Reprod. Nutr. Dev. 40 (2000) 113–125

Original article

Effect of dietary polyunsaturated fatty acids on contractile function of hearts isolated from sedentary and trained rats

Luc DEMAISON[a]*, James BLET[b], Jean-Pierre SERGIEL[a], Stéphane GREGOIRE[a], Doriane ARGAUD[b]

[a] Unité de Nutrition Lipidique, INRA, BV 1540, 17 rue Sully, 21034 Dijon Cedex, France
[b] Division Prospective et Développements, Laboratoire de Nutrition, SCERCAT, Quartier Étienne, BP 305, 11 rue Groussay, 78120 Rambouillet, France

(Received 23 September 1999; accepted 24 February 2000)

Abstract — Moderate physical training induced a decrease in arterial blood pressure in fish oil-fed rats as compared to sunflower seed oil-fed rats [14]. The purpose of this study was to determine if these changes were due to modifications of the left ventricular function of the heart. Forty rats were fed a semi-purified diet containing either 10% sunflower seed oil or 10% fish oil (EPAX 3000TG, Pronova). Each dietary group was assigned to two sub-groups, one being constituted by sedentary animals and the other by trained animals. Training was achieved by daily running for 60 minutes at moderate intensity for three weeks. At the end of the training period, the animals were sacrificed and their hearts were immediately perfused according to the working mode. The phospholipid fatty acid composition and parameters of the left ventricular function were determined. Feeding fish oil markedly reduced the proportion of n-6 polyunsaturated fatty acids (PUFA, 18:2 n-6, 20:4 n-6, 22:4 n-6 and 22:5 n-6) in cardiac phospholipids. The n-6 PUFA were replaced by n-3 PUFA (mainly docosahexaenoic acid). In sedentary animals, the fluid dynamic (aortic and coronary flow, cardiac output) was not modified by the diet. The heart rate was reduced (–10%) in n-3 PUFA-rich hearts. Physical training did not markedly alter the polyunsaturated fatty acid profile of cardiac phospholipids. Conversely, it reduced the heart rate, aortic flow and cardiac output (–11, –21 and –14%, respectively) at a similar extent in the two dietary groups. In a second set of experiments, the training period was repeated in animals fed a commercially available diet (A103, UAR) which simultaneously provided n-6 and n-3 fatty acids. In these dietary conditions, neither the aortic flow nor the heart rate was decreased by physical exercise. These results suggest that both n-6 and n-3 PUFA in the diet are necessary to ensure a good cardiac adaptation to moderate physical training. Furthermore, the fish oil-induced decrease in arterial blood pressure in trained animals was not related to changes in cardiac contractility, but to a decrease in vascular resistances. Moderate physical training + dietary n-3 PUFA might be used to prevent hypertension and cardiovascular diseases.

dietary polyunsaturated fatty acids / moderate physical training / cardiac function

* Correspondence and reprints
E-mail: demaison@dijon.inra.fr

Résumé — Effet des acides gras polyinsaturés sur la fonction contractile des cœurs isolés issus de rats sédentaires et entraînés. Une activité physique modérée réduit la pression artérielle chez les rats nourris avec de l'huile de poisson par rapport à ceux nourris avec de l'huile de tournesol [14]. Cette étude vise à déterminer si ces modifications sont liées à des variations de la fonction ventriculaire gauche. Quarante rats sont nourris avec des régimes semi-purifiés contenant soit 10 % d'huile de tournesol, soit 10 % d'huile de poisson (EPAX 3000TG, Pronova). Chaque groupe alimentaire est subdivisé en deux sous-groupes, l'un étant constitué d'animaux sédentaires, l'autre d'animaux entraînés physiquement. L'entraînement physique consiste en une course à intensité modérée d'une durée de soixante minutes tous les jours de la semaine. Il se prolonge sur une période de trois semaines. A la fin de cette période, les animaux sont sacrifiés et leur cœur est immédiatement perfusé selon le mode travaillant. La composition en acides gras des phospholipides et le fonctionnement du ventricule gauche sont déterminés. L'huile de poisson alimentaire réduit la proportion des acides gras polyinsaturés n-6 (AGPI, 18:2 n-6, 20:4 n-6, 22:4 n-6 et 22:5 n-6) dans les phospholipides cardiaques. Les AGPI n-6 sont remplacés par des AGPI n-3 (principalement de l'acide docosahexaénoique). Chez les animaux sédentaires, les débits aortique et coronaire ne sont pas modifiés par le régime. En revanche, la fréquence cardiaque est réduite par les AGPI n-3 (–10 %). L'activité physique n'a que peu d'influence sur le profil en AGPI des phospholipides cardiaques. En revanche, elle réduit la fréquence cardiaque et le débit aortique (–11 et –21 %, respectivement) d'une façon similaire dans les deux groupes alimentaires. Lors d'une seconde expérience, le protocole d'entraînement physique est répété chez des animaux nourris avec un régime commercial (UAR, réf. A103) qui apporte simultanément des AGPI n-6 et des AGPI n-3. Dans ces conditions, ni le débit aortique, ni la fréquence cardiaque ne sont réduits par l'entraînement physique. Ces résultats suggèrent qu'une bonne adaptation cardiaque à l'entraînement physique modéré nécessite l'application simultanée d'AGPI n-6 et d'AGPI n-3 dans l'alimentation. De plus, la diminution de la pression artérielle observée chez les rats nourris avec l'huile de poisson et entraînés physiquement n'est pas liée à des variations de la contractilité cardiaque, mais plutôt à une diminution des résistances vasculaires. L'association des acides gras polyinsaturés n-3 alimentaires et de l'entraînement physique modéré pourrait être employée pour prévenir l'hypertension artérielle et les maladies cardio-vasculaires.

