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Long-term effect of dietary α-linolenic acid or decosahexaenoic acid on incorporation of decosahexaenoic acid in membranes and its influence on rat heart in vivo

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Ayalew-Pervanchon A, Rousseau D, Moreau D, Assayag P, Weill P, Grynberg A. Long-term effect of dietary α-linolenic acid or decosahexaenoic acid on incorporation of decosahexaenoic acid in membranes and its influence on rat heart in vivo. *Am J Physiol Heart Circ Physiol* 293: H2296–H2304, 2007. First published May 25, 2007; doi:10.1152/ajpheart.00194.2007.—The present study was designed to evaluate whether long-term intake of dietary α-linolenic acid (ALA), supplied as whole grain-extruded linseed, can increase endogenous production of n-3 long-chain polyunsaturated fatty acids (FAs) in healthy adult rats and influence the heart rate (HR) and adrenergic response in the same way as docosahexaenoic acid (DHA)-rich diets. DHA enrichment was evaluated using FA analysis of tissue phospholipids after 8, 16, 24, and 32 wk of feeding in male Wistar rats randomly assigned to three dietary groups ($n = 8$ in each group): a reference fat diet (RFD), an ALA-rich (ALA) diet, and a DHA-rich (DHA) diet. At 1 wk before the animals were killed, under anesthesia, HR was measured from ECG recordings during an adrenergic stimulation challenge ($n = 8$). There was a significant increase of DHA in the cardiac membrane in the ALA group compared with the RFD group. DHA content in the cardiac membrane was ~10% in the ALA group vs. 20% in the DHA group and 4% in the RFD group. The cardiac FA profile was established after 2 mo and remained essentially unchanged thereafter. Regardless of the diet, DHA in the heart decreased with age. Nevertheless, DHA content in the heart remained at >15% in the DHA group and remained greater in older rats fed the ALA diet than in younger RFD-fed rats. Basal HR decreased in the ALA group (395 ± 24.9 beats/min) to a level between that of the DHA and RFD groups (375 ± 26.4 and 407 ± 36.7 beats/min, respectively). Both n-3 dietary intakes contribute to enhancement of the chronotropic response to adrenergic agonist stimulation. Regulation of HR by neurohumoral mediators may be controlled by lower content of DHA, e.g., by a dietary supply of extruded linseed (ALA).

cardiac docosahexaenoic acid; heart function

EPIDEMIOLOGICAL STUDIES have shown that nutrition is a major factor in the prevention of cardiovascular diseases. The risk of chronic diseases, especially coronary heart disease, is known to be reduced by consumption of dietary n-3 polyunsaturated fatty acid (PUFA) by humans (35, 51). Long-chain n-3 PUFAs, namely, docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3), are involved in a wide range of structural and functional modifications in the cardiovascular system in animals, as well as in humans, including improvement of cardiac function (6, 52, 53), decrease of blood pressure in humans (4, 60) as well as in rats (69, 70), arterial compliance (63), endothelial function and vascular reactivity (17, 18), inflammation and immunity (14, 75), and platelet aggregation (59). Dietary DHA has been reported to increase the DHA content in cardiac membranes and to modulate the electrophysiological status of myocytes in rats (39, 49, 71). This leads to various beneficial effects, such as cytoprotection during ischemia-reperfusion (28, 32), regulation of heart rate (HR) in rats (70, 71) as well as in humans (26), and enhanced adrenergic responsiveness in the marmoset monkey (66) as well as in rats (38). Moreover, DHA content reaching 18% of total fatty acids (FAs) in the heart phospholipids (PLs) was associated with antiarrhythmic effects (57). Although dietary DHA is supplied by fish oils, increasing the consumption of sea products for the prevention of heart disease has its limitations. The availability of fish for a daily intake of long-chain n-3 PUFAs that meets the recommendations (EPA and DHA intake of 850 mg/day for healthy adults) (73) is not sufficient for the worldwide population, inasmuch as intensive fishing has considerably reduced the global supply of wild fish. Therefore, epidemiological (23, 55) and animal (9, 36) studies have assessed whether shorter-chain n-3 essential FAs, namely, α-linolenic acid (ALA, *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid, 18:3 n-3) from vegetable oils (linseed, rapeseed, soybean, and walnuts), could be a valuable source of n-3 long-chain PUFAs. ALA has been investigated for its effect on the prevention of cardiovascular diseases (29, 30, 46). Despite an extremely low level of synthesis of DHA from its precursor ALA described in humans (12), the ALA-rich Mediterranean diet has been reported to reduce coronary events and cardiac deaths in humans (29). Animal experiments have suggested that dietary ALA, with a more efficient conversion process (54), can reduce cardiac arrhythmias in rats, but the reduction is significantly less than that induced by pure DHA or EPA (58). ALA has also been

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Table 1. *Whole composition of RFD, DHA, and ALA diets*

Components	RFD			ALA, g/kg	DHA, g/kg
	g/kg	kcal	% kcal		
Cornstarch	440	1,760	42.7	402	440
Sucrose	220	880	21.4	216	220
Extruded linseed flour	0	0	0	121	0
Protein				23 (19)	
Lipid				34 (28)	
Carbohydrate				42 (35)	
Soy protein isolate	180	720	17.5	157	180
l-Cysteine	1.8			1.8	1.8
Choline bitartrate	2.5			2.5	2.5
Cellulose	20	0	0	0	20
Salt mixture, including 20% sucrose	50	40	1.0	48	50
Vitamin mixture, including 96% sucrose	10	38.4	0.9	10	10
Sunflower seed oil	50	450	10.8	12	22
Cocoa butter	30	270	6.5	34	40
Purified fish oil	0	0	0	0	18
Total	1,000	4,120	100	1,000	1,000

