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Angiotensin Type 1 Receptor Blockade Prevents the Decrease in Conduit Artery Flow-Mediated Dilatation during NO-synthase Inhibition in Humans.

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ABSTRACT

Whether AT₁ receptor blockade can prevent the decrease in conduit artery flow-mediated dilatation during NO-synthase inhibition by endothelial alternative pathways has not previously been explored in humans. In 12 healthy subjects, we measured radial artery diameter (echotracking) and flow (Doppler) during flow-mediated dilatation induced by sustained reactive hyperemia in control period and following NO-synthase inhibition (L-NMMA: 1.5 mg.min⁻¹.L⁻¹), after single oral administration of telmisartan (80 mg) or placebo, during a double-blind, randomized, cross-over study. In 6 volunteers, we also assessed the roles of prostacyclin and endothelium-derived hyperpolarizing factor during radial flow-mediated dilatation after AT₁ receptor blockade by oral administration of aspirin (500 mg) alone and associated with L-NMMA and by the addition of the cytochrome epoxigenases inhibitor fluconazole (0.37 mg.min⁻¹.L⁻¹). Telmisartan did not affect radial artery flow-mediated dilatation in control period (placebo: 10.9±0.6% vs. telmisartan: 9.9±0.7%) but prevented its decrease after L-NMMA (placebo: 9.3±0.8% vs. telmisartan: 12.6±1.2%, *P*<0.05) with no modification in baseline parameters, hyperemia and radial artery endothelium-independent dilatation to sodium nitroprusside. Moreover, in telmisartan-treated subjects, the radial flow-mediated dilatation, compared with control (9.0±1.0%), was not modified by aspirin alone (9.4±0.7%) or associated with L-NMMA (9.5±0.5%) but in fact was reduced by the combination of aspirin, L-NMMA and fluconazole (7.5±0.6%, *P*<0.05). These results demonstrate that AT₁ receptor blockade prevents the decrease in conduit artery flow-mediated dilatation during NO-synthase inhibition in humans suggesting the development of an endothelial compensatory mechanism. This mechanism seems to be independent of prostacyclin and could possibly be related to an endothelium-derived hyperpolarizing factor release.

INTRODUCTION

Endothelium plays a crucial role in conduit artery flow-mediated dilatation (FMD) and participates in the control of arterial tone and trophicity by releasing vasoactive factors mainly nitric oxide (NO) [1,2]. A decrease in arterial NO availability is associated with abnormal vasomotion, smooth muscle cell proliferation and migration, monocyte adhesion and procoagulant state that characterize endothelial dysfunction and contribute to the pathophysiology of cardiovascular diseases [3,4]. Increasing reported data demonstrate that the local activation of the renin-angiotensin system participates in the development of endothelial dysfunction through the stimulation of angiotensin II type 1 (AT₁) receptor subtype and the inactivation of NO [5-7].

AT₁ receptor antagonist are specific agents that block the effects of angiotensin II mediated through AT₁ receptors thus preserving the potential beneficial activity of the angiotensin II type 2 (AT₂) receptors on vascular tone and blood pressure [6,8]. In humans, despite some conflicting results [9,10], the administration of AT₁ receptor antagonists enhances the FMD of conduit arteries in cardiovascular diseases associated with endothelial dysfunction [11-15]. However, the action mechanisms of these agents on endothelial function, to our knowledge, have not been fully investigated in humans. In patients with coronary artery disease, the beneficial effect of AT₁ receptor blockade on the endothelial function of peripheral conduit arteries is mainly related to the increase in both NO and bradykinin availability [12,14]. In addition, it has been demonstrated in animal models with hypertension or renal failure that chronic treatment with antagonists can also enhance the endothelium-dependent dilatation of isolated mesenteric arteries during combined inhibition of NO and prostacyclin (PGI₂) synthesis [16,17]. Under these experimental conditions, this beneficial effect of AT₁ receptor blockade is due to the release of an endothelium-dependent hyperpolarizing factor (EDHF) [16,17] that has not yet been identified but could be related to cytochrome P450 (CYP)-derived products [18]. In humans, whether AT₁ receptor blockade can prevent or not the decrease in conduit artery FMD during NO deficiency by an endothelial alternative pathway remains unknown.

In this context, the main objective of this work was to evaluate in humans the effect of the acute administration of an AT₁ receptor antagonist on the FMD of peripheral conduit arteries before and during NO-synthase inhibition. Moreover, in an exploratory approach, we tested whether the effect of AT₁ receptor antagonist on FMD during NO deficiency remains constantly during the inhibition of PGI₂ and CYP-related product synthesis.

METHODS

Subjects

A total of 18 healthy male volunteers were included in this study. None had evidence of hypertension (blood pressure >140/90 mmHg), hyperlipidemia (total serum cholesterol >200 mg/dl), or clinical evidence of cardiovascular disease and diabetes on the basis of previous medical history and complete medical examination, normal electrocardiogram and recent routine laboratory tests. No participants were smokers or taking medication at the time of the study. The forearm volume was measured in each subject by using the water displacement method based on the Archimedes' principle and drug infusion rates were normalized to 1 liter tissue forearm by alteration of the drug concentration in the solvent while the pump speed of infusion was maintained constant. The research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the Consultative Committee for the Protection of Persons Engaged in Biomedical Research of Haute-Normandie. All participants gave informed written consent after full explanation of the purpose, nature and risk of all procedures used.

