Preventing Hypertension: The Role of Oxidative Stress

Shawna D. Nesbitt MD, MS
Assistant Professor of Internal Medicine
Division of Hypertension
August 14, 2003

ABBREVIATIONS:

ACEI	Angiotensin converting enzyme inhibitor
Ang II	Angiotensin II
ARB	Angiotensin receptor blocker
AT_1-receptor	Angiotensin type 1 receptor
BMI	Body mass index
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal regulated kinase
GC	Guanyl cyclase
GSH	Oxidized glutathione
GSSG	Reduced glutathione
H_2O_2	Peroxide
JNC	Joint National Committee Report on High Blood Pressure
JNK	c-Jun N terminal kinase
L-Arg	L-arginine
MAPK	Mitogen activated protein kinase
NAD(P)H	Nicotinamide adenine dinucleotide phosphate, reduced form
NEM	N-ethylmaleimide
NO	Nitric oxide
$\cdot O_2^-$	Superoxide (reactive oxide species)
RAS	Renin angiotensin system
ROS	Reactive oxide species
SAPK	Stress activated protein kinase
SHR	Spontaneously hypertensive rats
SOD	Superoxide dismutase
TROPHY	Trial of Preventing Hypertension
VSMC	Vascular smooth muscle cells

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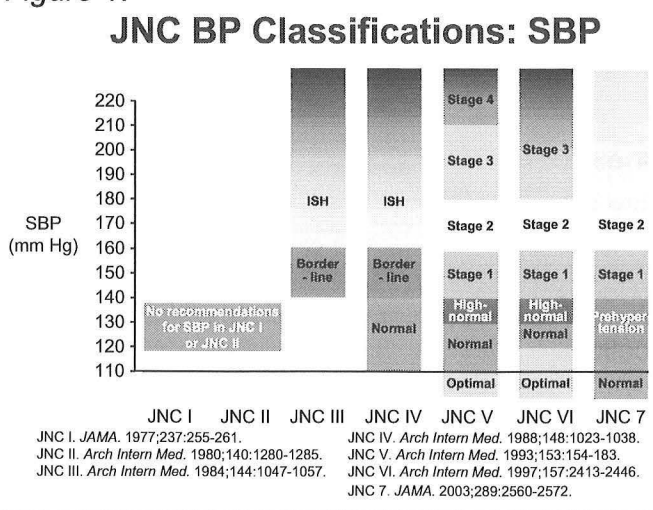
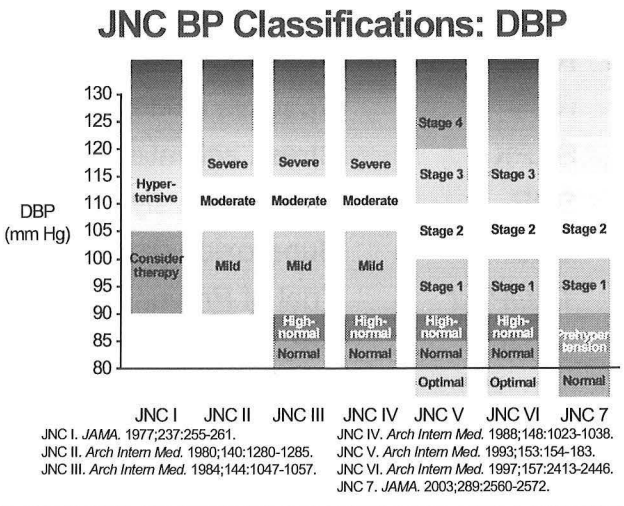
One of the highlighted changes in the recently published (JNC 7) Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure is the classification of blood pressure. This report now classifies blood pressures of 120-139/80-89 as "Prehypertensive."[1] Over the past 26 years, the previous expert committee reports have altered the classification of blood pressure considerably. Initially the focus of hypertension was purely on diastolic blood pressure. Blood pressures of 90-105 mm Hg (diastolic) were considered "mild" and drug treatment was only considered but not necessarily recommended. In 1977 in the first JNC report, hypertension was designated as diastolic blood pressures above 105 mm Hg, and therefore drug treatment was recommended while there were no guidelines for systolic blood pressure at that time.[2,3] By 1984, the Third JNC report introduced the class of "high normal blood pressure" further adjusting the classification of diastolic blood pressure to include the following stages; normal (<85 mm Hg), high normal (85-89mm Hg), mild elevation (90-105 mm Hg) and hypertensive (>105 mm Hg). In the 1984 report, for the first time, systolic blood pressure was included in the diagnosis of hypertension. Systolic blood pressures of 140-160 mm Hg were called "borderline" and >160 mm Hg was designated as "isolated systolic hypertension (ISH)."[4,5] In the Fifth JNC report published in 1993, hypertension staging was completely overhauled based largely on the data from the MRFIT study to include 4 stages of hypertension and 3 stages of normal blood pressure.[6] Both systolic and diastolic blood pressures were included in this staging process. The only change to this classification system in the Sixth JNC report was shifting the systolic criteria for normal blood pressure up from 110 to 120 mm Hg and consolidating hypertension stages 3 and 4. Thus in 1997, the nonhypertensive stages were designated as optimal (<120/80 mm Hg); normal (120-130/80-85 mm Hg); and high normal (130-129/85-89 mm Hg).[7] *(Fig 1)*

Figure 1.



JNC I. *JAMA.* 1977;237:255-261.
JNC II. *Arch Intern Med.* 1980;140:1280-1285.
JNC III. *Arch Intern Med.* 1984;144:1047-1057.
JNC IV. *Arch Intern Med.* 1988;148:1023-1038.
JNC V. *Arch Intern Med.* 1993;153:154-183.
JNC VI. *Arch Intern Med.* 1997;157:2413-2446.
JNC 7. *JAMA.* 2003;289:2560-2572.



JNC I. *JAMA.* 1977;237:255-261.
JNC II. *Arch Intern Med.* 1980;140:1280-1285.
JNC III. *Arch Intern Med.* 1984;144:1047-1057.
JNC IV. *Arch Intern Med.* 1988;148:1023-1038.
JNC V. *Arch Intern Med.* 1993;153:154-183.
JNC VI. *Arch Intern Med.* 1997;157:2413-2446.
JNC 7. *JAMA.* 2003;289:2560-2572.

The most recent change to this classification in the JNC 7 report has combined the normal and high normal classes into the newly titled class "prehypertension" with optimal blood pressure remaining as <120/80 mm Hg. In addition, the hypertension stages have been further consolidated with the combination of stages 2 and 3.[1] *(Fig 2)* The shifting in the staging process over time is related to

THE NEW JNC 7 BLOOD PRESSURE CLASSIFICATION

BP Classification	SBP mm Hg		DBP mm Hg
Normal	<120	and	<80
Prehypertension	120-139	or	80-89
Stage 1 Hypertension	140-159	or	90-99
Stage 2 Hypertension	≥160	or	≥100

Figure 2.
The new blood pressure clasification which highlights the new "prehypertensive" class and the consolidation of stage 2 hypertension. Data from Chobanian AV, et al. The JNC-7 Report. JAMA 2003;289:2560-2572.

increasing knowledge of the relationship of blood pressure to cardiovascular disease risk. Furthermore, the purpose of the JNC reports is to improve the awareness, diagnosis, evaluation and treatment of hypertension. To that extent, linking the stages of blood pressure to the recommendations for follow-up and treatment is appropriate for implementation of the guidelines. The most recent change to define Prehypertension as blood pressures of 120-139/80-90 mm Hg has caused some debate about the plausibility of such a class. The rationale for the "Prehypertension" stage is that the cardiovascular risk associated with blood pressure begins to increase from the level of 115 mm Hg systolic, and 75 mm Hg diastolic particularly for individuals aged 40-70 years old. Cardiovascular mortality increases 2 fold for every 20mm Hg in systolic and 10mm Hg in diastolic blood pressure.[8] *(Fig 3)* There is significant evidence of the hypertensive process beginning prior to the diagnosis of hypertension at >140/90 mm Hg.

