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# Coronary Circulation in Hypertension and Aging: An Experimental Study

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## ABSTRACT

**Purpose.** This study was undertaken to examine adverse changes in coronary hemodynamics associated with hypertension, aging, and excessive salt intake. To dissociate from the possible effects of atherosclerosis, the study was done in rats because they do not develop atherosclerosis. Moreover, this strain of spontaneously hypertensive rats (SHR) develops hypertension similar to essential hypertension in man.

**Methods.** Systemic and coronary hemodynamics, left ventricular mass, and collagen content in normotensive and SHR of various ages and given different treatments were determined.

**Results.** Compared with normotensive Wistar-Kyoto rats, coronary blood flow reserve was lower and minimal coronary vascular resistance was higher in SHR of all ages; an age-related decrease in flow reserve and an increase in minimal vascular resistance were observed for both strains of rats. In very old rats with isolated systolic hypertension, an increase in left ventricular collagen was associated with coronary insufficiency; antihypertensive therapy nearly normalized both measures. In SHR excessive salt intake increased pressure, increased collagen deposition in myocardial interstitium and perivascularly, and impaired coronary circulation; angiotensin II receptor blocker therapy prevented fibrosis and improved coronary hemodynamics.

**Conclusion.** In conclusion, these data indicate that considerable coronary insufficiency associated with hypertension, aging, and salt overload exists in the absence of atherosclerotic coronary changes. Perivascular fibrosis within myocardium may significantly contribute to the coronary vascular impairment.

Abnormalities in coronary circulation with consequent myocardial ischemia are well-established risk factors for cardiovascular morbidity and mortality.[1]

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Atherosclerosis of epicardial coronary arteries is certainly one of the major pathogenic factors causing myocardial ischemia, and a direct relationship between the severity of coronary artery disease and survival is well established.[2] In addition to atherosclerotic changes, functional and structural abnormalities of coronary vasculature occur in many clinical conditions.[2] Thus, besides atherosclerosis, hypertension and aging are the most common causes of structural and functional alterations in coronary microvasculature.[2–4] It should be noted that although abnormalities in coronary vasculature, regardless of cause, have essentially the same consequence (i.e., myocardial ischemia), atherosclerosis affects primarily the large coronary vessels, whereas hypertension- and aging-related changes in coronary vasculature affect the microvasculature. Vascular remodeling and rarefaction, perivascular fibrosis, and endothelial dysfunction are common causes of alterations in coronary vasculature in hypertensive and elderly persons.[2] Furthermore, atherosclerosis-related abnormalities and abnormalities from other causes often coincide, and in most cases it is very difficult to estimate the extent of their respective participation in the overall coronary insufficiency.

To examine hypertension- and aging-related changes in the coronary vasculature, we used rats as an experimental model because they do not develop atherosclerosis naturally, even when exposed to high cholesterol intake over a prolonged period.[5] Herein we report the results of studies from the last several years, including some of our most recent data. Spontaneously hypertensive rats (SHR), considered the best experimental model of essential hypertension,[6] and their normotensive counterparts, Wistar-Kyoto rats (WKY), were used throughout the study. They were studied at different ages and after exposure to various treatments.

## MATERIAL AND METHODS

### Animals

Male WKY and SHR were used throughout. Rats were purchased from Charles River Breeding Laboratories (Wilmington, Mass) and were maintained thereafter in temperature- and humidity-controlled rooms on a 12-hour light-dark cycle. They were given standard chow (PMI Nutrition International, St Louis, Mo) and tap water ad libitum unless stated otherwise. All rats were handled in accordance with National

Institute of Health guidelines, and all studies were approved by our institutional Animal Care and Use Committee.

## Experimental Design

The first experiment was designed to delineate hypertension- and age-related changes in coronary hemodynamics and to assess the role of cardiac hypertrophy and fibrosis in mediating the deterioration of coronary circulation associated with both hypertension and aging.[7] To this end, systemic and coronary hemodynamics, cardiovascular mass indexes, and ventricular hydroxyproline concentration (an estimate of collagen) were determined in WKY and SHR aged 22 (young adults), 35 (adults), and 65 (old) weeks.

