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Alteration in the availability of epoxyeicosatrienoic acids contributes with NO to the development of endothelial dysfunction in conduit arteries during aging

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ABSTRACT

Background and aims: The mechanisms involved in endothelial dysfunction in humans during aging are largely unknown at the level of conduit arteries. We aimed to asses the role of NO and CYP450 epoxygenases-derived epoxyeicosatrienoic acids (EETs) in the regulation of endothelium-dependent flow-mediated dilatation of conduit arteries during aging.
Methods: Radial artery diameter and mean wall shear stress were determined by echotracking coupled with Doppler in 83 subjects (19–71 years old) during a sustained flow increase induced by hand skin heating, with the brachial infusion of saline or NO-synthase and cytochrome P450 epoxygenase inhibitors (L-NNMA and fluconazole respectively). Local blood sampling was performed for the quantification of NO metabolite nitrite and EETs.
Results: The magnitude of flow-mediated dilatation was independently and negatively correlated with age, baseline artery diameter and systolic blood pressure, and positively correlated with the increase in shear stress induced by heating. There was an increase in nitrite level during heating until the age of 35–40 years, which declined thereafter. However, the inhibitory effect of L-NMMA on flow-mediated dilatation progressively decreased during aging, demonstrating a decrease in functional NO availability. Moreover, aging progressively reduced the increase in EET level during heating as well as the inhibitory effect of fluconazole on flow-mediated dilatation.
Conclusions: These results show that aging impairs the availability of EETs and NO and epoxyeicosatrienoic acids in peripheral conduit arteries, contributing to the development of endothelial dysfunction.

1. Introduction

A growing body of evidence shows that arterial aging is a major cause of morbidity and mortality, promoting the development of cardiovascular diseases and contributing to cognitive impairment and dementia [1–3]. Given the current and predicted rise in the number of older people, the search for new therapeutic strategies aiming to prevent arterial aging represents a critical healthcare challenge [1–3].

Endothelial dysfunction is an early change occurring in both resistance and conduit arteries during aging, even in humans free of major cardiovascular risk factors or clinical diseases [3–11]. In fact, advancing age shifts the endothelium to a vasoconstrictive, proinflammatory and prothrombotic phenotype, notably contributing to an increase in total peripheral resistance, blood pressure, arterial stiffeness and to the development of atherosclerosis [3-12–15]. Accordingly, the presence of endothelial dysfunction is predictive of future cardiovascular events even in healthy individuals, when assessed noninvasively in peripheral conduit arteries [14].

Regarding the mechanisms involved in aging-induced

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endothelial dysfunction, only a few studies have been performed *in vivo* in humans. In resistance arteries, the decreased availability of nitric oxide (NO) related to increased oxidative stress appears to play a major role. This is illustrated by the reduction in the inhibitory effect of the NO-synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) on forearm endothelium-dependent dilatation in response to acetylcholine, and its prevention by the acute administration of antioxidants such as vitamin C [6–8,10]. Vitamin C also improved brachial artery endothelium-dependent flow-mediated dilatation in response to post-ischemic hyperemia [9], but no evidence has yet been reported for an alteration in the availability of NO in conduit arteries during aging. In contrast, no change in cytochrome P450 (CYP450)-derived epoxyeicosatrienoic acids (EETs), which are endothelium-derived hyperpolarizing factors (EDHFs) interacting with NO to maintain the protective action of the endothelium [12], has been observed in the peripheral resistance arteries of older healthy adults [11]. Although EETs together with NO regulate the endothelium-dependent dilatation of conduit arteries in response to a sustained increase in blood flow [16–18], no study has explored the impact of aging on EETs availability at this level. Since the macrovascular and microvascular endothelium exhibit significant difference in function and do not respond similarly to risk factors [15], the mechanisms of endothelial dysfunction in conduit arteries during aging must be determined in order to develop specific preventive strategies.

In this context, the present work aimed to evaluate the impact of aging on the NO- and EETs-dependent regulation of sustained flow-mediated dilatation using functional and biological evaluations.

2. Patients and methods

2.1. Population

This study was performed in 83 patients selected from a total of 138 control participants of previous studies according to the following criteria, and classified into tertiles of age (Table 1) [17–19]. Subjects received no medication during the study and had no clinical evidence of atherosclerotic disease, heart failure or diabetes. Exclusion criteria were: patients who smoked more than 5 pack-years, with a supine systolic and diastolic blood pressure higher than 160/100 mmHg or with an estimated glomerular filtration rate (GFR) below 60 ml/min/1.73 m^2, according to the CKD-EPI equation. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee (Committee for the Protection of Persons of Normandy). All participants have provided written informed consent. Clinical trials were registered at https://eudract.ema.europa.eu under the unique identifiers RCB2007-A001-10-53 and RCB2010-A01279-30.