From a public health perspective, for individuals who live to be 55 years old, the lifetime risk of developing hypertension based on the Framingham population is 90%.[9] *(Fig 4)*

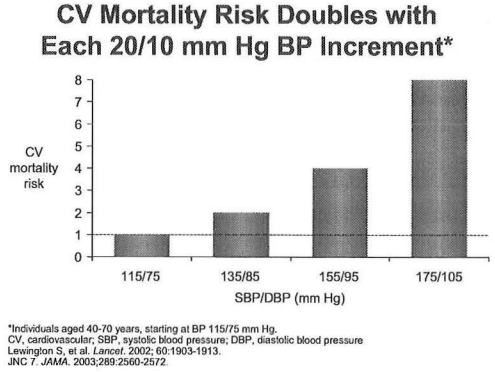


Figure 3.
Chobanian AV, et al. for the Joint National Committee. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The JNC-7 Report. JAMA 2003;289:2560-2572.

Lifetime Risk of Hypertension

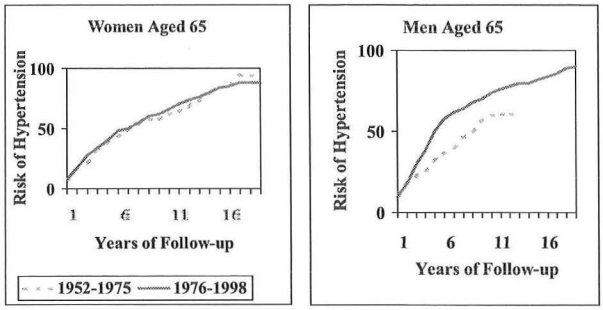


Figure 4.
Vasan RS, et al. Residual Lifetime Risk for Developing Hypertension in Middle-aged Women and Men: The Framingham Heart Study. JAMA 2002;287:1003-1010.

Furthermore, a decrease in systolic blood pressure of the population of the U.S. by 5 mm Hg would decrease stroke mortality by 14%, CHD mortality by 9% and total mortality by 7%.[1,10] *(Fig 5)* Lifestyle modifications are the primary mode of treatment for individuals in the prehypertensive class, while reserving drug treatment for those who have compelling indications for treatment such as renal disease, diabetes, and heart failure. Although clinical trials demonstrate benefits of lifestyle modifications, it is difficult to implement and maintain these effects over time.[11,12,13]

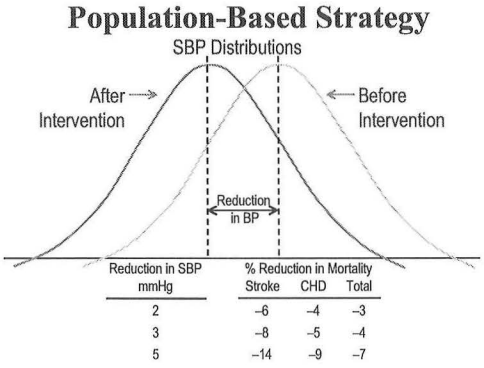


Figure 5.
The effect of shifting the mean blood pressure and distribution of the U.S. population by small increments may have dramatic effects on morbidity and mortality. JAMA 2003;289:2560.

However there is some difficulty with the assumption that all individuals in the new "prehypertensive" stage are the same. High normal blood pressure confers a higher level of risk than the previously designated normal blood pressures. Although the population theory is valid for reducing the overall burden of hypertension for the population, the risk of developing hypertension and cardiovascular events is clearly different for individuals who have blood pressures of 120-130/80-85(normal) mm Hg compared to those who have blood pressures of 130-139/85-89 mm Hg (high normal). Based on the Framingham and TOPH studies, the 4 year rate of progression to hypertension for those in the high normal blood pressure (130-139/85-89 mm Hg) is approximately 40% (37.3%-49.5%).[14] *(Fig 6)* This 4-year rate varies by age and is considerably higher than that of individuals with blood pressures of 120-130/80-85 mm Hg which is 17.6% for ages 35-64 and 25.5% for ages 65-94.[15,16] *(Table 1)* These rates have been adjusted for sex, age, BMI and baseline blood pressure. In addition, Vasan et al has shown that the risk of cardiovascular events is 1.6 fold higher for individuals in the high normal blood pressure range

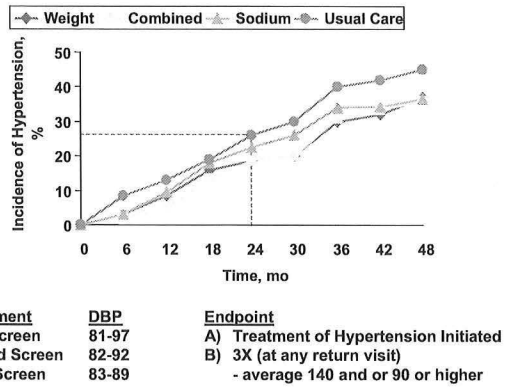


Figure 6.
In the NIH Trial of Preventing Hypertension (TOPH), high normal individuals in the usual care group developed hypertension at a rate of 21.1% at 18 months and 44.4% at 48 months. TOPH Study. Arch Intern Med 1997;157:657-667.

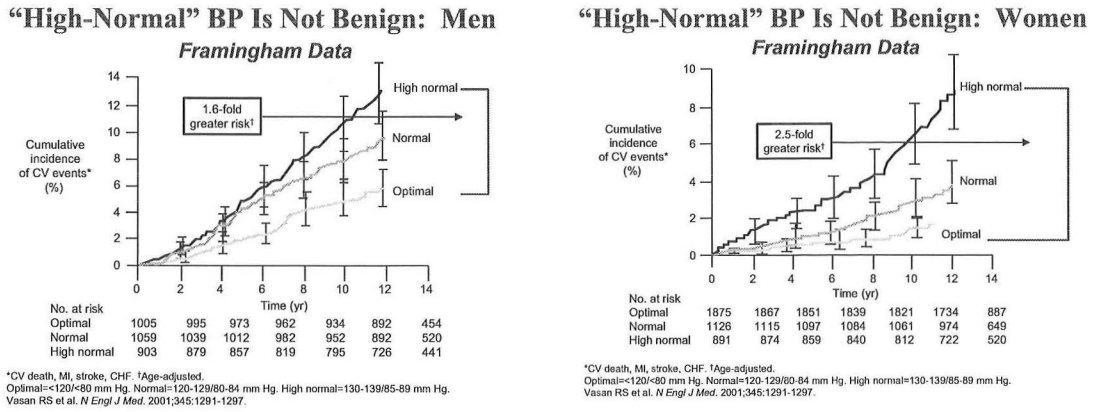
compared to optimal blood pressure for men, while there is a 2.5 fold increase in risk for women.[17] *(Fig 7)* The risk of cardiovascular events is also higher in the high normal blood pressure group than those in the normal blood pressure (120-130/80-85). The risk of cardiovascular events for normal individuals (120-130/80-85) reveals a positive trend, however it did not reach a level of statistical significance. This complicates the implementation of mass interventions in the entire group of "prehypertensives." It may be more prudent to concentrate focus on those in this group who are at clear risk for progressing to hypertension. Based on the Framingham data, the number needed to treat for 5 years to prevent one cardiovascular event in high normal individuals greater than 65 years old is 24-71 for men and 34-102 for women. In younger subjects, this number is 73-218 for men and 143-429 for women.[17] Better methods of characterizing those at risk will improve our success in implementing treatment to this group. For this discussion, we will limit our focus to those "prehypertensives" in the "high normal" blood pressure range (130-139/85-89 mm Hg).

