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Dietary n-3 polyunsaturated fatty acids and endothelium dysfunction induced by lysophosphatidylcholine in Syrian hamster aorta

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Abstract

This study investigated the influence of an eicosapentaenoic acid (EPA)– or a docosahexaenoic acid (DHA)–supplemented diet on the deleterious effects of lysophosphatidylcholine (LPC) on endothelium-dependent vasorelaxation of Golden Syrian hamster thoracic aorta. In a second step, LPC-modulated phospholipase A_2 (PLA_2)–derived ways of relaxation were investigated. Golden Syrian hamsters were fed for 6 weeks with a control diet or an EPA- or DHA-supplemented diet. Aortic fatty acid composition was analyzed by gas chromatography. Aortic rings were incubated for 20 minutes with LPC before constructing cumulative concentration-response curves for acetylcholine (ACh; 3 nmol/L-30 μmol/L) or sodium nitroprusside (3 nmol/L-30 μmol/L). The EPA- or DHA-supplemented diet increased n-3 polyunsaturated fatty acids in aortic fatty acids content because of the increase of EPA or DHA content, respectively, and decreased arachidonic acid aortic content. Lysophosphatidylcholine (1, 10, 15, and 20 μmol/L) induced a concentration-dependent inhibition of ACh-induced relaxation of preconstricted aortic rings in the control group, but did not influence sodium nitroprusside–induced aortic relaxation. The DHA- or EPA-supplemented diet worsened LPC (20 μmol/L) inhibitory effects on ACh-induced vasorelaxation. In the control diet group, ACh-induced relaxation was abolished by the nitric oxide synthase inhibitor (L-N^G-nitro-arginine methyl ester; 100μmol/L), whether LPC was added or not. The ACh-induced vasorelaxation was partially inhibited by PLA_2 inhibitors methyl arachidonyl fluorophosphonate (25 μmol/L) and arachidonyl trifluoromethyl ketone (20 μmol/L) as well as by the combination of 2 Ca^{2+}-dependent potassium (K_{Ca}) channel inhibitors charybdotoxin (0.1 μmol/L) plus apamin (0.3 μmol/L). In the presence of LPC (20 μmol/L), ACh-induced vasorelaxation was abolished by these inhibitors. These effects were not influenced by DHA or EPA diet. Our results suggested that EPA- or DHA-supplemented diet did not exhibit any beneficial effect against LPC-induced inhibition of endothelium-dependent aortic relaxation in Golden Syrian hamsters. These LPC effects were associated in our study not only with an inhibition of nitric oxide–dependent vasorelaxation, but also with a concomitant activation of a compensatory vasorelaxant pathway depending both on PLA_2 metabolites and on K_{Ca} channel opening.

1. Introduction

n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), found in seafood and fish oil complements, have attracted much attention because of their beneficial effects on cardiovascular diseases [1,2]. Dietary intake of n-3 PUFA has been reported to reduce hypertension [3,4], decrease the risk of cardiac arrest in patients [5], and reduce the risk of arrhythmia and myocardial infarction in animal models [6]. Clinical studies have also shown antiatherosclerotic effects of intake of fish or fish oils in patients [7,8]. These effects might involve changes in lipids and lipoproteins leading to a less atherogenic profile [9]. Moreover, incorporation of PUFA into vascular membranes may inhibit adhesion molecule expression and smooth muscle cell proliferation, decrease inflammation [10], and consequently contribute to the stabilization of atherosclerotic plaques [8].

Influence of EPA and DHA on endothelium-dependent vasorelaxation remains controversial, depending on their mode of administration (diet vs in vitro incubation), animal species, and pathology. The DHA-supplemented diet has been reported to improve the forearm blood flow in response

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to acetylcholine (ACh) in hyperlipidemic, overweight men [11]. However, short-term high-dose supplementation with EPA and DHA did not improve the endothelium-dependent vasodilation of peripheral arteries in patients with chronic heart failure [12]. Moreover, in animal models, EPA altered the endothelium-dependent vasorelaxation in healthy rats [13]; and DHA supplementation failed to induce any effect on aortic vasorelaxing and vasoconstricting responses in streptozotocin-induced diabetic rat [14].

Atherosclerosis is associated very early with abnormalities of vascular functions characterized by a marked attenuation of endothelium-dependent relaxation even before histologic evidence of plaque formation [15]. It is now well recognized that oxidized low-density lipoproteins contribute to this endothelial dysfunction. These deleterious effects have been shown to result mainly from the accumulation of oxidized lipid derivates in atherosclerotic lesions, and lysophosphatidylcholine (LPC) is thought to be one of the main active components mediating these effects [16,17]. Lysophosphatidylcholine was reported to inhibit nitric oxide (NO)–mediated and endothelium-dependent hyperpolarizing factor (EDHF)–mediated relaxation of different vessels, like porcine coronary arteries or rat aorta [18,19]. In these effects, LPC seems to desensitize endothelial nitric oxide synthase (eNOS) by interfering with Ca^{2+} signal and eNOS phosphorylation and to activate arachidonic acid (AA) release by phospholipase A_2 (PLA_2) stimulation [20]. The release of AA from phospholipids is regarded as an important step for the biosynthesis of eicosanoids by its transformation by cyclooxygenase (COX), lipoxygenase, and cytochrome P450. The increased activity of these enzymes may compensate for the decrease in NO availability observed in pathological conditions. However, the influence of LPC on these metabolic pathways has not yet been fully elucidated.

