Do incremental increases in blood pressure elicit neointimal plaques through endothelial injury?

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Ruiz-Feria, Ciro A., Yimu Yang, and Hiroko Nishimura. Do incremental increases in blood pressure elicit neointimal plaques through endothelial injury? Am J Physiol Regul Integr Comp Physiol 287: R1486–R1493, 2004. First published August 19, 2004; doi: 10.1152/ajpregu.00178.2003.—Fowl (males more than females) show maturation-dependent rises in blood pressure (BP) and formation of neointimal plaques (NPs), resembling balloon catheter injury-induced neointima, in the abdominal aorta (AbA) just above the bifurcation. The plaque comprises neointimal cells containing abundant endoplasmic reticulum and extracellular matrix. Hence, we investigated whether rapid incremental BP increases in male chicks trigger NP formation, possibly via endothelial injury in hemodynamically selective areas. In 6-wk-old chicks (n = 8) treated 4 wk with solvent (Sv; minipump) or arginine supplement (Arg; 0.3% in drinking water), BP increased from 140 ± 5 to 159 ± 4 (Sv) and from 138 ± 4 to 157 ± 3 (Arg) mmHg, whereas propranolol treatment (Prop, 8 mg·kg⁻¹·day⁻¹; minipump) prevented the rise. Arg and Prop groups had, respectively, 73% and 77% smaller (P < 0.05) NP areas and 19% and 25% less (P < 0.01) AbA medial thickness than Sv controls. In 16-wk-old cockerels, established BP remained high after Sv and Arg treatments. In the Prop group, BP decreased, but neither NP area nor medial thickness was lower than in the Sv group, whereas the Arg group showed greater NP area and medial thickness. Pulse pressure, determined by intravascular transducer, increased as the pulse wave descended the aorta. The results suggest that maturation-dependent rises in BP in chicks may trigger NP formation in the lower segment of the AbA, which was prevented by inhibition of BP increase, or via a possible increase in nitric oxide availability. BP reduction exerts no effect once BP reaches a plateau. Involvement of endothelial injury leading to NP formation and hemodynamic forces selective for the lesion-prone area remain to be determined.

arginine supplement; nitric oxide; hypertension; atherosclerosis; fowl model; pulse pressure; endothelial dysfunction; endothelial nitric oxide synthase uncoupling

Birds, particularly fowl, have higher blood pressure (BP) than most mammals. In both male and female fowl, BP is already ~150 mmHg at 2–3 wk after birth, and at plateau levels, mean BP is higher in males (194 ± 4.6 mmHg) than in females (169 ± 3.1 mmHg) (13, 29, 30). In males, BP tends to increase with maturation and/or age, whereas an age-dependent change is not clear in females (30). Before sexual maturation, vascular neointimal plaques (NPs), comprising deformed endothelial cells, proliferating neointimal cells of synthetic phenotype, increased endoplasmic reticulum, and abundant extracellular matrix, are spontaneously formed without a high-cholesterol or high-fat diet (30). The NP lesions are seen in the abdominal aorta, most frequently just before the aortic bifurcation into the ischiatric arteries (referred to as the lesion-prone area); the size of the NP area increases with maturation and/or age, whereas vascular smooth muscle (SM) exhibits adaptive hypertrophy (30). In humans, aging and hypertension are major factors in the induction of arterial hardening (6, 28), and increased brachial pulse pressure (PP), aortic pulse wave velocity (PWV), and elastic properties of central arteries have been identified as indexes for cardiovascular mortality (38). Using an intravascular microtransducer, we recently found (37) that PP shows stepwise increases during pulse wave transmission along the aorta and that this site-dependent increase in PP is largest in young male chickens.

