective tissue. Samples were weighed and immediately flash frozen in liquid nitrogen for future analysis. One study subject voluntarily withdrew from this testing session for the entire study.

Skeletal Muscle RNA extraction and quantitative real-time PCR (qRT-PCR). RNA extraction and quantification were determined using methods previously described by Frisard et al.[29] Briefly, an RNeasy Mini Fibrous Kit and DNase I treatment (Qiagen, Valencia, CA) were used according to the manufacturer's directions for mRNA extraction. qRT-PCR measured the expression of NADPH oxidase 4, TGF-β, IL-6, collagen III and MCP-1 using an ABI PRISM 7900 Sequence Detection System instrument and TaqMan Universal PCR Master Mix according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Human skeletal muscle gene expression was normalized to cyclophilinB RNA levels. Relative expression levels of the target genes were quantified using the $2^{-\Delta C_T}$ formula.

Statistical analysis. Repeated measures ANOVA was used to assess the effect of treatment (olmesartan treatment vs. control), time, and treatment by time interaction on the dependent variables of interest. Paired samples t-tests were used to compare the changes in dependent variables of interest. Possible pairwise associations among variables were analyzed using Pearson's Correlation. All statistical analyses were performed using SPSS 12.0 for Windows (SPSS, Chicago, IL). All data are presented as means ± SEM. Significance level was set a priori at $p<0.05$.

Results

Sixteen of 20 (80%) randomized subjects completed the study interventions. The study medication, olmesartan, was well tolerated and there were no adverse events reported. Adherence during the 8-week olmesartan intervention was 98.4% overall, with no subject missing more than 3 daily doses total.

Subject characteristics before and after each treatment are depicted in Table 1. There were no significant differences prior to the start of the treatment period with the exception of diastolic BP and triglycerides levels. Diastolic BP and triglycerides were higher in the ARB compared with the control at baseline (P=0.007 and P=0.042, respectively). As expected, there was a significant reduction in both systolic (-11.7 mmHg; -8.3%) and diastolic (-12.1 mmHg; -14.2%) BP in the ARB treatment. Body weight, BMI, body fat %, and total body fat mass increased (all $P<0.05$) during both treatment periods. Triglyceride levels remained higher throughout treatment in the ARB compared with control condition (P=0.038). No differences in lipoprotein measures or fasting plasma glucose levels were found between treatments (all $P>0.05$).

Habitual physical activity and dietary intake during ARB and control treatment are shown in Table 2. There were no significant changes in physical activity level or total calorie intake during either treatment period. The absolute (g) and relative (%) fat intake was lower (P=0.021 and P=0.047, respectively) during the ARB compared with control treatment. The % carbohydrate intake was concurrently higher (P=0.012) in the ARB compared with the control treatment. Protein and alcohol intake did not differ during the ARB or control treatment. Cholesterol, saturated fatty acid, fiber, and sodium intake all remained unchanged during the treatments.

The IVGTT variables before and after each treatment period are depicted in Figure 1. There were no significant differences for any of the IVGTT variables at baseline between the ARB and control treatments. A significant effect of time was detected for acute insulin response to glucose (AIRg) and disposition index (DI) (P=0.026 and P=0.045, respectively) during the treatment periods. Insulin sensitivity index (SI) and glucose effectiveness (Sg) did not change throughout the study.

Circulating cytokine and adiponectin concentrations are presented on Table 3. Baseline concentrations of all circulating inflammatory markers were not different between the treatment periods (P<0.05). There was a trend (P=0.065) for a higher MCP-1 concentration in the ARB compared to control treatment. There were no significant changes in IL-6, IL-10 or TNF-α with ARB treatment. Similarly, there was no significance detected for circulating total or high-molecular weight adiponectin concentrations in either treatment. No correlations were determined between circulating inflammatory markers and key variables (P>0.05).

Muscle mRNA levels are presented for only in the ARB treatment due to insufficient RNA yield for the majority of participants in the control treatment. There were no significant changes in any of the mRNA target genes from pre- to post-ARB treatment.

Table 5 shows the correlation between changes in IVGTT variables and dependent variables of interest in the ARB treatment. The change in fasting insulin was associated with the change in AIRg (P=0.011). No correlates of SI, Sg or DI were identified (all P>0.05).

Discussion

The results of our randomized crossover study showed that an 8-week period of olmesartan treatment did not affect insulin sensitivity in our overweight and obese subjects,

despite a significant reduction in blood pressure. We did not observe significant changes in circulating inflammatory markers or skeletal muscle inflammatory or collagen gene expression. There were no significant correlates of changes in insulin sensitivity in response to olmesartan treatment.

Recent reports from multiple large-scale clinical trials suggest a reduced incidence of new-onset diabetes with long-term RAS blockade use in at-risk hypertensive populations compared to other antihypertensive medications and placebo.[19-22] Numerous *in vivo* and *in vitro* studies have demonstrated improvements in whole-body insulin sensitivity after RAS blockade in nondiabetic hypertensive or diabetic animal models.[30-35] However, these promising outcomes have not been clearly translated to humans. The results of our study suggest that an improvement in insulin sensitivity is not among the additional benefits of ARBs that extend beyond their blood pressure-lowering capability.[36]

Studies investigating the changes in peripheral insulin sensitivity with RAS blockade in humans have led to conflicting results. The results of some randomized controlled trials have reported increases in insulin sensitivity with ARB treatment compared with other antihypertensive treatments.[37-44] Conversely, our study and others demonstrated no change in whole-body insulin sensitivity measures during RAS.[45-49] The reason for this apparent discrepancy is unclear but differences in the particular ARB used, duration of treatment, methods used to assess insulin sensitivity, and/or study population may contribute.

Some ARBs, such as telmisartan[25, 26], exhibit partial PPARγ agonist activity. PPARγ activation enhances insulin's effectiveness to promote glucose uptake in peripheral metabolic tissues.[50] Anti-diabetic medications such as thiazolidinediones are PPARγ agonists and are employed to reduce hyperglycemia in diabetic patients. Thus, partial PPARγ agonists can

influence insulin sensitivity in hypertensives via avenues independent of Ang II receptor blockade. This may contribute, in part, to the different outcomes observed in previous studies.[40, 42, 43, 51, 52] We used olmesartan in the present study because it is devoid of partial PPARγ agonist activity. Therefore, future studies are necessary to clarify determine whether the ability of ARBs to improve insulin sensitivity depend on intrinsic PPARγ agonist activity.

As with all randomized controlled studies in humans, there are some limitations of our study that should be considered. In the present study, the sample size was small and the majority of subjects were Caucasian. There was also large variability in glycemia, blood pressure, and body composition. Therefore, our study may have been underpowered and the findings may not be generalized. In addition, statistical analysis of the inflammatory and collagen gene targets by qRT-PCR was limited by the extraction of sufficient mRNA from the samples collected during each biopsy session. Further studies are needed to determine if RAS inhibition improve glucose homeostasis in populations at elevated risk of diabetes (e.g., prediabetics).

In conclusion, our findings suggest that RAS blockade with olmesartan for 8 weeks does not improve insulin sensitivity in overweight and obese individuals with elevated blood pressure. Thus, short-term olmesartan treatment may not be an effective treatment to improve insulin sensitivity in overweight/obese individuals with blood pressure in the pre- to hypertensive range. Furthermore, we did not observe significant changes in inflammatory and collagen mRNA expression in skeletal muscle. Nonetheless, evidence from clinical trials and animal experiments support the hypothesis that RAS inhibition prevents or delays insulin resistance and type 2 development via improved whole-body insulin sensitivity. Further research is needed to delineate which RAS antagonists improve whole-body insulin sensitivity during RAS inhibition and, if so, what population are likely to benefit.

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Disclosures

None.

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Table 1. Subject characteristics before and after the control and ARB treatment.

