Impaired Role of Epoxyeicosatrienoic Acids in the Regulation of Basal Conduit Artery Diameter During Essential Hypertension

Jeremy Bellien, Isabelle Remy-Jouet, Michele Iacob, Etienne Blot, Alain Mercier, Daniele Lucas, Yvonne Dreano, Laurence Gutierrez, Nathalie Donnadieu, Christian Thuillez, Robinson Joannides

Abstract—In young healthy subjects, epoxyeicosatrienoic acids synthesized by endothelial cytochrome P450 epoxygenases maintain basal conduit artery diameter during altered NO availability. Whether this compensatory mechanism is effective during essential hypertension is unknown. Radial artery diameter, blood flow, and mean wall shear stress were determined in 14 nontreated essential hypertensive patients and 14 normotensive control subjects during 8 minutes of brachial infusion for inhibitors of cytochrome P450 epoxygenases (fluconazole, 0.4 μmol/min) and NO synthase (N<sup>ω</sup>-monomethyl-l-arginine, 8 μmol/min) alone and in combination. In controls, the radial artery diameter was reduced by fluconazole (−0.034±0.012 mm) and N<sup>ω</sup>-monomethyl-l-arginine (−0.037±0.010 mm) and to a larger extent by their combination (−0.137±0.011 mm), demonstrating a synergic effect. In contrast, the radial diameter in hypertensive patients was not affected by fluconazole (0.010±0.014 mm) but was reduced by N<sup>ω</sup>-monomethyl-l-arginine (−0.091±0.008 mm) to a larger extent than in controls. In parallel, N<sup>ω</sup>-monomethyl-l-arginine decreased local plasma nitrite to a lesser extent in hypertensive patients (−14±5 mmol/L) than in controls (−50±10 mmol/L). Moreover, the addition of fluconazole to N<sup>ω</sup>-monomethyl-l-arginine did not further decrease radial diameter in patients (−0.086±0.011 mm). Accordingly, fluconazole significantly decreased local epoxyeicosatrienoic acid plasma level in controls (−2.0±0.6 ng/mL) but not in patients (−0.9±0.4 ng/mL). Inhibitors effects on blood flow and endothelium-independent dilatation to sodium nitroprusside were similar between groups. These results show that, in contrast to normotensive subjects, epoxyeicosatrienoic acids did not contribute to the regulation of basal conduit artery diameter and did not compensate for altered NO availability to maintain this diameter in essential hypertensive patients. (Hypertension. 2012;60:00-00.)

Key Words: hypertension ■ NO ■ cytochrome ■ arteries ■ epoxyeicosatrienoic acids

The endothelium regulates the vascular tone and prevents the appearance and progression of atherosclerosis through the dynamic release of various factors. Other than the protective actions of NO, other vasoactive substances may be involved, such as the epoxyeicosatrienoic acids (EETs). In fact, recent evidence indicates that EETs, which are vasodilatory eicosanoids synthesized by endothelial cytochrome P450 epoxygenases, share with NO many important anti-inflammatory and antiaggregating properties. In healthy humans, EETs contribute to the regulation of vasomotor tone in resistance and conduit arteries. We notably demonstrated that EET pathway compensates for the altered NO availability allowing for maintenance of the basal diameter of peripheral conduit arteries. This mechanism of compensation may be particularly relevant under pathophysiological conditions associated with reduced NO availability to maintain the continuous protective action of the endothelium.

Essential hypertensive patients are at increased risk of developing atherosclerosis lesions and related cardiovascular events even when blood pressure is well controlled. This increased risk is notably because of the presence of a dysfunctional endothelium, characterized by a decrease in NO availability. Furthermore, increasing experimental evidence suggests that an alteration in EET pathway also...
contributes to the cardiovascular complications of arterial hypertension. In human hypertension, few studies have been dedicated to assessment of the evolution of the EET pathway. One study reported an increased contribution of EETs during endothelial stimulation in forearm resistance arteries whereas, inversely, we recently demonstrated an impairment of this pathway during sustained flow-mediated dilatation of peripheral conduit arteries. However, it remains to be investigated whether arterial hypertension affects the balance between NO and EET pathways at baseline, which could contribute to acceleration of atherosclerosis development in conduit arteries of patients.