In this context, the goal of this study was to test the influence of an EPA- or a DHA-supplemented diet on the ACh-induced vasorelaxation in the presence of LPC in Golden Syrian hamster thoracic aorta. In a second step, we examined the PLA_2-derived way of relaxation activated in the presence of LPC and particularly the implication of COX and CYP450 epoxygenase metabolites and the one of small and large conductance Ca^{2+}-dependent K^+ channels.

2. Materials and methods

2.1. Animals and models

Male Golden Syrian hamsters were obtained from Janvier (a company providing laboratory animals; Le Genest Saint Isle, France) at 7 weeks of age, housed 3 per cage, and provided free access to food and water. After the 1-week acclimation period, hamsters were randomly divided into 3 dietary groups of 20 hamsters, receiving a control diet, the EPA-supplemented diet, or the DHA-supplemented diet for 6 weeks.

Table 1
Fatty acid composition (as percentage of total fatty acid) as analyzed in the lipid blend of the 3 dietary groups (3 blends were prepared for each group)

Fatty acid	Control diet	EPA diet	DHA diet
16:0	12.8	11.2	13.9
18:0	12.0	14.4	15.7
18:1 n-9	31.5	25.9	28.1
18:2 n-6	39.5	25.2	24.6
20:5 n-3	ND	17.9	0.3
22:5 n-3	ND	ND	2,4
22:6 n-3	ND	ND	9.5
Total SFA	26.1	26.7	29.1
Total MUFA	32.4	27.6	29.2
Total PUFA	41.5	45.7	41.7

ND indicates not detected.

2.2. Diets

The hamsters were fed a semipurified jellied diet [21] ad libitum in accordance with the AIN-93 recommendations. The food, which was prepared as a jellied mass, cut into cubes for feeding, and stored at −20°C, was distributed daily to the hamsters to maintain moisture content. Lipids (80 g/kg) were incorporated into a standard fabricated diet containing starch (526.2 g/kg), sucrose (100 g/kg), cellulose (50 g/kg), soy protein isolate (140 g/kg, ICN 905456), L-cystine (1.8 g/kg), gelatin (50 g/kg), salt mixture (40 g/kg, ICN 960401), vitamin mixture (10 g/kg, ICN 960402), and choline bitartrate (2 g/kg). For the dietary control groups (n = 15), the lipid part comprised 28 g/kg of cocoa butter (Cacao Barry, Meulan, France) plus 48 g/kg of sunflower seed oil (Fruidor, Asnières-sur-Seine, France) plus 4 g/kg of Oleisol (Lesieur, Antony, France). For the 2 other groups, DHA (n = 15) and EPA (n = 15), the experimental lipid part comprised 36 g/kg of cocoa butter plus 28 g/kg of sunflower seed oil plus 4 g/kg of Oleisol (Lesieur) plus 9.6 g/kg of DHA (ROPUFA “60”; Hoffmann-Laroche, Basel, Switzerland) or EPA (ROPUFA “70”; Hoffmann-Laroche). The fatty acid composition and particularly the content of DHA and EPA in these diets have been determined by gas chromatography after lipid extraction. As shown in Table 1, the diets were similar in their saturated, monounsaturated, and polyunsaturated fatty acid (SFA, MUFA, and PUFA, respectively) composition but differed qualitatively in their PUFA composition.

2.3. Biochemical investigations

At the end of the 6-week diet, 5 hamsters per group were anesthetized with pentobarbital (60 mg/kg). The thoracic aorta was quickly removed, cleaned of adhering tissues, longitudinally opened, rinsed with cold saline, immediately placed in dry ice, and then stored at −80°C until analysis. The lipids were extracted from the aorta in a mixture of chloroform-methanol (2:1). Phospholipids were separated from nonphosphorous lipids on silica acid cartridges [22,23], and the fatty acids were transmethylated with BF3-methanol [24]. Methyl esters were analyzed by gas chromatography on

