Vascular Consequences of Prehypertension

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VASCULAR CONSEQUENCES OF PREHYPERTENSION

By

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A Dissertation submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of
Doctor of Philosophy

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This dissertation entitled:
*Vascular Consequences of Prehypertension*
written by Brian Raymond Weil
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The final copy of this dissertation has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

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ABSTRACT

Weil, Brian Raymond (Ph.D., Integrative Physiology)

Vascular Consequences of Prehypertension

Dissertation directed by Professor Christopher A. DeSouza, Ph.D.

Prehypertension, defined as systolic blood pressure between 120-139 mm Hg and/or diastolic blood pressure between 80-89 mm Hg, is a common condition that affects ~30% of the US adult population. It is increasingly recognized that prehypertension is not only an independent risk factor for the development of clinical hypertension (blood pressure ≥ 140/90 mm Hg), but also cardiovascular disease and its clinical consequences, such as myocardial infarction, stroke, and congestive heart failure. The increased cardiovascular risk associated with prehypertension may be mediated, at least in part, by vascular endothelial dysfunction, a critical etiological event in the pathogenesis and progression of atherosclerotic vascular disease. However, the impact of blood pressure in the prehypertensive range on the vascular endothelium is currently unclear. Accordingly, the purpose of this dissertation was to determine: 1) if prehypertension is associated with impaired nitric oxide (NO)-mediated endothelium-dependent vasodilation; 2) whether endothelin (ET)-1 vasoconstrictor tone is elevated in prehypertensive adults; and, if so, 3) whether the increase in ET-1-mediated vasoconstriction contributes to endothelial vasodilator dysfunction in prehypertensive adults. To address these aims, venous occlusion plethysmography was used to measure forearm blood flow responses to intra-arterial acetylcholine, sodium nitroprusside, and selective and non-selective ET-1 receptor blockade in normotensive and prehypertensive adults. In addition, forearm blood flow responses to acetylcholine were determined with concomitant endothelial NO synthase inhibition and selective ET-1 receptor blockade. The results of these studies indicate that: 1) prehypertension is
associated with impaired NO-mediated endothelium-dependent vasodilation; 2) ET-1 vasoconstrictor tone is greater in prehypertensive compared with normotensive adults; and 3) the elevation in ET-1-mediated vasoconstriction contributes to impaired endothelium-dependent vasodilation with prehypertension. Collectively, these findings demonstrate that blood pressure in the prehypertensive range, independent of other cardiovascular risk factors, is associated with impaired endothelial vasomotor function. Reduced NO-mediated endothelium-dependent vasodilation and elevated ET-1-mediated vasoconstrictor tone represent an atherogenic endothelial phenotype that may contribute to the elevated risk of clinical hypertension and acute vascular events in prehypertensive adults.
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LITERATURE REVIEW

Prehypertension, Endothelial Function, and
Cardiovascular Disease Risk

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Introduction

Hypertension, defined as systolic blood pressure (BP) $\geq 140$ mm Hg systolic and/or diastolic BP $\geq 90$ mm Hg, is a major cardiovascular disease (CVD) risk factor that affects more than 25% of the worldwide adult population, with projections that this number will increase to $\sim 30\%$ by the year 2025 (1). Elevated BP in the hypertensive range is estimated to cause 7.1 million deaths each year, which accounts for 13% of all global deaths (2). Hypertension is a primary risk factor for premature cardiovascular and cerebrovascular morbidity and mortality (3-5). Furthermore, the World Health Organization has estimated that $\sim 62\%$ of cerebrovascular disease and $\sim 49\%$ of ischemic heart disease worldwide can be attributed to elevated BP levels (2). In the United States, one in three adults has hypertension (6) and the direct and indirect medical costs of high blood pressure in 2009 were approximately $73.4$ billion (7). Due to the high prevalence of hypertension and the significant health risks and medical costs associated with this condition, it has been suggested that high BP is the most important modifiable risk factor for cardiovascular, cerebrovascular, and renal disease (8,9).

In an attempt to assist clinicians in diagnosis, vascular risk assessment, and management of patients with elevated BP, the United States Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) and other international societies have categorized BP into discrete categories. The thresholds that have defined hypertension have evolved since the initial JNC report in 1976 due to the accumulation of new observational and epidemiologic data. While the first JNC report stated that adults with BP between 140/90 and 160/95 mm Hg should be monitored without specific treatment depending on age, it was not until JNC III, published in 1984, that it had become clear that a BP higher than 140/90 mm Hg required treatment, regardless of age (10). This threshold for hypertension has
remained in place throughout subsequent JNC reports, despite other changes in classification and treatment guidelines. However, several large observational investigations have demonstrated a continuous relation between elevated BP and cardiovascular risk that begins at BP ranges well-below the hypertension threshold of 140/90 mm Hg (5,11,12). For example, results from a worldwide meta-analysis of almost 1 million adults indicate that the risk of cardiovascular death doubles with every 20/10 mm Hg elevation in BP, starting at 115/75 mm Hg (5). As a reflection of the linear relation between elevated BP and cardiovascular risk, the guidelines set forth by the seventh and most recent report from the JNC include a new category termed “prehypertension” to describe individuals with a systolic BP between 120 and 139 mm Hg and/or a diastolic BP between 80 and 89 mm Hg (3).

The addition of the prehypertension category to the JNC guidelines was made in an effort to alert the public and health care providers to the increased risk associated with elevated BP levels, even at ranges that were previously considered normal (13). However, the new classification guidelines were not universally accepted; other international societies formulating guidelines for high BP did not adopt the term “prehypertension” into their recommendations (14-17). Furthermore, the addition of prehypertension to the JNC guidelines stimulated debate regarding the necessity for revising the classification system and the health impact of BP in the prehypertensive range (18). Nevertheless, those involved in formulating the JNC VII guidelines have stood by the consistent finding that prehypertensive individuals are at a greater risk of developing hypertension and experiencing adverse cardiovascular consequences (13).

A large body of evidence suggests that damage and dysfunction of the vascular endothelium is a key mechanism by which elevated BP promotes the development and
progression of atherosclerotic vascular disease (19). Indeed, hypertension is associated with abnormal endothelial function in the peripheral, coronary, and renal circulation (20-22). However, the impact of BP in the prehypertensive range on the vascular endothelium is currently unknown. Insight into the influence of prehypertension on endothelial function would provide a greater understanding of the mechanisms responsible for the increased risk of CVD in this population, as well as reinforce the importance of alerting society to the negative health implications of BP in the prehypertensive range.

**Prehypertension**

*Epidemiology*

Prehypertension is a common condition in the US and other countries (23). Data from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) indicate that ~25% of the US population above 20 years of age has prehypertension, including ~32 million men and ~21 million women (24). Other estimates of the prevalence of prehypertension in the US are even higher; there is evidence from the Framingham Heart Study that 43% of men and 39% of women between the ages of 35 and 63 years have BP in the prehypertensive range (25). The prevalence of prehypertension is similar across ethnicities, with only small variations between non-Hispanic whites (31.2%), non-Hispanic blacks (30.4%), Mexican-Americans (30.9%), and other ethnic groups (31%) (26). As with hypertension, prehypertension is commonly found in association with other traditional cardiovascular risk factors, particularly smoking, diabetes, obesity, and dyslipidemia (11,12,27,28). In addition to these traditional risk factors, several novel biomarkers are elevated in people with prehypertension, including markers of inflammation such as C-reactive protein and tumor necrosis factor-α (29-31).
Cardiovascular Risk

Individuals with prehypertension are at a greater risk of progressing to hypertension than those who are normotensive. Data from the Framingham Heart Study indicate that the four-year risk of developing hypertension for individuals aged 35-65 years is 18% for those with baseline BP 120-129/80-84 mm Hg (lower prehypertensive range) and 37% for those with BP 130-139/85-89 mm Hg (upper prehypertensive range), compared with 5% for subjects with normal BP (32). Corresponding rates of developing hypertension for adults aged 65 years and older were 26% and 50% in the lower prehypertensive range and upper prehypertensive range, respectively (32). Similar results were observed in a longitudinal study of over 2,000 British adults, which found that the risk of developing hypertension over a 7-year follow up period was two-fold greater for those in the lower prehypertensive range and 2.9-fold higher for those in the upper prehypertensive range compared with normotensive individuals (33). These findings of an increased risk of hypertension in prehypertensive individuals have been supported by several other investigations (25,34,35), providing support for the fact that prehypertensive adults are more likely to become hypertensive than normotensive adults.

In addition to the increased risk of progressing to hypertension, prehypertension is now considered to be an independent risk factor for CVD. Prior to publication of the JNC VII guidelines, Vasan and colleagues found that high-normal BP (i.e., 130-139/85-90 mm Hg; from the JNC VI guidelines) is an independent risk factor for major cardiovascular events, including myocardial infarction, stroke, and congestive heart failure (12). High-normal BP was associated with a significantly elevated hazard ratio for CVD of 2.5 in women (95% confidence interval (CI): 1.6 - 4.1) and 1.6 in men (95% CI: 1.1 – 2.2), even after adjustment for other cardiovascular
risk factors. Furthermore, the ten year absolute risk for a major cardiovascular event in individuals with high-normal BP above the age of 65 years was ~20% in both men and women (12). More recently, prehypertension was independently associated with an increased risk of CVD after an eighteen-year observation period in participants of NHANES I (1971-1975) (11). After adjustment for traditional CVD risk factors, including age, body mass index, and history of cigarette smoking, prehypertensive subjects had a 32% higher risk for major CVD events compared with those with normal BP (11). In the Women’s Health Initiative study, ~40% of the 60,785 postmenopausal women studied were prehypertensive and, after an eight year follow-up period, these women had a significantly higher risk of myocardial infarction (76%), stroke (93%), heart failure (36%), and cardiovascular death (58%) compared to normotensive women (36). Finally, Qureshi and colleagues found that prehypertension was significantly associated with an increased risk of coronary artery disease and myocardial infarction after a ten-year follow-up period (25). Strikingly, the population-attributable risk associated with prehypertension was 47% for myocardial infarction and 20% for coronary artery disease, nearly all of which was due to elevated systolic BP (25). These data emphasize the significant public health impact of prehypertension and the risk of CVD in this population.

