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Fatty acid–induced changes in vascular reactivity in healthy adult rats

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Abstract

Dietary fatty acids (FAs) are known to modulate endothelial dysfunction, which is the first stage of atherosclerosis. However, their exact role in this initial phase is still unclear. The effects of isolated or combined (by 2) purified FAs from the main FA families were studied on the vascular response of isolated thoracic aorta in healthy rats to get a better understanding of the mechanisms of action of dietary FAs in regulating vascular endothelial function. Cumulative contraction curves to phenylephrine and relaxation curves to carbachol and then to sodium nitroprusside were obtained in the absence or presence of the FAs studied allowing endothelium-dependent and endothelium-independent ability of the smooth muscle to relax to be assessed in each experimental group. The endothelium-dependent vasodilator response to carbachol was lowered by eicosapentaenoic acid, whereas it was not altered either by docosahexaenoic acid alone or by combined eicosapentaenoic acid–docosahexaenoic acid, oleic acid, or stearic acid, and it was increased by linoleic acid (LA). A decreased phenylephrine-induced contraction was observed after incubation with arachidonic acid and with stearic acid. On the other hand, the endothelium-dependent relaxation was reduced by the addition of combined LA–arachidonic acid and LA–oleic acid. In conclusion, these data point out the differential effects of different types of FAs and of FAs alone vs combined on vascular reactivity. The complex nature of these effects could be partially linked to metabolic specificities of endothelial cells and to interactions between some FAs.

1. Introduction

Dietary lipids have an important impact on cardiovascular disease (CVD) risk, and research in this field has placed particular emphasis upon the role played by fatty acids (FAs) in modulating vascular function [1,2]. In addition, several studies in humans as well as rodents have demonstrated that an experimental elevation in circulating nonesterified FA concentrations in healthy subjects led to impairment of endothelium-dependent and insulin-mediated vasodilation because of a reduced nitric oxide (NO) production [3]. However, the exact role of different types of FA on atherosclerosis, particularly during its initial phase dealing with an endothelial dysfunction [4], is still unclear.

It is currently believed that a diet enriched in long-chain $n-3$ polyunsaturated FAs ($n-3$ PUFAs) reduces the risk of developing CVD [5,6], that a long-term feeding of olive oil (OO), rich in oleic acid (OA), highly contributes to the very low prevalence of CVD in people of the Mediterranean region [7], and that γ-linolenic acid may also modulate the atherosclerosis process by inhibiting smooth muscle cell proliferation [8]. Several studies have shown that the main long-chain $n-3$ PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can prevent the activation of endothelial cells (ECs) either by inhibiting the expression of adhesion molecules or by improving endothelial NO synthase (eNOS) activity [9,10]. It has even been suggested that these effects of $n-3$ PUFA could be related to EC membrane characteristics or redox status [11,12]. Moreover, OA has been shown to inhibit the endothelium-dependent vasodilator response to acetylcholine in rabbit femoral artery rings preconstricted with phenylephrine by inhibiting eNOS activity [13]. However, this FA has also been shown to increase the relaxant response to acetylcholine in aortic rings from spontaneously hypertensive (SHR) as well as normal Wistar-Kyoto (WK) rats [7]. Thus, the respective

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effects of FAs appear rather controversial, which may be attributed to particular experimental conditions (animal species, cultured cells, etc). Moreover, in most of the aforementioned in vitro or ex vivo studies, FAs are generally considered separately, their possible interactions being not taken into account. It should be noted, however, that Herrera et al [7] have compared the effects of 2 OA-rich diets, containing OO and high–OA sunflower oil (HOSO), on vascular reactivity, and the results of their study suggest that only the long-term feeding of OO diet modulated the vascular response of rat aorta. As the OA concentration of OO and HOSO was similar, these authors considered that other components of OO, such as polyphenols, not present in HOSO, might be responsible for the beneficial effects of OO on the cardiovascular system. Without excluding this possibility, it may be hypothesized that the observed modulation results from an interaction between OA and other FAs present in these oils. Indeed, linoleic acid (LA) (18:2$n-6$) level was higher in HOSO than in OO (9.4% vs 3.5%), and this FA has been shown to be proatherogenic by promoting endothelial activation with enhanced expression of adhesion molecules and decreased eNOS activity [14,15]. The results obtained in this study could also be explained by a possible interaction between the effects of OA and LA in the HOSO treatment, whereas the effect of OA was predominant in the OO treatment. However, the mechanisms behind these beneficial or detrimental effects remain poorly understood. Most studies have focused on the effects of FAs under diseased conditions and not in healthy individuals. Therefore, the present study was undertaken to investigate the effects of isolated or combined FAs on endothelial function within the context of atherosclerosis prevention. In this study, we hypothesized that the modulating effect of FA on vascular reactivity in a healthy control animal could be very different whether FA were alone or in combination. This study should help us choose the FA to be tested in further mechanistic investigations addressing current hypotheses and questions recently raised [16,17].

2. Materials and methods

2.1. Animals

All surgical and experimental procedures followed institutional animal care guidelines and the investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (Publication No. 85-23, revised 1996). Male Sprague-Dawley rats (Charles River, St-Constant, Canada), weighing 300 to 350 g, were housed individually in stainless steel cages. They were placed in a temperature-controlled room (22°C ± 1°C) on a 12-hour light/dark cycle (lights on at 6:00 AM) and had free access to food and tap water. After the settling-in period, rats were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg/kg IP, respectively).

