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Endothelial dysfunction and improvement of the angiotensin II-reactivity in hypercholesterolemic rabbits: Role of cyclooxygenase metabolites

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Abstract

The aim of this paper was to study the effect of high cholesterol diet on endothelial function and vascular reactivity to angiotensin II and to test the role of vasoconstrictor cyclooxygenase metabolites in this experimental condition. Rabbits were fed with either normal chow or a diet containing 1% cholesterol for 6–7-week. Isometric contractions were measured in rubbed or unrubbed aortic rings. Arteries were contracted with noradrenaline and then exposed to one cumulative dose–response curve to acetylcholine in absence (control) or in presence of indomethacin, (*N*-[2-cyvlohexyloxy)-4-nitrophenyl]-methanesulfonamide) (NS 398) or 4-hydroxy-2,2,6,6-tetraethylpiperidine-*N*-oxyl (tempol). After washing the arteries, one cumulative dose–response curve to angiotensin II was constructed in absence or presence of indomethacin, NS 398, [1*S*-[1 alpha,2 beta (5*Z*),3 beta,4 alpha]-7-[3-[[2-[(phenylamino) carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1] hept-2-yl]-5-heptenoic acid (SQ29548) or 17-octadecynoic acid (17-ODYA). In other group, resting potential was recorded in basal and angiotensin II-stimulated conditions. Indomethacin, NS 398 or 17-ODYA were added to the bath before angiotensin II-stimulation. Rabbits fed on a diet enriched with cholesterol showed higher plasma levels of total cholesterol and LDL. Hypercholesterolemic diet impaired acetylcholine relaxation. Indomethacin normalized endothelium-dependent relaxation whereas NS 398 and tempol had no effect on this phenomenon. Angiotensin II-reactivity was increased in endothelium intact hypercholesterolemic aortic rings and indomethacin, SQ29548 or 17-ODYA blocked this effect. The resting potential of unrubbed hypercholesterolemic arteries was significantly less negative to control after angiotensin II-stimulation. 17-ODYA but not indomethacin prevented angiotensin II-depolarization. High cholesterol diet caused endothelial dysfunction and increased the angiotensin II-reactivity. Both effects were cyclooxygenase1-dependent. Deficit in the NO-production might improve 20-hydroxyeicosatrienoic acid availability, which induces depolarization and angiotensin II-sensitization. In addition, 20-hydroxyeicosatrienoic acid would be metabolized by cyclooxygenase1 to 20-endoperoxides which act through thromboxane A_2/prostaglandin H_2 receptors contributing to angiotensin II-reactivity increase.

Keywords: Angiotensin II; Endothelium; Cyclooxygenase; Hypercholesterolemia; Vascular reactivity; (Rabbit)

1. Introduction

It is well known that endothelial cells modulate the response of vascular smooth muscle to hormones, neuromediators and aggregating platelets by releasing relaxing and/or contracting factors. A number of functional abnormalities of the vascular endothelium are recognized as significant contributors involved in the genesis or perpetuation of pathological states such as hypertension. Under physiological conditions, relaxing factors appear to dominate. In contrast, in hypertensive and atherosclerotic arteries the release of endothelium-derived relaxing factors and/or the responsiveness of vascular smooth muscle cells to the relaxing factors are reduced. Whereas the release and the responsiveness of endothelium-derived contracting factors (EDCF) are augmented (Luscher, 1990; Poredos, 2002). In this regard numerous studies reported a dysfunction in nitric oxide (NO) and prostanoid production from endothelial cells, which in turn directly and indirectly may account for the alteration in

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vasomotor reactivity. Wang et al. (2006) demonstrated that intravenous injection of CYP4A2 adenovirus, an arachidonic acid omega hydroxylase which produces 20-hydroxyeicosatrienoic acid (20-HETE), promotes the development of hypertension and causes endothelial dysfunction, reduced levels of NO and increased levels of superoxide anion.