RFD, reference fat diet; ALA, α-linolenic acid; DHA, docosahexaenoic acid. Values in parentheses are percentages.

reported to reduce markedly HR in isolated working rat hearts (31). However, the level of synthesis of DHA from its precursor ALA before its incorporation in the heart is a subject of much discussion (10, 12, 37), depending on mammalian species and target tissues. The present study was designed to evaluate whether a high level and long-term supply of dietary ALA, as supplied in the diet by whole grain-extruded linseed, can increase the endogenous production of EPA and DHA in healthy adult rats and influence the HR and adrenergic response in the same way as a DHA-rich diet.

MATERIALS AND METHODS

Animals and Diets

All procedures were performed in accordance with institutional guidelines for the use of animals and the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, Revised 1996), and the protocol was approved by the Animal Care and Use Committee of the Faculty of Pharmacy, University of Paris XI. Five-week-old male Wistar rats (175 ± 10 g body wt) were obtained from Charles River (L'Arbresle, France). The animals were acclimatized to a standard diet (A04, UAR, Villemoisson-sur-Orge, France) for 4 days and then randomly assigned to 3 groups of 32 animals fed experimental diets ad libitum: reference fat (control) diet (RFD), ALA, and DHA (UPAE-INRA, Jouy-en-Josas, France; Table 1). All the rats were weighed every 2 wk. The RFD contained lipids (80 g/kg), which were incorporated into a basal mixture of cornstarch (440 g/kg), sucrose (220 g/kg), cellulose (20 g/kg), soy protein isolate (180 g/kg), salt mixture (50 g/kg), and vitamin mixture (10 g/kg). The diets mostly differed in their FA profile. The lipid part of the RFD was a mixture of 62.5% sunflower seed oil (Fruidor, Lesieur, Antony, France) and 37.5% cocoa butter (Barry Callebaut, Meulan, France). The lipid part of the DHA diet was a mixture of 27.5% sunflower seed oil, 50% cocoa butter, and 22.5% purified fish oil (ROPUFA DHA 60, Hoffmann-La Roche, Basel, Switzerland) as the n-3 PUFA supply. The lipid part of the ALA diet was 15% sunflower seed oil, 42.5% cocoa butter, and 42.5% extruded linseed flour (VALOMEGA, Valorex) as the ALA supply. Since linseed also has many nonlipid components, the linseed flour (121 g/kg) was incorporated into a flour base with a slight modification: cornstarch (402 g/kg), sucrose (216 g/kg), soy protein isolate (157 g/kg), salt mixture (48 g/kg), and vitamin mixture (10 g/kg). The final ALA diet contained 80 g/kg FAs and the same proportion of the other nonlipid components as the RFD and DHA diets. The total FA composition of the three diets was determined by gas chromatography (model GC 3400, Varian, Les Ulis, France), and the results are shown in Table 2. The rats received 25 g of dry diet per day, which provided 300 mg of ALA or DHA in the experimental (ALA and DHA) diets, i.e., 3% of the energy supply.

Physiological Investigations

Rats from each dietary group (n = 8) used for the assessment of cardiac β-adrenergic function were maintained on the experimental diets for 32 wk. At 8-wk intervals, the rats were anesthetized with a gas anesthesia device (Minerve, Esternay, France) that delivered 1.5:100 isoflurane-air to the rat at a rate of 0.6 l/min (up to 0.8 l/min

Table 2. *FA composition of RFD, DHA, and ALA diets*

FA	RFD		DHA		ALA	
	Lipids, %	g/kg diet	Lipids, %	g/kg diet	Lipids, %	g/kg diet
14:0	0.1	0.1	ND	0	ND	0
16:0	13.5	10.8	12.3	9.8	12.3	9.8
16:1 n-9	0.2	0.2	0.3	0.2	0.2	0.2
18:0	16.4	13.1	19.1	15.3	18.7	15.0
18:1 n-9	26.5	21.2	23.4	18.7	27.4	21.9
18:2 n-6 LA	41.5	33.2	21.3	17	19.6	15.7
18:3 n-6 GLA	0.1	0.1	0.1	0.1	0.1	0.1
18:3 n-3 ALA	0.3	0.2	0.3	0.2	19.8	15.8
20:0	0.6	0.5	0.7	0.6	0.7	0.6
20:1 n-9	0.2	0.2	0.1	0.1	0.2	0.2
20:5 n-3 EPA		0	0.4	0.3		0
22:0	0.5	0.4	0.4	0.3	0.4	0.3
22:5 n-6		0	0.6	0.5		0
22:5 n-3 DPA		0	3.8	3.0		0
22:6 n-3 DHA		0	16.8	13.4		0
Total	100.0	80	100.0	80	100.0	80
SFA	30.6	24.5	32.3	25.8	32.1	25.7
MUFA	26.9	21.5	23.9	19.1	27.9	22.3
PUFA	42.5	34	43.9	35.1	40.0	32
n-6 PUFA	41.7	33.4	22.1	17.7	19.8	15.8
n-3 PUFA	0.3	0.2	21.3	17	19.8	15.8
n-6/n-3	139	139	1.0	1.0	1.0	1

FA, fatty acid; LA, linoleic acid; GLA, γ-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; SFA, MUFA, and PUFA, saturated, monounsaturated, and polyunsaturated FAs.