The second experiment was designed to examine the preventability of progressive deterioration of cardiovascular structure and function in very old WKY rats with isolated systolic hypertension.[8] To this end, male 18-month-old WKY rats were given either placebo or L-arginine (nitric oxide precursor) (70 mg/kg/day) and an angiotensin-converting enzyme (ACE) inhibitor (enalapril, 30 mg/kg/day) for 6 months. Both control and treated rats were studied at the age of 2 years, including cardiovascular mass, collagen concentration, and systemic and coronary hemodynamics. A group of 35-week-old rats was also included for comparison.

In the third experiment we examined cardiovascular mass and function in SHR in which hypertension has been aggravated by salt overload.[9] To this end, the effects of 8 weeks of NaCl excess (8% in food) on systemic and coronary hemodynamics as well as collagen levels were examined in young adult SHR, aged 16 weeks by the end of the study.

Finally, the fourth experiment was designed to examine whether antihypertensive therapy might prevent cardiovascular damage induced by salt overload. To this end, 8-week-old SHR were then given either control (1%) (group 1; $n = 10$) or high-salt (8%; $n = 20$) diets for the ensuing 8 weeks. The salt-loaded SHR were randomized to 1 of 2 groups: group 2, ($n = 10$) untreated, and group 3 ($n = 10$), treated with an angiotensin II receptor blocker (ARB) (candesartan, 10 mg/kg/day). At the end of the study, cardiovascular mass indices, systemic and coronary hemodynamics, and myocardial collagen concentrations were examined.

## Experimental Protocols and Methods

All rats were anesthetized with pentobarbital (40 mg/kg) and instrumented for determination of systemic and coronary hemodynamics as detailed elsewhere.[10–12] A jugular vein was cannulated for the infusion of dipirydamole; femoral artery for arterial pressure measurement and reference blood sample withdrawal; whereas left ventricular cannula was used for the injection of radiolabeled microspheres. Catheters were filled with heparinized saline and exteriorized at the nape of the neck. Rats were then placed in nonrestrictive polyethylene cages and allowed to recover fully from anesthesia.

The baseline measurements of systemic and coronary hemodynamics were obtained in nonrestrained rats after their full recovery from anesthesia. A femoral artery catheter was connected to a multichannel recorder (Grass Instrument, Quincy, Mass) interfaced to an IBM computer, and arterial pressure was recorded using a digital data acquisition system (EMKA Technologies, Paris, France). Cardiac output (CO) was measured by the reference sample microsphere method as reported previously.[10–12] In brief, approximately 100 000 radiolabeled ($^{57}Co$) microspheres ($15 \pm 1$ μm in diameter; DuPont, Boston, Mass) suspended in 0.045 mL of saline containing Tween 80 (<0.01%) were injected into the left ventricle, followed by a 0.5-mL warm saline flush. The reference blood sample was withdrawn from the femoral artery by a pump (Harvard Apparatus, South Natick, Mass) at a rate of 0.45 mL/min over 60 seconds, starting 20 seconds before microsphere injection. The formula used to calculate CO was as follows: CO (mL/min) = sampling rate (mL/min) × injected radioactivity (counts/min)/reference sample radioactivity (counts/min). Cardiac index (CI) was calculated from CO and body weight and expressed in mL/min/kg. Total peripheral resistance index was calculated by dividing the mean arterial pressure (MAP) by the CI.

After completion of basal measurements, maximal coronary vasodilatation was produced by intravenous (IV) infusion of dipyridamole (4 mg/kg/min for 10 min) using a Harvard pump. The hemodynamic studies were then repeated using the second radionuclide ($^{113}Sn$) microspheres. The rats were killed with an overdose of pentobarbital, and the heart and aorta were removed; the atria were dissected from the ventricles, and the free wall of the right ventricle was separated from the left ventricle. Wet ventricular weights were recorded, normalized for body weight, and expressed as ventricular mass index. Tissue samples, as well as reference blood samples, were placed in plastic scintillation vials and counted for 15 min in a deep-well gamma scintillation spectrometer (Packard, Deep Grove, Ill) with a multichannel analyzer. Spillover correction between channels was achieved using matrix inversion software (Compusphere, Packard). Coronary blood flow was calculated by multiplying the fractional distribution of radioactivity to each ventricle by CO and normalized for the wet

