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# Prolonged L-Arginine on Cardiovascular Mass and Myocardial Hemodynamics and Collagen in Aged Spontaneously Hypertensive Rats and Normal Rats

Dinko Susic, Aloisio Francischetti, Edward D. Frohlich

**Abstract**—This study was designed to examine whether L-arginine could prevent hypertension- and age-related impairment of coronary hemodynamics and cardiac fibrosis in aged (80-week-old) rats. To differentiate between hypertension- and age-related changes, the study was performed in both normotensive Wistar-Kyoto rats (WKYs) and spontaneously hypertensive rats (SHR). Male 1-year-old rats of both strains were divided into 2 groups and given either placebo or L-arginine (1.2 g/L) in drinking water. After 6 months, systemic and coronary hemodynamics (radionuclide-labeled microspheres), right and left ventricular and aortic mass indexes, and ventricular hydroxyproline (an estimate of collagen) concentrations were determined. In the aged WKYs, L-arginine did not affect any of the examined variables except slightly reducing total peripheral resistance. In contrast, L-arginine diminished arterial pressure, total peripheral resistance, and left ventricular and aortic mass indexes in the SHRs; it also increased coronary flow reserve and reduced minimal coronary flow resistance and myocardial hydroxyproline concentration. These findings demonstrated that L-arginine ameliorated adverse cardiovascular effects of hypertension in aged SHRs, as demonstrated by reduced arterial pressure and total peripheral resistance, diminished left ventricular mass and collagen content, and improved coronary hemodynamics. There were no important effects in the old WKYs. (*Hypertension*. 1999;33[part II]:451-455.)

**Key Words:** hypertension ■ aging ■ mass, ventricular ■ mass, aortic ■ hemodynamics, coronary ■ myocardial collagen concentration ■ L-arginine

Morphological and functional changes that occur in the cardiovascular system with hypertension and aging are similar in many respects.<sup>1-4</sup> They include impaired cardiac performance and coronary hemodynamics and ventricular fibrosis.<sup>5-9</sup> In a recent study involving normotensive and spontaneously hypertensive rats (SHRs), aged 22, 35 and 65 weeks, we demonstrated that associated with aging per se there were progressive impairments in coronary hemodynamics with increased myocardial collagen deposition in both strains.<sup>9</sup> These changes were more pronounced in hypertensive rats at any age, as if hypertension might have induced premature aging alterations of cardiovascular system.

Much evidence exists that suggests that the endothelium, in autocrine/paracrine and endocrine manners, participates in regulating cardiovascular structure and function.<sup>10</sup> The endothelial cells produce and release a variety of vasoactive substances, including nitric oxide (NO).<sup>10</sup> NO is a powerful vasodilator which is derived from L-arginine by the action of NO synthase.<sup>11</sup> Many reports have demonstrated that endothelial dysfunction of NO synthesis and release is present, or even precedes cardiovascular changes associated with hypertension and aging.<sup>12-19</sup> Thus, endothelial dysfunction may participate the development of hypertension and age-related

changes in the coronary vasculature and myocardium. Therefore, the purpose of this study was to examine whether prolonged L-arginine administration, the initiator of NO production, could modulate cardiac fibrosis and deterioration of coronary hemodynamics in both old and hypertensive rats. To differentiate between hypertension- and age-induced changes, the results obtained in aging normotensive Wistar-Kyoto rats (WKYs) and SHRs were compared.

## Materials and Methods

### Animals

Male normotensive WKYs and SHRs were obtained from Charles River Breeding Laboratories Inc (Wilmington, Mass) at 16 weeks of age. They were maintained thereafter in temperature- and humidity-controlled rooms on a 12-hour light-dark cycle. All rats were given standard chow (PMI Nutrition International) and tap water ad libitum. All rats were handled in accordance with National Institutes of Health guidelines, and the protocol was approved by our institutional Animal Care and Use Committee.

### Experimental Design

One-year-old rats of both strains were divided randomly into 2 groups of 10 rats each. Control groups were given no therapy; the second received L-arginine (Sigma) in drinking water (1.2 g/L). We calculated that on the basis of their average daily fluid intake, the rats

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**Parameters and Indexes in Control and L-Arginine-Treated Normotensive Wistar-Kyoto and Spontaneously Hypertensive Rats**