according to body weight). The rats were placed on a heated operating table and maintained at 38°C internal temperature. Three electrodes, two in the forelimbs and one in the left hindlimb, were introduced subcutaneously and connected to an ECG device (Gould Windograph, Longjumeau, France). After stabilization, β-adrenergic receptors were stimulated by subcutaneous injection of increasing doses of isoproterenol (from 0.01 to 10 μg/kg in 0.2 ml of saline with ×3.3 progression steps). HR was assessed for 12 min after each injection. At the end of the experiment, the anesthesia mask was removed, and the rat was allowed to recover and returned to housing. The investigations were performed on a group of 6-wk-old rats before initiation of diet regimen and then repeated after 8, 16, 24, and 32 wk of dietary treatment. The highest HR obtained during the 12-min period after injection of a dose of isoproterenol was retained as the HR corresponding to the injected dose. Linear regression curves of HR vs. injected dose were then assessed and used to determine ED_{50} of the maximal chronotropic response (CR) to stimulation. The basal HR (BHR) and the maximal HR under stimulation [maximal stimulated HR (MSHR)] were also determined. The amplitude of the CR and the percentage of the CR were calculated as well.

Biochemical Investigations

At 8-wk intervals, rats from each experimental dietary group (n = 7) were killed, and FA composition of cardiac membranes and plasma was analyzed. The animals were anesthetized by an injection of pentobarbital sodium (50 mg/kg ip). A blood sample was collected from the abdominal aorta, and the heart was rapidly withdrawn and rinsed in cold saline (9% NaCl) solution. The ventricles were weighed, cut into two pieces, and stored at −20°C in 4 ml of 2:1 (vol/vol) chloroform-methanol. The blood was centrifuged, and plasma was stored at −20°C in 4 ml of 2:1 (vol/vol) chloroform-methanol for further lipid analysis. The lipids were extracted (34), and the PLs were separated from the non-PLs on silica cartridges (LC-Si SPE, Supelco, Sigma-Aldrich, Lyon, France) (47, 71). The FAs were *trans*-methylated with BF3-methanol (Sigma-Aldrich), as described by Morisson and Smith (60a), and the methylated FAs were analyzed by gas chromatography (model GC 3400, Varian), as previously described (69, 71).

Statistical Treatment of Results

Values are means ± SE of n determinations (number of animals per experimental group). Statistical significance was evaluated by a two-way ANOVA, with diet and time as fixed factors (24, 25). When significant, the means were compared by the Newman-Keuls test. The analyses were done with NCSS Statistical Analysis System (Kaysville, UT).

RESULTS

Morphometric Data

Body weight was 375 ± 5 g at 8 wk (14-wk-old rats) and reached 628 ± 6, 653 ± 11, and 629 ± 15 g in RFD, DHA, and ALA groups, respectively, after a 32-wk feeding period. After 32 wk, heart weight was 1,027 ± 15, 1,073 ± 26, and 1,002 ± 43 mg, respectively. Neither body weight nor heart weight was significantly affected by diet.

Physiological Data

The evolution of BHR is shown in Fig. 1*A*. The results show a slight decrease in beating rate with aging in the RFD group. Introduction of DHA into the diet provoked a significant decrease in beating rate that was strongly significant after 8 wk and plateaued after 16 wk (Fig. 1*A*). The decrease in beating rate in the ALA group was considerably slower and more

