Original Article

Regression of L-NAME–Induced Hypertension: The Role of Nitric Oxide and Endothelium-Derived Constricting Factor

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N^G-Nitro-L-arginine-methyl ester (L-NAME)–induced hypertension is a well established model of experimental hypertension. Although regression experiments are effective at approximating a clinical setting the reversal of already established L-NAME hypertension has not been intensively researched. We investigated whether spontaneous regression of L-NAME hypertension after discontinuing the drug administration was associated with recovery of endothelial dysfunction. Special attention was devoted to NO signaling and endothelium-derived constricting factor (EDCF) formation in various parts of the vascular tree. Male adult Wistar rats were divided into 4 groups: an L-NAME (5 weeks), a spontaneous recovery (5 weeks L-NAME + 3 weeks of recovery) and two age-matched control groups (a 5- and 8-week control group). The NO-mediated and EDCF-mediated components of acetylcholine-induced responses were evaluated in preconstricted small mesenteric and femoral arteries. The activity, mRNA and protein expression of NO synthase together with the mRNA expression of cyclooxygenase were determined in the aorta. L-NAME administration caused hypertension, impaired NO signaling (as indicated by the reduced NO component of acetylcholine-induced relaxation and decreased NO synthase activity) in all arteries investigated and reduced the inner diameter of the femoral artery. Moreover, we observed enhanced cyclooxygenase-dependent EDCF formation in the femoral arteries and enhanced cyclooxygenase-2 expression in the aortas of L-NAME–treated rats. During spontaneous recovery a functional restoration of NO signaling took place in all parts of the vascular tree. However, the increases in systolic blood pressure, EDCF formation, and cyclooxygenase expression and the reduction in femoral artery diameter were not completely restored. We conclude that impaired NO signaling was improved after the cessation of L-NAME administration. However, persisting arterial structural alterations and enhanced EDCF formation may decelerate blood pressure reduction even after the restoration of NO synthase activity. (*Hypertens Res* 2008; 31: 793–803)

***Key Words*: arteries, cyclooxygenase, endothelial factors, nitric oxide, vascular reactivity**

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Introduction

Vascular alterations including wall remodeling and endothelial dysfunction are common features in several forms of clinical and experimental hypertension (*1*–*5*). Once structural changes in arteries are developed, they are hard to regress and may participate in hypertension maintenance and target organ damage development (*6*, *7*). Thus the present experiments were designed to reveal functional aspects of persisting endothelial dysfunction and arterial wall remodeling, representing an attractive approach to experimental hypertension research.

N^G-Nitro-L-arginine-methyl ester (L-NAME)–induced hypertension is a well-established model of hypertension, and has also proven useful for studying endothelial dysfunction (*8*) and vascular wall remodeling (*9*). Because there have been few regression studies on L-NAME–induced hypertension, the mechanisms involved in its regression are not completely understood. The available studies on the reversal of L-NAME–induced hypertension have shown that, after the cessation of L-NAME administration, blood pressure (BP) decreases and NO synthase (NOS) activity improves, but impaired acetylcholine (ACh)-induced relaxations of the aorta and aortic wall remodeling are restored only partially (*8*).

However, not much is known about the regression of endothelial dysfunction and arterial remodeling in other parts of the vascular tree. Persistent alterations in conduit arteries and resistant arteries may contribute to the maintenance of an elevated peripheral vascular resistance, or favor the occurrence of complications and enhance the cardiovascular risk (*4*). Our preliminary experiments have suggested that endothelial dysfunction in the femoral arteries of L-NAME–treated rats was accompanied by ACh-induced release of endothelium-derived constricting factor (EDCF), which was abolished by the inhibition of cyclooxygenase (COX) with indomethacin (*10*), much like the EDCF production reported in spontaneous hypertension (*11*).

The aim of the present study was to investigate 3-week-long spontaneous regression of L-NAME–induced hypertension. This period may be the most appropriate to disclose asynchronous reversal of individual alterations in L-NAME–induced hypertension (*8*). In particular, we investigated the restoration of BP and vascular responses associated with disrupted NO signaling and enhanced EDCF formation in large, medium and small arteries.

Methods

Animals and Treatment

Four groups of male 10-week-old Wistar rats ($n=8$ each) were investigated: an L-NAME–treated group (LN-5; 40 mg/kg/d L-NAME in the drinking water for 5 weeks), a group in which a spontaneous recovery from L-NAME–induced hypertension was investigated (SR; 5 weeks of L-NAME administration followed by 3 weeks of water drinking), and two age-matched control groups, which were given free access to food and water for 5 (Ctr-5) or 8 (Ctr-8) weeks of experiment.

All four groups consisted of animals from our own colony (Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic) housed under standard laboratory conditions (temperature 23±1°C, 12-h light–dark cycle) were fed a standard ST-1 diet (% NaCl; Velaz, Prague, Czech Republic) and drank tap water ad libitum. If not stated, all chemicals were obtained from Sigma Chemicals Co. (Deisenhofen, Germany). All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic and conformed to the European Convention on Animal Protection and Guidelines on Research Animal Use (NIH Publication No. 85-23, revised 1996).

Blood Pressure Measurement and Tissue Sample Preparation

Systolic BP was measured each week using a tail-cuff plethysmograph (Hugo-Sachs Elektronik, Freiburg, Germany). Before the onset of the experiment, rats were acclimated to the tail-cuff plethysmography procedure for 2 weeks The BP measurement protocol consisted of 2 cycles separated by a 30 s interval. Each cycle comprised 5 measurements. The first cycle was considered to be the training cycle and the second cycle to be the representative cycle. The BP value was taken as the mean of the measurements in the representative cycle after removing the outliers. At the end of the experiment systolic BP (SBP) and diastolic BP (DBP) were determined by puncture of the carotid artery under light ether anesthesia. Thereafter the animals were sacrificed by overdosage of ether anesthesia. The body weight (BW) was measured, the heart removed, the left ventricular weight (LVW) determined and the relative left ventricular weight (LVW/BW) calculated.