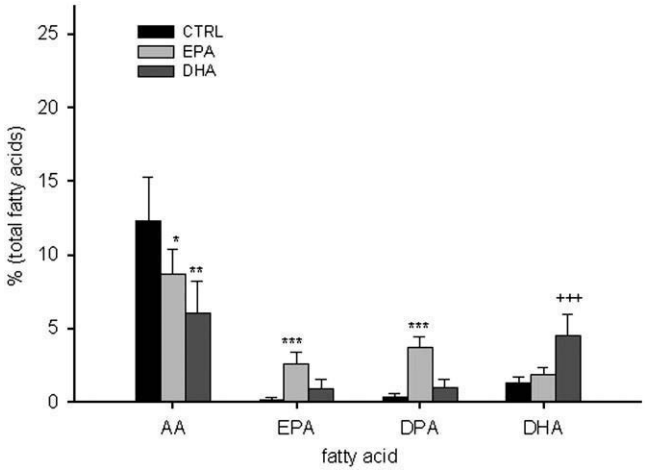

Fig. 1. Polyunsaturated fatty acid composition in the phospholipids fraction of aortic wall in hamsters fed the control diet (n = 5), EPA-supplemented diet (n = 5), or DHA-supplemented diet (n = 5). Results are expressed as mean ± SEM. *P < .05 vs control group; **P < .01 vs control group; ***P < .001 vs control group; +++P < .001 vs EPA group. CTRL indicates control.

an Econo-Cap EC-WAX capillary column (0.32 × 30 m; Alltech Associates, Deerfield, IL) coupled to a flame ionization detector using C17:0 as the internal standard.

2.4. Preparation of aortic rings and functional procedures

After the 6-week diet, 12 hamsters per group were anesthetized with pentobarbital (60 mg/kg). The thoracic aortas were dissected free and placed in ice-cold Krebs-Henseleit buffer containing the following (in millimoles per liter): NaCl 116.3, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 1.04, $MgSO_4$ 0.83, $NaHCO_3$ 19, glucose 5.5 (pH 7.4). The blood vessels were cleaned of connective tissue and cut into 2-mm rings. The rings were suspended isometrically between 2 stainless steel hooks in organ chambers containing 5 mL Krebs solution at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. The aortic rings were attached to a force-displacement transducer connected to an amplifier (Bionic Instrument; Phymep, Paris, France) and a data acquisition system (PowerLab; ADInstruments, Bella Vista, Australia) to record tension changes using Chart version 5 data analysis software (ADInstruments). After a stabilization period, the rings were stretched in a stepwise manner and were allowed to equilibrate at 1.5 g over a period of 1 hour during which the Krebs-Henseleit solution was changed every 15 minutes. The arterial rings were then challenged with KCl (60 mmol/L) to evaluate their functional integrity. When the developed tension had reached its peak value, the rings were washed with Krebs solution and then allowed to equilibrate for a 40-minute period. The rings were then contracted with increasing doses of phenylephrine (PE; 3 nmol/L-30 μmol/L; Sigma-Aldrich Chemicals, Saint-Quentin Fallavier, France). After stabilization of the maximal contraction, the presence of functional endothelial cells was confirmed by the relaxation induced by ACh (1 μmol/L; Sigma-Aldrich Chemicals). Endothelium was considered to be intact when ACh induced at least a 75% relaxation of the rings. The concentration of PE-inducing 80% maximal contraction of the rings was used to precontract the arteries after a washout and a 1-hour recovery period.

To assess the effects of LPC (Sigma-Aldrich Chemicals) on the vasorelaxant responses, the rings were incubated for 20 minutes with LPC (0, 1, 10, 15, and 20 μmol/L) before the contraction by PE (1 μmol/L) was repeated. After reaching a tension plateau value, the relaxation of aortic rings was evaluated by cumulative additions of ACh (3 nmol/L-30 μmol/L) or of the NO donor sodium nitroprusside (SNP; 3 nmol/L- 30 μmol/L). To test the involvement of NO, AA metabolites, or Ca^{2+}-dependent K^+ channels in the vasorelaxant response to ACh, arterial rings were incubated for 30 minutes with the NO synthase inhibitor L-N^G-nitro-arginine methyl ester (L-NAME; 100 μmol/L; Sigma-Aldrich), the phospholipase A_2 inhibitors methyl arachidonyl fluorophosphonate (25 μmol/L; Cayman Chemical, Ann Arbor, MI) and arachidonyl trifluoromethyl ketone ($AACOCF_3$; 20 μmol/L; ALEXIS Biochemicals, Plymouth Meeting, CA), the COX inhibitor indomethacin (10 μmol/L; Sigma-Aldrich), the CYP450 epoxygenase inhibitor sulfaphenazole (10 μmol/L; Sigma-Aldrich), or the large (BK_{Ca}) and small (SK_{Ca}) conductance Ca^{2+}-dependent K^+ channel inhibitor charybdotoxin (CHTX; 0.1 μmol/L; Sigma-Aldrich) plus apamin (APA; 0.3 μmol/L; Sigma-Aldrich) before contraction.

