Vascular Hyporeactivity to Angiotensin II and Noradrenaline in a Rabbit Model of Obesity

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Abstract: This study was conducted to explore the vascular reactivity of angiotensin II and noradrenaline and their relationship with endothelial function in rabbits fed a high-fat diet (HFD). The animals were fed either an HFD or regular chow [control diet (CD)]. After 12 weeks, the rabbits fed the HFD showed higher blood pressure, body weight, and insulin levels. Glucose tolerance was impaired and positively related to blood pressure. An endothelium-independent decrease of the sensitivity to angiotensin II [pD_2 endothelium-intact aortic rings (E+) in CD: 8.02 ± 0.07 vs. HFD: 7.60 ± 0.01; pD_2 endothelium-removed aortic rings (E−) in CD: 8.16 ± 0.11 vs. HFD: 7.83 ± 0.16] and noradrenaline (pD_2 E+ in CD: 6.36 ± 0.06 vs. HFD: 5.29 ± 0.06; pD_2 E− in CD: 6.11 ± 0.08 vs. HFD: 5.80 ± 0.08) was found. Noradrenaline desensitized the angiotensin II response (pD_2 with noradrenaline pretreatment in E+: 7.03 ± 0.16; in E−: 7.10 ± 0.02), but angiotensin II did not change the noradrenaline response. Acetylcholine maximal relaxation and basal nitric oxide (NO) release were comparable in both diet groups. The efficacy of angiotensin II (R_{max} CD: 4604 ± 574 mg vs. HFD: 3251 ± 533 mg) and noradrenaline (R_{max} CD: 11,675 ± 804 mg vs. HFD: 7975 ± 960 mg) was reduced in E+. L-N^G-nitroarginine methyl ester (L-NAME) recovered the efficacy of noradrenaline (R_{max} L-NAME: 12,015 ± 317 mg). In contrast, L-NAME had no effect on the angiotensin II response. Noradrenaline enhanced NO levels, but angiotensin II did not. Therefore, NO was associated with hyporeactivity to noradrenaline. The resting potential was more negative in E+, and the endothelium diminished the angiotensin II–induced depolarization. These findings demonstrated that the crosstalk and the endothelium may induce hyporeactivity to angiotensin II and noradrenaline as a mechanism to compensate the increase in the blood pressure in HFD-induced obesity.

Key Words: vascular reactivity, obesity, endothelium, angiotensin II, noradrenaline, rabbit aortae

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INTRODUCTION

High-fat diets (HFDs) have been mainly responsible for the dramatic increase in obesity that has occurred worldwide over the last 2 decades. Obesity is related to an increased cardiovascular risk, alteration in cardiovascular, and metabolic function and to an increased risk for developing diabetes, hypertension, and atherosclerosis. The Framingham Heart Study established that obesity can be directly responsible for about 78% of essential hypertension in men and about 65% in women. However, the mechanisms linking human obesity and essential hypertension remain unclear. Numerous factors such as insulin resistance/hyperinsulinemia, which increase sympathetic activity and increase renin–angiotensin system activity, have been suggested.[1]

Modan et al[2] demonstrated that insulin resistance and/or hyperinsulinemia constitutes a common pathophysiologic feature of obesity and glucose intolerance. Several studies have reported relations between arterial blood pressure (systolic, diastolic, or mean) and plasma insulin levels (fasting or post-glucose). Hyperinsulinemia is able to increase or to maintain high blood pressure by improving renal sodium reabsorption and by increasing the sympathetic activity.[3–5] Thus, insulin may be the link between hypertension and obesity.[6] The normal endothelium preserves the equilibrium between vasodilatation and vasoconstriction that determines arterial diameter, thereby influencing blood pressure control.[7] Endothelial dysfunction has a systemic nature, occurring both in peripheral and conduit arteries.[8] Dietary factors can be important, for example, the Western diet type, with its high salt and high-fat content, contributes to the progress of endothelial dysfunction, whereas low sodium or low fat intake has been shown to benefit the endothelial function as seen in animal and human studies.

Endothelial dysfunction is not completely equivalent to the inability of the endothelium to generate adequate amounts of bioactive nitric oxide (NO) and is linked to an imbalance between other endothelium-derived factors, such as prostacyclin (PGI2) and endothelium-dependent hyperpolarizing factor (EDHF), and endothelium-derived contracting agents.[9] The endothelial function is deficient in obese humans and animals; however, the mechanisms underlying this vascular dysfunction remain to be elucidated.[10]

Unlike studies on hypertension, the concern of investigators of obesity has only been recently directed to the sympathetic function. However, a lot of data on this topic has already been obtained. Obesity, even with normal blood pressure, is associated with symptoms of sympathetic activation, such as an increased resting heart rate and elevated

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plasma norepinephrine values.[11] Increased adrenergic activity is associated with human obesity and experimental obesity induced by overfeeding.[12]

Changes in the vascular expression and/or activity of angiotensin II have been found in obese animals[13] and precede pathological alterations related to obesity, which is often due to excessive dietary fat intake in humans.

Animal models represent an important component in uncovering the mechanisms involved in the pathological process of obesity. Over the last decade, diet-induced obese rabbit models have been used to study obesity. These obese models exhibit similar hemodynamic and neurohumoral alterations as seen in obese humans.[14,15] Additionally, Zhang et al[16] demonstrated that lipid metabolism in diet-induced obese rabbits is comparable with that of obese humans.

Considering the data mentioned above, our purpose was to explore the vascular reactivity of angiotensin II and noradrenaline and their relationship with endothelial function in a model of obesity in rabbits, which are commonly used to model human obesity.