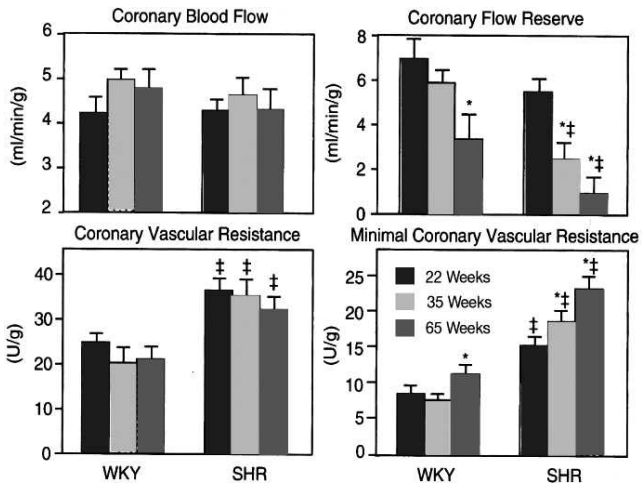


**Figure 1. Left ventricular coronary hemodynamics indexes for Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) aged 22, 35, and 65 weeks. Values are expressed as means ± SEM. *$P < .05$ compared with rats aged 22 weeks of the same strain; $^{+}P < .05$ compared with WKY rats of the same age. Each group comprised at least 10 animals.** (Reproduced with permisson from Susic D, Nunez E, Hosoya A, Frohlich ED. J Hypertens 1998;16:231–237.[7])

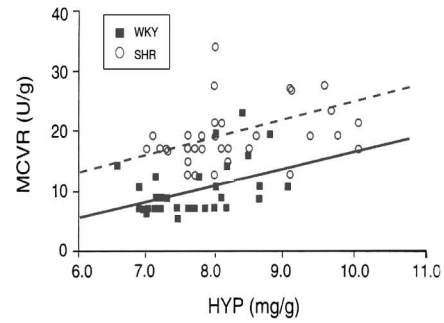


**Figure 2. The relationship between hydroxyproline concentration (HYP) and minimal coronary vascular resistance (MCVR) in the left ventricle of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)** (Reproduced with permisson from Susic D, Nunez E, Hosoya A, Frohlich ED. J Hypertens 1998;16:231–237.[7])

weight of the respective ventricle. Coronary flow reserve for each ventricle was calculated as the difference between flows during the baseline and dipyridamole-infusion periods. Coronary vascular resistance was determined by dividing mean arterial pressure by the flow to the ventricle, whereas minimal coronary vascular resistance was defined as vascular resistance achieved with dipyridamole.

## Myocardial Collagen Content

As an estimate of ventricular collagen content, hydroxyproline concentration was determined in the ventricular samples using a previously described procedure.[13]

## Statistical Analysis

Values are expressed as mean ± SEM. A two-way analysis of variance and Student-Newman-Keuls post hoc test were used to test the significance of differences between groups.[14] Correlation coefficients were computed using a least-squares linear regression analysis.[14] A probability value less than .05 was considered significant.

## RESULTS

In the first experiment, no difference was demonstrated between baseline coronary blood flow in SHR and normotensive WKY rats at any age (Figure 1). Furthermore, no age-related changes in baseline coronary blood flow were seen in rats of either group (Figure 1). Baseline coronary vascular resistance index was significantly ($P < .05$) higher in SHR than in WKY rats at all ages, but no age-related changes were noted for either group (Figure 1). Compared with WKY rats, blood flow reserve was significantly ($P < .05$) lower in SHR at all ages; and an age-related decrease in flow reserve was observed for both groups (Figure 1). Correspondingly, minimal coronary vascular resistance was greater in SHR at all ages, and a substantial increase in left ventricular minimal coronary vascular resistance was observed with aging for both SHR and WKY rats (Figure 1). Finally, a positive correlation and a linear regression between left ventricular hydroxyproline concentration and minimal coronary vascular resistance ($r = .508$, $P < .05$ for WKY rats and $r = 0.579$, $P < .05$ for SHR) were found for both strains (Figure 2).