Relaxation values were expressed as percentage of decreases of the PE-induced vasoconstrictor tone. E_{max} indicates the maximal relaxation obtained for ACh and SNP. Variation of E_{max} induced by the inhibitors was calculated for each hamster aorta in the absence or presence of LPC by the use of the following formula: $\Delta E_{max} = 100^* [E_{max\ (ACh\ ctrl)} - E_{max\ (ACh\ +\ inhibitor)}/E_{max\ (ACh\ ctrl)}]$, where $E_{max\ (ACh\ ctrl)}$ is the E_{max} value induced by ACh on the control ring and $E_{max\ (ACh\ +\ inhibitor)}$ is the E_{max} value induced by ACh on the ring preincubated with one inhibitor. Potencies were expressed as pD_2, which is the −log EC_{50}, in which EC_{50} is the concentration of each agonist producing half of its maximal response. The EC_{50} is derived from log-logit transformations of individual concentration-response curves.

2.5. Statistical analysis

The results are expressed as mean ± SEM. Concentrations-response curves were analyzed using a 2-way analysis of variance (ANOVA) followed by the Bonferroni-corrected t test. Diet and LPC treatment are fixed factors. Student unpaired t test or Mann and Whitney test (if one group failed the normality test) was used for comparison between E_{max} and pD_2 values of different groups. All differences were considered significant when P < .05.

3. Results

Initially, the mean body weight (BW) of hamsters did not differ significantly between the 3 groups of animals (BW =

Table 2
Maximal effects (E_{max}) and potencies (pD_2)

Test	Groups	n	E_{max}	pD_2
ACh	Control	11	87.1 ± 1.2%	7.4 ± 0.1
	DHA	11	86.4 ± 2.2%	7.5 ± 0.1
	EPA	10	80.8 ± 2.7%	7.5 ± 0.2
ACh + LPC	Control	9	52.8 ± 7.3%***	6.2 ± 0.2***
	DHA	10	29.5 ± 7.0%***	5.8 ± 0.2***
	EPA	10	24.8 ± 7.2%***	5.9 ± 0.2***
ACh + $AACOCF_3$	Control	6	70.0 ± 11%*	6.8 ± 0.2**
	DHA	7	70.8 ± 8.3%**	6.9 ± 0.1
	EPA	6	65.1 ± 5.4%*	7.0 ± 0.1
ACh + $AACOCF_3$ + LPC	Control	6	9.5 ± 3.4%†	
	DHA	7	3.51 ± 0.8%††	
	EPA	6	6.3 ± 2.7%†	–
ACh + APA + CHTX	Control	5	74.8 ± 8.9%*	7.34 ± 0.01
	DHA	5	71.7 ± 6.9%*	7.34 ± 0.01
	EPA	5	79.1 ± 3.9%	7.23 ± 0.01
ACh + APA + CHTX + LPC	Control	5	17.1 ± 4.3%‡	–
	DHA	5	7.2 ± 4.5%‡	
	EPA	5	7.3 ± 5.2%	

The E_{max} and the pD_2 values are for ACh alone or in the presence of LPC (20 μmol/L), $AACOCF_3$ (20μmol/L), LPC and $AACOCF_3$, APA (0.3 μmol/L) + CHTX (0.1 μmol/L), and APA + CHTX + LPC in the 3 groups of hamsters. Results are the means ± SEM for n hamsters. ***$P < .0001$, **$P < .01$, and *$P < .05$ vs ACh test of the same diet group of hamsters. $^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$ vs ACh + $AACOCF_3$ test of the same diet group of hamsters. $^{\ddagger}P < .05$ vs ACh + APA + CHTX test of the same diet group of hamsters.

82.7 ± 1.4, 83.1 ± 0.8, and 83.9 ± 1.0 g in control, EPA, and DHA groups of hamsters, respectively); and EPA or DHA feeding did not alter their BM gain (BW = 105.2 ± 2.2, 102.7 ± 2.5, and 103.9 ± 2.1 g after 6 weeks of diet in control, EPA, and DHA groups, respectively).

3.1. Fatty acid composition of aortic phospholipids

Fig. 1 shows that EPA-supplemented diet induced an increase in n-3 PUFA (9.4% ± 2.9% in EPA group vs 3.8% ± 1.9% in control group, $P < .05$) in the entire aortic wall phospholipids fraction because of the incorporation of EPA (2.6% ± 0.8% in EPA group vs 0.1% ± 0.2% in control group, $P < .001$) and its higher metabolite docosapentaenoic acid (3.7% ± 0.7% in EPA group vs 0.4% ± 0.2% in control group, $P < .001$). These modifications were associated with a significant decrease in AA ($P < .05$) without a significant decrease in total n-6 PUFA (19.8% ± 3.7% in EPA group vs 25.3% ± 6.1% in control group, not significant). The DHA-supplemented diet induced an increase in n-3 PUFA in entire aortic wall phospholipids fraction (11.3% ± 6.1% in DHA group vs 3.8% ± 1.9% in control group, $P < .01$) owing to the incorporation of DHA (5.0% ± 1.3% in DHA group vs 1.3 ± 0.4% in control group, $P < .001$) and a significant decrease in AA ($P < .01$) and 22:4w6 ($P < .01$), without a significant decrease in total n-6 PUFA (18.2% ± 6.2% in DHA group vs 25.3% ± 6.1% in control group, not significant). Neither EPA nor DHA feeding altered the fatty acid content of SFA, MUFA, and PUFA in aortic wall phospholipids fraction.