Instrumentation

Measurements were performed at 08:00 in the morning, one hour after a fat free breakfast without tea or coffee, while subjects were supine in a quiet air-conditioned room, maintained at a constant temperature (22-24°C). A 27-gauge needle was inserted under local anesthesia (lidocaine 1%) into the brachial artery of the non-dominant arm to allow the local infusion of physiological saline and pharmacological agents at constant rate (1 ml/min). Systemic blood pressure and heart rate were measured by means of a brachial cuff oscillometric device (Dinamap, 8103, Critikon, Johnson & Johnson Santé, France). Radial internal diameter, blood flow velocity and digital blood pressure were continuously measured using a high-precision echotracking device (Nius 02, Asulab, Switzerland; 10 MHz) coupled to a Doppler device (Doptek, Deltex, UK; 8 MHz) and a finger photoplethysmograph (Finapres 2300, Ohmeda, CO) as previously described [1,19-23]. This device allows the measurement of radial artery internal diameter with a resolution on the diameter evolution of <1 μ m [23]. Radial artery flow was based on the measurement of velocity and diameter. In absence of change in mean blood pressure, the variations of radial artery flow observed with the pharmacological agents reflects the changes in radial vascular resistance which is usually calculated as the ratio of mean blood pressure to radial artery flow [24].

Study protocols

Randomized double-blind placebo-controlled cross-over study: Effect of AT₁ receptor blockade on radial artery FMD before and during NO-synthase inhibition

Twelve healthy subjects (23 ± 1 years) were explored on 2 separate days with a two-week washout period between each day. Subjects were allowed to rest 30 minutes after instrumentation. Then heart rate and blood pressure were recorded and telmisartan (80 mg per os) or placebo were administered. After 2 hours, blood pressure, heart rate, radial artery blood flow and diameter were recorded for 5 minutes. Then, an arterial occlusion cuff placed on the wrist was inflated for 10 minutes at 180 mm Hg and was deflated to allow reactive hyperemia with the continuous measurements of all parameters. Based on preliminary results, we used a 10-min occlusion protocol to induce a maximal increase in radial artery diameter [22] with a significant participation of NO [19]. After radial artery blood flow and diameter had returned to baseline, the subjects received sodium nitroprusside (SNP) at the incremental doses of 1.31, 2.62, and 5.24 $\mu\text{g} \cdot \text{mg} \cdot \text{min}^{-1} \cdot \text{L}^{-1}$ (5, 10 and 20 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{L}^{-1}$), 3 min for each dose to assess the endothelium-independent dilatation [21]. Once radial artery diameter had returned to baseline, subjects received the NO-synthase inhibitor *N*^G-monomethyl-L-arginine (L-NMMA, Clinalfa, Switzerland) at the dose of 1.5 $\text{mg} \cdot \text{min}^{-1} \cdot \text{L}^{-1}$ (8 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{L}^{-1}$) for 7 min. This dose of L-NMMA induced a prolonged inhibition of NO-synthase of at least 40 min and blocks the forearm and radial artery vasodilatation to acetylcholine administrated at doses producing maximal regional effect without systemic hemodynamic change [21]. Five min after L-NMMA infusion, all parameters were again measured at baseline and the same occlusion-ischemia and SNP infusion protocol was repeated. Baseline and peak measurements represented means of values obtained during 15 and 3 cardiac cycles respectively. The continuous measurement of radial artery parameters allows to precisely estimate the maximal values of diameter and flow occurring after cuff deflation. Thus, radial artery FMD was calculated as the percent change of radial artery diameter in response to post-ischemic hyperemia with the peak diameter after cuff deflation and the basal diameter before cuff inflation (figure 1): $\text{FMD (\%)} = (\text{peak diameter} - \text{basal diameter}) \times 100 / \text{basal diameter}$.

Moreover, because the modifications in the hyperemic stimulus influence the magnitude of radial artery FMD, we also measured the parameters characteristic of this stimulus: the peak flow representing the maximal value of flow after cuff deflation and the area under the curve of flow (AUC of flow) representing the duration of the increase in flow (figure 1) [22].

The echotracking measures were performed by the same observer (MI) and were read with the assistance of 2 other investigators (JB, RJ) all blinded to the subjects and treatments.

Exploratory study: Role of PGI₂ and EDHF on radial artery FMD during AT₁ receptor blockade.

Six healthy subjects (23±2 years) participated in a complementary open study. Radial artery FMD was evaluated in response to four reactive hyperemias (wrist occlusion: 10 min) performed successively. First, the radial artery FMD was evaluated under control conditions prior to telmisartan administration. Then, each subject received a single oral dose of telmisartan (80 mg) followed, 90 min later, by a single oral dose of aspirin (500 mg) to block vascular cyclooxygenase activity 30 min after administration and during the next 6 hours [20,25]. Thirty minutes after aspirin, a second reactive hyperemia was performed. After an additional 30 min resting period and the return to baseline measurements, subjects received L-NMMA at 1.5 mg.min⁻¹.L⁻¹ (8 µmol/min/L) for 7 min followed by the third reactive hyperemia to evaluate a possible interaction between PGI₂ and NO during FMD. Finally, after obtaining baseline measurements again, a last reactive hyperemia was performed after the coinfusion during 7 min of L-NMMA at the same dose as previously with fluconazole, a potent inhibitor of endothelial cytochrome P450 (CYP) 2C9 located in human arteries in the endothelium and identified as EDHF synthase [18]. In fact, *ex-vivo* experiments have demonstrated that CYP 2C9 produces the 11,12-epoxyeicosatrienoic acid that induces the hyperpolarization and relaxation of vascular smooth muscle cells [26]. In addition, we have recently demonstrated using fluconazole that a CYP-derived EDHF is involved with NO in the control of the basal radial artery diameter [20]. Fluconazole was infused at the dose of 0.37 mg.min⁻¹.L⁻¹ (1.2 µmol.min⁻¹.L⁻¹) to obtain a calculated local concentration, according to a radial artery flow at baseline and during reactive hyperemia (10 to 100 ml/min), at least two times higher than the *in vitro* K_i of CYP 2C9 and with a weaker activity on other CYP enzymes [20,27].