It has been shown that the endothelium is the most important communication structure between the flowing blood and the vessel wall (10) and that endothelium-derived NO has antiatherosclerotic properties, including inhibition of cell growth and platelet/leukocyte aggregation and adherence (7, 17). In contrast, cardiovascular risk factors for atherosclerosis, such as hypertension, hypercholesterolemia, diabetes, and cigarette smoking, evoke endothelial dysfunction (3, 39). In fowl, endothelial function may also be impaired after exposure to high BP, with reductions in relaxation capacity (12). Arginine, the precursor of NO, is an essential amino acid in birds and has been reported to reduce the mortality of broiler chicks by attenuating the pulmonary hypertension syndrome, presumably through improved NO-dependent endothelial function (35, 43, 44). We therefore hypothesized that the incremental rises in BP from low pressure (embryonic life and neonatal period) to high pressure (rapid growth period after hatching) trigger endothelial injury leading to the development of NP lesions. The lower segments of the abdominal aorta, where local hemodynamic forces such as flow patterns and flow-imposed laminar shear stress may be altered because of aortic bifurcation, are selectively susceptible to this BP-triggered endothelial injury and dysfunction. As an initial approach to answering these questions, we intended, in the present study, 1) to investigate in male chicks the relationship between incremental increases in BP during maturation and spontaneous NP formation in the aorta, 2) to determine whether supplemental l-arginine prevents NP formation, and 3) to measure PP along the aorta as an indicator of vascular wall elasticity.

MATERIALS AND METHODS

Animals and maintenance. Male White Leghorn chickens (Sigma strain, DeKalb) were used. One-day-old chicks, vaccinated against Marek disease virus, were purchased at a commercial hatchery (Ozark Hatcheries, Neosho, MO) and raised at the University of Tennessee.
Health Science Center animal facilities. The chicks were housed in temperature-regulated brooders (32°C during the first week and gradually reduced to 25°C). At 4 wk, the chicks were moved to large indoor pens in temperature (22–24°C)- and light (12:12 h light-dark cycle)-controlled rooms. Chicks were fed commercial chicken feed (17% crude protein, 1% Ca; Start and Grow, Purina Mills, St. Louis, MO). Feed and water were provided ad libitum. Animal protocols were reviewed and approved by the University of Tennessee Health Science Center Institutional Animal Care and Use Committee.

**BP and PP measurement in conscious and anesthetized chickens.** Chicks were anesthetized with 75–100 mg/kg (im) of Ketaset (Aveco, Fort Dodge, IA), supplemented with a local anesthetic (2% lidocaine HCl; Abbott, Chicago, IL) at the incision site. A catheter (Tygon microbore tubing, inner diameter = 0.015 in., outer diameter = 0.030 in.; Norton Performance Plastics, Akron, OH) filled with heparinized saline was inserted into the left (before treatment) or right (after treatment) ischiadic artery and exteriorized in the thigh. At 24–48 h after recovery, BP was recorded with Digi-Med System Integrator 200 (DMSI) software (Micro-Med, Louisville, KY). To prevent excitement of the bird during BP measurement, the conscious bird was placed in a black Plexiglas box and loosely tied by the legs while the rest of the body remained unrestrained.

PP was measured at different locations along the aorta in anesthetized birds [Dial mixture, 60–75 mg/kg body wt (BW) 5,5-diallylbarbituric acid; Sigma, St. Louis, MO] with a Mikrotip PP transducer (1.4-French catheter for chicks and 3-F catheter for older birds; Millar Instruments, Houston, TX) (37). Briefly, a microtransducer was inserted into the ischiadic artery and advanced upward in a step-wise fashion to the respective location (see Experimental protocols), and the pulse waves were recorded with the DMSI software and Power Lab/8SP (AD Instrument, Colorado Springs, CO). The distance at which the transducer had to be placed (target site) for recording was determined in preliminary experiments by inserting a Tygon bore catheter with a known length in birds of similar ages.