	Control		ARB	
	Gender, Male=8, Female=8 Age, 49.5±2.9 y			
Variable	Pre	Post	Pre	Post
Body weight, kg	97.2±5.4	98.1±5.5	96.9±5.1	97.5±5.2†‡
Body mass index, kg/m^2	33.3±1.9	33.6±1.9	33.2±1.8	33.4±1.8†‡
Body fat, %	41.8±2.8	42.2±2.9	41.6±2.8	42.1±2.8†‡
Total fat mass, kg	39.3±3.7	40.1±3.8	38.9±3.6	39.7±3.8†‡
Fat-free mass, kg	56.5±3.4	56.7±3.6	56.3±3.3	56.3±3.3
Systolic BP, mmHg	138.5±4.1	140.7±4.6	140.6±3.2	128.9±2.5†‡
Diastolic BP, mmHg	79.3±1.7	82.9±2.0	85.5±2.2	73.4±1.6†‡
Triglycerides, mg/dL	100.6±11.1	104.0±12.6	117.9±15.3	116.0±14.5*
Total cholesterol, mg/dL	196.3±8.0	195.6±8.3	194.3±7.5	190.0±7.7
HDL cholesterol, mg/dL	50.6±5.5	49.0±5.3	48.1±4.5	49.1±4.9
LDL cholesterol, mg/dL	125.6±8.0	125.8±7.9	122.7±7.8	117.7±7.1
Glucose, mg/dL	89.4±2.8	89.8±2.9	91.2±3.3	89.6±3.1
Insulin, μIU/ml	4.3±0.7	6.0±1.2	5.6±1.1	5.7±1.1

All values are expressed as mean ± SEM. BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Effect of treatment (*), time (†) and treatment x time interaction (‡). $P<0.05$.

Table 2. Physical activity and dietary intake before and after the control and ARB treatment.

	Control		ARB	
	Gender, Male=8, Female=8			
Variable	Pre	Post	Pre	Post
Physical activity, counts/day x 10^3	236±26	213±30	189±21	210±25
Physical activity, steps/day	5991±573	5168±627	4889±565	5012±550
Kcal, per day	2137±203	1952±148	2070±176	1918±158
Fat, g	100±11	83±7	87±9	78±8*
%	39±1	38±1	37±1	35±2*
Carbohydrates, g	239±23	225±17	244±19	229±18
%	44±1	46±2	47±1	48±2*
Protein, g	82±8	74±7	77±6	73±6
%	16±1	15±1	15±1	15±1
Alcohol, g	6±3	6±3	5±3	5±2
%	2±1	2±1	2±1	2±1
SFA, g	31±3	30±3	30±3	27±3
Cholesterol, mg	270±33	297±43	270±24	259±35
Fiber, g	17±1	18±1	16±1	15±1
Sodium, mg	3596±273	3661±356	3607±346	3408±352

All values are expressed as mean ± SEM. SFA, saturated fatty acids. Effect of treatment (*), time (†) and treatment x time interaction (‡). $P<0.05$.

Table 3. Circulating plasma cytokine and adiponectin levels before and after the control and ARB treatment.

	Control		ARB	
	Gender, Male=8, Female=8			
Variable	Pre	Post	Pre	Post
IL-6, pg/mL	23.5±6.7	20.0±3.6	21.5±5.2	26.2±7.4
TNF-α, pg/mL	8.1±1.3	6.6±0.7	5.7±0.3	9.3±2.5
IL-10, pg/mL	23.7±2.0	22.8±2.2	22.2±1.0	24.9±1.4
MCP-1, pg/mL	190±20	177±14	217±21	197±21
Total adiponectin, ng/mL	3702±455	3820±560	3632±521	3824±575
HMW adiponectin, ng/mL	3299±441	3275±453	3360±389	3357±512

All values are expressed as mean ± SEM. IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; IL-10, interleukin-10; HMW, high molecular weight. Effect of treatment (*), time (†) and treatment x time interaction (‡). $P<0.05$.

Table 4. Skeletal muscle mRNA before and after ARB treatment.

	ARB	
Variable (A.U.)	Pre	Post
NOX4 (n=12)	1.1±0.2	1.4±0.2
TGF-β (n=10)	28.8±3.4	29.8±1.9
IL-6 (n=8)	23.0±6.2	25.3±10.0
COL3 (n=7)	1491±510	774±271
MCP-1 (n=7)	4.7±1.1	5.0±1.4

All values are expressed as mean ± SEM. NOX4, NADPH oxidase 4; TGF-β, transforming growth factor-beta; IL-6, interleukin-6; COL3, collagen III; MCP-1, monocyte chemoattractant protein-1. Effect of treatment (*). $P<0.05$.

Table 5. Correlation matrix – Change in IVGTT variables and subject characteristics in the ARB treatment.

	ΔSI	ΔSg	ΔAIRg	ΔDI	ΔBody wt	ΔBody fat %	ΔGluc	ΔIns	ΔSBP	ΔDBP
ΔSI	1.00									
ΔSg	-.194	1.00								
ΔAIRg	-.624*	.479	1.00							
ΔDI	.637*	-.007	-.251	1.00						
ΔBody wt	-.450	-.050	.286	-.367	1.00					
ΔBody fat %	-.506	-.352	.212	-.369	.521	1.00				
ΔFasting Glucose	.015	-.281	.010	-.018	-.571	.189	1.00			
ΔFasting Insulin	-.544	.474	.703*	-.240	.114	.009	-.016	1.00		
ΔSBP	.088	-.156	.137	.200	.166	-.041	.012	.251	1.00	
ΔDBP	.161	-.115	.451	.126	.434	.539	-.171	.266	.308	1.00

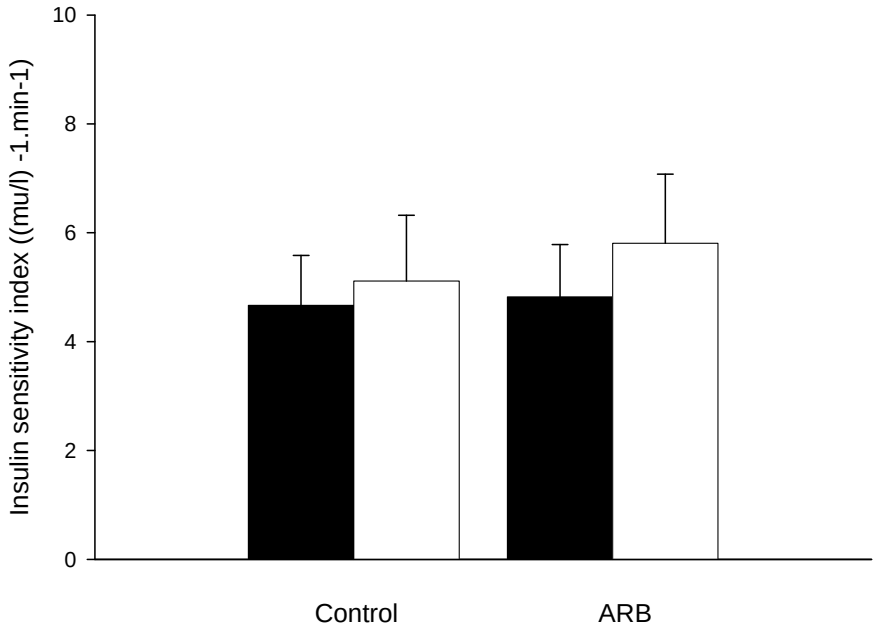
SI, insulin sensitivity index; Sg, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index; SBP, systolic blood pressure; DBP, diastolic blood pressure. Gender: Male=7, Female=5. *P<0.05 (for two-tailed test)