Table 1. **1–4-year incidence of hypertension according to baseline blood pressure category***

Baseline Blood Pressure Category	Age Group (35-64 Years)	Age Group (65-94 Years)
% Hypertension at 1 year (95% CI)		
Optimal blood pressure	1·3 (1·1-1·6)	4·3 (3·1-5·7)
Normal blood pressure	4·7 (4·0-5·5)	7·1 (5·5-9·0)
High normal blood pressure	11·0 (9·6-12·6)	15·7 (13·0-18·8)
% Hypertension at 4 years (95% CI)		
Optimal blood pressure	5·3 (4·4-6·3)	16·0 (12·0-20·9)
Normal blood pressure	17·6 (15·2-20·3)	25·5 (20·4-31·4)
High normal blood pressure	37·3 (33·3-41·5)	49·5 (42·6-56·4)

* Rates are per 100, and are adjusted for sex, age, BMI=baseline examinations, and baseline systolic and diastolic blood pressure.
Modified from Vasan RS, et al. Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: A cohort study. *Lancet* 2001;358:1682-1686.

Figure 7.



From Vasan RS et al. N Engl J Med. 2001;345:1291-1297.

In the Tecumseh Blood Pressure study, a cohort follow-up of Caucasian individuals who reside in Tecumseh Michigan, Julius et al has shown that individuals of mean age 29, with borderline or high normal blood pressures have risk profiles that differ from the normotensives. In fact, they are more similar to hypertensives. In this cohort follow-up, clinic and home blood pressures and blood samples were measured. This analysis revealed that clinic blood pressure correlates positively to total cholesterol, triglycerides, insulin, hematocrit, overweight and heart rate.[18] *(Fig 8 & Table 2)* Furthermore the trends in these risk factors are similar in the hypertensive and "borderline" or high normal blood pressure groups in this population.[19] Thus, the increase in the risk of cardiovascular events is mediated not only by elevated blood pressure but also through other risk factors.

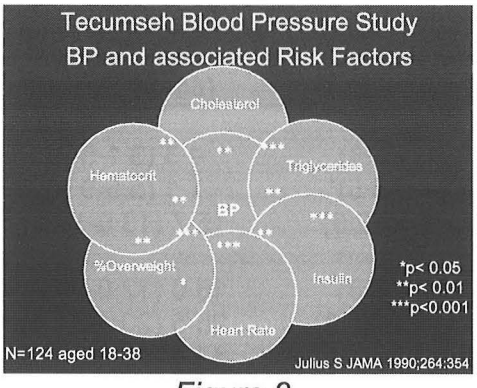


Figure 8.

The associations of blood pressure to other cardiovascular risk factors in the Tecumseh BP study. Data from the 601 subjects in the Tecumseh Blood Pressure Study. JAMA 1990:264:354.

Characteristics of Subjects with High Normal Blood Pressure in Tecumseh

	Normal N=577	High Normal N=31	P< 0.05
Cholesterol mg/dl	185	199	*
Triglycerides mg/dl	91	151	*
HDL mg/dl	44	38	*
Insulin µU/ml	12	18	NS
Glucose mg/dl	91	97	NS
Ins/Gluc	0.13	0.19	*

S Julius Hypertens 1990;16:617

Table 2.

Data from the 601 subjects in the Tecumseh Blood Pressure Study. Hypertension 1990;16:617.

The pathway to cardiovascular events and death begins with the appearance of cardio-vascular risk factors. The focus of most of the current interventions is on treating cardiovascular disease after it has already been manifested and treating risk factors once they appear. Over the course of a lifetime there is a slight gradual increase in blood pressure which also correlates to an increase in cardiovascular mortality and morbidity. As blood pressure increases, it contributes to the development of target organ damage such as left ventricular hypertrophy, renal insufficiency, coronary disease, diabetes and atherosclerosis. The additive risk of these target organ diseases to the underlying risk of elevated blood pressure alone leads to a dramatic rise in cardiovascular mortality. The underlying pathophysiology of hypertension and its progression suggests that early on there are structural changes in the blood vessel wall. While these early changes appear to be reversible, as further damage occurs these changes may become irreversible. ***(Fig 9)*** The early component of this process represent the period of "primary prevention of hypertension." In this time period, treating blood pressure reverses much of the increase in cardiovascular morbidity. However at some point the effects of elevated blood pressure on other vital organs and the resultant effect on cardiovascular mortality become irreversible. This is the period of "secondary prevention of hypertension." Shifting the focus of intervention to an earlier time point in the progression of hypertensive disease may be more effective in reducing the overall risk of cardiovascular mortality and morbidity.

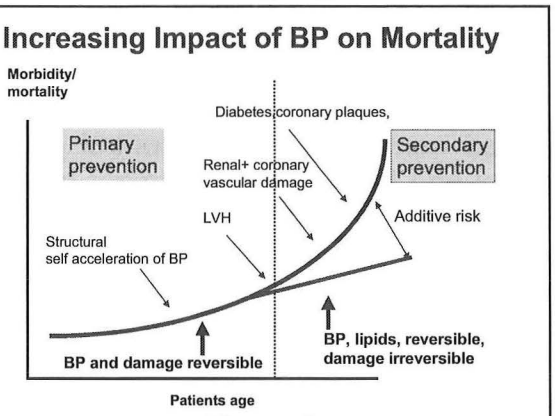


Figure 9.
Early treatment of blood pressure may limit the subsequent effects of blood pressure on mortality.

The Pathobiology of the Development of Hypertension

The pathway of the progression from normotension to hypertension as described by Alexander, is characterized by changes in the morphology of resistance blood vessels.[20-23] Increases in blood pressure lead to adaptive changes in the microvasculature. These changes manifest as an increase in the mass of the medial layer of vascular smooth muscle or through remodeling of the vascular smooth muscle medial layer. *(Fig 10)* This alteration in the vasculature further propagates hypertension through increased vascular resistance. Thus

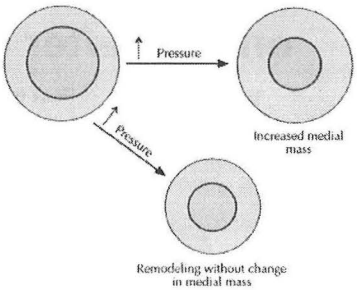


Figure 10.
From Alexander RW. Hypertension: Mechanisms and Therapy, 1998

interrupting these changes in the morphology may attenuate or perhaps ablate the progression of hypertension. We sought to find evidence of this possibility.

In an animal study of (SHR) spontaneously hypertensive rats, Giudicelli demonstrated that some antihypertensive agents have sustained blood pressure reduction beyond the treatment time period while others do not. In his experiment, SHR were treated for the first 20 weeks of life with 1 of 3 different antihypertensive agents or placebo control.[24,25] The agents in the study were hydralazine, a direct vasodilator; atenolol, a beta blocker; and captopril, an (ACEI) angiotensin converting enzyme inhibitor. All 3 treatment groups reduced blood pressure during the treatment period compared to the control group. However post treatment, in both the atenolol and hydralazine treatment groups, the blood pressure increased over the remaining 12 weeks of their lives to similar levels of blood pressure as the control group. In the captopril treatment group, although there was some increase in the post treatment, blood pressure was persistently lower than the control group. Thus it appears that renin angiotensin antagonism may confer some longer term antihypertensive effects not seen with other antihypertensive agents. *(Fig 11)* In a study by O'Sullivan, early treatment with the ACEI, ramipril, at 6 to 10 weeks of life in SHR led to persistent blood pressure reductions beyond the treatment period in comparison to the control animals.[26] *(Fig 12)* It is of particular interest whether this period of treatment could occur later in life and result in the similar sustained blood pressure reductions. In a study comparing early versus late treatment with the ACEI, Harrap showed that treatment with enalapril during weeks 6-10 resulted in persistent blood pressure reduction in the 12 weeks post treatment compared to controls. However later treatment during weeks 20-24 reduced blood pressure on treatment but this reduction was not sustained compared to the control group in the 12 weeks post treatment.[27] *(Fig 13)* Similar results were demonstrated using perindopril by Adams et al.[28,29] Thus these animal studies confirm that treatment with angiotensin antagonism with ACEI confers persistent reduction in blood pressure beyond the treatment period that is not seen with other classes of antihypertensives. This persistent blood pressure reduction is a time specific event, which occurs with early treatment and not with later treatment.