Statistical analysis

Results are expressed as mean ±SEM. In the randomized double-blind study, the effect of treatments and L-NMMA on measured parameters were analyzed by ANOVA with treatment (placebo and telmisartan), periods (control and L-NMMA) and subjects as factors and mean blood pressure as a covariate. In addition, the analysis of FMD was performed using the variation of radial artery flow during hyperemia as an additional covariate. Analysis was performed, only when ANOVA was considered significant, using a modified paired t-test adjusted for multiple comparisons. In the exploratory study, the effect of the inhibitors on radial artery FMD and peak flow were analyzed by ANOVA with inhibitors and subjects as

factors and basal values of flow and diameter as a cofactor. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Randomized double-blind study

Systemic hemodynamical parameters (table 1)

Two hours after treatment administration, a significant decrease in mean blood pressure ($P<0.05$) and heart rate ($P<0.001$) was observed in both treatment groups. However, this decrease was not significantly different between treatment groups. The local administration of L-NMMA did not modify the systemic hemodynamical parameters in either groups (table 2).

Radial artery parameters at baseline

In control period, compared with placebo, telmisartan did not modify the basal radial artery flow and diameter (table 2). L-NMMA did not modify the radial artery diameter but decreased radial artery flow similarly in either treatment groups (both $P<0.05$) suggesting, in absence of change in mean blood pressure, a similar increase in local radial vascular resistance.

Post-ischemic hyperemia

Deflation of the occluding cuff was associated, in all cases, with an increase in radial artery flow (figure 1A, $P<0.001$). Concerning the hyperemic stimulus, in control period, telmisartan did not modify either the peak or the AUC of flow and L-NMMA did not modify these parameters in both treatment groups.

Radial artery flow-mediated dilatation

Deflation of the occluding cuff was associated, in all cases, with an increase in radial artery diameter (figure 1B, $P<0.001$). In control period, the radial FMD was not modified by telmisartan ($9.9\pm0.7\%$) as compared with placebo ($10.9\pm0.6\%$). L-NMMA reduced the radial FMD in the placebo group ($P<0.05$) but not in the telmisartan group. Thus, the radial FMD after L-NMMA was enhanced by telmisartan ($12.6\pm1.2\%$) as compared with placebo ($9.3\pm0.8\%$, $P<0.05$).

Radial artery endothelium-independent dilatation

SNP induced a dose-dependent increase in radial artery blood flow (figure 2A, $P<0.05$) and diameter (figure 2B, $P<0.001$) in the two treatment groups that was not modified by telmisartan. L-NMMA enhanced the increase in radial artery diameter at each dose of SNP to the same extent in both treatment groups (both $P<0.05$) without affecting the increase in radial artery blood flow.

Complementary study (table 3)

None of the inhibitors administered affected the systemic hemodynamical parameters. Compared with control conditions before telmisartan and inhibitors administration, the basal radial artery flow was not modified in telmisartan-treated subjects by aspirin but was reduced to the same extent by aspirin+L-NMMA and aspirin+L-NMMA+fluconazole (both $P<0.05$). The basal radial artery diameter, compared with control conditions before telmisartan, was not modified in telmisartan-treated subjects by aspirin or aspirin+L-NMMA but in fact was reduced by aspirin+L-NMMA+fluconazole ($P<0.05$). Deflation of the occluding cuff was associated, in all cases, with an increase in radial artery flow and diameter (both $P<0.001$). The radial artery FMD, compared with control conditions before telmisartan, was not modified in telmisartan-treated subjects by aspirin or aspirin+L-NMMA but was decreased by aspirin+L-NMMA+fluconazole ($P<0.05$) with no modification in peak flow.

DISCUSSION

The main result of the present study performed with healthy subjects was that the acute angiotensin II type 1 receptor blockade by telmisartan maintains the peripheral conduit artery FMD during local NO-synthase inhibition which suggests the development of an endothelial compensatory mechanism to prevent the effects of a decrease in NO availability. This effect of AT₁ receptor blockade on the endothelium-dependent dilatation of conduit arteries appears to be independent of PGI₂ availability and could in fact be related to an increase in the release of an EDHF.

This double-blind study was designed to explore, in healthy subjects, the effect of AT₁ receptor blockade on the FMD of the radial artery, a model of peripheral conduit artery [1,2,12,19-22,28], before and during induced NO deficiency. In the placebo treated group, the local NO-synthase inhibition obtained using L-NMMA decreased the basal radial artery flow without modifying radial artery diameter confirming, in absence of change in mean blood pressure, the role of NO in the control of the basal vascular resistance in humans [1,12,19-21,28]. In addition, L-NMMA enhanced the radial artery dilatation to SNP demonstrating an hypersensitivity of the smooth muscle cells to exogenous NO which has been previously observed both *in vitro* [29] and *in vivo* [19-21]. In fact, removal of the endogenous NO-mediated vasodilator tone in conduit arteries has been shown to lead to a specific hypersensitivity to exogenous NO provided by nitrovasodilators [29]. Thus, this result demonstrates, under experimental conditions, an effective inhibition of NO release by L-NMMA in the radial artery wall. In contrast, the increase in radial artery blood flow in response to SNP infusion was not affected by L-NMMA. As previously stressed [21], this could be related to the weak vasodilator effect observed with SNP infusion in resistance arteries. Furthermore, L-NMMA decreased the radial artery FMD in the placebo group demonstrating the role of NO in the endothelium-dependent dilatation of peripheral conduit arteries in response to a sustained increase in flow induced by a prolonged period of ischemia of 10 min [19,21]. However, this decrease in the magnitude of FMD was only 15% when compared to experiments that have previously demonstrated a major role of NO in this response using shorter periods of ischemia of 3 to 8 min [1,2,28]. This reduced effect could result from the dose of L-NMMA that could have been insufficient to fully inhibit the endothelial NO-synthase. Moreover, as previously underlined [2], this moderate decrease in FMD after L-NMMA could also be explained by the decrease in the role of NO in peripheral conduit artery FMD during sustained hyperemic stimulation suggesting the involvement of other endothelial vasodilator factors under these experimental conditions.