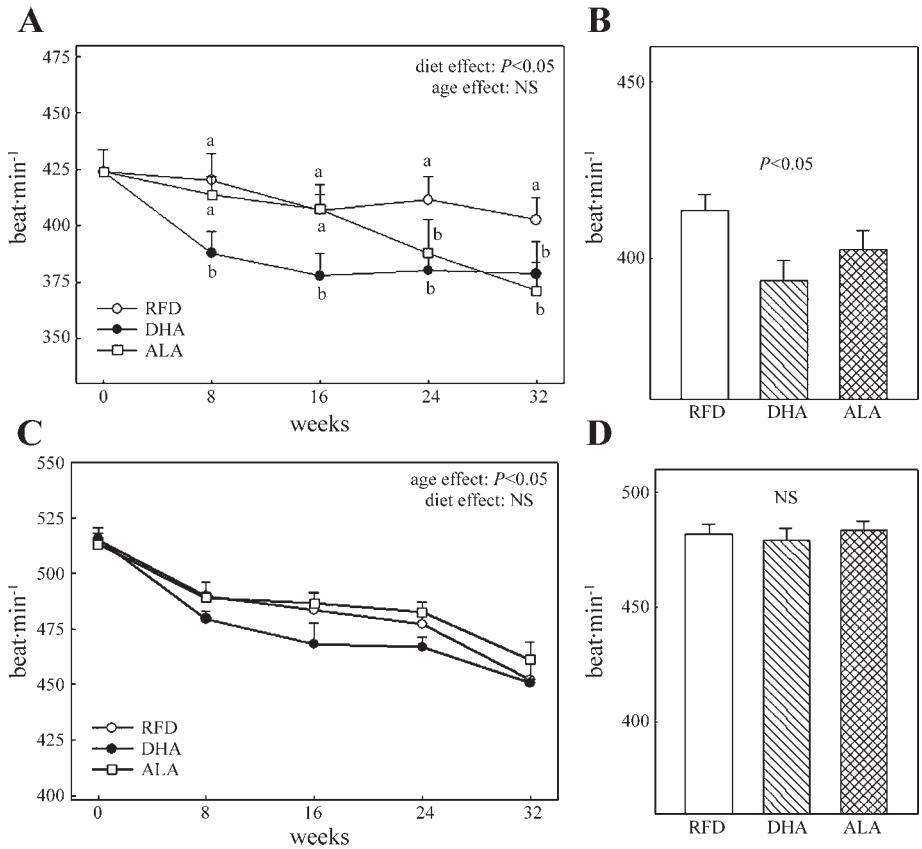

Fig. 1. *A* and *B*: basal heart rate (BHR) over 8–32 wk and overall in rats fed reference fat diet (RFD), diet rich in α-linolenic acid (ALA), and diet rich in docosahexaenoic acid (DHA). *C* and *D*: maximal heart rate over 8–32 wk and overall in RFD, ALA, and DHA groups. Values are means ± SE. NS, not significant. Different superscripts (a, b) denote statistically significant difference ($P < 0.05$, by 2-way ANOVA).

progressive. During the first 16 wk, the beating rate in the ALA group was significantly different from that in the DHA group, but not in the RFD group. After 16 wk, the beating rate continued to decrease in the ALA group, whereas it plateaued in the DHA group. At the end of the experiment, the beating rate in the ALA group was significantly different from that in the RFD group, but not the DHA group. The global effect of the diet on the beating rate throughout the 32 wk of experimentation is shown in Fig. 1*B*. Beating rate in the RFD group was 414 ± 5 beats/min and decreased to 394 ± 6 and 403 ± 5 beats/min in the DHA and ALA groups, respectively ($P < 0.05$).

MSHR after β-adrenergic stimulation is shown on Fig. 1*C*. Similar to BHR, MSHR also decreased with time in the three groups. However, evolution with time was not significantly influenced by diet. The global effect of diet on MSHR throughout the 32 wk of experimentation is shown in Fig. 1, *C* and *D*. MSHR was 482 ± 4, 479 ± 5, and 484 ± 4 beats/min in the RFD, DHA, and ALA groups, respectively; the differences were not statistically significant.

The amplitude of the CR, expressed in beats per minute to take in consideration the diet-related differences in BHR, is shown in Fig. 2. The amplitude of the maximal response decreased significantly with age in the RFD group. The amplitude of the CR was significantly higher in the DHA group than in the RFD group 8 wk after the onset of dietary change, and this difference remained constant thereafter. The amplitude of the CR in the ALA group showed a different evolution. During the first 16 wk, the CR was significantly different from that in the DHA group and similar to that in the RFD group. After 16 wk, the amplitude of the CR in the ALA group increased to reach that of the DHA group and became significantly different from that in the RFD group. The global effect of diet on the amplitude of the CR throughout the 32 wk of experimentation (Fig. 2*B*) confirms the significant increase associated with either n-3-containing diet. The CR was 71 ± 5, 87 ± 5, and 85 ± 4 beats/min in the RFD, DHA, and ALA groups, respectively ($P < 0.05$), representing 18 ± 1%, 23 ± 1.5%, and 22 ± 1% of the basal rate, respectively ($P < 0.05$).

Subcutaneous injections of increasing doses of isoproterenol were used for stimulation of β-adrenergic receptors and allowed the determination of ED_{50}. The individual ED_{50} values varied greatly among the rats in each group and with time in each rat (data not shown). For this reason, the evolution with time was not significantly different among the three groups. The global effect of the diet throughout the 32 wk of experimentation shows a slight decrease in ED_{50} in the ALA and DHA groups, but the difference was not statistically significant (data not shown).

Biochemical Data

Heart. The FA composition of the cardiac PL fraction was determined after 8, 16, 24 and 32 wk. The maximal changes occurred between 1 and 8 wk (Table 3). The proportions of saturated FA (SFA), monounsaturated FA, and PUFA were not (or weakly) influenced by diet or time. The n-6 and n-3 PUFA contents were not significantly affected by time but were significantly affected by diet, with no significant cross-interaction between these factors (Table 3). The n-6 PUFA content in cardiac PLs was 49 ± 1% in the RFD group and significantly decreased ($P < 0.01$) by the incorporation of n-3 PUFAs in the diet (35 ± 5% and 30 ± 3% in the ALA and DHA groups, respectively) within the first 8 wk and remained unchanged thereafter. Conversely, the n-3 PUFA content in cardiac PLs was 2 ± 1% in the RFD group and significantly increased ($P < 0.01$) by the incorporation of n-3 PUFAs in the diet (14 ± 3% and 17 ± 1% in the ALA and DHA groups, respectively) within the first 8 wk and did not change significantly thereafter. EPA slightly increased in the heart of the DHA or ALA groups but never exceeded 1% after 8 wk and 1.3% after 32 wk (Table 3).