Plasma levels of total cholesterol and low density lipoproteins (LDL) play a significant role in atherosclerosis development and subsequent coronary heart disease (Martin et al., 1986; Kannel, 1995). This is one of the main causes of cardiovascular morbidity and mortality. Lipid accumulation in blood vessel walls during hypercholesterolemia produces endothelium dysfunction, even before morphological changes occur (Sorensen et al., 1994). This condition is characterized by an impairment of endothelium-dependent vasodilation, and it suggests a reduced NO availability. However, an increase in vasoconstrictor factors could also be involved since thromboxane A_2 (TXA_2) biosynthesis increase has been found in hypercholesterolemic patients (Davi et al., 1992; Akimova, 1994).

Several studies have demonstrated that hypercholesterolemia and atherosclerosis are associated with augmented angiotensin II production through the enhancement of both angiotensin-converting enzyme (ACE) and chymase activities as well as the upregulation of angiotensin type I (AT_1) receptor gene expression (Yang et al., 1998). The improvement of endothelial dysfunction, inhibition of the NADPH-oxidase and reduction of early plaque formation by an AT_1-receptor antagonist suggest a crucial role of angiotensin II-mediated O_2-production in the early stage of atherosclerosis (Warnholtz et al., 1999).

Endothelium-dependent contractions to acetylcholine in the aorta of spontaneously hypertensive rat (SHR) involve reactive oxygen species that activate the cyclooxygenase (COX) pathway with the production of endoperoxides. These endoperoxides stimulate thromboxane receptors on the aortic vascular smooth muscle.

Both endothelial cells and smooth muscle cells express COX and thromboxane receptors. Mistry and Nasjletti (1988) and Dellipizzi et al. (1997) reported that treatment with a blocker of thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptor lowers the blood pressure of rats with angiotensin II — salt induced hypertension and it showed the same effect on rats in the early stage of the aortic coarctation-hypertension. Furthermore, Qi et al. (2002) have demonstrated that selective inhibition of COX-2 activity in mice enhanced the pressor effects of angiotensin II on blood pressure while inhibiting COX-1 attenuated angiotensin II-induced hypertension.

Jerez et al. (2005) have demonstrated, in a previous work, that angiotensin II stimulates endothelium release of COX-dependent vasoconstrictor prostanoids during nitric oxide synthase (NOS) inhibition. The increase of free radical production would be responsible for the activation of the COX-dependent pathway, which leads to the vasoconstrictor prostanoid synthesis in this experimental condition. The released metabolites act through TXA_2/PGH_2 receptors and its action mechanism would imply K_{ca} channels-activity decrease. The present work intends to demonstrate, by taking into account these data previously mentioned, that high cholesterol diet induces endothelial dysfunction and increase the reactivity to angiotensin II caused by vasoconstrictor COX-dependent metabolites. Furthermore, the present study intends to discriminate the COX-isoform involved.

2. Materials and methods

Male hybrid-Flanders rabbits from a slaughterhouse initially weighing 850–1000 g were used in this study. The animals were maintained under controlled light and temperature conditions and fed with either normal rabbit chow or a diet containing 1% cholesterol for 6–7 weeks with free access to tap water. Only male rabbits were used to avoid the secondary variability to sex differences in this experimental model. At the end of the 6–7-week dietary intervention, food was withdrawn for 12 h, and the rabbits were weighed and then anesthetized with Ketamin (75 mg/kg). All experimental procedures comply with the European Community guidelines for the use of experimental animals and protocols were approved by the Bioethical and Research Committee of Medicine from National University of Tucumán.

2.1. Blood pressure measurement

Arterial blood pressure was directly measured in the carotid artery through a catheter connected to a pressure transducer (Gould, USA) and recorded on a data acquisition system (BIOPAC MP100). After arterial pressure measurement, blood samples were collected in prechilled glass tubes containing EDTA 10^{-7} M through the catheter inserted in the carotid artery. Plasma cholesterol, High Density Lipoproteins (HDL), LDL, triglycerides and glucose were measured by use of enzymatic reaction with commercial kits (Wiener, Argentina).

2.2. Isometric tension measurement

After blood samples were taken, 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 of the following composition (mM): NaCl 128, KCl 4.7, $NaHCO_3$ 14.4, NaH_2PO_4 1.2, Na_2-EDTA 0.1, $CaCl_2$ 2.5, glucose 11.1, pH 7.2. Krebs solution was kept at 37 °C and aerated with 95% O_2 and 5% CO_2.