FIGURE LEGEND

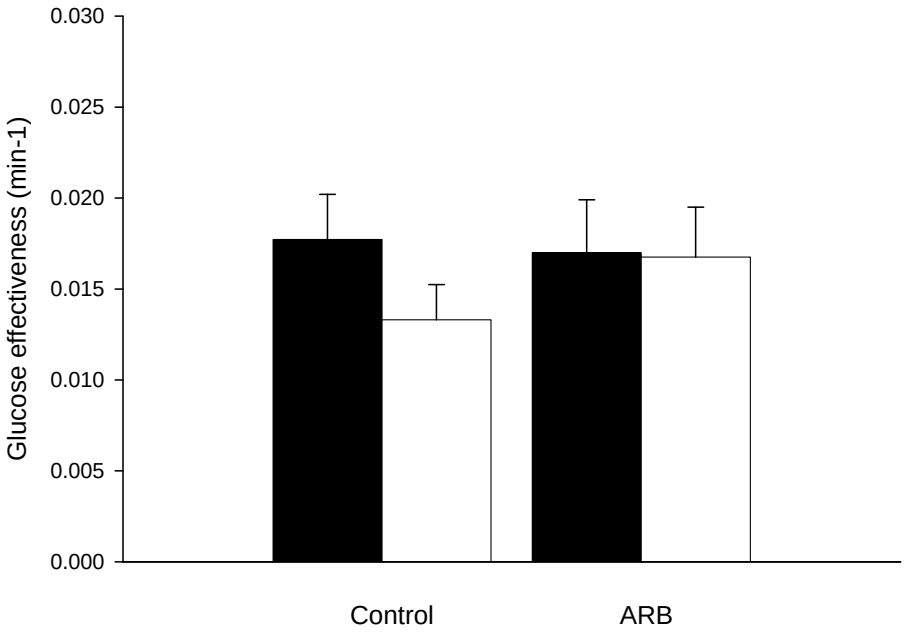
Figure 1. Absolute values of insulin sensitivity index (A), glucose effectiveness (B), acute insulin response to glucose (C) and disposition index (D) before and after the control and ARB treatment. Gender: Male=7, Female=5. Effect of treatment (*), time (†) and treatment x time interaction (‡). Values are mean ± SEM. $P<0.05$ vs. control treatment.

Figure 1

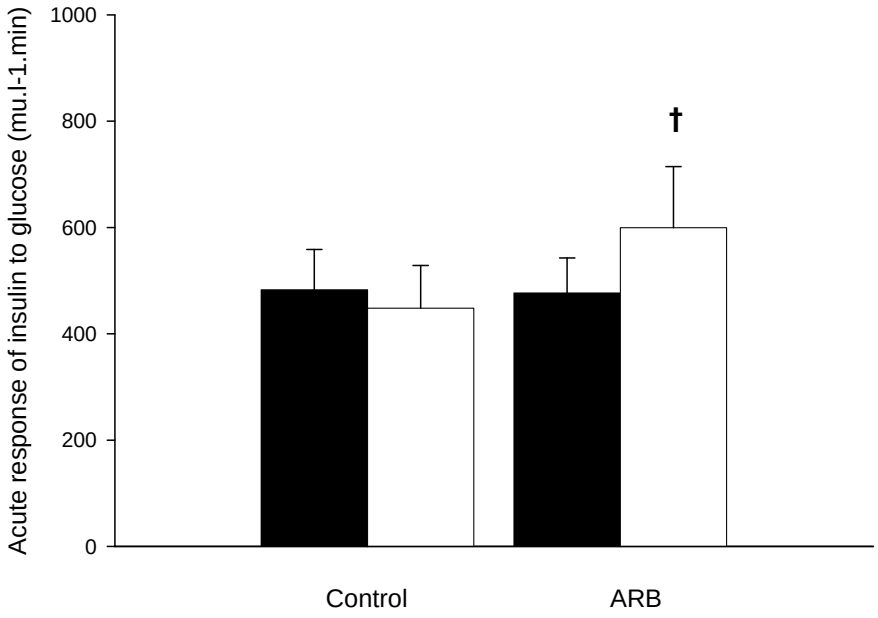
A


Insulin sensitivity index ((mu/l) -1.min-1)
10
8
6
4
2
0
Control
ARB

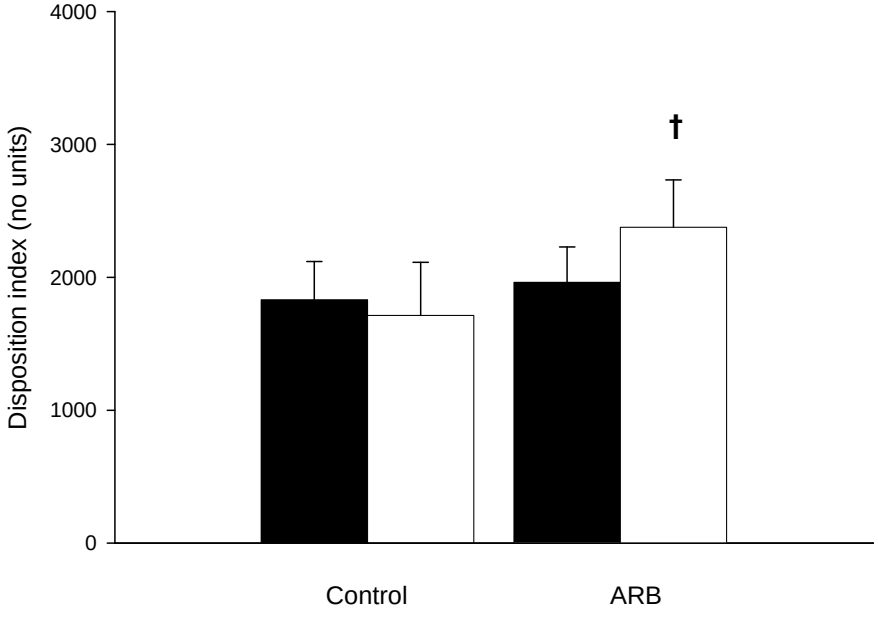
B


Glucose effectiveness (min-1)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Control
ARB

C


Acute response of insulin to glucose (mu.l-1.min)
1000
800
600
400
200
0
†
Control
ARB

D


Disposition index (no units)
4000
3000
2000
1000
0
†
Control
ARB

CHAPTER IV

Conclusions and Future Directions

Obesity is a major risk factor for the development of cardiovascular disease (CVD), type 2 diabetes, liver and kidney diseases, cancers as well as other chronic diseases. Overweight and obesity rates have risen steadily over the last few decades. Currently, 68% of the U.S. population is overweight, with ~34% classified as obese. Furthermore, the prevalence of hypertension has concomitantly risen to unprecedented figures and is present in ~60-75% of obese individuals. Moreover, obesity hypertension commonly leads to an insulin resistant state which, in turn, augments the progression of type 2 diabetes.

Intensive lifestyle modifications (e.g., diet and exercise) for weight loss are initially suggested to improve insulin action and reduce the incidence of type 2 diabetes in obese individuals. However, these interventions are limited by low rates of adoption and adherence over long periods. Thus, clinicians need alternative strategies, such as pharmacological approaches, for those patients in who lifestyle modifications have failed. Identification and utilization of effective agents is of utmost importance to reduce type 2 diabetes and CVD risk in obesity.

A primary mediator of insulin resistance in obese and hypertensive states is the renin-angiotensin system (RAS). Expression of all RAS components is increased in obesity hypertension and chronic activation of the RAS contributes to CVD development. However, researchers have begun to evaluate therapeutic effects of long-term RAS inhibition on CVD outcomes. It has been noted that RAS inhibitors, such as angiotensin converting enzyme inhibitors and angiotensin II receptor blockers, may have protective properties that extend beyond their blood pressure-lowering capabilities. Multiple large-scale clinical trials indicate

reduced cardiovascular events as well as reduced incidence of new-onset diabetes in at-risk hypertensive populations treated with RAS inhibitors. Thus, RAS treatment may prevent or delay the development of type 2 diabetes, though the role of RAS blockade needs to be clarified in obese hypertensives.

