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Hypercholesterolemia blunts the oxidative stress elicited by hypertension in venules through angiotensin II type-2 receptors

Alper Yildirim, Elena Senchenkova, and D. Neil Granger

Department of Molecular & Cellular Physiology, LSU Health Sciences Center, Shreveport, Louisiana 71130

Abstract

OBJECTIVE—Hypertension and hypercholesterolemia elicit inflammatory and thrombogenic responses in the microvasculature. However, little is known about whether and how risk factor combinations alter microvascular function. We examined how the actions of HTN+HCh on the microvasculature differ from the responses elicited by either risk factor alone.

METHODS—Intravital microscopy was used to monitor the adhesion and emigration of leukocytes and dihydrorhodamine oxidation in cremaster muscle venules of wild type mice that were infused with angiotensin II for 2 wks (HTN), placed on a high cholesterol diet (HCD), or both.

RESULTS—Either HTN or HCh alone enhanced the production of reactive oxygen species and promoted the recruitment of leukocytes in venules. However, the combination of HTN and HCh produced changes in ROS production and leukocyte recruitment that were greatly attenuated compared to HTN alone. The inhibitory effects of HCh on the AngII mediated responses were also observed in genetically-induced HCh (ApoE-deficient mice). Treating HCh + HTN mice with an antagonist to AT2r reversed the HCh-dependent protection against oxidative stress and inflammation during HTN.

CONCLUSIONS—These findings indicate that HCh blunts the oxidative stress and inflammatory cell recruitment elicited by hypertension in venules through a mechanism that involves AT2 receptor activation.

Keywords

Angiotensin II; hypercholesterolemia; neutrophils; oxidative stress; blood vessels; microcirculation

Address correspondence to: D. Neil Granger, PhD, Department of Molecular & Cellular Physiology, LSU Health Science Center – Shreveport, 1501 Kings Hwy, Shreveport, LA 71130-3932, Phone: (318) 675-6011, Fax: (318) 675-6005, dgrang@lsuhsc.edu.

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Introduction

Hypertension and hypercholesterolemia are two major factors that increase the risk of cardiovascular disease. Long-term exposure to either of these risk factors results in significant changes in the structure and function of large and microscopic blood vessels. Oxidative stress, increased leukocyte adhesion, accelerated thrombus formation, reduced endothelial barrier function, and impaired vasomotor responses are common manifestations of risk factor-induced vascular dysfunction(5, 12, 14).

Angiotensin II, a product of the renin-angiotensin system, has received much attention as a circulating soluble factor that mediates the elevated blood pressure as well as other phenotypic changes in blood vessels that accompany HTN and other risk factors (e.g., hypercholesterolemia)(2, 8,21). Oxidative stress, which results from an imbalance between the generation of reactive oxygen species and antioxidant defense mechanisms, is known to result when AngII levels are elevated, and this response has been implicated as a central underlying cause of the vascular abnormalities that accompany most CVD risk factors. Risk factor-induced oxidative stress usually results from an increased production of ROS by NADPH oxidase in vascular endothelial cells and in circulating neutrophils, and there is evidence linking these two cellular sources of ROS to risk factor-induced microvascular dysfunction(4, 11). Similarly, both the enhanced ROS production and altered microvascular function have been linked to activation of angiotensin II type-1 receptors(21,32). For example, we have previously reported that wild type mice treated with an AT1r antagonist as well as NADPH oxidase deficient mice are protected against the microvascular inflammation and enhanced ROS production induced by HTN(33). The pro-oxidative and pro-inflammatory effects of AT1 receptor stimulation may be attenuated if AT2 receptor activation also occurs, as reported in animal models of hypertension and obesity(7,25). Since hypercholesterolemia has been shown to increase the expression of AT2 receptors in the vascular wall(31), it is possible that these receptors have a protective role in blunting the oxidative stress, inflammation and microvascular dysfunction that accompanies this risk factor.