Isometric contractions were measured by 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 preparations were allowed to equilibrate for 120 min and washed with Krebs solution at 15 min intervals. The endothelium was kept intact in some rings (unrubbed arteries) and was removed by rubbing the luminal surface (rubbed arteries) in other groups.

In order to check endothelial function after equilibration, aortic rings were contracted with noradrenaline 5×10^{-6} M and then exposed to the endothelium-dependent vasorelaxant acetylcholine (10^{-9} to 5×10^{-6} M). Thus, one cumulative dose–response

curve was constructed. In other group, the endothelium-independent vasodilator sodium nitroprusside 5×10^{-6} M was used in a similar protocol.

Indomethacin 10^{-5} M (COX-1 inhibitor) or NS 398 (*N*-[2-cyvlohexyloxy)-4-nitrophenyl]-methanesulfonamide) 10^{-7} M (COX-2 inhibitor) were added to the bath 30 min before noradrenaline stimulation in order to evaluate the role of COX products and to discriminate the possible differential role of two different isoform involved in the response to acetylcholine. Furthermore, 4-hydroxy-2,2,6,6-tetraethylpiperidine-*N*-oxyl (tempol) 10^{-6} M (superoxide dismutase mimetic) was used in a similar protocol with the aim of checking the role of free radicals. The relaxation was expressed as a percent change from preexisting tone (before addition of vasorelaxant).

Aortic rings were washed twice with Krebs solution and then were exposed to increasing doses of angiotensin II (10^{-10} to 10^{-6} M) to construct one cumulative dose–response curve. Indomethacin 10^{-5} M, NS 398 10^{-7} M, SQ29548 ([1*S*-[1 alpha,2 beta (5*Z*),3 beta,4 alpha]-7-[3-[[2-[(phenylamino) carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1] hept-2-yl]-5-heptenoic acid) 10^{-6} M (TXA_2/PGH_2 receptor antagonist) or 17-ODYA (17-octadecynoic acid) 10^{-6} M (omega hydroxylase inhibitor) were added to the bath 30 min before the cumulative dose–response curve to angiotensin II in order to study the role of prostanoids in the increase of the contractile response observed in arteries from hypercholesterolemic rabbits. Results are expressed as mg of isometric contraction.

2.3. Electrophysiological studies

Aortic arteries were removed from rabbits as described above and they were then cut open along the long axis before being pinned, intimal surface upwards, 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. Unrubbed segments were immersed in Krebs solution. Smooth muscle cells impalement were 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) which were connected to the headstage of a recording amplifier equipped with capacitance neutralization (Intra 767, World Precision Instruments Ltd, USA); 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, USA). Successful impalements were signaled by a sudden negative drop in potential from the baseline (zero potential reference) followed by a stable negative potential for at least 10 min and were held under current clamp conditions.

The resting membrane potentials (Pm) were measured in basal conditions (B0) and next to angiotensin II 10^{-6} M stimulation (1 min: A0; 10 min: A10). Arteries were washed with Krebs solution and Pm was measured again to check its recovery. Depolarizing KCl solution was finally added.

Indomethacin 10^{-5} M or 17-ODYA 10^{-6} M were added to the bath after the basal Pm measurement in order to study the role of COX-1 and omega hydroxylase metabolites on the resting potential. Following to an incubation period of 20 min with these inhibitors, angiotensin II-stimulation was performed in this experimental group.

Values are expressed in mV as differences: (B)=B0 minus A0, and (Ang II)=A0 minus A10.

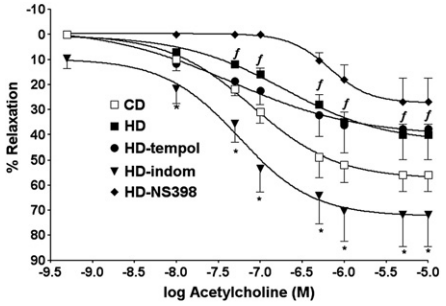

Fig. 1. Vasorelaxation effect induced by acetylcholine (10^{-8}–10^{-5} M) in rabbit aortic rings precontracted with submaximal dose of noradrenaline (5×10^{-6} M) from rabbits fed on a hypercholesterolemic (HD) or a control diet (CD). A group of arteries from hypercholesterolemic rabbits were treated with indomethacin 10^{-5} M (HD-indom), NS 398 10^{-7} M (HD-NS 398) or tempol 10^{-5} M (HD-tempol). Each data point shows the mean of 8 experiments and vertical lines indicate S.E.M. $^{f}P<0.05$ indicates differences statistically significant between HD and CD. $^{*}P<0.05$ between HD vs HD-indom. (ANOVA and Duncan's test).