In this sense, the purpose of our research was to determine if RAS inhibition with the olmesartan, a non-PPARγ agonist Ang II receptor blocker (ARB), would improve whole-body insulin sensitivity in overweight and obese individuals with elevated blood pressure. In contrast to the hypothesis, we observed no significant changes in insulin sensitivity measures after 8-week olmesartan treatment in our subjects. Our study may have been underpowered to determine significant changes in insulin sensitivity or using an ARB without intrinsic PPARγ agonist activity may not have affected insulin sensitivity in our study population. Nonetheless, it is imperative to continue investigating the effect of RAS blockade on whole-body insulin sensitivity in this population. Accumulating evidence from animal and clinical studies supports that RAS inhibition has beneficial effects on insulin sensitivity in addition to reducing blood pressure in hypertensive populations.

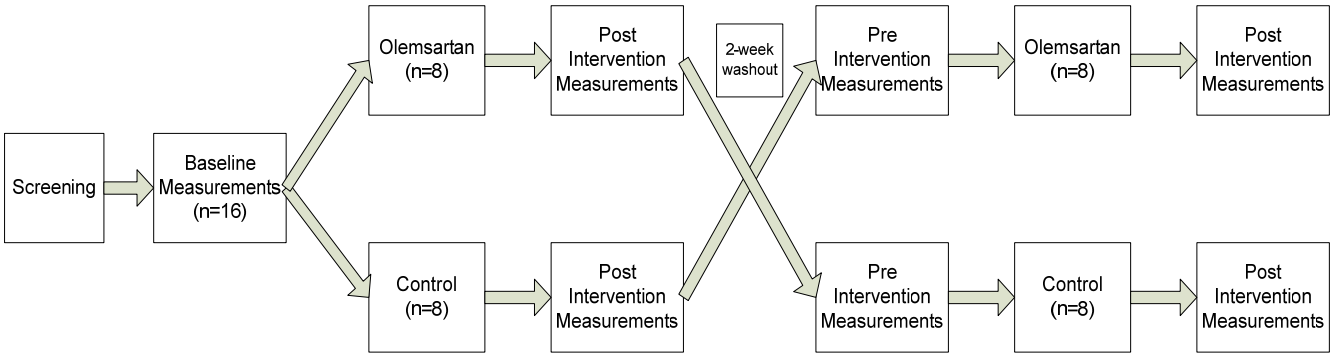
Additional studies are needed to confirm that whole-body insulin sensitivity is improved with the use of individual RAS inhibitors, with or without partial PPARγ activation, in hypertensive populations. If so, future studies could then determine if RAS antagonism augments glucose regulation in populations at an increased risk for developing diabetes (e.g., impaired fasting glucose or impaired glucose tolerance). In addition, RAS-related mechanisms contributing to the progression of type 2 diabetes could then be identified. Another consideration is whether lone RAS inhibitors are as effective as the combination of multiple RAS antagonists for improving insulin sensitivity in at-risk populations. Furthermore, additional long-term

prospective investigations are necessary to examine the effect of RAS inhibition-only interventions on the progression of type 2 diabetes in at-risk populations.

APPENDICES

Appendix A: Study design

Figure A1. Study design flowchart



Appendix B: Health History Questionnaire

Virginia Tech
Department of Human Nutrition, Foods, and Exercise

HEALTH HISTORY QUESTIONNAIRE

STUDY_____ **DATE**_____

SUBJECT ID #_____

PLEASE PRINT

1. Address:_____

   City:_____ State:_____ Zip Code_____

   Home Phone: _____ Work Phone:_____

   E-mail address:_____

   Emergency Contact:_____ Phone:_____

   Relation to you:_____

2. Employer:_____ Occupation:_____

3. Date of Birth:_____ Age:_____ Sex:___

   Race and/or Ethnic Origin

   ☐ American Indian or Alaskan Native ☐ Asian or Pacific Islander ☐ Black, not of Hispanic Origin

   ☐ Hispanic ☐ White, not of Hispanic Origin

   ☐ Other

4. **GENERAL MEDICAL HISTORY**

Do you have any current medical conditions? YES ☐ NO ☐
If Yes, please explain:

Are you allergic to any medications? YES ☐ NO ☐
If Yes, please explain:

Have you had any major illnesses in the past? YES ☐ NO ☐
If Yes, please explain:

Have you ever been hospitalized or had surgery? YES ☐ NO ☐
If Yes, please explain: (include date and type of surgery, if possible)

Are you currently taking any medications or supplements, including aspirin, hormone replacement therapy, or other over-the-counter products?
YES ☐ NO ☐
If Yes, please explain:

Medication/Supplement	**Reason**	**Times taken per Day**	**Taken for how long?**

Have you ever had an EKG? YES ☐ NO ☐
If Yes, please explain:

Have you been diagnosed with diabetes? YES ☐ NO ☐
If Yes, please explain:

Age at diagnosis_____

5. **FAMILY HISTORY**

	Age (if alive)	Age of Death	Cause of Death
Father	_____	_____	_____
Mother	_____	_____	_____
Brothers/Sisters	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

		YES	NO	Relation	Age at Diagnosis
a.	High blood pressure	☐	☐		
b.	Heart Attack	☐	☐		
c.	Coronary bypass surgery	☐	☐		
d.	Stroke	☐	☐		
e.	Diabetes	☐	☐		
f.	Obesity	☐	☐		

6. **TOBACCO/ALCOHOL HISTORY** (check one)

None ☐
Quit ☐ (when)_____
Cigarette ☐
Cigar ☐
Pipe ☐
Chew Tobacco ☐
Snuff ☐

CURRENT TOBACCO USE
(if applicable)
 # per day

Cigarette _____
Cigar _____
Pipe _____
Chew Tobacco _____
Snuff _____

Total years of tobacco use_____

Do you consume alcohol? Drinks per day ____ Drinks per week ____

7. **CARDIORESPIRATORY/METABOLIC HISTORY**

	YES	NO
Are you presently diagnosed with heart disease?	☐	☐
Do you have any history of heart disease?	☐	☐
Do you have a heart murmur?	☐	☐
Occasional chest pain or pressure?	☐	☐
Chest pain or pressure on exertion?	☐	☐
Episodes of fainting?	☐	☐
Daily coughing?	☐	☐
High blood pressure?	☐	☐
Shortness of breath?		
At rest?	☐	☐
lying down?	☐	☐
After 2 flights of stairs?	☐	☐
Do you have asthma?	☐	☐
Do you have a history of bleeding disorders?	☐	☐

	YES	NO
Do you have a history of problems with blood clotting?	☐	☐
Do you have high cholesterol? Or, low good (HDL) cholesterol?	☐	☐
Do you have thyroid problems?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

8. **MUSCULOSKELETAL HISTORY**

	YES	NO
Any current muscle injury or illness?	☐	☐
Any muscle injuries in the past?	☐	☐
Do you experience muscle pain at rest?	☐	☐
Do you experience muscle pain on exertion?	☐	☐
Any current bone or joint (including spinal) injuries?	☐	☐
Any previous bone or joint (including spinal) injuries?	☐	☐
Do you ever experience painful joints?	☐	☐
Do you ever experience swollen joints?	☐	☐
Do you ever experience edema (fluid build up)?	☐	☐
Do you have pain in your legs when you walk?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

9. **NUTRITIONAL HABITS**

Have you ever dieted? YES ☐ NO ☐

If YES, have you dieted within the past 12 months or are you currently on a diet?