METHODS

Animals

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Tucuman. All animal care and use programs were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication 86 to 23, revised 1985). Male hybrid Flanders rabbits from a slaughterhouse initially weighing 850–1000 g were used in this study. They were housed individually in a humidity- and temperature-controlled room with a 12-hour light cycle and fed 100 g/d of standard rabbit chow. After a 1-week acclimation period, they were randomly divided into 2 groups: one designated to remain lean (n = 12) and the other to become obese (n = 12). The lean group continued with the same dietary regimen, which is an appropriate maintenance diet for a normal adult rabbit. The obese groups were given an HFD ad libitum, which consisted of standard rabbit chow with 10% added fat. The excess fat in the diet consisted of two-thirds corn oil and one-third lard. Experiments were performed after the rabbits had been on their respective diets for 12 weeks.

Only male rabbits were used to avoid secondary variability related to sex differences in this experimental model.

Experimental Procedures

Intraperitoneal Glucose Tolerance Test

An intraperitoneal glucose tolerance test (GTT) was performed in accordance with the methods of Georgiev et al.[17] Before the beginning of the experiment, the animals were deprived of food but not of water for 12 hours during the night. The GTT was performed on the next morning through an intraperitoneal injection of 2 g/kg of glucose using a 22-G needle.

Blood samples were obtained from the ear vein (v. auricularis externa) using sterile lancets (Vitrex Medical Aps, Denmark) before glucose injection (0 minutes) and at minutes 60, 90, and 120 after glucose injection. The blood concentrations of glucose were measured immediately after blood collection by using a glucometer (Home Diagnostics, Inc, United States) based on the glucose oxidase method using 1 drop of whole blood.

Blood Pressure and Measurement of Lipids and Insulin

At the end of the 12-week dietary intervention, food was withdrawn for 12 hours, and the rabbits were weighed and then anesthetized with 75 mg/kg of ketamine.

Arterial blood pressure was measured directly in the carotid artery through a catheter connected to a pressure transducer (Gould, United States) and recorded using a data acquisition system (Biopac MP100). After mean arterial pressure measurement, blood samples were collected in prechilled glass tubes containing ethylenediaminetetraacetic acid 10^{-7} M through a catheter inserted in the carotid artery.

Plasma cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and glucose were measured using colorimetric reactions with commercial kits (Wiener, Argentina). Plasma insulin concentrations were measured by radioimmunoassay.

Isometric Tension Measurement

After blood samples were collected, the descending thoracic aorta was exposed through a midline incision and excised. It was carefully dissected, and all adherent fat and connective tissue were removed. Five-millimeter-wide rings were cut and mounted in a 10-mL organ bath containing Krebs solution with the following composition: 128 mM NaCl, 4.7 mM KCl, 14.4 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 0.1 mM Na_2-ethylenediaminetetraacetic acid, 2.5 mM $CaCl_2$, and 11.1 mM glucose at pH 7.2. Krebs solution was kept at 37°C and aerated with 95% O_2 and 5% CO_2.

Isometric contractions were measured using force–displacement transducers and recorded under an initial tension of 2 g, which was found to be the optimal tension for KCl-induced contraction (100 mM). All the preparations were allowed to equilibrate for 120 minutes and washed with Krebs solution at 15-minute intervals. The endothelium was kept intact in some rings (endothelium-intact arteries) and was removed by rubbing the luminal surface (endothelium-removed arteries) in the other group.

To check endothelial function after equilibration, aortic rings were contracted with noradrenaline 5×10^{-6} M. Although the resultant tone in aortic rings from the HFD rabbits was lower than the tone obtained with the same dose in the control diet (CD), we added cumulatively to the bath a dose of noradrenaline 10^{-5} M to reach a similar tone. Then arteries were exposed to the endothelium-dependent vasorelaxant acetylcholine (10^{-8}–5×10^{-6} M). Thus, a concentration–response curve (CRC) was constructed.

Endothelium-intact and endothelium-removed aortic rings (E+ and E−) were separated into 2 groups. One group was exposed to increasing doses of angiotensin II (10^{-10}–10^{-6} M) to construct a CRC. The other group was exposed to increasing doses of noradrenaline (10^{-8}–10^{-4} M) to construct a CRC.

Both endothelium-independent and endothelium-dependent marked declines were observed in the reactivity to

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noradrenaline and angiotensin II in aortic rings from rabbits fed an HFD. To investigate whether the decrease in the vascular reactivity to noradrenaline and angiotensin II reflects crosstalk between α1-adrenoceptors and angiotensin AT1 receptors, endothelium-intact and endothelium-removed arteries from rabbits fed a CD or an HFD were treated with concentrations of noradrenaline equivalent to the noradrenaline CRC (10^{-8}–10^{-4} M) or with concentrations of angiotensin II equivalent to the angiotensin II CRC (10^{-10}–10^{-6} M). The rings were rinsed, and a 30-minute recovery period was allowed before performing the following treatments: (1) administration of a subsequent CRC for angiotensin II in the rings previously treated with noradrenaline or (2) a subsequent CRC for noradrenaline in arteries pretreated with angiotensin II.

To study the role of endothelium-relaxing factors in the endothelium-dependent decrease of the contractile response to angiotensin II and noradrenaline, the NO synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME, 10^{-4} M) was added to the bath 30 minutes before administering the CRC for angiotensin II or the CRC for noradrenaline.