Table 1
Lipids and blood glucose levels, arterial blood pressure and weight values from rabbits fed on a control diet or a diet containing 1% cholesterol

	Control diet	Hypercholesterolemic diet
Cholesterol total	0.49±0.10 g/l	6.41±2.20 g/l[a]
LDL-cholesterol	0.14±0.04 g/l	2.85±0.97 g/l[a]
HDL-cholesterol	0.28±0.11 g/l	0.35±0.09 g/l
Triglycerides	1.74±0.38 g/l	1.08±0.18 g/l
Blood pressure	81±10 mm Hg	88±8 mm Hg
Blood glucose	105±13 mg/dl	98±4 mg/dl
Weight	2.072±0.178 g	2.198±0.115 g

Data are expressed as means±S.E.M. of 10 rabbits. LDL: low density lipoproteins. HDL: high density lipoproteins.
[a]$P<0.05$ indicates statistically significant differences between rabbits fed on a control diet and rabbits fed on a diet enriched with cholesterol (unpaired *t* test).

2.4. Statistical analysis

Data are presented as mean values±S.E.M. The pEC_{50} (negative log of molar concentration inducing 50% of the maximal contraction), the maximal contractile response (E_{max}) and the maximal relaxation were calculated using a curve-fitting analysis program. Student's *t* test for paired or unpaired data was used to compare pEC_{50} values or maximal response. $P<0.05$ was considered statistically significant (two-tail test). The significance of the differences between and within the groups was examined with an analysis of variance (ANOVA) for repeated measures followed by a Duncan's test.

3. Results

Rabbits fed on a diet enriched with cholesterol showed higher plasma levels of total cholesterol and LDL-cholesterol

Table 2
pEC_{50} and maximal relaxation to acetylcholine in rabbit aortic rings with endothelium

	Maximal relaxation (%)		pEC_{50}	
	Control diet	Hypercholesterolemic diet	Control diet	Hypercholesterolemic diet
Control	54.5±5.8	33.3±3.4 [a]	7.08±0.09	6.67±0.11 [a]
Tempol	53.4±9.9	37.8±12.0 [a]	8.16±0.29 [b]	7.28±0.39 [a, b]
Indomethacin	58.3±5.4	71.8±12.8	8.00±0.08 [b]	7.32±0.10 [a, b]
NS 398	71.0±8.8	26.7±9.6 [a]	7.20±0.17	6.58±0.10 [a]

Values are expressed as means±S.E.M. of 8 experiments.
Arteries from rabbits fed on either a control or an enriched with cholesterol diet were stimulated with noradrenaline $5 \cdot 10^{-6}$ M and when maximal contraction was achieved one cumulative doses response curve to acetylcholine was performed (control). In other groups, aortic rings were incubated with indomethacin 10^{-5} M, NS 398 10^{-7} M or tempol 10^{-5} M. pEC50 and maximal relaxation values were obtained by using a curve-fitting analysis program.
[a] $P<0.05$ indicates statistically significant differences between rabbits fed on a control diet and rabbits fed on a diet enriched with cholesterol (ANOVA and Duncan's test).
[b] $P<0.05$ indicates statistically significant differences between arteries treated with indomethacin or tempol and arteries without treatment (control) (ANOVA and Duncan's test).

than animals fed on a control diet. By contrast, HDL, triglycerides and glucose plasma levels were similar in both groups. At the end of the experiment, no differences were found either in body weight or blood pressure between both diet groups (Table 1).

3.1. Response to acetylcholine

Acetylcholine (10^{-8}–10^{-5} M) caused endothelium-dependent relaxation in a concentration–response manner in both diet groups. However, endothelium-dependent relaxation and affinity to acetylcholine decreased significantly in aorta from rabbits fed on cholesterol-enriched diet (Fig. 1, Table 2). The maximal relaxation was induced by acetylcholine 10^{-5} M. Incubation with indomethacin 10^{-5} M restored endothelium-dependent relaxation to normal whereas NS 398 10^{-7} M or tempol 10^{-5} M did not (Fig. 1). Nevertheless, not only indomethacin but also tempol improved acetylcholine affinity with respect to control in both diet groups (Table 2).