Systolic arterial pressure (SAP) and MAP were significantly ($P < .05$) greater in 2-year-old control WKY rats with isolated systolic hypertension (middle) than in younger 35-week-old adults (left) or in the 2-year-old WKY rats treated with L-arginine and ACE inhibitor (right) (Figure 3). No difference in CI was found between the groups (Figure 3). Total peripheral resistance index was greater in the old control WKY rats (middle) than in the other two groups (Figure 3). Left ventricular coronary blood flow under basal conditions was similar in all groups, whereas coronary vascular resistance was increased in old control WKY rats (middle) (Figure 4). Compared with 35-week-old WKY rats (left), minimal vascular resistance was increased in both groups of 2-year-old rats; it improved with treatment but did not reach the level seen in younger animals (Figure 4). Likewise, coronary flow reserve was diminished in both groups of old rats; and treatment produced a partial beneficial

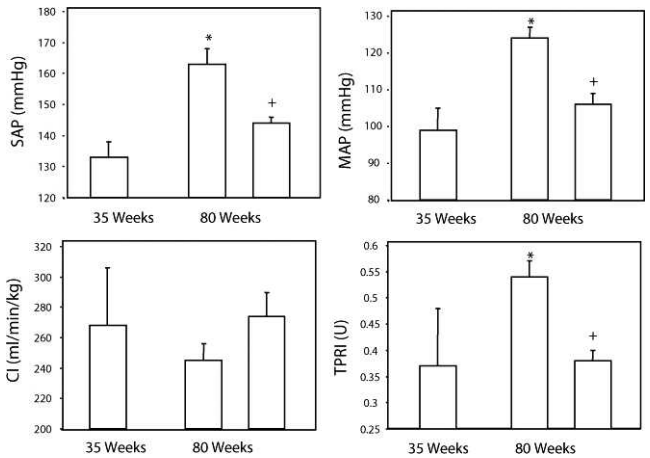


**Figure 3. Systolic (SAP) and mean (MAP) arterial pressure, cardiac index (CI), and total peripheral resistance index (TPRI) in 35- (left) and 80-week-old (controls: middle, treated: right) Wistar-Kyoto rats (WKY). *$P < .05$ compared with rats aged 35 weeks; $^{+}P < .05$ compared with control 2-year-old WKY rats. Each group comprised at least 7 animals.**

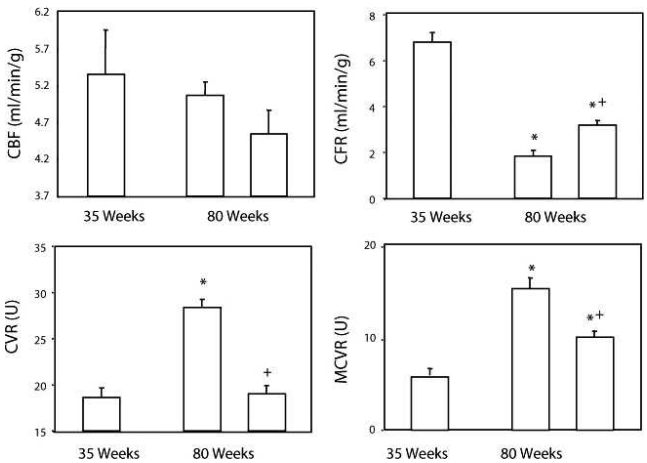


**Figure 4. Coronary blood flow (CBF) and coronary vascular resistance (CVR) at baseline, coronary flow reserve (CFR) and minimal coronary vascular resistance (MCVR) in 35- (left) and 80-week-old (controls: middle, treated: right) Wistar-Kyoto rats (WKY). *$P < .05$ compared with rats aged 35 weeks; $^{+}P < .05$ compared with control 2-year-old WKY rats. Each group comprised at least 7 animals.**

effect (Figure 4). Compared with younger adult WKY rats (3.73 ± 0.26 mg/g), left ventricular hydroxyproline concentration was significantly ($P < .05$) increased in both groups of 2-year-old WKY rats (7.08 ± 0.17 mg/g in controls and 6.43 ± 0.17 mg/g in treated rats); again, treatment partially decreased collagen content.