The present study was thus designed to compare the relative role of NO and the EET pathway in the maintenance of the radial artery diameter between essential hypertensive patients and normotensive control subjects.

**Methods**

An expanded description of the Methods section is available in the online-only Data Supplement.

**Subjects**

The study was performed in 14 nontreated essential hypertensive patients (13 never-treated patients and 1 patient reporting a history of discontinued pharmacological antihypertensive treatment) and 14 healthy volunteers. The study was conducted according with the Principles of Good Clinical Practice and the Declaration of Helsinki and was approved by the local ethical committee (Committee for the Protection of Persons of Normandy). All of the participants gave written informed consent.

**General Procedure**

Radial internal diameter (d), blood flow (Q), and digital arterial pressure were continuously obtained using a high-precision echo-tracking device coupled to a Doppler system (NIUS 02, Asulab) and a finger photoplethysmograph (Finapres System, Ohmeda), as described previously. Total blood viscosity (µ) was measured using a cone-plate viscometer (Ex100 CTB, Brookfield) at a shear rate of 241 s⁻¹ at 37°C allowing the calculation of the mean arterial wall shear stress (τ), assuming a Poiseuillean model, that is: τ = (4pQ)/(πr³), (r = d/2), Oral aspirin (500 mg, BMS Laboratory) was administered to block the production of vasomotor prostanoids, and subject received during 8 minutes, using a 27-gauge needle inserted into the brachial artery, either the inhibitor of cytochrome P450 epoxygenases flucnazol (0.4 µmol/min per liter of forearm, Pfizer Holding), the NO synthase inhibitor N'-monomethyl-L-arginine (L-NMMA; 8 µmol/min per liter of forearm, Bachem), or their association. Endothelium-independent dilatation was assessed using sodium nitroprusside (5, 10, and 20 nmol/min per liter, SERB Laboratory). Furthermore, a 4F catheter was inserted into the distal portion of the antecubital vein of the infused arm, when accessible, allowing blood sampling in the venous return and the quantification of EET plasma level by gas chromatography-mass spectrometry with negative-ion chemical ionization and nitrate plasma level, used as an indicator of NO bioavailability, by a tri-iodide/ozone-based chemiluminescence assay. In addition, because NO synthase and cytochrome P450 epoxygenases can produce superoxide anions, additional blood samplings were performed to quantify the whole blood level of reactive oxygen species (ROS) by electron paramagnetic resonance spectroscopy.

**Statistical Analysis**

All the results are expressed as mean±SEM. The effects of the inhibitors on radial artery and biological parameters were analyzed by ANOVA with time (before and after inhibition) and group as factors. In the whole population, Spearman rank correlation coefficient was calculated to assess the correlation between blood pressure levels and the degree of vasoconstriction induced by the inhibitors. In addition, we compared the degree of vasoconstriction induced by the inhibitors between quartiles of systolic and diastolic blood pressures, using a Kruskal-Wallis test followed, in case of significance, by a Dunn test for post hoc pairwise comparisons. A value of P≤0.05 was considered statistically significant.

**Table. Demographic and Biological Characteristics of Essential Hypertensive Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Subjects (n=14)</th>
<th>Hypertensive Patients (n=14)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>48 ± 3</td>
<td>51 ± 3</td>
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<tr>
<td>Male, n (%)</td>
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<td>10 (71)</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>Heart rate, bpm</td>
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<td>73 ± 3*</td>
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<td>Total cholesterol, mg/dL</td>
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<td>Creatinemia, mg/dL</td>
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<td>Estimated GFR, ml/min per 1.73 m²</td>
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<td>Blood viscosity, cP</td>
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</tbody>
</table>

Values are mean±SEM. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; GFR, glomerular filtration rate. *P<0.05 vs control subjects.

**Results**

There was no difference between groups for demographic and biological characteristics except higher systolic and diastolic blood pressures, as well as a higher heart rate in hypertensive patients as compared with control subjects (Table). None of the inhibitors affected systolic or diastolic blood pressures or heart rate in either groups. Radial artery diameter, blood flow, and mean wall shear stress were similar between groups before the infusion of flucnazol, L-NMMA, and their combination (Table S1).

Flucnazol did not modify significantly radial artery blood flow in control subjects or in hypertensive patients (Figure 1). L-NMMA similarly reduced radial artery blood flow in both groups. L-NMMA+flucnazol reduced radial artery blood flow to a similar extent as during L-NMMA alone in both groups.