	WKY		SHR	
	Control (n=8)	L-Arginine (n=9)	Control (n=7)	L-Arginine (n=8)
Body weight, g	417±12	422±14	409±13	426±10
Left ventricular weight, g	0.92±0.01	0.94±0.02	1.46±0.03†	1.27±0.03*†
Left ventricular weight index, mg/g	2.21±0.05	2.23±0.06	3.45±0.07†	2.98±0.06*†
Right ventricular weight index, mg/g	0.56±0.03	0.55±0.03	0.61±0.03	0.59±0.02
Aortic weight index, mg · mm <sup>-1</sup> · kg <sup>-1</sup>	2.89±0.11	2.78±0.09	4.63±0.14†	3.99±0.12*†
Mean arterial pressure (mm Hg)	98.7±2.4	92.9±3.7	141.3±4.6†	127.4±2.3*†
Heart rate, bpm	386±11	411±13	405±15	418±11
Cardiac index, mL · min <sup>-1</sup> · kg <sup>-1</sup>	307±16	339±14	316±18	345±16
Total peripheral resistance index, U/kg	0.32±0.01	0.27±0.02†	0.45±0.03†	0.37±0.02*†

Values are mean±SE. WKY indicates Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

\*P<0.05 versus control rats of the same strain; †P<0.05 versus similarly treated WKY rats.

consumed about 35 mg of L-arginine per day. Before the beginning of the treatment, indirect determinations of systolic pressure (tail-cuff) were made in all rats. Systolic pressure was 125±6 mm Hg in control WKYs, 119±5 in L-arginine-treated WKYs, 196±7 in control SHRs, and 198±6 in L-arginine-treated SHRs. Rats received their respective protocol treatments for the ensuing 6 months. At the end of the 6 months of treatment, when rats were about 80 weeks old, systemic and coronary hemodynamics, cardiac and aortic masses, and myocardial hydroxyproline concentration were determined.

### Procedures and Techniques

At the end of treatment, rats were anesthetized with ketamine (10 mg/kg) and acepromazine (50 mg/kg) and instrumented for determination of systemic and coronary hemodynamics (using the reference standard microsphere method), as described previously.<sup>9,20–22</sup> In brief, a jugular vein, femoral artery, and left ventricle were cannulated with polyethylene catheters, which were exteriorized at the nape of the neck through a subcutaneous tunnel. Rats were then placed in nonrestrictive polyethylene cages and allowed to recover, and baseline measurements of systemic and coronary hemodynamics were obtained when they had fully recovered from anesthesia. The femoral artery catheter was connected to a pressure transducer (P23Db, Statham Instruments), and mean arterial pressure was recorded on a multichannel physiograph (Sensor Medics R612) while simultaneously heart rate was derived through a tachometer coupler. Cardiac output was measured by the reference sample microsphere method as reported previously, using the first radionuclide (<sup>57</sup>Co) microspheres.<sup>20–22</sup>

After basal measurements were obtained, maximal coronary vasodilation was induced by dipyridamole infusion (4 mg · kg<sup>-1</sup> · min<sup>-1</sup> IV for 10 minutes)<sup>22,23</sup> using a Harvard infusion/withdrawal pump (Harvard Apparatus). The hemodynamic measurements were then repeated using a second radionuclide microspheres (<sup>113</sup>Sn). At the end of the study, the rats were killed by an overdose of pentobarbital, and the heart, aorta, lungs, and kidneys were removed immediately. After the heart was removed, the atria were dissected free from the ventricles and discarded; the free wall of the right ventricle was separated carefully from the left ventricle (the septum remained with left ventricle). Wet weights of the ventricles were recorded, normalized for body weight and expressed as ventricular mass indexes (mg/g). A measured segment of the descending aorta (3 cm long, starting from a point just distal to the origin of subclavian artery) was also removed, weighed, normalized for its length and body weight, and expressed as aortic mass index. Tissue samples, as well as blood reference samples, were placed in plastic scintillation vials and counted for 15 minutes in a deep-well gamma scintillation spectrometer (Packard) with a multichannel analyzer. Spillover correction between channels was achieved using matrix inversion software (Compusphere, Packard). Coronary blood flow (for each ventricle) was calculated by multiplying

the fractional distribution of radioactivity to each ventricle by cardiac output; it was normalized for the wet weight of the respective ventricle and expressed as mL · min<sup>-1</sup> · g<sup>-1</sup>. Coronary flow reserve for each ventricle was calculated as the difference between flow during dipyridamole infusion and baseline flow. Coronary vascular resistance was calculated by dividing the mean arterial pressure with the blood flow to the respective ventricles; it was normalized for ventricular weight and expressed as mm Hg/mL per minute per gram (or U/g). Minimal coronary vascular resistance was defined as vascular resistance determined during dipyridamole infusion.