2.2. Measurement of aorta reactivity

The thoracic aortas from the rats were quickly excised and placed in chilled Krebs buffer solution (in mmol/L: NaCl, 118; KCl, 4.7; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 25; dextrose, 11.1; $CaCl_2$, 2.5; EDTA, 0.06) containing bovine serum albumin (90 μmol/L). The fat and adventitia were dissected free, and the aorta was cut into 3-mm rings. The rings were mounted under 2-g resting tension on stainless steel hooks in 15-mL organ baths filled with Krebs buffer and gassed with 95% O_2 and 5% CO_2 at 37°C. Tension was recorded with a Grass force transducers

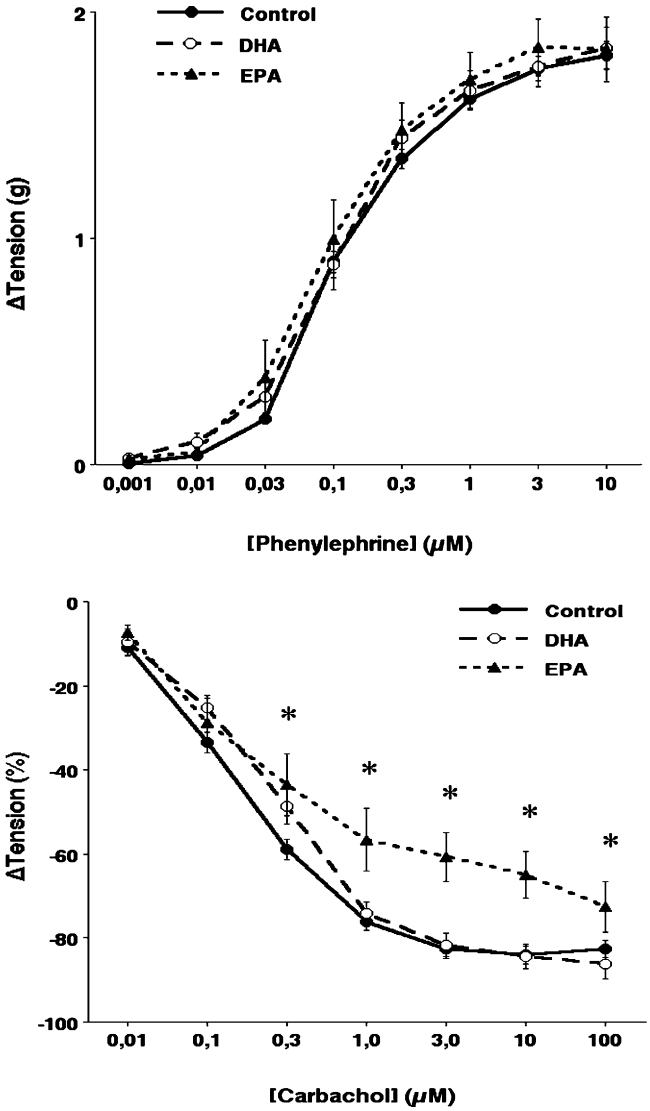


Fig. 1. Top, Cumulative DRCs to increasing concentrations of phenylephrine (10^{-9} to 10^{-5} mol/L) in rat isolated aortic rings incubated in the absence (control, n = 26) or presence of DHA (90×10^{-6} mol/L, n = 10) or EPA (90×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. Bottom, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of DHA (90×10^{-6} mol/L, n = 9) or EPA (90×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and EPA-exposed aortic rings.

on a 4-channel multipen recorder. The rings were allowed to equilibrate in the chamber for 1 hour, during which time the Krebs buffer was changed at 15-minute intervals. Contractions were elicited to 18 mmol/L KCl and then to 10^{-6} mol/L phenylephrine. While the rings were contracted with phenylephrine, endothelium-dependent dilator responses to 10^{-5} mol/L carbachol were obtained to ensure that the endothelium of each vascular ring was functioning. After washout of the phenylephrine and carbachol, the rings were allowed to equilibrate in the presence or absence (control) of a specific FA (90 μmol/L) in the chamber for 45 minutes, during which the Krebs + bovine serum albumin buffer solution was changed at 15-minute intervals. The FAs were added in the chamber after each washout. Then, a cumulative dose-response curve (DRC) to phenylephrine (10^{-9} to 10^{-5} mol/L) was obtained in the absence or presence of FA (the same FA that was present before). After a washout period, rings were precontracted with 10^{-6} mol/L phenylephrine, and cumulative relaxation curves to carbachol (10^{-8} to 10^{-4} mol/L) and then to sodium nitroprusside (SNP; 10^{-10} to 10^{-7} mol/L) were quantified (in the absence