Prehypertension is also associated with accelerated progression of atherosclerosis (37) and elevated markers of subclinical CVD (38-40). Data from the Coronary Artery Risk Development in Young Adults (CARDIA) study indicate that the development of prehypertension between the ages of 18 and 35 is associated with greater levels of coronary artery calcium, which is also a strong predictor of future coronary heart disease, after a twenty year follow-up period (39). In addition, individuals with “borderline hypertension” (BP = 130-140/85-90 mm Hg) have a greater intima-media thickness of the carotid and brachial arteries
compared with subjects with a BP less than 130/85 mm Hg (40). Using JNC VII guidelines, Manios and colleagues measured intima-media thickness in individuals with prehypertension and normal BP. Prehypertensive individuals had a higher carotid artery intima-media thickness and a greater left ventricular mass than subjects with normal blood pressure, indicating that the atherosclerotic burden associated with high BP begins to develop during the prehypertensive state (38).

However, not all studies have found prehypertension to be an independent CVD risk factor. Mainous and colleagues (41) found prehypertension to be associated with a significant risk of all-cause and CVD-related mortality only in individuals below the age of 55 years. Furthermore, prehypertension was no longer associated with a significant increase in risk when the authors controlled for baseline cardiovascular risk factors and markers for concomitant CVD (41). Similarly, Lee et al. (42) recently published data from the Singapore Cardiovascular Cohort Study indicating that prehypertension is only associated with an increased risk of all-cause or CVD-related mortality in the presence of diabetes, smoking, or pre-existing CVD. These findings have fueled debate regarding the cardiovascular risks associated with prehypertension, and it remains controversial as to whether prehypertensive BP alone or its common association with other CVD risk factors is responsible for the increased risk observed in this population.

Summary

The inclusion of prehypertension in the guidelines set forth by the JNC VII identifies a new population of individuals at a higher risk for developing CVD. In a majority of epidemiological studies, prehypertension has been shown to be an independent risk factor for
CVD, in addition to being associated with a higher risk of developing hypertension. Furthermore, evidence of elevated markers of subclinical CVD in individuals with prehypertension provides compelling evidence to suggest that the development of atherosclerotic vascular disease associated with high BP occurs prior to the progression to essential hypertension. However, evidence that prehypertension is only associated with increased risk in the presence of other CVD risk factors has encouraged debate as to whether prehypertensive BP alone is harmful to the cardiovascular system.

The Vascular Endothelium

The mechanisms contributing to the development of atherosclerotic vascular disease are largely mediated through damage to the vascular endothelial monolayer, resulting in its dysfunction (43). The vascular endothelium is a monolayer of cells that lines the luminal surface of blood vessels. Originally considered to be simply a passive interface between the blood and tissues, the vascular endothelium is now recognized as a metabolically active layer of cells that serve several structural, metabolic, and signaling functions that maintain vascular homeostasis (43). Healthy endothelium is capable of sensing physical and chemical stimuli and responding to these factors by generating a wide range of substances that modulate vasomotor tone and hemostatic balance, protect the vessel from potentially harmful substances circulating in the blood, and regulate innate and adaptive immunity (44). Thus, the normal vascular endothelium provides a protective, non-thrombogenic surface with homeostatic vasomotor and anti-inflammatory properties (43).

Due to its location and exposure to pathogenic stimuli in the circulating blood, the endothelium is a primary target for injury (43). Endothelial injury can increase cell permeability,
alter the release of vasoactive substances, and interfere with its inherent anti-thrombotic properties (45,46). This impairment of function represents one of the earliest events in the pathogenesis of atherosclerotic vascular disease, and contributes to plaque progression and thrombus formation (47,48). Therefore, maintenance of endothelial function is essential in preventing the development and progression of atherosclerotic disease. Vasomotor control and fibrinolytic regulation are two key components of vascular endothelial function and play an integral role in maintaining the vasoprotective and thromboresistant properties of the endothelium (44,49).

**Endothelial Vasomotor Function: Role of Nitric Oxide**

Regulation of vasomotor tone is a key function of the vascular endothelium and is accomplished through the synthesis and release of substances that modulate contraction and relaxation of vascular smooth muscle cells (VSMC) in the underlying medial layer of blood vessels.

Endothelium-dependent vasodilation occurs primarily through the secretion of nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). NO is the primary regulator of endothelium-dependent vasodilation and plays a particularly important role in maintaining the health of the vessel wall (47). NO is a labile, lipid-soluble gas synthesized in endothelial cells (ECs) from the amino acid L-arginine via the action of endothelial NO synthase (eNOS) (50). Physiologic (e.g., shear stress) and pharmacologic (e.g., thrombin, acetylcholine) stimuli are capable of increasing enzymatic activity of eNOS via increases in intracellular \([\text{Ca}^{2+}]\). Following diffusion from ECs to VSMCs, NO activates guanylate cyclase, resulting in increased intracellular concentrations of cyclic guanosine monophosphate (cGMP). cGMP activates
protein kinase G, which in turn leads to VSMC relaxation through mechanisms that involve a reduction in cytosolic [Ca\textsuperscript{2+}] (43).

Activation of protein kinase G also results in the phosphorylation of a number of proteins that are involved in the inhibition of smooth muscle cell proliferation, expression of adhesion molecules, and platelet aggregation (51). Thus, in addition to its vasodilatory properties, NO has anti-thrombogenic effects. As a result, reductions in the bioavailability of endothelium-derived NO can cause a shift toward a pro-thrombotic and vasoconstrictive state, which contributes to the development of atherosclerotic vascular disease (44).

**Endothelial Vasomotor Function: Role of Endothelin-1**

The vascular endothelium is also capable of releasing vasoconstrictor substances. The primary vasoconstrictor peptides produced by the endothelium are the endothelins (ETs). The ET family consists of four 21-amino-acid peptides: ET-1, ET-2, ET-3, and ET-4 (52,53). Of these peptides, ET-1 is the major vascular isoform and of most importance in the cardiovascular system (54). Large pre-proendothelin peptides are cleaved by endopeptidases to form big ET-1, which is converted to ET-1 by ET-converting enzymes (ECE) (55). Factors that modulate expression of ET-1 include shear stress, angiotensin II, thrombin, hypoxia, and inflammatory cytokines such as tumor necrosis factor (TNF)-\(\alpha\) and interleukin (IL)-2 (55). The majority of ET-1 produced by the endothelium (>80%) is released abluminally toward the vascular smooth muscle (56).

The vascular actions of ET-1 are mediated by two distinct receptor subtypes. ET\(_A\) and ET\(_B\) receptors located on the vascular smooth muscle mediate the vasoconstrictor effects of ET-1. Binding of ET-1 to these receptors activates the phospholipase C-inositol triphosphate
pathway, which results in an increase in intracellular \([\text{Ca}^{2+}]\), leading to phosphorylation of myosin kinase and subsequent smooth muscle cell contraction \((52,57)\). ET\(_B\) receptors are also found on ECs, where their activation results in vasodilation mediated primarily by NO \((58)\). Thus, activation of ET\(_B\) receptors can lead to dual vasoregulatory actions.

ET-1 system activation has been implicated in the initiation and progression of atherosclerotic vascular disease \((59)\). Increased expression of ET-1 and ECE has been observed in human arteries at different stages of atherosclerosis \((60,61)\), and elevated levels of ET-1 have been found in human atherosclerotic lesions \((60,62,63)\). There are several potential mechanisms by which ET-1 may contribute to atherogenesis. ET-1 stimulates platelet aggregation, cell adhesion molecule expression, and smooth muscle cell proliferation, all of which are characteristic features of atherosclerotic lesion development \((64,65)\). ET-1 is also involved in the inflammatory component of atherosclerosis via activation of the transcription factor NF-\(\kappa\)B, which stimulates the production of proinflammatory cytokines and activates leukocyte chemotaxis \((64)\). As a result, ET-1 system activation is involved in both vasomotor regulation and atherosclerotic disease progression.

*Endothelial Fibrinolytic Regulation*

In addition to the control of vasomotor tone, the endothelium regulates the endogenous fibrinolytic system. The fibrinolytic system serves as a counterbalance against coagulation and protects against thrombus formation by regulating fibrin deposition on the surface of the endothelium \((49)\). The modulation and resolution of thrombi associated with unstable atherosclerotic plaques are dependent on the effectiveness of the fibrinolytic system \((49)\).
Dysfunction of endothelial fibrinolytic regulation leads to a prothrombotic state and an increased risk of atherothrombotic vascular disease (49).

The vascular endothelium regulates fibrinolysis through the synthesis and release of activators and inhibitors, including the pro-enzyme plasminogen, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor (PAI)-1, and plasmin (49). Following the initiation of thrombus formation, the endothelium acutely releases t-PA in response to several factors related to the coagulation cascade, including thrombin and factor Xa (66). Upon its release, t-PA binds to thrombus-bound plasminogen and catalyzes its conversion to plasmin, which facilitates thrombus dissolution through the proteolytic degradation of fibrin to soluble fibrin degradation products (49). Synthesis and release of t-PA is a critical aspect of endothelial function, as it is the primary activator of the fibrinolytic system.