**Tissue collection and fixation.** In anesthetized chickens, a catheter (PE-50 or PE-60; Intramedic, Sparks, MD) was inserted into the right ischiadic artery, into which heparin (50 U/ml, 1 ml/kg BW) and then the fixative, containing 4% pararformaldehyde in PBS (pH 7.4), were perfused under constant pressure simulating fowl mean arterial BP. Before fixative perfusion, the lower abdominal cavity was opened and the right ischiadic artery (~1 cm off bifurcation) was occluded to effectively deliver the fixative to the aorta in a retrograde fashion. Rapid bleaching of the visceral organs indicated a successful perfusion. The aorta was pinned on a board (overnight in fixative), simulating the architectural organization in vivo. Three consecutive segments of abdominal aorta (referred to as A1, A2, and A3, at intervals of 3 mm in chicks and 5 mm in cockerels; see diagram in Fig. 1) were excised just above the bifurcation of the abdominal aorta into the ischiadic arteries and processed for dehydration and embedding for histological examination. Cross-sectioning and staining (hematoxylin and eosin) were conducted at the Integrative Microscopy Center of the University of Memphis.

**Experimental protocols.** Six-week-old chicks (BW 325 ± 4 g; n = 24) and twelve-week-old cockerels (BW 1,223 ± 14 g; n = 24) were used. Chicks were adapted to individual wire cages 3 days before the start of the experiment and were divided into three groups (n = 8 each) after the initial BP measurement. Because the BP of maturing chickens varies, the birds were assigned to treatment groups having an equal distribution of BP levels. The first group received tap water and served as the control group (solvent; Sv group); the second group received supplemental l-arginine (Sigma) in the drinking water ad libitum (0.3% wt/vol; Arg group); and the third group received propranolol (8 mg·kg⁻¹·day⁻¹; Sigma), a β-adrenergic blocker, via an osmotic minipump (Alza, Palo Alto, CA) implanted subcutaneously in the bird’s back (Prop group). Osmotic minipumps containing deionized distilled water were implanted in the Sv and Arg groups to expose all the birds to the same surgical handling. Treatment lasted 4 wk.

BP was recorded in conscious chickens before and at the end of treatment for two consecutive days. The pressure transducer was connected to the chronically implanted catheter, and recording began after the bird had relaxed and BP had stabilized. BP was recorded for 10 min each day for 2–3 days and averaged. After the second BP measurement (end of treatment), the birds were fully anesthetized and the intravascular transducer was inserted into the aorta via the same ischiadic artery incision. Pulse wave, systolic BP, diastolic BP, mean arterial BP, and heart rate were recorded in the ischiadic artery, the lower segment of the abdominal aorta, the thoracic aorta, the aortic arch, and the heart. After 1 min of stabilization, BP and heart rate were recorded every 6 s for five to seven consecutive minutes. The results...

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**Fig. 1.** Effects of solvent (control), arginine supplement, and propranolol on mean arterial pressure (top), neointimal plaque (NP) area (middle), and aortic smooth muscle (SM) width (bottom). Mean arterial pressure was measured in conscious chicks (n = 8 in each group) before treatment (pre; 6 wk of age) and after 4 wk of treatment (post; 10 wk of age) with solvent (distilled water sc; via minipump), arginine supplement (0.3% in drinking water ad libitum), or propranolol (8 mg·kg⁻¹·day⁻¹ sc; via minipump). NP area and aortic SM width independent of NP were measured in histological sections of aortic tissues collected near the bifurcation as shown in the inset (A1, A2, A3). The mean of the 3 aortic sections. For each morphometric measurement, 2 tissue slices per location (A1, A2, and A3) were examined (each was measured twice), and the mean of the measurements and then of the treatment groups was calculated. Significantly different (P < 0.05) within group by paired t-test (mean arterial pressure) or by ANOVA (width of aortic SM under the NP); **significantly different (P < 0.01) between groups by ANOVA (mean NP area and mean aortic SM width).
of each variable were averaged for analysis. PP was calculated as systolic BP minus diastolic BP. Pulse waves were recorded by the DMSI-200 acquisition system and then exported to a Photoshop file. The polyethylene catheter for infusing fixative was inserted at the same ischiadic artery incision.