To investigate the influence of vasodilator prostaglandins or EDHFs in the endothelium-dependent decrease of the contractile response to angiotensin II, an inhibitor of cyclooxygenase (10^{-5} M indomethacin) or an inhibitor of cytochrome P_{450} epoxygenase (10^{-6} M miconazole) was added to the bath 30 minutes before administering the CRC for angiotensin II.

The results are expressed in milligrams of isometric contraction.

Determination of Nitrite Release

Nitrites were measured using the Griess reaction. Arteries from rabbits fed either a CD or an HFD were placed in a 1 mL organ bath containing Krebs solution and aerated with 95% O_2 and 5% CO_2. Samples (500 μL) were collected from the incubation medium at different experimental times. Two sets of standard curves were prepared for each experiment. Tubes contained 500 μL of 0, 1, 2.5, 5, 7.5, and 10 μmole/L of $NaNO_2$; then, *N*-1-naphthylethylenediamine (50 μL of a 0.2% solution) and sulfanilamide (450 μL of a 0.1% solution) were added to each tube containing 500 μL of a standard or experimental sample. Each tube was then mixed for a few seconds before incubating at room temperature until a pink color developed (15 minutes). Absorbance was measured at 540 nm with a spectrophotometer (Metrolab 1000) calibrated to zero with a blank solution. Nitrite absorbance was calculated through regression analysis ($y = a + bx$) and converted into a straight line. Only curves with a correlation coefficient >0.95 were used.

To detect angiotensin II- or noradrenaline-stimulated nitrite release, samples were collected 20 minutes after the equilibration period (Basal) and 30 minutes after 10^{-6} M of angiotensin II or 10^{-4} M of noradrenaline stimulation.

Electrophysiological Studies

Aortae were removed, and they were cut open along the long axis before being fixed, with the intimal surface upward, to the silicone rubber base of an organ chamber (volume 5 mL) with a temperature controller (37°C) and aerated with 95% O_2 and 5% CO_2. Segments were immersed in Krebs solution. Smooth muscle cell impalement was performed from the intimal side of the vessels. The transmembrane potential was recorded with glass electrodes filled with 3 M KCl (tip resistance 50–80 Ω) that were connected to the headstage of a recording amplifier equipped with capacitance neutralization (Intra 767, World Precision Instruments Ltd, United States); an Ag/AgCl pellet in contact with the bath solution and directly connected to the amplifier served as the reference electrode. The electrophysiological signal was continuously monitored on an oscilloscope and simultaneously recorded on paper (Gould chart recorder). Successful impalements were signaled by a sudden negative drop in the potential from the baseline (zero potential reference), followed by a stable negative potential for at least 10 minutes and were held under current clamp conditions.

The resting membrane potentials (Pm) were measured in E+ and E− under basal conditions (B0) and under incubation with 10^{-6} M angiotensin II (10 minutes). In another group, the Pm was measured under basal conditions and under incubation with 10^{-6} M miconazole (10 minutes) or 10^{-6} M miconazole plus 10^{-6} M angiotensin II (10 minutes). Arteries were washed with Krebs solution, and the Pm was measured again to assess its recovery. A depolarizing KCl solution was finally added.

Values are expressed in millivolts or as differences between the Pm under basal conditions and the Pm with angiotensin II stimulation.

Statistical Analyses

Data are presented as the mean values ± SEM. The pD_2 (negative log of the molar concentration of angiotensin II inducing 50% of the maximal contraction) and the maximal contractile response (R_{max}, in milligrams) were calculated using a curve-fitting analysis program. Single-variable comparisons were made with a 1-way analysis of variance (ANOVA). CRC or multiple comparisons were analyzed by a 2-way multivariate analysis of variance (MANOVA) followed by the Duncan test. $P < 0.05$ was considered statistically significant.

RESULTS

General

Rabbits fed an HFD showed a higher body weight than did control rabbits (Table 1). Plasma levels of total cholesterol, LDL cholesterol, and HDL cholesterol were similar in both groups (Table 1). The mean blood pressure and heart rate of the rabbits fed an HFD was higher than that of the controls. Although feeding the HFD did not alter fasting glucose (Table 1), glucose tolerance was significantly impaired (Fig. 1). Plasma levels of insulin were significantly higher in rabbits fed an HFD (Table 1). Glucose levels at 120 minutes were positively related to blood pressure levels ($r = 0.59$, n = 12, $P < 0.05$).

Response to Acetylcholine

Acetylcholine (10^{-8}–10^{-5} M) caused similar maximal endothelium-dependent relaxation in both diet groups (Fig. 2).

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TABLE 1. Plasma Levels of Lipids, Glucose, and Insulin; Mean Arterial Blood Pressure; Heart Rate; and Weight Values From Rabbits Fed a CD or an HFD

	CD	HFD
Weight (g)	2172 ± 178	3250 ± 180*
Basal glucose (mg/dL)	98.5 ± 3.2	102 ± 3
Insulin (μUI/mL)	3.2 ± 1	15.5 ± 1.5†
Cholesterol (mg/dL)	49 ± 10	37.9 ± 3.9
LDL cholesterol (mg/dL)	14 ± 4.0	9.2 ± 2.1
HDL cholesterol (mg/dL)	45.5 ± 10	56.4 ± 3.6
Blood pressure (mm Hg)	62.7 ± 5.1	75.8 ± 2.3*
Heart rate (bpm)	175 ± 8	210 ± 7*

Data are expressed as mean ± SEM of 12 rabbits.
*$P < 0.05$.
†$P < 0.01$ indicates statistically significant differences between rabbits fed a CD and rabbits fed an HFD (Student unpaired *t*-test).