The endothelium is the principal site of t-PA generation (49). Endothelial cells synthesize and constitutively secrete t-PA in vitro (67). This continuous release contributes to the ~5 ng/mL concentration of t-PA antigen that is typically found in human plasma (49). However, only a small amount is active due to the presence of serine protease inhibitors, particularly PAI-1. The interaction of PAI-1 and t-PA occurs rapidly and PAI-1 concentrations are several-fold higher than t-PA concentrations in plasma, yielding a large amount of plasma t-PA inactive (68,69). Upon stimulation, however, the endothelium is capable of substantially increasing t-PA release. The precise signaling pathway has yet to be elucidated, but evidence suggests that the primary factor responsible for the acute release of t-PA from intracellular storage vesicles is a large increase in cytosolic [Ca^{2+}] (70,71). Acute release of t-PA is crucial to the effectiveness of the fibrinolytic system as it allows active unbound t-PA to reach the surface of a developing thrombus (49). Indeed, fibrinolysis is much more effective if t-PA is
incorporated during, as opposed to after, thrombus formation (72). As a result, the capacity of the vascular endothelium to rapidly release t-PA represents a vital component of the endogenous fibrinolytic system (73).

Summary

The vascular endothelium serves several functions that maintain vascular homeostasis and preservation of endothelial function is a key component of cardiovascular health. Endothelial dysfunction, including dysregulation of vasomotor and fibrinolytic function, represents a key mechanism contributing to the development of atherosclerotic vascular disease in at-risk populations.

Hypertension and Vascular Endothelial Dysfunction

Because of the significant cardiovascular risks associated with elevated BP, vascular endothelial function has been a target of extensive study in hypertensive patients. These investigations indicate that hypertension is associated with endothelial dysfunction in the peripheral, coronary, and renal circulation (20-22). Endothelial dysfunction has been suggested to represent an important mechanism by which hypertension promotes the development and progression of CVD (19). In fact, Perticone et al. observed that impaired endothelium-dependent vasodilation in the forearm was significantly associated with the development of clinical cardiovascular events after a mean follow-up period of ~32 months in a group of patients with essential hypertension (74).

Hypertension and Endothelial Vasomotor Function: Nitric Oxide

Early studies showing impaired endothelium-dependent vasodilation in animal models of hypertension provided the rationale for further research examining the role of endothelial
dysfunction in hypertensive humans (75-77). Two studies that were published in 1990 provided the first evidence of endothelial dysfunction in patients with essential hypertension in vivo (22,78). Panza and colleagues studied the forearm blood flow response to intra-arterial infusions of acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, an endothelium-independent vasodilator, in 18 hypertensive patients and 18 non-hypertensive control subjects (22). The forearm blood flow response to acetylcholine was significantly lower in the hypertensive subjects compared with control subjects, while no significant differences in the response to sodium nitroprusside were observed between groups, indicating that endothelium-dependent vasodilation is impaired in patients with essential hypertension, without any loss of vascular smooth muscle function (22). These findings have been replicated in several subsequent studies in the human forearm circulation, reinforcing the idea that hypertension is associated with impaired endothelium-dependent vasodilation (79-84). Importantly, this dysfunction in endothelium-dependent vasodilation extends to the coronary vascular bed in patients with essential hypertension (20,85).

Due to the importance of NO in maintaining normal endothelial function, the effects of hypertension on NO bioavailability have been studied. Calver and colleagues infused the eNOS inhibitor $N^G$-monomethyl-L-arginine (L-NMMA) intra-arterially to study basal NO activity in hypertensive patients and non-hypertensive controls (86). Patients with essential hypertension had a significantly blunted vasoconstriction response to L-NMMA compared with control subjects, indicating reduced NO availability with hypertension (86). Furthermore, urinary excretion of $^{15}$N nitrate, the metabolic oxidation product of NO, following administration of $^{15}$N-labelled arginine was reduced in hypertensive patients compared with non-hypertensive controls, indicating that whole body NO production is diminished under basal conditions (87). While the
authors of these studies concluded that hypertension results in impaired NO bioavailability and subsequent endothelial dysfunction, it has also been postulated that reductions in basal NO production may contribute to the development of high BP. This idea is supported by results from Gamboa et al., who used systemic L-NMMA infusion during complete autonomic blockade to show that endothelial-derived NO tonically restrains BP by approximately 30 mm Hg in humans (88). Thus, in addition to being a consequence of hypertension, a loss of NO bioavailability may also be a potential cause of elevated BP.

*Hypertension and Endothelial Vasomotor Function: Endothelin-1*

In an attempt to gain further understanding of the pathophysiology of endothelial vasomotor dysfunction associated with hypertension, Cardillo and colleagues performed a series of investigations examining the role of ET-1 in regulating vasomotor tone in patients with essential hypertension (89,90). First, FBF responses to intra-arterial infusion of an ET<sub>A</sub> receptor antagonist (BQ-123) and an ET<sub>B</sub> receptor antagonist (BQ-788) were measured in hypertensive patients and normotensive control subjects (90). In control subjects, BQ-123 alone or in combination with BQ-788, had no effect on FBF. In hypertensive patients, infusion of BQ-123 alone led to a significant (~35%) increase in FBF, indicating that ET<sub>A</sub>-mediated vasoconstriction is enhanced in these subjects. In addition, the combination of BQ-123 and BQ-788 resulted in an even greater increase in FBF (~65%) in subjects with hypertension. Moreover, while infusion of BQ-788 alone caused vasoconstriction in normotensive subjects, it caused vasodilation in subjects with hypertension. Collectively, these results indicate that hypertension is associated with increased vascular ET-1 activity and dysfunctional ET<sub>B</sub>-mediated vasodilation, which may contribute to the increased vascular tone observed in this population (90).
In a subsequent investigation, the FBF response to intra-arterial infusion of acetylcholine was measured in hypertensive patients and non-hypertensive control subjects before and after non-selective blockade of ET\textsubscript{A} and ET\textsubscript{B} receptors (89). In accordance with previous studies (22,91), the vasodilator response to acetylcholine was blunted in hypertensive patients compared with non-hypertensive controls. Furthermore, non-selective blockade of ET receptors resulted in a significant increase in the FBF response to acetylcholine in hypertensives, while no change was observed in control subjects (89). These findings indicate that the increased ET-1 activity observed in hypertensive patients contributes to the impaired endothelium-dependent vasodilator response that is observed in this population.

In addition to influencing endothelial vasomotor function, enhanced ET-1 system activity is thought to contribute to the etiology of CVD in hypertension through additional mechanisms, including the promotion of inflammation and oxidative stress, as well as vascular smooth muscle cell hypertrophy (55). In a genetically engineered mouse with endothelium-restricted over-expression of ET-1, increased ET-1 production was associated with enhanced activity of NADPH oxidase, as well as structural remodeling and endothelial dysfunction of resistance vessels (92). ET-1-mediated hypertrophic remodeling of resistance arteries has also been demonstrated in salt-dependent models of experimental hypertension (93,94).

Clinical trials examining the effects of ET receptor antagonists as potential anti-hypertensive medications have provided evidence that ET-1 is also involved in the pathogenesis of hypertension. Daily administration of the combined ET\textsubscript{A}/ET\textsubscript{B} antagonist bosentan (500 to 2000 mg/day) lowered diastolic BP by ~6 mm Hg following 4 weeks of treatment in patients with mild-to-moderate hypertension (diastolic BP 95 – 115 mm Hg), a reduction that was
comparable to the effects of the angiotensin converting enzyme (ACE) inhibitor enalapril (95). Also, 6 weeks of treatment with the selective ET$_A$ receptor antagonist darusentan (100 mg/day) reduced systolic BP by 11.3 mm Hg in hypertensive patients (96). Although negative side effects and inconvenient dosing patterns of ET receptor antagonists have limited enthusiasm for their use as anti-hypertensive medication (97), the BP-lowering effects shown in these studies provide convincing evidence that ET-1 is involved in the pathophysiology of high BP.

Hypertension and Endothelial Fibrinolytic Regulation

The high prevalence of thrombotic complications associated with hypertension has directed research towards examination of thrombogenic abnormalities that may be linked to elevations in BP, including alterations in fibrinolytic function. The first indication that hypertension may be associated with an altered fibrinolytic balance came with the observation of a significant correlation between plasma PAI-1 levels and systolic BP in a group of healthy middle-aged men and women (98). Hrafnkelsdottir and colleagues were the first to examine stimulated endothelial t-PA release in the forearm vasculature of hypertensive humans (99). In response to intra-arterial infusions of desmopressin, an established stimulator of endothelial t-PA release, hypertensive patients released a significantly lower amount of t-PA than non-hypertensive control subjects (99). Importantly, endothelium-independent vasodilation by sodium nitroprusside had no stimulatory effect on t-PA release, indicating that this difference in t-PA release was not due to differences in FBF between the two groups. These investigators later replicated this finding in a group of hypertensive patients with chronic kidney disease (100). Collectively, these results provided the first in vivo evidence that patients with essential
hypertension have an impaired capacity for stimulated t-PA release from the vascular endothelium.

Hypertensive patients also display impaired t-PA release in response to acetylcholine (101,102) and adrenergic stimulation with epinephrine (103). It has been suggested that the blunted capacity for endothelial t-PA release associated with hypertension is linked to the decrease in NO bioavailability that characterizes this condition, and that patients with essential hypertension depend exclusively on t-PA release via an endothelium-derived hyperpolarizing factor-dependent pathway (102). However, the suggested role of NO in modulating t-PA release is debatable, as other laboratories have shown t-PA release to either increase (104) or not change (105) during eNOS blockade in the forearm vasculature of healthy subjects.