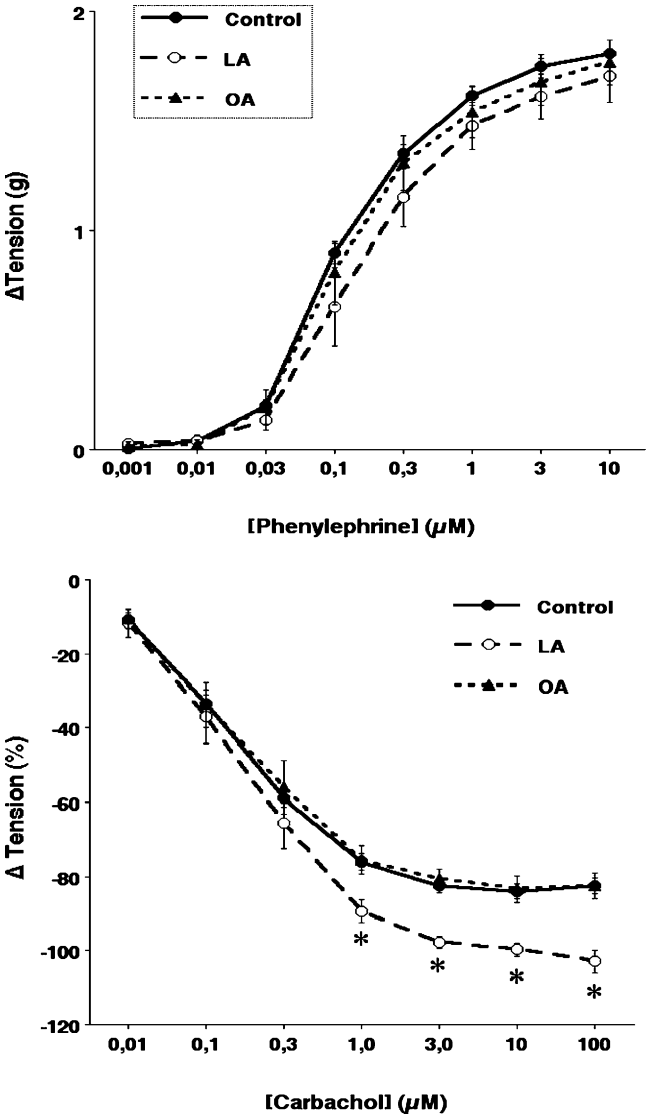


Fig. 2. Top, Cumulative DRCs to increasing concentrations of phenylephrine (10^{-9} to 10^{-5} mol/L) in rat isolated aortic rings incubated in the absence (control, n = 26) or presence of LA (90 × 10^{-6} mol/L, n = 8) or OA (90 × 10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. Bottom, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of LA (90 × 10^{-6} mol/L, n = 8) or OA (90 × 10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. *P < .05 indicates a difference between control and LA-exposed aortic rings.

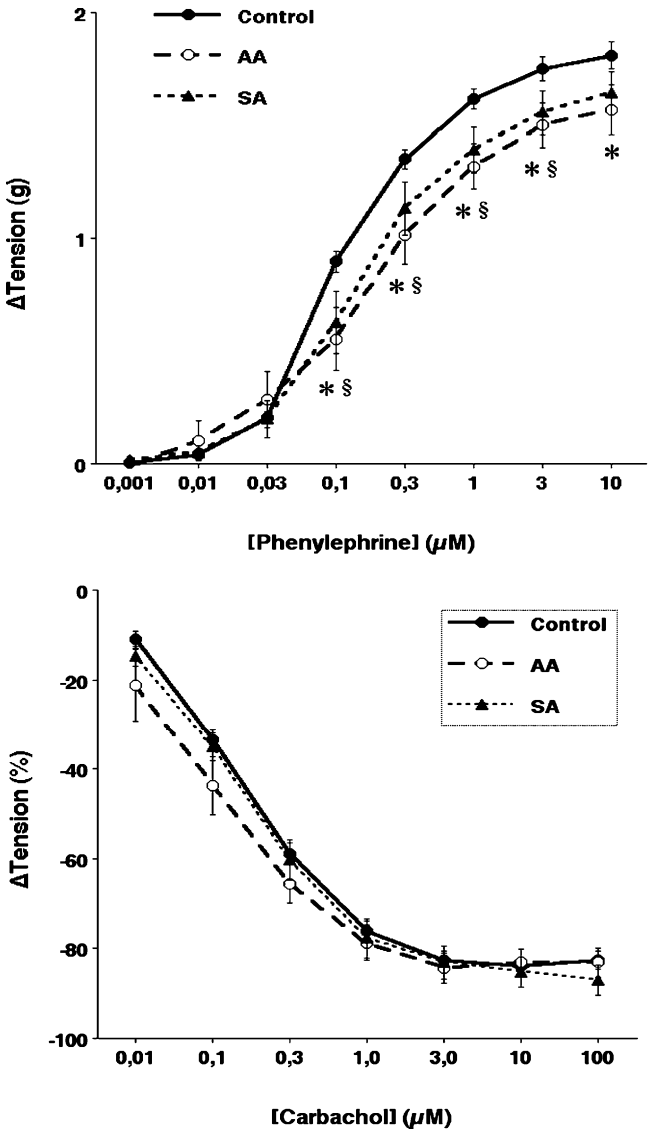


Fig. 3. Top, Cumulative DRCs to increasing concentrations of phenylephrine (10^{-9} to 10^{-5} mol/L) in rat isolated aortic rings incubated in the absence (control, n = 26) or presence of AA (90 × 10^{-6} mol/L, n = 9) or SA (90 × 10^{-6} mol/L, n = 12). Values are means ± SE shown by vertical lines. *P <.05 indicates a difference between control and AA-exposed aortic rings. §P < .05 indicates a significant difference between control and SA-exposed aortic rings. Bottom, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of AA (90 × 10^{-6} mol/L, n = 9) or SA (90 × 10^{-6} mol/L, n = 11). Values are means ± SE shown by vertical lines.