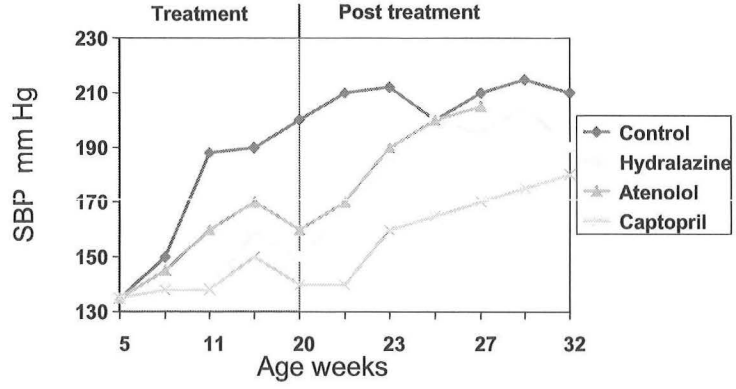


Figure 11.
Effects of early short term treatment with antihypertensive agents on blood pressure in SHR during and after treatment. Giudicelli et al. Clin Exp Hypertens 1980;2:1083-1096.

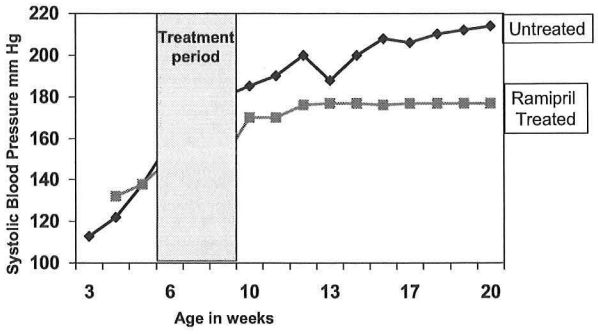
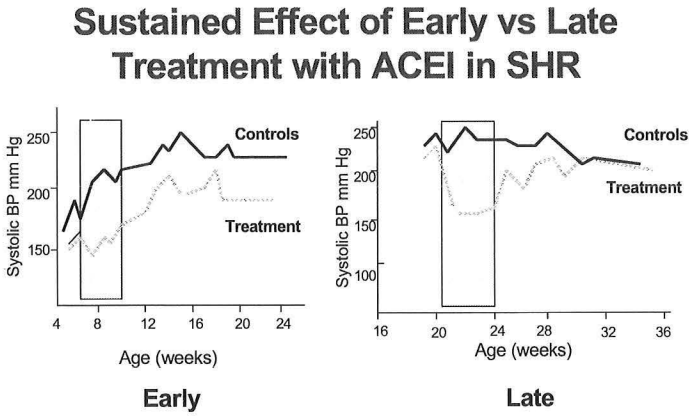


Figure 12.
Effects on systolic BP in SHR treated with ACEI (ramipril) versus control with early treatment. O'Sullivan JB. Hypertension 1995;25:162

Figure 13.
Early ACEI treatment in SHR causes persistent BP reduction 12 weeks post-treatment that are not seen with later treatment period. Harrap SBG. Hypertension 1990;16:603.



Furthermore, human investigations also demonstrate the unique effect of angiotensin antagonism on the vasculature. Schiffrin studied the vascular changes in a group of stage 1 hypertensives treated over a 1 year period with the beta blocker, atenolol, or the ACEI, cilazapril. Resistance arteries were dissected from gluteal fat biopsies taken at baseline and after 1 year of treatment in these hypertensives. Despite the same level of blood pressure reduction with atenolol and cilazapril, only cilazapril reduced the media to lumen ratio of the resistance arteries after 1 year.[30] The media layer of these blood vessels was reduced in size as demonstrated by Mulvany in a similar study.[31] *(Table 3, Fig 14)* Thus it is reasonable to speculate that vascular changes begin early in the development of hypertension. These vascular changes are modulated by angiotensin antagonists. These reversible effects may be limited to a critical period in the development of hypertension. Reversing these changes confers persistence to the blood pressure reduction. This result strongly implicates the RAS system in the development of vascular hypertrophy and remodeling, and provides important rationale for the Trial Of Preventing Hypertension Study (TROPHY).

Table 3.

Structural and Functional Effects of Treatment on Human Resistance Arteries in Hypertensive Patients at 1 Year

	Atenolol		Cilazapril		Normal
FACTORS	Pre	Post	Pre	Post	
BP mm Hg	146/99	131/85*	147/99	132/87*	
Media/Lumen Ratio (%)	7.97	8.07	7.54	6.31*	5.1
Active Wall Tension	2.86	3.46	3.01	4.34*	4.7
Active Media Stress	1.78	1.74	1.39	2.82*	2.7

All values are means
*Statistically significant from pretreatment means

Schiffrin EL. Hypertens 1994;23:83

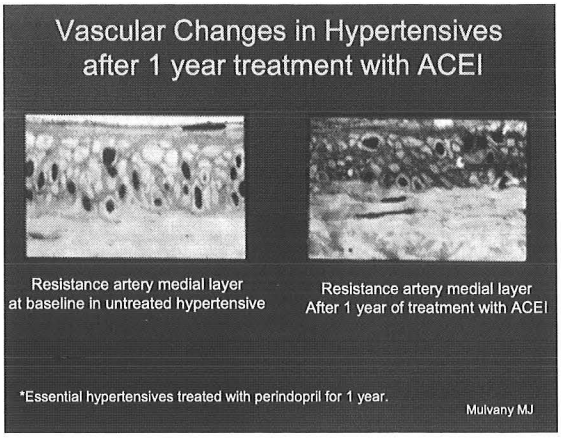


Figure 14.
From Mulvany MJ. J Hypertens 1996;14:S21-S24.

The TROPHY Study is a randomized, placebo-controlled trial, designed to test the hypothesis that treatment of high normal blood pressure with a low dose of an AT_1-receptor blocker (candesartan cilexitil 16 mg per day) will delay or prevent the progression to hypertension. Between June 1999 and June 2001, 809 individuals were randomized to placebo or low dose candesartan cilexitil for 2 years followed by 2 years of placebo. They were qualified for the study by the average of 3 seated blood pressure measurements taken on 3 separate clinic visits by automated device (OMRON 706). Untreated individuals with systolic blood pressures between 130-139 mm Hg and diastolic 85-89 mm Hg were included in the study. The primary outcome of the trial is the incidence of hypertension determined by clinic blood pressures greater than 140 mm Hg and/or 90 mm Hg systolic and diastolic respectively on three visits during the study follow-up; or greater than 160 mm Hg and/or 100 mm Hg on one occasion; or development of target organ damage requiring blood pressure treatment.[32] *(Fig 15)* The primary analysis of the TROPHY Study will focus on the comparison of the incidence slopes. *(Fig 16)*

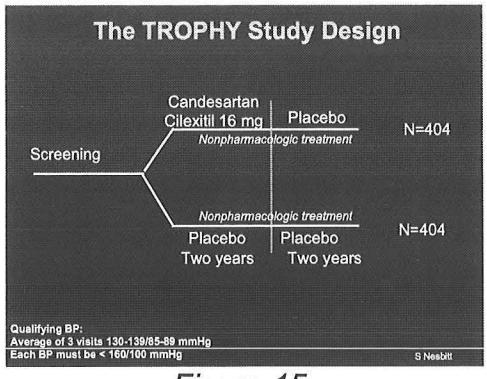


Figure 15.
The Trial of Preventing Hypertension (TROPHY) is a randomized, placebo controlled trial of ARB treatment in high normal blood pressure.

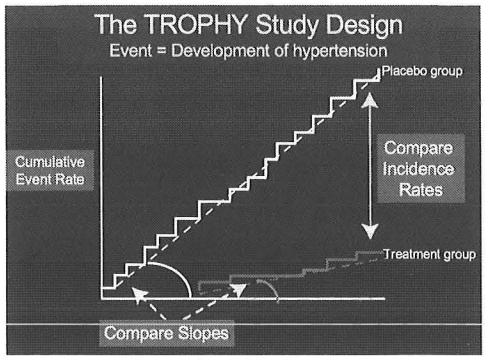


Figure 16.
The design analysis plan for the TROPHY study.