Furthermore, in accordance with previous results [1,2,27,30,31], L-NMMA did not modify the radial artery peak flow during hyperemia supporting the absence of contribution of NO in the maximal arteriolar vasodilatation in response to forearm ischemia. In addition, L-NMMA did not further decrease the duration of hyperemia in the present study in contrast to previous results obtained with brief duration of ischemia [1,2,28]. A similar negative result was also reported after a duration of ischemia of 15 minutes [2] and could be related to the decrease in the relative contribution of NO compared with the direct effect of adenosine in the late phase of flow increase during sustained hyperemia [30].

As regards the acute blockade of the AT₁ receptor, this was due to telmisartan, a high affinity antagonist, administered orally at a single dose of 80 mg which represents the maximal therapeutic dose [32,33]. Systemic hemodynamical and vascular parameters were obtained two hours after treatment intake corresponding to the time of the maximal inhibitory effect of the drug and during the first two hours of its maximal activity [32]. In fact, a single administration of this dose of telmisartan has been shown to prevent in healthy volunteers 90% of the pressure increase in response to exogenous angiotensin II demonstrating under similar conditions than ours an effective and near maximal degree of AT₁ receptor blockade [32]. As expected from our population, the acute AT₁ receptor blockade caused a mild but non significant decrease in blood pressure [32]. In fact, this absence of decrease in blood pressure, which was also observed by Stangier and coworkers [32], is not an indicator of a non significant AT₁ receptor blockade but confirms the absence or the low involvement of the renin-angiotensin system in the short-term regulation of blood pressure in sodium-replete healthy subjects [34,35]. However, as a negative relationship was demonstrated between blood pressure and the magnitude of conduit artery FMD, we used blood pressure as a covariate into the statistical analysis to avoid any passive effect of this parameter on the responses observed [36]. During the control period, telmisartan did not significantly modify the radial artery flow at baseline nor the hyperemic response confirming that the renin-angiotensin system, under physiological conditions, was not involved in the acute control of basal and post-ischemic peripheral vascular tone [14,28,34,35]. Moreover, telmisartan did not modify the magnitude of radial artery FMD as previously reported in healthy subjects using repeated administration of losartan and the angiotensin converting enzyme inhibitor quinapril [37]. In contrast, during endothelial NO-synthase inhibition, the acute AT₁ receptor blockade prevented the decrease in the radial artery FMD observed in the placebo-treated group. This effect was not related to a modification in the smooth muscle cells sensitivity after AT₁ receptor antagonist administration because the endothelium-independent dilatation assessed

by SNP was comparable in both treatment groups before and after L-NMMA. In addition, it appears unlikely that telmisartan could have increased the release of NO during FMD by enhancing endothelial NO-synthase activity to a level which makes this enzyme insensitive to the dose of L-NMMA administered. In fact, the persistence of the hypersensitivity to SNP observed in the telmisartan group after L-NMMA demonstrated the effective decrease of NO availability in the arterial wall. Moreover, the magnitude of this hypersensitivity was similar as that after placebo which demonstrated that, even if not maximal, a stable level of NO-synthase inhibition was achieved in both treatment groups. This last point shows that maintaining FMD during NO-synthase inhibition following AT₁ receptor blockade was not related to the increase in NO availability. Furthermore, this effect was not related to an increase in the hyperemic stimulus because telmisartan did not significantly affect the peak flow nor the duration of the increase in flow. Therefore, this result suggests the development of an endothelial compensatory mechanism after AT₁ receptor antagonists administration, probably the increase in the release by the endothelium, in response to flow, of an other vasodilator factor to compensate for the loss of NO synthesis. It is unlikely that telmisartan could have increased the release of PGI₂ under our conditions as we observed, in the complementary study, that the cyclooxygenase inhibitor aspirin administered alone or associated with L-NMMA no longer affected the radial artery FMD in the healthy subjects treated with the AT₁ receptor antagonist when compared to control. This result is consistent with previous reported data showing that a compensatory increase in PGI₂ release was only observed after chronic NO-synthase inhibition in animals [38] and that the inhibition of cyclooxygenase did not modify radial artery FMD in response to sustained and non sustained hyperemic stimulations even in presence of L-NMMA [1,2].

Since the combined inhibition of NO and PGI₂ synthesis did not affect radial artery FMD in the telmisartan-treated volunteers, this beneficial effect of AT₁ receptor blockade on the conduit artery endothelial vasomotor function during induced NO deficiency could be explained by the increase in the release of an EDHF. This hypothesis is consistent with previous experiments performed in aged and hypertensive animals and in experimental renal failure demonstrating that AT₁ receptor antagonists administration can enhance the EDHF-mediated dilatation of mesenteric arteries in response to acetylcholine during NO-synthase and cyclooxygenase inhibition [16,17,39]. Although the EDHF involved in this effect has not been identified, we coinjected fluconazole with L-NMMA to evaluate the role of CYP epoxygenases in radial artery FMD during AT₁ receptor blockade. In fact, previous *ex-vivo* studies have shown that FMD is mediated by CYP-related metabolites during NO deficiency

in human coronary and mice mesenteric arterioles [40,41]. In addition, an interaction between NO and an endothelial CYP-dependent vasodilator pathway participates in the regulation of exercise-induced skeletal muscle blood flow in humans *in vivo* [42]. Under these conditions, the basal radial artery diameter was reduced by the combination of aspirin, L-NMMA and fluconazole according to our previous study performed in healthy subjects without treatment that showed the role of a CYP-related EDHF in the regulation of radial artery diameter at baseline [20]. During reactive hyperemia, with combined blockade of both NO and PGI₂ synthesis, the infusion of fluconazole was associated with a lesser increase in diameter with no modification in peak flow compared to the control experiments. This demonstrates for the first time that a CYP-dependent vasodilator pathway is involved under these experimental conditions in human peripheral conduit artery FMD. Although the reduction in the magnitude of radial artery FMD via the addition of fluconazole in subjects treated with telmisartan was similar with that observed after L-NMMA in the placebo group, additional dedicated experiments are required to confirm whether the administration of AT₁ receptor antagonists increases the influence of a CYP-dependent vasodilator pathway to maintain conduit artery FMD during the loss of NO synthesis in humans.