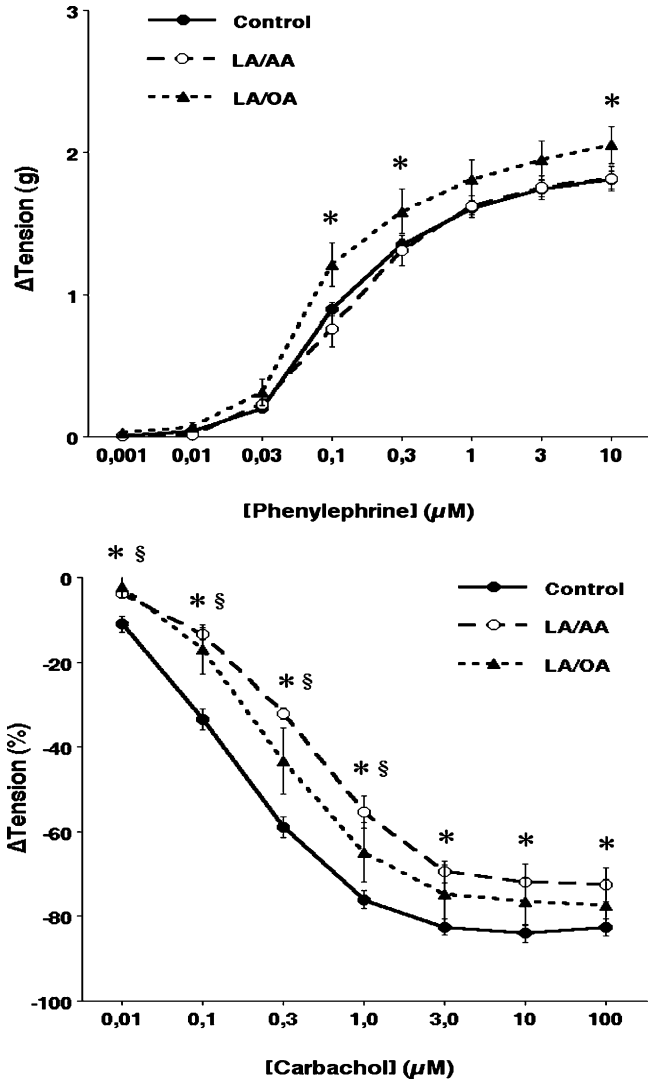


Fig. 4. Top, Cumulative DRCs to increasing concentrations of phenylephrine (10^{-9} to 10^{-5} mol/L) in rat isolated aortic rings incubated in the absence (control, n = 26) or presence of a mixture made of LA/AA (both at 90×10^{-6} mol/L, n = 7) or LA/OA (both at 90×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and LA/OA-exposed aortic rings. Bottom, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made of LA/AA (both at 90×10^{-6} mol/L, n = 7) or LA/OA (both at 90×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and LA/AA-exposed aortic rings. §$P < .05$ indicates a significant difference between control and LA/OA-exposed aortic rings.

or presence of FA) to assess endothelium-dependent and endothelium-independent ability of the smooth muscle to relax in each experimental group. After a washout period, the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) (100 μmol/L) was added in each organ baths and rings again were precontracted with phenylephrine (10^{-6} mol/L), and cumulative relaxation curves to carbachol (10^{-8} to 10^{-4} mol/L) were evaluated in the absence or presence of FAs. The studied FAs belong to the main FA families, EPA and DHA of the $n - 3$ PUFA family, LA and arachidonic acid (AA) of the $n - 6$ PUFA family, OA of the monounsaturated $n - 9$ family, and stearic acid (SA) of the saturated family (sodium salts, Sigma-Aldrich Canada Ltd, Ontario, Canada). When provided by the diet, these FAs reach the cell membrane phospholipids in significant proportions and may affect lipid-lipid and/or lipid-protein interactions and therefore protein functions [18]. Moreover, they are very often chosen for studies dealing with the effect of FA on different stages of CVD [1]. FAs were tested alone as well as in combination of 2: LA/AA, LA/OA, and DHA/EPA. These combinations were chosen based on metabolic relationships between them or related to their current coexistence in several foods. Indeed, in the context of

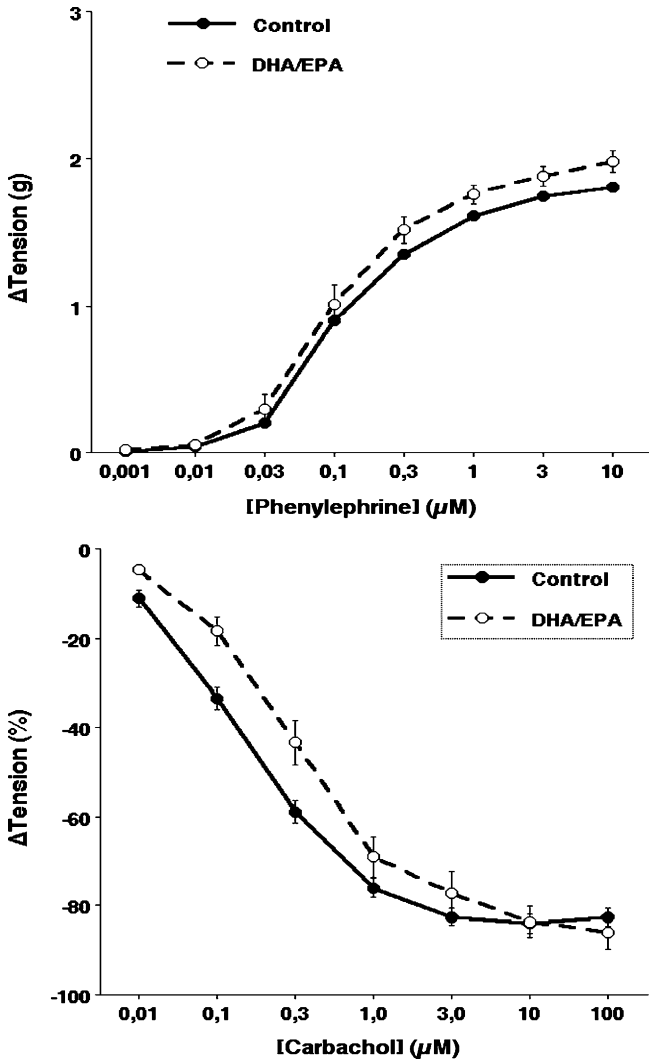
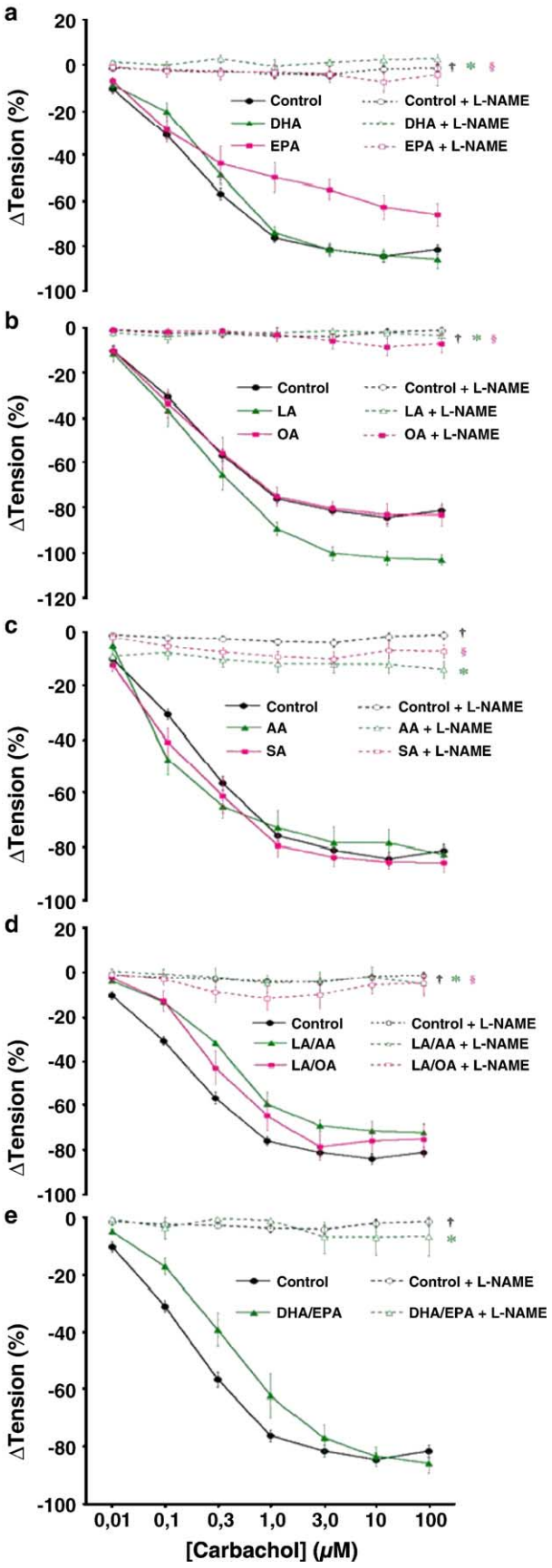