2.2. General procedure

Measurements were performed in the morning, 1 h 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). Systolic and diastolic blood pressures were measured on the dominant arm by means of a brachial cuff oscillometric device (Omron HEM-705 C P). Radial artery internal diameter was measured continuously with a high-precision A-mode echo-tracking device (NIUS 02, Asulab) with a resolution on the diameter evolution of <1 μm [19–23]. Briefly, a 10-MHz focused transducer was positioned over the radial artery. The probe was set perpendicular to the artery by a stereotaxic arm with micrometric screws, while proper positioning was adjusted with a stereo Doppler mode. After switching to A-mode, the echoes from both anterior and posterior walls of the artery were visualized on a screen and tagged by electronic trackers, allowing continuous recording of artery internal diameter. The processed radio-frequency line was visualized on a computer screen, and the operator selected the peaks corresponding to the interfaces, after which the exact position of each selected peak was determined by an interpolation technique. Radial artery blood velocity was continuously recorded by an 8-MHz Doppler probe (Doptek, 2002, Deltex). Radial artery blood flow was calculated from the measurements of velocity and internal diameter. Total blood viscosity was measured using a cone-plate viscometer (Ex100 CTB, Brookfield) at a shear rate of 241 s^{-1} at 37 °C [17–19]. From the individual values of radial artery diameter (d), blood flow (Q), and total blood viscosity (μ), the mean arterial wall shear stress, which is the stimulus of the flow-dependent release of vasoactive endothelial factors, was calculated based on a Poiseuillean model, *i.e.* $\tau = [(4\mu Q)/(\pi r^3)$, $(r = d/2)]$ [17–19]. The patient's hand was introduced into a thin watertight glove fixed in a thermo-controlled tank [17–19]. The tank was then filled with water and the temperature was set to 34 °C for 20 min. Then, hand skin heating was performed by gradually increasing the water temperature from 34 to 37, 40, and 44 °C. Each temperature step was maintained for 7 min and measurements of radial artery parameters were taken during the

Table 1
Baseline characteristics of the subjects according to tertiles of age.

Parameters	Age tertile 1 (<30 years) n = 28	Age tertile 2 (30–46 years) n = 28	Age tertile 3 (>46 years) n = 27
Age, years	24.1 ± 0.6	38.2 ± 0.9	54.6 ± 1.2
Male, n (%)	18 (64%)	18 (64%)	17 (63%)
Body mass index (kg/m^2)	22.8 ± 3.9	25.9 ± 4.1*	25.6 ± 3.6*
Systolic blood pressure, mmHg	118 ± 11	125 ± 15	135 ± 13*†
Diastolic blood pressure, mmHg	69 ± 8	76 ± 10*	84 ± 9*†
Heart rate, bpm	67 ± 10	64 ± 10	68 ± 11
Total cholesterol, mg/dL	172 ± 29	198 ± 28*	220 ± 26*†
LDL cholesterol, mg/dL	104 ± 29	131 ± 29*	144 ± 26*
HDL cholesterol, mg/dL	56 ± 19	56 ± 14	51 ± 11
Triglycerides, mg/dL	79 ± 60	81 ± 41	119 ± 69*†
Fasting glucose, mg/dL	85 ± 8	92 ± 10*	98 ± 10*
Creatinemia, mg/dL	80 ± 12	78 ± 14	78 ± 15
Estimated GFR, mL/min	108 ± 12	99 ± 14	89 ± 12*†
Blood viscosity, cP	4.2 ± 0.9	3.8 ± 0.4	4.0 ± 0.6
Flow-mediated dilatation, %	26.8 ± 7.4	20.3 ± 6.4*	19.2 ± 7.2*
Shear stress variation, dynes/cm^2	11.5 ± 6.9	13.0 ± 6.3	14.1 ± 8.9
Endothelium-independent dilatation, %	26.9 ± 7.6	28.3 ± 8.2	25.8 ± 6.3

Values are mean ± SD or number (%). GFR: glomerular filtration rate. *$p<0.05$ *vs.* age tertile 1; †$p<0.05$ *vs.* age tertile 2.