Prehypertension: The New Frontier in Cardiovascular Disease Prevention

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Abbreviations:

ACEI	Angiotensin converting enzyme inhibitor
Ang II	Angiotensin II
ARB	Angiotensin receptor blocker
ARIC	Atherosclerosis Risk in Communities Study
AT_1-receptor	Angiotensin type 1 receptor
BMI	Body mass index
EGFR	Epidermal growth factor receptor
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal regulated kinase
GC	Guanyl cyclase
GSH	Oxidized glutathione
GSSG	Reduced glutathione
H_2O_2	Peroxide
IGF-1R	Insulinlike growth factor-1 receptor
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
$\bullet O_2^-$	Superoxide (reactive oxide species)
PDGFR	Platelet derived growth factor
RAAS	Renin angiotensin aldosterone 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 (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]

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 1.
The new blood pressure classification 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.

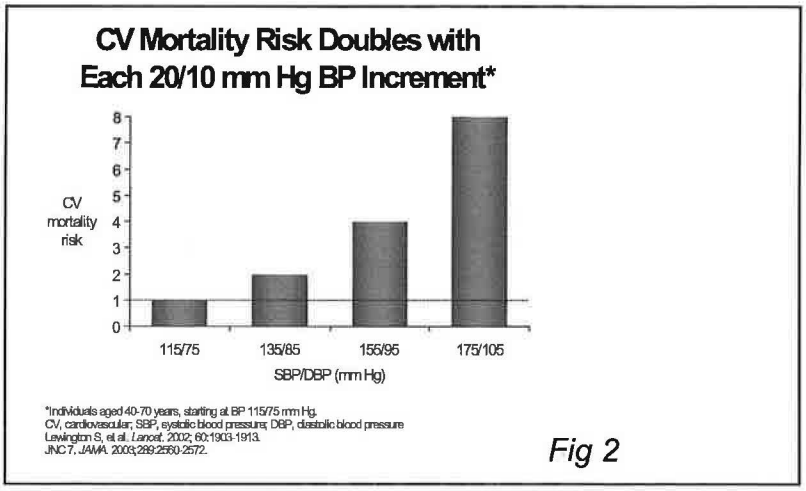
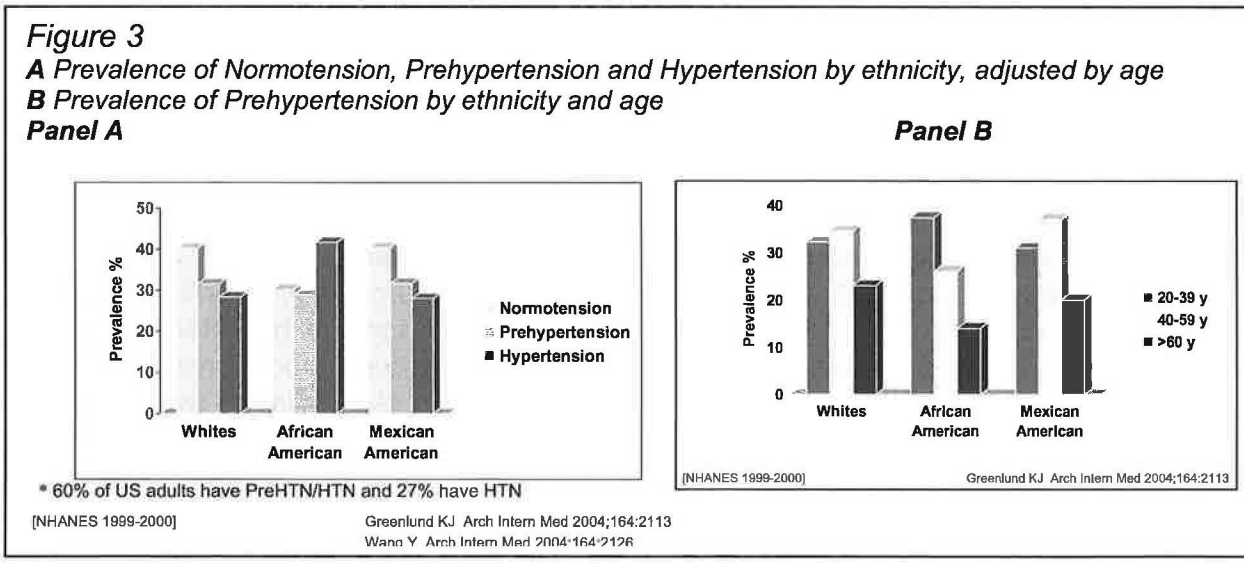


Fig 2

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] The shifting in the staging process over time is related to 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 2)* There is significant evidence of the hypertensive process beginning prior to the diagnosis of hypertension at >140/90 mm Hg.