YES ☐ NO ☐

If YES, please describe the diet:

a). Name (if applicable):_____

b). Prescribed by a Physician/nutritionist? YES ☐ NO ☐

c). Have you lost weight? YES ☐ NO ☐

d). Duration of diet_____

What was your weight 24 months ago? _____ 12 months ago? _____ 6 months ago? _____

Have you dieted other than in the past 12 months? YES ☐ NO ☐

If YES, please answer the following:

a). How many times have you dieted?

b). How old were you?

c). Weight loss (amount)?

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

10. **PHYSICAL ACTIVITY SURVEY**

Compared to a year ago, how much regular physical activity do you get? (Check one)

Much less ☐

Somewhat less ☐

About the same ☐

Somewhat more ☐

Much more ☐

Have you been exercising regularly for the past three months? YES ☐ NO ☐

If YES, what type of exercise do you regularly participate in? (check those that apply)

		Days per week	Minutes per session	Intensity (1=easy, 10=very hard)
Walking	☐	_____	_____	_____
Running	☐	_____	_____	_____
Cycling	☐	_____	_____	_____
Swimming	☐	_____	_____	_____
Aerobics	☐	_____	_____	_____
Weight Training	☐	_____	_____	_____
Martial Arts	☐	_____	_____	_____
Other (describe)		_____	_____	_____

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

11. **OBSTETRIC/GYNECOLOGICAL HISTORY**

	YES	NO
Do you have a normal menstrual cycle (1 menses each ~1 month)?	☐	☐

If no, please indicate frequency_____

Do you take any kind of contraceptive (oral, injectable, implant)?	☐	☐

If yes, please indicate type and name_____

How many full term pregnancies have you had? _____ How long ago was your more recent pregnancy?_____

Have long since you have last breast fed?_____

12. **SLEEP HISTORY**

Please answer yes/no or check appropriate answer.

	YES	NO
Do you snore?	☐	☐
	Don't Know ☐	

Snoring loudness

- ☐ Loud as breathing
- ☐ Loud as talking
- ☐ Louder than talking
- ☐ Very loud. Can be heard in nearby rooms.

Snoring frequency

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Does your snoring bother other people?	☐	☐
Has anyone told you that you quit breathing during your sleep?	☐	☐

How often have your breathing pauses been noticed?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Are you tired after sleeping?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Are you tired during waketime?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Have you ever fallen asleep while driving?

- ☐ Almost every day
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 1-2 times per month
- ☐ Never or almost never

Sleepiness Assessment

0 (zero) = would never doze off
1 (one) = slight chance of dozing
2 (two) = moderate chance of dozing
3 (three) = high chance of dozing

Situation	**Chance of Dozing**
Sitting and reading	___
Watching TV	___
Sitting, inactive in a public place (e.g., a theatre or meeting)	___
As a passenger in a car for an hour without a break	___
Lying down to rest in the afternoon when circumstances permit	___
Sitting quitely after lunch without alcohol	___
Sitting and talking to someone	___
In a car, while stopped for a few minutes in traffic	___

13. **EDUCATION**

Please check the highest degree obtained:

Grade School ☐
Junior High ☐
High School ☐
College Degree ☐
Master's Degree ☐
Doctorate ☐

14. **FAMILY PHYSICIAN**

Name:_____

Address:_____

_____

Phone: _____

Should it be necessary, may we send a copy of your results to your physician? YES☐ NO☐

Signature:_____ Date:_____

Witness:_____ Date:_____
Print Name Signature

Reviewer:_____ Date:_____
Print Name Signature

Appendix C: Infection/Inflammation Questionnaire

INFECTION/INFLAMMATION QUESTIONNAIRE

Evaluator Script: I would like you to think if you had a cold, the flu, a dental infection or other infection during the past month. I am going to ask you about some symptoms that may have accompanied those types of conditions.

1) Did you have a cold, the flu, a dental infection or other infection in the past month?
   ( ) Yes ( ) No ( ) Refused ( ) Don't Know
   If yes, ( ) Within 1 week ( ) 2 weeks prior ( ) 3 weeks prior ( ) 4 weeks prior

In the prior month did you experience any of the following symptoms? [Note to examiner: If symptom was present, the timing of symptom onset and resolution (# days) prior to interview is recorded. If symptom is still present on the day of interview, place 0 in "Resolved___days ago".]

2) Did you feel feverish or have a fever? ( ) Yes ( ) No
   If Yes, Symptom Started ___days ago. Resolved_____days ago.
   Did you take your temperature? ( ) Yes ( ) No

3) Chills? ( ) Yes ( ) No
   If Yes, Started____days ago. Resolved____days ago.

4) Sore throat ? ( ) Yes ( ) No
   If Yes,.Started____days ago. Resolved____days ago.

5) Coughing? ( ) Yes ( ) No
   If Yes, Started____days ago. Resolved____days ago.

6) Sputum? ( ) Yes ( ) No
   If Yes,.Started____days ago. Resolved____days ago.

7) Sneezing? ( ) Yes ( ) No
   If Yes, Started____days ago. Resolved____days ago.

8) Runny nose or nasal congestion? ( ) Yes ( ) No
If Yes, Started____days ago. Resolved____days ago.

If Yes to (5), (6), (7), or (8). Do you have seasonal allergies? ( ) Yes ( ) No
Do you have a chronic lung or sinus condition? ( ) Yes ( ) No
If Yes, are these symptoms typical for your chronic lung or sinus condition?
( ) Yes ( ) No

9) Ear pain or discharge? ( ) Yes ( ) No
If Yes, Started____days ago. Resolved____days ago.

10) Run down feeling or achy muscles you feel may have been due to a cold or flu?
( ) Yes ( ) No
If Yes, Started____days ago. Resolved____days ago.

11) Tooth/Gum pain? ( ) Yes ( ) No
If Yes, Started____days ago. Resolved____days ago.
If Yes, did you seek dental care?( ) Yes ( ) No
If Yes, did a Dentist find a cavity or other dental infection? ( ) Yes ( ) No

12) Mouth/gum (Y N), Skin (Y N), or Joint (Y N) redness or swelling?
If Yes, Started____days ago. Resolved____days ago.

13) Skin infection? ( ) Yes ( ) No
If Yes, Started____days ago. Resolved____days ago.

14) Nausea/Vomiting? ( )Yes( )No
If Yes, Started____days ago. Resolved____days ago.

15) Diarrhea? ( )Yes ( )No
If Yes, Started____days ago. Resolved____days ago.

16) Pain upon urination or urgency? ( )Yes ( )No
If Yes, Started____days ago. Resolved____days ago.

17) Cloudy discolored urine? ( )Yes ( )No
Urinalysis showing evidence of infection? ( )Yes ( )No
If Yes, Started____days ago. Resolved____days ago.

18) Did you seek medical care for any sort of cold, flu, or infection in the prior month?
( )Yes ( )No
If yes, diagnosis given_____

19) Did you take any over the counter or prescription medications for a cold, flu, or any infection in the prior month?
( )Yes ( )No
If yes, names of medication_____
_____
_____

Appendix D: Informed Consent for Subjects

Informed Consent for Participants of Investigative Projects
Department of Human Nutrition, Foods and Exercise
Virginia Tech

TITLE: Angiotensin II Receptor Blockade and Adipose Tissue Inflammation in Obesity

INVESTIGATORS: **Kevin P. Davy, Ph.D.**
Madlyn Frisard, Ph.D.
Matthew Hulver, Ph.D.

MEDICAL DIRECTOR: Jose Rivero, M.D.