### Exclusion Criteria

The results obtained in any particular rat were completely discarded (a) if the fractional distribution of radioactivity to the lungs was >5%, suggesting arteriovenous shunting,<sup>24</sup> or (b) if the difference in radioactivity between the 2 kidneys was >15%, suggesting uneven mixing or distribution of microspheres.<sup>21</sup> Three rats were excluded from the study based on these criteria; 2 rats died during treatment period; and the cannulation procedure was unsuccessful in 3 animals.

### Myocardial Collagen Content

As an estimate of ventricular collagen content, hydroxyproline concentration was determined in samples of both ventricles, using a previously described procedure.<sup>9</sup> Myocardial samples were dried to constant weight and lipids were then extracted. Collagen was hydrolyzed with 6N hydrochloric acid (at 110°C) and, after extraction with activated charcoal, samples were treated with Chloramine-T and paradimethylaminobenzaldehyde solution. Absorbance was read at 560 nm; hydroxyproline concentration was determined from standard curve and was expressed as mg/g dry wt.

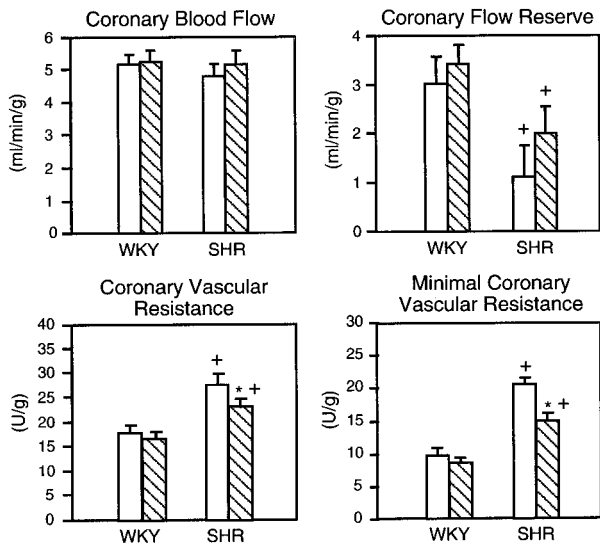
### Statistical Analysis

Values are expressed as the mean±SEM. A 2-way ANOVA and Student-Newman-Keuls post hoc tests were used to test the significance of differences between the groups.<sup>25</sup> The 5% confidence level was considered to be statistically significant.

## Results

### Body, Cardiac, and Aortic Masses

No differences in body, cardiac, and aortic weight indexes were found between the control and L-arginine-treated groups in the WKYs (Table). Body weight was similar in the control and L-arginine-treated SHRs; however, both, left ventricular and aortic weight and weight indexes were significantly (*P*<0.05) lower in L-arginine-treated SHRs than in



**Figure 1.** Left ventricular coronary hemodynamic indexes in control (open rectangles) and L-arginine-treated (shaded rectangles) normotensive rats (WKYs) and hypertensive rats (SHRs). Values are expressed as mean±SEM. \* $P<0.05$  versus control rats; + $P<0.05$  versus similarly treated WKYs.

controls; there was no difference in right ventricular mass between these 2 SHR groups (Table). When compared with similarly treated WKYs, left ventricular and aortic weights and weight indexes were significantly ( $P<0.05$ ) higher in SHRs (Table).

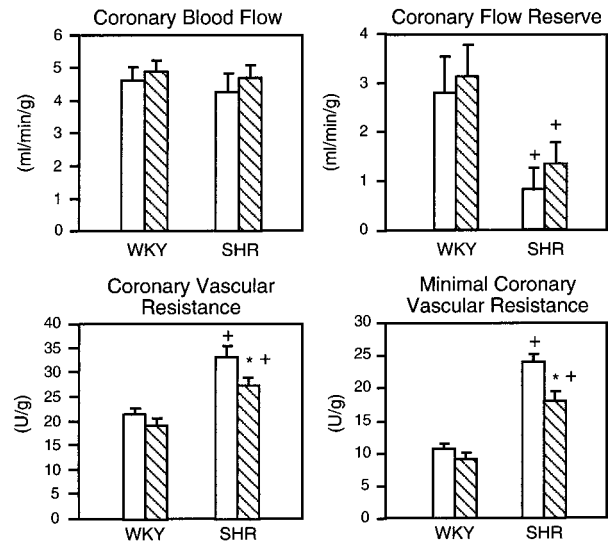
**Systemic Hemodynamics**

Compared with similarly treated WKYs, mean arterial pressure and total peripheral resistance were significantly higher in the SHRs, but cardiac output and heart rate were similar (Table). Therapy with L-arginine slightly but not significantly reduced arterial pressure in the WKYs, although cardiac output was slightly increased so that total peripheral resistance was just significantly ( $P<0.05$ ) lower in L-arginine-treated rats than in controls (Table). On the other hand, L-arginine significantly reduced arterial pressure and total peripheral resistance in the SHRs associated with a slight but insignificant increase in cardiac output (Table).