Oxidative Stress as a Cause of Progressive NO Deficient Hypertension

Recent work from David Harrison's group and others implicates nitric oxide and oxidative stress in the underlying mechanism of the progression to hypertension.[33-35] In rats, the slow pressor response to infused angiotensin provides an experimental model of Angiotensin II type 1(AT_1) receptor-mediated progressive blood pressure elevation. Acutely infused high dose intravenous Angiotensin II (Ang II) leads to a sharp rise in blood pressure. However, it turns out that this mechanism probably has little to do with chronic hypertension. Twenty years ago, Brown showed that in rats, prolonged intravenous infusion of Ang II to cause a steady state 2-3 fold increase in plasma Ang II levels (mimicking the plasma levels in human renovascular hypertension) does not cause an immediate rise in blood pressure. Rather, the blood pressure increases progressively over 3-5 days to a new steady-state level of hypertension.[36] Although this slow pressor response has been known for two decades, the underlying mechanism has been elucidated only recently. It is now clear that this experimental hypertension is mediated by deficiency of the endothelial-dependent vasodilator substance nitric oxide (NO). Interestingly, this NO-deficient hypertension is not caused by impaired NO production but rather by enhanced NO destruction, i.e., conversion to peroxynitrite by its interaction with superoxide anion ($\cdot O_2^-$). ***(Fig 17)*** A key factor in the regulation of this mechanism is Ang II. AT_1 receptor stimulation activates a family of NAD(P)H oxidases in the vessel wall that generate $\cdot O_2^-$ and other reactive oxygen species that quench NO, resulting in NO deficient hypertension.[34] The vascular NAD(P)H oxidases are similar in many respects to the well-known NAD(P)H oxidases that mediate the oxidative burst in leukocytes and macrophages.[35]

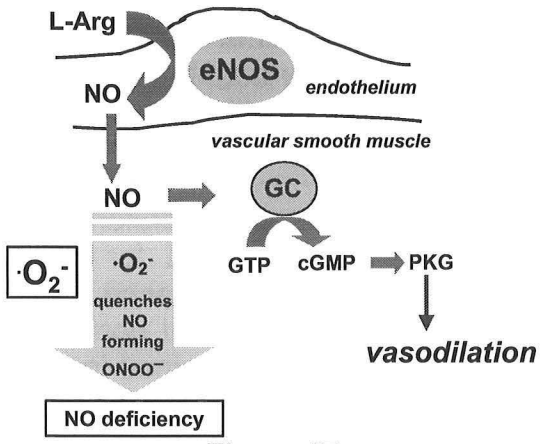


Figure 17. Mechanisms of nitric oxide deficient hypertension.

Some of the key experimental findings from this animal model demonstrate the proposed mechanism in hypertension. First, the production of vascular $\cdot O_2^-$ in the Ang II infused rat is specific for Ang II and is not secondary to elevated blood pressure. Mimicking the slow pressor effect of Ang II with a continuous infusion of norepinephrine does not yield vascular $\cdot O_2^-$. In addition, given the ARB losartan, with Ang II eliminates the increase in $\cdot O_2^-$.[37,38] *(Fig 18)* Secondly, in Ang II-infused rats the slow pressor response can be rescued by administering superoxide dismutase (SOD) in liposomes or by SOD mimetics that reduce the circulating concentration of $\cdot O_2^-$, thus increasing the bioavailability of NO.[38] There are 3 forms of SOD in the blood vessel, including extracellular

SOD, mitochondrial SOD, and cytosolic SOD. All 3 forms constitute candidate genes for further investigation. Thirdly, some of the same mechanisms appear to be operative in standard rat models of hypertension such as the SHR and recently in cultured cells from human vasculature.[39-41]

While this is elegant basic research which may have important translational implications for human hypertension, it is important to point out that this effect in rats develops over a matter of days whereas human hypertension progresses over years and involves hypertrophy and remodeling of the vessel wall.

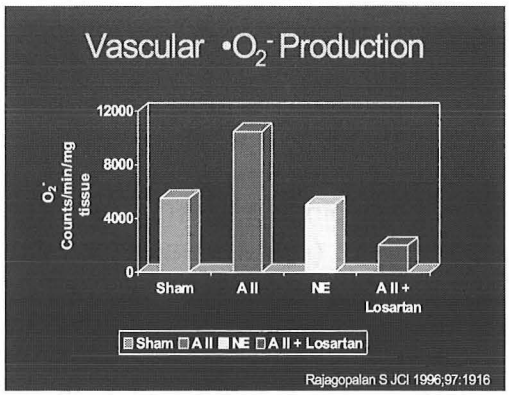


Figure 18.
$\cdot O_2^-$ is increased by Ang II and this access is limited by the addition of ARB, while norepinephrine has no effect.

Oxidative Stress as a Cause of Progressive Vascular Hypertrophy

In this regard, a second mechanism by which NAD(P)H oxidase leads to hypertension is through vascular hypertrophy and cell growth. Vascular hypertrophy is thought to set off a vicious cycle by which hypertension induced vascular hypertrophy begets more hypertension.[21,42-45] By increasing the media-to-lumen ratio and amplifying peripheral vascular resistance, these processes are both the consequence and the cause of progressive hypertension. In such vessels, endogenous vasoconstrictor substances elicit exaggerated increases in vascular resistance and therefore blood pressure. Touyz and Schiffrin have developed a human model for studying the regulation of small resistance vessels. In this model, resistance vessels are dissected from gluteal fat biopsies taken from hypertensive and normotensive individuals. These vascular smooth muscle cells are then examined in primary culture. Some of the salient findings from this model are relevant to the discussion of the role of oxidative stress in the development of hypertension.

First, similar to the responses noted in rat models, human vascular smooth muscle cells subjected to Ang II, also lead to a slow production of $\cdot O_2^-$ and subsequent exposure to SOD yields the conversion of $\cdot O_2^-$ to H_2O which is freely diffusible.[46] *(Fig 19)* In culture, Ang II increases the production of H_2O_2 and SOD from vascular smooth muscle cells. Furthermore, both inhibiting SOD and providing excess catalase (the enzyme which stimulates the degradation of H_2O_2) lead to reduction in protein synthesis.[35] *(Fig 20)* This highlights the important role of both $\cdot O_2^-$ and H_2O_2 in the pathway to vascular hypertrophy.

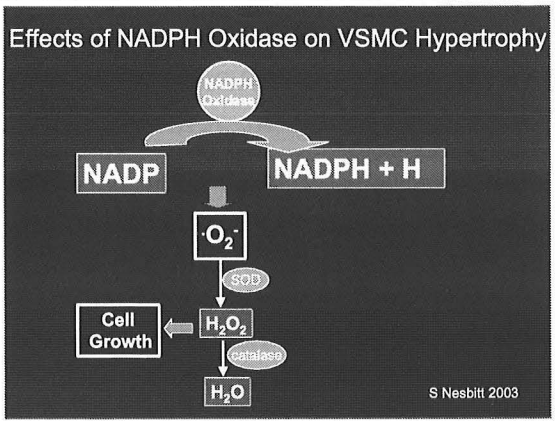


Figure 19.
NADPH oxidase cause release of $\cdot O_2^-$ which is degraded to H_2O_2 thereby leading to cell growth.

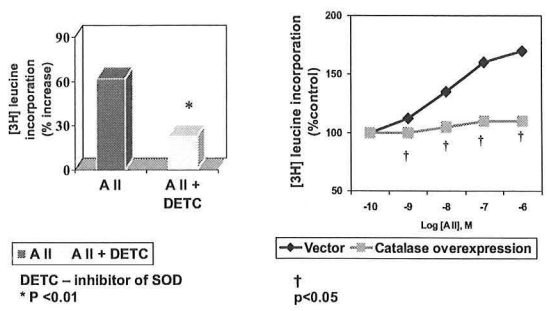


Figure 20.
Inhibition of SOD and catelase overexpression reduce protein synthesis in VSMC. From Zafari AM. Hypertension 1998; 32:488.