The n-6-to-n-3 ratio in the RFD group significantly increased from 8 to 32 wk (Table 3). This change resulted from the slight increase of n-6 and slight decrease of n-3 in the RFD group; these changes were not individually significant. In the two groups receiving n-3 PUFAs, the n-6-to-n-3 ratio was significantly decreased ($P < 0.001$) and exhibited no further evolution with time (Table 3). The time evolution of the two major n-6 PUFAs, linoleic acid (LA) and arachidonic acid (AA), is shown in Fig. 3, *A* and *B*. The diet did not influence the LA content in cardiac PLs. Moreover, the AA content remained unchanged in the RFD group throughout the study. As expected, dietary n-3 PUFAs significantly decreased cardiac AA, which was significantly more pronounced in the DHA group than in the ALA group. These modifications were achieved within 8 wk and remained unchanged thereafter. The time evolution of the two major n-3 long-chain PUFAs, docosapentaenoic acid (DPA, C22:5 n-3) and DHA, is shown in Fig. 3, *C* and *D*, for the three dietary groups. The cardiac DHA

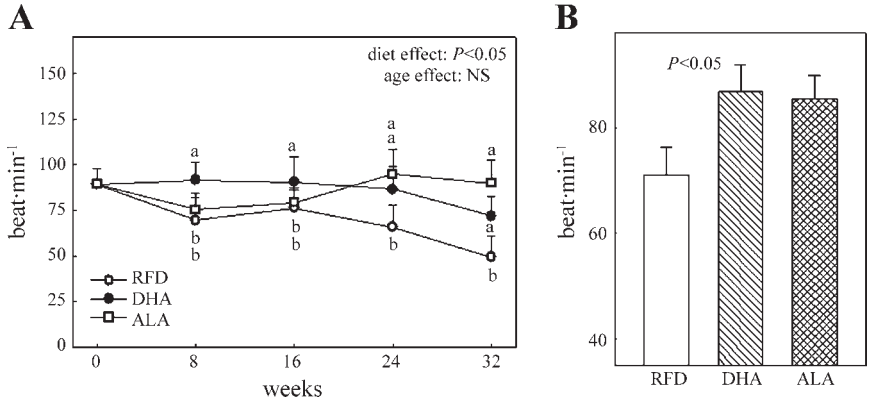

Fig. 2. *A* and *B*: chronotropic response (CR) over 8–32 wk and overall in RFD, ALA, and DHA groups. Values are means ± SE. Different superscripts (a, b) denote statistically significant difference ($P < 0.05$, by 2-way ANOVA).

Table 3. *FA profiles of heart phospholipids after 8 and 32 wk of RFD, DHA, and ALA diet*

	8 wk			32 wk		
	RFD	DHA	ALA	RFD	DHA	ALA
LA	21.0±0.6	24.5±2.2	25.4±1.3	23.4±2.0	24.3±1.4	28.3±2.0
ALA	ND	ND	0.7±0.1*	ND	0.1±0.0	0.8±0.1*
AA	24.6±0.3	7.9±0.4†‡	15.7±0.3*	23.2±1.4	7.5±0.7†‡	13.7±1.9*
EPA	0.0±0.0	1.0±0.2	0.8±0.2	ND	1.3±0.1	1.1±0.3
22:4 n-6	1.6±0.2	ND†	0.2±0.0*	1.2±0.1	ND†	0.1±0.0*
22:5 n-6	5.2±0.7	0.5±0.2†	0.3±0.1*	2.6±0.6	0.4±0.1†	ND*
DPA	0.6±0.1	1.8±0.1†‡	3.9±0.2*	0.5±0.1	1.8±0.2†‡	3.6±0.5*
DHA	4.1±0.6	20.5±1.2†‡	11.1±0.6*	2.3±0.2	17.6±0.3†‡	5.8±0.3*
SFA	34.3±0.7	34.7±2.8	32.3±1.1	37.6±2.7	38.1±1.4	35.1±2.3
MUFA	6.2±0.5	6.1±0.3	6.6±0.1	6.9±0.8	6.1±0.4	8.1±0.3
PUFA	57.8±0.9	56.9±2.9	58.7±1.0	53.8±1.7	53.5±1.7	54.1±1.1
n-6 PUFA	53.0±1.3	33.6±2.0†‡	42.2±1.2*	51.0±1.6	32.7±1.4†‡	42.8±1.6*
n-3 PUFA	4.8±0.7	23.4±1.2†‡	16.5±0.3*	2.8±0.3	20.8±0.3†‡	11.3±0.6*
n-6/n-3	11.3±1.9	1.4±0.1†‡	2.6±0.1*‡	18.2±2.1	1.6±0.04†‡	3.8±0.3*‡

Values are means ± SE. AA, arachidonic acid; ND, not detected. *$P < 0.001$, ALA vs. RFD. †$P < 0.001$, DHA vs. RFD. ‡$P < 0.001$, DHA vs. ALA.

content did not significantly change in the RFD group but increased significantly in the ALA group and further in the DHA group. Only a dietary intake of DHA caused a sustained and very large increase of DHA in cardiac membranes, whereas a dietary intake of ALA moderately increased DHA and total n-3 PUFA content. Again, these changes were achieved within 8 wk. Moreover, the evolution of the DHA content showed a very slight decrease with aging, which was significant ($P < 0.05$) from 8 to 32 wk in the three groups. However, despite a decrease with time, DHA content in cardiac membranes remained higher in aged ALA-fed rats and aged DHA-fed rats than in young RFD rats. Similarly, the DPA content in heart PLs in the RFD group remained very low (~0.5%) throughout the study, significantly increased in the DHA group (~2%), and increased further in the ALA group (~4%). Again, the changes were maximal after 8 wk and remained constant thereafter. The n-3 family also includes ALA and EPA; regardless of the dietary group, their incorporation remained very low (0.04 ± 0.01 and 0.65 ± 0.05 for ALA and 1.0 ± 0.2 and 0.8 ± 0.2 for EPA after 8 wk for DHA- and ALA-fed rats, respectively).