While much has been learned about the responses of the vasculature to individual risk factors, less is known about whether and how combinations of CVD risk factors (e.g., HTN + HCh) result in microvascular dysfunction. While it might be expected that risk factor combinations would exert an additive or synergistic effect on the vessel dysfunction elicited by the presence of individual risk factors, recent studies from our laboratory have revealed that mild diet-induced hypercholesterolemia blunts the recruitment of adherent leukocytes and platelets in venules, and protects against the impaired endothelium-dependent dilation in arteries elicited by hypertension(18, 22). It remains unclear whether this protective effect of HCh on HTN-induced vascular dysfunction is also manifested in genetically hypercholesterolemic (e.g, ApoE^{-/-}) mice placed on a normal diet. Similarly, the role of oxidative stress in the protective action of HCh towards HTN has not been previously addressed. It is also not clear whether the blunted recruitment of inflammatory cells and platelets in HTN mice with HCh is associated with a corresponding reduction in ROS production and whether prevention of such a reduction in ROS generation would reverse the actions of HCh on HTN-induced blood cell recruitment.

The overall objectives of this study were to better understand the influence of HCh on AngII-induced microvascular dysfunction and to address the contribution of ROS production towards the protective effect afforded by HCh. Our findings indicate that both diet- and genetically-induced HCh blunts both the ROS production and inflammation elicited by HTN. This protective effect of HCh appears to depend on AT2 receptor activation.

Materials and Methods

All mice used in the experimental studies were on a C57BL/6 background. Male wild type C57BL/6J mice (n = 62), and apolipoprotein E knockout (n = 12), mice were purchased from Jackson Laboratory (Bar Harbor, ME). All of the experimental procedures involving the use of animals were approved by the LSU Health Sciences Center Institutional Animal Care and Use Committee and were in accordance with the guidelines of the American Physiological Society.

Osmotic pump implantation

Saline or AngII (1 µg/kg/min) was infused over 14 days using micro-osmotic pumps (Alzet, Cupertino, CA, model 1002), which were implanted subcutaneously in the intrascapular region of isofluorane-anesthetized mice WT (n=11), as previously described(20). Blood pressure was measured by tail-cuff plethysmography (model SC-1000, Hatteras Instruments, Inc., North Carolina, USA) in non-anesthetized animals.

Hypercholesterolemia

Mice (6 to 8 weeks old) were placed either on a normal (n=10) or high cholesterol diet without cholate (Teklad 94059, containing 1.25% cholesterol, 15.8% fat, Harlan Teklad) for 3 weeks (n=12). The AngII groups were placed on the diet 1 week before pump implantation. The ApoE^{-/-} mice (8 wk old) were placed on a normal diet (n=7).

Surgical Protocol

Mice were anesthetized with ketamine hydrochloride (150 mg/kg body wt. IP) and xylazine (7.5 mg/kg body wt. IP). The right carotid artery was cannulated for systemic arterial pressure measurement. The cremaster muscle was prepared for intravital fluorescence microscopic observation and leukocyte adhesion and emigration in cremaster muscle venules was determined, as previously described (1,29).

Intravital microscopic methods—Ketamine/xylazine anesthetized mice were placed in a semi-upright position on a Plexiglas microscope stage(30). Venules with diameters between 20–40 µm and wall shear rates $>500 \text{ sec}^{-1}$ were selected for study. An optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University, College Station, TX) was used to measure red blood cell velocity in the microvessels and wall shear rate was calculated from the product of mean red blood cell velocity [$V_{\text{mean}} = V_{\text{RBC}}/1.6$] and microvascular cross-sectional area, assuming cylindrical geometry. Wall shear rate was calculated assuming $\text{WSR} = 8(V_{\text{mean}}/D_v)$. Adherent leukocytes in postcapillary venules were determined, over a 2 min period, by light microscopy. The microscopic images were

recorded on DVD and blood cell adhesion was analyzed offline. Adhesion values were recorded in 100 μm length vessel segments at 300 μm intervals along the length of the vessel, beginning as near to the source of the venule as possible. The mean value of each variable within a single venule was calculated, and comparisons were made between the experimental groups. A leukocyte was considered adherent if it remained stationary for 30 s (number per millimeter square). Leukocyte emigration was measured online at the end of each observation period and expressed as the number of interstitial leukocytes per high-powered field of view adjacent to the observed vascular segment (number per field).