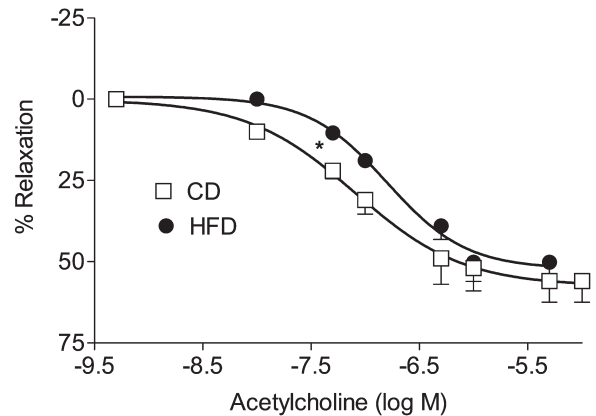

FIGURE 2. Endothelium-dependent vasodilation to acetylcholine (10^{-8}–5×10^{-6} M) in rabbit aortic rings precontracted with a submaximal dose of noradrenaline (5×10^{-6} M) from rabbits fed an HFD or a CD. *$P < 0.05$ indicates statistically significant differences in pD_2 between arteries from rabbits fed a CD and rabbits fed an HFD.

Maximum relaxation was induced by 10^{-5} M acetylcholine. However, the potency for acetylcholine decreased significantly in the aortae of rabbits fed an HFD (pD_2 control: 7.08 ± 0.09 vs. pD_2 HFD: 6.82 ± 0.14, n = 12, $P < 0.05$).

Basal NO release was similar in both diet groups (rabbits fed a CD: 131.8 ± 26 pmole/mg/mL and rabbits fed an HFD: 104 ± 19 pmole/mg/mL).

Contractile Response to Angiotensin II and Noradrenaline in Endothelium-intact and Endothelium-removed Rabbit Aortae

Angiotensin II (10^{-9}–10^{-7} M) induced contractions in the E+ and E− of control rabbits and rabbits fed the HFD. The maximal contraction (R_{max}) and potency (pD_2) were similar in endothelium-intact and endothelium-removed arteries from rabbits fed a CD (Table 2). In E+ from rabbits fed an HFD, the R_{max} and pD_2 were significantly decreased compared with that in rabbits fed a CD (Table 2). The R_{max} in response to angiotensin II in arteries from rabbits fed an HFD was enhanced by endothelium removal, reaching values almost identical to those obtained in E− from rabbits fed a CD (Fig. 3A). However, endothelium removal did not recover the loss of potency that had been observed in endothelium-intact arteries from rabbits fed an HFD (Table 2).

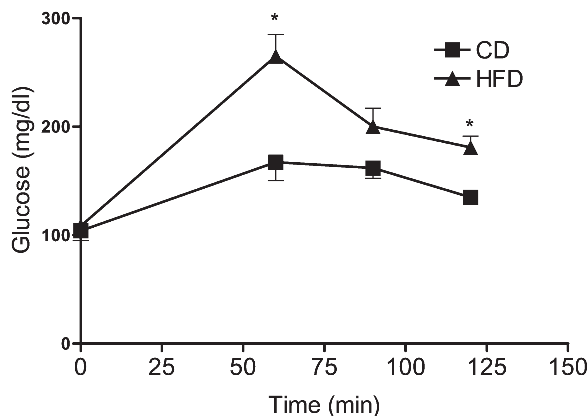

FIGURE 1. Plasma glucose levels at base line (0 minutes) and at indicated time points after injection of 2g/kg of glucose (GTT) from rabbits fed a CD and rabbits fed an HFD. Data are mean ± SEM of 12 animals. *$P < 0.05$ indicates statistically significant differences compared with arteries from rabbits fed a CD.

Noradrenaline (10^{-8}–10^{-4} M) induced contractions in the endothelium-E+ and E− of control and HFD rabbits. Contractile response was slightly higher in endothelium-intact aortae from control rabbits (Table 2). Nevertheless, the differences observed in R_{max} and pD_2 between endothelium-intact removed arteries from rabbits fed a CD were not statistically significant. Contractile response of aortic rings to noradrenaline was lower in E+ from rabbits fed an HFD than in those fed a CD (Table 2). Endothelium disruption improved R_{max} and potency in response to noradrenaline in rabbits fed an HFD (Fig. 3B). However, the loss of potency compared with arteries from rabbits fed a CD was not recovered by endothelium removal (Table 2).

Crosstalk Between Adrenoceptors and Angiotensin II Receptors

Noradrenaline stimulation before exposure to angiotensin II did not modify the R_{max} or pD_2 in the aortic rings from control rabbits (Table 2). In contrast, noradrenaline potentiated the desensitization to the angiotensin II response in E+ and E− from rabbits fed an HFD (Table 2).

Administration of one CRC to angiotensin II induced an important shift to the right of the subsequent CRC to noradrenaline in endothelium-intact and endothelium-removed control arteries (Table 2). In contrast, angiotensin II pretreatment did not modify the CRC to noradrenaline in E+ or E− from rabbits fed an HFD (Table 2).