Figure 3
***A** Prevalence of Normotension, Prehypertension and Hypertension by ethnicity, adjusted by age*
***B** Prevalence of Prehypertension by ethnicity and age*



* 60% of US adults have PreHTN/HTN and 27% have HTN

[NHANES 1999-2000] Greenlund KJ Arch Intern Med 2004;164:2113

Wang Y Arch Intern Med 2004;164:2126

According to NHANES 1999-2000, in the U.S. population the prevalence of prehypertension is 31%, hypertension is 29%, and normotension is 39%. The age adjusted prevalence of prehypertension is greater in men (39%) than women (23.1%). Furthermore, in younger adults (20-39 y/o), the prevalence of prehypertension is greater in African Americans (37.4%) than whites (32.2%) and Mexican Americans (30.9%); while among adults ages 40-59 y/o and ≥60 y/o, the prevalence of prehypertension is higher in whites and Mexican Americans than African Americans[9]. From a public health perspective, there is a 90% lifetime risk of developing hypertension for individuals who live to be 55 years old, based on the Framingham population.[10] *(Fig 4)*

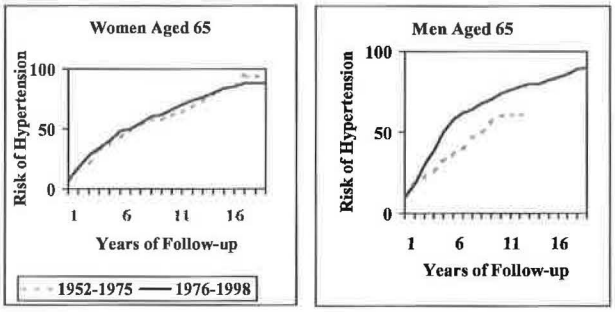


Figure 4.
Vasan RS, et al. Residual Lifetime Risk for Developing Hypertension in Middle-aged Women and Men: The Framingham Heart Study. JAMA 2002;287:1003-1010.

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%.[1,11] *(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.[12,13,14,15] There is no doubt that the success of large scale, long term lifestyle modifications requires societal changes and the support of public policy.

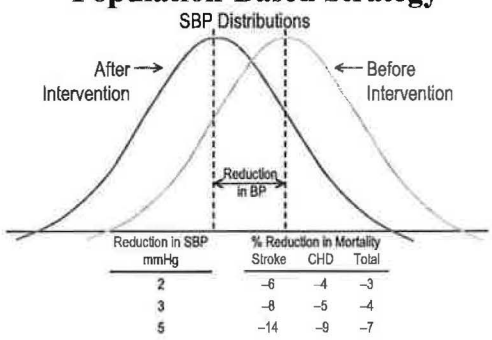


Figure 5.
The effect of shifting the mean blood pressure and distribution of the U.S. population by small increments may have dramatic effects on morbidity and mortality. JAMA 2003;289:2560.

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-

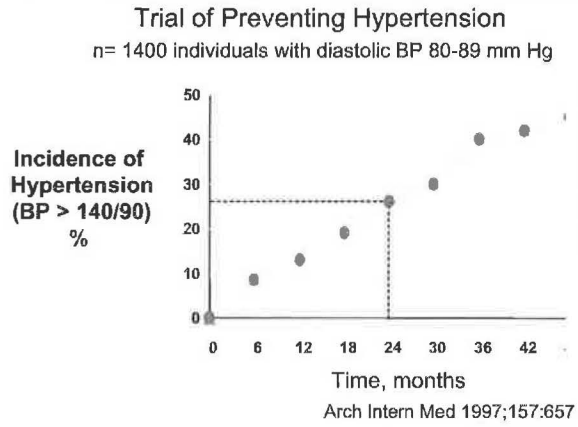
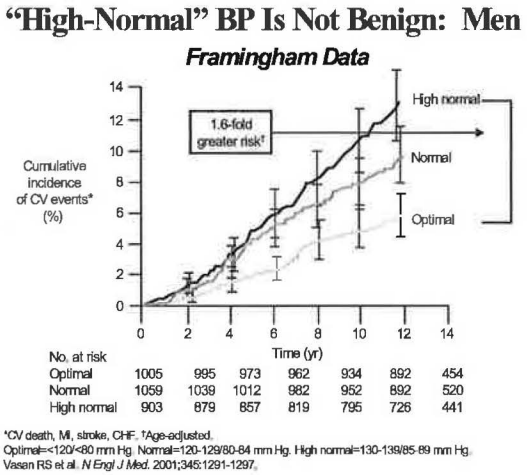
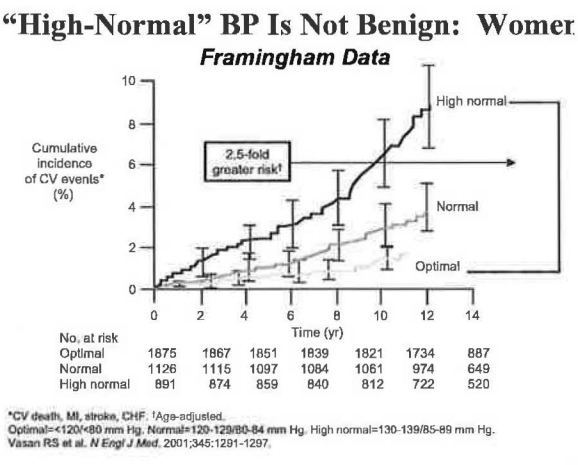


Figure 6.
In the NIH Trial of Preventing Hypertension (TOPH), high normal individuals in the usual care group developed hypertension at a rate of 21.1% at 18 months and 44.4% at 48 months. TOPH Study. Arch Intern Med 1997;157:657-667.