Fig. 5. Top, Cumulative DRCs to increasing concentrations of phenylephrine (10^{-9} to 10^{-5} mol/L) in rat isolated aortic rings incubated in the absence (control, n = 26) or presence of a mixture made of DHA/EPA (both at 90×10^{-6} mol/L, n = 6). Values are means ± SE shown by vertical lines. Bottom, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made of DHA/EPA (both at 90×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines.



CVD nutritional prevention, their coexistence in several foods is important. Concerning the LA/OA combination, it should be noted that LA and OA are among the most common unsaturated FA present in foods and sometimes in nonnegligible proportions in certain vegetable oils. Moreover, these 2 FAs have the same chain length but differ in their unsaturation level and in the fact that LA is an essential FA whereas OA is not. The LA/AA combination was chosen because of the metabolic links between these 2 PUFAs and

Fig. 6. A, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of DHA (90×10^{-6} mol/L, n = 9), DHA + L-NAME (100×10^{-6} mol/L, n = 9), EPA (90×10^{-6} mol/L, n = 9), or EPA + L-NAME (100×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between DHA-exposed aortic rings without L-NAME and DHA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between EPA-exposed aortic rings without L-NAME and EPA-exposed aortic rings with L-NAME. B, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of LA (90×10^{-6} mol/L, n = 8), LA + L-NAME (100×10^{-6} mol/L, n = 8), OA (90×10^{-6} mol/L, n = 9), or OA + L-NAME (100×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between LA-exposed aortic rings without L-NAME and LA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between OA-exposed aortic rings without L-NAME and OA-exposed aortic rings with L-NAME. C, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of AA (90×10^{-6} mol/L, n = 9), AA + L-NAME (100×10^{-6} mol/L, n = 9), SA (90×10^{-6} mol/L, n = 11), or SA + L-NAME (100×10^{-6} mol/L, n = 11). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between AA-exposed aortic rings without L-NAME and AA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between SA-exposed aortic rings without L-NAME and SA-exposed aortic rings with L-NAME. D, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with LA/AA (both at 90×10^{-6} mol/L, n = 7), LA/AA + L-NAME (100×10^{-6} mol/L, n = 7), LA/OA (both at 90×10^{-6} mol/L, n = 7), or LA/OA + L-NAME (100×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between LA/AA-exposed aortic rings without L-NAME and LA/AA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between LA/OA-exposed aortic rings without L-NAME and LA/OA-exposed aortic rings with L-NAME. E, Cumulative DRCs to increasing concentrations of carbachol (10^{-8} to 10^{-4} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with DHA/EPA (both at 90×10^{-6} mol/L, n = 7) or DHA/EPA + L-NAME (100×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between the DHA/EPA-exposed aortic rings without L-NAME and the DHA/EPA-exposed aortic rings with L-NAME.

especially because ECs have been shown to be deficient in FA $\delta - 6$ desaturase and, as a consequence, are not able to convert large amounts of LA (18:2$n - 6$) into AA (20:4$n - 6$) [19]. Finally, DHA and EPA are the most used FAs in studies on the dietary modulation of CVD, and they are naturally associated in their main source, which is fish oil. Therefore, DHA/EPA combination has to be studied, like the individual DHA and EPA, to better understand the effects of these 2 FAs and of fish oil on the disease.