Secondly, the generated H_2O_2 initiates hypertrophic signaling through a family of mitogen activated protein kinases (MAPK) specifically, extracellular signal-regulated kinase ERK1(p44)/ERK2(p42), ERK 5, JNK (c-Jun N terminal kinase)/SAPK (stress activated protein kinase) and p38 MAPK. *(Fig 21)* Phosphorylation of these protein kinases is required to activate protein synthesis. In studies of human vascular cells in culture, Touyz has demonstrated that while Ang II increases ERK phosphorylation, angiotensin receptor blockade with losartan results in similar reduction in phosphorylation as a direct inhibitor of ERK in vascular smooth muscle cells.[49] *(Fig 22)* These kinases regulate a number of intracellular pathways, which culminate in cellular growth. ERK pathways activate nonspecific early response genes controlling cell growth and differentiation, DNA

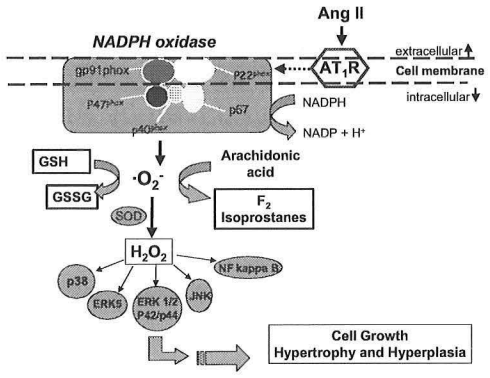


Figure 21.
The pathway from Ang II to vascular cell hypertrophy and hyperplasia involves NADPH oxidase stimulation providing excess H_2O_2 then activating protein kinase signaling.

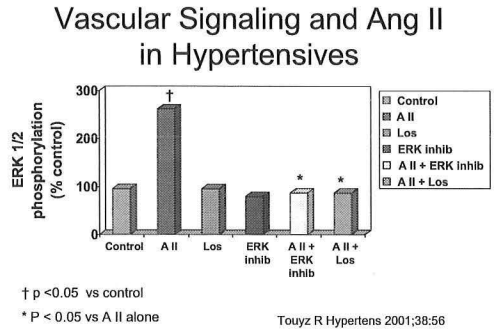


Figure 22.
Ang II stimulates protein synthesis in vascular smooth muscle cells through the ERK 1/2 kinases. This synthesis is inhibited by ERK inhibition and ARB (losartan) treatment. Touyz RM et al. Hypertens 2001;38:56-64.

synthesis, and cytoskeleton organization such as c-fos, c-myc, and c-jun. The JNK/SAPK pathway regulates VSMC growth by promoting apoptosis or inhibiting growth.[47-52] These MAPK's are also candidate genes which warrant further investigation.

Thirdly, all 5 subunits of leukocyte NAD(P)H oxidase are also present in human vascular smooth muscle cells.[53,54] This is important because at least one of the subunits is not found in rat aortic cells.[53,54] There are 2 membrane bound and 3 cytoplasmic subunits which, when phosphorylated, form a functional enzymatic unit. While p22phox and gp91phox are located in the cell membrane and must form a complex prior to the activation of the oxidative function of NAD(P)H oxidase, the p40phox, p47phox, and p67phox subunits are located in the cytoplasm.[55-57] When human vascular smooth muscle cells from hypertensives were treated with Ang II, the expression of gp91phox, p22phox, and p47phox was increased more strikingly in hypertensives compared to those from normotensives.[58] Candidate genes for each of these subunits have been identified. (*See Fig 21)*

Fourthly, when exposed to exogenous Ang II, vascular smooth muscle cells from hypertensive subjects produced more $\cdot O_2^-$ than those from normotensive subjects.[59] Importantly, the $\cdot O_2^-$ generation in both groups is blocked by the addition of an ARB. These findings taken in view of the findings by Schiffrin,[30] permits the clear transition from basic research to clinical medicine. The previously discussed trial demonstrated that vascular remodeling in the small resistance vessels of hypertensives was improved only by the ACE inhibitor and not beta blockers. *(See Fig 14)* These findings clearly implicate the RAS system, NO pathway and oxidative stress pathway in the development of vascular hypertrophy and remodeling. ***(Fig 23)*** The novel design of the TROPHY Study presents an excellent opportunity to further study these mechanisms in a clinical trial setting.

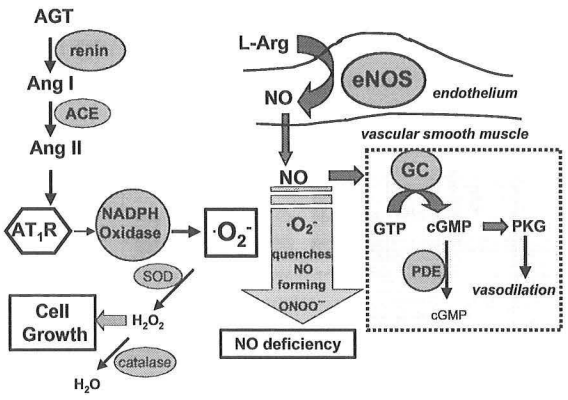


Figure 23.
Mechanism of NO deficient hypertension driven by Ang II stimulated increased $\cdot O_2^-$ which quenches NO and activates cell growth in VSMC.

The Mechanistic Substudy of the TROPHY Study

We will measure biomarkers of oxidative stress at 2, 3, and 4 years of follow-up in the participants of the TROPHY study *(see Fig 15)*. In this mechanistic substudy, we will assess the pattern of oxidative stress in the placebo and treatment groups to test whether individuals with high normal blood

pressure and high oxidative stress are at especially high risk for progressing to hypertension. Further, in the ARB treatment group, we will test whether ARB treatment alters this pattern. Finally, we will assess the role of genetic sequence variables in the NO, RAS, and oxidative stress pathways in explaining the progression to hypertension and the response to early treatment.

Biomarkers of Oxidative Stress

It is central to this design to identify stable and measurable biomarkers of the oxidative stress pathway in humans. In the setting of a multicenter trial, it is important that these markers be easily processed samples, and amenable to transport to a reference laboratory for analysis. In previous studies of oxidative stress, several markers have been utilized. At present, there is no perfect single way to measure oxidative stress in human subject. Therefore, for the present study, we have chosen the two best methods currently available. The most reliable of these is urine isoprostanes and plasma glutathione ratio (GSH:GSSG). In the majority of subjects, both methods will be used.

1. F_2-Isoprostanes as a Biomarker.

Concurrent with the increase in the production of $\cdot O_2^-$, arachidonic acid is converted to F2 Isoprostanes. This metabolite is measurable in both plasma and urine. Morrow et al have studied the biochemical properties of this metabolite extensively. He found that it is reliable and stable over time.[60-66] Urinary isoprostanes will be measured by HPLC in our study. This is probably the most well studied measure of oxidative stress which is translatable to human research in a large clinical trial setting. Isoprostanes are the product of nonenzymatic free radical-induced peroxidation of arachidonic acid. High concentrations of glutathione may augment the formation of isoprostanes.[67] Isoprostane level serves as an index of the presence of superoxide as well as having its' own biological properties. These properties are direct or receptor mediated, and includes both vasoconstriction and cell growth. Of the isoprostane classes, F_2-Isoprostanes are the most stable and thus ideal candidates for measurement as a marker of lipid peroxidation. The intra-subject variability in the measurement is 5%.[68]

In a study of lean normotensives and obese hypertensives, Stojiljkovic et al. found that urinary F2-isoprostanes are higher in the hypertensives than in the normotensive group. Furthermore there is a positive, continuous relationship of systolic blood pressure to urinary isoprostanes level.[69] *(Fig 24)*

2. Oxidized-to-Reduced Glutathione.

The ratio of oxidized to reduced glutathione (GSH/GSSG ratio) in blood will be measured according to the method of Jones et. al.[70] Glutathione is one of the central agents in the cellular antioxidant defense system. GSH is present intracellularly in millimolar concentrations, in human plasma in micromolar levels. Acting as an antioxidant, GSH is oxidized to its disulfide form (GSSG); thus, this ratio may be used to identify oxidative stress in tissue.[71,72] The validity of this ratio hinges on the prevention of spurious GSH oxidation after the sample is taken. To prevent this, N-ethylmaleimide (NEM) is added to the sample and subsequently derivatized by dinitrofluorobenzene and analyzed by HPLC.