Dihydrorhodamine oxidation

The production of reactive oxygen species in cremaster venules was determined by monitoring the oxidation of DHR, as previously described(29). DHR oxidation was monitored in cremaster preparations separate from those used to monitor blood cell adhesion. Background fluorescence (I_{Bgrd}) of the first 100 μm of every 300 μm vessel length was recorded along the length of selected postcapillary venules with a xenon light source and a SIT camera system (Hamamatsu). Then, freshly prepared dihydrorhodamine-123 (1 mmol/L, a nonfluorescent dye that is oxidized by ROS to the fluorescent compound rhodamine-123) in bicarbonate-buffered saline was superfused over the cremaster for 15 minutes. The tissue was then washed with BBS, and the fluorescent image of each section recorded (I_{DHR}). Images were captured onto a computer, and an area 100 μm long and 7 μm wide along the vessel wall was analyzed in each vascular segment using NIH Image 1.62 software. The ratio of $I_{\text{DHR}}:I_{\text{Bgrd}}$ was calculated for each vascular segment and the average ratio for each animal was determined.

Blood cell analyses

Whole mixed-blood samples (20–25 μL) were obtained by cardiac puncture in each mouse for determination of leukocyte (stained with 3% acetic acid and 10% crystal violet) and platelet (stained with 1% buffered ammonium oxalate) counts using a hemocytometer.

Experimental protocols

The adhesion of leukocytes, leukocyte emigration, and DHR oxidation (oxidative stress) in cremaster muscle venules, and blood pressure were monitored in every experiment on days 14 (AngII alone) or day 21 (HCh alone or HCh + HTN) using the intravital video microscopic procedures described above. The AT2r antagonist PD123319 (10mg/kg) (Sigma Chemicals, St Louis, MO) administered both i.p. at 2 hrs prior and i.v. at 30 min. prior to the experiment (n=9).

Some WT mice with AngII pumps were rendered thrombocytopenic via an intraperitoneal injection of anti-platelet serum (125 uL/kg in 200 uL PBS IP; rabbit anti-mouse thrombocyte antiserum, Accurate Chemicals, Westbury, NY) 24 h before intravital microscopy (n=5). Treatment with antiplatelet serum (APS) reduced the number of circulating platelets by ~91%, without significantly reducing the number of circulating leukocytes, as confirmed by measurements of blood cells with a hemocytometer. The effects of neutrophil depletion on AngII induced microvascular responses were examined in mice using anti-granulocyte receptor-1 (Gr-1) RB6-8C5 (eBioscience, 100 μL per mouse) (n=5). Gr-1 was delivered by

intraperitoneal injection 24h before intravital microscopy. Treatment with RB6-8C5 reduced the number of circulating neutrophils by ~90%, while showing no effect on the number of circulating platelets. Upon completion of each experiment, the mice were sacrificed with an intravenous overdose of sodium pentobarbital (200 mg/kg).

Serum cholesterol levels

At the end of each experiment, blood was drawn from the tail vein, centrifuged, and the plasma was frozen for the subsequent measurement of cholesterol levels using a spectrophotometric assay kit (Stanbio Laboratory, Boerne, TX, USA).

Statistics

Data were analyzed using standard statistical analyses, i.e., one-way ANOVA and Bonferroni post-hoc test. The control and HTN data shown in Figures 1–4 represent the same groups. However, the statistical outcomes of the specific interventions described in the Results section were based on a simultaneous comparison of all control and experimental groups. A multivariate analysis revealed no influence of group body weight/age on the statistical differences noted. All values are reported as means $\pm$ SE, and statistical significance was set at $P < 0.05$.