Prolonged salt excess in young SHR elevated MAP only modestly (163 ± 4 mm Hg in controls and 181 ± 5 mm Hg in salt-loaded rats), but it markedly ($P < .05$) increased left ventricular mass (2.85 ± 0.05 mg/g vs 4.70 ± 0.22 mg/g in controls and in salt-loaded rats, respectively) and left ventricular hydroxyproline concentration (4.32 ± 0.22 vs 5.37 ± 0.21 mg/g in control and salt-loaded rats, respectively). Collagen deposition in myocardium (both in extracellular matrix and perivascular spaces) was increased (Figure 5). Basal coronary blood flow and vascular resistance were unaffected by salt overload, but minimal vascular resistance significantly increased ($P < .05$; 18 ± 2 vs 12 ± 1 U/g), whereas coronary flow reserve decreased (3.88 ± 0.62 vs 6.38 ± 1.22 mL/min/g) in the salt-loaded SHR. Therefore, salt overload resulted in an accumulation of ventricular collagen as well as altered myocardial perfusion.

In salt-loaded SHR, therapy with an ARB (candesartan 10 mg/kg/day) failed to reduce the salt-induced rise in MAP (151 ± 4, 173 ± 4, and 169 ± 5 mm Hg in control SHR, salt-loaded SHR, and salt-loaded SHR given candesartan, respectively). However, it significantly ($P < .01$) attenuated left ventricular mass (944 ± 10, 1212 ± 40, and 1096 ± 56 mg in control SHR, salt-loaded SHR, and salt-loaded SHR given candesartan, respectively) and myocardial fibrosis as estimated by reduced hydroxyproline concentration (4.27 ± 0.20, 4.27 ± 0.20, and 4.6 ± 0.20 mg/g in control SHR, salt-loaded SHR, and salt-loaded SHR given candesartan, respectively). Perivascular collagen deposition in the myocardium of salt-loaded SHR given the ARB was markedly reduced (Figure 6). The ARB also prevented the salt-induced deterioration of coronary hemodynamics as

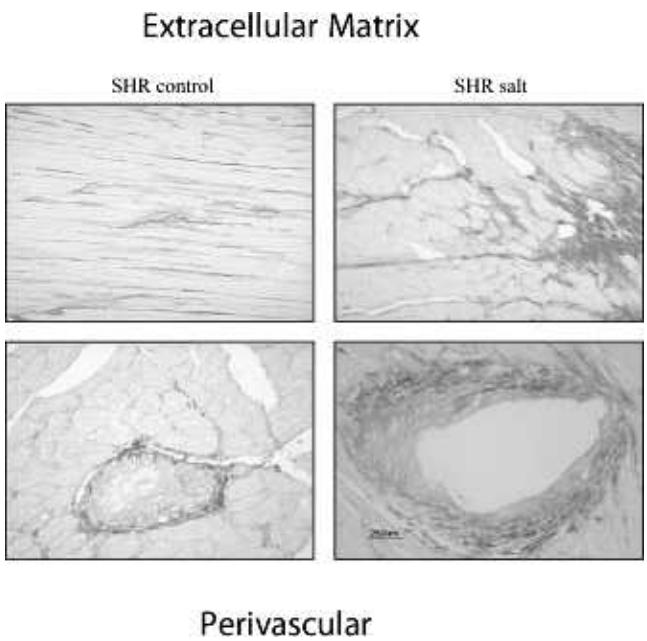


**Figure 5. Collagen deposition in extracellular matrix and in perivascular spaces in control and salt-loaded spontaneously hypertensive rats (SHR).**

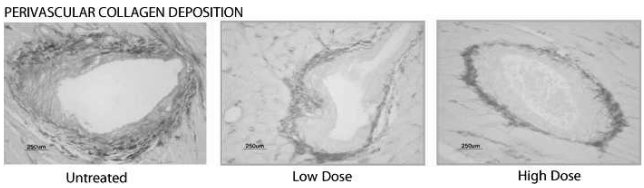


**Figure 6. Perivascular collagen deposition in untreated salt-loaded spontaneously hypertensive rats (SHR) and in salt-loaded SHR given a low (1 mg/kg) or high (10 mg/kg) dose of candesartan, an angiotensin II receptor blocker.**

indicated by significantly ($P < .05$) reduced minimal coronary vascular resistance (11.3 ± 0.6, 17.2 ± 0.5, and 14.4 ± 1.1 U/g in control SHR, salt-loaded SHR, and salt-loaded SHR given candesartan, respectively) and significantly increased coronary flow reserve (6.38 ± 0.51, 2.76 ± 0.49, 5.76 ± 0.56 mL/min/g in control SHR, salt-loaded SHR, and salt-loaded SHR given candesartan, respectively).