Radial artery diameter was reduced by flucnazol (−1.5±0.5%), L-NMMA (−1.5±0.4%) and, to a larger extent, by L-NMMA+flucnazol (−5.7±0.5%) in control subjects demonstrating a synergistic effect of the inhibitors (Figure 2). In contrast, radial artery diameter was not affected by flucnazol (0.4±0.5%) in hypertensive patients but was reduced.
by 1-NMMA (−3.8±0.3%) to a larger extent than in control subjects. Moreover, radial artery diameter was not further decreased by the addition of fluconazole to 1-NMMA (−3.6±0.5%) in hypertensive patients.

As a consequence of the decrease in diameter, fluconazole slightly increased radial artery mean wall shear stress in control subjects but not in hypertensive patients (Figure 3). 1-NMMA similarly reduced radial artery mean wall shear stress in both groups. 1-NMMA+fluconazole reduced radial artery mean wall shear stress to a similar extent as that during 1-NMMA alone in both groups. Endothelium-independent dilatation to sodium nitroprusside was similar between groups (Table S2).

In the whole population, the magnitude of vasoconstriction induced by fluconazole decreased with the increase in diastolic and systolic blood pressures (Figure 4A and 4B). In contrast, the magnitude of vasoconstriction induced by 1-NMMA increased with blood pressure. Finally, the magnitude of vasoconstriction induced by 1-NMMA+fluconazole decreased with the increase in blood pressure.

Regarding the biological markers, there was no significant difference between groups for the basal plasma level of EETs (Figure 5A), whereas nitrite basal plasma level was lower in hypertensive patients than in control subjects (Figure 5B). Fluconazole significantly decreased EET plasma level in controls (−16.5±5.4%) but not in patients (−5.0±2.0%). In addition, 1-NMMA decreased nitrite plasma level in both groups, but this decrease was more marked in control subjects (−32±6%) than in patients (−11±3%). Finally, ROS blood level was similar in control subjects and hypertensive patients at baseline and was not affected in either group by 1-NMMA (Figure 5C) or fluconazole (Figure 5D).

**Discussion**

The major finding of the present study is that EET pathway, which physiologically contributes under basal conditions and during the loss of NO synthesis to the maintain of peripheral conduit artery diameter, is altered in essential hypertensive patients.

This study was designed to investigate the impact of arterial hypertension on the role of EETs at rest and during decreased NO availability at the level of the radial artery, a model of peripheral conduit artery. In this way, we infused, into the brachial artery, potent inhibitors of the EETs-synthesizing enzymes cytochrome P450 epoxygenases, fluconazole, and of NO-synthase, 1-NMMA, at doses producing a local effect without modifying systemic hemodynamics. We demonstrated previously in mice that fluconazole is equipotent as N-methylsulfonyl-6-(2-propargyloxyphenyl)-hexanamide, a specific inhibitor of cytochrome P450 epoxygenases, to decrease the endothelium-dependent relaxation of coronary arteries, without direct effects on calcium-activated potassium channels, which mediate the hyperpolarizing effect of EETs.

In a subset of normotensive control subjects, we observed that fluconazole and 1-NMMA effectively decreased the plasma levels of EETs and nitrite, respectively, confirming that these agents are adequate pharmacological tools to study the role of EETs and NO in human conduit arteries. In addition, neither fluconazole nor 1-NMMA altered ROS level, suggesting the absence of a relevant production of superoxide anions by CYP450 epoxygenase or NO synthase at baseline under physiological conditions. In this context, fluconazole...
However, these results were obtained in younger healthy volunteers, suggesting that the role of EETs in the control of vascular resistance at baseline may be physiologically relevant.6 In contrast, fluconazole reduced radial artery diameter showing the physiological role of EETs in the maintenance of basal peripheral conduit artery diameter in humans. This result was observed despite a significant increase in mean wall shear stress, as expected from the decrease in diameter without change in flow, thus identifying a direct effect of fluconazole on the conduit artery wall. We observed previously a decrease in diameter of similar magnitude with fluconazole in healthy volunteers, but statistical significance was not reached, probably because of the lower sample size.3 Furthermore, l-NMMA significantly reduced radial artery diameter in control subjects. Such effect, demonstrating the role of NO in the regulation of basal conduit artery diameter, has been reported previously by some studies10,20,21 but not all.3,22,23 In particular, we observed previously a vasoconstrictor effect of l-NMMA in healthy volunteers, but only after blockade of the cytochrome P450 epoxygenase pathway and not when l-NMMA was infused alone.5 However, these results were obtained in younger healthy volunteers, suggesting that aging may alter the physiological balance between NO and other vasodilatory mechanisms, notably the cytochrome P450 epoxygenases pathway, which help to maintain basal conduit artery diameter during altered NO availability. Nonetheless, during the concomitant infusion of l-NMMA and fluconazole, a decrease in radial artery diameter twice that obtained by adding the decrease obtained with the inhibitors when infused alone (i.e., −0.140 versus −0.070 mm) was observed in our control subjects. This synergistic effect of the dual inhibition, observed in presence of a similar decrease in mean wall shear stress compared with during l-NMMA alone, confirms that a functional interaction between NO and EET pathways maintaining the basal peripheral conduit artery diameter is still present in this population of healthy controls.5