SPONSOR: National Institutes of Health

PURPOSE:
Angiotensin II receptor blockers are commonly-used medicines to treat high blood pressure. The results of recent studies show that this and other similar medicines may also prevent type 2 diabetes, a disease associated with an increase in blood sugar. However, the biological reasons for this protective effect are not known. One possibility is that this medication increases the ability of fat and muscle to take up glucose by reducing inflammation in these tissues. Therefore, the purpose of this study is to determine if a particular angiotensin II receptor blocker, Benicar, reduces inflammation and improves the ability of the hormone insulin to help the body take up glucose, at least in part, by altering the inflammation in fat and muscle tissue. We will also determine how Benicar affects your blood vessels. Forty eight people will be included in this study.

METHODS:
You are being asked to participate in all of the sessions of the study described below. If you agree to participate in this study, you will first be required to complete a personal health history questionnaire and undergo blood and urine tests. The results of your medical history and blood and urine tests may be discussed with the study medical director to determine your eligibility. Based on our evaluation of the questionnaire and your current health, you may then be eligible to become a study subject. Eligible candidates will be males or females between 18 and 75 years of age. If you are female, you must be postmenopausal and not receiving hormone replacement to participate. You must have a body mass index greater than or equal to 25 kg/m^2 or a measured body fat of at least 20% for men and 25% for women to be included in the study. You should have a blood pressure greater than or equal to 120/80 mmHg but less than 160/100 mmHg. You will not be eligible to participate in the study if you have diabetes, secondary hypertension (hypertension with a known cause), a history of stroke, myocardial infarction or chronic kidney disease (or renal artery stenosis), or cardiovascular (e.g., chronic heart failure), respiratory (e.g., chronic obstructive pulmonary disease), neurological (e.g., Parkinson's), metabolic (e.g., hyperthyroidism), oncological (e.g., active cancer) diseases or other diseases that would make participation unsafe. The medical director of the study must approve your participation. You will not be able to participate if you take medications or nutritional supplements that might influence the study variables or make participation unsafe. You will not be able to participate if you exercise three or more times a week at a moderate to hard level (e.g., exercise that causes you to breathe hard and sweat).

You may be eligible to participate if you are currently taking blood pressure medications or vitamins/nutritional supplements. However, your blood pressure prior to starting medications must have been greater than or equal to 140/90 mmHg but less than 160/100 mmHg. Your own doctor must also approve your participation and agree to safely stop your current blood pressure medication for at least 2 weeks prior to starting the study as well as while you are participating in the study. You must also agree to have your blood pressure measured every other day for the 2-week period. If you are taking vitamins or nutritional supplement, you may be asked to stop taking these for at least 2 weeks before beginning the study. If your doctor has prescribed or recommended these

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supplements, we will need his/her approval for you to stop taking the vitamins or supplements. You will need to discuss the best way to start your medication with your doctor after completing the study. If your doctor does not approve or you are unwilling to stop your medication for the study, then you will not be allowed to participate in this study.

You are being asked to participate in both a Benicar treatment group and a group that does not receive Benicar. Each will require 10 weeks of your time with 2 weeks in between for a total of 22 weeks. The actual amount of time may differ by as much as one to two weeks depending on your schedule for testing. You will be assigned to participate in one of these groups first and then the other. The order will be determined by a process similar to a coin toss. During the Benicar treatment period, you will take one 20 mg Benicar pill every day for 2 weeks and then two 20 mg pills (40 mg of Benicar) for another 6 weeks. You will be provided with 1-2 weeks of medication at a time to take home with you. You will need to come back to the laboratory every 1-2 weeks to return unused medication as well as to obtain more medication for the next 1-2 week period. If your blood pressure falls below 110/70 mmHg during the first two weeks, then you will continue to take only one pill each day for the remainder of the study. You will continue to take Benicar during all of the follow-up measurements which take approximately 2 weeks. You will be asked to not change your daily diet and physical activity throughout the entire study. After the Benicar treatment period, there will be a 2-week period before you begin the next treatment period. This is to give your body a chance to get rid of all the Benicar. After each treatment period, you are being asked to participate in follow-up testing, which is a repeat of the testing sessions described below. Forty-eight people will be included in this study.

At the completion of the study, you will be instructed to see your own physician for follow-up care as soon as possible. Individuals who do not have a primary care physician will see Dr. Rivero for further evaluation. Dr. Rivero, the medical director of the study, is a board certified cardiologist specializing in the treatment of high blood pressure. At the end of the study, you will also be provided with additional information on lowering your blood pressure. You will be able to visit with a trained member of our staff on two occasions to discuss individual strategies for accomplishing these goals. If you withdraw from the study before it is completed, you will be referred back to your physician (or Dr. Rivero) for follow-up care so that your ongoing treatment can be evaluated.

You are being asked to participate in all of the testing sessions four times (except the medical history and Session 2), once at baseline and again after each study period. There will be approximately 50 visits if you participate in this study. The actual number and order of visits may depend on your schedule and the availability of the study staff. The session order may differ from the order of appearance in this document.

Session 1

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Urine Test:** You will be asked to urinate in a small cup that we provide to you. We will measure the amount of sodium and other electrolytes, glucose, protein, pH and whether there are blood cells present to determine whether it is safe for you to participate in the study.
- **Pregnancy Test:** If you are female who has not been postmenopausal for at least 2 years, you will be required to have a pregnancy test. This will require collection of 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant, you will not be able to participate in this study.
- **Medical History:** You will be asked to complete a medical history questionnaire. This procedure is used to screen for health problems or reasons you should not participate in this study.
- **Body Mass and Height:** Your height and weight will also be measured at this time. Your body weight will be measured on a standard digital scale and will include the weight of light indoor

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clothing or hospital gown without your shoes. Your waist, hip, and neck circumference will be measured using a measuring tape.

- **Body Composition:** This test is to measure your body fat. You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 5 minutes and there is no pain associated with the procedure. This procedure will be performed once at the beginning and a second time at the end of each treatment.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Blood Draw:** A small venipuncture needle will be inserted into a large forearm or hand vein to draw blood (approximately 3 tablespoons). The blood collected will be used to measure your blood sugar, cholesterol and other hormones that influence your health.

Approximately time required: 1.5 hours

Session 2

- **Health and Physical Exam:** The medical director (Jose Rivero, M.D.) will listen to your heart and lungs with a stethoscope, measure your blood pressure, review your blood and urine tests and ask you basic questions about your health history. This test will take place at Dr. Jose Rivero's medical office in Christiansburg. Directions will be provided to you.

Approximate time required: 15 minutes. There may be up to one hour of waiting time depending on the number of patients in Dr. Rivero's office.

Session 3

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Diet Records:** You will be asked to write down everything you eat for a 4-day period (3 consecutive weekdays and 1 weekend day) four times, at the beginning and end of each treatment period. This will be used to determine what and how much you eat on a daily basis.
- **Physical Activity Monitor and Questionnaire:** You will be asked a series of questions to estimate your usual physical activity level, which will require about 15 minutes to complete. You will also be asked to wear a small monitor to measure your physical activity performed during 3 consecutive weekdays (72 hrs) and 1 weekend day (24 hrs). The monitor is slightly larger than a watch and will clip to your belt or waistband and will not interfere with your normal daily activity.

Approximate time required: 60 minutes.

Session 4

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. This is to make sure that the test results will not be influenced by the food you eat or by the normal digestion process.
- **Blood Pressure:** You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an automated blood pressure monitor.
- **Arterial Stiffness**: To measure arterial stiffness, the blood flow and diameter in the arteries in your neck and leg will be measured with an ultrasound machine. An ultrasonic machine is sort of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a "TV-like" screen. A mobile hand unit used will be pressed gently against an artery in your neck

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and leg. The amount of blood that your heart pumps in one beat and in one minute will be measured with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest.

Approximate time required: 1 hour.