acides gras polyinsaturés alimentaires / entraînement physique modéré / fonction cardiaque

1. INTRODUCTION

Dietary fish oil increases n-3 polyunsaturated fatty acids (mainly docosahexaenoic acid, but also eicosapentaenoic acid) in the heart [8, 20, 24], liver [12] and blood [5] at the detriment of n-6 polyunsaturated fatty acids (arachidonic acid, 22:4 n-6 and 22:5 n-6). These changes in membrane lipid composition can influence the biochemical and physiological functions of the organs. The n-3 polyunsaturated fatty acid-induced modifications of heart function are now well documented. They include a decreased heart rate under physiological conditions associated with an increased systolic ejection volume [7, 16]. In pathological conditions, the changes are even more obvious: for example, n-3 PUFA are cardioprotective during ischemia/reperfusion. They favour the recovery of mechanical activity during post-ischemic reperfusion [8, 23, 27]. The occurrence of severe arrhythmias is decreased during ischemia and reperfusion [3, 15, 17, 19]. The cardioprotective effects of n-3 polyunsaturated fatty acids could occur through upholding of calcium homeostasis. Membrane n-3 polyunsaturated fatty acids prevent the uncoupling action of calcium in isolated cardiac mitochondria [25]. Furthermore, they modify prostanoid synthesis [22] which are known to modulate adenylate cyclase activity and, thereby, intracellular free calcium concentration.

A recent advance in the understanding of the physiological effects of n-3 polyunsaturated fatty acids has been provided by the study of Lortet and Verger [14]. These

authors reported that the association of fish oil-feeding and moderate physical training reduces mean aortic pressure and left ventricular blood pressure in rats. This decrease was associated with a reduced heart rate. It was not seen in sunflower seed oil- and lard-fed rats subjected to physical training nor in sedentary animals fed fish oil, sunflower seed oil or lard. The experimental physical training carried out in the study of Lortet and Verger [14] can not be considered as a model of effort adaptation. It seems to intensify the effects of n-3 PUFA. Physical training is commanded and it does not result of psychological or physical desire. This model could mimic the situation of people forced to follow physical training, such as certain middle-aged people or patients with myocardial infarction. The decreased blood pressure observed with the association of fish oil-feeding and physical training is fundamental in the field of cardiovascular diseases. Hypertension is a pre-requisite to hypertrophy and cardiac failure. Furthermore, it is a well-known risk factor for atherosclerosis. Decreasing blood pressure by eating n-3 PUFA and performing moderate physical training might be of first importance for preventing cardiovascular diseases. On the other hand, the reduced blood pressure noticed by Lortet and Verger [14] in trained rats fed fish oil may be explained by a reduction of cardiac mechanical function or by a decrease in peripheral resistances. If the lower blood pressure was due to a reduction of cardiac mechanical function, the association of n-3 PUFA and moderate physical training would be deleterious for health. Conversely, if reduced peripheral resistances explain the lower blood pressure, dietary n-3 PUFA + physical training are beneficial. To further understand the mechanism involved in the lower blood pressure observed in trained rats fed fish oil, it appears necessary to estimate the influence of polyunsaturated fatty acids and physical training on the cardiac mechanical activity. Such a study can not be performed in vivo, since heart functioning is modulated by numerous factors (blood volume, blood pressure, hormonal status, nervous regulation, etc.). Conversely, it can be carefully carried out in a model of isolated perfused working rat hearts.

The aim of this study was to determine whether the decrease in blood pressure induced by n-3 PUFA feeding and moderate physical training is due to a reduction of the left ventricular mechanical performances or a decrease in peripheral resistances. To further understand, rats were fed either 10% sunflower seed oil or 10% fish oil and they were subjected or not to moderate physical training. Thereafter, the mechanical and biochemical functions of the isolated working hearts were determined. Since cardiac adaptation is characterised by changes in energy metabolism, we evaluated myocardial oxygen consumption, β-oxidation rate (determined by the intracellular fate of radiolabelled palmitate) and cardiac metabolic efficiency, which was not estimated in the study of Lortet and Verger [14]. Furthermore, since PUFA supply was either only n-3 PUFA or only n-6 PUFA, we also evaluated the effect of moderate physical training on the functioning of isolated working hearts of rats fed a commercial diet known to supply equilibrated amounts of n-3 and n-6 PUFA.

2. ANIMALS AND METHODS

2.1. Animals, diets and physical activity

In a first set of experiments, we tested the effect of dietary PUFA and moderate physical training. The protocol was performed in the SCERCAT laboratory where was done that of Lortet and Verger [14]. Since we wanted to determine the reasons for the decreased blood pressure in trained rats fed fish oil, the protocol was strictly similar. The composition of the diet was the same and the fatty acid composition of the oils tested was similar. Furthermore, the duration and intensity of physical training