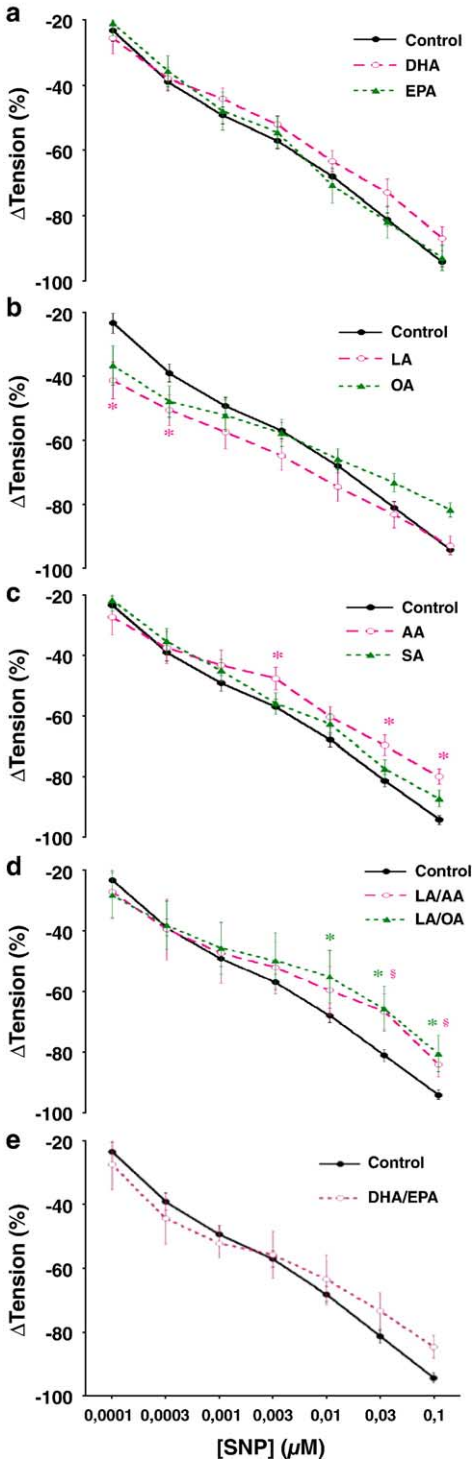


Fig. 7. A, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of DHA (90×10^{-6} mol/L, n = 9) or EPA (90×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. B, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of LA (90×10^{-6} mol/L, n = 9) or OA (90×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and LA-exposed aortic rings. C, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of AA (90×10^{-6} mol/L, n = 9) or SA (90×10^{-6} mol/L, n = 11). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and AA-exposed aortic rings. D, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with LA/AA (both at 90×10^{-6} mol/L, n = 7) or LA/OA (both at 90×10^{-6} mol/L, n = 8). Values are means ± SE shown by vertical lines. *$P < .05$ indicates a significant difference between control and LA/OA-exposed aortic rings. §$P < .05$ indicates a significant difference between control and LA/AA-exposed aortic rings. E, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with DHA/EPA (both at 90×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines.

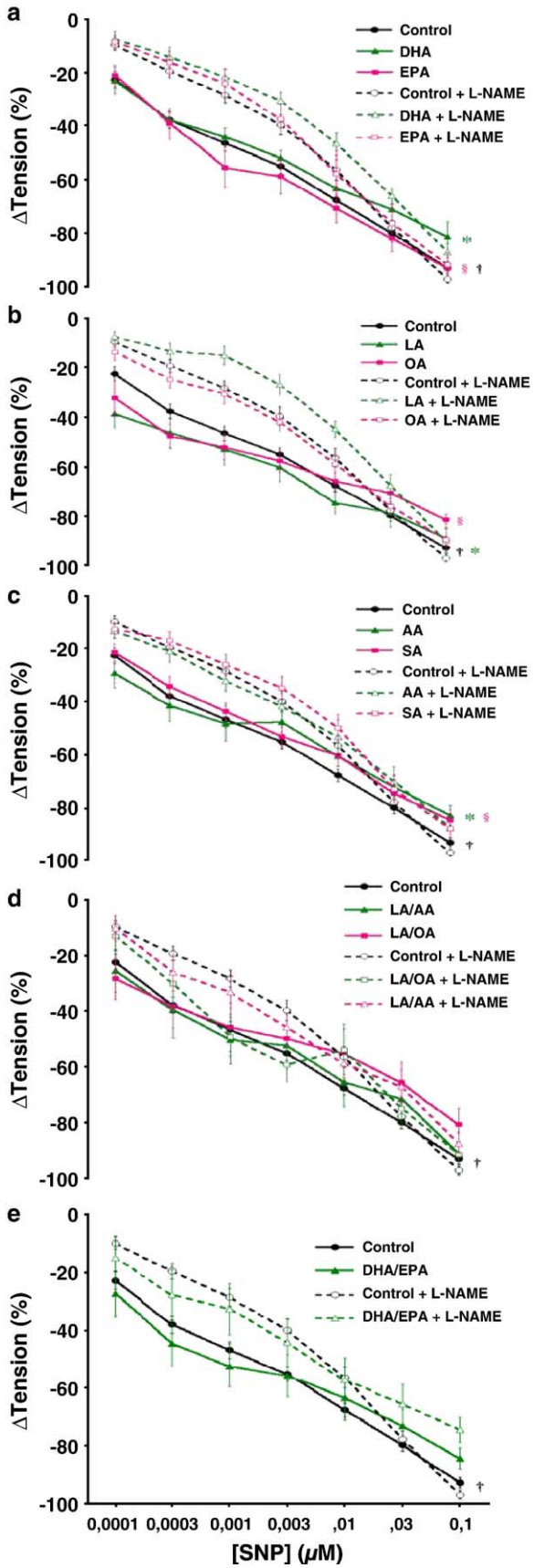
2.3. Data analysis

Data are expressed as means ± SE; n is the number of rats in each group. Multiple tracings from each rat were averages for each intervention. The DRCs were compared using analysis of variance for repeated measurements. Post hoc comparisons were made using Fisher exact test. A P value of less than .05 was considered to be statistically significant.

3. Results

3.1. Effects of FAs on endothelium-dependent vascular reactivity in rat aortic rings

DRCs for phenylephrine-induced contractions (10^{-9} to 10^{-5} mol/L) in endothelium-intact preparations were used as an indicator of Ca^{2+} release through the inositol 1,4,5-triphosphate receptor pathway, whereas cumulative relaxation curves to carbachol (10^{-8} to 10^{-4} mol/L) allowed smooth muscle cell relaxation after endothelial NO production to be assessed. Figs. 1-5 mainly show that EPA significantly reduced and LA significantly increased the relaxation response to carbachol in rat aortic rings preconstricted with 1 μmol/L phenylephrine (Figs. 1 and 2, bottom), whereas DHA, AA, OA, or SA had no significant effect on the DRCs to carbachol. However, AA and SA were found to decrease the contraction response to phenylephrine (Fig. 3, top), whereas EPA, DHA, LA, and OA did not change it (Fig. 2, top). The aortic reactivity was also altered by the FA combinations, the contraction response to



phenylephrine being increased by LA/OA (Fig. 4, top), whereas the endothelium-dependent relaxation was significantly reduced by LA/AA and LA/OA (Fig. 4, bottom). The combined DHA/EPA had no significant effect on the contraction response to phenylephrine or relaxation response to carbachol (Fig. 5).