Session 5:

- **Overnight Fast:** You will be asked to avoid eating or drinking for 12 hours prior to your visit. Please also avoid strenuous exercise for 36 hours prior and aspirin or other non-steroidal anti-inflammatory medications (e.g., Tylenol, Ibuprofen) for 72 hours prior to this visit. This is to make sure that the test results will not be influenced by the food you eat, recent exercise or anti-inflammatory medication use.
- **Infection / Inflammation Questionnaire:** You will be asked to complete a questionnaire about any recent illnesses or infections that you may have had in the past month. You will also be asked to complete this questionnaire before the post testing sessions following each study period.
- **Fat Biopsy:** You should not take aspirin, ibuprofen or other non-steroidal, anti-inflammatory medications (such as Advil, Motrin, Naprosen, Celebrex), or other medications or substances that may affect bleeding or bruising, for 72 hours prior to and after this procedure. This procedure is used to sample a small amount of fat (about 2-4 g) from underneath the skin of the abdomen. The actual biopsy site will be on either the right or left side of the abdomen just above the level of where you would wear a belt. You will be asked to undergo this procedure twice during each treatment, once at baseline and once following the study period. This procedure will be performed by a study investigator (Kevin P. Davy, Ph.D.) or co-investigator (Matthew Hulver, Ph.D.) who has been specifically trained to perform the biopsy. Neither a physician nor a nurse will be onsite during this procedure. You will be lying down and your skin will be cleansed with an iodine-type solution (Providine or Betadine). If you are allergic to iodine, we will use chlorhexadine which does not contain iodine. A sterile drape will be placed over the area and your skin and fat tissue will be numbed by injecting numbing medication (lidocaine/bipivicaine) into the area with a small needle. If you allergic to lidocaine or bipivicaine, you cannot participate in this study. Then, a small incision (about 1/4 of an inch) will be made in the skin and a needle (a little thinner than a pencil) will be placed under the skin to remove a small amount of fat. Some suction will be applied to the other end of the needle to help remove the fat. After the biopsy is completed, pressure will be applied and the skin will be closed with sterile tape. To ensure cleanliness, the skin will be cleaned with saline and covered with gauze and a clear adhesive dressing. The site will then be wrapped with an ACE bandage. You will be asked to keep the ACE bandage on for at least 10-15 minutes. You may take Tylenol for any discomfort you may experience following the biopsy. We will use the biopsy samples to measure factors which contribute to inflammation. The biopsy will take place at the Human Integrative Physiology Laboratory (228 War Memorial Hall).

  You will be provided with instructions on how to care for the biopsy site as well as what to look for if a problem were to occur.

- **Muscle Biopsy:** You should not take aspirin, ibuprofen or other non-steroidal, anti-inflammatory medications (such as Advil, Motrin, Naprosen, Celebrex), or other medications or substances that may affect bleeding or bruising, for 72 hours prior and after this procedure. This procedure is used to sample a small amount of muscle (about 50-250 mg) from underneath the skin of the thigh. The actual biopsy site will be on the top of either the right or left leg half way between the knee and the hip. You will be asked to undergo this procedure twice during each treatment, once at baseline and once following the study period. This procedure will be performed by a study investigator (Kevin P. Davy, Ph.D.) or co-investigator (Matthew Hulver, Ph.D.) who has been specifically trained to perform the biopsy. Neither a physician nor a nurse will be onsite during the procedure. You will be lying down and your skin will be cleansed with an iodine-type solution (Providine or Betadine). If you are allergic to iodine, we will use chlorhexadine which does not contain iodine. A sterile drape will be placed over the area and your skin and fat tissue will be

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numbed by injecting numbing medication (lidocaine/bipivicaine) into the area with a small needle. If you allergic to lidocaine or bipivicaine, you cannot participate in this study. Then, a small incision (about 1/4 of an inch) will be made in the skin and a needle (a little thinner than a pencil) will be inserted to remove a small amount of muscle. Some suction may be applied to the other end of the needle to help remove the muscle. After the biopsy is completed, pressure will be applied and the skin will be closed with sterile tape. To ensure cleanliness, the skin will be cleaned with saline and will be covered with gauze and a clear adhesive dressing. The site will then be wrapped with an ACE bandage. You will be asked to keep the ACE bandage on for at least 10-15 minutes. You may take Tylenol for any discomfort you may experience following the biopsy. We will use the biopsy samples to measure factors which contribute to inflammation. The biopsy will take place at the Human Integrative Physiology Laboratory (228 War Memorial Hall).

Session 6:

- **Infection / Inflammation Questionnaire:** You will be asked to complete a questionnaire about any recent illnesses or infections that you may have had in the past month. You will also be asked to complete this questionnaire before the post testing sessions following the study period.
- **Intravenous Glucose Tolerance Test:** Two small plastic tubes (catheters) will be placed in each of two arm veins (different arms). The test involves injecting small amounts of glucose (0.3 mg/kg of body weight) and insulin (0.03 unit/kg body weight) into your veins (insulin is a hormone which helps your body's cells metabolize glucose). We will draw a small amount of blood (less than one half teaspoon) approximately 28 times over a 3-hour period. A registered nurse will be present to perform this test with the assistance of the investigators.

The total amount of blood drawn is equal to about one-half cup or 100 cc. The catheters will remain in your arms throughout the entire test. This test measures the ability of the hormone insulin to help your body cells take up glucose.

Approximate time required: 4 hours.

In addition to the above visits, you will be asked to return up to 20-25 additional times to have your blood pressure measured. We will measure your blood pressure at least once every week for the duration for the study. These will be scheduled at a time that is convenient for you and, if possible, at a time when you are scheduled to pick up additional medication when you are on the Benicar treatment. We will take an additional blood sample 1 week and at 6 or 7 weeks after beginning Benicar to make sure you are not developing kidney problems as a result of the medication. Each of these visits may take up to 30 minutes.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Provide an accurate history of any health problems or medications you use before the study begins.
- Inform the experimenters of any discomfort or unusual feelings including dizziness.
- Be on time and attend all of the scheduled testing sessions.
- Follow all participant instructions for each session.
- Maintain your current diet and daily physical activity level.
- Take only the number of pills of the medicine each day and return the unused portion at scheduled visits.
- Inform the study investigators if you are pregnant or intend on becoming pregnant.

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RISKS OF PARTICIPATION

- Benicar: Benicar is approved by the Food and Drug Administration (FDA) for the treatment of high blood pressure. Benicar is considered a very safe and effective medication. However, you should not take Benicar if you are pregnant, intend to become pregnant, or are nursing. If you are hypersensitive or allergic to this medication or any of its ingredients, you will not be permitted to participate in this study. You should report any thing new or different to the investigator regardless of whether you think it might be related to the study medicine. The most common side effect of this medication is dizziness. If you feel dizzy, faint, lightheaded or have an increased heart rate, you should sit or if possible lie down immediately. If these feeling do not go away soon after sitting or lying down, you should call 911 or have someone take you to the nearest emergency room. If you experience signs of severe allergic reaction (including difficulty breathing, tightness in the chest, or swelling of the mouth, face, lips, or tongue), you should call 911 or have someone take you to the nearest hospital emergency room. Do not take any more medication until this issue is discussed with the medical director. If you have an allergic response to the medication, you will not be able to continue. However, you may be able to continue if you have experienced dizziness but this will need to be determined by the medical director of the study. You should know that there have been extremely rare cases of acute kidney failure and/or death in patients who may be susceptible to the effects of angiotensin II receptor blockers such as those with kidney problems or congestive heart failure. However, if you have kidney problems, congestive heart failure or other diseases which may make participation unsafe, then you will not be allowed to participate. If you have any concerns while participating in the study, you can contact the investigator immediately (phone numbers are at the end of the document). He will notify Dr. Rivero immediately and get instructions for what you should do next.

