PHOSPHOLIPID COMPOSITION OF IMMATURE RAT MYOCARDIUM EXPOSED TO CHRONIC HYPOXIA AND THE EFFECT OF NORMOXIC RECOVERY

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Dedicated to the 50th anniversary of the foundation of the Department of Biochemistry, the first biochemical department in Czechoslovakia.

Four-day-old male Wistar rats were exposed to intermittent high-altitude (IHA) hypoxia of 7000 m simulated in a hypobaric chamber (8 h/day, 5 days/week, 25 exposures). The concentration of individual phospholipids (PL) and fatty acid (FA) composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) were determined in right (RV) and left (LV) ventricles of rats adapted to chronic hypoxia (40-day-old), rats after 30 days of recovery from hypoxic to normoxic conditions (70-day-old) and both age-matched controls. The adaptation to IHA hypoxia decreased the concentration of DPG in LV (by 10%) in comparison with normoxic control. In hypoxic group the proportion of linoleic acid (18:2n-6) decreased; on the contrary, the proportion of arachidonic (20:4n-6), docosapentaenoic (22:5n-3) and docosahexaenoic (22:6n-3) acids increased in PC and PE of both RV and LV. As to DPG, IHA hypoxia caused a significant decrease in the n-6/n-3 ratio due to the increase in the 22:6n-3 proportion in RV. Thirty-day-long recovery from hypoxic to normoxic conditions led to complete regression of the hypoxic effect on FA composition in all PL. No difference in FA composition of PL was observed between RV and LV in any experimental group. Numerous dietary studies with fish oil supplements confirmed cardioprotective effect of n-3 polyunsaturated FA. We suppose that their increased content in heart-membrane PL observed in this study independently on a diet might contribute to higher tolerance of chronically hypoxic myocardium to ischemic injury.

Keywords: Chronic hypoxia; Neonatal rat heart; Phospholipids; n-3 PUFA; Fatty acids; Phosphatidylcholine; Biomembranes.

Chronic myocardial hypoxia is the major pathophysiological feature of various cardiopulmonary diseases, like chronic obstructive pulmonary disease and cyanotic congenital heart defects. It is also naturally encountered in fetuses and in populations living at high altitudes. Permanent or intermittent hypobaric hypoxia simulated in a low-pressure chamber is one of the relevant experimental models of chronic hypoxia. It was shown that the adaptation to chronic hypoxia led to a variety of morphological, biochemical and functional changes in order to maintain homeostasis with minimal energy expenditure[1]. Pulmonary hypertension and right ventricular hypertrophy, the characteristic features of adaptation to chronic hypoxia, were observed in both adults and newborn rats exposed to intermittent high-altitude (IHA) hypoxia in the first postnatal week[2,3].

Besides an adverse influence on the cardiopulmonary system, it was well established that the heart of animals adapted to chronic hypoxia exhibits an increased tolerance to acute ischemic injury manifested as a reduction of myocardial infarction size, improvement of post-ischemic contractile dysfunction and limitation of life-threatening ventricular arrhythmias[4,5]. The molecular mechanism of these phenomena is still unclear. Qualitative and quantitative alterations of both extracellular matrix and myofibrillar proteins in hypoxic myocardium together with a remodelling of cardiac metabolism, activation of mitochondrial K_{ATP} channels and role of protein kinase C should be taken in consideration[6–10]. Cardiac protection by the adaptation to chronic hypoxia may persist in adults as well as in newborn rats long time after regression of other hypoxia-induced adaptive changes, such as polycythemia, pulmonary hypertension and right ventricular hypertrophy[11].

Recently we have published that the adaptation of adult rats to IHA hypoxia substantially increased content of n-3 polyunsaturated fatty acids (PUFA) in cardiac phospholipids[12] (PL) that are known to be cardioprotective from dietary studies[13]. The great majority of experimental data indicate that immature mammalian heart is more resistant to oxygen deficiency as compared with adults[1]. Therefore the aim of this study was to analyse the PL composition in cardiac membranes of young rats exposed to IHA hypoxia during early postnatal period. We also analysed the reversibility of hypoxia-induced remodelling of membrane phospholipids in young rats recovering from IHA hypoxia for one or four months under normoxic conditions.