In the presence of L-NAME, cumulative relaxation curves to carbachol in rat aortic rings preconstricted with phenylephrine remained to baseline whatever the FAs tested (Fig. 6A-E), meaning no visible relaxation occurred. The blockade of NO synthesis using the NOS inhibitor L-NAME suppressed the vasodilator responses to carbachol described above as well as the vascular relaxation effects of LA and EPA and therefore confirmed their endothelial dependence [20].

Fig. 8. A, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of DHA (90×10^{-6} mol/L, n = 9), DHA + L-NAME (100×10^{-6} mol/L, n = 9), EPA (90×10^{-6} mol/L, n = 9), or EPA + L-NAME (100×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between DHA-exposed aortic rings without L-NAME and DHA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between EPA-exposed aortic rings without L-NAME and EPA-exposed aortic rings with L-NAME. B, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of LA (90×10^{-6} mol/L, n = 9), LA + L-NAME (100×10^{-6} mol/L, n = 9), OA (90×10^{-6} mol/L, n = 9), or OA + L-NAME (100×10^{-6} mol/L, n = 9). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between LA-exposed aortic rings without L-NAME and LA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between OA-exposed aortic rings without L-NAME and OA-exposed aortic rings with L-NAME. C, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of AA (90×10^{-6} mol/L, n = 9), AA + L-NAME (100×10^{-6} mol/L, n = 9), SA (90×10^{-6} mol/L, n = 11), or SA + L-NAME (100×10^{-6} mol/L, n = 11). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. $^{*}P < .05$ indicates a significant difference between AA-exposed aortic rings without L-NAME and AA-exposed aortic rings with L-NAME. $^{\S}P < .05$ indicates a significant difference between SA-exposed aortic rings without L-NAME and SA-exposed aortic rings with L-NAME. D, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with LA/AA (both at 90×10^{-6} mol/L, n = 7), LA/AA + L-NAME (100×10^{-6} mol/L, n = 7), LA/OA (both at 90×10^{-6} mol/L, n = 8), or LA/OA + L-NAME (100×10^{-6} mol/L, n = 7). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings. E, Cumulative DRCs to increasing concentrations of SNP (10^{-10} to 10^{-7} mol/L) in rat isolated aortic rings precontracted with phenylephrine (10^{-6} mol/L) and incubated in the absence (control, n = 26) or presence of a mixture made with DHA/EPA (both at 90×10^{-6} mol/L, n = 7) or DHA/EPA + L-NAME (100×10^{-6} mol/L, n = 6). Values are means ± SE shown by vertical lines. $^{\dagger}P < .05$ indicates a significant difference between control and L-NAME–exposed aortic rings.

3.2. Effects of FAs on endothelium-independent vascular relaxation in rat aortic rings

Cumulative relaxation curves to the NO donor SNP (10^{-10} to 10^{-7} mol/L) were used to assess endothelium-independent ability of the smooth muscle to relax. Indeed, SNP releases NO after intracellular metabolism in the vascular smooth muscle cells (VSMCs), leading to relaxation via a cyclic guanosine monophosphate–dependent mechanism [21]. Fig. 7A to E mainly shows an enhanced endothelium-independent relaxation response with LA at low doses of SNP (Fig. 7B), but a reduced relaxation response with AA (Fig. 7C), LA/OA, and LA/AA at SNP doses of 0.03 to 0.1 μmol/L (Fig. 7D). DHA and EPA, alone or combined, had no significant effect on the endothelium-independent relaxation response (Fig. 7A, E). In the presence of L-NAME, cumulative relaxation curves to SNP (Fig. 8A, E). The addition of L-NAME in the incubation medium was found to slightly but significantly reduce the vasorelaxations elicited by low doses of SNP (10^{-10} to 10^{-9} mol/L) in control vessels, as well as in aortic rings incubated in the presence of DHA or EPA (Fig. 8A), LA or OA (Fig. 8B), or SA or AA (Fig. 8C). However, the vasorelaxing responses elicited in the presence of LA/OA, LA/AA, or DHA/EPA were not altered by the presence of L-NAME (Fig. 8D, E).

4. Discussion

This study clearly showed that certain individual as well as combined FAs alter the endothelial-dependent reactivity of thoracic aorta in healthy rats, whereas some FA combinations alter both endothelium-dependent and endothelium-independent vascular reactivity. Moreover, it is noteworthy that some of these FAs exhibited distinct modulating effect depending on whether they were alone or combined with another.

Individually, EPA, AA, LA ,and SA, but not DHA or OA, markedly altered the endothelial-dependent aorta reactivity as measured by phenylephrine- and carbachol-stimulated responses. The most striking findings concerned the significant effects of both EPA, which reduced the relaxation response to carbachol, and LA, which increased it. These results are in good agreement with some studies on healthy individuals [22] or ECs [23], but they deeply differed from other studies, mainly performed under diseased conditions [1,24-26]. Indeed, in an in vivo study conducted in healthy, nonsmoking men, with highly purified EPA and DHA, Hansen et al [22] found that neither the dietary intake of EPA or DHA nor the concentration of these FAs in serum phospholipids at baseline were correlated with PAI-1 activity (plasminogen activator inhibitor type 1, product of endothelial origin). The decrease in vascular relaxation observed here in response to EPA probably means that, under our conditions, this FA led to a reduced endothelial NO production. However, most of the studies dealing with the possible effects of $n-3$ FA in at-risk patients found increased NO production [9,27,28] and improved endothelium-dependent dilation with DHA, EPA, or a combination of both [2]. As DHA did not alter the vascular reactivity of aorta in our healthy rats, some controversy exists concerning the experimental data dealing with the effects of these FAs on vascular reactivity. On the other hand, the antiatherogenic effects of LA have been demonstrated, assuming that this FA inhibits cytokine-induced adhesion molecule expressions in ECs [23]. Thereafter, this could induce an increased NO production, which would explain the LA-induced increase in vasodilator response to carbachol observed here. However, LA has also been shown to decrease NO production in bovine artery ECs [13] as well as in healthy humans [15] and to exhibit atherogenic properties [14,29]. A proinflammatory and atherogenic nature, combined with a proactivating effect on ECs, has even been generalized to the whole $n-6$ FA series, including LA and AA [14]. Therefore, some controversy also exists for the modulatory effects of LA on the endothelium-dependent vasodilation.