Results

Table 1 provides a summary of all the data collected in the study, including the results of the ANOVA for each measured variable and the number of mice in each experimental group and for each measured variable. The results show that AngII pump implantation (HTN) yielded significant elevations (~40 mmHg) in blood pressure (compared to control WT mice) and that placement on a cholesterol-enriched diet did not significantly alter the blood pressure response to AngII. Venular shear rate was reduced by chronic AngII infusion, and this response was not significantly altered by any treatment or intervention. Total plasma cholesterol concentration was elevated in all mice placed on the cholesterol-enriched diet.

The presence of HTN did not alter plasma cholesterol concentration (Figure 1). Mice implanted with an AngII-loaded pump exhibited a significant increase in the number of leukocytes adhering in postcapillary venules, compared to the control group. Increased leukocyte adhesion was also noted in mice placed on a high-cholesterol diet. However, the combination of AngII-induced HTN and placement on HCD resulted in a blunted blood cell recruitment response, compared to HTN alone (Figure 1A). The number of emigrated leukocytes was also significantly elevated in the AngII infused mice (vs controls) but this was not observed in HCD and HTN+HCD group (Figure 1B). The DHR oxidation measurements revealed a large and significant increase in ROS production in venules of mice with AngII-induced HTN. HCD alone did not elicit a significant increase in DHR oxidation compared to controls. HCD and the combination of HTN and HCD produced a highly blunted DHR oxidation response compared to HTN alone.

Another objective of this study was to determine whether mice with genetically-induced hypercholesterolemia, i.e., ApoE^{-/-} mice placed on a normal diet, exhibit the same responses to AngII-induced HTN as noted in diet-induced hypercholesterolemia. Figure 2

illustrates that although the ApoE^{-/-} mice develop a greater elevation in plasma cholesterol concentration than noted in WT mice placed on HCD (Figure 1), a very similar attenuation of the leukocyte recruitment and ROS production responses to HTN is observed in ApoE^{-/-} mice, compared to control (normotensive) ApoE^{-/-} mice. The number of adherent leukocytes as well as ROS production induced by AngII was significantly reduced in ApoE^{-/-} mice. The reduction of emigrated leukocytes (Figure 2B) wasn't statistically significant compare to HTN.

To address the potential role of AT2 receptors in mediating the anti-inflammatory and anti-oxidative effect of hypercholesterolemia in the presence of hypertension some HCh + HTN mice were treated with the AT2 receptor antagonist PD123319 (Figure 3). The results of these experiments revealed a significant reversal of the protection afforded by HCH on HTN-induced leukocyte adhesion (Figure 3A), and DHR oxidation (Figure 3C) in mice treated with the AT2r antagonist but the protection wasn't statistically significant for emigrated leukocytes (Figure 3B). AT2r blockade did not alter the increase in plasma cholesterol concentration caused by placement of a cholesterol-enriched diet (data not shown).

Figure 4 summarizes the results of experiments designed to assess the contribution of neutrophils and platelets to the leukocyte recruitment and oxidative stress induced by the combination of AngII-induced HTN and HCh. In mice rendered neutropenic (neutrophil-depleted group), leukocyte adhesion was significantly reduced (Figure 4A) and emigration (Figure 4B) was reduced (to control levels) compared to HTN group. In platelet-depleted animals, leukocyte adhesion (Figure 4A) and emigration (Figure 4B) remained elevated, compared to control mice. Thrombocytopenia did not significantly alter leukocyte adhesion compared to the untreated HTN + HCD group. Mice rendered neutropenic exhibited a significant reduction in ROS production compared to HTN alone but not compared to the untreated HTN + HCD group (Figure 4C), while thrombocytopenia did not alter the ROS response to HTN + HCh. Neither neutrophil nor platelet depletion changed the plasma cholesterol concentration resulting from placement of the mice on a cholesterol rich diet (Figure 4D).