**Assessment of NP area, aortic SM width, and cell density.** We measured the NP area (between the endothelium and internal elastic lamina/inner edge of the vascular SM), the width of the vascular SM layers underlying the NP (we measured the central area and 2 areas at the edges and then averaged the 3 measurements), and the width of the vascular SM in areas independent of NP (3 locations per section) with the computerized NIH Image program (1.62). The number of cells (number of nuclei/unit of area) was also quantified with a light microscope. These variables were determined in two tissue slices per location (A1, A2, and A3); each was measured twice, and the mean of the measurements and treatment groups was calculated.

**Statistical analysis.** Data were analyzed with one-way or two-way ANOVA followed by Newman-Keuls post hoc multiple-comparison test (Quick Statistica software; StatSoft, Tulsa, OK). BP measurements (before and after treatment) were compared with paired t-tests. Morphometric data were analyzed with a randomized 3 × 3 factorial design, with treatments (Sv, Arg, and Prop) and locations (A1, A2, and A3) as main factors. The changes of location-dependent PP were analyzed by a repeated-measure analysis, and the interactions were compared with orthogonal contrasts (JMP software, SAS Institute). Significance was declared at *P* ≤ 0.05.

**RESULTS**

**Animal growth.** The BW (g) of the birds at the beginning and end, respectively, of each experiment was as follows: chicks: 324 ± 6 and 661 ± 15 (Sv), 328 ± 7 and 666 ± 10 (Arg), and 324 ± 9 and 650 ± 21 (Prop); cockerels: 1,210 ± 28 and 1,532 ± 38 (Sv), 1,240 ± 22 and 1,580 ± 28 (Arg), and 1,220 ± 25 and 1,521 ± 36 (Prop). BW and BW gain (during 4 wk of treatment) did not differ significantly among treatments in either chicks or cockerels. The birds were all active and in good condition.

**BP, NP area, and aortic SM width in chicks.** BP showed maturation-dependent increases (*P < 0.05*) during 4 wk of treatment in the Sv (from 140 ± 5 to 159 ± 4; *n* = 8) and Arg (from 138 ± 4 to 157 ± 3; *n* = 8) groups, whereas BP in the Prop-treated birds showed no significant changes (from 142 ± 3 to 134 ± 6; *n* = 8) before and after treatment (Fig. 1). The mean NP area (A1, A2, and A3) was significantly lower (*P < 0.05*) in the Arg- and Prop-treated groups than in the Sv group, but it did not differ between Arg and Prop birds. In the Sv group, the NP area in the A1 segment was significantly higher than in the A3 segment (*P < 0.05*, ANOVA); no difference was found among A1, A2, and A3 in the Arg and Prop groups. In the areas free of NP, the Sv group showed a larger mean SM thickness than the Arg and Prop groups; no differences were found between the last two groups (Fig. 1). In all groups, the aortic SM width was similar in the three aortic locations. The width of the aortic SM underlying the NP was significantly (*P < 0.05*) smaller than the width of the SM not associated with NP. The mean heart rate of conscious chicks before the start of treatment was 395 ± 11 beats/min (*n* = 23). No significant difference was noted in heart rate responses among the three treatment groups (data not shown).

**BP, NP area, and aortic SM width in cockerels.** In more mature male chicks (cockerels), BP reached a plateau and did not change during 4 wk of treatment with Sv or Arg but decreased in the Prop-treated birds (Fig. 2). The mean NP area (A1, A2, and A3) was similar in the Sv- and Prop-treated groups but was larger (*P < 0.05*) in the Arg group (Fig. 2). The mean SM thickness measured in the areas independent of NP was not significantly different between the Sv- and Arg-treated groups but was slightly higher in the Arg-treated groups compared with the Prop-treated birds. The width of the aortic SM underlying the NP was significantly (*P < 0.05*) smaller than the width of the SM not associated with NP. No differences were found in the aortic SM width of A1, A2, and A3 regions within the groups. The mean heart rate of conscious cockerels was 278 ± 8 beats/min (*n* = 24) before the start of treatment and did not change significantly after treatment within the same group or among the three treatment groups (data not shown).