In essential hypertensive patients, fluconazole did not alter radial artery blood flow, whereas l-NMMA and, to a similar extent, l-NMMA+fluconazole reduced it in controls. These results, suggesting that NO but not EETs are involved in the control of basal vascular resistance, are in accordance with most of the previous studies reporting no effect or nonsignificant increase in blood flow with cytochrome P450 inhibitors.3,8,14 However, a recent study involving an higher number of volunteers has shown that fluconazole slightly reduced basal forearm blood flow and that this effect tended to be more marked after l-NMMA, suggesting that the role of EETs in the control of vascular resistance at baseline may be physiologically relevant.6

In hypertensive patients, fluconazole reduced radial artery blood flow, whereas l-NMMA, but statistical significance was not reached, probably because of the lower sample size.3 Furthermore, l-NMMA significantly reduced radial artery diameter in control subjects. Such effect, demonstrating the role of NO in the regulation of basal conduit artery diameter, has been reported previously by some studies10,20,21 but not all.3,22,23 In particular, we observed previously a vasoconstrictor effect of l-NMMA in healthy volunteers, but only after blockade of the cytochrome P450 epoxygenase pathway and not when l-NMMA was infused alone.5 However, these results were obtained in younger healthy volunteers, suggesting that aging may alter the physiological balance between NO and other vasodilatory mechanisms, notably the cytochrome P450 epoxygenases pathway, which help to maintain basal conduit artery diameter during altered NO availability. Nonetheless, during the concomitant infusion of l-NMMA and fluconazole, a decrease in radial artery diameter twice that obtained by adding the decrease obtained with the inhibitors when infused alone (i.e., −0.140 versus −0.070 mm) was observed in our control subjects. This synergistic effect of the dual inhibition, observed in presence of a similar decrease in mean wall shear stress compared with during l-NMMA alone, confirms that a functional interaction between NO and EET pathways maintaining the basal peripheral conduit artery diameter is still present in this population of healthy controls.5

In essential hypertensive patients, fluconazole did not alter radial artery blood flow, whereas l-NMMA and the combination of l-NMMA and fluconazole reduced it to a similar
extant as in control subjects. These results suggest that basal NO availability is preserved in our patients at the arteriolar level, as already reported in 1 study\(^\text{24}\) or more probably, that hyperpolarizing compensatory pathways other than EETs are upregulated to maintain basal vascular resistance, as shown in patients with hypercholesterolemia or diabetes mellitus.\(^\text{6}\)

Regarding the conduit artery, in contrast to the effect observed in control subjects, fluconazole did not decrease radial artery diameter or local EET plasma level in hypertensive patients. Because endothelium-independent dilatation was similar between groups, this result shows for the first time in humans that essential hypertension is associated with an alteration in the physiological role of EETs in the control of basal conduit artery diameter. This result extends our recent data demonstrating an abrogation of this pathway during flow stimulation in essential hypertensive patients.\(^\text{15}\)