were rigorously identical. As compared to the work of Lortet and Verger [14], the two subgroups of rats (sedentary and trained) fed lard were not studied, since it did not seem to be different to those with n-6 PUFA. Since we were interested to evaluate the effect of dietary PUFA, we compared the effect of dietary fish oil and sunflower seed oil in sedentary and trained animals. Forty 25-day-old male Wistar rats were used. They had continuous access to food and water. The rats were randomly assigned to four groups: a group of sedentary animals fed a semi-purified diet containing 10% fish oil (FO) as a sole source of fats; a group of exercise-trained rats fed a semi-purified diet containing 10% FO; a group of sedentary animals fed a semi-purified diet containing 10% sunflower seed oil (SSO); a group of exercise-trained rats fed a semi-purified diet containing 10% SSO. Exercise was performed every day on a motor-driven treadmill at moderate intensity (16 $m \cdot mn^{-1}$) for 60 minutes, 7 days per week, for 3 weeks. An electric grid, located at the rear of each compartment, prevented the animals from stopping to run. During the three-week oil alimentation (concomitant to the exercise protocol in the exercised-rats), the animals were fed special diets composed of a common protein-carbohydrate basis, ad libitum. The diets contained casein (20%), commercial grade sucrose (50%), corn starch (15.3%), salt mixture (4%), cellulose powder (2%), vitamins (1%) and fat (10%). The 10% fat were either 10% fish oil (EPAX 3000TG, Pronova) or 10% sunflower seed oil. Since cardiac mechanical performances were slightly reduced by physical training in the two dietary groups, we were led to conduct a second set of experiments. A commercially available diet with an equilibrated supply of n-6 and n-3 PUFA (Ref A103, UAR) was used, but the physical training characteristics were similar to those of the first set of experiments. The commercially available diet contained 5% lipids, which was low as compared to the amounts (10%) used in the first set of experiments. The fatty acid composition of the diets used in the two sets of experiments is presented in Table I. The sunflower seed oil-rich diet provided only n-6 PUFA. The fish oil-rich diet was rich in n-3 PUFA (mainly 20:5 n-3 and 22:6 n-3). The commercially available diet contained large amounts of n-6 PUFA, but also n-3 PUFA in noticeable proportions. As in the fish oil diet, 20:5 n-3 and 22:6 n-3 were present, but their precursor (18:3 n-3) was the more abundant.

Table I. Fatty acid composition of the different diets.

Fatty acid	SSO	FO	UAR
14:0	–	6.7	0.4
16:0	6.5	14.5	16.3
18:0	3.1	2.4	3.3
20:0	0.1	0.4	0.3
16:1	0.1	7.8	0.8
18:1	24.1	14.2	19.9
20:1	0.1	3.6	0.7
18:2 n-6	65.1	1.1	51.0
20:3 n-6	–	1.3	–
20:4 n-6	–	0.8	–
18:3 n-3	–	0.7	5.7
18:4 n-3	–	2.8	–
20:5 n-3	–	18.2	0.6
22:5 n-3	–	2.2	–
22:6 n-3	–	13.7	0.9
Other fatty acid	0.9	9.6	0.1

The results are expressed as percents of total fatty acids. SSO: sunflower seed oil-rich diet; FO: fish oil-rich diet; UAR: commercially available diet.

2.2. Heart perfusion

After three weeks on the experimental diets, the animals were anaesthetised with diethyl ether vapors and heparinised (1 000 $IU \cdot kg^{-1}$) via the saphenous vein. After rapid thoracotomy, the hearts were removed, stopped in cold medium and immediately perfused according to the Langendorff mode at 37 °C with a

Krebs-Heinselett buffer continuously gassed with 95% O_2–5% CO_2 (pH 7.4). The perfusion medium contained NaCl (118 mM); KH_2PO_4 (2 mM); $NaHCO_3$ (25 mM); $MgCl_2$, 6 H_2O (1.2 mM); KCl (5.6 mM); $CaCl_2$, 2 H_2O (2.4 mM); caprylic acid (25 µM) as a source of hydrosoluble fatty acid, glucose (11 mM) and insulin (10 IU·L^{-1}). During the perfusion according to the Langendorff mode, the left atrium was canulated and a tubing was introduced in the pulmonary artery for collecting the coronary effluent. After 15 minutes of Langendorff perfusion, the hearts were perfused according to the working mode [21] in normoxic conditions for twenty minutes. The preload and afterload were settled to 12 and 80 cm of water, respectively and were constant throughout the perfusion procedure. At the end of the twenty-minute-perfusion, a bolus (0.6 mL, 2.8 Bq·mM^{-1}) of uniformly labelled ^{14}C-palmitate (31.5 GBq·mM^{-1}) bound to albumin (0.9 mM, fatty acid/albumin ratio = 2) was injected through the coronary arterial vessel via the left atrium. The perfusion with non-radioactive Krebs-Heinselett buffer was maintained according to the working mode for one minute and the hearts were freeze-clamped. The hearts were stored at –80 °C for further biochemical analysis. During the perfusion according to the working mode, the aortic and coronary flows were collected at five-minute intervals for 30 seconds and their value was assessed by weight determination. The aortic pressure and electrocardiogram were continuously monitored. Myocardial oxygen consumption was determined by evaluating the oxygen concentration in the arterial and venous perfusates. The cardiac output was the sum of aortic and coronary flows. The cardiac work was calculated according to the following formula: $WC = 0.0167 \times (AP / 7.6) \times CO$ where WC, AP and CO are the cardiac work (mW), the peak of aortic pressure (mmHg) and the cardiac output (mL·min^{-1}), respectively. The metabolic efficiency was calculated as the cardiac work to oxygen consumption ratio.

2.3. Intracellular fate of radiolabelled palmitate

The frozen hearts were ground in a mortar in liquid nitrogen. Perchloric acid (1 M, 5 mL·g^{-1} of tissue) was added to the powder of myocardial tissue and the suspension was homogenised with a polytron (2 × 12 s, rheostat = 5, 4 °C). The homogenate was neutralised with KOH (2 M) and centrifuged (2000 RPM, 10 min, 4 °C). The radioactivity of the supernatant (acid-soluble products resulting from β-oxidation) was determined using a liquid scintigraphy counter (2000 CA, Packard) and ASC II as a scintillator. The lipids were extracted from the pellet resulting from the perchloric acid extraction according to Folch et al. [9]. The lipid fraction was collected, dried and redissolved in chloroform-methanol (2:1, v/v). An aliquot was used for the determination of total radioactivity. The remaining lipid fraction was used for separation of the different lipid classes by thin-layer chromatography according to Bizerte et al. [1]. Separation of the lipid classes was performed on silica gel layers (Kieselgel 60F254, Merck) using a hexane-diethyl ether-acetic acid mixture (60:40:4, v/v) as an eluant system. Polar lipids, free fatty acids, diglycerides and triglycerides were determined with pure standards. The distribution of radioactivity in the different lipid classes was determined with a radiochromatograph (Berthold).