139/85-89 mm Hg (high normal). First, 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%).[16] *(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.[17,18] *(Table 1)* These rates have been adjusted for sex, age, BMI and baseline blood pressure. Second, it is important to assess the risk of cardiovascular events in prehypertension. 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 compared to optimal blood pressure for men, while there is a 2.5-fold increase in risk for women.[19] *(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 focus on those in this group who are at clear risk for progressing to hypertension. In the Strong Heart Study population, prehypertension increased the risk of cardiovascular

events 1.8-fold compared with their normotensive counterparts, with an absolute increase of 6 events per 1000 person years.[20] Similar effects have been demonstrated in the ARIC study, also highlighting the excess effect of prehypertension on cardiovascular events in African Americans compared to Whites.[21] Based on the

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.

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.[18] Recent estimates of the effect of prehypertension on hospital admissions and death suggest that eliminating prehypertension would reduce hospitalization by 3.4% and death by 9.1%[22]. Better methods of characterizing those at

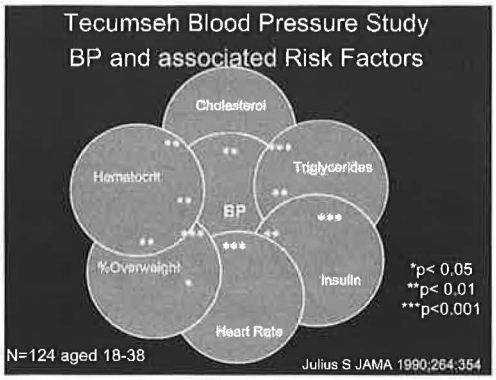


Figure 8.
The associations of blood pressure to other cardiovascular risk factors in the Tecumseh BP study. Data from the 601

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.

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).

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.[23] *(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.[24] Similarly, prehypertensives in the NHANES population had 1.83-fold increased likelihood of having at least one additional cardiovascular risk factor compared to normotensives.[25] Furthermore, the prevalence of novel risk factors such as CRP is also higher in prehypertensives compared to normotensives.[26, 27] The recent increasing trend in overweight and obesity may have direct effects on prehypertension. In particular, abdominal obesity significantly increases the risk of prehypertension, an effect which is most potent in African American women.[28] Thus, the increase in the risk of cardiovascular events is mediated not only by elevated blood pressure but also through other risk factors.

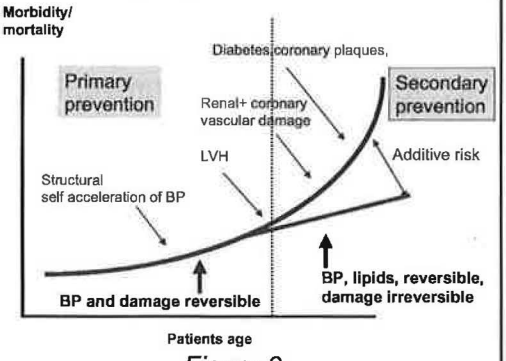


Figure 9.
Early treatment of blood pressure may limit the subsequent effects of blood pressure on mortality.

The pathway to cardiovascular events and death begins with the appearance of cardiovascular 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 steady 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

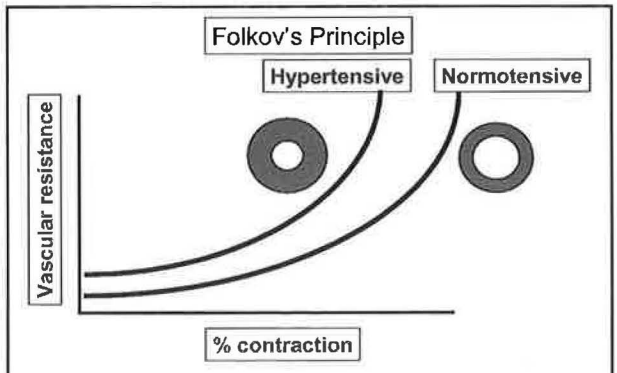


Figure 10. Hypertensive blood vessels have an altered contraction, vascular resistance and morphology.

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.

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.[29-32] Increases in blood pressure lead to adaptive changes in the microvasculature. These changes manifest as an increase in the mass of the medial