Discussion

Hypertension and hypercholesterolemia are the most common risk factors for cardiovascular diseases and are associated with an increased risk of coronary and cerebrovascular diseases (3,28). While much has been learned about the influence of each risk factor on vascular function, less attention has been devoted to addressing the combined actions of HTN and HCh on the vasculature. Such an effort is justified based on epidemiological studies that reveal a high prevalence of co-existing HTN and HCh in the human population, with approximately 30–40% of men and women showing both risk factors(10). Clinical evidence also indicates that the concurrence of HTN and HCh increases the incidence of atherosclerosis and cardiac events, relative to the changes noted with either risk factor alone(27). Animal studies have generally confirmed these clinical observations by demonstrating accelerated atherosclerotic lesion development and more pronounced large artery dysfunction in response to HTN + HCh, compared to either risk factor alone (9,23).

While we have recently reported that mild diet-induced hypercholesterolemia appears to protect against the inflammation and enhanced platelet recruitment in the cerebral microvasculature elicited by hypertension induced by either DOCA-salt or AngII infusion (21), it appears that the combination of hypertension and large (4-fold) increases in plasma cholesterol concentration results in a severe, but reversible, inflammatory and thrombogenic phenotype in the cerebral microvasculature (23). The results of the present study extend these observations to the skeletal muscle microvasculature, demonstrating a protective effect against AngII-induced leukocyte recruitment by HCh, and further reveal that the beneficial effects of HCh are also evident in both wild type mice placed on a cholesterol enriched diet and in ApoE^{-/-} mice placed on a normal diet. In addition, our findings implicate AT2 receptor activation as a potentially important component of the mechanism that underlies the protection afforded by HCh.

The accelerated production of ROS that accompanies both hypercholesterolemia and hypertension has been linked to angiotensin II, the angiotensin II type-1 receptor, and NADPH oxidase(6, 13, 19). In both situations, there is evidence implicating AngII mediated activation of AT1 receptors in the increased ROS production. Consequently, it might be expected that the combination of HTN and HCh would yield more ROS production (perhaps additive) than the responses elicited by either risk factor alone (24). Our findings do not support that expectation and demonstrate an inhibitory influence of hypercholesterolemia on HTN-induced ROS production, which was evident with both diet- and genetically-induced HCh.

There are several possible explanations for the observed protective effect of HCh against HTN-induced oxidative stress. One possibility is that a sustained elevation in plasma cholesterol alters the expression and/or function of angiotensin II receptors in the vasculature and/or on circulating blood cells. Hypercholesterolemia has been shown to elicit an increased expression of both AT1 and AT2 receptors(26, 31). Since activation of AT1 and AT2 receptors promotes increases and decreases in vascular ROS production respectively(20,31), the protective effect that we noted on AngII-induced ROS production by HCh may reflect the influence of this risk factor on the expression of AT2 receptors. Our findings in HCh + HTN mice treated with an AT2r antagonist are consistent with HCh mediated stimulation of AT2r and a consequent attenuation of both ROS production and leukocyte recruitment in the microvasculature. The ability of AT2r activation to down-regulate NADPH oxidase, blunt oxidative stress, and protect tissues against the end-organ damage caused by HTN is well documented(7, 17,25).

Our effort to detect AT2r expression in aortic tissue under the conditions assessed in this study yielded equivocal results and we were unable to confidently demonstrate either basal or increased AT2r expression. This may reflect low protein levels that are not detected by immunostaining or limited specificity of the AT2r antibody. The latter possibility is supported by a recent report that demonstrated several commercially available AT2R antibodies lack sufficient specificity to warrant meaningful conclusions regarding the localization and expression of the receptor (15). In this regard, it is noteworthy that others have been similarly unable to demonstrate AT2r expression using commercially available antibodies while showing a major effect of AT2r blockade in the same target tissue (16).