Sodium nitroprusside 5×10^{-6} M caused a relaxation in control and hypercholesterolemic arteries in a similar magnitude (38.2±5.8% vs 41.0±10%, $n=6$, N.S.), respectively.

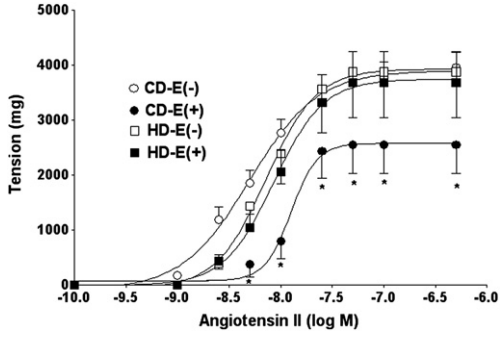

Fig. 2. Cumulative dose–response curves to angiotensin II in aortic rings with intact endothelium (E+) or without endothelium (E−) from rabbits fed on a hypercholesterolemic (HD) or a control diet (CD). Each data point shows the mean of 8 experiments and vertical lines indicate S.E.M. *$P<0.05$ indicates differences statistically significant with respect to arteries with intact endothelium from rabbits fed on a control diet (CD−E+) (ANOVA and Duncan's test).

3.2. Response to angiotensin II

Angiotensin II-contractile response and affinity were increased in endothelium intact arteries from rabbits fed on a hypercholesterolemic diet compared with rabbits fed on a control diet. There were no differences in the contractile response between rubbed arteries in both diet groups (Fig. 2). By contrast, the response of aortic rings to noradrenaline 5×10^{-6} M was lower in unrubbed rings from hypercholesterolemic rabbits than in those ones from unrubbed control or rubbed hypercholesterolemic rabbits (2000±178 mg vs 3365±544 mg vs 3477±634 mg, $n=24$, $P<0.05$, ANOVA and Duncan's post test).

Incubation of aortic rings with indomethacin 10^{-5} M, NS 398 10^{-7} M or SQ29548 10^{-6} M have not modified the contractile response to angiotensin II in rabbits fed a control diet. However, indomethacin 10^{-5} M and SQ29548 10^{-6} M were capable of blocking the increase in the contractile response which had been observed in rabbits fed on an enriched cholesterol diet (Fig. 3).

NS 398 10^{-7} M had no effect in this phenomenon, but as well as indomethacin diminished the affinity to angiotensin II in hypercholesterolemic rabbit aortic rings (Table 3). Treatment of arteries from rabbits fed on a control diet with 17-ODYA

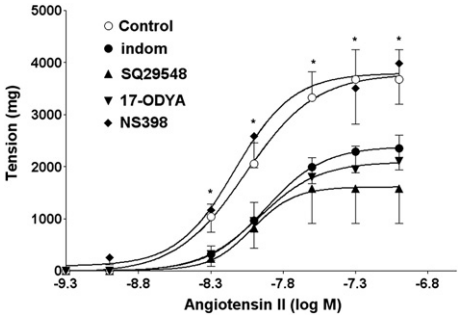

Fig. 3. Effects of indomethacin (indom), NS 398, SQ29548 and 17-ODYA on the contractions induced by angiotensin II in aortic rings from rabbits fed on a diet containing 1% cholesterol. Each data point shows the mean of 8 experiments and vertical lines indicate S.E.M. *$P<0.05$ indicates differences statistically significant between arteries without treatment (control) and arteries treated with indomethacin, SQ29548 or 17-ODYA (ANOVA and Duncan's test).

10^{-6} M increased the reactivity and affinity to angiotensin II (Table 3). This effect was blocked by incubation with indomethacin 10^{-5} M (Non treated arteries: R_{max}= 2740±251 mg vs 17-ODYA: R_{max}= 5237±935 mg vs 17-ODYA plus indomethacin: R_{max}= 2334±371 mg; n= 8, P<0.05, ANOVA with Duncan's test). However, 17-ODYA was able to prevent the increase in the contractile response and to diminish the affinity to angiotensin II in aortic rings from hypercholesterolemic rabbits (Fig. 3 and Table 3).