**Coronary Hemodynamics**

There were no differences in baseline coronary blood flows between all groups studied (Figure 1). Moreover, there were no differences in coronary flow reserve, coronary vascular resistance, and minimal coronary vascular resistance between L-arginine-treated and control WKY groups. Both baseline coronary vascular resistance index and minimal coronary vascular resistance index were significantly ( $P<0.05$ ) reduced in L-arginine-treated SHR group, but there was no difference in coronary flow reserve between the 2 SHR groups. Coronary flow reserve was significantly ( $P<0.05$ ) higher, whereas basal and minimal coronary vascular resistance were lower in normotensive rats than in similarly treated SHRs.

Right ventricular coronary hemodynamics paralleled those of the left ventricle (Figure 2). L-arginine did not affect coronary hemodynamics in WKYs; it reduced basal and

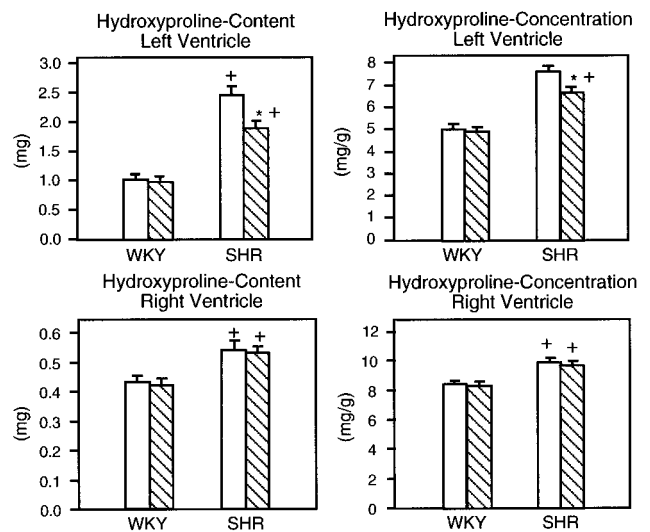


**Figure 2.** Right ventricular coronary hemodynamic indexes in control (open rectangles) and L-arginine-treated (shaded rectangles) WKYs and SHRs. Values are expressed as mean±SEM. \* $P<0.05$  versus control rats; + $P<0.05$  versus similarly treated WKYs.

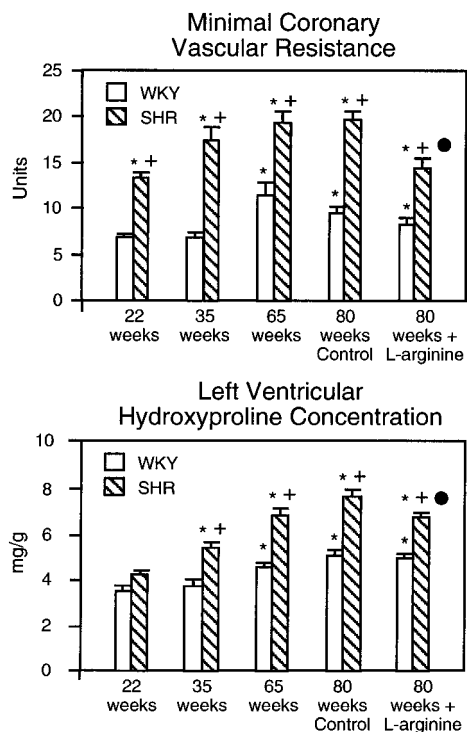
minimal coronary vascular resistance in SHRs (Figure 2). Moreover, as in the left ventricle, coronary flow reserve was significantly ( $P<0.05$ ) higher, whereas basal and minimal coronary vascular resistance were lower in normotensive rats than in similarly treated SHRs.

**Hydroxyproline Concentration**

L-arginine did not affect either hydroxyproline concentration or content in the left or right ventricle of normotensive rats; it decreased hydroxyproline content and concentration in the left, but not in the right, ventricle of SHRs (Figure 3). Hydroxyproline content and concentration were significantly ( $P<0.05$ ) higher in both ventricles of SHRs than in similarly treated WKYs (Figure 3).