Our observation that HCh protects the microvasculature from both the increased ROS production and recruitment of adherent leukocytes induced by AngII-induced HTN raises a question about whether and how the two responses are linked. Neutrophil depleted AngII infused mice do not exhibit increases in leukocyte adherence/emigration and ROS production in cremaster muscle venules(33). Since AngII induced ROS production and leukocyte adherence/emigration are also inhibited in gp91phox^{-/-} bone marrow chimeras with circulating cells deficient in NADPH oxidase(33), it appears likely that neutrophils attached to the venular wall are the dominant source of superoxide produced in response to AngII infusion. In the presence of both risk factors, neutrophil depletion significantly reduced the already blunted leukocyte adherence response observed in HTN + HCh mice, while no further suppression of ROS production was observed in neutropenic mice with HTN +HCh. The observation that neutrophil depletion did not add to the protection against ROS production that was afforded by HCh (without neutropenia) is consistent with two possibilities: 1) HCh inhibits ROS production by AngII stimulated neutrophils, consequently the elimination of circulating neutrophils offers no additional benefit, and 2) the principal target of HCh mediated inhibition of AngII-induced ROS production are endothelial cells or blood cells other than neutrophils.

In conclusion, the results of this study indicate that both diet- and genetically-induced hypercholesterolemia protects postcapillary venules against the leukocyte recruitment and accelerated ROS production observed during AngII-induced hypertension. Furthermore, our findings implicate AT₂ receptor activation as a key-initiating event in the HCh mediated protection of the microvasculature against oxidative stress and blood cell recruitment. These results justify an increased research focus on the responses of the vasculature to cardiovascular risk factor combinations and suggest that such work may reveal novel therapeutic strategies to lessen the morbidity and mortality associated with cardiovascular disease.

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List of Abbreviations

HTN	Hypertension
HCh	Hypercholesterolemia
HCD	High cholesterol diet
WT	Wild type
AngII	Angiotensin II
ROS	Reactive oxygen species
AT₂r	Angiotensin II type- 2 receptor
CVD	Cardiovascular disease

RAS	Renin-angiotensin system
AT1r	Angiotensin II type-1 receptors
ApoE^{-/-}	Apolipoprotein E knockout
DHR	Dihydrorhodamine
BBS	Bicarbonate-buffered saline
PD123319	Angiotensin II type- 2 receptor antagonist
APS	Antiplatelet serum
Gr-1	Anti-granulocyte receptor-1 (RB6-8C5)
ND	Normal diet
WSR	Wall shear rate
V_{RBC}	Red blood cell velocity
I_{Bgrd}	Background fluorescence
I_{DHR}	Fluorescent image of each section recorded
i.p	Intra peritoneal
i.v	Intra venular

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Highlights

- Hypercholesterolemia protects against hypertension-induced leukocyte recruitment.
- Hypercholesterolemia blunts oxidant stress during AngII-induced hypertension.
- Angiotensin-II type 2 receptors mediate these responses to hypercholesterolemia.

Figure 1.

Changes in leukocyte adhesion (panel A) and emigration (panel B), DHR oxidation (panel C) and plasma cholesterol concentration (panel D) in wild type mice elicited by angiotensin II-infusion (HTN), a high cholesterol diet (HCD) or the combination of HTN and HCD (HTN + HTN), compared to control mice. See table 1 for n values in each group. * indicates $p < 0.05$ relative to Control, # indicates $p < 0.05$ relative to HTN alone.

Figure 2.

Comparison of angiotensin II mediated changes in leukocyte adhesion (panel A), emigration (panel B), DHR oxidation (panel C) and plasma cholesterol concentration (panel D) in wild type (HTN) (n=11), and ApoE^{-/-} mice (ApoE^{-/-} + HTN) mice. Data also shown for control (Control) wild type (no AngII infusion) and control ApoE^{-/-} (ApoE^{-/-} control) mice. See table 1 for n values in each group. * indicates p<0.05 relative to WT control, # indicates p<0.05 relative to HTN.