3.3. Electrophysiological studies

The P_m of rubbed and unrubbed arteries from rabbits fed on a control diet was similar in basal and angiotensin II-stimulated conditions. No differences were found in basal conditions in rubbed and unrubbed arteries from rabbits fed on an enriched cholesterol diet. However, in this diet group the P_m of arteries with endothelium was significantly less negative 10 min after angiotensin II-stimulation (A10) (Fig. 4). P_m values were not modified with the incubation of arteries from control or hypercholesterolemic diet with indomethacin 10^{-5} M. By contrast, 17-ODYA was able to prevent the depolarization caused by angiotensin II in unrubbed arteries from hypercholesterolemic rabbits (Fig. 4).

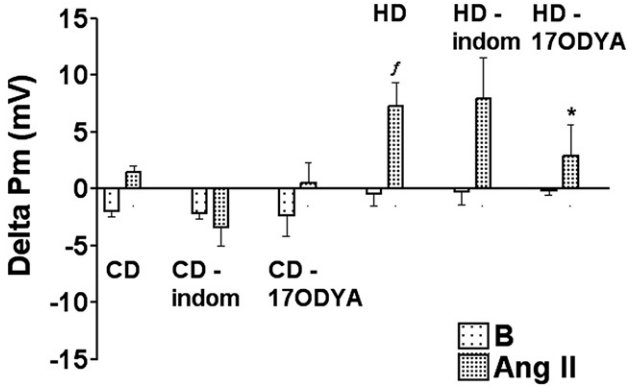

Fig. 4. Effects of indomethacin (indom) and 17-ODYA on the resting membrane potentials (Pm) of unrubbed arteries from rabbits fed either on a control diet (CD) or hypercholesterolemic diet (HD). Values are expressed as differences (Delta) between Pm before angiotensin II-stimulation and after 1 min of angiotensin II-stimulation (B), as well as between Pm after 1 min and after 10 min of angiotensin II-stimulation (Ang II). Each bar shows the mean of 8 experiments and vertical lines indicate S.E.M. *P<0.05 indicates differences statistically significant between arteries from rabbits treated with 17-ODYA and control arteries (ANOVA with Duncan's test). ^{f}P<0.05 indicates differences statistically significant between arteries from hypercholesterolemic rabbits and arteries from rabbits fed on a control diet.

4. Discussion

In the present study, rabbits fed on a diet enriched with cholesterol showed higher plasma levels of cholesterol and LDL-cholesterol than animals fed a control diet. No differences were observed in HDL-cholesterol, triglycerides, blood glucose levels, body weight increase and mean arterial blood pressure among both diet groups at the end of the experiment. These results indicate that the present was a non-obese, non-diabetic, hypercholesterolemic model without risk factors associated.

According to other studies (Verbeuren et al., 1986; Chappel et al., 1987), the present findings show endothelial dysfunction in aortic rings from hypercholesterolemic rabbits. Relaxation in response to acetylcholine, an endothelium-dependent vasodilator, was clearly blunted in arteries from animals with experimental hypercholesterolemia. However, Sodium nitroprusside (an endothelium-independent vasodilator), caused similar relaxation in both diet groups.

Thus, these data confirm previously reported studies in experimental animals and humans. It indicates that during hypercholesterolemia and during early stages of atherosclerosis, the most common vascular functional alteration is a reduction of endothelium-dependent relaxation (Sorensen et al., 1994). This phenomenon would be caused by a diminished availability of NO as the result of enhanced-production of oxidized-low density lipoproteins. This enhanced-production has been demonstrated to reduce the expression of nitric oxide synthase (NOS) by endothelial cells (Blair et al., 1999).

As it was stated in the Introduction, a previous work (Jerez et al., 2005) has shown increase in the endothelium release of COX-dependent vasoconstrictor prostanoids during NOS inhibition. Enhancement of free radical production might be responsible for the COX-dependent activation pathway towards the vasoconstrictor prostanoid synthesis in this experimental condition.