**Histological observation and cell density.** Cross sections of representative abdominal aortas with and without NP are shown in Fig. 3, and the approximate size (% obstruction of aortic lumen) and number of NPs in three aortic segments (A1, A2, and A3) are summarized in Table 1 for each treatment and
Fig. 3. Histological sections of the abdominal aorta above the bifurcation (A1 area) (male chickens). A: propranolol-treated chick (10 wk old) showing no NP formation. B: solvent-treated chick (10 wk old) showing thin-medium NP growth (occupies 15.7% of luminal area). C: large NP growth (occupies 35.8% of luminal area) from a 16-wk-old cockerel that received L-arginine supplement; vascular smooth muscle underlying the plaque shows thickening at the edge of the plaque (arrows) and thinning in the middle of the plaque. Hematoxylin-eosin staining. Horizontal bar, 500 μm (for all images).

The aortas from Prop- or Arg-treated chicks showed well-organized, intact muscle layers for the most part (Fig. 3A). In Sv-treated chicks, large or medium-sized NPs (Fig. 3B) that bulged to the lumen were seen in the A1 and A2 areas (Table 1), whereas in Prop- or Arg-treated chicks, NPs, when there were any, were smaller and thinner than those of the Sv group (Table 1). The number of cockerels with larger NPs (Fig. 3C) was higher in the Arg-treated group (Table 1). NPs consist of a randomly arranged heterologous (size and shape) population of cells that have abundant extracellular matrix. They appeared initially in the most distal segment of the abdominal aorta and, in some birds, extended to the upper area. The aortic SM layers underneath the large NPs were compressed in the middle and slightly thicker at the edges (Fig. 3C).

Cell densities (number of nuclei/mm²) measured in the NP, SM underlying the NP, and SM independent of NP are summarized in Fig. 4. The cell densities in the NPs were not measurable when the NP was very thin; hence, the number of birds varies among groups in Fig. 4. Cell densities were significantly higher in the NP and in the SM underlying the NP than in the SM layers independent of NP in the three experimental groups and at both ages (Fig. 4). In chicks the cell density of the aortic SM underlying the NP was similar among the three treatment groups, whereas in cockerels it was slightly lower in the Arg-treated group than in the other two groups.

Changes in PP along aorta. PP measured in birds after 4 wk of treatment is shown in Fig. 5. PP showed stepwise increases from the ascending aorta to the descending aorta and ischiadic arteries in all three treatment groups and in both chicks and cockerels. The increases in PP at the ischiadic artery relative to the PP at the aortic arch (%) were 27.8 ± 2.5 (Sv), 17.1 ± 4.3 (Arg), and 27.0 ± 6.0 (Prop) in chicks and 53.2 ± 13.9 (Sv), 40.5 ± 9.2 (Arg), and 38.2 ± 9.4 (Prop) in cockerels (PPs were significantly different between aortic arch and ischiadic artery; P < 0.01 in all treatment groups in 2 age groups). This site-dependent widening of PP is primarily due to a reduction in diastolic BP, whereas systolic BP remained stable (Table 2). The site-dependent increase in PP in the Sv group was steeper in cockerels than in chicks (P < 0.01). There was no significant difference among treatments in the slope in either age group (Fig. 5). As the pulse wave traveled down the aorta, the contour of the pressure wave also slightly changed in cockerels: in the proximal region of the aorta the pulse wave was smooth in the ascending and descending limbs, whereas in more peripheral sites the slope in the ascending and descending limbs during systole became steeper and the magnitude of the systolic pressure peak was larger; the contour became smooth in