Role of the Endothelium in the Decrease of the Vascular Reactivity to Angiotensin II and Noradrenaline

Incubation of aortic rings with 10^{-4} M L-NAME increased the contractile response to angiotensin II in the aortic

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TABLE 2. Maximal Contractile Response and pD_2 of Angiotensin II and Noradrenaline in Rabbit Aortic Rings

	E+				E−			
	HFD		CD		HFD		CD	
	R_{max}	pD_2	R_{max}	pD_2	R_{max}	pD_2	R_{max}	pD_2
Ang II	3251 ± 533	7.60 ± 0.10	4604 ± 574	8.02 ± 0.07	4250 ± 550*	7.83 ± 0.16	4293 ± 431	8.16 ± 0.11
Ang II/NA	3291 ± 408	7.03 ± 0.16†	4654 ± 652	8.25 ± 0.09	5375 ± 682*	7.10 ± 0.20†	5138 ± 398	8.03 ± 0.08
NA	7738 ± 894	5.29 ± 0.08	10,474 ± 639	6.36 ± 0.06	14,681 ± 1047*	5.80 ± 0.10*	8959 ± 894	6.11 ± 0.13
NA/Ang II	7630 ± 798	5.30 ± 0.10	11,572 ± 1104	4.77 ± 0.08	13,361 ± 1257*	5.73 ± 0.10*	11,232 ± 1244	4.75 ± 0.14

Values are expressed as mean ± SEM of 8 experiments.
*$P < 0.05$ indicates statistically significant differences between endothelium-intact and endothelium-removed arteries.
†$P < 0.05$ indicates statistically significant differences between arteries treated with a CRC to noradrenaline before angiotensin II stimulation and untreated arteries.
Ang II, angiotensin II; Ang II/NA, arteries treated with a CRC to NA hefore angiotensin II stimulation; NA, noradrenaline; NA/Ang I, arteries treated with a CRC to angiotensin II before NA stimulation.

rings from rabbits fed a CD (Fig. 4A). In aortic rings from rabbits fed an HFD, L-NAME had no effect on R_{max}, but it induced a shift to the left of the CRC to angiotensin II (pD_2 untreated: 7.63 ± 0.08 vs. pD_2 L-NAME: 8.08 ± 0.11, n = 8, $P < 0.05$). The quantity of the leftward shift was similar to that observed in arteries from rabbits fed a CD.

Miconazole (10^{-6} M) did not change the efficacy of angiotensin II treatment either in rabbits fed a CD (R_{max} untreated: 4717 ± 280 mg; R_{max} miconazole: 4780 ± 708 mg) or in rabbits fed an HFD (Fig. 4B). Indomethacin administered at 10^{-5} M did not modify the angiotensin II response in the aortic rings from rabbits fed a CD (R_{max} untreated: 4604 ± 574 mg; R_{max} indomethacin: 4689 ± 639 mg) or an HFD (Fig. 4B).

The endothelium did not change the Pm in arteries from rabbits fed a CD (Fig. 5). However, in aortic rings from the rabbits fed an HFD, the Pm was lower in arteries with endothelium. Angiotensin II induced a modest but significant depolarization in aortae from rabbits fed an HFD. This depolarization was lower in endothelium-intact (Delta Pm Basal minus Pm angiotensin II = 3 ± 1 mV) than in endothelium-removed aortae (Delta Pm Basal minus Pm angiotensin II = 9 ± 2 mV; n = 12, $P < 0.05$). Incubation of E+ with 10^{-6} M of miconazole did not modify either the basal Pm or the Pm in response to angiotensin II stimulation.

The NO level in the perfusate of aortic rings was increased by angiotensin II compared with the basal NO level in control[18] but not in the rabbits fed an HFD (Fig. 6).

Incubation of aortic rings with 10^{-4} M L-NAME did not modify the contractile response to noradrenaline in rabbits fed a CD (R_{max} untreated: 11,675 ± 804 mg vs. R_{max} L-NAME: 9926 ± 700 mg; n = 12; $P < 0.05$). In contrast, L-NAME recovered the contractile response to noradrenaline in aortic rings from rabbits fed an HFD (R_{max} untreated: 7630 ± 798 mg vs. R_{max} L-NAME: 12015 ± 317 mg; n = 12; $P < 0.05$).

Noradrenaline increased NO levels compared with the basal NO level in aortic rings from rabbits fed a CD (Basal: 52.7 ± 8.8 pmole/mg vs. noradrenaline stimulated: 401 ± 125 pmole/mg, n = 8, $P < 0.05$). Noradrenaline also increased NO levels in rabbits fed an HFD (Fig. 6), but the NO increase was lower than the NO increase observed in rabbits fed a CD (Delta Basal-stimulated CD: 341 ± 63 pmole/mg/mL vs. Delta Basal-stimulated HFD: 62 ± 15 pmole/mg/mL, n = 8, $P < 0.05$).

DISCUSSION

The principal findings of this study are as follows: (1) The HFD-induced glucose intolerance was positively related to blood pressure levels. (2) The HFD was associated with reduced sensitivity to noradrenaline and angiotensin II. This phenomenon was endothelium independent. (3) Adrenoceptor stimulation induced desensitization to angiotensin II that was endothelium independent in rabbits fed an HFD. (4) An endothelium-intact state is related with lower efficacy of noradrenaline and angiotensin II in obese rabbits. (5) NO is associated with reduced vascular reactivity to noradrenaline.