Plasma. The qualitative evolution with time of plasma PUFA is presented in Table 4 and was roughly similar to that described in the heart. However, the SFA content in plasma increased with age, whereas the total PUFA content decreased. The PUFA-to-SFA ratio was close to 1.7 at 8 wk, regardless of

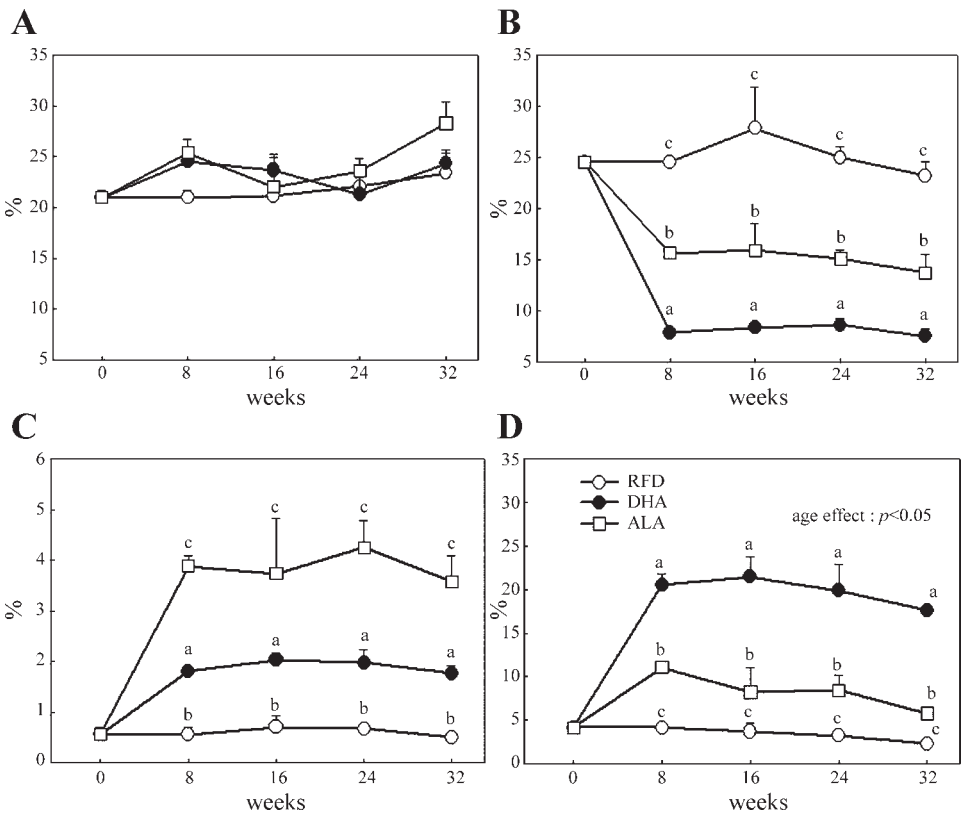

Fig. 3. Percentage of linoleic acid (LA, *A*), arachidonic acid (AA, *B*), docosapentaenoic acid (DPA, *C*), and DHA (*D*) in heart phospholipids of RFD, DHA, and ALA groups at 8, 16, 24, and 36 wk. Values are means ± SE. Different superscripts (a, b, c) denote statistically significant difference ($P < 0.05$, by 2-way ANOVA).

Table 4. *FA profiles of plasma lipids after 8 and 32 wk of RFD, DHA, and ALA diet*

	8 wk			32 wk		
	RFD	DHA	ALA	RFD	DHA	ALA
LA	23.4±2.9	21.0±2.9	26.2±2.5	28.5±2.1	22.4±1.0	20.6±2.0
ALA	0.4±0.2	1.1±0.9	5.9±2.7*	0.3±0.1	0.2±0.0	7.6±1.8
AA	22.6±2.9	5.5±1.8†‡	7.2±1.7*	19.4±3.4	5.0±1.0†‡	8.1±2.3*‡
EPA	0.1±0.05	3.9±0.9	2.9±0.4	0.1±0.0	6.6±0.9	4.0±0.6
22:4 n-6	0.6±0.2	0.1±0.0†	0.1±0.0*	0.3±0.0	ND†‡	0.1±0.0*‡
22:5 n-6	1.2±0.2	0.4±0.3†	ND*	0.4±0.1	ND†	ND*
DPA	0.2±0.0	1.5±0.2†‡	1.6±0.2*	0.1±0.0	1.7±0.1†	1.9±0.9*
DHA	1.3±0.4	10.1±0.9†‡	3.2±0.7*	0.8±0.2	9.5±1.2†	3.0±1.6*
SFA	30.6±2.0	34.8±0.3	30.3±1.9	27.3±4.6	39.5±1.1	31.5±1.2
MUFA	17.8±2.4	18.6±3.0	21.3±2.5	19.4±1.5	16.0±2.3	21.3±0.9
PUFA	51.2±0.4	46.2±2.9	48.3±1.9	51.9±5.2	47.0±2.8	46.8±2.1
n-6 PUFA	49.2±0.6	29.6±3.4†‡	34.7±3.5*	50.6±4.9	29.0±2.3†	30.3±1.1*
n-3 PUFA	2.0±0.5	16.5±0.5†‡	13.6±3.1*	1.3±0.3	17.9±0.5†	16.5±1.6*
n-6/n-3	25.6±4.1	1.8±0.3†‡	2.7±0.9*‡	41.8±5.5	0.6±0.5†	1.9±0.2*

Values are means ± SE. *$P < 0.001$, ALA vs. RFD. †$P < 0.001$, DHA vs. RFD. ‡$P < 0.001$, DHA vs. ALA.