Table 1. Number and size of neointimal plaques at the three aortic locations

<table>
<thead>
<tr>
<th></th>
<th>No. of Birds</th>
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<tr>
<td></td>
<td>Large</td>
<td>Medium</td>
<td>Small</td>
<td>None</td>
<td>Large</td>
<td>Medium</td>
<td>Small</td>
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<tr>
<td>Chicks</td>
<td>Solvent</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<tr>
<td></td>
<td>Arginine</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Propranolol</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Cockerels</td>
<td>Solvent</td>
<td>8</td>
<td>0</td>
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<td></td>
<td>Arginine</td>
<td>8</td>
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<td>Propranolol</td>
<td>8</td>
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Large, >20% obstruction of respective abdominal lumen area; medium, 10–20% obstruction, small; <10% obstruction.
Fig. 4. Cell density (no. of nuclei/mm²) in the NP, aortic SM underlying the NPs, and aortic SM independent of NP of chicks (A) and cockerels (B) after a 4-wk treatment with solvent (distilled water sc; minipump), arginine supplement (0.3% in drinking water ad libitum), or propranolol (8 mg·kg⁻¹·day⁻¹ sc; minipump). Mean (A1, A2, A3) cell density of the designated area (2 slices per location, each measured twice) was calculated in each bird; the mean ± SE of each treatment group is shown. Numbers in the columns indicate the number of birds used. Note that not all birds had NPs and that cell density was not measurable in some thin NPs. *Significantly different (P < 0.05) from cell density in aortic SM; †significantly different (P < 0.05) between groups, analyzed by 1-way ANOVA and compared with Newman-Keuls method.

Fig. 5. Pulse pressure (PP) along the aorta of anesthetized chicks (A) and cockerels (B) after a 4-wk treatment with solvent (distilled water sc; minipump), arginine supplement (0.3% in drinking water ad libitum), or propranolol (8 mg·kg⁻¹·day⁻¹ sc; minipump). Each point represents the mean ± SE. PP was measured by inserting an intravascular microtransducer via the ischiadic artery and advancing it into the aorta in a retrograde fashion. See text for statistical evaluation.

diastole. This change in pulse wave contour along the aorta was not seen clearly in chicks. Heart rates were relatively stable during the experimental course, and no clear changes were noted by location (Table 2).

**DISCUSSION**

*Animal growth and conditions.* The similarity in behavior and growth rates among the groups indicates that the surgical procedures and treatments caused minimal distress to the birds. The dose of arginine used in the present experiments was the same as that used for a previous study, which slightly elevated plasma arginine levels with no change in growth performance compared with control birds (35). The hypotensive effect of propranolol was similar to the finding we obtained previously (13, 29); chronic and acute administration of propranolol, as well as other β-adrenoceptor blockers such as atenolol and practolol, decreased BP in conscious chickens (13).

*Relationships among BP, NP formation, and medial thickening.* In the present study, chicks treated with solvent showed a maturation-dependent rise in BP and the development of NP lesions and medial hypertrophy at the lower segment of the abdominal aorta, similar to those in our previous observations (30). The BP rise, NP formation, and medial thickening were prevented by maintaining a lower BP with propranolol, suggesting that incremental rises in BP may trigger the initiation of vascular lesions. The size of the NP area correlates positively with the BP level (30). Despite the significant reduction in BP, however, this preventive effect of propranolol was not seen in more mature chickens (cockerels, 12–16 wk of age) in which BP had already plateaued; this indicates that relatively rapid increases in BP, rather than BP levels per se, may be important for evoking NPs. The study also suggests that NP lesions, once developed, are not reversible during the relatively short time (4 wk) of antihypertensive treatment. Treatment with nifedipine for 7 wk reduced neointimal lesions of carotid arteries induced by endothelial denudation in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (20); enhanced apoptosis independent of nifedipine’s effect on BP appears to account for its antihypertrophic effect. At present, there is no evidence showing that chicks are more susceptible to endothelial damage than more mature birds. Endothelial function, such as magnitude of endothelium-dependent relaxation, is similar in 2- to 3-wk-old chicks and pullets/cockerels (31).