*In vitro* investigation has provided insight into the mechanisms by which hypertension is associated with impaired stimulated t-PA release (106). Using a computerized vascular perfusion model to regulate intraluminal pressure in human umbilical vein segments, six hours of elevated pressure led to downregulation of t-PA gene and protein expression, as well as reduced vascular t-PA secretion (106). These findings indicate that the impaired capacity for stimulated t-PA release in hypertensive patients may be an effect of elevated intraluminal pressure *per se*. Support for this concept has been established *in vivo* with the observation of an improved capacity for acute t-PA release in hypertensive patients following 8 weeks of BP-lowering therapy (107). Anti-hypertensive treatment with either an ACE inhibitor or a calcium channel blocker augmented stimulated t-PA release, suggesting that the improvement in t-PA release is not drug-class dependent and that BP reduction alone enhances endothelial fibrinolytic function.
Summary

There is convincing evidence that essential hypertension is associated with abnormal endothelial vasomotor and fibrinolytic function. Specifically, hypertension is associated with impaired endothelium-dependent vasodilation due in part to reduced NO bioavailability and enhanced ET-1 system activation, as well as an impaired capacity for stimulated endothelial t-PA release. Importantly, endothelial dysfunction contributes to the increased risk of CVD in patients with essential hypertension (74).

Prehypertension and Endothelial Function

Limited information is currently available regarding the influence of prehypertension on endothelial function. An early study comparing individuals with “borderline hypertension” (defined as BP = 130-140/85-90 mm Hg) to subjects with BP < 130/85 mm Hg found no difference in flow-mediated dilation (FMD) between groups (40). However, the inclusion of prehypertensive individuals in the “control” group may have confounded the results of that study. In contrast to these findings, subjects with high-normal BP (130-139/85-89 mm Hg) had a ~30% lower FMD response than subjects with BP below 120/80 mm Hg (108). Moreover, when the high-normal BP group was stratified by systolic BP, subjects with systolic BP between 135 and 140 mm Hg had a significantly lower FMD than subjects with a systolic BP between 125 and 135 mm Hg (108). These results indicate that impairments in conduit artery endothelial function are apparent at BP levels in the prehypertensive range. Finally, coronary flow reserve is impaired in prehypertensive subjects compared with normotensive subjects, indicating that prehypertension is associated with impaired coronary microvascular function (109). However, the function of the endothelium was not directly examined in this investigation.
The capacity for stimulated endothelial t-PA release has been studied in young subjects with “borderline hypertension” (defined as SBP 140-160 mm Hg or DBP 85-95 mm Hg) (110). Methacholine was administered intra-arterially to ten young men with borderline hypertension and ten normotensive controls. Net t-PA release in response to methacholine was not different between groups. However, the individuals included in this study were not classified using JNC VII guidelines and all subjects were healthy men between the ages of 22 and 27 years. As a result, the findings of this study may not be representative of the population of prehypertensive individuals in whom an elevated CVD risk has been observed.

Summary

Although little is known regarding the influence of prehypertension on vascular endothelial function, there is evidence to suggest that impairments in vascular function may occur prior to the development of hypertension. Future studies are necessary to determine if specific aspects of vascular endothelial function, including vasomotor control and fibrinolytic regulation, are impaired in individuals with prehypertension.

Future Research Directions

While there is substantial evidence to support the idea that prehypertension is associated with an increased risk of CVD, the mechanisms responsible for this heightened cardiovascular risk are not completely understood. Specifically, very little information is available regarding the impact of prehypertension on endothelial function. Considering the established role of endothelial dysfunction in the development of CVD associated with hypertension, as well as evidence of an elevated cardiovascular risk in individuals with prehypertension, it is possible that
abnormalities in endothelial function associated with elevated BP may actually develop during
the prehypertensive state. Moreover, many of the investigations that have studied endothelial
function in hypertensive populations have used a control group that includes subjects with BP in
the prehypertensive range. Therefore, the endothelial phenotype associated with prehypertension
has not been accurately characterized. If endothelial dysfunction is occurring earlier in the
development of elevated BP, specifically during the prehypertensive state, this would provide a
therapeutic target for the prevention of atherosclerotic vascular disease that is associated with
elevated BP and emphasize the importance of alerting the public to the health risks associated
with BP in the prehypertensive range.

Investigations designed to determine whether endothelial vasomotor and/or fibrinolytic
function are impaired in prehypertensive adult humans will provide insight to advance our
understanding of the increased cardiovascular risk in this population. Furthermore, if
impairments in vasomotor and/or fibrinolytic function are indeed found in prehypertensive
adults, it would be important to determine the mechanisms by which BP in the prehypertensive
range causes endothelial dysfunction. These experiments will help elucidate the role that
endothelial dysfunction may play in the increased cardiovascular risk associated with
prehypertension and provide the experimental rationale for future studies aimed at improving
endothelial function and, in turn, reducing the risk of CVD in prehypertensive adults.
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PREHYPERTENSION IS ASSOCIATED WITH IMPAIRED NITRIC OXIDE-MEDIATED ENDOTHELIUM-DEPENDENT VASODILATION IN SEDENTARY ADULTS

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ABSTRACT

Background: Endothelial vasodilator dysfunction contributes to the development of hypertension (blood pressure (BP) ≥ 140/90 mm Hg) and cardiovascular disease (CVD). Prehypertension (BP 120-139/80-89 mm Hg) has recently been identified as an independent risk factor for hypertension and CVD. It is currently unclear whether BP in the prehypertensive range is associated with endothelial vasodilator dysfunction. We tested the hypothesis that blood pressure in the prehypertensive range, independent of other cardiovascular risk factors, is associated with impaired NO-mediated endothelium-dependent vasodilation.

Methods: Forearm blood flow (FBF) responses to intra-arterial acetylcholine (ACh; 8.0-32.0 µg/100 mL tissue/min) and sodium nitroprusside (SNP; 1.0-4.0 µg/100 mL tissue/min) were measured in 20 normotensive (age: 56±1 yrs; BP: 110/70±1/2 mm Hg) and 20 prehypertensive (56±2 yrs; 128/79±2/2 mm Hg) adults. In addition, FBF responses to ACh were determined in the absence and presence of the endothelial NO synthase inhibitor L-NMMA (5mg/min).

Results: FBF responses to ACh were significantly lower (~30%) in prehypertensive (from 4.2 ± 0.3 to 11.4 ± 0.7 mL/100 mL tissue/min) compared with normotensive (from 4.6 ± 0.2 to 14.5 ± 0.7 mL/100 mL tissue/min) adults. There were no group differences in FBF responses to SNP. Co-infusion of L-NMMA significantly reduced the FBF response to ACh in the normotensive (~30%; P<0.05) but not the prehypertensive adults.

Conclusions: Prehypertension is associated with impaired NO-mediated endothelium-dependent vasodilation. The endothelial vasodilator dysfunction that characterizes hypertension is present at BP levels in the prehypertensive range and may contribute to the increased risk of hypertension and CVD in this population.
INTRODUCTION

Several epidemiological studies have demonstrated a continuous positive relation between blood pressure (BP) and cardiovascular disease (CVD) risk that begins at BP levels well-below the traditional threshold for clinical hypertension (systolic BP ≥140 and/or diastolic BP ≥ 90 mm Hg)(1-3). As a reflection of the linear relation between elevated BP and CVD risk, the guidelines set forth by the most recent report from the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of Hypertension (JNC-7) included a new category termed “prehypertension” to describe individuals with systolic BP 120-139 and/or diastolic BP 80-89 mm Hg (4). Although the inclusion of this category was not intended to introduce a new disease category, several subsequent studies have indicated that prehypertension is an independent risk factor for the development of clinical hypertension, as well as coronary artery disease and its clinical consequences such as myocardial infarction and stroke (2,5-7). Moreover, ~50% of BP-related deaths occur in individuals with systolic BP between 115 and 140 mm Hg, suggesting that prehypertension accounts for a significant proportion of CVD-related mortality attributed to high BP (8).

Dysfunction of the vascular endothelium, especially impaired endothelium-dependent vasodilation, is etiologically involved in the pathogenesis of atherosclerotic vascular disease and occurs early in atherogenesis, prior to histological or angiographic evidence of disease (9,10). Impairments in endothelium-dependent vasodilation are largely mediated by a reduction in the bioavailability of endothelium-derived nitric oxide (NO) (11). Diminished NO-mediated endothelium-dependent vasodilation has been linked to an increased risk of future coronary artery disease, cerebrovascular disease, and atherothrombotic events (12,13). In addition, clinical hypertension is associated with impaired NO-mediated endothelium-dependent
vasodilation (14-16). Given the elevated CVD risk associated with prehypertension, it is possible that impairments in NO-mediated endothelium-dependent vasodilation arise at BP levels in the prehypertensive range. If so, this may be an important underlying mechanism contributing to the development of clinical hypertension and atherosclerotic vascular disease in this population.

Accordingly, we tested the hypothesis that blood pressure in the prehypertensive range is associated with impaired NO-mediated endothelium-dependent vasodilation. To address this hypothesis, we measured forearm blood flow responses to intra-arterial infusion of acetylcholine, in the absence and presence of the endothelial NO synthase inhibitor L-NMMA, in normotensive and prehypertensive adults.