The Relationship of Blood Pressure and Urine F2 isoprostanes

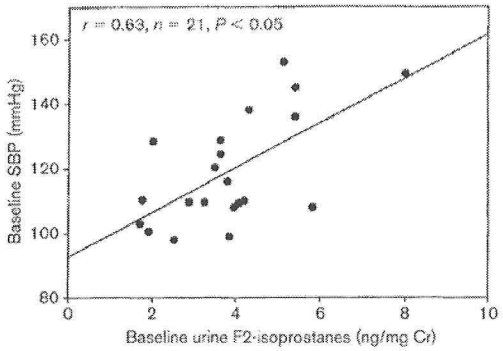


Figure 24.
Urine F2-isoprostanes correlate positively to systolic blood pressure. From Stojiljkovic MP. J Hypertens 2002;20:1215.

In animal studies of carbon tetrachloride induced liver failure, the GSH:GSSG ratio is reduced as the dose of CCl_4 increases as well as time of exposure to the toxin, thus reflecting the level of liver damage.[73] *(Fig 25)* In another human study, Samiec found differences in the glutathione ratio by age. Plasma GSH levels were similar in young and old individuals, while GSSG levels were lower in the younger group. Thus older individuals had a higher GSH:GSSG ratio. Furthermore individuals with diabetes had reduced GSH compared to normal individuals both young and old, with higher levels of GSSG. Therefore diabetics have higher GSH:GSSH ratio indicating higher levels of oxidative stress than normal individuals.[71] *(Fig 26)*

Pharmogenomics of Human Hypertension. This field is in its infancy. While there are some single nucleotide polymorphisms in signaling molecules associated with greater blood pressure lowering with certain antihypertensive agents, none of the reported results to date are of sufficient magnitude to make clinical treatment recommendations.

One of the best studies to date, (Turner et al.) investigated a C825T polymorphism of the G protein β_3-subunit that is involved in signaling via the thiazide-sensitive Na+/H+ cotransporter in the distal convoluted renal tubule and

GSH/GSSG Ratio as a Biomarker of Oxidative Stress

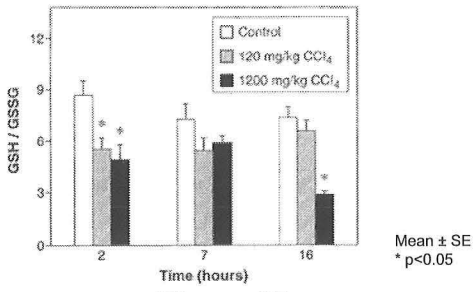
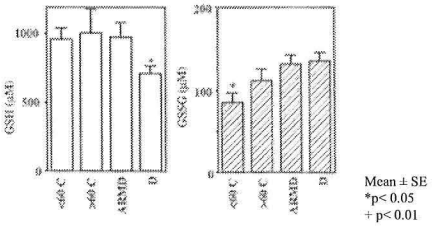


Figure 25.
Glutathione ratio decreases with higher levels of oxidative stress. From Kadiiska MB. Free Radical Biol & Med 2000;28:838.

Glutathione in Human Plasma



<60 c: < 60 y.o.
>60 c: >60 y.o.
ARMD: age related macular degeneration
D: Diabetes

Figure 26.
Glutathione levels vary by age and disease state. From Samiec PS. Free Radical Biol & Med 1998;24:699.

thus associated with low-renin hypertension.[74] In a group of black and white hypertensives who received one month of monotherapy with hydrochlorothiazide, this single nucleotide polymorphism alone accounted for only 3-4% of the overall variability in the blood pressure response. Although the mutant allele was more prevalent in blacks than whites (76% vs. 28%), the net predictive effect on blood pressure response to diuretic treatment was similar in both races. In multivariate analysis, genotype added very little additional predictive value to standard clinical variables. For example, the baseline level of systolic pressure explained 24% of the variance in the systolic blood pressure response to diuretic treatment, which is a nonspecific finding with any drug. Building on the pioneering study of Turner et al., the mechanistic substudy of the TROPHY study has been designed to: (1) test for combinations of polymorphisms that might produce synergistic effects on progression to (or protection against) hypertension via an AT_1 receptor mechanism; (2) extend the sample size and observation period substantially beyond that of previous pharmacogenetic studies; and (3) restrict the baseline blood pressure to a very narrow range of high normal values. Additional distinctive features of this proposal include: (4) translation of an emerging body of basic research on oxidative stress and vascular biology to a large clinical trial; and (5) utilization of the two best available markers of oxidative stress, isoprostanes and glutathione reduction, to examine the strength of correlation between prospective changes in blood pressure and oxidative stress.

In the TROPHY Mechanistic Study, we will examine genetic sequences in the specific pathways involved in the progression from high normal blood pressure to hypertension. *(Table 4)*

Table 4.

Genes in Selected Hypertension Pathways

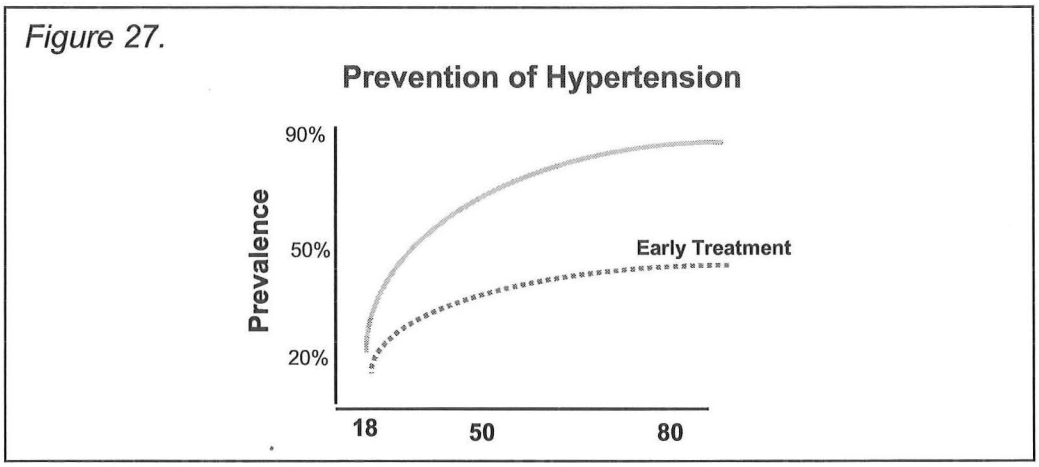
RAS Pathway	Oxidative Stress/ Vascular Pathways		Nitric Oxide Pathway
Angioten-sinogen	ERK 5	P67phox	Endothelial NOS
Renin	P38 MAPK	NFκB	Cu, Zn-SOD (Cytosol)
ACE	P22phox	C-fos	Mn-SOD (mitochondrial)
Type 1 AII receptor	Gp91 phox	C-myc	Ec-SOD (extracellular)
	P47phox	C-jun	
	p40phox		

Hypertension is a highly prevalent condition with significant complications and high human and economic costs. The current modes of treatment focus on "secondary prevention" of hypertension. Perhaps this model is flawed in the central theory that fails to recognize that the process of vascular change associated with hypertension has already begun prior to the onset of "established hypertension." Furthermore, the opportunity to have persistent effects beyond acute blood pressure treatment is lost in the time that we do not offer drug

treatment. Improving our understanding of the mechanisms which underly the progression to hypertension, will help to clarify the role of early treatment in the evolution of high blood pressure. The oxidative stress pathway respresents a promising mechanism to further describe the interaction between what is already known about nitric oxide deficient hypertension and renin angiotensin stimulated hypertension.

The ultimate goal of the TROPHY Study and the mechanistic substudy is to further our understanding of hypertension and to test whether early treatment alters the progression of high blood pressure. *(Fig 27)*

Figure 27.