EXPERIMENTAL

Animal Model

Male Wistar rats were exposed to IHA hypoxia on postnatal day 4. The IHA hypoxia was simulated in a hypobaric chamber for 8 h/day, 5 days a week. Barometric pressure (P_B) was lowered stepwise so that the level equivalent to the altitude of 7000 m (P_B = 308 mm Hg, 41 kPa; P_{O2} = 65 Torr, 8.7 kPa) was reached after 13 exposures. The total number of exposures was 25 (5 weeks). The age-matched control (normoxic) group of animals was kept for the corresponding period at P_B and P_{O2} equivalent to altitude of 200 m (742 mm Hg, 98.9 kPa; 155 Torr, 20.7 kPa). All animals were fed the same vitamin-enriched, low fat (3.5% by weight) standard diet ST1 (Velaz). The diet contained, by our analyses, these fatty acids (FA): 1.5% of 14:0; 19.2% of 16:0; 2.2% of 16:1n-7; 5.5% of 18:0; 25.4% of 18:1n-9; 1.9% of 18:1n-7; 38.7% of 18:2n-6 and 4.2% of 18:3n-3. One third of adapted animals were employed 24 h after the last hypoxic exposure (40-day-old rats) whereas remaining animals were kept under normoxic conditions for another 30 days (hypoxic recovery I, 70-day-old rats) and for 4 months (hypoxic recovery II, 160-day-old rats). The groups of age-matched control animals (control, 40-day-old; recovery control I, 70-day-old; recovery control II, 160-day-old rats) were kept for corresponding period of time under normoxic conditions. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, rev 1996).

Lipid Analysis

Frozen samples of ventricular tissue were pulverized and homogenized. Phospholipids (from 100 mg wet tissue) were extracted in three consecutive steps according to the modified method of Folch et al.[14] The first extraction was performed with three portions (0.25 ml each) of a chloroform–methanol mixture (1:3, 2:1 and 2:1) in a chilled mortar. Subsequent extractions were performed with a mixture 2:1 (0.6 ml each), 0.9% NaCl in water (20% of the volume of extract) was added and after vigorous shaking the lower lipid layer was dried at 40 °C under a stream of nitrogen.

Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) were separated by two-dimensional thin layer chromatography. Silica Gel H (Merck) as a slurry of 22.5 g in 62 ml water containing 2.5 g of magnon (Merck) was spread in a 0.25 mm layer with a spreader (Desaga) on glass plates (20 × 20 cm). Solvent mixtures were used according to the method of Rouser et al.[15] Plasmalogen components of PC (PLPC) and PE (PLPE) were analysed by the method of Horrocks et al.[16] Silica Gel G (Merck) in 0.5 mm layers was used for their separation. Phospholipid spots were detected with iodine vapours, scraped off and analysed for phosphorus[15].

For FA analyses, phospholipids were separated on plates with Silica Gel H (0.5 mm) by the method of Rouser et al.[15] Spots were observed under UV light after staining with 0.005% 2,7-dichlorofluorescein in methanol, scraped off and stored in nitrogen atmosphere at –20 °C until the next day when the methyl esters were prepared. For the preparation of FA methyl esters, sodium methanolate was added to tubes with silica gel, the tubes were then incubated at room temperature for 60 min in the dark, methyl esters were extracted with hexane and the extracts were evaporated under a stream of nitrogen and stored at –20 °C. FA methyl esters were separated with a gas chromatograph Chrompack CP 438 A (Chrompack, Middelburg, The Netherlands) using a medium-polar column CP WAX 52 CB

(25 m × 0.25 mm i.d.). The oven temperature was programmed from 145–230 °C at 2 °C/min. Hydrogen was used as carrier gas. FAs were identified using a mixture of FA methyl esters (Sigma–Aldrich Co., St.Louis, MO, U.S.A.).

Statistical Analyses

All results are expressed as means ± S.E.M. The statistical significance of differences between groups was determined by the two-way ANOVA and subsequent Student–Newman–Keuls test. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Body and Heart Weight