Our finding of HFD-induced glucose intolerance that was positively related to blood pressure levels is in agreement with and extends previous evidence and strengthens the hypothesis that hyperinsulinemia represents the link between obesity and hypertension.[6]

In our attempt to elucidate the role of the vascular mechanisms involved in this phenomenon, we found that obesity was associated with an endothelium-independent decrease in the vascular reactivity to noradrenaline and angiotensin II. These results obtained in our work disagree with those of other studies[19,20] that found increased response to angiotensin II in vivo and increased vascular responsiveness to angiotensin II, phenylephrine, and serotonin in obese Zucker rats. The reason for such differences is not evident but may be linked to the method of producing obesity in different animal species. We generated an obese model by feeding the rabbits an HFD, which often induces an increment in sympathetic activity.[21] The obese Zucker rats showed a genetic abnormally of the leptin receptor in the hypothalamus and may not exhibit enhanced sympathetic activity. The vascular hyporeactivity of noradrenaline observed in the rabbits fed an HFD in our study is in agreement with those of previous reports of reduced vascular resistance and enhanced regional blood flow in other obese models in which obesity has been induced by feeding an HFD.[22] In accord with our results, Bhattacharya et al[23] found that increasing the percentage of fat in the diet decreased angiotensin II contractile response in carotid arteries from mice.

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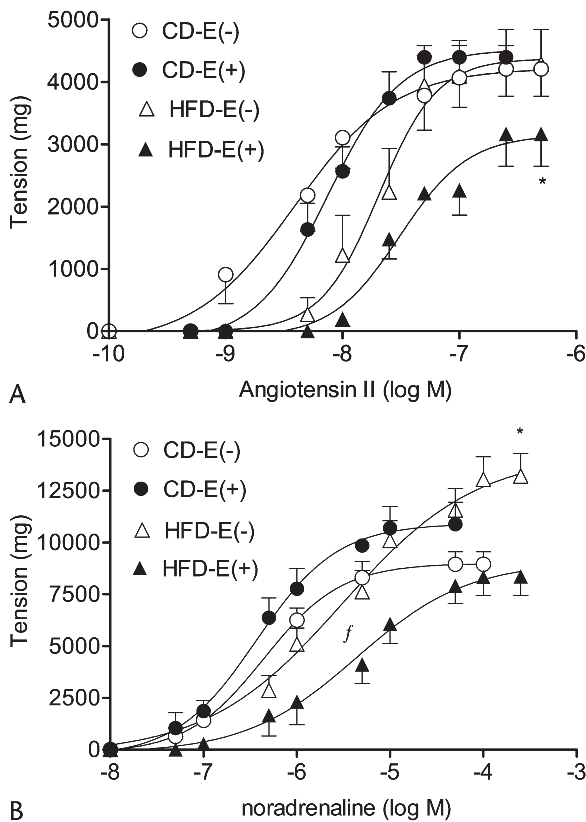

FIGURE 3. A, CRCs to angiotensin II in E+ or E– from rabbits fed an HFD or a CD. Each data point shows the mean of 8 experiments and vertical lines indicate SEM. $^{*}P < 0.05$ indicates statistically significant differences compared with endothelium-intact arteries from rabbits fed a CD [2-way repeated measures ANOVA (RMANOVA) with the Duncan post test]. B, CRCs to noradrenaline in E+ or E– from rabbits fed an HFD or a CD. $^{*}P < 0.05$ indicates statistically significant differences compared with endothelium-intact arteries from rabbits fed an HFD. ƒ$P < 0.05$ indicates statistically significant differences in pD_2 between E+ or E– arteries from HFD and CD rabbits (2-way RMANOVA and Duncan post test).

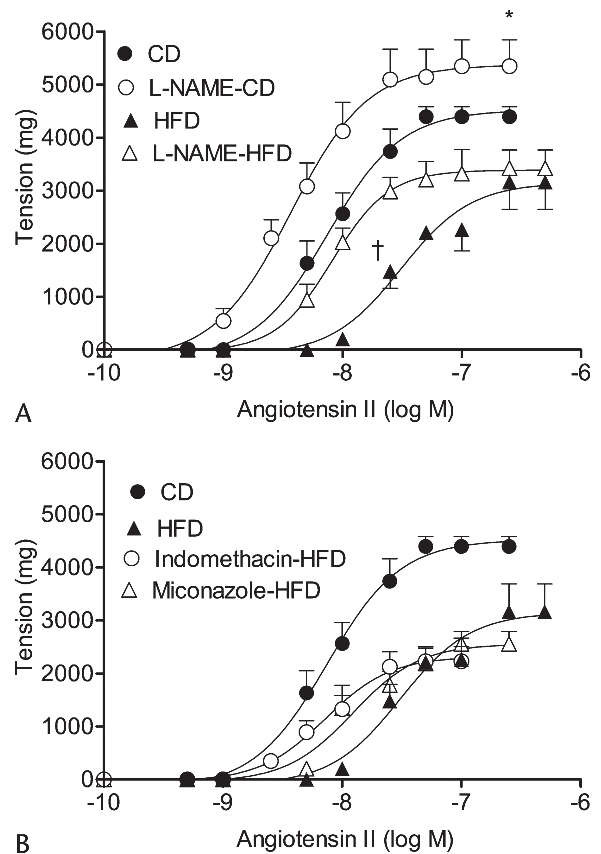

FIGURE 4. CRCs to angiotensin II in E+ from rabbits fed an HFD or a CD. A, Arteries were incubated with L-NAME 10^{-4} M 30 minutes before the curve to angiotensin II. B, Arteries were incubated with 10^{-5} M indomethacin or 10^{-6} M miconazole 30 minutes before the curve to angiotensin II. Each data point shows the mean of 8 experiments and vertical lines indicate SEM. * $P < 0.05$ indicates statistically significant differences in maximal contraction of L-NAME treated arteries compared with untreated arteries from the rabbits fed a CD (2-way RMANOVA with the Duncan post test). †$P < 0.05$ indicates statistically significant differences in pD_2 of L-NAME treated arteries compared with untreated arteries from rabbits fed an HFD.