Furthermore, it would be interesting to evaluate if the endothelial alternative mechanism involved in the beneficial effect of AT₁ receptor blockade could compensate a greater magnitude of decrease in NO-dependent FMD similar to those provided by L-NMMA during shorter occlusion periods. In addition, whether the development of this NO- and PGI₂-independent mechanism following the administration of AT₁ receptor antagonists is effective in pathological states characterized by a decrease in NO availability warrants further investigation. This effect could be particularly important to maintain an adequate vascular conductance and cardiovascular coupling and to limit the progression of cardiovascular diseases in particular atherosclerosis.

Conclusion

These results demonstrate that acute AT₁ receptor blockade opposes the decrease in conduit artery flow-mediated dilatation during induced NO deficiency in humans suggesting the development of an endothelial compensatory mechanism. Although this mechanism is independent of PGI₂, it may be related to the release of an EDHF.

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REFERENCES

1. Joannides R., Haefeli W., Linder L., et al. (1995) Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* **91**, 1314-1319.
2. Mullen M.J., Kharbanda R.K., Cross J., et al. (2001) Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo. Relevance to endothelial dysfunction in hypercholesterolemia. *Circ. Res.* **88**, 145-151.
3. Mombouli J.V., and Vanhoutte P.M.(1999) Endothelial dysfunction: from physiology to therapy. *J. Mol. Cell. Cardiol.* **31**, 61-74.
4. Quyyumi A.A. (2003) Prognostic value of endothelial function. *Am. J. Cardiol.* **91**, 19H-24H.
5. Fleming I. (2006) Signaling by the angiotensin-converting enzyme. *Circ. Res.* **98**, 887-896.
6. Nickenig G., and Harrison D.G. (2002) The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis. Part I: Oxidative stress and atherogenesis. *Circulation* **105**, 393-396.
7. Mollnau H., Wendt M., Szocs K., et al. (2002) Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ. Res.* **90**, e58-e65.
8. You D., Loufrani L., Baron C., Levy B.I., Widdop R.E., and Henrion D. (2005) High blood pressure reduction reverses angiotensin II type 2 receptor-mediated vasoconstriction into vasodilation in spontaneously hypertensive rats. *Circulation* **111**, 1006-1011.
9. Anderson T.J., Elstein E., Haber H., and Charbonneau F. (2000) Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilatation in patient with coronary disease (BANFF study). *J. Am. Coll. Cardiol.* **35**, 60-66.
10. Ghiadoni L., Magagna A., Versari D. et al. (2003) Different effect of antihypertensive drugs on conduit artery endothelial function. *Hypertension* **41**, 1281-1286.
11. Giannattasio C., Achilli F., Grappiolo A. et al. (2001) Radial artery flow-mediated dilatation in heart failure patients. Effects of pharmacological and nonpharmacological treatment. *Hypertension* **38**, 1451-1455.
12. Hornig B., Kohler C., Schlink D., Tatge H., and Drexler H. (2003) AT1-receptor antagonism improves endothelial function in coronary artery disease by a bradykinin/B2-receptor-dependent mechanism. *Hypertension* **41**, 1092-1095.

13. Koh K.K., Han S.H., Chung W.J., et al. (2004) Comparison of effects of losartan, irbesartan, and candesartan on flow-mediated brachial artery dilation and on inflammatory and thrombolytic markers in patients with systemic hypertension. *Am. J. Cardiol.* **93**, 1432-1435.
14. Prasad A., Tupas-Habib T., Schenke W.H., et al. (2000) Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. *Circulation* **101**, 2349-2354.
15. Wassmann S., Hilgers S., Laufs U., Bohm M., and Nickenig G. (2002) Angiotensin II type 1 receptor antagonism improves hypercholesterolemia-associated endothelial dysfunction. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1208-1212.
16. Goto K., Fujii K., Onaka U., Abe I., and Fujishima M. (2000) Renin-angiotensin blockade improves endothelial dysfunction in hypertension. *Hypertension* **36**, 575-580.
17. Koobi P., Kalliovalkama J., Jolma P., et al. (2003) AT1 receptor blockade improves vasorelaxation in experimental renal failure. *Hypertension* **41**, 1364-1371.
18. Fleming I. (2004) Cytochrome P450 epoxygenase as EDHF synthase(s). *Pharmacol. Res.* **49**, 525-533.
19. Bellien J., Joannides R., Iacob I., and Thuillez C. (2003) Role of nitric oxide in sustained peripheral conduit artery FMD. *Arch. Mal. Coeur Vaiss.* **96**, 832-835.
20. Bellien J., Joannides R., Iacob I., Arnaud P., and Thuillez C. (2006) Evidence for a basal release of a cytochrome-related endothelium-derived hyperpolarizing factor in the radial artery in humans. *Am. J. Physiol. Heart. Circ. Physiol.* **290**, H1347–H1352.
21. Joannides R., Richard V., Haefeli W.E., Linder L., Lüscher T.F., and Thuillez C. (1995) Role of basal and stimulated release of nitric oxide in the regulation of radial artery caliber in humans. *Hypertension* **26**, 327-331.
22. Joannides R., Bakkali E.L.H., Richard V., Moore N., and Thuillez C. (1997) Evaluation of the determinants of flow-mediated radial artery vasodilatation in humans. *Clin. Exper. Hypertens.* **12**, 813-826.
23. Tardy Y., Meister J.J., Perret F., Brunner H.R., and Arditi M. (1991) Non invasive estimate of the mechanical properties of peripheral arteries from ultrasonic and photoplethysmographic measurements. *Clin. Phys. Physiol. Meas.* **12**, 39-54.
24. Benjamin N., Calver A., Collier J., Robinson B., Vallance P., Webb D. (1995). Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension.* **25**, 918-923.