Figure 3.

Effects of treatment with an AT2r antagonist on leukocyte adhesion (panel A), emigration (panel B), and DHR oxidation (panel C) in wild type mice exposed to a combination of angiotensin II-induced HTN and a cholesterol-enriched diet (HCD). Data are also shown for responses in HTN, HCD and HCD + HTN mice without antagonist treatment. See table 1 for n values in each group. * indicates $p < 0.05$ relative to WT, † indicates $p < 0.05$ relative to WT + PD, # indicates $p < 0.05$ relative to HTN, ψ indicates $p < 0.05$ relative to HCD, ‡ indicates $p < 0.05$ relative to HTN + HCD.

Figure 4.

Effects of neutrophil (Neutrophil-D) or platelet (Platelet-D) depletion on the changes in leukocyte adhesion (panel A), leukocyte emigration (panel B), DHR oxidation (panel C) and plasma cholesterol concentration (panel D) induced by the combination of AngII induced hypertension (HTN) and hypercholesterolemia (HCh) in wild type mice. See table 1 for n values in each group. * indicates $p < 0.05$ relative to WT controls, # indicates $p < 0.05$ relative to HTN, ‡ indicates $p < 0.05$ relative to WT+HTN+HCD, φ indicates $p < 0.05$ relative to WT +HTN+HCD+ Neutrophil-D.

Mean data for all measured variables in all experimental groups. The results from the analysis of variance (ANOVA) is provided along with the number of animals included in each group and for each measured variable.

Table 1

Values	Experiment groups										
	Control	HTN	HCD	HCD+H TN	ApoE ^{-/-} Control	ApoE ^{-/-} + HTN	WT + PD	HCD + HTN + PD	WT + HTN + HCD + Neutrop hil D.	WT + HTN + HCD + Platelet D.	P value
Leukocyte Adhesion (#/mm ²)	123.9±20.82 n=10	865.6±21.55 n=11	603.9±54.04 n=12	565.2±33.01 n=5	361.7±123.3 n=5	487.8±78.6 n=7	137.9±31.94 n=5	893.9±60.15 n=9	156.7±52.69 n=5	661.7±74.06 n=5	p<0.0001
Leukocyte Emigration (#/per field)	0.39±0.10 n=10	1.27±0.13 n=11	0.96±0.15 n=12	0.73±0.19 n=5	0.66±0.18 n=5	0.75±0.14 n=7	1±0.27 n=5	0.99±0.11 n=9	0.72±0.16 n=5	1.13±0.16 n=5	p<0.048
DHR (I _{DHR} :I _{Bgrd})	0.90±0.43 n=9	33.01±5.02 n=11	13.15±5.04 n=7	10±3.15 n=5	3.1±0.88 n=5	7.46±4.15 n=7	3.3±1.73 n=5	27.4±4.92 n=9	6.72±3.53 n=5	16.78±6.01 n=4	p<0.0001
Cholesterol (mg/dL)	91±5.7 n=5	88.6±4.4 n=5	129.3±4.11 n=5	146.8±2.53 n=5	346.1±60.85 n=5	330.1±68.08 n=3			178.9±9.06 n=5	162.9±7.34 n=5	p<0.0001
Systolic Blood Pressure (mmHg)	98±2.2 n=5	138.7±2.9 n=4	109.1±5.3 n=5	155.1±8.9 n=4		128.1±11.8 n=3		142±3.54 n=3	158.3±2.7 n=5	139.8±8 n=2	p<0.0001
Wall Shear Rate	932.5 ±162.5 n=10	587.1± 20.19 n=11	766.8± 29.86 n=12	578.9± 23.69 n=5	732.4 ±31.5 n=5	616.2±18.27 n=7	922.9±188.3 n=5	634.2±25.41 n=9	619.6±26.89 n=5	665.5± 42.57 n=5	p<0.0001