3.2. Effects of LPC on aortic relaxation induced by ACh and SNP: influence of diet

Lysophosphatidylcholine (1, 10, 15, and 20 μmol/L) induced a concentration-dependent inhibition of ACh-induced relaxation of preconstricted hamster aortic rings (ANOVA, $P < .001$) associated with a decrease in ACh sensitivity and in its maximal relaxant effect (Table 2). These effects were endothelium dependent because LPC (1, 10, 15, and 20 μmol/L) did not affect the aortic relaxation induced by SNP, an endothelium-independent NO donor (Fig. 2).

The EPA- or DHA-supplemented diet did not modify the relaxant effects of ACh on hamster aortic rings in control conditions. However, in the presence of LPC (20 μmol/L), this relaxation was significantly lower in the EPA and DHA groups than in the control group (ANOVA, $P < .001$, respectively) (Table 2, Fig. 3).

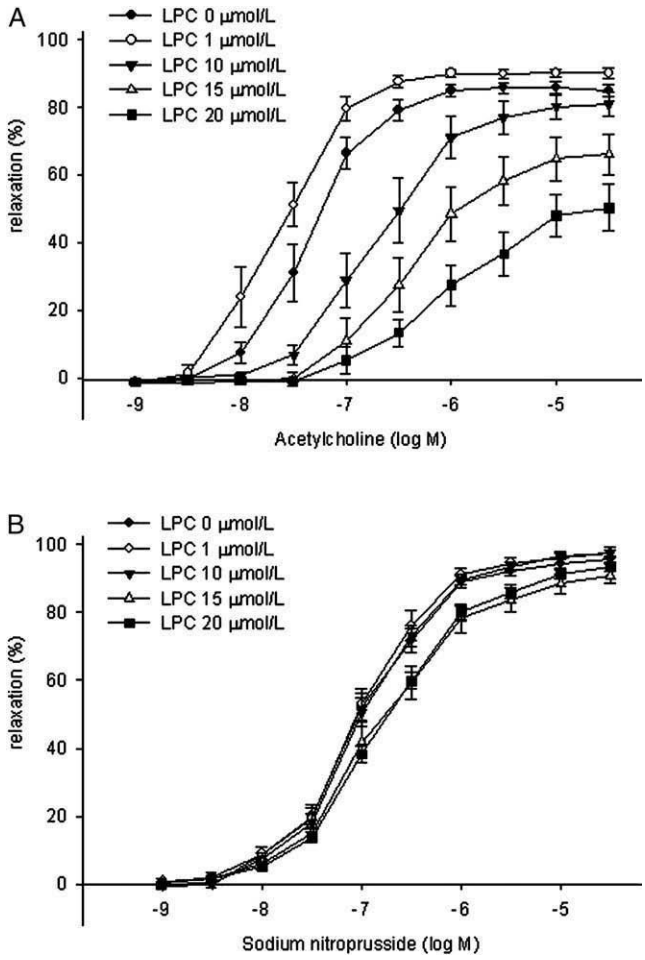

Fig. 2. Concentration-response curves for ACh (0.001-30 μmol/L, n = 8) (A) or SNP (0.001-30 μmol/L, n = 8) (B) in PE-contracted aortic rings of control hamsters incubated in the absence or the presence of LPC (1, 10, 15, and 20 μmol/L; n = 8). Results are expressed as mean ± SEM.

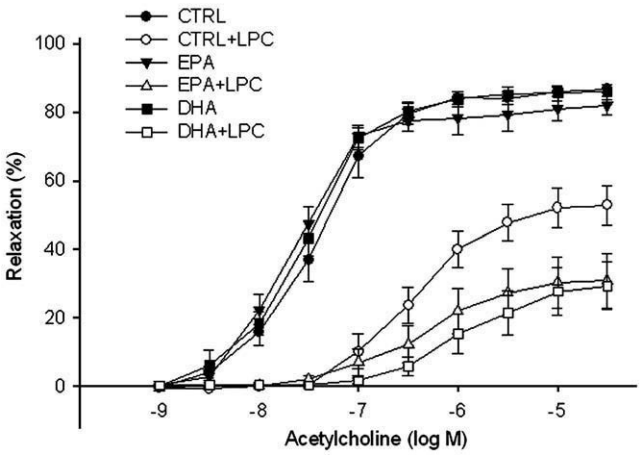

Fig. 3. Concentration-response curves for ACh (0.001-30 μmol/L) in PE-contracted (1 μmol/L) aortic rings in control hamsters (n = 11, 9), EPA-supplemented hamsters (n = 10, 10), and DHA-supplemented hamsters (n = 11, 10) in the absence (full symbols) or presence (empty symbols) of LPC (20 μmol/L), respectively. Results are expressed as mean ± SEM.