Wire-Myograph Experiments

Arterial segments were isolated and mounted on a Mulvany-Halpern-style wire-myograph (M 510A; Danish Myo Technology A/S, Aarhus, Denmark), filled with modified Krebs-Henseleit solution (KHS; in mmol/L: 119 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 25 $NaHCO_3$, 1.18 KH_2PO_4, 0.03 EDTA, 2.5 $CaCl_2$, 11.51 glucose and 200 mg/L ascorbic acid) bubbled with carbogen (95% O_2 and 5% CO_2) (*12*). Arteries were allowed to equilibrate for 60 min and stretched to 90% of their normalized inner diameter (ID, corresponding to a pressure of 100 mmHg) as determined by a function incorporated into the myograph system. The constriction force was measured using a PowerLab Chart 5.0 system (ADInstruments Ltd., Bella Vista, Australia), and the change in wall tension was calculated as force/segment length and expressed as a percentage of the maximal

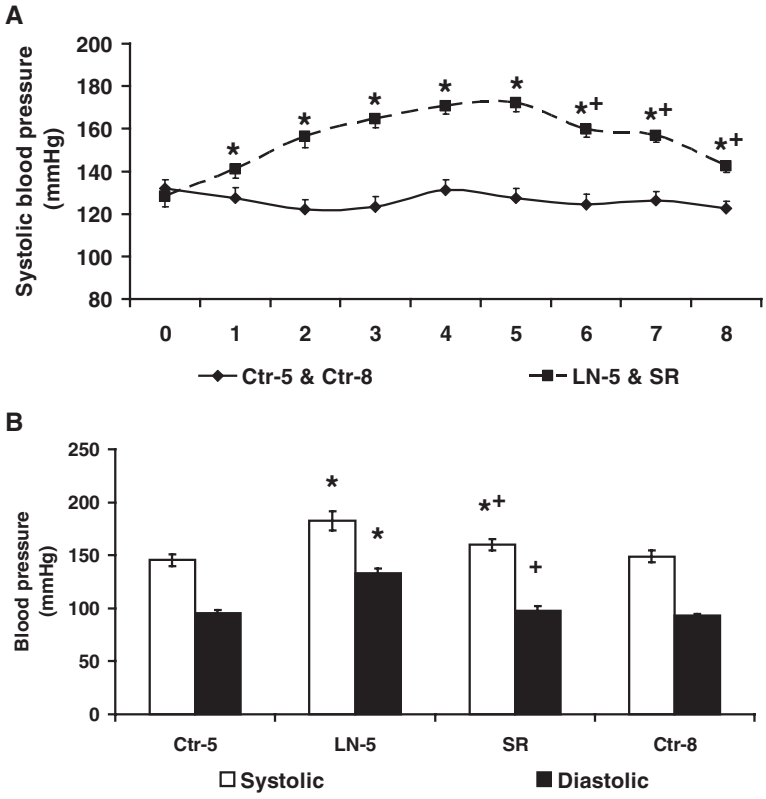

Fig. 1. *The influence of L-NAME administration and its cessation (in the 5th week) on systolic blood pressure during experiment (A) and systolic and diastolic blood pressure at the end of experiment (B). The horizontal axis represents the duration of treatment in weeks. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week water drinking, *$p < 0.05$ vs. the respective controls, †$p < 0.05$ vs. LN-5. Values are the means ± SEM. p, repeated measures ANOVA (A) and one-way ANOVA Bonferroni (B).*

wall tension change. The segment length was measured using a light microscope (50×) with a calibrated objective.

Small Mesenteric Arteries

Our preliminary experiments on preconstricted small mesenteric arteries showed sustained relaxations in response to ACh at concentrations from 100 nmol/L to 3 μmol/L, which were not influenced by incubation with indomethacin (50 μmol/L). The experimental protocol for the mesenteric arteries consisted of the following steps: change of KHS to KHS in which NaCl was exchanged for an equimolar concentration of KCl (120 mmol/L for 2 min), norepinephrine (NE) addition (10 μmol/L for 2 min), three times wash-out (8 min), preconstriction (3 μmol/L phenylephrine [PhE] for 2 min), and determination of responses to increasing concentrations of ACh (10 nmol/L to 1.5 μmol/L, each for 2 min). To evaluate the role of decreased NO release in L-NAME–treated rats, the response to a single concentration of ACh (5 μmol/L) was investigated in preconstricted (3 μmol/L NE for 2 min) arteries before and after 10 min of incubation with L-NAME (1 mmol/L). The difference between these two responses was considered to be the NO-dependent component of ACh-induced response.

Femoral Arteries

Our preliminary experiments revealed that the preconstricted femoral arteries from L-NAME–treated rats responded to ACh with transient relaxation (early response) followed by constriction (delayed response). Both responses were dose-dependent and were abolished after removing the endothelium by rubbing the inside of the artery with human hair. Delayed responses (constrictions) were also abolished after incubation with indomethacin (50 μmol/L for 30 min). We considered these constrictions to have been caused by EDCF-release (*10*, *13*). Therefore, in addition to the femoral arteries that were subjected to the same protocol as the mesenteric arteries, a group of NE-preconstricted (3 μmol/L) femoral artery segments was tested for the delayed response to a single concentration of ACh (5 μmol/L) before and after 30 min of incubation with indomethacin (50 μmol/L) under the condition of NOS inhibition (by 1 mmol/L L-NAME). The difference between these two responses was considered to be the EDCF-mediated component of the ACh-induced responses.

Table 1. Basic Cardiovascular Parameters after 5 Weeks of L-NAME Administration (LN-5) and 3 Weeks after Its Cessation (SR)

	Ctr-5	LN-5	SR	Ctr-8
BW (g)	386±10	371±8	380±14	405±12
LVW (mg)	429.9±11.3	472.4±6.8*	460.8±14.4*	435.9±6.8
LVW/BW (mg/g)	1.12±0.02	1.28±0.04*	1.22±0.04*,†	1.08±0.03
ID (μm)	675.0±23.2	502.5±19.1*	540.7±43.6*	652.5±24.9

Values are mean±SEM. Ctr-5, control after 5 weeks of experiment; Ctr-8, control after 8 weeks of experiment; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME followed by 3-week water drinking; LVW, left ventricular weight; BW, body weight; ID, normalized inner diameter of femoral artery. *$p<0.05$ *vs*. respective control; †$p<0.05$ *vs*. LN-5: Two-tailed one-way ANOVA-Bonferroni.