- Stopping Medicines: You will not receive any health benefit from stopping your current medications. In fact, there is the potential for you to experience an increased risk of health complications when you stop taking your current blood pressure medicine particularly. However, you should know that this risk of health complications is extremely small given your blood pressure level and the short length of the study. We will measure your blood pressure every week during the study. We will let you and your doctor know immediately if you blood pressure increases too much. You should tell the study investigators immediately if have any concerns or experience anything new or different while participating in the study.

- Catheter and Blood Draw: Some pain or discomfort may be experienced when the catheter is inserted in the vein, but this persists for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200 (less than 0.5%), while the risk of infection or significant blood loss is 1 in 1000 (less than 0.1%). There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the catheter is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in catheter placement and blood draws will minimize these risks.

- Intravenous Glucose Tolerance Test: Because this procedure requires the placement of a catheter in an arm vein, the risks here are identical to that stated above. In addition, there is a small risk of low blood sugar occurring during or after the test. We will be monitoring your blood sugar frequently and can usually anticipate this before your blood sugar drops too low. If this happens, orange juice (with table sugar) or some other simple sugar containing food will be given to you. We will monitor your glucose until it returns to normal. A registered nurse will perform the test with the assistance of the investigators.

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- HIV/AIDS: Your blood will be tested for the presence of HIV if one of the study investigators is exposed to your blood. There will not be any cost to you for this test. The results will be sent to your primary care physician or the study medical director, Dr. Jose Rivero, if you do not have a primary care physician. He/she will discuss them with you and provide you with the necessary referral for further evaluation and/or counseling if your results are positive. The results of your test will remain confidential.

- DEXA Scan: The amount of radiation that you will receive in the DEXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.

- Pregnancy: You should not become pregnant during this study because of the exposure to x-rays and study drugs. If you are capable of having a child, you must have a negative pregnancy test before each session that may pose a risk to an embryo or fetus (x-ray exposure or medication injection). You must agree to use an effective method of birth control, such as abstinence, condom use, or use of an intrauterine device, to ensure that you will not get pregnant. If you become pregnant during this study, you must notify your study investigator immediately. There may be unforeseen risks to the embryo or fetus in the event that you become pregnant.

- Arterial Stiffness: There are no known risks associated with this procedure.

- Fat and Muscle Biopsies: If you are allergic to iodine, we will use another product called chlorhexidine to clean the biopsy site. If you are allergic to lidocaine/bipivicaine, you will not be allowed to participate in this procedure but you may participate in the rest of the study. There may be slight discomfort and burning when the local anesthetic is injected prior to the biopsy, but you are not expected to experience discomfort during the biopsy procedure. Bruising in the area of the fat biopsy for 1-2 weeks will likely occur, but local pressure and ice are applied to the site immediately after the procedure to limit this potential effect and its accompanying tenderness. You should not perform any strenuous exercise for 24 hours following this procedure. You should not take aspirin or ibuprofen for any discomfort you feel for at least 3 days following the procedure. You may use Tylenol as a pain reliever if necessary. You may also apply an ice pack for 20 min every 2 hours until the pain subsides. If you have pain that lasts longer than one day, you should contact the study investigator immediately. If you have any bleeding from the biopsy site you should contact the study investigator immediately. There is a slight risk of infection at the biopsy site. There is a small risk that you will become lightheaded, dizzy, or anxious before or during the procedure. All of these reactions are temporary and resolve within a short time after completing or stopping the procedure. If you have pain, swelling, redness, pus or foul-smelling drainage at the biopsy site with or without a fever, you should contact the study investigator immediately. These risks are minimized by having a trained individual perform the procedure and by using aseptic procedures and sterile instruments. You will be asked to return within 5 days after the biopsy to have the site checked to ensure proper healing.

You will likely receive a scar from each of the biopsies performed but these are expected to be very small. These scars usually turn a purple color in the weeks to months following the biopsy and then fade considerably over time. The study staff will show you several pictures of examples of the scarring (greater than 1 year old) that can occur following similar biopsy procedures. It is important that you understand that these are just examples of the scarring that can occur. The actual scar you receive may be smaller or larger or differ in coloring. Individuals with darker skin (e.g., African Americans, Hispanics and Asians) tend to scar more than those with lighter skin. You should consider this before you agree to participate.

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It is not possible to identify all potential risks in an experimental study; however, the study investigators and staff will take all possible safeguards to minimize any known and potential risks to your well-being.

Side effects are possible in any research study despite high standards of care and could occur through no fault of your own or the study doctors or the study staff.

BENEFITS OF PARTICIPATION

You will receive the following as part of your participation:

- Health and physical examination by a physician.
- Information on your blood pressure, cholesterol and other risk factors for cardiovascular disease.
- Information on healthy lifestyle habits including increased physical activity, weight loss and reducing sodium intake.
- Improve general medical knowledge

COMPENSATION

You will receive $25 for each time you complete session 5 and another $25 for each time you complete session 6. You are being asked to complete these sessions four times. The total amount of compensation you can receive is $200. If you do not complete the study, you will be compensated for the sessions you have completed.

CONFIDENTIALITY

The data from this study will be kept strictly confidential. In the event that any of your tests indicate you may have a health problem, Dr. Rivero and the investigators may need to discuss this information with your doctor. The Food and Drug Administration may require that your identifying information be released to them in the event that you are injured as a result of participating in this study. For all other situations, your study information will be identified only by a code of numbers and letters, without anything to identify you by name.

FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time for any reason. Simply inform the experimenters of your intention to cease participation. You should understand that circumstances may come up that the researcher will determine that you should not be a subject in the study. For example, lack of compliance to instructions, failure to take the medication or placebo, and illness could be reasons for the researchers to stop your participation in the study. If you withdraw, it is important that you consult your personal physician on how to change from the study medication back to your original prescription.

INJURY DURING PARTICIPATION IN THIS STUDY

Neither the researchers nor the University have money set aside to pay for medical treatment that would be necessary if you are injured as a result of your participation in this study. Any expenses that you incur including emergencies and long term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

REVIEW OF RESEARCH

This research protocol has been submitted to and reviewed by the Virginia Tech Institutional Review Board For Research Involving Human Subjects, and was found to meet the requirements set forth in federal laws and regulations governing the protection of human subjects. You will receive a copy of this form to take with you.

SUBJECT PERMISSION

I have read the informed consent and fully understand the procedures and conditions of the project. I have had all my questions answered, and I hereby give my voluntary consent to be a participant in

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this research study. I agree to abide by the rules of the project. I understand that I may withdraw from the study at any time.

If you have questions, you may contact:

- Principal Investigator: Kevin Davy, Associate Professor, Department of Human Nutrition, Foods, and Exercise. (540) 231-3487; After hours: 540-230-0486
- Chairman, Institutional Review Board for Research Involving Human Subjects: David Moore, (540) 231-4991

Name of Subject (please print)_____

Signature of Subject_____ Date_____

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Appendix E: Institutional Review Board Approval

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24060
540/231-4606 Fax 540/231-0959
e-mail irb@vt.edu
Website: www.irb.vt.edu

MEMORANDUM

DATE: October 12, 2011

TO: Kevin P. Davy, Madlyn Frisard, Matthew Hulver, Jose Rivero, Elaina Marinik, Kristin Osterberg

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: Angiotensin II Receptor Blockade and Adipose Tissue Inflammation in Obesity

IRB NUMBER: 07-555

Effective November 9, 2011, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the continuation request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at http://www.irb.vt.edu/pages/responsibilities.htm (please review before the commencement of your research).

PROTOCOL INFORMATION:
Approved as: **Full Board Review**
Protocol Approval Date: **11/9/2011 (protocol's initial approval date: 12/10/2007)**
Protocol Expiration Date: **11/8/2012**
Continuing Review Due Date*: **10/25/2012**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:
Per federally regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Date*	OSP Number	Sponsor	Grant Comparison Conducted?
12/17/2007	07266106	NIH	yes on 12/17/2007

*Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.

cc: File
OSP