2.4. Fatty acid composition of heart phospholipids

The determination of fatty acid composition of cardiac phospholipids was carried out in three hearts randomly selected from each group. The total lipids were extracted from the whole ventricles according to Folch et al. [9]. The phospholipids were purified on a silicic acid column (Sep-pack, Waters) according to Juaneda and Rocquelin [11]. After transmethylation, the phospholipid fatty acid methyl esters were separated and

analysed by gas chromatography on a Carbowax 20 M capillary column.

2.5. Statistical analysis

The results are presented as mean ± S.E.M. For the first set of experiments (effect of dietary PUFA and physical training), the results of cardiac left ventricular function, phospholipid fatty acid composition and intracellular fate of radiolabelled palmitate were submitted to a two-way analysis of variance [6] which described the effect of dietary polyunsaturated fatty acids (D ef), that of physical training (PT ef) and the cross-interaction between these two factors (CI). The experiment carried out with the commercially available diet was performed with a six-month interval as compared to the first set of experiments. Because of eventual problems of chronobiology, the results were presented separately. For this experiment, the results were submitted to a one-way analysis of variance describing the effect of physical training (PT ef).

3. RESULTS

In the first set of experiments, food intake was similar in each group (19 g of dried food per animal per day). The weight gain of the animals was not modified by the diet and by physical training. At the end of the experiment, the animal weight was similar. The heart weight of sedentary animals was not modified by dietary PUFA. It was not significantly affected by physical training. The fatty acid composition of cardiac phospholipids is presented in Table II. The results are expressed in percent of major fatty acids, although we know that minor fatty acids such as 20:3 n-6 can be modulated by the diet. Substituting the SSO diet by the FO diet markedly modified the fatty acid composition of myocardial membranes. The proportion of palmitic acid was noticeably increased (+33%), whereas that of stearic acid was slightly reduced (–8%). This contributed to a moderate increase in the percentage of saturated fatty acids (+3%). The monounsaturated fatty acids were detected in higher proportions (+54%), since both palmitoleic and oleic acids were increased (+766 and +40%, respectively). The proportion of total polyunsaturated fatty acids was reduced (–10%). Their nature was also markedly affected. The n-6 polyunsaturated fatty acids were decreased (–59%) and substituted by n-3 polyunsaturated fatty acids (+833%). Linoleic acid, 22:4 n-6 and 22:5 n-6 were considerably reduced (–79, –100 and –98%, respectively), whereas arachidonic acid was decreased less (–28%). All the n-3 polyunsaturated fatty acids were increased, but docosahexaenoic acid remained the more abundant. Physical training did not modify the proportion of saturated and polyunsaturated fatty acids and the percentage of individual fatty acids. Its effect on the proportion of monounsaturated fatty acids depended on the diet. In the SSO group, physical training increased palmitoleic acid, oleic acid and total monounsaturated fatty acids (+ 50, + 15 and + 16%, respectively). Conversely, in the FO group, moderate physical activity reduced these three values (–23, –8 and –10%, respectively).

The changes in dietary lipids and physical activity were associated with changes in physiological parameters of isolated working hearts (Tab. III). Enriching membrane phospholipids with n-3 polyunsaturated fatty acids did not alter the aortic flow, aortic developed pressure and cardiac work, but reduced the heart rate (–11%). The parameters of oxidative metabolism (myocardial oxygen consumption and metabolic efficiency) were not affected. Physical training also altered the heart rate. It contributed to reducing this parameter, similarly in the two dietary groups (–10 and –11% in the SSO and FO groups, respectively). This did not increase the systolic ejection volume. The aortic flow and cardiac output were thus reduced (–21 and –14%, respectively).

Table II. Effects of dietary polyunsaturated fatty acids and physical training on the fatty acid content (%) of cardiac phospholipids.

	SSO		FO		ANOVA		
	–PT	+ PT	–PT	+ PT	D ef	PT ef	CI
16:0	11.6 ± 0.5	11.9 ± 0.7	15.9 ± 0.3	15.4 ± 0.6	$p < 0.001$	NS	NS
18:0	31.0 ± 0.5	29 ± 0.7	27.3 ± 0.5	27.9 ± 0.9	$p < 0.010$	NS	NS
SFA	42.6 ± 0.4	40.9 ± 0.1	43.2 ± 0.6	43.3 ± 0.5	$p < 0.050$	NS	NS
16:1	0.1 ± 0.1	0.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	$p < 0.001$	NS	$p < 0.05$
18:1	6.2 ± 0.1	7.1 ± 0.4	9.7 ± 0.3	8.9 ± 0.5	$p < 0.001$	NS	$p < 0.05$
MUFA	6.3 ± 0.1	7.3 ± 0.5	11.0 ± 0.3	9.9 ± 0.5	$p < 0010$	NS	$p < 0.05$
18:2 n-6	18.9 ± 1.0	20.0 ± 0.2	3.8 ± 0.1	4.3 ± 0.7	$p < 0.001$	NS	NS
20:4 n-6	21.8 ± 0.7	21.1 ± 0.5	15.1 ± 0.3	16.0 ± 0.1	$p < 0.001$	NS	NS
22:4 n-6	2.1 ± 0.1	2.2 ± 0.1	nd	nd	nc	nc	nc
22:5 n-6	5.2 ± 0.6	6.1 ± 0.2	0.2 ± 0.1	nd	nc	nc	nc
n-6 PUFA	48.0 ± 0.8	49.4 ± 0.5	19.1 ± 0.4	20.3 ± 0.7	$p < 0.001$	NS	NS
20:5 n-3	0.1 ± 0.1	nd	4.1 ± 0.2	4.0 ± 0.1	nc	nc	nc
22:5 n-3	0.1 ± 0.1	nd	2.8 ± 0.1	2.8 ± 0.1	nc	nc	nc
22:6 n-3	3.0 ± 0.5	2.5 ± 0.1	19.8 ± 0.2	19.6 ± 1.0	$p < 0.001$	NS	NS
n-3 PUFA	3.2 ± 0.5	2.5 ± 0.1	26.7 ± 0.1	26.4 ± 0.9	$p < 0.001$	NS	NS
PUFA	51.2 ± 0.3	51.9 ± 0.4	45.8 ± 0.5	46.7 ± 0.2	$p < 0.001$	NS	NS
n-6/n-3	15.0 ± 2.9	19.8 ± 1.0	0.7 ± 0.1	0.8 ± 0.1	$p < 0.001$	NS	NS