NO Synthase Activity and Expression in the Aorta

Total NOS activity was determined in crude aortic homogenates by measuring the formation of L-[^{3}H]citrulline from L-[^{3}H]arginine (Amersham, Little Chalfont, UK) (*14*), with some modifications (*15*). NOS activity was expressed as picokatals per gram of tissue protein (pkat/g) as determined by the Lowry method (*16*). NOS protein expression was determined by regular 10% SDS-PAGE. Lanes were loaded with the same amount of protein as determined by the Lowry method. The equivalent protein loading was confirmed by Ponceau staining. Membranes were stained with polyclonal antibodies directed against endothelial NOS and inducible NOS (both Santa Cruz Biotechnology Inc., Santa Cruz, USA) and peroxidase-conjugated secondary anti-rabbit IgG antibodies (Pierce, Rockford, USA). The protocol was standardized using a control protein supplied by the manufacturer with the antibodies. The bands were identified using chemiluminescence (ECL) and densitometrically evaluated.

NO Synthase and Cyclooxygenase mRNA Expression in the Aorta

Gene expression was analyzed using real time polymerase chain reaction. mRNA isolated from 20 mg of tissue using Trizol (Molecular Research Center, Cincinnati, USA) was used as a template. A Qiagen Sybr Green RT-PCR kit was used for one-step real-time polymerase chain reaction on a Bio-Rad IQ5 cycler (Bio-Rad Laboratories, Hercules, USA). β-Actin was chosen as a housekeeping gene for normalization of the results. The obtained threshold cycle values were corrected for β-actin threshold cycle values and expressed as a percentage of the controls.

Statistics

Data are presented as the means±SEM. Values of $p<0.05$ were considered to indicate statistical significance. One-way, two-tailed analysis of variance (ANOVA) with Bonferroni post-test was used for unpaired values and repeated measures ANOVA was used for paired values. Normality of distribution was tested according to Kolmogoroff and Smirnoff and the difference in standard deviations was tested by Barlett's test.

Results

Blood Pressure

L-NAME administration caused a progressive increase in SBP measured non-invasively. After the cessation of L-NAME administration, the SBP began to decrease. However, 3 weeks after the cessation SBP still remained higher than in the controls ($p<0.01$) (Fig. 1A).

The invasively measured SBP and DBP after L-NAME administration were increased by 26% and 40%, respectively (both $p<0.001$). Spontaneous recovery decreased both SBP and DBP (both $p<0.001$). However, SBP remained elevated by 10% above the control values ($p<0.01$) (Fig. 1B).

Left Ventricular Weight

L-NAME administration increased relative left ventricular weight by 14% ($p<0.001$). The spontaneous recovery decreased relative LVW ($p<0.05$), but it remained 9% higher than the control values ($p<0.05$) (Table 1).

Small Mesenteric Arteries

In all investigated groups, ACh induced a dose-dependent sustained relaxation of PhE-preconstricted arteries (Fig. 2A). These responses were endothelium-dependent and were not influenced by the incubation with indomethacin. In arteries from L-NAME–treated rats, the dose-response curve for ACh was shifted to the right (representing a decreased sensitivity to ACh, with significant relaxations starting typically from 500 nmol/L *vs*. 50 nmol/L in controls). The dose-response curve was partially restored in arteries from rats in which L-NAME administration was withdrawn (spontaneous recovery) (Fig. 2B).

The NO-dependent component of ACh-induced relaxation, estimated by comparing responses before and after incubation with L-NAME, was decreased in L-NAME–treated rats (7.2% relaxation compared to 28.4% in controls), but after the