Body and heart weight parameters of rats adapted to IHA hypoxia and those at the recovery to normoxia for another 30 days and 4 months after the last hypoxic exposure are presented in Table I. The adaptation to IHA hypoxia significantly increased the heart weight as compared with age-matched normoxic controls whereas the body weight was not changed. This rise was due to the hypertrophy of right ventricles (RV; by 36%) and left ventricles (LV; by 22%) to a moderate extent. The enlargement of heart corresponds to the data of previous studies using the same experimental model[17]. Whereas both ventricles are under the influence of chronic hypoxia, there is an RV-LV difference in hypertrophy stemming from increased afterload of RV due to elevated cardiopulmonary resistance[18]. We did not find any retardation of body growth which was mostly observed in other studies[12,17]. A probable explanation of this difference may be a higher tolerance of young animals to the hypoxic stress in our experimental sets. Thirty days after the termination of hypoxia (hypoxic recovery I), RV and LV weights remained significantly elevated (by 11 and 13%, respectively). Complete regression of all weight parameters was reached 4 months after the termination of hypoxia (hypoxic recovery II). Previous reports demonstrated that some changes induced by IHA hypoxia (polycythemia, pulmonary hypertension, RV hypertrophy) reversed in 4–5 weeks after removal from the hypoxic atmosphere[18]. However, an increased ratio of collagenous/contractile proteins persisted in the period when the ventricular weights had been already normal[11]. On the other hand, an increased resistance of isolated RV to acute anoxia remained significantly higher even 4 months after removal of animals from hypoxic environment as compared with normoxic controls[18].

TABLE I
Weight parameters of rats adapted to intermittent high-altitude hypoxia and rats recovering from hypoxia in comparison with corresponding age-matched controls

Parameter	Control	Hypoxic	Control recovery I	Hypoxic recovery I	Control recovery II	Hypoxic recovery II
BW (g)	142.17 ± 4.18	142.67 ± 3.89	340.00 ± 4.57	340.29 ± 4.57	565.67 ± 21.14	562.50 ± 13.05
HW (mg)	421.17 ± 13.94	514.17 ± 16.26[a]	789.00 ± 18.08	865.29 ± 9.79[a]	1129.50 ± 56.67	1135.33 ± 17.15
RV (mg)	91.17 ± 1.38	124.17 ± 5.73[a]	188.43 ± 3.58	209.57 ± 5.27[a]	234.50 ± 11.55	252.17 ± 10.11
LV (mg)	238.50 ± 12.02	291.33 ± 12.08[a]	416.00 ± 12.52	471.57 ± 8.29[a]	633.33 ± 52.09	658.83 ± 8.63
HW/BW ($\times 10^{-2}$)	0.296 ± 0.004	0.361 ± 0.006[a]	0.232 ± 0.002	0.254 ± 0.004[a]	0.198 ± 0.003	0.202 ± 0.005
RV/BW ($\times 10^{-3}$)	0.643 ± 0.014	0.869 ± 0.025[a]	0.555 ± 0.010	0.617 ± 0.021[a]	0.414 ± 0.011	0.451 ± 0.025
RV/HW	0.217 ± 0.005	0.240 ± 0.005[a]	0.240 ± 0.005	0.240 ± 0.005[a]	0.208 ± 0.006	0.222 ± 0.008
LV/BW ($\times 10^{-2}$)	0.168 ± 0.004	0.204 ± 0.005[a]	0.123 ± 0.003	0.138 ± 0.002[a]	0.111 ± 0.005	0.118 ± 0.002
LV/HW	0.567 ± 0.014	0.567 ± 0.011	0.529 ± 0.013	0.543 ± 0.010	0.557 ± 0.019	0.581 ± 0.009
RV/LV	0.387 ± 0.018	0.428 ± 0.017	0.454 ± 0.015	0.444 ± 0.017	0.377 ± 0.023	0.383 ± 0.019

Values are mean ± S.E.M. of 6 hearts in each group. BW, body weight; HW, heart weight; RV, right ventricle; LV, left ventricle.
[a] $p < 0.05$, hypoxic vs corresponding control tissue.

Phospholipid Concentration

Phospholipid concentration in LV and RV of rats adapted to IHA hypoxia, that of rats recovering from hypoxia and of corresponding controls are summarized in Table II. PC, PE and DPG are major phospholipids either in LV (accounting for 38.9, 38.7 and 13.3% of total PL, respectively) or RV (accounting for 39.6, 37.8 and 13.2% of total PL, respectively) in the normoxic heart tissue of 40-day-old rats. The proportion of PC and PE plasmalogen component was similar in both LV and RV (4.9% of PC, 22.5% of PE and 5.4% of PC, 22.6% of PE, respectively). These proportions correspond to our previously published data[19,20]. The proportion of minor phospholipids (PI, PS and SM) was 4.1, 2.8 and 2.2%, respectively, in LV and 3.9, 3.0 and 2.3%, respectively, in RV. The concentration of PL shows a mild increasing trend between 40- and 70-day-old control rats in both ventricles. This is consistent with the results of Nováková et al.[21] describing the developmental rise in heart PL concentration in the period between postnatal day 2 and adulthood in rats.