The number of animals was three per group. SSO: sunflower seed oil-fed rats; FO: fish oil-fed rats; - PT: without physical training; + PT: with physical training; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; D ef: diet effect; PT ef: physical training effect; CI: cross-interaction; nd: not detected; nc: non-computerised; NS: not significant.

Despite these changes in fluid dynamics, the oxygen consumption and metabolic efficiency were not altered by physical training. The intracellular fate of radiolabelled palmitate was also investigated (data not shown). In both dietary groups, physical training did not affect the rate of β-oxidation, as evidenced by the similar labelling of the acid-soluble compounds. The incorporation of ^{14}C-palmitate in phospholipids, diglycerides and triglycerides was not affected by the diet and by physical training.

In a second set of experiments, the effect of moderate exercise on cardiac functioning was evaluated in rats fed a commercially available diet. The fatty acid composition of cardiac phospholipids is presented in Table IV. After the three-week diet, cardiac phospholipids contained more n-6 polyunsaturated fatty acids than n-3 polyunsaturated fatty acids. The main n-6 polyunsaturated fatty acids were linoleic and arachidonic acids, since they constituted 23 and 16% of total fatty acids, respectively. Both 22:4 n-6 and 22:5 n-6 were low. One fifth of the polyunsaturated fatty acids was n-3 fatty acids with docosahexaenoic constituting more than 85% of them. Eicosapentaenoic

Table III. Effects of dietary polyunsaturated fatty acids and physical training on the parameters of cardiac functioning in physiological conditions.

	SSO		FO		ANOVA		
	–PT	+ PT	–PT	+ PT	D ef	PT ef	CI
AF	32.7 ± 1.3	27.6 ± 2.9	32.6 ± 2.1	24.3 ± 3.6	NS	$p < 0.05$	NS
CF	12.0 ± 1.3	12.2 ± 0.9	11.6 ± 1.5	12.0 ± 1.4	NS	NS	NS
CO	44.7 ± 1.8	39.9 ± 2.8	44.2 ± 1.8	36.3 ± 3.4	NS	$p < 0.05$	NS
AP	89 ± 6	86 ± 6	85 ± 4	83 ± 6	NS	NS	NS
CW	8.8 ± 0.7	7.6 ± 0.8	8.3 ± 0.6	6.8 ± 1.0	NS	NS	NS
Rate	234 ± 10	210 ± 8	209 ± 11	185 ± 14	$p < 0.05$	$p < 0.05$	NS
SEV	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.21 ± 0.03	NS	NS	NS
QO_2	179 ± 21	148 ± 12	170 ± 23	134 ± 9	NS	NS	NS
ME	53 ± 6	57 ± 6	55 ± 8	48 ± 10	NS	NS	NS

The number of animals was 9, 11, 9 and 8 for the sunflower seed oil-fed rats not subjected to physical training, sunflower seed oil-fed rats subjected to physical training, fish oil-fed rats not subjected to physical training and fish oil-fed rats subjected to physical training, respectively. SSO: sunflower seed oil; FO: fish oil; –PT: without physical training; + PT: with physical training; AF: aortic flow expressed as $mL \cdot min^{-1}$; CF: coronary flow expressed as $mL \cdot min^{-1}$; CO: cardiac output expressed as $mL \cdot min^{-1}$; AP: aortic pressure expressed as mmHg; CW: cardiac work expressed as mW; Rate: heart rate expressed as $beats \cdot min^{-1}$; SEV: systolic ejection volume expressed as $mL \cdot beat^{-1}$; QO_2: oxygen consumption expressed as µL of oxygen per minute; ME: metabolic efficiency expressed as $\times 10^{-3}$ $mW \cdot \mu L^{-1}$ of oxygen; D ef: diet effect; PT ef: physical training effect; CI: cross-interaction; NS: not significant.

acid was almost not detected, but 22:5 n-3 was present in noticeable amounts (approximately 1.5% of total fatty acids). Physical training did not alter the fatty acid profile of cardiac phospholipids. The physiological behavior of these hearts was assessed in the same conditions as those used for the hearts of the SSO and FO groups (Tab. V). Physical training did not depress the aortic flow and the heart rate. The hearts of the trained rats functioned like those of the rats not subjected to physical training.