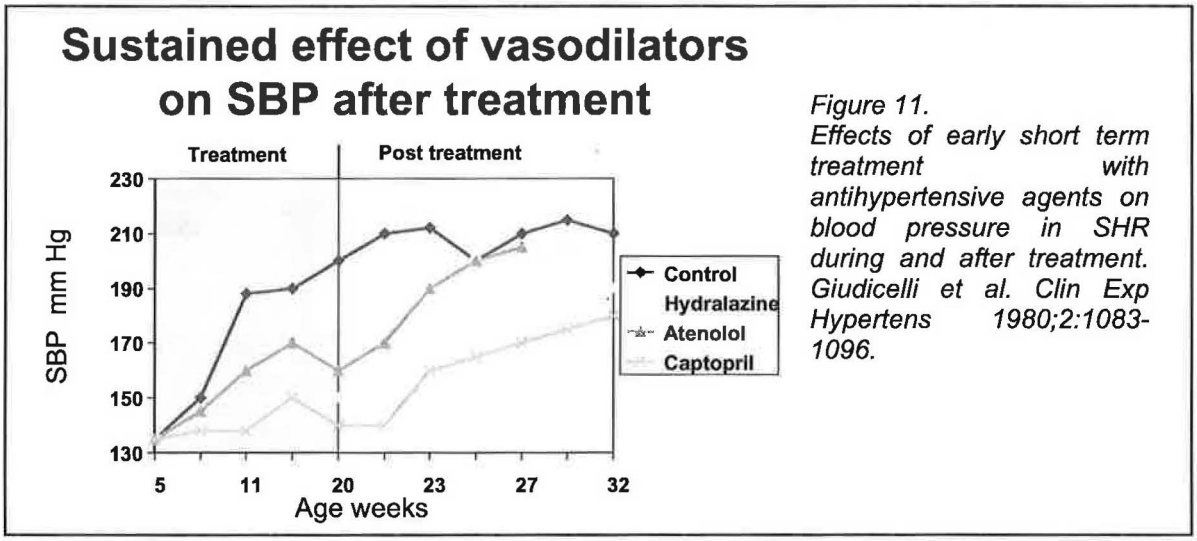


Figure 11.
Effects of early short term treatment with antihypertensive agents on blood pressure in SHR during and after treatment. Giudicelli et al. Clin Exp Hypertens 1980;2:1083-1096.

layer of vascular smooth muscle or through remodeling of the vascular smooth muscle medial layer. This alteration in the vasculature further propagates hypertension through increased vascular resistance which is consistent with Folkov's principle. Thus interrupting these changes in the morphology may attenuate or perhaps ablate the progression of hypertension. We sought to find evidence of this possibility.

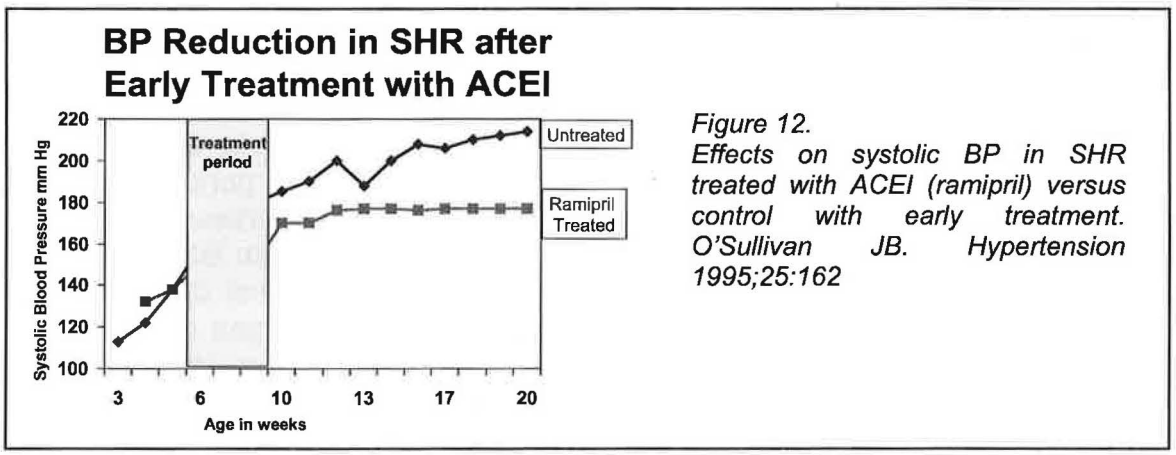
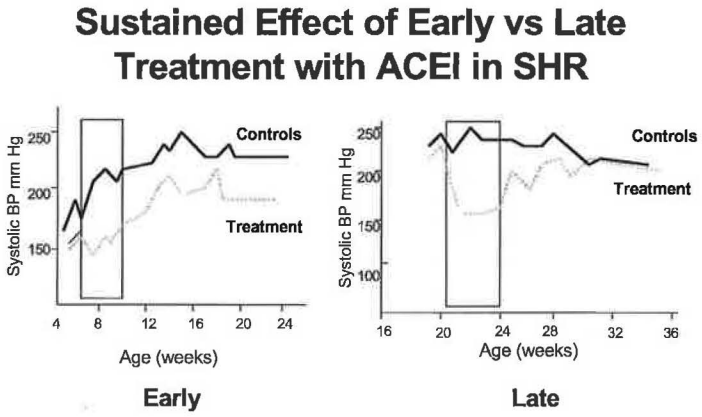


Figure 12.
Effects on systolic BP in SHR treated with ACEI (ramipril) versus control with early treatment. O'Sullivan JB. Hypertension 1995;25:162

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.[33,34] 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

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.