The NP lesions spontaneously developing in chickens before sexual maturation have a morphological appearance resembling balloon catheter injury-induced NP in rats (41) and chickens (Ref. 22 and unpublished observations) but differ from atheromatous plaques in lacking the fatty streaks that are
frequently found in human atherosclerosis and in models of experimentally induced atherosclerosis (for review, see Ref. 24). The chicken model is unique because NP lesions develop concomitantly with incremental rises in BP at young ages in the selected lesion-prone area without a diet of excess fat or cholesterol (Refs. 25, 26, and 30 and present study). The endothelium injury-induced neointima has been used extensively as a model for vascular remodeling and atherosclerosis (11, 41), but the animals are usually normotensive. SHR and rats with genetically or experimentally induced hypertension do not exhibit spontaneous formation of neointimal/atheromatous lesions (atherosclerosis resistant). Furthermore, mammalian models for atherosclerosis usually require a dietary fat/cholesterol supplement or genetic manipulation, such as apolipoprotein-knockout mice (18). Fowl models are therefore useful for examining interrelations among high-BP-induced hemodynamic forces, endothelial injury, and vascular lesions.

**Effects of L-arginine supplementation in NP formation.** We intended to determine whether supplemental L-arginine inhibits NP formation during maturation-dependent rises in BP, possibly by increasing NO bioavailability. In the present study, the total NP area, the incidence of vascular lesions, and the aortic SM thickness, were lower in chicks with arginine supplementation than in Sv control chicks, whereas BP similarly increased with maturation in both groups. Although the results support our hypothesis, it is necessary to determine whether L-arginine supplementation in chicks indeed increases NO and/or cGMP production in the aortic wall. It has been reported that the uptake of L-arginine into platelets from hypertensive humans and the transport of L-arginine into red blood cells of hypertensive rats are lower than those in normotensive controls (27); L-citrulline production was also reduced in platelets from hypertensive humans (25). In contrast, L-arginine treatment increased the NO content of ischemic myocardium and reduced apoptosis of myocytes (21). L-Arginine decreased elevated BP and restored urinary NO level and renal NO-generating capacity in rats on a low-protein diet (5). L-Arginine improves endothelial function and induces regression of intimal lesions in various clinical or experimental cardiovascular disorders (1, 23), whereas reduced vascular endothelial NO production and endothelial dysfunction likely play an important role in the vascular pathology of atherosclerosis, hypertension, and diabetes mellitus (for review, see Refs. 3 and 4).

Accumulating evidence suggests that hypertension increases vascular production of reactive oxygen species, specifically superoxide anion (O$_2^-$), via the activation of NAD(P)H oxidase (3, 46), which is critically involved in the breakdown of NO and in endothelial dysfunction (45, 46). Increased O$_2^-$ also increases the production of H$_2$O$_2$, which appears to contribute to medial hypertrophy (46). L-Arginine supplementation in hypercholesterolemic rabbits reduces vascular oxidative stress (1) and also normalizes leukocyte adhesion to nonendothelial matrix (2). In SHR with established high BP, flow-dependent vasodilation of arterial wall is attenuated compared with that of age-matched normotensive control rats (14), suggesting that shear stress-mediated NO release is reduced in hypertension. We previously reported (30) that the plasma arginine level of 3-wk-old chicks was 307 ± 10 nmol/ml and that it significantly decreased around 12–27 wk when NP formation in the abdominal aorta became evident. In the present study, medial thickness was smaller in Arg-treated chicks than in Sv-treated chicks despite the similar increases in BP in both groups. NO
inhibits vascular SM proliferation and mobilization and maintains vascular wall integrity (4, 7), whereas a balloon catheter-induced endothelial injury evokes vascular SM hypertrophy and hyperplasia. If incremental rises in BP induce endothelial injury in chicks, supplemental arginine may prevent or inhibit medial hypertrophy by a similar mechanism.

Cockerels (12–16 wk of age) had an established high BP that was unchanged by Arg or Sv treatment. In contrast to chicks, the mean (A1, A2, A3) NP area of the Arg-treated cockerels was nearly four times larger than that of the Sv group. The mechanism of these enhanced vascular lesions evoked by arginine supplementation is not clear at present. It has been shown that tetrahydrobiopterin (H4B) is a critical cofactor for endothelial NO synthase (eNOS) and that when H4B is reduced or oxidized by O2·− in injured endothelium eNOS is uncoupled (3, 9, 19, 23). Uncoupled eNOS produces, on stimulation, large amounts of O2·− and peroxynitrite (ONOO−), leading to further enhancement of endothelial dysfunction and vascular lesions (9, 19, 40). Further studies are needed to determine whether arginine supplementation in maturing chickens enhances production of reactive oxygen species including O2·−, ONOO−, and H2O2.