## DISCUSSION

The present study demonstrates that, compared with normotensive rats, coronary hemodynamics in SHR of all ages is significantly impaired, as evidenced by findings of a lower coronary flow reserve and a higher minimal coronary vascular resistance. Moreover, the results demonstrate that the aging process per se impairs coronary hemodynamics. Thus, a progressive deterioration in coronary flow reserve and minimal coronary vascular resistance were observed in normotensive aging rats, and these changes were exacerbated by hypertension. The findings that both hypertension and aging adversely affect coronary circulation are in agreement with previous data.[2,4,15–17]

Furthermore, the presented data demonstrate that normotensive WKY, which usually serve as controls for SHR, develop isolated systolic hypertension with advancing age. Compromised coronary hemodynamics, as demonstrated by increased minimal coronary vascular resistance and decreased coronary flow reserve, and myocardial fibrosis, as evidenced by increased left ventricular hydroxyproline concentration, were also found in these very old WKY. Combined therapy with L-arginine (nitric oxide precursor) and an ACE inhibitor ameliorated the adverse cardiovascular effects of systolic hypertension in old WKY. However, compared with 35-week-old WKY rats, old rats, regardless of therapy, still exhibited signs of deteriorating cardiovascular structure and function. This finding is in agreement with the previously described studies in rats of different ages. Furthermore, all these results are in accord with the notion that adverse cardiovascular effects of hypertension and aging are similar in appearance, but they seem to be brought about by different mechanisms. Several reports from other laboratories support this concept.[18–20]

Excessive salt intake affects cardiovascular structure and function adversely.[21–23] Our results demonstrating aggravated myocardial fibrosis and impaired coronary hemodynamics in salt-loaded SHR are in agreement with these studies. Moreover, our data extended earlier observations by demonstrating that in salt-loaded SHR coronary vasodilatory response to pharmacologic stimuli is impaired, thereby resulting in reduced coronary flow reserve. Furthermore, the finding that treatment with an ARB did not reduce arterial pressure, but it did reduce myocardial fibrosis and improve coronary hemodynamics, clearly suggests that the adverse cardiovascular effects of salt overload are not mediated solely by hemodynamic factors (ie, increased pressure). Thus, the involvement of local tissue renin-angiotensin-aldosterone system (RAAS) is suggested.[24]

Finally, our results suggest that myocardial fibrosis, particularly perivascular fibrosis, may contribute significantly to the development of coronary deficiency associated with hypertension, aging, and salt excess. Several factors are pertinent. First, a good correlation between the extent of myocardial fibrosis, as estimated by left ventricular hydroxyproline concentration and minimal coronary vascular resistance, was found in both SHR and WKY rats of various ages. Furthermore, development of isolated systolic hypertension in very old WKY is accompanied by myocardial fibrosis and impairments in coronary hemodynamics; therapy aimed at improving endothelial function and inhibiting the RAS somewhat reduced fibrosis and improved hemodynamics but did not restore either to the level seen in younger rats. Excessive salt intake aggravated myocardial fibrosis, showing abundant perivascular collagen deposition in SHR, and simultaneously induced impairment in coronary circulation. Finally, therapy with an ARB did not affect blood pressure but improved coronary hemodynamics in parallel with a reduction in perivascular fibrosis in the left ventricle of salt-overload SHR. The concept that perivascular fibrosis may impair coronary vasodilatation is further supported by the results obtained in a study using a different hypertensive model.[25]

## CONCLUSION

The data presented here indicate that considerable coronary insufficiency associated with hypertension, aging, and salt overload can exist in the absence of atherosclerotic coronary changes. Furthermore, perivascular fibrosis within the myocardium can significantly contribute to the development of coronary vascular impairment. These findings may help to

achieve a better understanding of the pathophysiology of coronary insufficiency and also might help in formulating novel therapeutic strategies.[26]

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