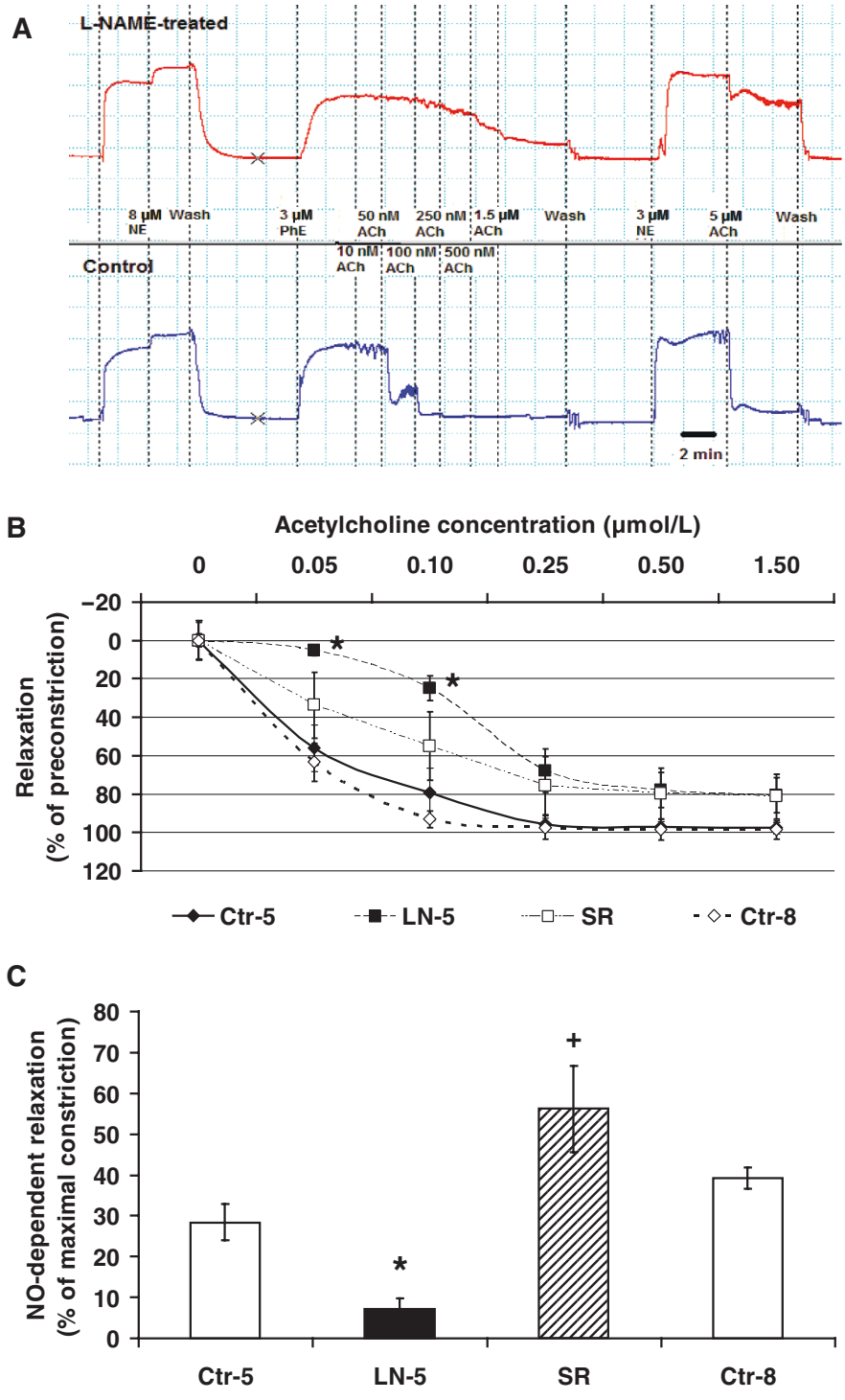

Fig. 2. *The influence of L-NAME administration and its cessation (in the 5th week) on vascular responses to acetylcholine (ACh) of isolated small mesenteric arteries. Representative charts depicting impaired relaxations in L-NAME–treated rats (upper record) compared to controls (lower record) (A), averaged dose-dependent relaxations (B), and the NO-dependent component of ACh-induced relaxation (difference in response to ACh before and after incubation with L-NAME) (C) are shown. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week placebo. *$p < 0.05$ vs. the respective controls, $^{\dagger}p < 0.05$ vs. LN-5. Values are the means ± SEM. p, one-way ANOVA Bonferroni.*

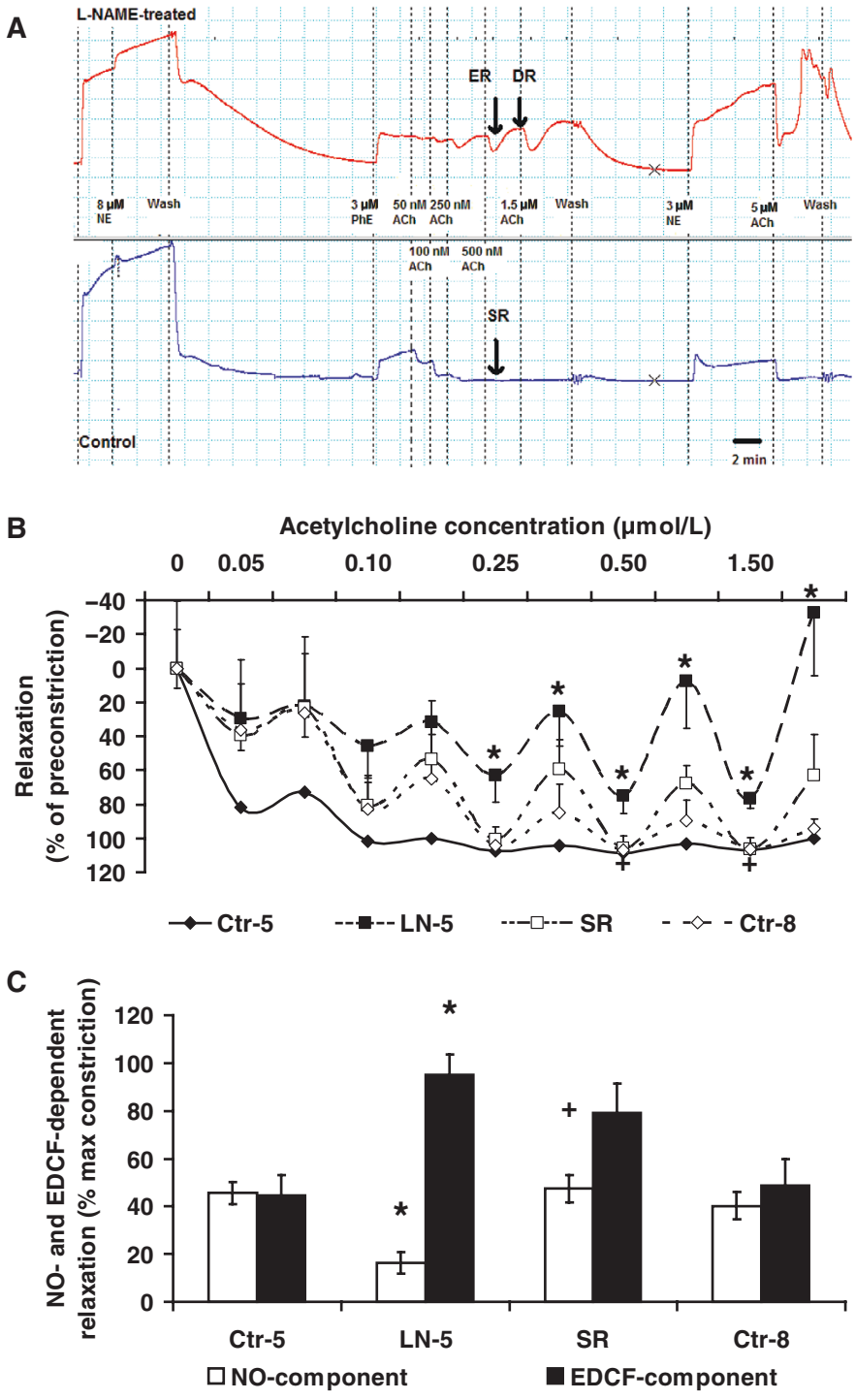

Fig. 3. *The influence of L-NAME administration and its cessation (in the 5th week) on vascular acetylcholine (ACh)-induced responses of isolated femoral arteries. Representative charts depicting only transient ACh-induced relaxations (early response, ER) in L-NAME–treated rats (upper record) followed by dose-dependent constrictions (delayed response, DR) in contrast to sustained relaxations (SR) of control arteries (lower record) (A), averaged dose-dependent responses to ACh (B), and the NO-dependent (difference in response to ACh before and after with L-NAME) and the EDCF-dependent (difference in response to ACh before and after incubation with indomethacin) components of ACh-induced relaxation (C) are shown. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week placebo. *$p < 0.05$ vs. the respective controls, †$p < 0.05$ vs. LN-5. Values are the means ± SEM. p, one-way ANOVA Bonferroni.*