Furthermore, we observed that L-NMMA induced a lesser decrease in nitrite plasma level, supporting a decreased basal NO bioavailability, but a more marked decrease in radial artery diameter in hypertensive patients than in control subjects. In addition, we observed in the whole population studied a significant positive relationship between the degree of vasoconstriction induced by L-NMMA and blood pressure: the higher the pressure, the higher the constriction. These last results are at first sight surprising, contrasting with what would be expected from a decrease in NO availability, that is, a negative correlation between the degree of vasoconstriction induced by L-NMMA and blood pressure. In fact, this more marked decrease in diameter is the consequence of the alteration in EET pathway, which does not compensate for the decrease in NO availability to maintain conduit artery diameter under conditions of high blood pressure. Indeed, the addition of fluconazole to L-NMMA did not further decrease radial artery diameter in hypertensive patients than during L-NMMA alone and, in the whole population, the magnitude of vasoconstriction induced by the combination decreases with the increase in blood pressure thus unmasking the reduction in the availability of EETs and NO.

Concerning the effect of NO synthase inhibition, in contrast to our results, Ghiadoni et al\(^\text{10}\) reported a reduction in the vasoconstrictor effect of L-NMMA in essential hypertensive patients as compared with normotensive controls. This difference may be related to the twice higher dose of L-NMMA that we used in the present work.\(^\text{10}\) In addition, although NO and EET pathways were not explored concomitantly in the study of Ghiadoni et al,\(^\text{10}\) this may be related to differences in the population studied regarding the presence of associated risk factors or to the severity and duration of the disease, which interact to alter NO and EET availability.\(^\text{1}\) Thus, the evolution with time of NO and EET availability in nontreated hypertension

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**Figure 5.** Bar graphs show the variations in the plasma level of epoxyeicosatrienoic acids (EETs) before and at the end of fluconazole infusion (A), nitrite before and at the end of N\(^\text{ω}\)-monomethyl-L-arginine (L-NMMA; B), and in the whole blood level of reactive oxygen species (ROS) before and after infusion of fluconazole (C) and L-NMMA (D) in control subjects and in hypertensive patients. *P*<0.05 vs saline; †*P*<0.05 vs corresponding value in control subjects; ‡*P*<0.05 vs variation in control subjects.
may be, first, a moderate decrease in NO availability partially compensated by EETs, as observed in human experiments at the arteriolar level,14 followed by the development of NO/EET imbalance as demonstrated in the present study, and finally an abrogation of both NO and EET pathways, as suggested from the study by Ghiadoni et al.10 This hypothesis remains difficult to confirm in humans but is consistent with animal data showing that a cytochrome epoxygenase pathway is upregulated in young spontaneously hypertensive rats to maintain the renal artery endothelium-dependent relaxation, whereas this mechanism of compensation is completely lost in old hypertensive animals.25

Furthermore, the mechanisms involved in the alteration of EET pathway during essential hypertension are difficult to investigate in vivo in humans. The decrease in EET bioavailability may be notably related to an increased degradation of EETs by soluble epoxide hydrolase, for which expression is enhanced by angiotensin II, as shown in animals.1,11,26,27 In contrast, a decreased EET production by cytochrome P450 epoxygenases probably contributes less to this alteration, and an additional modification in the ability of Kca channels to mediate the vasorelaxing effect of EETs appears unlikely.1,11,26,27

Finally, a generation of superoxide anions by either CYP450 epoxygenases or NO synthase does not appear to contribute to the alteration in the control of basal conduit artery tone in our hypertensive patients. In fact, as observed in normotensive controls, fluconazole and 1-NMMA did not affect ROS level. Similarly, an increased release of ROS by CYP450 epoxygenases does not contribute to the impairment of the endothelial-dependent flow-mediated dilation in essential hypertensive patients.15 In contrast, during these conditions of endothelial stimulation, an uncoupling of NO synthase and subsequent generation of ROS became apparent.15

In conclusion, our results demonstrate that the role of EETs in maintaining conduit artery diameter under basal conditions and altered NO availability is impaired in essential hypertensive patients. This alteration may alter the conducting and buffering function of these arteries, contributing to the impairment in cardiovascular coupling and inadequate perfusion to organs in hypertension. In addition, given the anti-inflammatory and anti-hypertrophic properties of EETs,4 this alteration in conduit arteries may contribute to the development of atherosclerosis and the high incidence of ischemic events in hypertensive patients.