METHODS

Subjects

Forty adults were stratified based on BP according to JNC-7 guidelines (4): 20 normotensive [BP < 120/80 mmHg; 12 males/8 females] and 20 prehypertensive (BP 120-139/80-89 mmHg; 11 males/9 females). All subjects were non-obese and free of overt cardiovascular, metabolic, and chronic inflammatory disease as assessed by medical history, physical examination, and fasting blood chemistries. All subjects were free of recent infection/inflammation (<2 weeks), as determined by questionnaire (17). Men over the age of 40 years and women over the age of 50 years were further evaluated for clinical evidence of coronary artery disease with electrocardiograms and BP at rest and during incremental exercise performed to exhaustion. All of the women were at least 1 year postmenopausal and had never taken or had discontinued use of hormone replacement therapy at least 1 year before the start of
the study. None of the subjects smoked, were taking medications, or performed regular physical exercise for at least 6 months before the start of the study. Daily physical activity was assessed by the Stanford Physical Activity Questionnaire and used to document the sedentary status (i.e., absence of regular aerobic and other types of exercise) of all subjects (18,19). Family history of hypertension was assessed by questionnaire. Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

**Blood Pressure Measurement**

BP measurement was completed in strict accordance with American Heart Association guidelines as established by the Council for High Blood Pressure Research (20). Resting BP measurements were performed in the sitting position between 8 a.m. and 10 a.m. on at least two separate days one week apart. Caffeinated beverages were avoided for at least 30 minutes prior to measurement. The recordings were made under quiet, comfortable ambient (~24°C) laboratory conditions. To avoid any possibility of investigator bias, measurements were made with a semi-automated device (Dinamap, Critikon, FL) that uses an oscillometric technique over the brachial artery. An appropriately sized cuff (cuff bladder encircling at least 80 percent of the arm) was used. Recordings were made in triplicate in the upright sitting position and the average recorded.

**Body Composition and Metabolic Measurements**

Body mass was measured to the nearest 0.1 kg using a medical beam balance. Percent body fat was determined by dual energy X-ray absorptiometry (Lunar Corp., Madison, WI).
Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines (21). Fasting plasma lipid, lipoprotein, glucose, and insulin concentrations were determined using standard techniques as previously described (22). The presence of the metabolic syndrome was established according to the National Cholesterol Education Program ATP III criteria (23,24).

Maximal Oxygen Consumption ($VO_2$ max)

To assess aerobic fitness, subjects performed incremental treadmill exercise with a modified Balke protocol. Maximal oxygen consumption ($VO_2$ max) was measured with on-line computer-assisted open circuit spirometry as described previously (25).

Intra-Arterial Infusion Protocol

All studies were performed between 7 am and 10 am in a temperature-controlled room following a 12-hour overnight fast as previously described (22). Under local anesthesia (1% lidocaine), a 5-cm, 20-gauge catheter was inserted in the brachial artery of the non-dominant arm. Forearm blood flow (FBF) was measured in both the experimental (non-dominant) and contralateral (dominant) forearm using strain-gauge venous occlusion plethysmography. FBF was measured at baseline and in response to acetylcholine (ACh; IOLAB Pharmaceuticals) infused intra-arterially at 4.0, 8.0, 16.0 µg/100 mL tissue/min and sodium nitroprusside (SNP; Abbott Laboratories) at 1.0, 2.0 and 4.0 µg/100 mL tissue/min for 3 to 5 minutes at each dose. The sequence of drug administration was randomized to avoid an order effect.

To determine the contribution of NO to ACh-mediated vasodilation, FBF responses to ACh were determined before and after administration of the endothelial NO synthase (eNOS)
inhibitor $N^G$-monomethyl-L-arginine (L-NMMA; Clinalfa) in 16 of the 20 normotensive (9 males/7 females) and 16 of the 20 prehypertensive (10 males/6 females) adults. After ACh was infused at the doses noted above and FBF was allowed to return to resting levels, L-NMMA was infused at 5 mg/min for 5 min. Immediately thereafter, the ACh dose response was repeated with the continuous infusion of L-NMMA.

**Statistical Analysis**

Differences in subject characteristics and area under the curve data were determined by analysis of variance (ANOVA). Group differences in FBF responses to ACh, SNP, and ACh+L-NMMA were determined by repeated measures ANOVA. When indicated by a significant F value, a post hoc test using the Newman-Keuls method was performed to identify differences among the groups. Pearson correlations were determined between variables of interest. There were no significant sex differences with respect to the main effect of BP on any of the key outcome variables; therefore, the data were pooled and are presented together. Power calculations were performed *a priori* to determine the appropriate number of subjects per group. All data are presented as mean ± SEM. Statistical significance was set at $P<0.05$.

**RESULTS**

Selected subject characteristics are presented in Table 1. Anthropometric characteristics were similar between groups. By design, both systolic and diastolic BP were significantly higher ($p<0.05$) in the prehypertensive group compared with the normotensive controls. There were no differences between the groups in maximal oxygen consumption or plasma lipid and lipoprotein, glucose, and insulin concentrations. Four subjects in the prehypertensive group met the criteria
for the metabolic syndrome, and eight subjects (5 prehypertensive; 3 normotensive) reported a family history of hypertension.
Table 1: Selected subject characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=20)</th>
<th>Prehypertensive (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females</td>
<td>12/8</td>
<td>11/9</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>56 ± 1</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.5 ± 2.4</td>
<td>79.5 ± 2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 0.7</td>
<td>27.1 ± 0.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.5 ± 2.2</td>
<td>34.4 ± 2.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.3 ± 1.8</td>
<td>90.8 ± 2.4</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.87 ± 0.02</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>VO₂ max (L/min)</td>
<td>2.5 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>110 ± 1</td>
<td>128 ± 2*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70 ± 2</td>
<td>79 ± 2*</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.1 ± 0.2</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L)</td>
<td>3.2 ± 0.1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>29.4 ± 2.6</td>
<td>34.1 ± 3.9</td>
</tr>
</tbody>
</table>

BMI = body mass index; BP = blood pressure; VO₂ max = maximal oxygen consumption; HDL = high-density lipoprotein; LDL = low-density lipoprotein
Values are means ± SEM
*P<0.05 vs. normotensive
Resting FBF was not different between the normotensive (4.6 ± 0.2 mL/100 mL tissue/min) and prehypertensive (4.2 ± 0.3 mL/100 mL tissue/min) subjects. The vasodilator response to ACh was markedly blunted (~25%; P<0.05) in the prehypertensive (from 4.2 ± 0.3 to 11.4 ± 0.7 mL/100 mL tissue/min) compared with normotensive (from 4.6 ± 0.2 to 14.5 ± 0.7 mL/100 mL tissue/min) adults (Figure 1). As a result, total FBF to ACh (area under the curve) was significantly lower in the prehypertensive (53.3 ± 5.6 mL/min) than normotensive group (71.9 ± 5.9 mL/min). FBF responses to SNP were not significantly different between the groups (Figure 1). Heart rate, mean arterial BP and FBF in the non-infused arm, remained constant throughout the infusion protocol in both the prehypertensive and normotensive adults (data not shown). Systolic BP (r = -0.31; P < 0.05) was the only correlate of the FBF response to ACh in the overall study population. Group differences in FBF responses to ACh were maintained when subjects with the metabolic syndrome or a family history of hypertension were excluded from analysis.

Co-infusion of L-NMMA significantly reduced the FBF responses to ACh in the normotensive but not the prehypertensive adults (Figure 2). For example, in the normotensive group, FBF at the highest dose of ACh declined from 14.3 ± 0.8 mL/100 mL tissue/min to 10.6 ± 0.9 mL/100 mL tissue/min with L-NMMA. In contrast, FBF at the highest dose of ACh in the prehypertensive group was largely unaffected by L-NMMA (from 10.8 ± 1.0 mL/100 mL tissue/min to 9.0 ± 1.2 mL/100 mL tissue/min). Consequently, total FBF to ACh was ~30% lower (P < 0.05) in the presence of L-NMMA in the normotensive adults compared with a modest ~15% reduction (P = 0.43) in total FBF to ACh in the prehypertensive adults.
Figure 1: FBF responses (panel A) and total FBF (area under the curve) (panel B) to acetylcholine and sodium nitroprusside (panel C) in normotensive and prehypertensive adults.

Values are means ± SEM. *P < 0.05 vs. normotensive.
**Figure 2:** FBF responses and total FBF (area under the curve) to acetylcholine in the absence and presence of the nitric oxide (NO) synthase inhibitor N\(^G\)-monomethyl-L-arginine (L-NMMA) in normotensive and prehypertensive adults.

Values are means ± SEM. *P < 0.05 vs. saline.
DISCUSSION

The seminal findings of the present study are as follows: 1) otherwise healthy adults with BP in the prehypertensive range exhibited impaired endothelium-dependent vasodilation compared with normotensive adults of similar age and body composition; and 2) the contribution of NO to endothelium-dependent vasodilation was significantly diminished in adults with prehypertension. Taken together, these results indicate that prehypertension is associated with impaired NO-mediated endothelium-dependent vasodilation. Diminished NO-mediated endothelial vasodilator function may contribute to the increased risk of hypertension and atherosclerotic vascular disease in this population.

Because of the significant cardiovascular risk associated with elevated BP and the well-established role of the vascular endothelium in maintaining cardiovascular health, endothelial vasodilator function has been a target of extensive study in adults with hypertension (14-16,26). These studies have repeatedly demonstrated that BP in the clinical hypertensive range is associated with impaired endothelium-dependent vasodilation in several vascular beds, including the coronary, peripheral, and renal circulation (14-16). Moreover, impaired acetylcholine-mediated endothelium-dependent vasodilation predicts future cardiovascular events in hypertensive adults, underscoring the clinical importance of this aspect of endovascular health in individuals with hypertension (27). In light of recent data demonstrating that prehypertension is an independent risk factor for CVD (2,5-7), it has been suggested that the cardiovascular consequences of hypertension may already be apparent in the prehypertensive state (26,28). The results of the present study support this notion. Indeed, FBF responses to acetylcholine were significantly blunted (~25%) in prehypertensive compared with normotensive adults. Furthermore, co-infusion of L-NNMA did not significantly alter FBF responses to acetylcholine
in the prehypertensive adults, indicating that a reduction in NO bioactivity contributes to
impaired acetylcholine-mediated vasodilation with prehypertension. Interestingly, the magnitude
of impairment in the FBF response to acetylcholine in prehypertensive compared with
normotensive adults in the present study was comparable to that observed in hypertensive adults
reported in previous studies that utilized similar experimental procedures. For example, Taddei
et al. (29) reported that FBF responses to acetylcholine were ~25% lower in hypertensive (BP = 154/100 mm Hg) compared with normotensive adults. In addition, Panza et al. (30) have shown
that co-infusion of L-NMMA does not significantly affect FBF responses to acetylcholine in
hypertensive adults. This finding is similar to that observed in prehypertensive adults in the
present study. Thus, our results indicate that the impairment in NO-mediated endothelium-
dependent vasodilation that characterizes hypertension is already present in the prehypertensive
state.