The adaptation of young rats to IHA hypoxia did not influence the concentration of heart phospholipids with the exception of a minor but significant decrease in DPG (marker of mitochondrial membranes) concentration in LV. On the other hand, our previous study using adult rats for adaptation to IHA hypoxia had demonstrated a greater effect on phospholipid concentration, namely in RV (decrease in DPG of 19% and in total PL of 7%)[12]. It is evident that the chronic hypoxia is more effective in membrane remodelling in adult heart, in particular of mitochondrial membrane as the greatest effect on DPG indicates. The relative stability of membrane PL concentration in newborn hearts exposed to chronic hypoxia is in a good accordance with other studies that have confirmed greater resistance of newborn rat heart to stress conditions than that of adult one[1]. It is noteworthy that the absence of a decrease in the concentration of major PL despite a significant rise in the heart weight, which was observed in young rats, might reveal that during hypertrophy induced by IHA hypoxia the proportional rise in synthesis of membrane components (phospholipids and proteins) and other components of myocardial tissue (collagenous and contractile proteins) takes place. We have published that in the cardiomegaly induced by pressure overload in rats in early postnatal period, the concentration of all heart phospholipids decreased[19]. This might be an example of the disproportional rise in the synthesis of different tissue components with the predominance of collagenous and contractile proteins. On the other hand, we have also shown that in hypertrophied heart of young

TABLE II

Phospholipid concentration (in μmol/g w.w.) in left and right ventricles of rats adapted to intermittent high-altitude hypoxia and rats recovering from hypoxia in comparison with corresponding age-matched controls

Parameter	Right ventricle				Left ventricle			
	control	hypoxic	control recovery I	hypoxic recovery I	control	hypoxic	control recovery I	hypoxic recovery I
PC	10.49 ± 0.21	10.82 ± 0.17	10.83 ± 0.31	10.75 ± 0.29	10.63 ± 0.26	11.17 ± 0.30	11.47 ± 0.31	11.01 ± 0.22
PE	10.00 ± 0.28	10.01 ± 0.20	10.06 ± 0.17	9.91 ± 0.24	10.59 ± 0.32	10.25 ± 0.23	10.81 ± 0.25	10.39 ± 0.21
DPG	3.50 ± 0.12	3.33 ± 0.07	4.06 ± 0.13	4.03 ± 0.13	3.64 ± 0.07	3.27 ± 0.13[a]	4.24 ± 0.12	4.10 ± 0.08
PI	1.04 ± 0.03	1.02 ± 0.04	1.13 ± 0.03	1.07 ± 0.03	1.12 ± 0.06	1.06 ± 0.04	1.14 ± 0.03	1.14 ± 0.02
PS	0.81 ± 0.06	0.76 ± 0.05	0.69 ± 0.04	0.66 ± 0.02	0.77 ± 0.04	0.83 ± 0.05	0.67 ± 0.02	0.72 ± 0.08
SM	0.62 ± 0.06	0.66 ± 0.04	0.51 ± 0.04	0.53 ± 0.03	0.59 ± 0.05	0.69 ± 0.06	0.49 ± 0.02	0.52 ± 0.05
Total PL	26.46 ± 0.58	26.60 ± 0.51	27.27 ± 0.67	26.96 ± 0.68	27.36 ± 0.65	27.27 ± 0.62	28.81 ± 0.68	27.89 ± 0.50
PLPC[b]	0.57 ± 0.08	0.55 ± 0.08	0.64 ± 0.09	0.68 ± 0.08	0.52 ± 0.07	0.48 ± 0.04	0.69 ± 0.10	0.70 ± 0.09
PLPE[b]	2.26 ± 0.05	2.23 ± 0.05	2.57 ± 0.08	2.26 ± 0.09	2.38 ± 0.09	2.42 ± 0.10	2.69 ± 0.16	2.46 ± 0.11

Values are mean ± S.E.M. of 6 hearts in each group, w.w., wet weight. PL, phospholipids; PC, choline phosphoglycerides; PE, ethanolamine phosphoglycerides; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PS, phophatidylserine; SM, sphingomyelin; PLPC, choline plasmalogen; PLPE, ethanolamine plasmalogen. [a] $p < 0.05$, hypoxic vs corresponding control tissue. [b] Choline and ethanolamine plasmalogens are included in choline and ethanolamine phosphoglycerides, respectively.

hyperthyroid rats, the concentration of all major PL, namely DPG, increased. This is in a good accordance with accelerated maturation of myocytes that is associated with intensive proliferation of sarcoplasmic reticulum and mitochondrial membranes[20].