Study Limitations

Although basal levels were similar, fluconazole decreased EET plasma level in normotensive controls but not in hypertensive patients. The reasons for these discrepancies remain to be elucidated, but one possible explanation is that the majority of circulating EETs are esterified in phospholipids, and the quantity of EET in these pools may be very stable in the long term, even when their release by endothelial cell decreases.28 In addition, it was not possible to perform blood sampling in all of the study participants, and we cannot exclude that small variations in EETs exist in hypertensive patients. Furthermore, although not tested in the present study, an increased smooth muscle responsiveness to vasoconstrictors may be involved in the more marked decrease in radial artery diameter obtained with 1-NMMA in hypertensive patients, as reported in some studies but not all.8,29,30

This difference of behavior, together with the limited sample size of the present study, may notably modify the relationships between blood pressure and the responses to fluconazole and 1-NMMA assessed in the whole population. However, whatever the change in the responsiveness to vasoconstrictors, our results clearly establish the absence of compensation by EETs to maintain the basal conduit artery diameter.

Perspectives

Normalizing blood pressure may be not sufficient to prevent vascular dysfunction and the development of cardiovascular complications in essential hypertensive patients, and new therapeutic targets are needed.7 In this context, restoring EET bioavailability using pharmacological inhibitors of soluble epoxide hydrolase, which are currently under development, represents an attractive pharmacological approach.3 Indeed, in addition to lowering blood pressure, the use of soluble epoxide hydrolase inhibitors may be useful to restore the physiological function of EETs at the level of the conduit arteries and, thus, may help to decrease cardiovascular morbidity and mortality during essential hypertension.

Acknowledgment

We thank the general practitioners of the research network of the General Medicine Department of Rouen University Hospital for the recruitment of the essential hypertensive patients.

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Disclosures

None.

References

9. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent...


Novelty and Significance

What Is New?

• Investigations into mechanisms that contribute to alteration in conduit artery tone in essential hypertension are very limited.

• This study evaluates whether alteration in EET pathway may be involved.

What Is Relevant?

• This study shows that the maintenance of peripheral conduit artery diameter by EETs is altered in essential hypertensive patients at rest and during the acute reduction of NO availability.
Impaired Role of Epoxyeicosatrienoic Acids in the Regulation of Basal Conduit Artery Diameter During Essential Hypertension
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Impaired role of epoxygenesatrienoic acids in the regulation of basal conduit artery diameter during essential hypertension

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METHODS

Subjects
The study was performed in a total of 28 subjects. All subjects were non-smokers and did not receive any medication. Subjects with cardiac and/or cerebrovascular ischemic vascular disease, heart failure, impaired renal function (estimated glomerular filtration rate<60 ml/min/1.73 m² according to the Cockcroft-Gault equation), and other major pathologies were excluded from the study. Fourteen non-treated essential hypertensive patients were recruited from general practitioners of the Research Network of the General Medicine Department of Rouen University, if supine systolic and diastolic blood pressure (SBP, DBP) measured, after 10 min rest, were consistently found to be ≥140/90 mm Hg 3 times at 1 to 2-week intervals. Secondary forms of hypertension were excluded by routine diagnostic procedures. Patients were enrolled if never treated (n=13) or reporting a history of discontinued pharmacologic antihypertensive treatment (n=1), interrupted for at least 3 months before the day of inclusion. Moreover, 14 volunteers were recruited by the CIC-Inserm 0204 and the Department of Pharmacology of Rouen University Hospital based on the same inclusion and exclusion criteria, and were deemed healthy according to the absence of familial history of essential hypertension and SBP/DBP values below 140/90 mm Hg, as well as complete medical examination and routine laboratory tests. On the day of inclusion, SBP and DBP were measured after 10 rest, on the dominant arm by mean of a brachial cuff oscillometric device (Omron HEM-705CP) 3 times at 5-minutes intervals. The study was conducted according with the Principles of Good Clinical Practice and the Declaration of Helsinki, and was approved by the local ethical committee (Committee for the Protection of Persons of Normandy). All participants gave written informed consent. The study was registered at https://eudract.ema.europa.eu under the unique identifier RCB2007-A001-10-53.