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.[35] *(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.[36] *(Fig 13)* Similar results were demonstrated using perindopril by Adams et al.[37,38] Thus these animal studies confirm that treatment with an angiotensin antagonist 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. 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.[39] The media layer of these blood vessels was reduced in size as demonstrated by Mulvany in a similar study.[40] *(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 RAAS 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

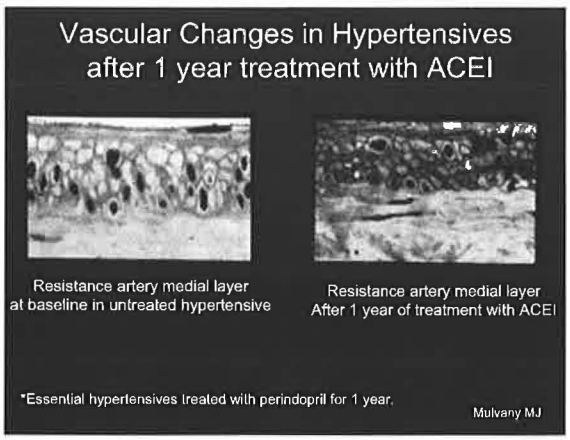


Figure 14.
From Mulvany MJ. J Hypertens 1996;14:S21-S24.

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 1999 and 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

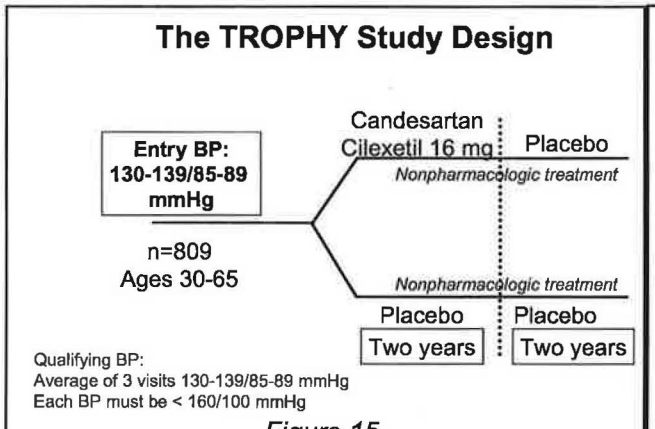


Figure 15.
The Trial of Preventing Hypertension (TROPHY) is a randomized, placebo controlled trial of ARB treatment in high normal blood pressure.

Baseline Characteristics of the Intention to Treat Group

	Candesartan N=391	Placebo N=381
Age (yrs)	48.6± 7.9	48.3± 8.2
Men	231 (59.1%)	229 (60.1%)
Race		
Caucasian	312 (79.8%)	321 (84.3%)
Black	48 (12.3%)	31 (8.1%)
Other	31 (7.9%)	29 (7.6%)
Weight (Kg)	89.0±17	88.8±17.7
BMI (Kg/m^2)	30.0±5.5	29.9±5.5
Office BP Omron device**	133.9±4.3 / 84.8±3.8	134.1±4.2 / 84.8±4.1
Office BP standard device **	130.9±7.2 / 85.0±4.8	131.5±7.1 / 84.9±5.6
Home BP Omron device **	133.9±8.5 / 82.7±5.9	133.9±8.5 / 82.7±5.9

Note: Values represent mean ± SD where noted. ** (mm Hg)

Table 4

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.[41] *(Fig 15)*

At baseline the demographics and characteristics of the study participants were similar in both randomized groups.*(Table 4)* In general, they were a middle aged,

Risk Factor	TROPHY Prevalence %	Excess Compared to NHANES %
↑ Cholesterol	53	+5
↓ HDL	35	-6
↑ Triglycerides	39	+26
↑BMI	84	+30
↑Glucose	7	0
↑ Insulin	11	+78
↑ Heart Rate	20	N/A
↑ Hematocrit	29	+10

Table 5

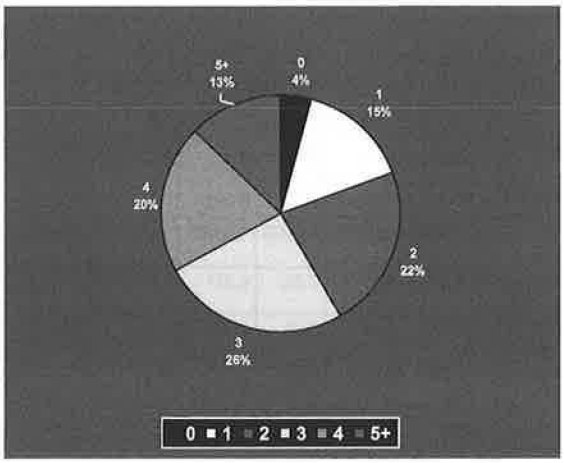


Figure 16

primarily Caucasian, overweight group which was approximately 60% male. Although the trial aimed to include healthy individuals with high normal (prehypertensive) blood pressure, this group had more cardiovascular risk factors than expected. In an assessment of risk status, using traditional risk factors as well as insulin level , heart rate and hematocrit which have been validated as indicators of increased cardiovascular risk the participants of the TROPHY study (mean age of 49 ± 8.1) with high normal blood pressure (mean 134 ±4 / 85 ±4 mmHg) had excess additional cardiovascular risk factors.[43,44,45] Compared to a similar aged group of