According to these data, the current study has checked the hypothesis that increased production of free radicals would cause enhancement in COX-dependent metabolites availability. These metabolites might participate in the reduction of the acetylcholine relaxation observed in aortic rings from hypercholesterolemic rabbits. It has been found that treatment with indomethacin normalized endothelium-dependent relaxation in hypercholesterolemic rabbits whereas neither tempol nor NS 398 had any effect in this phenomenon. These results would show that COX-1 dependent metabolites but not free radicals might be the responsible for the reduction in the endothelium-dependent relaxation observed in the present model of hypercholesterolemia. Considering the data from the literature

Table 3
pEC_{50} to angiotensin II in aortic rings from rabbits fed on either a control diet or a diet containing 1% cholesterol

	Control diet	Hypercholesterolemic diet
Control	7.91±0.05	8.05±0.06 [a]
Indomethacin	8.00±0.07	7.90±0.07 [b]
NS 398	8.08±0.10	7.76±0.15 [b]
SQ29548	8.00±0.08	8.02±0.04
17-ODYA	8.18±0.08	7.86±0.13 [a, b]

Values are expressed as means±S.E.M. of 8 experiments.
[a] P<0.05 indicates statistically significant differences between rabbits fed on a control diet and rabbits fed on an hypercholesterolemic diet.
[b] P<0.05 indicates statistically significant differences between arteries from hypercholesterolemic rabbits treated with indomethacin, NS 398 or 17-ODYA and non treated arteries (control) (ANOVA and Duncan's test).

previously mentioned, it might be hypothesized that reduced expression of NOS but not NO-degradation increase caused by free radicals account for the endothelial dysfunction observed. This lower expression of NOS might be caused, in addition to enhanced-production of oxidised-low density lipoproteins, by an increase in the availability of COX-1 constricting factors. In agreement with these data, Vasalle et al. (2003) found that indomethacin significantly stimulated the release of NO and eNOS expression. The mechanism for such effect of COX-1 dependent metabolites has not been yet elucidated.

Furthermore, indomethacin and tempol improved acetylcholine affinity in arteries from rabbits fed on both diets. This would indicate a diet-independent effect of these drugs on the receptor-affinity to acetylcholine.

The present study demonstrated that contractile response and sensitivity to angiotensin II was enhanced in hypercholesterolemic rabbits. These data are in agreement with Yang et al. (1998) who found that supplementation of the diet with cholesterol increased AT_1 receptor expression and constrictor response to angiotensin II in rabbit aortic rings. In addition, van der Linde et al. (2006) reported that low density lipoprotein-cholesterol levels directly influence angiotensin II sensitivity in healthy, young subjects with isolated hypercholesterolemia. By contrast, the contractile response to noradrenaline was decreased in arteries from hypercholesterolemic rabbits, which is in agreement with data from the literature (Verbeuren et al., 1986; Du and Woodman, 1992).

However, the contractile response to angiotensin II was enhanced in endothelium intact aortic rings from hypercholesterolemic rabbits compared with those rings from control diet rabbits. The response to angiotensin II in hypercholesterolemic aortic rings with endothelium had the same magnitude as control arteries without endothelium. Therefore, the lack of differences between angiotensin II-contractile response in hypercholesterolemic arteries with and without endothelium support the view that endothelial dysfunction would have a role in the angiotensin II-reactivity increase.

The effect of indomethacin and NS 398 on the angiotensin II-reactivity increase was checked according to previously mentioned results about endothelial dysfunction in hypercholesterolemic rabbits caused by an increase in the availability of COX-1 constricting factors and the reduced production of NO. Indomethacin and NS 398 had no effect on the contractile response to angiotensin II either in rubbed or unrubbed arteries from control rabbits, which is in agreement with Gruetter et al. (1988). However, indomethacin but not NS 398 blocked the increase on the contractile response to angiotensin II in endothelium intact aortic rings from hypercholesterolemic rabbits. These results suggest a role of COX-1 dependent metabolites in the angiotensin II-reactivity improvement. This is in agreement with the findings of Qi et al. (2006). They found that COX-1 is the major isozyme in vasculature and it is responsible for the basal aortic production of TXA_2 and prostacyclin. Furthermore, they demonstrated that aortic vasodilator prostaglandins have not shown significant increase after acute angiotensin II infusion. This suggests that the aorta may not be the major site where COX-2 plays an important role in regulating blood pressure.