General procedure
The subjects were explored on two separate days. Measurements were performed in the morning while subjects were in a supine position, in a quiet air-conditioned room, maintained at a constant temperature (22°C to 24°C). A 27-gauge needle was inserted, under local anesthesia
(1% lidocaine), into the brachial artery of the non-dominant arm to permit infusion of saline (0.9%) and pharmacological agents at a constant rate (1 ml/min) using dual programmable syringe pumps (Vial Program 2, Becton Dickinson). Radial internal diameter (d), blood flow (Q) and digital arterial pressure were continuously obtained using a high-precision echotracking device coupled to a Doppler system (NIUS 02, Asulab) and a finger photoplethysmograph (Finapres System, Ohmeda). Total blood viscosity (µ) was measured using a cone-plate viscometer (Ex100 CTB, Brookfield) at a shear rate of 241 sec⁻¹ at 37°C allowing the calculation of the mean arterial wall shear stress (τ), assuming a Poiseuille model, i.e. τ=[(4µQ)/(πr³)], (r=d/2)].

The forearm volume of each volunteer was measured by using the water displacement method to adjust the doses of the pharmacological agents to be infused. Oral aspirin (500 mg, BMS Laboratory) was administered to block the production of vasomotor prostanoids, and subject received during 8 min either the inhibitor of cytochrome P450 epoxygenases fluconazole (0.4 µmol/min/L, Pfizer Holding), the NO-synthese inhibitor N⁴-monomethyl-L-arginine (L-NMMA: 8 µmol/min/L of forearm, Bachem), or their association. These doses and duration of infusion have been used to demonstrate in healthy volunteers the role of EETs and NO in the regulation of basal radial artery diameter, in absence of modification in systemic hemodynamics. Endothelium-independent dilatation was assessed using sodium nitroprusside (5, 10 and 20 nmol/min/L, SERB Laboratory). Inhibitor sequences and sodium nitroprusside administration were performed in a random order during the two days of exploration with a one hour wash-out period between two procedures of infusion.

Furthermore, a 4-F catheter was inserted into the distal portion of the antecubital vein of the infused arm, when accessible, allowing blood sampling in the venous return. Blood sampling was performed using prechilled 2 mL-syringes directly connected to the catheter, before and at the end of fluconazole infusion for the quantification of EET plasma level by gas chromatography-mass spectrometry with negative-ion chemical-ionization, as well as before and at the end of L-NMMA infusion for the quantification of nitrite plasma level, used as indicator of NO bioavailability, by a tri-iodide/ozone-based chemiluminescence assay. Additional blood sampling were performed using 1 mL-syringe containing the spin probe 1-hydroxy-3-methoxy carbonyl-2,2,5,5-tetramethylpyrrolidine, before and at the end of L-NMMA and fluconazole infusion, for the quantification of the whole blood level of reactive oxygen species by electron paramagnetic resonance spectroscopy.
## SUPPLEMENTARY TABLE

### Supplementary Table S1. Basal radial artery parameters before inhibitor infusion

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<th>Inhibitor</th>
<th>Group</th>
<th>Radial artery diameter (mm)</th>
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<td></td>
<td>Hypertensive patients</td>
<td>2.411±0.082</td>
<td>11.9±1.5</td>
<td>6.1±0.9</td>
</tr>
<tr>
<td>Before fluconazole</td>
<td>Control subjects</td>
<td>2.470±0.085</td>
<td>10.4±1.3</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td></td>
<td>Hypertensive patients</td>
<td>2.383±0.080</td>
<td>10.8±1.0</td>
<td>5.5±0.5</td>
</tr>
<tr>
<td>Before L-NMMA + fluconazole</td>
<td>Control subjects</td>
<td>2.447±0.095</td>
<td>10.7±1.0</td>
<td>5.6±1.0</td>
</tr>
<tr>
<td></td>
<td>Hypertensive patients</td>
<td>2.408±0.082</td>
<td>11.7±1.2</td>
<td>5.8±0.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

### Supplementary Table S2. Endothelium-independent dilatation to sodium nitroprusside

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Sodium nitroprusside (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial artery diameter (mm)</td>
<td>Control subjects</td>
<td>2.464±0.057 (12.0±1.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.738±0.039 (18.7±2.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.896±0.046 (22.2±2.7%)</td>
</tr>
<tr>
<td></td>
<td>Hypertensive patients</td>
<td>2.415±0.056 (10.1±1.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.658±0.072 (17.7±2.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.825±0.072 (24.8±2.0%)</td>
</tr>
</tbody>
</table>

Values are mean±SEM.