The obesity-related vascular hyporeactivity could be explicated by different possible mechanisms, such as lower affinity to noradrenaline or angiotensin II at the α-adrenoceptor or AT-receptor level, respectively; lower vascular elasticity; or lower Ca^{2+} influxes. Khalil et al[22] reported that contractile response to phenylephrine or caffeine is similar in normal-salt obese and control rabbits, which indicates similar levels of Ca^{2+} release from and/or Ca^{2+} uptake to intracellular stores. They did find impairment in phenylephrine- and depolarization-induced Ca^{2+} influxes, which indicates that the Ca^{2+} permeability of receptor-operated and voltage-gated Ca^{2+} channels may be diminished in obesity.[22] However, Watkins and Davidson[24] reported observing in rabbits aortic strips 2 components of noradrenaline-induced contraction (tonic and phasic), whereas angiotensin II–induced contractions exhibit only one term (phasic), which was independent of extracellular Ca^{2+}. Thus, decreased Ca^{2+} permeability of receptor-operated and voltage-gated Ca^{2+} channels in obese animals may explain decreases in the contractile response to noradrenaline but may not explain the decreased contractile response to angiotensin II.

As noted in the Introduction, obesity is related to sympathetic activation.[11,12] It is well known that there are multiple interactions between the renin–angiotensin system and the sympathetic nervous system. Heterologous desensitization between angiotensin II and noradrenaline may be a significant mechanism for regulating the blood pressure. Accordingly, modifications in the desensitization of the angiotensin AT1 receptor by angiotensin II and/or noradrenaline may be present under pathophysiological conditions.[25,26] In a previous study, we found heterologous desensitization between the α1-adrenoceptor and AT1 receptors in rabbit aortae. α1-Adrenoceptor or AT1-receptor stimulation induces angiotensin II or noradrenaline (respectively) desensitization in E+.[27] Additionally, we reported that a high cholesterol diet modifies the reciprocal interactions between the α1-adrenoceptor and angiotensin II

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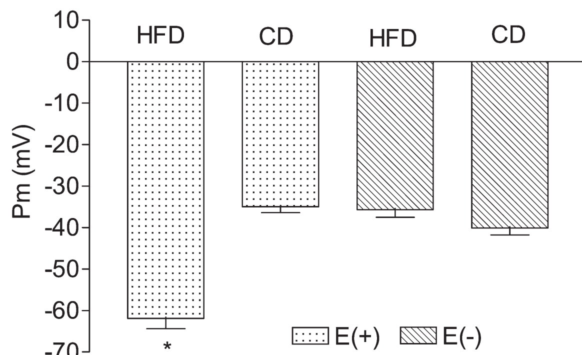

FIGURE 5. Resting Pm from rabbits fed an HFD and rabbits fed a CD. Each column represents the mean ± SEM for 8 experiments. $*P < 0.05$ indicates statistically significant differences compared with arteries from rabbits fed a CD (2-way RMANOVA with the Duncan post test).

receptors.[28] Considering these findings, we hypothesized that the endothelium-independent decrease of vascular reactivity involves mechanisms of desensitization induced by HFD activation of sympathetic activity. Sustained adrenoceptor stimulation may cause angiotensin II desensitization. The present results show that the stimulation of adrenoceptors with noradrenaline before angiotensin II stimulation shifted the angiotensin II curve to the right in both unrubbed and rubbed arteries.

In hypercholesterolemic rabbits, we found that angiotensin II potentiates the effect of noradrenaline through AT1 receptors.[28] However, in rabbits fed the HFD, stimulation of AT-receptors with angiotensin II before noradrenaline stimulation did not alter the contractile response to noradrenaline. These findings suggest that obesity modified the reciprocal interaction between adrenoceptors and angiotensin II receptors and support the hypothesis that sympathetic activation may desensitize the angiotensin II response in the present animal model.

Endothelial dysfunction is characterized by the reduced endothelium-dependent vasodilation. Mechanisms that participate in this phenomenon include reduced NO production, oxidative excess, and minor production of hyperpolarizing factor.[29] In addition, Campbell et al[30] demonstrated that NO is not the only mediator of acetylcholine-induced relaxations in the rabbit aorta. We found that despite the lower sensitivity to acetylcholine in the obese rabbits, the maximal relaxation and the basal NO release were similar between the 2 diet groups. In agreement with this finding, Mundy et al[13] found that acetylcholine relaxation was not modified by increasing the percentage of fat in the diet in mice. These results suggest an early vascular dysfunction in the present model of obesity. In such conditions, the endothelium would attempt to compensate this dysfunction through increased agonist-stimulated NO release and mechanisms independent of NO.

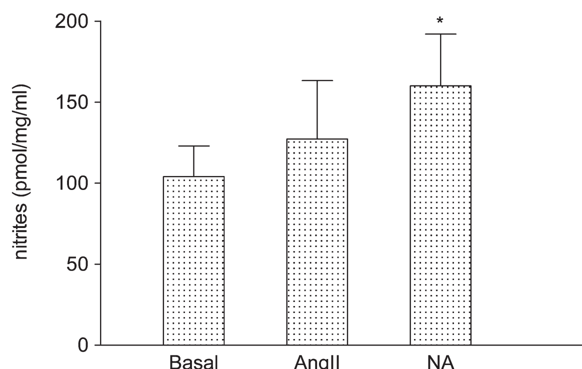

FIGURE 6. NO release from aortic rings of rabbit fed an HFD. Basal NO release, NA: noradrenaline-stimulated NO release; Ang II, angiotensin II–stimulated NO release. Each column represents the mean of 8 experiments and vertical lines indicate SEM. $*P < 0.05$ indicates statistically significant differences compared with basal NO release (one way-ANOVA and Duncan test).