the group, and decreased after 32 wk to ~1.4 in the three groups. Plasma n-6 PUFAs were significantly decreased by dietary n-3 PUFA intake, much more in the DHA group than in the ALA group (Table 4). Overall, dietary ALA caused a small production of n-3 FA upper metabolites, including EPA, DPA, and DHA. These modifications were dependent on the duration of the dietary treatment. The data show that n-3 PUFA intake induced a very significant increase in circulating n-3 PUFAs, especially EPA and DHA, the latter being more pronounced in the DHA group than in the ALA group. Nevertheless, both supplies induced a significant increase in EPA. In contrast to n-6 PUFAs, the plasma content of n-3 PUFAs was significantly lower in older rats (32 wk of diet) than in younger rats (8 wk of diet). In DHA- and ALA-fed animals, plasma DHA decreased with age, whereas EPA increased (from 3.9% to 6.6% and from 2.9% to 4.0%, respectively).

DISCUSSION

The aim of the present study was to compare the long-term effect of dietary ALA and DHA intake on the DHA content of cardiac membranes and the functional consequences on the cardiac β-adrenergic response. We used FA analysis along with measurement of BHR and evaluation of the β-adrenergic CR to assess the evolution of DHA incorporation into cardiac membranes at 8-wk intervals. The dietary lipid intake (8%) was in the range of that used in other studies (5–10%) to investigate the functional effects of n-3 PUFAs (41–43).

The FA composition of the plasma was affected by dietary n-3 FA intake and reflected the FA composition of the diet. The conversion of ALA to higher metabolites is a slow process in mammals for several reasons. Dietary ALA quickly accumulates in adipose tissue and skin and is directed to β-oxidation. Many studies showed that ALA conversion in humans is highly variable and mainly limited to EPA (and DPA), with very little further transformation to DHA (11, 12). Most of the bioconversion of ALA to DHA may occur in the liver, and preformed DHA may be taken from plasma by other tissues, especially the heart and brain. The results on conversion in humans have been based only on plasma lipid data and may underestimate conversion of ALA to DHA, which is rapidly removed from plasma to meet the n-3 long-chain PUFA requirements of various tissues, including the brain and heart (33).

Our results confirmed the strong impact of dietary FA intake on the FA profile of cardiac PLs and other tissues. The DHA content increased 2- and 5-fold in the ALA and DHA groups, respectively, whereas the n-6-to-n-3 ratio decreased 2- and 10-fold, respectively, compared with the RFD group. In response to dietary DHA, DHA rapidly accumulated in cardiac PLs and reached its maximum content within 8 wk (e.g., 18–20%), in accordance with data previously reported by us (69–71) and others (16, 65). Within this period, only trace amounts of ALA (<1%) accumulated in cardiac membranes of the ALA group, whereas the DHA content in cardiac PLs increased noticeably (e.g., 10%) in this group, although the increase was significantly less than in the DHA group. Similar results reported in guinea pigs show less effect of dietary ALA than dietary DHA on the increase in tissue DHA content (1). The time evolution of cardiac membrane FA composition exhibited different profiles depending on fat intake. Beyond the 8-wk period of feeding, no further DHA enrichment was observed in cardiac membranes, regardless of the n-3 PUFA supplied. Similarly, Gudbjarnason et al. (40, 43, 44) reported the highest level of DHA in cardiac PLs after 12 wk of DHA intake in the rat and a decrease after 36 wk. This study demonstrated that dietary ALA resulted in a significant increase in cardiac DPA, probably to compensate for the lack of DHA, since this was not observed in the DHA-fed rats. To our knowledge, the specific functional effect of DPA is poorly documented in the heart. Rissanen et al. (68) reported that a high level of DHA and DPA (3.6% of total FAs) in men is associated with a 44% reduction of the risk of an acute coronary event compared with a lower content (2.4% of total FAs). However, the specific effects of DHA and DPA were not identified. In a comparison of the effects of DPA, EPA, and DHA on platelet aggregation and AA metabolism (3), DPA was the most potent inhibitor of collagen- or AA-stimulated platelet aggregation by virtue of its ability to interfere with the cyclooxygenase pathway and accelerate the lipoxygenase pathway. DPA may thus display more than a basal structural function when incorporated into the membranes and may be considered in the future to explain some biological effects of n-3 PUFAs. A significant increase of EPA was observed in plasma, more in DHA- than in ALA-fed rats, whereas the increase in EPA was very weak in the heart, regardless of the

n-3 intake, up to 1% and with almost no evolution (+0.2% in 6 mo). On the contrary, in plasma, EPA continued to increase with time, reaching the same level in the ALA group at 32 wk as in the DHA group at 8 wk. Therefore, if EPA did not exert a direct effect on the heart in terms of cardiac FA enrichment and physiological function, it may act at the systemic level, improving the inflammatory status (15, 21, 55) and/or eicosanoid balance (13) and finally, indirectly, enhancing heart function.

The n-3 FAs are highly concentrated in the brain because of their involvement in cognitive and behavioral function (7, 8). In the brain, the PLs are naturally rich in PUFAs (50% of total FAs), and we observed a high DHA content (~12%) in the RFD group. In both n-3 PUFA-fed groups, DHA content was raised to 15% and AA content was slightly, but significantly, decreased (data not shown). Several studies showed that only dietary DHA can fulfill the DHA requirements of several tissues. In baboons, dietary DHA was seven times more effective for DHA in nervous tissue than dietary ALA (74). DHA produced from ALA could be incorporated into brain membranes before cardiac membranes. The observation that brain membranes contained 16.8% and 15.4% DHA (in the DHA and ALA groups, respectively) suggested that the ALA-rich diet was able to meet the demand of the brain for DHA, despite a weak plasma DHA content (3% and 2.5% at 8 and 32 wk, respectively) compared with the DHA group (10% and 9.5% at 8 and 32 wk, respectively). These results support the statement that health benefits associated with DHA supplementation not only result from a reduced accretion of n-6 PUFAs and metabolites, but also from specific long-chain n-3 PUFA levels in tissue lipids (33).