Previous studies on the influence of BP in the prehypertensive range on endothelial
vasodilator function have yielded conflicting results. An early study comparing individuals with
“borderline hypertension” (defined as BP = 130-140/85-90 mm Hg) to those with BP < 130/85
mm Hg reported no differences in flow-mediated dilation (FMD) between the groups (31).
However, the inclusion of prehypertensive individuals in the “control” group may have
confounded these results. In contrast to these findings, Plavnik et al. (32) reported that adults
with high-normal BP (130-139/85-89 mm Hg) demonstrated a ~30% lower FMD response than
adults with BP below 120/80 mm Hg, suggesting that impairments in conduit artery endothelial
function are apparent at BP levels in the prehypertensive range. Although differences in BP
stratification likely contributed to the discrepancy in results between these studies, the results of
Plavnik et al. (32) are supported by data from Giannotti and colleagues (33) who demonstrated a
significantly lower FMD response in prehypertensive compared with normotensive adults. The present study significantly extends these earlier findings by demonstrating impaired NO-mediated endothelium-dependent vasodilation in prehypertensive adults free of other cardiometabolic risk factors who were strictly classified based on JNC-7 guidelines (4).

The mechanisms underlying impaired NO-mediated endothelium-dependent vasodilation with prehypertension are unclear. A number of factors may play a role, however, including oxidative stress and inflammatory cytokines. Prehypertension is associated with elevated levels of inflammatory markers such as C-reactive protein and tumor necrosis factor-α (34), as well as higher levels of oxidative stress markers such as oxidized LDL (35). These inflammatory and oxidative factors can contribute to endothelial cell dysfunction. For example, oxidized LDL impairs NO bioavailability by reducing eNOS activity directly, as well as promoting superoxide anion formation and the subsequent oxidative inactivation of NO (36,37). Another possibility is that impairments in endothelium-dependent vasodilation with prehypertension are the result of enhanced endothelin (ET)-1 vasoconstrictor activity. ET-1 system activity is involved in the regulation of blood pressure and has been shown to be elevated in hypertensive adults (38-40). It is currently unknown whether ET-1 vasoconstriction is enhanced with prehypertension. If so, elevated ET-1 system activity might contribute to the impairment in NO-mediated endothelium-dependent vasodilation in prehypertensive adults.

From a public health perspective, it is important to emphasize that we studied non-medicated and non-smoking adults free of cardiometabolic abnormalities that are commonly associated with prehypertension (41) and known to influence endothelial function, such as obesity (22), dyslipidemia (42), and diabetes (43). Although our cross-sectional study design does not allow conclusions regarding causality, the findings of the present study demonstrate that
BP in the prehypertensive range, independent of other cardiovascular risk factors, is associated with impaired NO-mediated endothelium-dependent vasodilation. These results provide support for the emerging argument for earlier intervention in the treatment and prevention of high BP (28,44,45). Considering prehypertension is associated with accelerated progression of coronary atherosclerosis (46), carotid artery intima-media thickening (47,48) and endothelial dysfunction, interventions directed at lowering BP and reducing cardiovascular risk may need to be more strictly implemented at BP levels below the traditional clinical cut-off for hypertension.

In conclusion, the results of the present study demonstrate that prehypertension is associated with impaired NO-mediated endothelium-dependent vasodilation. These data indicate that prehypertension is not a benign condition and provide further evidence that the endothelial vasodilator dysfunction that characterizes hypertension is apparent in the prehypertensive range. Impaired NO-mediated endothelium-dependent vasodilation may contribute to the increased risk of CVD in prehypertensive adults.
Acknowledgements

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ELEVATED ENDOTHELIN-1 VASOCONSTRICTOR TONE
IN PREHYPERTENSIVE ADULTS

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March, 2011.
**ABSTRACT**

**Background:** Prehypertension (blood pressure (BP): 120-139/80-89 mmHg) is an independent risk factor for hypertension and cardiovascular disease (CVD). Currently, it is unknown whether ET-1-mediated vasoconstrictor tone is elevated with BP in the prehypertensive range. We determined whether endothelin (ET)-1 vasoconstrictor tone is elevated in prehypertensive adults and, if so, whether ET-1-mediated vasoconstriction contributes to endothelial vasodilator dysfunction in this population. **Methods:** Forearm blood flow (FBF) responses to selective ET_A receptor blockade (BQ-123; 100 nmol/min) were determined in 26 normotensive (age: 55±1 yrs; BP: 112/72±1/1 mmHg) and 30 prehypertensive (57±1 yrs; 130/80±1/1 mmHg) adults. In a subset of subjects, FBF responses to non-selective ET-1 receptor blockade (BQ-123+BQ-788) were determined. FBF responses to acetylcholine (ACh; 8.0-32.0 µg/100 mL tissue/min) were measured in the absence and presence of selective ET_A receptor blockade. **Results:** BQ-123 elicited a significantly greater increase in FBF in prehypertensive (~20%) than normotensive (~5%) adults. Addition of BQ-788 resulted in a further increase (P<0.05) in FBF in prehypertensive but not normotensive adults. FBF responses to ACh were lower (P<0.05) in prehypertensive (4.6 ± 0.3 to 12.6 ± 0.5 mL/100 mL tissue/min) than normotensive (4.9 ± 0.3 to 14.7 ± 0.8 mL/100 mL tissue/min) adults. Co-infusion of BQ-123 did not affect ACh-induced vasodilation in normotensive adults, but resulted in an ~20% increase (P<0.05) in prehypertensive adults. **Conclusions:** ET-1-mediated vasoconstrictor tone is elevated with prehypertension, contributing to impaired endothelium-dependent vasodilation. ET-1 vasoconstriction may underlie the increased risk of hypertension and CVD in prehypertensive adults.
INTRODUCTION

Prehypertension, defined as systolic blood pressure between 120-139 mm Hg and/or diastolic blood pressure between 80-89 mm Hg, is a common condition that affects ~30% of the US adult population (1,2). A growing body of evidence indicates that prehypertension is not only an independent risk factor for the development of clinical hypertension (blood pressure ≥ 140/90 mm Hg) (3,4), but also cardiovascular disease (CVD) and its clinical consequences, such as myocardial infarction, stroke, and congestive heart failure (1,5-7). The increased cardiovascular risk associated with prehypertension may be mediated, at least in part, by vascular endothelial dysfunction. Endothelial damage and dysfunction is considered to be a critical early event in the pathogenesis and progression of atherosclerotic vascular disease (8,9). Recent data indicate that blood pressure in the prehypertensive range is associated with impaired nitric oxide (NO)-mediated endothelium-dependent vasodilation (10,11). In addition to NO, endothelin (ET)-1 plays an important role in vascular health and vasomotor regulation (12,13).

ET-1 is the most potent vasoconstrictor released by the endothelium and has been linked to the etiology of hypertension (12,13). Acute and chronic blockade of ET-1 receptors have been shown to reduce blood pressure and ameliorate endothelial vasodilator dysfunction in patients with clinical hypertension (14-17). In addition to its vasoregulatory actions, ET-1 also promotes the development of atherosclerotic vascular disease by stimulating inflammatory cytokine release, platelet aggregation, cell adhesion molecule expression, and vascular smooth muscle cell proliferation (12,18,19). Although elevations in ET-1 system activity have been demonstrated in patients with clinical hypertension (14,15), it is currently unknown whether increased ET-1 vasoconstrictor tone is apparent at blood pressure levels in the prehypertensive range. If so,
enhanced ET-1 system activity with prehypertension may contribute to elevations in blood pressure and the development of CVD in this population.

The aims of the present study were to determine whether ET-1 vasoconstrictor tone is elevated in prehypertensive adults and, if so, whether the increase in ET-1-mediated vasoconstriction contributes to endothelial vasodilator dysfunction in this population. To address these aims, forearm blood flow responses to selective and non-selective ET-1 receptor antagonists were determined in normotensive and prehypertensive adults. Additionally, acetylcholine-mediated endothelium-dependent vasodilation was assessed in the absence and presence of selective ET-1 receptor blockade. We hypothesized that ET-1 vasoconstrictor tone is greater in prehypertensive compared with normotensive adults and that the elevation in ET-1-mediated vasoconstriction contributes to impaired endothelium-dependent vasodilation with prehypertension.