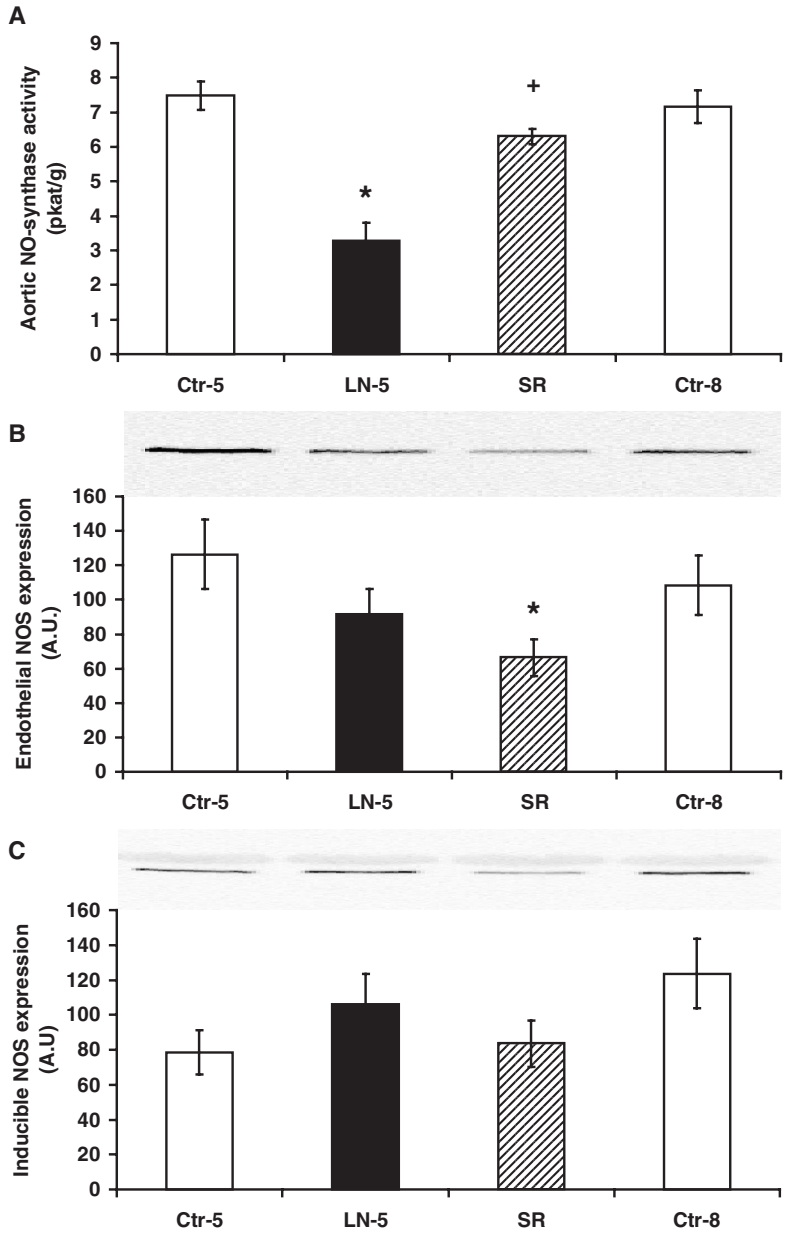

Fig. 4. *The influence of L-NAME administration and its cessation (in the 5th week) on aortic NO synthase activity (A), endothelial NO synthase expression (B) and inducible NO synthase expression (C) with representative Western blots. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week placebo. *$p < 0.05$ vs. the respective controls, $^{\dagger}p < 0.05$ vs. LN-5. Values are the means ± SEM. p, one-way ANOVA Bonferroni.*

cessation of L-NAME administration this component was recovered to even above control levels (56.2%) (Fig. 2C).

Femoral Arteries

The investigation of dose-dependent response to ACh indicated that femoral arteries from L-NAME–treated rats started to constrict again (delayed response) after a transient relaxation (early response) (Fig. 3A). The delayed responses (constrictions) were typically present at the ACh concentration of 250 nmol/L or higher, and these constrictions were abolished by the incubation with indomethacin (50 μmol/L for 30 min). Both responses were abolished by endothelium removal. The early responses (relaxations) were almost completely normalized after the cessation of L-NAME administration; but the delayed responses (EDCF-mediated constrictions) were diminished only partially (Fig. 3B).