A complete recovery from the hypoxic effect on both concentration and FA pattern in phospholipids was already reached after 30 days of recovery to normoxic conditions (recovery I). There was no difference in phospholipid concentration and their FA pattern among any experimental groups after 4 months of recovery to normoxic conditions (data are not presented).

Fatty Acid Composition in Major Phospholipids

The FA composition of PC, PE and DPG in LV and RV of all experimental groups is demonstrated in Tables III–V. In control normoxic tissue, the predominant saturated FA (SFA) in PC were palmitic (16:0) and stearic (18:0) acids accounting for about 43% of total FA; in PE the most abundant was 18:0, which together with 16:0 were equivalent to about 40% of total FA. The main monounsaturated FA (MUFA) were oleic (18:1n-9) and vaccenic (18:1n-7) acids in both PC and PE. The most plentiful polyunsaturated FA (PUFA) were linoleic (18:2n-6), arachidonic (20:4n-6), docosahexaenoic (22:6n-3) and docosapentaenoic (22:5n-3) acids. The higher content of 18:2n-6 in PC was counterbalanced by higher contents of 20:4n-6, 22:5n-3 and 22:6n-3 PUFA in PE. A comparison of 40- and 70-day-old normoxic animals reveals moderate ontogenetic changes in FA composition either in PC (increase in n-6 and decrease in n-3 PUFA) or PE (increase in n-3 PUFA and decrease in SFA) with the same trend in both ventricles. This is in a good agreement with results of Gudmundsdottir et al.[22] showing similar changes in FA composition of PC and PE in rat heart during the same developmental period. The remodelling of FA composition in PL has been ascribed to important nutritional, hormonal and functional changes that are in progress immediately after birth as well as in the period of the suckling/weaning transition[23].

The SFA proportion of DPG in ventricles of normoxic rats was very low in comparison with that in PC and PE (ca. 12% of total FA). The most dominant FA in cardiac DPG was 18:2n-6, contributing by almost 70% of total FA in 40-day-old rats. As to developmental changes in FA composition of DPG between postnatal days 40 and 70, the most prominent observation was a rise in the 18:2n-6 content and fall in 20:4n-6 and MUFA. Similar developmental changes in FA composition of DPG in rat heart between postnatal days 2 and 60 have been already presented[21].

TABLE III

Fatty acid composition (in mole %) of choline phosphoglycerides in left and right ventricles of rats adapted to intermittent high altitude hypoxia and rats recovering from hypoxia in comparison with corresponding age-matched controls