METHODS

Subjects

Fifty-six middle-aged and older adults (age range: 42 – 65 years) were studied: 26 normotensive (systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg; 15 males/11 females) and 30 prehypertensive (systolic blood pressure 120-139 mmHg and/or diastolic blood pressure 80-89 mmHg; 20 males/10 females). Blood pressure classification was based on guidelines from the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) and established by two or more seated blood pressure readings from two separate laboratory visits over 10-14 days (20).
All subjects were sedentary and had not participated in a regular aerobic exercise program for at least 1 year prior to the start of the study. Subjects were excluded from the study if they presented a history or evidence of: hepatic, renal, or hematological disease; peripheral vascular disease; stroke; diabetes (fasting plasma glucose > 7.0 mmol/L) (21); dyslipoproteinemia (22); and hypertension (arterial blood pressure ≥ 140/90 mmHg) (20). All subjects were screened for clinical evidence of cardiovascular disease by medical history, physical examination, fasting blood chemistries, and electrocardiograms and blood pressure at rest and during incremental exercise performed to exhaustion. None of the subjects smoked or were taking medications including vitamins. All of the women were at least 1 year postmenopausal and had never taken or had discontinued use of hormone replacement therapy at least 1 year before the start of the study. Prior to participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

**Body Composition and Metabolic Measurements**

Body mass was measured to the nearest 0.1 kg using a medical beam balance (Detecto, Webb City, MO). Percent body fat was determined by dual energy X-ray absorptiometry (Lunar Radiation Corporation, Madison, WI). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines (23). Fasting plasma lipid, lipoprotein, glucose, and insulin concentrations were determined using standard techniques as previously described (24).
Intra-arterial Infusion Protocol

All studies were performed between 7:00 am and 10:00 am after a 12-hour overnight fast in a temperature-controlled room. Under strict aseptic conditions a 5-cm, 20-gauge catheter was inserted into the brachial artery of the non-dominant arm under local anesthesia (1% lidocaine). Heart rate and arterial blood pressure were continuously measured throughout the infusion protocol. Forearm blood flow (FBF) at rest and in response to each pharmacological agent was measured in both the experimental (non-dominant) and contralateral (dominant) forearm using strain-gauge venous occlusion plethysmography (D. E. Hokanson, Bellevue, WA), as previously described by our laboratory (25).

Following measurement of baseline FBF (5 minutes), BQ-123 (Clinalfa, AG), a selective ET\(_A\) receptor antagonist, was infused at a rate of 100 nmol/min for 60 minutes. FBF was measured every 10 minutes throughout the infusion period. The selected dose of BQ-123 has been shown to completely inhibit the vasoconstrictor effect of ET-1 in the human forearm of healthy adults (15,25). After 60 minutes of BQ-123 infusion, the FBF response to non-selective ET-1 receptor blockade was assessed by the co-administration of BQ-123 and BQ-788 (Clinalfa, AG) for an additional 60 minutes. FBF was measured every 10 minutes during the combined BQ-123 and BQ-788 infusion. BQ-788, a specific antagonist of ET\(_B\) receptors, was infused at a rate of 50 nmol/min, a dose shown to effectively inhibit ET\(_B\) receptors (15,25). Because of limited drug availability, studies involving BQ-788 were only performed in a subset of the total study population (16 prehypertensive and 10 normotensive adults).

To assess the influence of ET-1 activity on endothelium-dependent vasodilation, FBF responses to acetylcholine (ACh) were measured in the absence and presence of BQ-123 in 12 of
the 26 normotensive (blood pressure: 110±2/70±2; 8 M/4 F) and 12 of the 30 prehypertensive (blood pressure: 129±2/80±2; 8 M/4 F) subjects. Following the measurement of resting blood flow for 5 minutes, FBF was assessed in response to infusions of ACh (IOLAB pharmaceuticals, Duluth, GA) at 4.0, 8.0, and 16.0 µg/100 mL tissue/min and sodium nitroprusside (SNP; Nitropress, Abbott Laboratories) at 1.0, 2.0, and 4.0 µg/100 mL tissue/min. Each dose of ACh and SNP was infused for ~5 minutes and sufficient time (~20 minutes) was allowed for FBF to return to resting levels between each vasoactive agent. To avoid an order effect, the sequence of drug administration was randomized. After the initial infusion of ACh and SNP, BQ-123 was infused in an identical manner to Study 1. After 60 minutes, infusion of BQ-123 was continued at the same dose and FBF was reassessed during co-administration of ACh as performed earlier.

Statistical Analysis

Differences in subject baseline characteristics were determined by between-groups analysis of variance (ANOVA). Group differences in FBF responses to BQ-123, acetylcholine, and sodium nitroprusside were determined by repeated-measures ANOVA. Pearson correlations were determined between variables of interest. There were no significant gender interactions, therefore the data were pooled and presented together. All data are expressed as mean ± SEM. Statistical significance was set a priori at P <0.05.

RESULTS

Selected subject characteristics are presented in table 1. There were no differences between the groups in anthropometric characteristics or plasma lipid and lipoprotein, glucose, and insulin concentrations. By design, systolic blood pressure and diastolic blood pressure were greater (P<0.05) in the prehypertensive compared with the normotensive group. FBF in the non-
infused arm and mean arterial blood pressure remained constant throughout the infusion protocol and did not differ significantly between groups.
Table 2: Selected Subject Characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n=26)</th>
<th>Prehypertensive (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females</td>
<td>15/11</td>
<td>20/10</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55 ± 1</td>
<td>57 ± 1</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.0 ± 2.7</td>
<td>80.3 ± 2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 0.7</td>
<td>26.7 ± 0.6</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>32.3 ± 2.1</td>
<td>32.0 ± 1.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.3 ± 2.7</td>
<td>91.6 ± 1.9</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.88 ± 0.02</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>112 ± 1</td>
<td>130 ± 1*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>72 ± 1</td>
<td>80 ± 1*</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>5.0 ± 0.2</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/L)</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L)</td>
<td>3.2 ± 0.2</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>39.9 ± 4.1</td>
<td>39.1 ± 3.2</td>
</tr>
</tbody>
</table>

BMI = body mass index; BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein
Values are mean ± SEM
*P<0.05 vs. normotensive
The FBF responses to selective ET\textsubscript{A} receptor blockade (BQ-123) were markedly different (P<0.05) between groups. BQ-123 elicited a significantly greater increase in FBF in prehypertensive (~20%) than normotensive (~5%) adults (Figure 1). FBF responses to BQ-123 at the 60-minute time point were significantly related to systolic blood pressure (r=0.55), diastolic blood pressure (r=0.36), and mean arterial pressure (r=0.50). The addition of BQ-788 to BQ-123 did not significantly affect the FBF responses to BQ-123 in the normotensive adults. However, the prehypertensive adults demonstrated a further increase (~10%; P < 0.05) in FBF to BQ-123 + BQ-788 beyond that observed with BQ-123 alone (Figure 2). As a result, significant group differences remained in the FBF responses to non-selective ET\textsubscript{A}/ET\textsubscript{B} blockade.

FBF responses to ACh were ~20% lower (P<0.05) in the prehypertensive (from 4.6±0.2 to 12.6±0.5 mL/100mL tissue/min) compared with the normotensive (from 5.0±0.2 to 14.7±0.8 mL/100mL tissue/min) group. There were no significant differences between the groups in FBF responses to SNP (Figure 3). Co-infusion of BQ-123 with ACh did not significantly affect ACh-mediated vasodilation in the normotensive subjects (Figure 4). However, FBF to ACh increased (~30%; P < 0.05) in the prehypertensive adults with BQ-123 (Figure 4). In fact, the co-infusion of BQ-123 abolished the blood pressure -related difference in ACh-mediated vasodilation between the groups. FBF responses to ACh in the presence of BQ-123 were not significantly different between the normotensive (from 5.1±0.4 to 16.6±0.8 mL/100mL tissue/min) and prehypertensive (from 5.1±0.2 to 15.3±0.8 mL/100mL tissue/min) adults.
Figure 3: Forearm blood flow (FBF) responses to BQ-123 (100 nmol/min) in normotensive and prehypertensive adults.

Values are mean ± SEM. *P<0.05 refers to the difference in FBF responses to BQ-123 in prehypertensive vs. normotensive adults.
**Figure 4:** FBF responses to BQ-123 (100 nmol/min) alone and BQ-123 combined with BQ-788 (50 nmol/min) in normotensive and prehypertensive adults.

Values are mean ± SEM. *P<0.05 refers to the difference in FBF responses to BQ-123 and BQ-123+BQ-788 in prehypertensive vs. normotensive adults. †P<0.05 refers to the difference in FBF responses to BQ-123 vs. BQ-123+BQ-788 among the prehypertensive adults.
Figure 5: FBF responses to acetylcholine (panel A) and sodium nitroprusside (panel B) in normotensive and prehypertensive adults.

Values are mean ± SEM. *P<0.05 vs. normotensive.
Figure 6: FBF responses to acetylcholine in the presence and absence of ET\textsubscript{A} receptor blockade with BQ-123 in normotensive (panel A) and prehypertensive (panel B) adults.

Values are mean ± SEM. *P<0.05 vs. saline.
DISCUSSION

The novel findings of the present study are as follows: (1) selective ET\textsubscript{A} receptor blockade elicited a significantly greater vasodilator response in prehypertensive compared with normotensive adults; (2) non-selective ET\textsubscript{A} and ET\textsubscript{B} receptor blockade produced significantly greater vasodilation in prehypertensive adults than that produced by selective ET\textsubscript{A} antagonism; and (3) selective ET\textsubscript{A} receptor blockade increased ACh-mediated endothelium-dependent vasodilation in prehypertensive adults to levels similar to that of normotensive adults.

Collectively, these findings indicate that ET-1-mediated vasoconstrictor tone is elevated with prehypertension independent of other CVD risk factors and contributes to the impairment in endothelium-dependent vasodilation in prehypertensive adults.