L-NAME administration decreased the L-NAME–sensi-

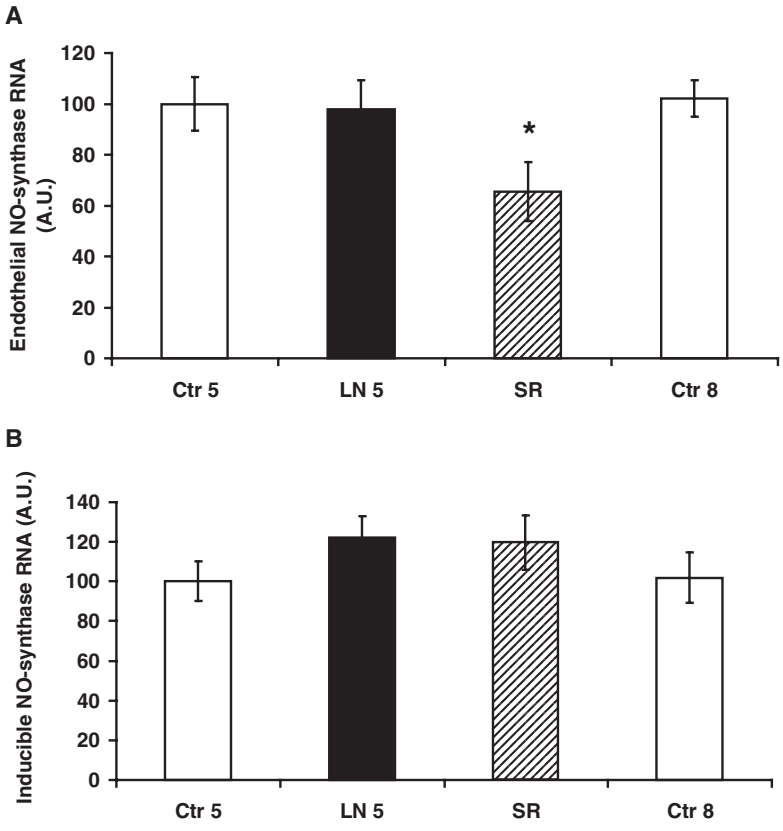

Fig. 5. *The influence of L-NAME administration and its cessation (in the 5th week) on aortic endothelial (A) and inducible (B) NO synthase mRNA expression. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week placebo. *$p < 0.05$ vs. the respective controls. Values are the means ± SEM. p, one-way ANOVA Bonferroni.*

tive, NO-dependent component of the early response (relaxation) (16.3% relaxation compared to 45.5% in controls). L-NAME administration also increased the indomethacin-sensitive, EDCF-dependent component of delayed response (constriction) (94.9% preconstriction compared to 44.6% in controls). After the cessation of L-NAME administration, only the NO-dependent component was normalized (47.5% compared to 40.1% in age-matched controls), while the EDCF-component remained elevated (79.0% compared to 48.6%) (Fig. 3C).

L-NAME administration decreased ID by 34% compared to controls ($p < 0.001$). Three weeks after the cessation of L-NAME administration, ID in the spontaneous recovery group remained lower than that in the age-matched controls (Ctr-8). However, the difference to age-matched controls (Ctr-8) was diminished due to non-significant decrease of ID in this control group (Table 1).

NO Synthase Activity and Protein Expression in the Aorta

L-NAME administration diminished NOS activity by 66% ($p < 0.001$). The cessation of L-NAME administration in the spontaneous recovery group restored NOS activity to the control levels (Fig. 4A).

Decrease in NOS activity was not accompanied by significant changes of endothelial (Fig. 4B) or inducible (Fig. 4C) NOS expression after 5 weeks of L-NAME administration. On the other hand, the spontaneous recovery was accompanied by 60% reduction of endothelial NOS expression ($p < 0.01$) (Fig. 3A) and no change in inducible NOS expression (Fig. 4B and C).

NO Synthase and Cyclooxygenase mRNA Expression in the Aorta

The lack of change in endothelial NOS protein expression after L-NAME treatment was confirmed by the unaltered endothelial NOS mRNA levels. In the spontaneous recovery group, endothelial NOS mRNA was reduced by 36.6% ($p < 0.05$) compared to the respective controls (Fig. 5A).

The mRNA expression of the inducible NOS (Fig. 5B) or COX-1 (Fig. 6A) was not altered during the experiment.

L-NAME administration enhanced COX-2 mRNA by 35% ($p < 0.05$). After the cessation of L-NAME treatment, the COX-2 mRNA expression remained increased by 31.7%

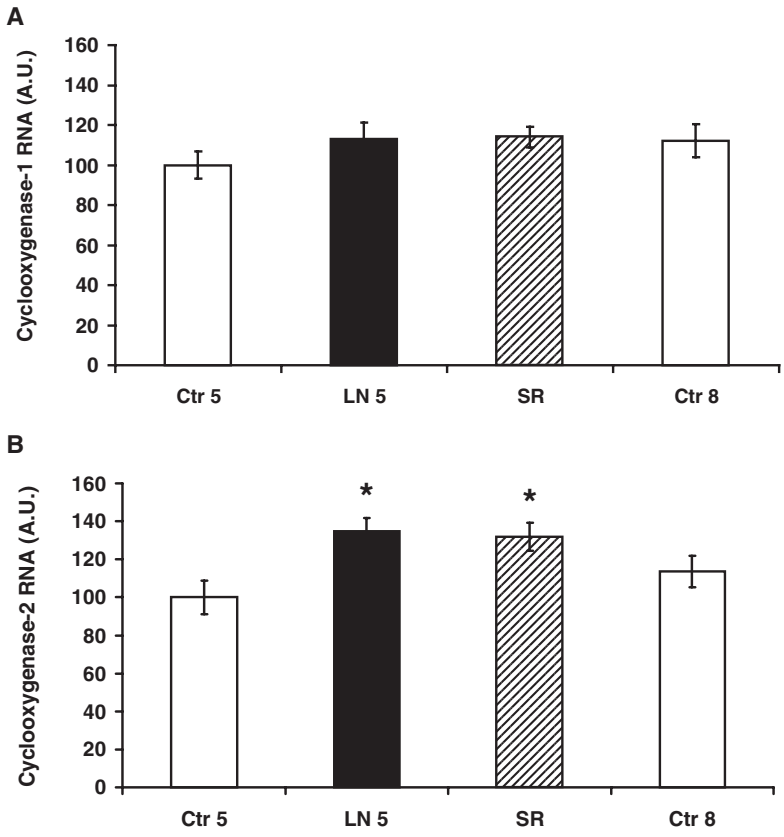

Fig. 6. *The influence of L-NAME administration and its cessation (in the 5th week) on aortic cyclooxygenase-1 (A) and cyclooxygenase-2 (B) mRNA expression. Ctr-5 and Ctr-8, controls; LN-5, 5-week 40 mg/kg/d L-NAME; SR, 5-week L-NAME + 3-week placebo. *$p < 0.05$ vs. the respective controls. Values are the means ± SEM. p, one-way ANOVA Bonferroni.*

($p < 0.05$) (Fig. 6B).

Multiple Regression Analysis

No significant correlations were found between SBP levels and ECDF formation in the experimental groups. Multiple regression analysis showed that neither NO nor ECDF could explain the differences in SBP between the groups. However, the relatively low number of animals employed in these experiments does not allow definitive conclusions regarding the source of variance in SBP.