25. Heavey .DJ., Barrow S.E., Hickling N.E., and Ritter J.M. (1985) Aspirin causes short-lived inhibition of bradykinin-stimulated protacyclin production in man. *Nature* **318**, 186-188.
26. Archer S.L., Gragasin F.S., Wu X., et al. (2003) Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation* **107**, 769-776.
27. Venkatakrishnan K., von Moltke L.L., and Greenblatt D.J. (2000) Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. *Clin. Pharmacokinet.* **38**, 111-180.
28. Hornig B., Arakawa N., Kohler C., and Drexler H. (1998) Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* **97**, 363-368.
29. Moncada S., Rees D.D., Schulz R., and Palmer R.M. (1991) Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2166-2170.
30. Costa F., Sulur P., Angel M., Cavalcante J., Haile V., Christman B., and Biaggioni I. (1999) Intravascular source of adenosine during forearm ischemia in humans. Implications for reactive hyperemia. *Hypertension* **33**, 1453-1457.
31. Tagawa T., Imaizumi T., Endo T., Shiramoto M., Harasawa Y., and Takeshita A. (1994) Role of nitric oxide in reactive hyperemia in human forearm vessels. *Circulation* **90**, 2285-2290.
32. Stangier J., Su C.A., Van Heiningen P.N., et al. (2001) Inhibitory effect of telmisartan on the blood pressure response to angiotensin II challenge. *J. Cardiovasc. Pharmacol.* **38**, 672-685.
33. Timmermans P.B. (1999) Pharmacological properties of angiotensin II receptor antagonists. *Can. J. Cardiol.* **15**, F26-F28.
34. Newby D.E., Masumori S., Johnston N.R., Boon N.A., and Webb D.J. (1997) Endogenous angiotensin II contributes to basal peripheral vascular tone in sodium deplete but not sodium replete man. *Cardiovasc. Res.* **36**, 268-275.
35. Baan J. Jr., Chang P.C., Vermeij P., Pfaffendorf M., and van Zwieten P.A. (1999) Effects of sodium depletion on the role of AT1- and alpha-adrenergic receptors in the regulation of forearm vascular tone in humans. *J. Hypertens.* **17**, 229-235.
36. Iiyama K., Nagano M., Yo Y., et al. (1996) Impaired endothelial function with essential hypertension assessed by ultrasonography. *Am. Heart J.* **132**, 779-782.

37. Wilmink H.W., Banga J.D., Hijmering M., Erkelens W.D., Stroes E.S., and Rabelink T.J. (1999) Effect of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor antagonism on postprandial endothelial function. *J. Am. Coll. Cardiol.* **34**, 140-145.
38. Puybasset L., Bea M.L., Ghaleh B., Giudicelli J.F., and Berdeaux A. (1996) Coronary and systemic hemodynamic effects of sustained inhibition of nitric oxide synthesis in conscious dogs – evidence for cross talk between nitric oxide and cyclooxygenase in coronary vessels. *Circ. Res.* **79**, 343-357.
39. Kansui Y., Fujii K., Goto K., Abe I., and Iida M. (2002) Angiotensin II receptor antagonist improves age-related endothelial dysfunction. *J. Hypertens.* **20**, 439-446.
40. Huang A., Sun D., Jacobson A., Carroll M.A., Falck J.R., and Kaley G. (2005) Epoxyeicostrienoic acids are released to mediate shear stress-dependent hyperpolarization of arteriolar smooth muscle. *Circ. Res.* **96**, 376-383.
41. Miura H., Wachtel R.E., Liu Y., et al. (2001) Flow-induced dilatation of human coronary arterioles. Important role of Ca^{2+} -activated K^{+} channels. *Circulation* **103**, 1992-1998.
42. Hillig T., Krstrup P., Fleming I., Osada T., Saltin B., and Hellsten Y. (2003) Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. *J. Physiol.* **546**, 307-314.

FIGURE LEGENDS

Figure 1. Typical recording of the mean values of radial artery diameter (A) and blood flow (B) at baseline before cuff occlusion, during hand ischemia and after deflation of the cuff during hyperemia. Radial artery flow-mediated dilatation (FMD) was calculated as the percent change of radial artery diameter during hyperemia. In addition, peak flow and the area under the curve of flow (AUC of flow) were determined to characterize the hyperemic stimulus.

Figure 2. Bar graphs show the values of radial artery peak flow (A) and the area under the curve (AUC) of flow (B) during reactive hyperemia in the placebo (open bars) and telmisartan groups (solid bars) before (control) and after infusion of L-NMMA. All results are mean \pm SEM of 12 subjects.

Figure 3. Bar graphs show the percent change from baseline of radial artery diameter in response to reactive hyperemia in the placebo (open bars) and telmisartan groups (solid bars) before (control) and after infusion of L-NMMA. All results are mean \pm SEM of 12 subjects. *: $P < 0.05$.

Figure 4. Line graphs show radial artery blood flow (A) and diameter (B) measured at baseline and during the incremental dose of sodium nitroprusside in the placebo group before (○) and after (●) infusion of L-NMMA and in the telmisartan group before (□) and after (■) infusion of L-NMMA. All results are mean \pm SEM of 12 subjects. P : dose effect and *: $P < 0.05$ vs before L-NMMA.

Table 1. Randomized double-blind study: systemic hemodynamical parameters measured at baseline before and 2 hours after placebo and telmisartan administration

Parameters	Treatment	Before	2h after	ANOVA (<i>P</i>)		
				Time	Treatment	Time x treatment
SBP	Placebo	119 ± 2	116 ± 2	0.06	0.90	0.07
(mmHg)	Telmisartan	122 ± 2	114 ± 4			
DBP	Placebo	63 ± 1	63 ± 2	0.13	0.58	0.51
(mmHg)	Telmisartan	63 ± 1	60 ± 2			
MBP	Placebo	82 ± 1	80 ± 1	0.03	0.75	0.07
(mmHg)	Telmisartan	83 ± 2	78 ± 2			
HR	Placebo	68 ± 2	59 ± 2	0.001	0.36	0.54
(bpm)	Telmisartan	70 ± 3	62 ± 2			

Values are mean ±SEM of 12 subjects. SBP, DBP and MBP indicate respectively, systolic, diastolic and mean blood pressure, HR, heart rate.