Although the introduction of the prehypertension blood pressure classification in the JNC-7 report was not initially intended to designate a new disease category (20), several studies have shown that prehypertension is an independent risk factor for clinical hypertension and adverse cardiovascular events, such as myocardial infarction and stroke (1,4-6). These findings have led to the suggestion that the pathogenesis of blood pressure-related cardiovascular disorders begins at blood pressure levels in the prehypertensive range (26,27). This notion is supported by recent data demonstrating that otherwise healthy adults with blood pressure in the prehypertensive range exhibit impaired endothelium-dependent vasodilation, similar to that observed in hypertensive adults (10,11). The results of the present study significantly extend our understanding of the endothelial vasomotor dysfunction associated with prehypertension by demonstrating, for the first time, that prehypertensive adults exhibit elevated ET-1-mediated vasoconstrictor tone.
ET-1 vasoconstrictor activity is mediated by two distinct receptor subtypes, ET_A and ET_B. ET_A receptors are located exclusively on vascular smooth muscle cells, where ET-1 binding activates the phospholipase C-inositol triphosphate pathway resulting in an increase in intracellular calcium, subsequent phosphorylation of myosin kinase and, in turn, long-lasting smooth muscle cell contraction (12,13). In contrast to ET_A receptors, ET_B receptors are expressed by both the endothelium and vascular smooth muscle and can mediate dual vasoregulatory actions of ET-1 (28). Activation of ET_B receptors expressed by the vascular smooth muscle elicits vasoconstriction, while ET-1 binding to ET_B receptors on endothelial cells results in NO-mediated vasodilation (12,13). The development of pharmacologic agents that selectively block the ET_A and ET_B receptors has provided a means of assessing the contribution of ET-1 to vasomotor regulation in vivo. In the present study, prehypertensive adults exhibited a significantly greater vasodilator response to selective ET_A receptor blockade than their normotensive counterparts, indicating that blood pressure in the prehypertensive range is associated with elevated ET-1 vasoconstriction mediated by the ET_A receptor. Furthermore, non-selective ET_A/B receptor antagonism resulted in a further increase in FBF above that observed with ET_A blockade alone in the prehypertensive adults only, demonstrating that the ET_B receptor also contributes to the elevation in ET-1 vasoconstrictor tone with prehypertension. Thus, prehypertension is associated with enhanced ET-1 vasoconstrictor tone that is mediated by both the ET_A and ET_B receptor subtypes.

Elevated ET-1 system activity in prehypertensive adults may have important pathophysiological implications. Given ET-1’s potent vasoconstrictor actions, elevated ET-1-mediated vasoconstriction may lead to increased vascular tone and further elevations in blood pressure in prehypertensive adults, thereby contributing to the elevated risk of progression to
clinical hypertension. This idea is supported by the finding that chronic ET receptor blockade prevents blood pressure elevation in various animal models of hypertension (29-31). In addition to inducing vasoconstriction, ET-1 receptor stimulation promotes fibrosis, vascular hypertrophy, inflammatory cytokine production, and smooth muscle cell proliferation, all of which contribute to atherogenesis (12,32,33). In fact, chronic ET\textsubscript{A} receptor blockade has been shown to reduce plaque formation in an experimental model of atherosclerosis (34), underscoring the adverse effects of ET-1 activity on vascular health beyond vasoconstriction. As such, elevated ET-1-mediated vasoconstriction may contribute to increased vascular tone and the subsequent risk of clinical hypertension, as well as the development and progression of atherosclerotic vascular disease in prehypertensive adults.

A functional consequence of the elevated ET-1 vasoconstrictor tone with prehypertension appears to be impaired endothelium-dependent vasodilation. The prehypertensive adults in the present study demonstrated significantly lower (~30\%) acetylcholine-mediated vasodilation than normotensive adults, confirming that prehypertension is associated with endothelial vasodilator dysfunction (10,11). However, blockade of the ET\textsubscript{A} receptor restored acetylcholine-mediated endothelium-dependent vasodilation in prehypertensive adults to levels similar to that of normotensive adults. There are a number of potential mechanisms that may be involved in this improvement in endothelium-dependent vasodilation following ET\textsubscript{A} receptor blockade. For example, it is possible that inhibition of ET\textsubscript{A}-mediated ET-1 vasoconstriction reversed an imbalance in vasoconstrictor and vasodilator influences on the vascular smooth muscle in prehypertensive adults, thereby allowing endothelium-derived relaxing factors to act without opposition and dilate the vessel appropriately in response to acetylcholine stimulation. Alternatively, ET\textsubscript{A} receptor blockade may improve endothelium-dependent vasodilation by
increasing the bioavailability of NO (35). ET-1 binding to the ET\(_A\) receptor can reduce NO bioavailability directly via inhibition of endothelial NO synthase or indirectly through the production of superoxide anion and subsequent inactivation of NO (32,35,36). Thus, enhanced NO bioavailability may underlie the improvement in endothelium-dependent vasodilation with ET\(_A\) receptor blockade in prehypertensive adults. Unfortunately, in the present study we did not address this issue by infusing the endothelial NO synthase antagonist N\(^G\)-monomethyl-L-arginine with acetylcholine and BQ-123 to assess the contribution of NO to the observed increase in vasodilation.

It is important to note that the FBF responses observed in the prehypertensive adults in the present study are similar to those reported in adults with clinical hypertension (14,15,37). For example, Cardillo and colleagues (15) observed greater forearm vasodilation to intra-arterial BQ-123 and BQ-788 in hypertensive compared with normotensive adults. Additionally, ET-1 receptor blockade improved endothelium-dependent vasodilation in hypertensive adults (14), similar to our findings in prehypertensive adults. These similarities in ET-1-mediated vasoconstrictor activity between prehypertensive and hypertensive adults support the notion that vascular abnormalities linked with hypertension may already be apparent in the prehypertensive state (26). Indeed, blood pressure in the prehypertensive range is also associated with carotid artery intima-media thickening (38). Taken together, these findings indicate that prehypertension is not a benign condition and support recent editorials and reviews advocating the implementation of therapeutic interventions at blood pressure levels in the prehypertensive range in order to minimize blood pressure-related cardiovascular risk (26,27,39).

There are a few experimental considerations regarding the present study that merit discussion. Firstly, given our cross-sectional study design, we cannot discount the possibility
that genetic and/or lifestyle behaviors may have influenced our results. To minimize the influence of lifestyle behaviors, we studied sedentary adults who were non-smokers and not currently taking any medication that could influence endothelial vasomotor function. Moreover, to isolate the primary influence of blood pressure in the prehypertensive range, we studied adults free of cardiometabolic abnormalities that are commonly associated with prehypertension (40) and known to influence endothelial function, such as obesity (24), dyslipidemia (41), and diabetes (42). Secondly, the vast majority (~95%) of adults in the present study were Caucasian. Thus, any generalizations to other racial groups must be made with caution, given that marked elevations in ET-1 vasoconstrictor activity have been reported in African-American adults (43,44). Finally, we did not measure circulating plasma levels of ET-1 in the present study. The physiological significance of plasma ET-1 levels is questionable because ET-1 is predominantly (>80%) released abluminally toward the vascular smooth muscle (37,45). As a result, circulating plasma concentrations of the peptide are largely the result of variable spillover into, and clearance from, the bloodstream and do not accurately reflect local vascular production or action (15). The measurement of forearm blood flow responses to intra-arterial infusion of ET-1 receptor antagonists affords a more direct biological assessment of ET-1 system activity in vivo (15,25,46).

In conclusion, the results of the present study demonstrate that prehypertension is associated with enhanced ET-1 vasoconstrictor tone that is mediated by both the ET\(_A\) and ET\(_B\) receptor subtypes. In addition, the elevation in ET-1-mediated vasoconstrictor activity contributes to diminished endothelium-dependent vasodilation in prehypertensive adults. Enhanced ET-1 system activity may be involved in the increased risk of hypertension and atherosclerotic vascular disease in prehypertensive adults. Given the fact that endothelial
abnormalities that characterize hypertension appear to be already apparent with blood pressure in the prehypertensive range, it may be prudent to more aggressively advocate intervention strategies aimed at managing blood pressure and reducing vascular risk in prehypertensive adults.
Acknowledgements

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REFERENCES


The primary new findings of the studies presented herein are as follows:

1) prehypertension is associated with impaired endothelium-dependent vasodilation. Compared with normotensive adults, prehypertensive adults exhibited significantly lower forearm blood flow responses to the endothelium-dependent vasodilator acetylcholine;

2) impaired acetylcholine-mediated endothelium-dependent vasodilation with prehypertension is mediated, in part, by a reduction in nitric oxide bioactivity. Co-infusion of the endothelial nitric oxide synthase inhibitor L-NMMA significantly reduced forearm blood flow responses to acetylcholine in normotensive but not prehypertensive adults;

3) prehypertensive adults exhibit elevated endothelin-1 vasoconstrictor tone that is mediated by both the ET_A and ET_B receptor subtypes. Selective ET_A receptor blockade elicited a significantly greater vasodilator response in prehypertensive compared with normotensive adults. Furthermore, non-selective ET_A and ET_B receptor blockade produced significantly greater vasodilation in prehypertensive adults than that produced by selective ET_A antagonism;

4) the elevation in endothelin-1-mediated vasoconstrictor activity contributes to diminished endothelium-dependent vasodilation in prehypertensive adults. Selective ET_A receptor blockade increased acetylcholine-mediated endothelium-dependent vasodilation in prehypertensive adults to levels similar to that of normotensive adults;

Collectively, these studies indicate that prehypertension is associated with impaired vascular endothelial vasomotor function. These endothelial abnormalities may contribute to the increased
risk of clinical hypertension and atherosclerotic vascular disease in prehypertensive adults.

Given the fact that alterations in endothelial function that characterize clinical hypertension are already apparent in adults with prehypertension, it may be necessary to more strictly implement interventions aimed at lowering blood pressure and reducing cardiovascular risk at blood pressure levels below the traditional cut-off for hypertension.
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