4. DISCUSSION

The purpose of this study was to evaluate the effect of dietary polyunsaturated fatty acids on the cardiac functioning of rats subjected or not to moderate physical training. The fatty acid composition of heart phospholipids was considerably modified by the diet. Substituting dietary sunflower seed oil by fish oil increased saturated and monounsaturated fatty acids at the detriment of polyunsaturated fatty acids. This might be due to the high degree of unsaturation of n-3 polyunsaturated fatty acids that could contribute to enhancing membrane fluidity. To maintain membrane fluidity, saturated fatty acids may be increased. All the n-6 polyunsaturated fatty acids were reduced, but the diminution was less for arachidonic acid than for 18:2 n-6, 22:4 n-6 and 22:5 n-6. The PUFA profile of the sunflower seed oil group indicates that the animals might be deficient in n-3 PUFA, since the 22:5 n-6 to 22:6 n-3 ratio was higher than 1 [10]. Although exacerbated, the diet could mimic that of industrialised countries, since it is well known that the n-3 PUFA supply is too low in those societies. On the contrary, the fish oil diet was very rich in n-3 PUFA. It could mimic the diet absorbed by Greenland Eskimos, since this population eats mainly seafood products. It appears difficult to perform it in industrialised country, since the n-6 PUFA supply is high.

Table IV. Effects of physical training on the fatty acid composition of cardiac phospholipids in rats fed the commercially available diets.

Fatty acid	Without physical training	With physical training	ANOVA
16:0	14.5 ± 0.3	15.0 ± 0.3	NS
18:0	26.0 ± 0.3	26.6 ± 1.6	NS
SFA	40.4 ± 0.1	41.6 ± 1.7	NS
16:1	0.3 ± 0.1	0.3 ± 0.1	NS
18:1	8.8 ± 0.8	9.1 ± 0.4	NS
MUFA	9.1 ± 0.9	9.4 ± 0.4	NS
18:2 n-6	23.1 ± 1.2	21.8 ± 0.8	NS
20:4 n-6	15.7 ± 0.6	15.2 ± 0.5	NS
22:4 n-6	0.8 ± 0.1	0.7 ± 0.1	NS
22:5 n-6	0.4 ± 0.1	0.2 ± 0.1	NS
n-6 PUFA	40.0 ± 0.6	38 ± 0.9	NS
20:5 n-3	0.1 ± 0.1	nd	nc
22:5 n-3	1.5 ± 0.1	1.4 ± 0.2	NS
22:6 n-3	8.8 ± 1.4	9.6 ± 0.8	NS
n-3 PUFA	10.4 ± 1.5	11.0 ± 1.0	NS
PUFA	50.4 ± 0.9	49.0 ± 1.5	NS
n-6/n-3	4.0 ± 0.6	3.5 ± 0.3	NS

The number of animals was three per group. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-6/n-3: n-6/n-3 polyunsaturated fatty acid ratio; nd: not detected; nc: non-computerised; NS: not significant.

Table V. Effects of physical training on the parameters of cardiac functioning in rats fed the commercially available diet.

Fatty acid	Without physical training	With physical training	ANOVA
AF	26.1 ± 1.4	23.0 ± 2.7	NS
CF	14.7 ± 1.5	15.7 ± 1.7	NS
CO	40.8 ± 2.4	38.7 ± 3.5	NS
AP	98 ± 6	90 ± 7	NS
CW	8.7 ± 0.5	7.8 ± 1.0	NS
Rate	220 ± 18	219 ± 18	NS
SEV	0.21 ± 0.01	0.19 ± 0.03	NS
QO_2	191 ± 38	185 ± 18	NS
ME	52 ± 6	53 ± 8	NS

The number of animals was 8 per group. AF: aortic flow expressed as $mL \cdot min^{-1}$; CF: coronary flow expressed as $mL \cdot min^{-1}$; CO: cardiac output expressed as $mL \cdot min^{-1}$; AP: aortic pressure expressed as mmHg; CW: cardiac work expressed as mW; Rate: heart rate expressed as $beat \cdot min^{-1}$; SEV: systolic ejection volume expressed as $mL \cdot beat^{-1}$; QO_2: oxygen consumption expressed as µL of oxygen per min; ME: metabolic efficiency expressed as $\times 10^{-3}$ $mW \cdot \mu L^{-1}$ of oxygen; NS: not significant.

However, the n-3 PUFA supply in industrialised society might be increased to achieve an equilibrated diet. This is the reason why we performed the second set of experiments with the commercial diet. Since the two sets of experiments were carried out at different period of the year, the physiological parameters were analysed separately. Furthermore, the lipid content of the diets was different (10% for the semi-synthetic diets and only 5% for the commercial diet).

The changes in membrane lipid composition noticed in the first set of experiments did not alter the fluid dynamics of the isolated perfused working hearts. Neither the aortic flow, nor the coronary flow was modified. The oxidative metabolism (oxygen consumption, β-oxidation rate and metabolic efficiency) was similar in the two dietary groups. The incorporation of palmitate in diglycerides and phospholipids was not modified. Changes in fatty acid turnover in phospholipids could reflect abnormalities of membrane homeostasis, which could bring about alterations of cardiac function. Similarly, diglycerides are second messengers involved in calcium homeostasis. Changes in their synthesis rate could modify the intracellular calcium concentration and cardiac mechanical activity. The sole parameter being significantly modified was the heart rate that was reduced (–11%) by the n-3 polyunsaturated fatty acid-rich diet. This was already reported in other studies carried out in mature marmosets and isolated working rat hearts [4, 7]. We previously attributed this phenomenon either to the high level of docosahexaenoic acid in membrane phospholipids or to the reduction of the arachidonic acid proportion [26]. Both modifications could contribute to altering prostaglandin synthesis [22] and calcium homeostasis.