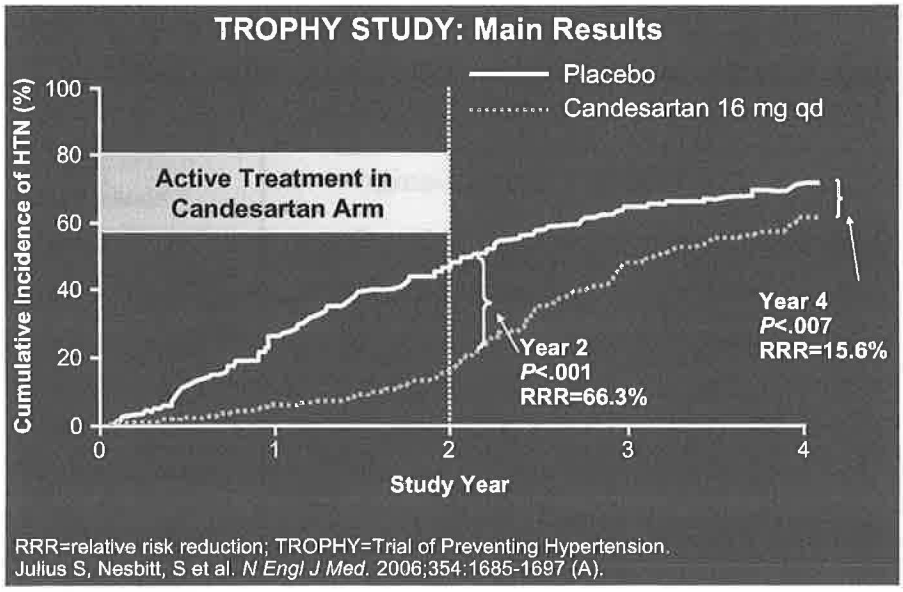


Figure 17

normotensives from NHANES, the TROPHY group were more overweight, had higher cholesterol, triglycerides, glucose, insulin and hematocrit. Ninety-five percent of the group had at least 1; 80% ≥ 2; and 31% had ≥ 4 additional risk factors.[46]

The TROPHY Study is the first randomized clinical trial of pharmacologic treatment in prehypertensives. Initially there was concern for adverse events, such as hypotension in the treatment group, however adverse events in the study were rare and similar in both study groups.

New onset of hypertension was suppressed in the candesartan group at two years ($p<0.0001$), and after four years ($p< 0.007$), by Fisher exact test. This was further tested by logistic regression, adjusted for the following significant baseline covariates; home diastolic pressure, clinic systolic by automated device, hematocrit, plasma insulin/glucose ratio, and age. The significance values were <0.0001 at two and <0.009 at four years respectively. There was a 66.3% relative and a 26.8% absolute reduction in the candesartan group at year two. At year four, two years after discontinuing candesartan, there was a 15.6% relative and a 9.8% absolute reduction of new onset hypertension in the former candesartan group. In the analyses above, we assumed that participants who prematurely discontinued the study did not develop hypertension. A sensitivity analysis assuming that all dropouts developed hypertension did not change the results. Removal of 49 entry criteria violators did not alter the results ($p<0.0001$ year two and <0.0085 year four by Fisher exact). The median hypertension-free-period time was 2.2 years (95 percent confidence interval = 2.0- 2.5) in the placebo and 3.3 (95 percent confidence interval = 3.0-3.8) in the candesartan group.

The relative hazards were significantly different throughout the study ($p<0.0001$ by log-rank test, $p< 0.0001$ by Cox proportional hazard with covariate adjustments) as illustrated in the Kaplan Meier curves. (Figure 17) After conversion to placebo, the incidence of hypertension in the candesartan group increased, but the curves remained separated until the study end. Hazard ratios in various subgroups (Figure 18) favored the candesartan group.