Table 2. Randomized double-blind study: systemic hemodynamical and radial artery parameters measured at baseline before and during NO-synthase inhibition in the placebo- and telmisartan-treated groups

Parameters	Treatment	ANOVA (<i>P</i>)				
		Control	L-NMMA	Period	Treatment	Period x Treatment
Mean blood pressure (mmHg)	Placebo	84 ± 2	86 ± 2	0.35	0.40	0.99
	Telmisartan	83 ± 2	85 ± 2			
Heart rate (bpm)	Placebo	60 ± 2	60 ± 2	0.30	0.91	0.90
	Telmisartan	64 ± 3	65 ± 3			
Radial artery flow (ml/min)	Placebo	13.7 ± 1.7	9.7 ± 0.8*	0.03	0.90	0.70
	Telmisartan	15.4 ± 2.0	12.3 ± 1.6*			
Radial artery diameter (mm)	Placebo	2.56 ± 0.06	2.56 ± 0.05	0.13	0.58	0.51
	Telmisartan	2.54 ± 0.06	2.53 ± 0.08			

Values are mean ±SEM of 12 subjects. *: *P*<0.05 vs control.

Table 3. Complementary study: systemic hemodynamical and radial artery parameters measured in control conditions before telmisartan and two hours after the administration of telmisartan in the presence of the pharmacological inhibitors

Parameters	Control	Aspirin	Aspirin+ L-NMMA	Aspirin+ L-NMMA+ fluconazole
Mean blood pressure (mmHg)	82 ± 6	81 ± 5	85 ± 5	85 ± 4
Heart rate (bpm)	64 ± 3	63 ± 5	62 ± 5	63 ± 5
Basal radial artery flow (ml/min)	16.4 ± 4.1	17.8 ± 4.2	12.6 ± 2.2*	11.4 ± 2.4*
Basal radial artery diameter (mm)	2.74 ± 0.11	2.77 ± 0.09	2.70 ± 0.11	2.63 ± 0.10*
Peak flow (ml/min)	83±16	89±10	88±18	82±17
Flow-mediated dilatation (%)	9.0±1.0	9.4±0.7	9.5±0.5	7.5±0.6*

Values are mean±SEM of 6 subjects. *: $P<0.05$ vs control.