Physical training did not tremendously modify the fatty acid profile of cardiac phospholipids. The polyunsaturated fatty acid profile was not altered. It was similar for saturated and monounsaturated fatty acids. The sole significant difference observed depended on the diet. In the sunflower seed oil-fed rats, the monoenes were slightly increased by physical training, whereas they were reduced in the fish oil-fed rats. The weak training-induced changes in phospholipid fatty acid composition could be due to the low training intensity that did not bring about any left ventricular hypertrophy. Despite the absence of major change in the phospholipid fatty acid profile of cardiac membranes, several changes in heart function were seen. The heart rate was reduced by physical training at a similar extent in the sunflower seed oil and fish oil groups. The aortic flow and cardiac output were thus markedly decreased. The reduced heart rate induced by physical training was already observed in isolated perfused working rat hearts trained by swimming [18]. However, it was counterbalanced by an increased systolic ejection volume that provoked an enhancement of cardiac output and aortic flow. These changes in mechanical performance have been associated with an increased coronary flow and oxygen consumption, to compensate for the enhanced energy demand. In the present study, the coronary flow, β-oxidation rate (as estimated by the intracellular fate of radiolabelled palmitate) and myocardial oxygen consumption were not increased by physical training, because the cardiac work was not enhanced. The physical training-induced changes in fluid dynamics and heart rate were probably not due to modifications in the monoene composition of membrane phospholipids, since the monounsaturated fatty acids varied differently in the sunflower seed oil and fish oil groups and the heart rate and aortic flow decreased in both dietary groups. The reduced aortic flow might be related to the effect of psychological stress due to undesired physical training. In the present study, physical training reduced the heart rate in the two dietary groups. This was different as compared to the in vitro study [14], where the heart rate was decreased only in the fish oil group. This indicates that

undefined regulation mechanisms (hormonal or nervous factors) prevented the decrease in heart rate in the rats fed sunflower seed oil. This would impede the beneficial reduction of blood pressure in these animals. On the other hand, the dietary oil-induced changes in blood pressure might be due to changes in blood volume. Physical training could contribute to modify blood volume. This would alter end diastolic ventricular volume and modify intraventricular pressure development according to the Frank-Starling length-tension relationship. Dietary fish oil could contribute to altering blood volume in a way that the intraventricular pressure development is reduced. Such changes in end diastolic ventricular volume would not be noticed in the isolated perfused working heart, since ventricular filling depends mainly on the constant atrial perfusion pressure. However, the involvement of changes in blood volume and Frank-Starling relationship would not explain the reduced heart rate that might be the main determinant of the reduced blood pressure in trained animals of the fish oil group.

In the study of Bowles and Starnes [2], rats were subjected to exercise training through a 60-min·day^{-1} run. The isolated working hearts of these animals did not display a different left ventricular function than those of sedentary animals. The aortic flow and cardiac output were not reduced. Since the semi-purified diets used in the present study could be responsible for the changes in left ventricular function, we repeated the experiment with a commercially available diet. This diet provided sufficient amounts of n-6 and n-3 polyunsaturated fatty acids, which differed with the first set of experiments where the diet contained either n-6 polyunsaturated fatty acids or n-3 polyunsaturated fatty acids. The fatty acid composition of cardiac phospholipids was characterised by an equilibrated n-6/n-3 polyunsaturated fatty acid ratio that ranged between those found in the first set of experiments. The parameters of left ventricular function were not significantly altered by physical training. The heart rate, the aortic flow and the cardiac output were not reduced. This suggests that the semi-purified diets used in the first set of experiments were not satisfying to allow the heart to adapt to changes in physical activity. Both n-6 and n-3 polyunsaturated fatty acids should be present in the diet in sufficient amounts to allow the good adaptation of myocardium to moderate physical training.

Recently, Lortet and Verger [14] reported interesting data concerning the effect of dietary polyunsaturated fatty acids and physical training. In the same dietary and training conditions as those used in the first set of experiments, they reported a reduced arterial blood pressure in the trained animals fed fish oil. The study was carried out in vivo. It was not observed for the sunflower seed oil group. Because of the results obtained in the present study, one can consider that the decreased blood pressure observed by Lortet and Verger [14] after training in the fish oil-fed rats was not due to changes in cardiac function. A n-3 polyunsaturated fatty acid-induced modification of vascular resistance might rather be responsible for the changes in cardiovascular function in the whole animal. Lawson et al. [13] reported an increase in acetylcholine-mediated vasorelaxation of aortic rings by n-3 polyunsaturated fatty acids.

In conclusion, a moderate but sustained physical exercise performed by 60 min·day^{-1} running induced a decrease in aortic flow, cardiac output and heart rate without hypertrophy in the isolated heart of rats fed either n-6 polyunsaturated fatty acids or n-3 polyunsaturated fatty acids. The decrease was similar whatever the dietary polyunsaturated fatty acid ingested. It appears to be related to the polyunsaturated fatty acid composition of the diet. The simultaneous presence of n-6 and n-3 polyunsaturated fatty acids in the diet appears necessary to provide adequate adaptation of the heart to physical training. A reduced vascular tone in the fish oil-fed rats might explain the

decreased aortic blood pressure noticed in vivo. Thus, dietary fish oil associated with moderate physical training might be used to prevent hypertension and cardiovascular diseases (hypertrophy, cardiac failure and atherosclerosis). Further studies should be carried out to verify if such a decreased blood pressure occurs in the human being with a diet equilibrated in polyunsaturated fatty acids. Furthermore, the effect of dietary n-3 PUFA + moderate physical exercise on blood pressure should be determined in hypertensive subjects.

ACKNOWLEDGMENTS

The Conseil Régional de Bourgogne supported this work.

REFERENCES

[1] Bizerte C., Sezille G, Bertrand M., Jaillard J., Chromatographie en couche mince des principales classes de lipides, Ann. Biol. Clin. 22 (1964) 861–897.

[2] Bowles D.K., Starnes J.W., Exercise training improves metabolic response after ischemia in isolated working rat heart, J. Appl. Physiol. 76 (1994) 1608–1614.