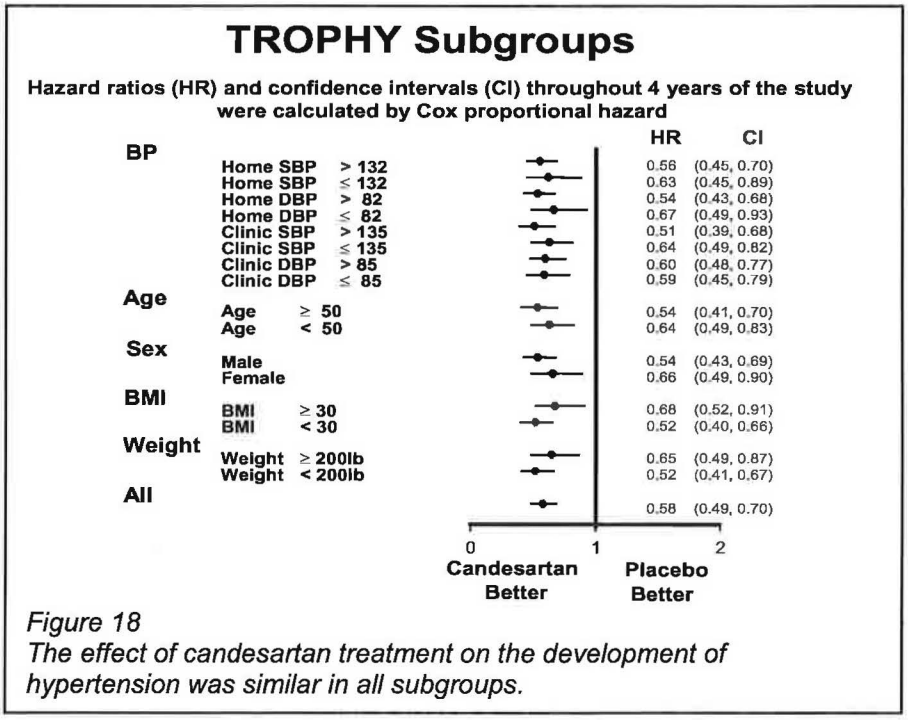


Figure 18
The effect of candesartan treatment on the development of hypertension was similar in all subgroups.

TROPHY results support our primary hypothesis that pharmacological treatment of prehypertension can prevent or postpone the development of hypertension. At four years, two years after discontinuing candesartan, there was a significant reduction

of hypertension in the candesartan pretreated group. The relative proportion of hypertension-free participants was 26.5 percent greater in the candesartan group. The median hypertension-free time was 1.1 year longer in the candesartan group.

The mean age of 48.5 years in TROPHY participants is younger than in other recent studies of hypertension. Whether treatment in even younger subjects could maximize prevention of hypertension is unknown. It is also not known if longer periods of treatment or a larger degree of blood pressure lowering would yield different results. Whether TROPHY results reflect only the blood pressure lowering actions of the drug or other effects of angiotensin blockade has not been resolved. Potentially the largest impact would come from a study of clinical outcomes with pharmacological intervention in prehypertension. Finally, the issue of cost effectiveness has not been resolved.[47]

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.[48-50] 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.[51] 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 19)* 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.[49] 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.[50]

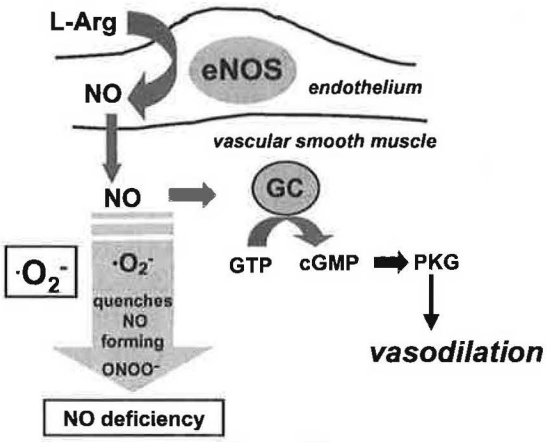


Figure 19. Mechanisms of nitric oxide deficient hypertension.

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 $\cdot O_2^-$.[52, 53] Second, 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.[53] 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. Third, 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.[54-56]

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.

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.[30, 57-60] 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_2 that is subsequently degraded to H_2O which is freely diffusible.[61] *(Fig 20)* 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.[50] *(Fig 19)* In addition, H_2O_2 stimulates and $\cdot O_2^-$ activate growth factors such as PDGFR, EGFR, and IGF-1R which may in turn modulate the effect of Ang II on ROS.[62] This highlights the important role of both $\cdot O_2^-$ and H_2O_2 in the pathway to vascular hypertrophy.

Second, 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. 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.[63] 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 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.[64-68] These MAPK's are also candidate genes which warrant further investigation.

Third, all 5 subunits of leukocyte NAD(P)H oxidase are also present in human vascular smooth muscle cells.[69,70] This is important because at least one of the subunits is not found in rat aortic cells.[69,70] 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.[71-73] 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.[74]

Fourth, in VSMC from SHR, NADPH driven ROS generation progressively increases with blood pressure from 4 to 16 weeks of life.[62] Similarly, in humans when exposed to exogenous Ang II, vascular smooth muscle cells from hypertensive subjects produced more $\cdot O_2^-$ than those from normotensive subjects.[75] 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,[39] permit 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 RAAS system, NO pathway and oxidative stress pathway in the development of vascular hypertrophy and remodeling. *(Fig 20)* The novel design of the TROPHY Study presents an excellent opportunity to further study these mechanisms in a clinical trial setting.