3.3. Effects of LPC on NO- and PLA_2-dependent relaxation induced by ACh: influence of diet

Acetylcholine-induced aortic relaxation was completely inhibited by L-NAME (100 μmol/L) in control conditions or in the presence of LPC (20 μmol/L), suggesting that NO was the main way of relaxation in both conditions. Similar results were obtained in EPA and DHA groups (Fig. 4).

In the 3 diet groups, when the aortic rings were preincubated with LPC (20 μmol/L), ACh-induced relaxation was strongly inhibited by the PLA_2 inhibitor $AACOCF_3$ (20 μmol/L) (ANOVA, $P < .001$), whereas in the absence of LPC (20 μmol/L), $AACOCF_3$ (20 μmol/L) shifted moderately but significantly to the right the ACh-induced relaxation curve (ANOVA, $P < .001$; Fig. 4); and these results were confirmed by the decrease of the E_{max} values (Tables 2 and 3). Similar data were obtained by the use of a second PLA_2 inhibitor, methyl arachidonyl fluorophosphonate (25 μmol/L) (ANOVA, $P < .01$; data not shown). Our data suggest that pretreatment of the aortic rings with LPC (20 μmol/L) stimulates a PLA_2-dependent way of relaxation.

Aortic pretreatment with the COX inhibitor indomethacin (10 μmol/L) or the CYP2C9 inhibitor sulfaphenazole (10 μmol/L) did not further modify the ACh-induced relaxation in the absence or presence of LPC (20 μmol/L), as illustrated by the variations of the E_{max} values (Table 3), suggesting that the activation of PLA_2 is not associated with the one of COX or CYP2C9. However, pretreatment of the rings with the combination of the K_{Ca} channel inhibitor CHTX (0.1 μmol/L) plus APA (0.3 μmol/L) mimicked the inhibitory effects of the PLA_2 inhibitors on the ACh-induced relaxation in presence of LPC (ANOVA, $P < .001$, $P < .01$, and $P < .01$ in control, EPA, and DHA groups, respectively) (Fig. 4, Tables 2 and 3), whereas CHTX plus APA shifted moderately to the right the ACh-induced relaxation curve in