PP along aorta. We recently reported (37) that the magnitude of the PP increases as the pulse wave descends the aorta of chickens and that this site-dependent amplification of PP is more marked in young adult males than in chicks or adult females. Increase in PP is primarily due to the reduction of diastolic BP (37). In the present study, a similar site-dependent widening of PP was noted in all treatment groups for both chicks and cockerels (more marked in cockerels). The site-dependent increases in PP are due to the decrease in diastolic BP, whereas systolic BP remains relatively stable. It has been shown in normotensive humans that the elastic properties of the arterial wall, such as the ratio of elastin over collagen, determine the distensibility/compliance of the vessels (6, 33). The abdominal aorta and its immediate descending branches, such as the femoral arteries, are less distensible than the proximal part of the aorta, resulting in lower diastolic BP and, accordingly, higher PP in the latter (for review, see Refs. 28, 32, and 33). In humans, systolic BP starts to rise after adolescence and continuously increases with age. Diastolic BP, in contrast, initially increases with age and levels off at age ~50 yr. After age ~60 yr, loss of elasticity and stiffness of central arteries, rather than systemic vascular resistance, become the dominant hemodynamic factor; thus diastolic BP tends to become lower and PP widening occurs (6, 28). Our preliminary study indicates that PWV, which is a better index for arterial stiffness, increases with maturation and aging, particularly in male chickens (36).

Propagation of the PP wave along the arterial tree is influenced by various factors, including the effect of wave frequency on wave velocity, the length of blood vessels, and the rate of damping; high-frequency waves are transmitted faster than low-frequency waves, and this enhances the systolic peak and changes the contour of the pulse wave (more steep) in the distal part of the aorta (6, 32, 33). When the pulse wave travels further to the periphery, its contour changes because of the damping effect. Furthermore, a rise in systolic BP depends on left ventricular performance as well as the stiffness of the central conduit arteries and the degree of systemic resistance (6, 32, 33). When hardening/stiffness further increases and PWV becomes higher, the difference in contour and magnitude of pulse wave between the proximal and distal aorta becomes small because the wave reflection from the periphery (faster return) enhances the steepness and height of the ascending limb of the pulse wave in the ascending aorta (32). Because in the present study systolic BP was relatively stable in all groups, the widening of PP may primarily be ascribed to the reduction in compliance/hardening of the aortic (arterial) wall.

The mechanism by which NP formation occurs most frequently in the distal segment of the aorta is not clear at present. Because atherosclerotic plaques more frequently appear at sites of branching and arterial curvature and because these locations are expected to harbor complex flow patterns, it has been postulated that fluid dynamics, via an endothelial mechanism, may play an initiating role in atherogenesis (8, 42). Fluid flow-imposed laminar shear stress induces structural changes in endothelial cells in culture and influences vascular wall function, including NO production, prostacyclin release, vascular relaxation, monocyte recruitment, and intimal thickening (34, 42). In the human carotid artery (16) and common femoral artery (15), the mean wall shear rate is lower, whereas the intimal-medial thickness is greater, at the posterior wall proximal to the bifurcation than at the downstream wall, suggesting that the areas with low shear stress with nonlaminar flow may be subject to more endothelial damage and dysfunction.

In summary, NP formation and medial thickening in the lower part of the abdominal aorta above the bifurcation occurred in accordance with incremental increases in BP during maturation of intact chicks, and these vascular wall alterations were prevented by antihypertensive treatment. Oral L-arginine supplement also reduced NP formation while BP increased, suggesting that BP-triggered endothelial damage/dysfunction may be involved in the mechanism of NP formation. Once BP reaches and maintains a plateau, however, reducing BP does not reduce/restore structural changes within 4 wk of treatment. It remains to be determined whether fluid dynamics such as laminar shear stress are altered and reactive oxygen species are produced in the NP-prone regions.

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