Figure 1

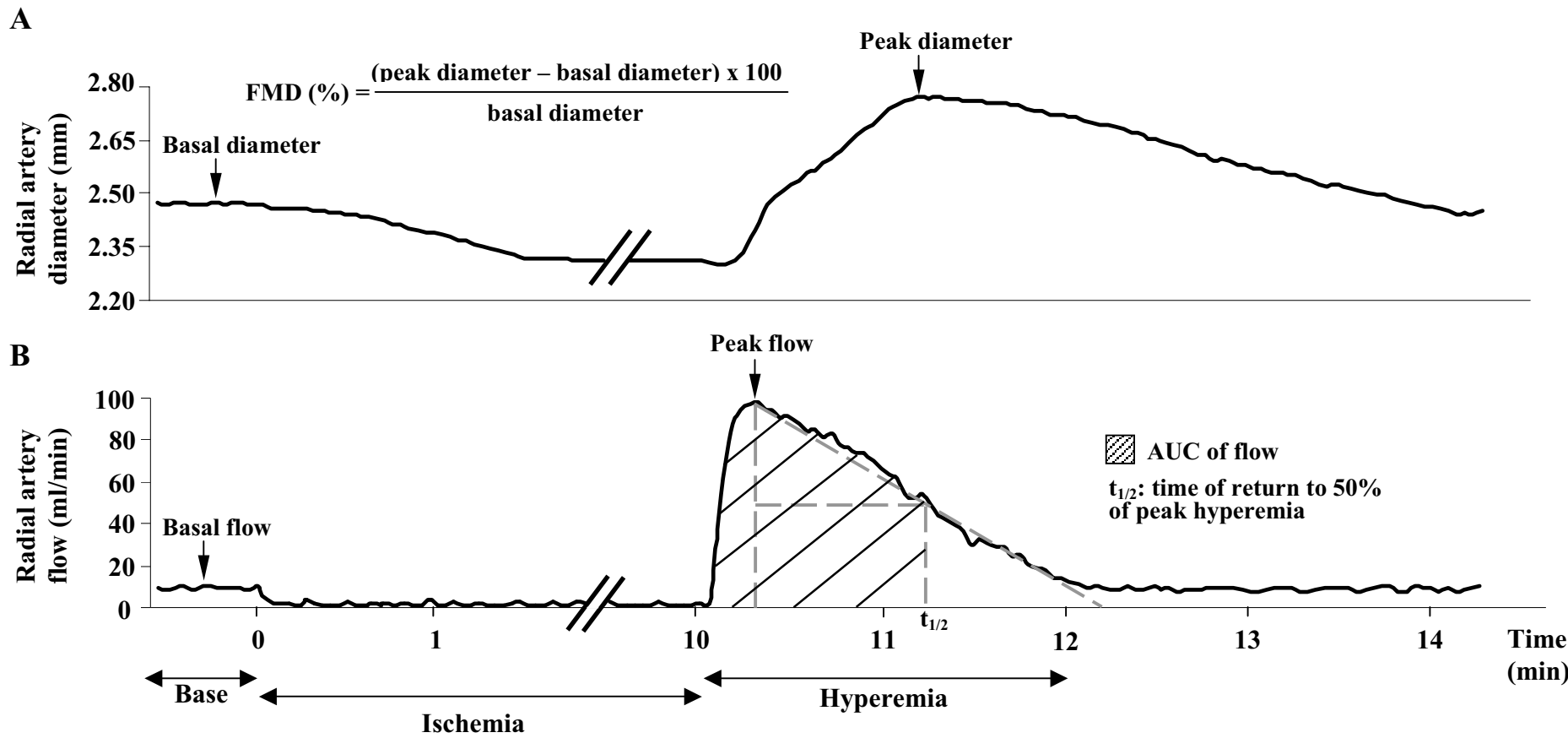


Figure 2

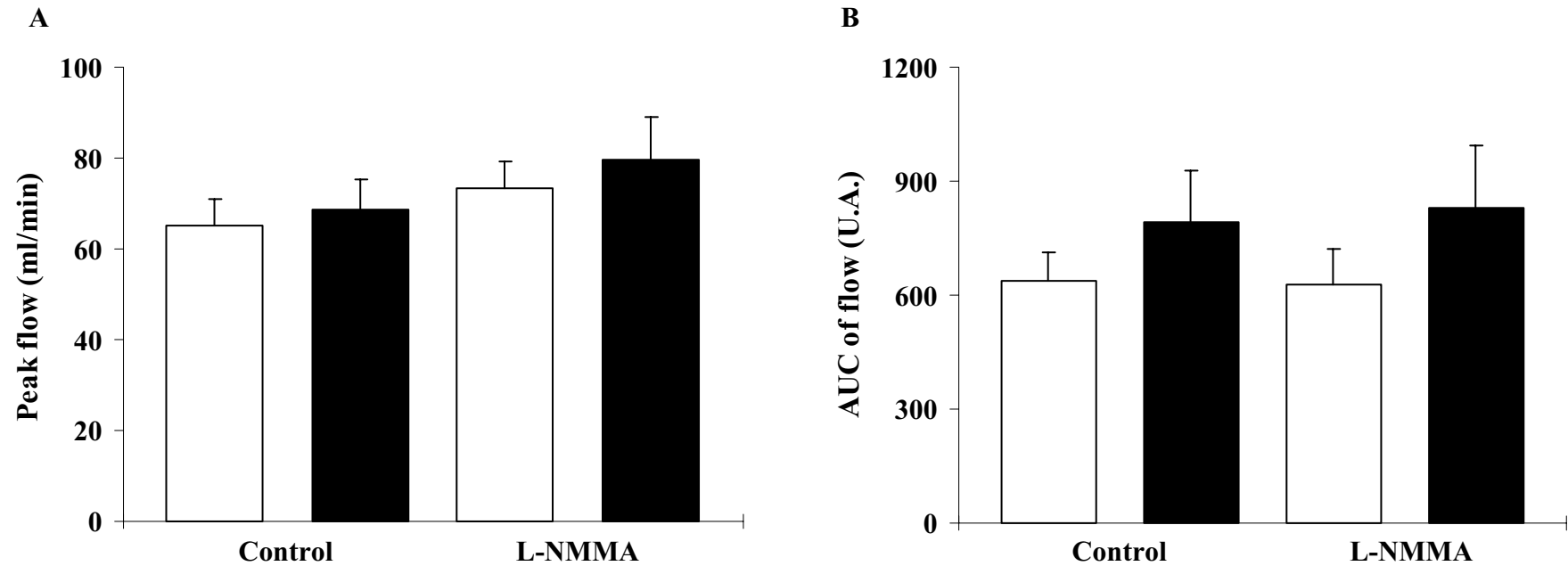


Figure 3

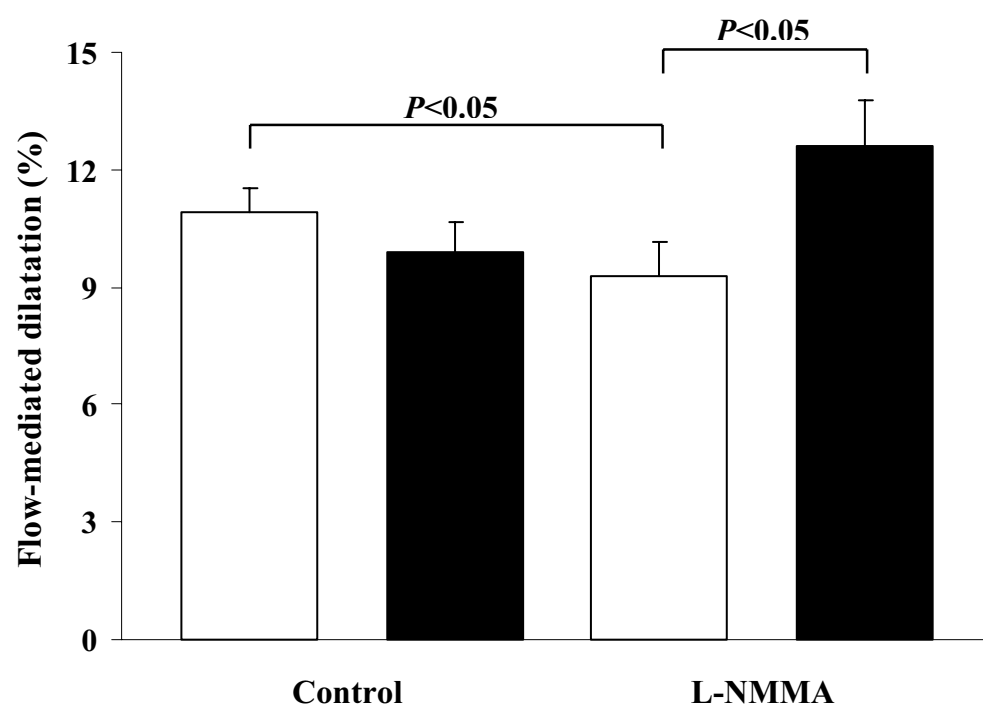


Figure 4