**Figure 3.** Hydroxyproline concentration and content in the left (LV) and right (RV) ventricle of control (open rectangles) and L-arginine-treated (shaded rectangles) WKYs and SHRs. Values are expressed as mean±SEM. \* $P<0.05$  versus control rats; + $P<0.05$  versus similarly treated WKYs.



**Figure 4.** Minimal coronary vascular resistance (top) and left ventricular hydroxyproline concentration (bottom) in WKYs and SHRs of different ages. Combined data from this study and reference 9. Values are expressed as mean  $\pm$  SEM. \* $P < 0.05$  versus 22-week-old rats; + $P < 0.05$  versus WKYs of the same age; ● $P < 0.05$  versus control rats of the same age.

## Discussion

The results of the present study demonstrated that prolonged (oral) administration of L-arginine differed in its effects on systemic and coronary hemodynamics, cardiovascular mass, and myocardial collagen between WKYs and SHRs. Thus, in the aged WKYs, L-arginine failed to affect any of the variables used to assess coronary hemodynamics and cardiac fibrosis whereas, in the SHRs, it significantly improved coronary hemodynamics and reduced ventricular collagen content. Since we have previously shown that a progressive deterioration in coronary hemodynamics associated with an increased myocardial collagen occur with aging in normotensive WKYs,<sup>9</sup> the present findings also indicate that prolonged administration of L-arginine did not prevent the adverse cardiovascular effects of aging. The effect of L-arginine administration on progressive age and hypertension related changes in coronary hemodynamics and myocardial collagen is illustrated in Figure 4. NO synthase levels and NO production have been reported to be reduced in old normotensive rats.<sup>26,27</sup> In addition, L-arginine administration was found to increase NO production and reduce blood pressure in young hypertensive rats with renal failure.<sup>28</sup> In this context, failure of L-arginine to improve coronary hemodynamics in the old WKYs of this present study may indicate that alterations in NO pathway might become irreversible after a certain age. Alternatively, the present results may indicate that alterations in NO production or its availability may not be involved in the pathogenesis of age related cardiovascular

changes. We did not measure NO production in the present study. However, previous results from our laboratory,<sup>28</sup> together with the results of other studies,<sup>29</sup> and our results in the SHRs, support the assumption that oral L-arginine administration stimulates NO production in rats.

Prolonged administration of L-arginine decreased arterial pressure and total peripheral resistance in old SHRs in the present study. These findings are in agreement with earlier reports which demonstrated that L-arginine reduced arterial pressure in rats with renal ablation hypertension<sup>29</sup> as well as in hypertensive Dahl rats.<sup>30</sup> Furthermore, prolonged treatment with L-arginine improved coronary hemodynamics in aged SHRs, as demonstrated by significant decreases in basal and minimal coronary vascular resistances. These findings are supported by clinical and experimental reports that L-arginine improved endothelial function.<sup>31–33</sup> Moreover, acute L-arginine administration restored coronary vascular response to acetylcholine in elderly<sup>31</sup> and hypertensive patients<sup>33</sup> and in hypercholesterolemic rabbits.<sup>32</sup> It, therefore, is tempting to speculate that L-arginine improved the coronary hemodynamics in the SHRs in our study by improving endothelial function. However, we cannot exclude the possibility that the improved coronary hemodynamics in the SHR was secondary to a decreased arterial pressure, since various antihypertensive agents have been shown to improve coronary hemodynamics in hypertensive rats.<sup>34–37</sup> Finally, we found that L-arginine reduced cardiac fibrosis in the SHR, as demonstrated by reduction in left ventricular hydroxyproline content and concentration. Our results do not indicate whether the effect of L-arginine on myocardial collagen was direct or was mediated by hemodynamic changes. In fact, the finding that L-arginine decreased hydroxyproline in the left but not in the right ventricle could favor the concept that pressure overload, directly or indirectly, promotes myocardial fibrosis.

It is also worth noting that the present study is among the few that examined effects of therapy on hypertension related changes in old animals. As already mentioned, numerous studies in young animals have shown that cardiovascular consequences of hypertension are reversible.<sup>34–37</sup> On the other hand, only a few studies have addressed this issue in old animals, their results have been variable, although all demonstrated some degree of improvement.<sup>38–40</sup> Interestingly, the present study demonstrated that treatment with L-arginine improved coronary hemodynamics and myocardial fibrosis in hypertensive rats, but only partially, since none of the examined variables returned to the level seen in normotensive rats of the same age (Figure 4).

In conclusion, prolonged (6 months) administration of L-arginine ameliorated adverse cardiovascular effects of hypertension in aged SHRs but not in WKYs, indicating that adverse cardiovascular effects of hypertension and aging although similar in appearance may have different underlying mechanisms.

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