The present study investigated in vivo every 2 mo the cardiac response to subcutaneous injections of increasing doses of isoproterenol. The aim of these experiments was to observe the CR in basal conditions and the maximal HR values for each injected dose of isoproterenol. BHR and CR were influenced by the diet. After 8 wk of feeding, BHR was significantly lower in the DHA group than in the ALA and RFD groups. A significant decrease of HR after dietary DHA intake has been described in a rodent model of psychosocial stress in vivo (71), in isolated perfused heart from fish oil-fed rats (72), and in other species, including humans (17, 19, 20, 26). After 8 wk of feeding, only the DHA group exhibited a decreased BHR and an increased CR. In cultured cardiomyocytes, the increase in membrane n-3 content was reported to increase the positive chronotropic effect induced by isoproterenol but to decrease the production of cAMP compared with n-6 PUFA-rich cells (22, 38). Murphy (61) reported that saturated vs. unsaturated FA diets over a short period were able to affect the β-receptor activity in young rats. Other authors reported the relationship between n-3 PUFAs and cardiac adrenergic activity in aged rats (42, 45). Their findings are consistent with our results, which showed a relationship between β-adrenergic response and the enrichment of cardiac membrane PLs with DHA. Regardless of the experimental dietary group, the β-receptor-mediated maximum HR was higher in young rats than in older rats, an observation consistent with the literature (2). In this study, BHR and CR elicited by isoproterenol stimulation decreased with aging, which is consistent with the literature (45). This observation may be related to an age-dependent evolution of the DHA-to-AA ratio reported by others (5, 41, 45). Compared with DHA intake, ALA intake had a delayed effect on the CR and BHR that was significantly different from control only after 24 and 32 wk of feeding. After this long-term period of experimental nutrition (24–32 wk), changes in the CR and BHR were similar in the DHA and ALA groups, despite a significant difference in DHA (and DPA) membrane content.

Although the functional alterations in the DHA group are clearly correlated with the increase in membrane DHA, our results pointed out a discrepancy between physiological and biochemical parameters in the ALA group. In this group, the functional alterations were observed only after 24 wk, although the total DHA content did not change significantly after 8 wk. In the ALA group, there was a significant difference in HR and responsiveness to β-adrenergic stimulation between 8 and 32 wk, despite a roughly similar n-3 membrane composition. This delay suggests that the CR and BHR are not strictly correlated with the level of DHA in the total membrane pool and that other specific membrane PUFAs (possibly DPA) or the membrane AA-to-total long-chain n-3 PUFA ratio may contribute to the regulation of HR and adrenergic responsiveness. Long-term feeding of an ALA-rich diet has been reported by others to lower plasma AA (56), a finding that is also consistent with our results. In our study, consumption of high amounts of n-3 PUFAs resulted in a large decrease of cardiac and plasma AA (and n-6 PUFA) availability. A DHA-rich diet decreases AA content in plasma and heart more efficiently than an ALA-rich diet; therefore, n-3 PUFA intake via an ALA-rich diet might be an intermediate way of reducing plasma and cardiac AA and increasing DPA and DHA.

DHA, which is known for its antiarrhythmic properties (27), was suggested to act through the depression of surface membrane electrical excitability (48, 50) and the inhibition of Ca^{2+} release from the sarcoplasmic reticulum (62). The differential effects of DHA on contractions and L-type Ca^{2+} current in adult cardiac myocytes may constitute an expanding field of investigation. This may involve the combination of a direct inhibition of the Ca^{2+} release channel of the plasma membrane and a decrease in intracellular Ca^{2+} (67). According to O'Neill et al. (64), the antiarrhythmic effect of long-chain n-3 PUFAs involves the sarcoplasmic reticulum and the sarcolemma, since these FAs significantly increase the time required to refill the cell between two waves. Our functional parameter results are consistent with this view. The interaction of DHA with the cardiac adrenergic system has been reported in vitro (22, 28, 32) and in vivo (69), as has been the effect on HR (26, 70, 71). In the present study, dietary n-3 PUFA intake did not affect the catecholamine content of the heart, adrenals, and plasma (data not shown), as reported previously (69, 70). These observations suggest that the mechanism did not depend on differences in adrenergic stimulation level but, rather, the response to stimulation. In addition, our results show that long-term ALA intake has the same effect as short-term DHA intake. The differences in the mechanism may be a different time evolution of specific membrane composition or a slow evolution of the regulation of membrane proteins involved in the cardiac adrenergic system.

In conclusion, the ALA-rich diet allowed the incorporation of less DHA in cardiac membranes than was allowed by the DHA-rich diet. Moreover, dietary intake of ALA and DHA resulted in a decrease in BHR (more efficiently with the DHA-rich diet) and better responsiveness to β-adrenergic stim-

ulation, which may appear as a protective effect in the course of aging in rats. However, the ALA-rich diet induced a functional alteration only after long-term feeding, and these functional alterations may not be strictly correlated with the total membrane DHA content, but also with the other n-3 PUFA, which demonstrated a possible role of DPA.

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