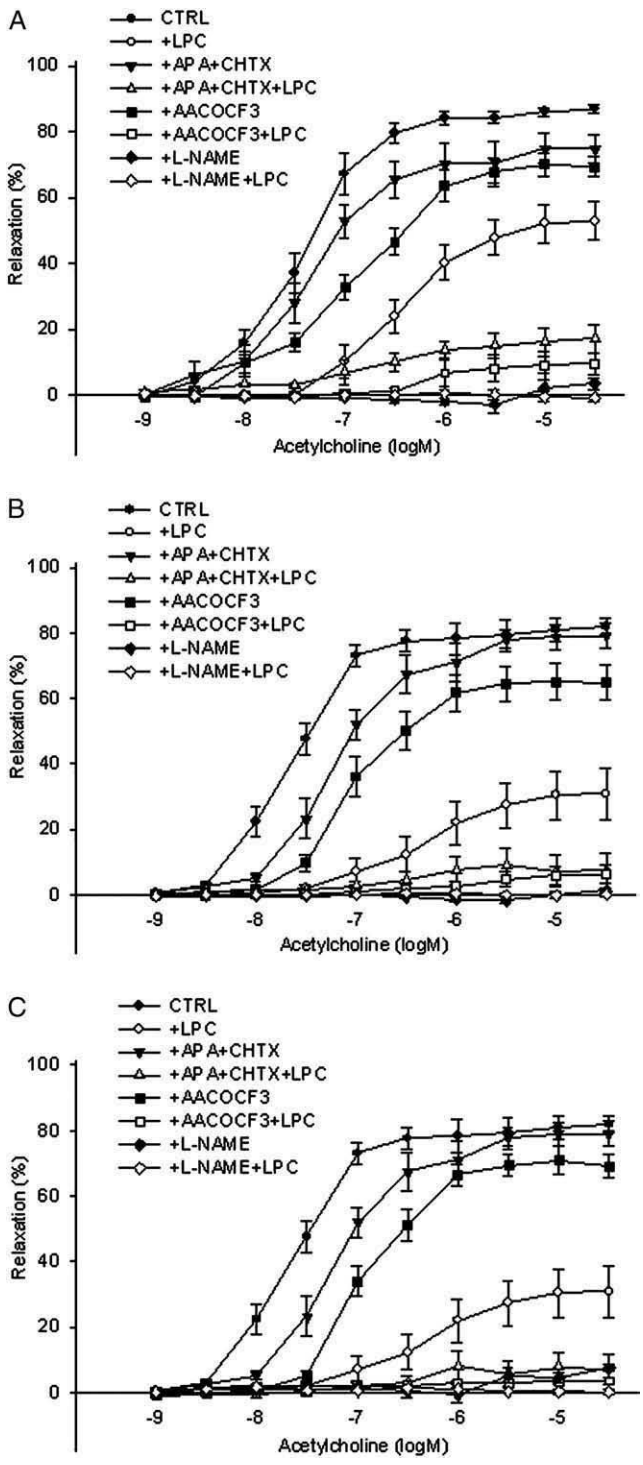

Fig. 4. Concentration-response curves for ACh (0.001-30 μmol/L) in PE-contracted (1 μmol/L) aortic rings in control hamsters (A), EPA-supplemented hamsters (B), and DHA-supplemented hamsters (C) incubated alone (n = 9, 8, 12) or in the presence of LPC (20 μmol/L; n = 9, 10, 10), L-NAME (100 μmol/L), LPC (20 μmol/L) + L-NAME (100 μmol/L), $AACOCF_3$ (20 μmol/L; n = 6, 7, 6), LPC (20 μmol/L) + $AACOCF_3$ (20 μmol/L) (n = 6, 7, 6), APA (0.3 μmol/L) + CHTX (1 μmol/L; n = 5, 5, 5), and LPC (20 μmol/L) + APA (0.3 μmol/L) + CHTX (1 μmol/L) (n = 5, 5, 5). Results are expressed as mean ± SEM.

Table 3
Variations of E_{max} values (ΔE_{max}) induced by the inhibitors in the absence or presence of LPC in the control diet group of hamsters

Test	n	ΔE_{max} in the absence of LPC	ΔE_{max} in the presence of LPC
$AACOCF_3$	11	−30.0% ± 3.2%	−85.7% ± 5.3%*
Indomethacin	7	−0.9% ± 0.1%	−8.81% ± 1.2%
Sulfaphenazole	6	−3.9% ± 1.9%	−4.5% ± 1.6%
APA + CHTX	5	−13.9% ± 5.0%	−62.6% ± 3.3%*

Percentage of decrease of the E_{max} value induced by each inhibitor in the presence or not of LPC was determined for each hamster aorta. Results are means ± SEM for n hamsters. $P < .05$ vs ΔE_{max} in the absence of LPC of the same test.

the absence of LPC (ANOVA, $P < .001$, in control, EPA, and DHA groups, respectively) that was associated with a decrease in the E_{max} values (Tables 2 and 3). Influence of L-NAME, indomethacin, sulfaphenazole, and CHTX plus APA was not different between the 3 groups of diet (Fig. 4).

4. Discussion

The purpose of the present study was to evaluate the effects of a DHA- or EPA-supplemented diet on the LPC-induced decrease of endothelium-dependent vasorelaxation on Syrian hamster aortic rings and, in a second step, to evaluate the AA-derived ways of relaxation modulated by LPC. Golden Syrian hamster has been shown to be a good experimental model to study human lipid metabolism because it presents important aspects of cholesterol metabolism similar to those in humans, particularly concerning the mechanisms of hepatic and nonhepatic plasmatic cholesterol concentration regulation [25]. Furthermore, the Syrian hamster hepatic cholesterol metabolism is sensitive to the type of nutritional fatty acids. Finally, it develops atherosclerotic lesions very similar to those in humans [26].

Our results showed that pretreatment of the rings with LPC altered the endothelium-dependent aortic relaxation in a dose-dependent manner, as reported in other experimental studies [16,19]. The absence of any effect of LPC on SNP-induced aortic relaxation indicated that its action is specifically due to endothelium-dependent mechanisms and excluded nonspecific cytotoxic effects or changes in the response of smooth muscle cells, in accordance with others [27]. In rodents, NO release of large elastic arteries like aorta constitutes the main way of relaxation, as illustrated by the complete inhibition of ACh-induced relaxation by L-NAME in our study. Our results confirm the concentration-dependent inhibitory effects of LPC on endothelium-dependent vasorelaxation, as extensively described in the literature [27].

The deleterious effects of LPC were not prevented by dietary supplementation of the hamsters over a period of 6 weeks with 17.9% EPA or 9.5% DHA. By contrast, impairment of the vasorelaxation induced by LPC was more important in EPA- and DHA-supplemented groups than in the control group of hamsters. The EPA- and DHA-supplemented diets, in our study, disturbed the composition of PUFA by increasing the n-3 PUFA aortic phospholipids content. The EPA-supplemented diet increased the aortic tissue content in EPA and its higher metabolite docosapentaenoic acid, and the DHA-supplemented diet the one of DHA; and these modifications were associated with a decrease in AA content. The level of DHA incorporation in our study was similar with that obtained in spontaneously hypertensive rats, where DHA-supplemented diet increased DHA aortic tissue content from 0.7% to 3.5% [28], but less than that in cardiac tissue, where the same diet increased DHA content from 5% to 15% [4].