Summary of concepts in the progression to hypertension:

- The relationship of blood pressure to cardiovascular risk is continuous.
- High normal blood pressure carries significant cardiovascular risk.
- High normal blood pressure progresses to hypertension in 40% of individuals over a 4 year period.
- This progression to hypertension features vascular changes which are reversible.
- Ang II but not norepinephrine increases the production of ROS in vascular smooth muscle, which is reduced by ARB treatment.
- The production of ROS in vascular smooth muscle is mediated by NADPH oxidase which is limited by SOD.
- ROS increases the production of H_2O_2 and SOD activity through stimulation from Ang II.
- Decreasing ROS by inhibiting SOD and decreasing H_2O_2 by excess catalase, limits the increased protein synthesis stimulated by Ang II. *(See Figure 23)*
- Therefore to attenuate the effects of Ang II on VSMC, it is necessary to have sufficient levels of SOD and catalase present, facilitating the degradation of ROS to H_2O.
- Touyz has shown similar findings in humans as noted in animals.
- Extracellular signal regulated kinases (ERK) mediates the pathway from Ang II to smooth muscle hypertrophy by phosphorylation.

- Ang II enhances ERK phosphorylation in hypertensives and normotensives.
- Inhibitors of ERK reduce the protein synthesis associated with Ang II.
- ARB limits ERK phosphorylation.
- Isoprostanes have been validated in humans with hypertension from both plasma and urine as a measurable marker of oxidative stress.
- GSH/GSSG ratio has been validated as a marker of oxidative stress in humans with vascular changes from aging, diabetes, and smoking.
- Although individual genes identified in hypertension have not been enormously clinically helpful, perhaps assessing sequence variations in distinct phenotypes of hypertension may offer more substantive information.

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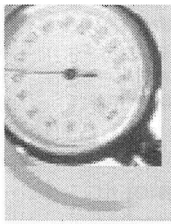

Reference Card From the

Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7)

EVALUATION

Classification of Blood Pressure (BP)*

Category	SBP mmHg		DBP mmHg
Normal	<120	and	<80
Prehypertension	120–139	or	80–89
Hypertension, Stage 1	140–159	or	90–99
Hypertension, Stage 2	≥160	or	≥100

* See *Blood Pressure Measurement Techniques* (reverse side)

Key: SBP = systolic blood pressure DBP = diastolic blood pressure

Diagnostic Workup of Hypertension

- Assess risk factors and comorbidities.
- Reveal identifiable causes of hypertension.
- Assess presence of target organ damage.
- Conduct history and physical examination.
- Obtain laboratory tests: urinalysis, blood glucose, hematocrit and lipid panel, serum potassium, creatinine, and calcium. Optional: urinary albumin/creatinine ratio.
- Obtain electrocardiogram.

Assess for Major Cardiovascular Disease (CVD) Risk Factors

- Hypertension
- Obesity (body mass index ≥30 kg/m^2)
- Dyslipidemia
- Diabetes mellitus
- Cigarette smoking
- Physical inactivity
- Microalbuminuria, estimated glomerular filtration rate <60 mL/min
- Age (>55 for men, >65 for women)
- Family history of premature CVD (men age <55, women age <65)

Assess for Identifiable Causes of Hypertension

- Sleep apnea
- Drug induced/related
- Chronic kidney disease
- Primary aldosteronism
- Renovascular disease
- Cushing's syndrome or steroid therapy
- Pheochromocytoma
- Coarctation of aorta
- Thyroid/parathyroid disease

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TREATMENT

Principles of Hypertension Treatment

- Treat to BP <140/90 mmHg or BP <130/80 mmHg in patients with diabetes or chronic kidney disease.
- Majority of patients will require two medications to reach goal.

Algorithm for Treatment of Hypertension

Lifestyle Modifications

Not at Goal Blood Pressure (<140/90 mmHg)
(<130/80 mmHg for patients with diabetes or chronic kidney disease)
See *Strategies for Improving Adherence to Therapy*

Initial Drug Choices

Without Compelling Indications

Stage 1 Hypertension (SBP 140–159 or DBP 90–99 mmHg)

Thiazide-type diuretics for most. May consider ACEI, ARB, BB, CCB, or combination.

Stage 2 Hypertension (SBP ≥160 or DBP ≥100 mmHg)

2-drug combination for most (usually thiazide-type diuretic and ACEI, or ARB, or BB, or CCB).

With Compelling Indications

Drug(s) for the compelling indications
See *Compelling Indications for Individual Drug Classes*

Other antihypertensive drugs (diuretics, ACEI, ARB, BB, CCB) as needed.

Not at Goal Blood Pressure

Optimize dosages or add additional drugs until goal blood pressure is achieved. Consider consultation with hypertension specialist.

See *Strategies for Improving Adherence to Therapy*

Blood Pressure Measurement Techniques

Method	Notes
In-office	Two readings, 5 minutes apart, sitting in chair. Confirm elevated reading in contralateral arm.
Ambulatory BP monitoring	Indicated for evaluation of "white coat hypertension." Absence of 10–20 percent BP decrease during sleep may indicate increased CVD risk.
Patient self-check	Provides information on response to therapy. May help improve adherence to therapy and is useful for evaluating "white coat hypertension."

Causes of Resistant Hypertension

- Improper BP measurement
- Excess sodium intake
- Inadequate diuretic therapy
- Medication
  - Inadequate doses
  - Drug actions and interactions (e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), illicit drugs, sympathomimetics, oral contraceptives)
  - Over-the-counter (OTC) drugs and herbal supplements
- Excess alcohol intake
- Identifiable causes of hypertension (see reverse side)

Compelling Indications for Individual Drug Classes

Compelling Indication	Initial Therapy Options
Heart failure	THIAZ, BB, ACEI, ARB, ALDO ANT
Post myocardial infarction	BB, ACEI, ALDO ANT
High CVD risk	THIAZ, BB, ACEI, CCB
Diabetes	THIAZ, BB, ACEI, ARB, CCB
Chronic kidney disease	ACEI, ARB
Recurrent stroke prevention	THIAZ, ACEI

Key: THIAZ = thiazide diuretic, ACEI= angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker, ALDO ANT = aldosterone antagonist

Strategies for Improving Adherence to Therapy

- Clinician empathy increases patient trust, motivation, and adherence to therapy.
- Physicians should consider their patients' cultural beliefs and individual attitudes in formulating therapy.

The National High Blood Pressure Education Program is coordinated by the National Heart, Lung, and Blood Institute (NHLBI) at the National Institutes of Health. Copies of the JNC 7 Report are available on the NHLBI Web site at **http://www.nhlbi.nih.gov** or from the NHLBI Health Information Center, P.O. Box 30105, Bethesda, MD 20824-0105; Phone: 301-592-8573 or 240-629-3255 (TTY); Fax: 301-592-8563.

Principles of Lifestyle Modification

- Encourage healthy lifestyles for all individuals.
- Prescribe lifestyle modifications for all patients with prehypertension and hypertension.
- Components of lifestyle modifications include weight reduction, DASH eating plan, dietary sodium reduction, aerobic physical activity, and moderation of alcohol consumption.

Lifestyle Modification Recommendations

Modification	Recommendation	Avg. SBP Reduction Range†
Weight reduction	Maintain normal body weight (body mass index 18.5–24.9 kg/m^2).	5–20 mmHg/10 kg
DASH eating plan	Adopt a diet rich in fruits, vegetables, and lowfat dairy products with reduced content of saturated and total fat.	8–14 mmHg
Dietary sodium reduction	Reduce dietary sodium intake to ≤100 mmol per day (2.4 g sodium or 6 g sodium chloride).	2–8 mmHg
Aerobic physical activity	Regular aerobic physical activity (e.g., brisk walking) at least 30 minutes per day, most days of the week.	4–9 mmHg
Moderation of alcohol consumption	Men: limit to ≤2 drinks* per day. Women and lighter weight persons: limit to ≤1 drink* per day.	2–4 mmHg

* 1 drink = 1/2 oz or 15 mL ethanol (e.g., 12 oz beer, 5 oz wine, 1.5 oz 80-proof whiskey).

† Effects are dose and time dependent.

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