[3] Charnock J.S., McLennan P.L., Sundram K., Abeywardena M.Y., Omega-3 PUFA's reduce the vulnerability of the rat heart to ischaemic arrhythmia in the presence of a high intake of saturated animal fat, Nutr. Res. 11 (1991) 1025–1034.

[4] Charnock J.S., McLennan P.L., Abeywardena M.Y., Dietary modulation of lipid metabolism and mechanical performance of the heart, Mol. Cell Biochem. 116 (1992) 19–25.

[5] Conquer J.A., Holub B.J., Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects, J. Nutr. 126 (1996) 3032–3039.

[6] Dagnélie P., in: Dagnélie P. (Ed.), Théories et méthodes statistiques, Presses Agronomiques de Gembloux, Gembloux, 1995.

[7] Demaison L., Sergiel J.P., Moreau D., Grynberg A., Influence of dietary fish oil on the mechanical and biochemical function of isolated working rat hearts before and after ischemia, in: Devron C.A., Baksaas I., Krokan H.E., (Eds.), Omega-3 Fatty Acids: Metabolism and Biological Effects, Birkäuser Verlag, Basel, 1993, pp. 115–123.

[8] Demaison L., Sergiel J.P., Moreau D., Grynberg A., Influence of the phospholipid n-6/n-3 polyunsaturated fatty acid ratio on the mitochondrial oxidative metabolism before and after myocardial ischemia, Biochim. Biophys. Acta 1227 (1994) 53–59.

[9] Folch J., Lees M., Sloane-Stanley G.H., A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1957) 497–509.

[10] Galli C., Agradi E., Paoletti R., The (n-6) pentaene: (n-3) hexane fatty acid ratio as an index of linolenic acid deficiency, Biochim. Biophys. Acta 369 (1974) 142–145.

[11] Juaneda P., Rocquelin G., Rapid and convenient separation of phospholipids and non phosphorus lipids from rat hearts using silica cartridge, Lipids (1985) 40–41.

[12] Kanazawa A., Shirota Y., Fujimoto K., Increase hepatic β-oxidation of docosahexaenoic acid, elongation of eicosapentaenoic acid, and acylation of lysophosphatidate in rats fed a docosahexaenoic acid-enriched diet, Lipids 32 (1997) 737–744.

[13] Lawson D.L., Mehta J.L., Saldeen K., Mehta P., Saldeen T.G., Omega-3 polyunsaturated fatty acids augment endothelium-dependent vasorelaxation by enhanced release of EDRF and vasodilator prostaglandins, Eicosanoids 4 (1991) 217–223.

[14] Lortet S., Verger P., Alteration of cardiovascular function in trained rats fed with fish oil, Int. J. Sports Med. 16 (1995) 519–521.

[15] McLennan P.L., Abeywardena M.Y., Charnock J.S., Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion, Am. Heart. J. 16 (1988) 709–717.

[16] McLennan P.L., Barnden L.R., Bridle T.M., Abeywardena M.Y., Charnock J.S., Dietary fat modulation of left ventricular ejection fraction in the marmoset due to enhanced filling, Cardiovasc. Res. 26 (1992) 871–877.

[17] McLennan P.L., Bridle T.M., Abeywardena M.Y., Charnock J.S., Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys, Am. J. Clin. Nutr. 58 (1993) 666–669.

[18] Moreau D., Guilland J.C., Noirot P., Malval M., Étude chez le rat des modifications nutritionnelles, cardiaques et adrénergiques induites par l'entraînement, J. Physiol. Paris 75 (1979) 755–764.

[19] Nair S.S.D., Leitch J.W., Falconer J., Garg M.L., Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action, J. Nutr. 127 (1997)–393.

[20] Nalbone G., Termine E., Leonardi J., Portugal H., Lechene P., Calaf R., Lafont R., Lafont H., Effect of dietary salmon oil feeding on rat lipid status, J. Nutr. 118 (1988) 809–817.

[21] Neely J.R., Leibermeister H., Battersby E.J., Morgan H.E., Effect of pressure development on oxygen consumption by isolated rat hearts, Am. J. Physiol. 212 (1967) 804–814.

[22] Oudot F., Grynberg A., Sergiel J.P., Eicosanoid synthesis in cardiomyocytes: influence of hypoxia, reoxygenation and polyunsaturated fatty acids, Am. J. Physiol. 26 (1995) H308–H315.

[23] Paulson D.J., Smith J.M., Zhao J., Bowman J., Effects of dietary fish oil on myocardial ischemic/reperfusion injury of Wistar Kyoto and stroke-prone spontaneously hypertensive rats, Metabolism 41 (1992) 533–539.

[24] Ruiz-Gutierrez V., Molina M.T., Vasquez C.M., Comparative effects of feeding different fats on fatty acid composition of major individual phospholipids of rat hearts, Ann. Nutr. Metab. 34 (1990) 50–358.

[25] Sentex E., Grynberg A., Sergiel J.P., Demaison L., Influence of the phospholipid n-6:n-3 polyunsaturated fatty acid ratio on the oxidative phosphorylation of isolated cardiac mitochondria, Exp. Clin. Cardiol. 1 (1996) 37–44.

[26] Sergiel J.P., Martine L., Raederstorff D., Grynberg A., Demaison L., Individual effects of dietary EPA and DHA on the functioning of the isolated working rat heart, Can. J. Physiol. Pharmacol. 76 (1998) 728–736.

[27] Yang B.C., Saldeen T.G.P., Bryant J.L., Nichols W.W., Mehta J.L., Long-term dietary fish oil supplementation protects against ischemia-reperfusion-induced myocardial dysfunction in isolated rat hearts, Am. Heart. J. 126 (1993) 1287–1292.