The endothelium significantly potentiated the decrease in the efficacy of noradrenaline and angiotensin II in obese rabbits. Possible mechanisms to explain the decreased efficacy of agonist vasoconstrictors may include a compensatory increased release of endothelium-dependent vasodilators. Siddiqui and Hussain[20] reported enhanced NO release to counteract the effect of angiotensin II in the aortae of obese Zucker rats. Kamata et al[31] found that an increase in NO release may account for the decrease in the noradrenaline contractile response observed in fructose-fed mice. Considering these data, we studied the influence of NO in our model. Noradrenaline sensitivity and efficacy were markedly increased by treatment with L-NAME, suggesting that the intrinsic activity to noradrenaline is modulated by NO released from the endothelium. The increased NO release induced by noradrenaline stimulation supports this hypothesis.

L-NAME increases contractile response to angiotensin II in control rabbits. This increase was higher than endothelium disruption because L-NAME blocked vasodilator NO, but it did not block vasoconstrictor release. Endothelium disruption abolished both vasodilators (NO, EDHFs, PGI2) and vasoconstrictors (endothelin, TXA2, free radicals) factors. In contrast to the effect of L-NAME treatment in the contractile effect of noradrenaline, L-NAME did not recover the efficacy of angiotensin II. L-NAME treatment shifted the CRC for angiotensin II to the left in both control and HFD rabbits with a similar magnitude. This result indicates that basal NO release may be responsible for the decrease in the sensitivity to noradrenaline and angiotensin II, but NO is not expected to be associated with the attenuation of the efficacy of angiotensin II. In agreement with this result, we found that angiotensin II did not stimulate NO release in arteries from rabbits fed an HFD.

Consequently, we investigated the role of other endothelium-relaxing factors in the reduced efficacy of angiotensin II. Considering that PGI2 is the main endothelium-vasodilator prostaglandin, we assessed the effect of the cyclooxygenase-inhibitor indomethacin. We found no effects of indomethacin on the contractile response to angiotensin II. These results imply that the reduced efficacy of angiotensin II was independent of cyclooxygenase metabolites.

By definition, an EDHF is a substance and/or electrical signal that is generated or synthesized in and released from the endothelium that hyperpolarizes smooth muscle cells, followed by relaxation. Unless the EDHF is found to be

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most important in small arteries, the rabbit aorta exhibits EDHF-mediated responses.[32] We found that the basal Pm of aortae with endothelium from rabbits fed an HFD was more negative than the basal Pm of endothelium-removed arteries. Moreover, angiotensin II-induced depolarization was weaker in endothelium-intact aortae. Considering that metabolites from cytochrome P_{450} epoxygenase represent a putative EDHF,[33] we investigated their role in the endothelium-dependent hyperpolarization and the minor angiotensin II–induced depolarization of the smooth muscle. Miconazole failed to modify the basal Pm and to block the endothelium effect on the angiotensin II-induced depolarization. Miconazole also failed to recover the intrinsic activity of angiotensin II in rabbit aortic rings. These results imply that hyperpolarization of the rabbit aortae induced by the endothelium may have blunted the contractile response to angiotensin II in rabbits fed an HFD. However, metabolites from cytochrome P_{450} epoxygenase would not be involved in this process.

Bhattacharya et al[23] found that increases in body fat and/or even obesity per se may stimulate a protective effect of the endothelium in some vascular beds. The observed decrease in the efficacy of angiotensin II in aortic rings with endothelium was independent of NO because L-NAME had no effect, was independent of cyclooxygenase metabolites because indomethacin had no effect, and was independent of cytochrome P_{450} epoxygenase metabolites because miconazole had no effect. Despite the evidence that endothelium induced the hyperpolarization of the smooth muscle in rabbits fed an HFD, the mechanisms that account for this phenomenon remain to be clarified.

Although blood pressure was significantly higher in rabbits fed the HFD, the increase was modest compared with that of renal hypertension or hypertension after angiotensin II infusion. Aylward et al[34] reported that mean arterial pressure was increased by 57% in rabbits with renal hypertension produced by bilateral wrapping with cellophane, and Cuniberti et al[35] found in a one-kidney/one-clip hypertensive model that systolic blood pressure was enhanced by 36.3% and that diastolic blood pressure was increased by 47%. Van der Buuse and Malpas[36] found that chronic intravenous infusion of angiotensin II significantly increases blood pressure levels by 40%. In our model, in agreement with the findings of Carroll et al,[14] blood pressure was increased by 20% in rabbits fed an HFD. Antic et al[21] found that mean arterial blood pressure was enhanced by 14% in obese rabbits. Thus, we may assume that the observed reduced reactivity to noradrenaline and angiotensin II may be a compensatory mechanism to preserve blood pressure homeostasis in our obesity model.

Our study provides first evidence that the increased blood pressure observed in HFD-induced obesity may be partially compensated by endothelium-dependent and endothelium-independent decreases in the reactivity to angiotensin II and noradrenaline. Because we chose to perform our experiments using the rabbit thoracic aorta, we cannot come to a definitive conclusion regarding whether the observed changes in the reactivity to noradrenaline and angiotensin II also occur in resistance vessels.

We believe that an interesting subject for subsequent studies will be to determine whether the decreased reactivity to noradrenaline and angiotensin II is a compensatory mechanism in response to the increase in the blood pressure induced by other mechanisms related to obesity.

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