Discussion

In the present study, L-NAME administration elevated BP, decreased NO signaling and reduced femoral artery diameter. Altered NO signaling in the aorta was indicated by the decreased NOS activity, and in femoral and mesenteric arteries by a shift of the dose-response curve to higher ACh doses and by a decrease in the NO-dependent component of ACh-induced relaxations. L-NAME–induced hypertension was also associated with augmented COX-dependent EDCF formation in the femoral arteries and increased COX-2 mRNA expression in the aorta. Spontaneous recovery after the cessation of L-NAME administration led to rapid functional restoration of NO signaling in all investigated arteries. However, at that time point, enhanced EDCF formation, COX-2 expression and arterial remodeling were restored only partially. Consequently, DBP but not SBP was restored during recovery of L-NAME hypertensive rats, suggesting that the stiffness of the conduit arteries persisted.

The present findings confirm previously reported effects of L-NAME administration on BP (*17*), cardiac hypertrophy (*18*), vascular remodeling (*19*) and endothelial dysfunction (*20*), which in this study were associated with disruptions to the NO-pathway in all parts of the vascular tree. In the present study, such disruptions were indicated by the impaired NO-mediated responses to ACh in the mesenteric and femoral arteries as well as by decreased NOS activity in the aorta. The finding that NO release made a smaller contribution to ACh-induced relaxation in the control mesenteric arteries than in the femoral arteries is congruent with previous reports that in smaller arteries the role of NO is replaced by endothelium-derived hyperpolarizing factor (*5*, *21*).

Our experiments provide evidence that endothelial dysfunction in L-NAME–treated rats is also associated with

increased production of EDCF following ACh stimulation in the femoral arteries. Previously reported endothelium-derived constricting substances include endothelin (*22*), angiotensin II (*23*), arachidonic acid derivates (mainly TXA_2/PGH_2) (*13*, *24*), or free radicals (*25*, *26*). Free radicals may not only serve as a vasoconstriction agent (*27*) but may also further decrease the availability of NO by converting it to peroxynitrite (*28*, *29*). The lack of an inhibitory effect of NO on cyclooxygenase activity in L-NAME–induced hypertension may, in addition to augmenting COX expression, enhance EDCF formation (*30*) and thus contribute to endothelial dysfunction in L-NAME–induced hypertension.

In preconstricted femoral arteries from L-NAME–treated rats in the present study, endothelium-dependent EDCF formation was usually present at ACh concentrations of 250 nmol/L and higher. This EDCF formation was completely abolished after the incubation with indomethacin. In addition, COX-2 expression in the aorta was enhanced. On the other hand, we did not observe any EDCF-mediated responses on quiescent (not preconstricted) arteries or on mesenteric arteries. Thus, we demonstrated for the first time that the differences in EDCF-mediated responses of the vessels from L-NAME hypertensive rats were dependent on the vessel diameter. This finding is in contrast with EDCF formation in spontaneous hypertension, which has been reported in large (*11*) as well as in resistant arteries (*31*). L-NAME–treatment seems to exert a larger effect on the femoral arteries, in which the role of NO-dependent vasodilation is more pronounced compared to small mesenteric arteries (*5*, *21*).

During the phase of established hypertension, the expected beneficial effect of antihypertensive treatment may be overridden by persisting functional and structural alterations of the conduit and resistance vasculature bed. "Self-sustaining" mechanisms enabling preservation of high BP have been reported in several forms of experimental hypertension, including L-NAME–induced hypertension (*6*), Dahl rats (*32*) or renovascular hypertension (*33*). In our previous studies on L-NAME hypertension we have observed complete (*34*) or partial restoration of SBP (*8*), no regression of left ventricular hypertrophy (*8*, *34*), complete restoration of NOS activity and partial restoration of ACh-induced relaxations and vascular remodeling in the aorta (*8*) after discontinuing L-NAME. Other than the functional restoration of NOS activity, we have not observed any changes in the expression of inducible NOS and decreased expression of endothelial NOS after the cessation of L-NAME administration. The reduced NOS protein and mRNA expression might represent a tendency to equilibrate the specific endothelial NOS protein activity, which seems to be enhanced after L-NAME withdrawal. However, until highly specific inhibitors for all three isoforms of NOS are available, the exact quantification of NOS activity generated by a certain NOS isoform will remain difficult.

Expanding the findings of previous experiments (*8*, *34*), we have investigated the reversal of vascular responses in medium-sized conduit arteries and small resistance arteries. The present study provides unique evidence that despite the functional restoration of NO signaling during hypertension regression, the increased EDCF formation in femoral arteries and COX-2 expression in the aorta were not normalized. The persisting elevation of EDCF production in arteries of medium diameter may help to explain the incomplete normalization of SBP and previously reported partial restoration of ACh-induced relaxations in the aorta, despite the restoration of NOS activity (*8*). Although EDCF production was not observed in small arteries from the mesenteric bed, we cannot exclude the possibility that it might occur in smaller arteries from other parts of the vascular bed, including the striated muscles. Moreover, contractile responses to ACh in specific vascular beds, *e.g.*, in renal arteries (*35*) or basilar arteries (*36*), may promote target organ damage in these organs and participate in increased morbidity after the reduction of high BP.

The difficulty of reversing functional endothelial alterations may be an additional explanation for the effect of the combination of acetylsalicylic acid, as an inhibitor of COX, with antihypertensive treatment (*36*). Addition of acetylsalicylic acid to antihypertensive treatment effectively diminished the number of cardiovascular accidents without affecting BP in the HOT trial, while simple BP decrease led only to a moderate decrease in ischemic heart disease mortality (*37*). However, future studies will be needed to elucidate the details of the functional aspects of EDCF formation in various parts of the vascular tree and at different time points of hypertension regression.

We demonstrate that L-NAME administration led to a disruption of NO signaling in arteries with large and small diameter. The impaired NO signaling was fully restored 3 weeks after the cessation of L-NAME administration, but persisting structural alterations and enhanced formation of EDCF in the conduit arteries decelerated the SBP reduction even after the restoration of NO formation.