Moreover, a decreased phenylephrine-induced contraction was obtained with AA and SA, suggesting that both FAs might induce a reduction in phenylephrine sensitivity. The precise mechanism involved in this reduction remains to be elucidated, but might be consistent with NO up-regulation in vascular tissues exposed to AA and SA. Indeed, in normotensive animals, norepinephrine was reported to act on ECs to increase NO production and/or release, thus attenuating its contractile effect on vascular smooth muscle [30]. Therefore, we cannot exclude that in vivo, at an early stage of atherosclerosis, NO up-regulation might provide a compensatory mechanism to preserve endothelial function when high levels of some FAs are present in the plasma. This mechanism would have contributed to the reduced contracting response to phenylephrine observed in the present study. However, the effects of AA and SA on the endothelial function remain insufficiently documented. Concerning the effect of OA on the endothelial-dependent aorta reactivity, our results showing no effect of this FA individually are partly in agreement with those of Herrera et al [7]. Indeed, these authors have shown that in rats fed the OO diet (reflecting a proper effect of OA), phenylephrine-induced contraction remained unchanged in normotensive WK rats whereas it decreased in SHR rats, and the acetylcholine-induced relaxation increased in both rat breeds, however, more slowly in WK than in SHR rats.

On the other hand, the reduction of endothelium-dependent relaxation by combined LA/AA and LA/OA revealed surprising interactions. Indeed, although LA alone significantly increased the relaxation response to carbachol in our healthy control rats, the effects of combined LA/OA and LA/AA both led to reduced endothelium-dependent relaxation. Moreover, LA/OA combination increased phenylephrine-induced contraction response. Considering the

latter FA combination, it may be noted that although the results previously reported with HOSO-fed rats [7] were different from our observations with combined LA/OA, they were also clearly different from those obtained with OO (ie, with OA alone) by these authors.

Very few explanations have been provided for these different effects. The mechanism of action of FAs in modulating vascular physiology is not known, but it could be linked to changes in cell membrane homeostasis, at least partly because of their incorporation into the EC membrane phospholipids [7,14,23]. Indeed, it has previously been demonstrated that all FAs are able to be incorporated into membrane phospholipids of ECs within less than 1 hour [19] and even after 10 minutes of incubation with the cells [31]. This could have occurred here because the incubation time used was 45 minutes. Furthermore, selective incorporation mechanisms for specific FAs into various phospholipids via the deacylation-reacylation pathway have been demonstrated in ECs [31,32]. Eicosanoid precursors—that is, AA, eicosatrienoic acid (20:3$n-6$), and EPA—have been shown to be incorporated at comparable rates and more rapidly than other FAs, namely, DHA, LA, and OA [31]. According to this hypothesis, the unique effect of EPA in our experimental conditions and the apparent inaction of DHA may partly result from the retroconversion of the latter [31-33] and the subsequent increase in EPA content in cell membrane phospholipids. Moreover, the respective effects of AA and LA could be related to the competition for esterification into position 2 of glycero-phospholipids, which likely takes place between these FAs, as PUFA are known to compete much more than saturated FA for this 2-acyltransferase reaction [19]. The potential negative effect of AA could predominate on the positive effect of LA on vasorelaxation, possibly because of the more rapid incorporation of AA than LA into EC phospholipids [31], leading to the observed reduction in the vasodilator response induced by combined LA/AA. The effect of LA/OA combination could result from a similar mechanism because OA has been shown previously to reduce the vasodilator response [13].

Furthermore, the reduced endothelium-independent relaxation provoked by combined LA/OA and LA/AA at the highest SNP doses might be because of some modulation of the NO production signaling pathway in VSMCs. Indeed, our data showed that AA alone significantly reduced the relaxing effect of SNP, whereas LA alone tended to increase it and OA was shown to have no effect [13]. Metabolic interactions between FA and especially the competition evoked above for LA/AA combination could likely be different in VSMC than in ECs and that led to the observed effects with the 2 combinations. The mechanism involved could be attributed to the vasodilator response to carbachol we observed here with LA, which may be related rather to its action on VSMC than on ECs.

In summary, the most striking finding of this study was the effect of both EPA and LA on the vascular reactivity of healthy animals. Although they were obtained in an ex vivo study, these results revealed unexpected effects of some FAs as compared with those reported in the literature, which were generally obtained in vascular preparations from animals or in humans who have CVD or cells exposed to inflammatory cytokines. We also observed for the first time the modulating effect of combining several purified FAs, which might result from possible interactions between their respective relaxing or contracting role. Above all, this study clearly emphasized the complexity of the metabolic phenomena involved in the modulation of endothelial function by FAs. This complexity might be higher because the final effect of FAs could occur via the regulation of gene expression, either directly by such FA [34] or by their derived products [35]. Further studies are needed to elucidate the mechanism of action of individual or combined dietary FAs in the modulation of vascular reactivity, perhaps taking into account the possible participation of an endothelium-derived hyperpolarizing factor, which seems to be required to promote relaxation in certain rat arteries [36].

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