FA composition	Right ventricle				Left ventricle			
	control	hypoxic	control recovery I	hypoxic recovery I	control	hypoxic	control recovery I	hypoxic recovery I
14:0	0.47 ± 0.04	0.55 ± 0.08	0.37 ± 0.04	0.27 ± 0.03[b]	0.40 ± 0.07	0.42 ± 0.08	0.27 ± 0.03	0.30 ± 0.02
16:0	**20.38 ± 1.04**	**20.61 ± 1.62**	**17.81 ± 0.67**[b]	**18.18 ± 0.58**	**18.96 ± 0.97**	**20.09 ± 1.13**	**18.79 ± 0.42**	**17.84 ± 0.64**
16:1	1.30 ± 0.11	1.19 ± 0.15	1.25 ± 0.11	1.22 ± 0.07	1.22 ± 0.15	1.09 ± 0.09	1.13 ± 0.06	1.44 ± 0.07[b]
18:0	**22.38 ± 0.87**	**26.44 ± 0.65**[a]	**23.41 ± 0.33**	**24.10 ± 0.94**	**23.83 ± 1.50**	**26.05 ± 0.63**	**23.63 ± 0.43**	**23.99 ± 0.91**
18:1n-9	7.00 ± 0.46	5.88 ± 0.72	5.60 ± 0.12[b]	5.25 ± 0.20	6.03 ± 0.35	4.47 ± 0.21	5.50 ± 0.15	5.32 ± 0.21[b]
18:1n-7	5.98 ± 0.24	4.84 ± 0.48	6.82 ± 0.13[b]	7.56 ± 0.33[b]	5.46 ± 0.37	4.79 ± 0.37	7.43 ± 0.28[b]	7.29 ± 0.21[b]
18:2n-6	**23.56 ± 1.70**	**12.23 ± 1.12**[a]	**23.49 ± 0.92**	**20.85 ± 1.50**	**21.63 ± 1.86**	**12.90 ± 1.41**[a]	**21.52 ± 1.01**	**20.30 ± 1.71**[b]
20:3n-6	0.67 ± 0.04	0.54 ± 0.10	0.52 ± 0.02[b]	0.53 ± 0.01	0.70 ± 0.03	0.51 ± 0.07	0.52 ± 0.03	0.51 ± 0.02
20:4n-6	**14.11 ± 1.33**	**21.25 ± 1.86**[a]	**18.55 ± 0.76**[b]	**19.79 ± 1.52**	**17.57 ± 1.64**	**24.22 ± 0.61**[a]	**18.87 ± 0.85**	**20.41 ± 1.55**
20:5n-3	0.16 ± 0.08	1.14 ± 1.07	0.01 ± 0.01	0.01 ± 0.01	0.14 ± 0.04	0.13 ± 0.06	0.01 ± 0.01	0.03 ± 0.03[b]
22:5n-3	**0.96 ± 0.11**	**1.96 ± 0.21**[a]	**0.81 ± 0.07**	**0.87 ± 0.09**[b]	**1.24 ±0.13**	**2.25 ± 0.14**[a]	**0.84 ± 0.06**[b]	**0.98 ± 0.09**[b]
22:6n-3	**1.60 ± 0.29**	**2.53 ± 0.32**	**1.34 ± 0.07**	**1.35 ± 0.13**[b]	**1.89 ± 0.19**	**3.10 ± 0.26**[a]	**1.47 ± 0.09**	**1.54 ± 0.17**[b]
SFA	43.22 ± 1.02	47.59 ± 1.46	1.59 ± 0.77	42.54 ± 0.65[b]	43.19 ± 1.26	46.55 ± 1.19	42.69 ± 0.25	42.13 ± 0.60[b]
MUFA	14.29 ± 0.74	11.91 ± 1.19	13.67 ± 0.23	14.03 ± 0.56	12.71 ± 0.65	10.34 ± 0.52	14.06 ± 0.34	14.05 ± 0.40[b]
n-6	**38.34 ± 1.11**	**34.01 ± 2.12**	**42.55 ± 0.74**[b]	**41.16 ± 0.44**[b]	**39.90 ± 1.25**	**37.63 ± 1.26**	**40.91 ± 0.44**	**41.23 ± 0.39**[b]
n-3	**2.72 ± 0.39**	**5.64 ± 1.35**	**2.15 ± 0.14**	**2.23 ± 0.21**[b]	**3.27 ± 0.33**	**5.48 ± 0.36**	**2.31 ± 0.15**[b]	**2.55 ± 0.26**[b]
SFA/UFA	0.78 ± 0.04	0.93 ± 0.05	0.71 ± 0.02	0.74 ± 0.02[b]	0.78 ± 0.04	0.88 ± 0.04	0.75 ± 0.01	0.73 ± 0.02[b]
n-6/n-3	**15.90 ± 2.67**	**7.38 ± 1.21**[a]	**20.17 ± 1.32**	**19.33 ± 1.83**[b]	**12.96 ± 1.57**	**7.08 ± 0.64**	**18.08 ± 1.11**	**16.99 ± 1.72**[b]
20:4/18:2	0.63 ± 0.09	1.83 ± 0.24[a]	0.80 ± 0.06	1.00 ± 0.14[b]	0.86 ± 0.13	2.03 ± 0.28[a]	0.89 ± 0.07	1.07 ± 0.16[b]
UI	135.02 ± 4.52	153.68 ± 10.17	148.49 ± 2.76[b]	148.94 ± 4.05	146.59 ± 5.25	165.02 ± 1.83	147.13 ± 2.14	152.16 ± 3.95[b]

Values are mean ± S.E.M. of 6 hearts in each group. SFA, saturated fatty acids (FA); MUFA, monounsaturated FA; UFA, unsaturated FA; UI, unsaturation index calculated as mole % of individual unsaturated FA multiplied by number of double bonds. The values for FA discussed in the text are in bold scripts. [a] $p < 0.05$, hypoxic vs corresponding control tissue. [b] $p < 0.05$, control recovery I vs control and hypoxic recovery I vs hypoxic.