The deleterious effects of DHA- and EPA-supplemented diet on the AC-induced vasorelaxation in the presence of LPC in the present study disagree with several clinical or experimental studies exhibiting beneficial effects of these fatty acids on endothelium-dependent vasorelaxation. Continuous intake of n-3 PUFA has been reported to ameliorate the NO-mediated endothelium-dependent vasodilation in patients with coronary artery disease [29]. In experimental models, the influence of EPA and DHA on the endothelium-dependent vasorelaxation has been controversial and seems to depend mostly on the experimental conditions and the animal species. According to our results, preincubation of aortic rings with EPA reduced the vasorelaxing response to carbachol in healthy rats [13]. In the same way, DHA-supplemented diet failed to improve the altered endothelium-dependent aortic vasorelaxation induced by hypertension in spontaneously hypertensive rats [30] or by diabetes in streptozotocin-treated rats [14]. Determination of the way of relaxation modulated by n-3 PUFA remains a subject of debate. Several studies have described modulator effects of n-3 PUFA on NOS function. In human culture endothelial cells, incorporation of EPA in the culture medium has been reported to increase the NO production [31]. Furthermore, Omura et al [32] have suggested that EPA induced NOS translocation from the caveolin to the cytosol in endothelial cells. However, by increasing the basal NO production, EPA may also induce eNOS desensitization, which may contribute to the deleterious effects of the diet observed in our study. Further explorations of this hypothesis would be useful to improve the comprehension of EPA and DHA effects in the presence of LPC.

Our results clearly showed the activation of a PLA_2-dependent way of relaxation in the presence of LPC. Activation of PLA_2 by LPC and subsequent AA release have been reported in endothelial cells [20], cardiac cells [33], or monocytes-macrophages cells [34]. In our study, the PLA_2-derived relaxing pathway did not implicate COX derivates because indomethacin failed to modulate the aortic relaxation in the presence of LPC. By contrast, LPC was shown to increase COX_2 expression and subsequent prostacyclin synthesis in culture endothelial cells [35]. Our results did not support this hypothesis, although the

nonspecificity of indomethacin does not permit us to dissect more, specifically whether our results are the consequences of a modified production of vasodilator, vasoconstrictor, or both COX derivates. A decrease in NO availability observed in pathological conditions, such as atherosclerosis, was shown to be associated with a compensatory increase in the activity of an EDHF. In the presence of LPC, more specifically, activation of EDHF remains controversial [18,19]. Several authors support that EDHF may result from the transformation of AA by cytochrome P450–dependent monoxygenases (MOX) into several compounds as epoxyeicosatrienoic acids [36]. In hamster arterioles, the importance of sulfaphenazole-inhibited CYP2C9-MOX derivates in the vasorelaxation has been demonstrated [37]. That is not the case in our study, either in the absence or in the presence of LPC. The variability of CYP-MOX expression and activity, according to the vascular size or vascular bed, may explain these discrepancies. Finally, we focused on the role of BK_{Ca} and SK_{Ca} channels, inhibited by CHTX and APA, respectively, because EDHF-induced relaxation was also reported to involve their opening in vascular smooth muscle cells [36,38]. Our results strongly suggest that K_{Ca} channel activation is implicated in ACh-induced aortic relaxation and mostly in the presence of LPC. Facilitation of an NO-cyclic guanosine monophosphate–dependent vasorelaxation by K_{Ca} channel opening in the presence of LPC cannot be ruled out in our experiments. According to this hypothesis, it has been shown that LPC stimulates the opening of K_{Ca} channels in endothelial culture cells and, in doing so, increases cyclic guanosine monophosphate levels [38]. As the deleterious effects of EPA and DHA diets on ACh-induced relaxation appeared only in the presence of LPC, we might speculate that EPA and DHA diets disturbed the LPC-induced potentiation of AA and/or K_{Ca} channel–dependent ways of relaxation. Indeed, the decrease in AA content in the vascular wall of EPA- and DHA-fed hamster may be involved. However, this hypothesis needs further explorations to be confirmed.

In conclusion, our results showed that DHA- or EPA-supplemented diet did not exhibit any beneficial effect on the decrease in endothelium-dependent vasorelaxation induced by LPC in the Golden Syrian hamster aorta. The LPC-induced endothelium dysfunction may be mostly associated with a decrease in NO endothelial release, but might also be limited by a concomitant activation of a compensatory vasorelaxation pathway depending both on PLA_2 metabolites and on K_{Ca} channel opening.

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