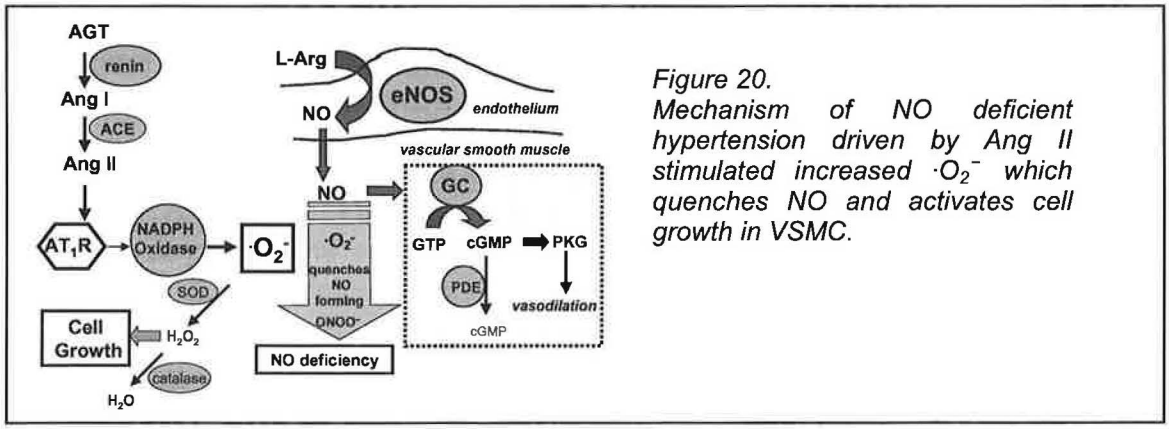


Figure 20.
Mechanism of NO deficient hypertension driven by Ang II stimulated increased $\cdot O_2^-$ which quenches NO and activates cell growth in VSMC.

The Mechanistic Substudy of the TROPHY Study

We measured 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, RAAS, 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 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.

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.[76-82] 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.[83] Isoprostane level serves as an index of the presence of superoxide as well as having unique 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

The Relationship of Blood Pressure and Urine F2 isoprostanes

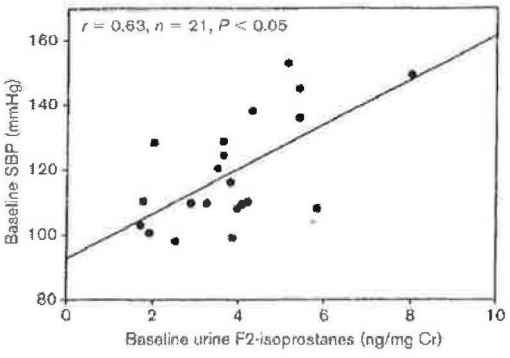


Figure 21.
Urine F2-isoprostanes correlate positively to systolic blood pressure. From Stojiljkovic MP. J Hypertens 2002;20:1215.

peroxidation. The intra-subject variability in the measurement is 5%.[84]

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.[85] *(Fig 21)*

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.[86] 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.[87, 88] 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.

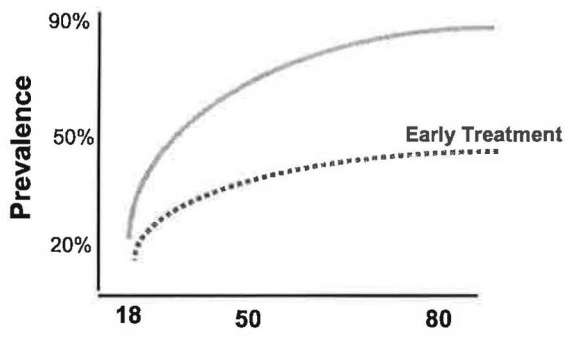
In animal studies of carbon tetrachloride induced liver failure, the GSH:GSSG ratio is reduced as the dose of CCl_4 increases as well as time of exposure to the toxin, thus reflecting the level of liver damage.[89] In another human study, Samiec found differences in the glutathione ratio by age. Plasma 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.[87]

Summary

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." The TROPHY Study has demonstrated that short term treatment with an ARB in prehypertensives leads to delayed onset of hypertension. Improving our understanding of the mechanisms which underlie the progression to hypertension, will help to clarify the role of early treatment in the evolution of high blood pressure. The oxidative stress pathway represents a promising mechanism to further describe the interaction between what is already known about nitric oxide deficient hypertension and renin angiotensin stimulated hypertension.

The TROPHY Study is the first of hopefully many investigations into the role of pharmacologic with nonpharmacologic interventions in prehypertension. Investigating the multiple questions which the TROPHY Study raises is undoubtedly the next frontier in reducing the ravages of hypertension.

Figure 22



Summary of concepts in the progression to hypertension:

The Risk of Prehypertension

- 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.
- Prehypertension is accompanied by an increased prevalence of other cardiovascular risk factors.

The TROPHY Study Results

- Prehypertensives have an excess of additional cardiovascular risk factors.
- The four year incidence of hypertension among prehypertensives (high normal) is 63%, which is higher than previously observed.
- Low dose treatment with the ARB candesartan is safe and well tolerated.
- The 2 year incidence of hypertension is reduced by 66.3% after 2 years of ARB treatment in prehypertension.
- The 4 year incidence of hypertension is reduced by 15.8% after only 2 years of ARB treatment in prehypertension.

Mechanisms of Prehypertension

- Ang II increases the production of ROS in VSMC, which is reduced by ARB treatment.
- Activation of NADPH oxidase is the key mechanism in the activation of Ang II in the production of ROS.
- NADPH oxidase activation promotes production of ROS ($\cdot O_2^-$ which is degraded to H_2O_2)
- H_2O_2 stimulates vascular hypertrophy and hyperplasia through extracellular signaling and growth factor stimulation.

Oxidative Stress Measures

- Oxidative stress is measured by biomarkers such as Isoprostanes and GSSG:GSH ratio. These markers have been validated in humans.

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