The role of TXA_2/PGH_2 receptors in the present model of hypercholesterolemia has been checked by taking into account that COX-dependent vasoconstrictor prostanoid released during NOS inhibition acts through these receptors (Jerez et al., 2005). In that sense, it was found that SQ29548 was able to avoid angiotensin II-reactivity increase.

Numerous data in the literature reported the role of the cytochrome P_{450} metabolites of arachidonic acid in the control of cardiovascular function (Roman, 2002). Kaide et al. (2003) reported that cytochrome P_{450} 4A1 metabolites increases vascular reactivity; Escalante et al. (1993) demonstrated that 20-HETE is an endothelium-dependent vasoconstrictor in rabbit arteries and Carroll et al. (1987) reported that the 20-HETE is converted by COX to a vasoconstrictor prostaglandin H_2 (PGH_2) analogue (20-OH PGH2). Schwartzman et al. (1989) found that 20-HETE metabolites constrict rat aortic rings and the contractile response was partially dependent on the presence of endothelium and was abolished by pretreatment of the rings with either indomethacin or SQ29548. In agreement with these data, the present study found that 17-ODYA, in addition to indomethacin and SQ29548, was able to block the increase on the contractile response to angiotensin II in unrubbed arteries from hypercholesterolemic rabbits.

By taking all these data into account, the results of the present work suggest that in conditions of endothelial dysfunction caused by high cholesterol diet there would be an improvement either in the COX-1 and/or omega hydroxylase metabolites production. In such condition, an increase in 20-HETE levels would sensitize smooth muscle cells to angiotensin II. Furthermore, 20-HETE partially might be metabolized by COX to 20-hydroxyendoperoxides, which act thought TXA_2/PGH_2 receptors. Moreover, Sun et al. (1998) demonstrated that NO inhibits 20-HETE-synthesis, therefore an improvement in 20-HETEs production may be caused by diminished levels of NO. Wang et al. (2006) supported this point of view and they found that augmentation in vascular 20-HETE promotes the development of hypertension and causes endothelial dysfunction.

On the other hand, it has been found that 17-ODYA induced increase on the contractile response to angiotensin II in unrubbed and rubbed control arteries (data not shown). This effect disappears when 17-ODYA-treated arteries were incubated with indomethacin. According to these results, it would be stated that in physiological conditions, the inhibition of omega hydroxylase would shift the metabolic pathway of arachidonic acid angiotensin II-stimulated towards vasoconstrictor COX-derived metabolites. This effect is endothelium-independent and it would account for the increase in the contractile response to angiotensin II observed in the present conditions.

Numerous authors reported that 20-HETE increases intracellular Ca^{2+} and promotes vasoconstriction. This omega hydroxylase metabolite blocks the large-conductance Ca^{2+}-activated K+ channels (Zou et al., 1996; Lange et al., 1997) and it has a direct effect on L-Type Ca channels (Gebremedhin et al., 1998). These effects cause depolarization of vascular smooth

muscle membrane. Jerez et al. (2005) demonstrated previously that prostanoid released in L-NAME treated arteries may act by blocking Ca^{2+}-activated K+ channels. According to these data and those mentioned above about the improvement in the 20-HETE production in hypercholesterolemic arteries, it has been checked the hypothesis that the depolarization effect of angiotensin II-treatment in smooth muscle cells from hypercholesterolemic rabbits is higher than control arteries.

Angiotensin II caused no effect on Pm of arteries from control diet rabbits but was able to depolarize unrubbed aortic rings from hypercholesterolemic rabbits. This effect was blocked by 17-ODYA but not by indomethacin. These findings show that the mechanism of sensitization to angiotensin II-response involves changes in the membrane potential caused by an endothelial product of arachidonic acid metabolism.

In conclusion, results of the present work show COX-1-dependent endothelial dysfunction caused by high cholesterol diet. In these conditions, there was an increase of the angiotensin II-reactivity induced by COX-1 and/or omega hydroxylase vasoconstrictor metabolites. Deficit in the NO-production might improve 20-HETE-synthesis, which induces sensitization of the smooth muscle to angiotensin II. In addition, 20-HETE would be metabolized by COX-1 to 20-endoperoxides which act through TXA_2/PGH_2 contributing to angiotensin II-reactivity increase.

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