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References

1. Konishi M, Su C: Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. *Hypertension* 1983; **5**: 881–886.
2. Lockette W, Otsuka Y, Carretero O: The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension* 1986; **8** (Suppl II): II-61–II-66.
3. Lüscher TF, Raij L, Vanhoutte PM: Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* 1987; **9**: 157–163.
4. Vanhoutte PM, Boulanger CM: Endothelium-dependent responses in hypertension. *Hypertens Res* 1995; **18**: 87–98.
5. Mori Y, Ohyanagi M, Koida S, Ueda A, Ishiko K, Iwasaki

T: Effects of endothelium-derived hyperpolarizing factor and nitric oxide on endothelial function in femoral resistance arteries of spontaneously hypertensive rats. *Hypertens Res* 2006; **29**: 187–195.
6. Morton JJ, Beattie EC, Speirs A, Gulliver F: Persistent hypertension following inhibition of nitric oxide formation in the young Wistar rat: role of renin and vascular hypertrophy. *J Hypertens* 1993; **11**: 1083–1088.
7. Haudenschild CC, Prescott MF, Chobanian AV: Effects of hypertension and its reversal on aortic intima lesions of the rat. *Hypertension* 1980; **2**: 33–44.
8. Bernatova I, Pechanova O, Babal P, Kysela S, Stvrtina S, Andriantsitohaina R: Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am J Physiol Heart Circ Physiol* 2002; **282**: H942–H948.
9. Kristek F: Different structure of coronary wall in two types of hypertension: NO deficiency and SHR, in Moncada S, Gustafsson LE, Wiklund NP, Higgs EA (eds): The Biology of Nitric Oxide (Pt 7). London, Portland Press Ltd, 1997, p 69.
10. Paulis L, Zicha J, Kunes J, *et al*: Regression of L-NAME-induced hypertension: the role of NO-pathway and endothelium-derived constricting factor. *J Hypertens* 2006; **24** (Suppl 4): S7 (Abstract).
11. Lüscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986; **8**: 344–348.
12. Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res* 1977; **41**: 19–26.
13. Koga T, Takata Y, Kobayashi K, Takishita S, Yamashita Y, Fujishima M: Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat. *Hypertension* 1989; **14**: 542–548.
14. Bredt DS, Snyder SH: Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci U S A* 1990; **87**: 682–685.
15. Bernatova I, Pechanova O, Kristek F: Mechanism of structural remodeling of the rat aorta during long-term N^G-nitro-L-arginine methyl ester treatment. *Jpn J Pharmacol* 1999; **81**: 99–106.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
17. Ribeiro MO, Antunes E, de-Nucci G, Lovisolo SM, Zatz R: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* 1992; **20**: 298–303.
18. Pechanova O, Bernatova I, Pelouch V, Simko F: Protein remodeling of the heart in NO deficient hypertension: the effect of captopril. *J Mol Cell Cardiol* 1997; **29**: 3365–3374.
19. Simko F, Matuskova J, Luptak I, *et al*: Effect of simvastatin on remodeling of the left ventricle and aorta in L-NAME–induced hypertension. *Life Sci* 2004; **74**: 1211–1224.
20. Rossi MA, Colombini-Netto M: Chronic inhibition of NO synthesis *per se* promotes structural intimal remodeling of the rat aorta. *J Hypertens* 2001; **19**: 1567–1579.
21. Nagao T, Illiano S, Vanhoutte PM: Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. *Am J Physiol* 1992; **263**: H1090–H1094.
22. Yanagisawa M, Kurihara H, Kimura S, *et al*: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411–415.
23. Kifor I, Dzau VJ: Endothelial renin-angiotensin pathway: evidence for intracellular synthesis and secretion of angiotensin. *Circ Res* 1987; **60**: 422–428.
24. Kato T, Iwama Y, Okumura K, Hashimoto H, Ito T, Satake T: Prostaglandin H_2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat. *Hypertension* 1990; **15**: 475–481.
25. Vanhoutte PM, Katusic ZS: Endothelium-derived contracting factor: endothelin and/or superoxide anion? *Trends Pharmacol Sci* 1988; **9**: 229–230.
26. Jameson M, Dai FX, Lüscher T, Skopec J, Diederich A, Diederich D: Endothelium-derived contracting factors in resistance arteries of young spontaneously hypertensive rats before development of overt hypertension. *Hypertension* 1993; **21**: 280–288.
27. Katusic ZS, Vanhoutte PM: Superoxide anion is an endothelium-derived contracting factor. *Am J Physiol* 1989; **257**: H33–H37.
28. Pearson PJ, Lin PJ, Schaff HV: Production of endothelium-derived contracting factor is enhanced after coronary reperfusion. *Ann Thorac Surg* 1991; **51**: 788–793.
29. Beckman JS, Beckman TW, Chen J, Marshall PA: Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990; **87**: 1620–1624.
30. Kanner J, Harel S, Granit R: Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin. *Lipids* 1992; **27**: 46–49.
31. Luscher TF, Aarhus LL, Vanhoutte PM: Indomethacin improves the impaired endothelium-dependent relaxations in small mesenteric arteries of the spontaneously hypertensive rat. *Am J Hypertens* 1990; **3**: 55–58.
32. Dahl LK: Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. *J Exp Med* 1961; **114**: 231–236.
33. Herrera-Acosta J, Gabbai F, Franco M, *et al*: Glomerular hemodynamics in persistent renovascular hypertension in the rat. *Hypertension* 1983; **5**: V110–V114.
34. Bernatova I, Pechanova O, Pelouch V, Simko F: Regression of chronic L-NAME–treatment–induced left ventricular hypertrophy: effect of captopril. *J Mol Cell Cardiol* 2000; **32**: 177–185.
35. Lüscher TF, Diederich D, Weber E, Vanhoutte PM, Bühler FR: Endothelium-dependent responses in carotid and renal arteries of normotensive and hypertensive rats. *Hypertension* 1988; **11**: 573–578.
36. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contractions to stretch in canine basilar arteries. *Am J Physiol* 1987; **252**: H671–H673.
37. Hansson L, Zanchetti A, Carruthers SG, *et al*: Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomized trial. HOT Study Group. *Lancet* 1998; **351**: 1755–1762.