TABLE IV

Fatty acid composition (in mole %) of ethanolamine phosphoglycerides in left and right ventricles of rats adapted to intermittent high-altitude hypoxia and rats recovering from hypoxia in comparison with corresponding age-matched controls

FA composition	Right ventricle				Left ventricle			
	control	hypoxic	control recovery I	hypoxic recovery I	control	hypoxic	control recovery I	hypoxic recovery I
14:0	0.87 ± 0.19	0.87 ± 0.16	0.57 ± 0.08	0.63 ± 0.08	0.76 ± 0.25	0.74 ± 0.20	0.99 ± 0.32	0.47 ± 0.05
16:0	**11.45 ± 0.36**	**12.36 ± 0.63**	**9.22 ± 0.14**^{b}	**9.26 ± 0.35**^{b}	**11.00 ± 0.65**	**12.88 ± 0.96**	**10.04 ± 0.23**	**9.90 ± 0.67**
16:1	1.08 ± 0.14	1.07 ± 0.13	0.94 ± 0.06	1.06 ± 0.07	0.83 ± 0.13	0.89 ± 0.12	1.12 ± 0.09^{b}	1.04 ± 0.05
18:0	**28.68 ± 0.43**	**31.33 ± 0.41**^{a}	**28.07 ± 0.51**	**28.47 ± 0.68**^{b}	**28.83 ± 0.70**	**31.87 ± 0.56**^{a}	**27.89 ± 0.36**	**27.88 ± 0.72**^{b}
18:1n-9	6.94 ± 0.44	5.77 ± 0.36	5.93 ± 0.16	6.16 ± 0.23	6.33 ± 0.37	5.13 ± 0.26	6.27 ± 0.18	6.28 ± 0.31^{b}
18:1n-7	2.81 ± 0.11	2.47 ± 0.14	3.71 ± 0.09^{b}	3.89 ± 0.18^{b}	2.91 ± 0.20	2.48 ± 0.26	3.93 ± 0.26^{b}	3.89 ± 0.21^{b}
18:2n-6	**11.70 ± 0.60**	**6.76 ± 0.68**^{a}	**11.63 ± 0.44**	**11.56 ± 0.68**^{b}	**12.16 ± 0.98**	**6.89 ± 0.92**^{a}	**11.41 ± 0.59**	**11.72 ± 1.37**
20:3n-6	0.40 ± 0.02	0.33 ± 0.03	0.28 ± 0.01^{b}	0.30 ± 0.01	0.42 ± 0.03	0.32 ± 0.04	0.28 ± 0.01^{b}	0.31 ± 0.02
20:4n-6	**25.97 ± 0.42**	**23.68 ± 1.07**	**27.79 ± 0.58**	**27.00 ± 0.71**	**25.29 ± 0.62**	**22.71 ± 1.29**	**25.08 ± 0.63**	**25.88 ± 0.98**^{b}
20:5n-3	0.10 ± 0.05	0.09 ± 0.04	0.12 ± 0.03	0.29 ± 0.17	0.10 ± 0.03	0.09 ± 0.03	0.25 ± 0.11	0.09 ± 0.04
22:5n-3	**2.21 ± 0.17**	**3.29 ± 0.16**^{a}	**2.14 ± 0.05**	**2.26 ± 0.14**^{b}	**2.51 ± 0.19**	**3.40 ± 0.14**^{a}	**2.29 ± 0.12**	**2.49 ± 0.26**
22:6n-3	**7.77 ± 0.67**	**11.95 ± 1.07**^{a}	**9.56 ± 0.56**^{b}	**9.08 ± 0.54**^{b}	**8.84 ± 0.80**	**12.56 ± 0.93**^{a}	**10.41 ± 0.6**	**10.01 ± 1.02**
SFA	41.00 ± 0.65	44.57 ± 0.05^{a}	37.86 ± 0.46^{b}	38.36 ± 0.87^{b}	40.60 ± 1.13	45.49 ± 1.56	38.92 ± 0.69	38.25 ± 1.00^{b}
MUFA	10.83 ± 0.61	9.30 ± 0.51	10.59 ± 0.25	11.11 ± 0.39^{b}	10.07 ± 0.44	8.50 ± 0.32	11.32 ± 0.32	11.20 ± 0.48^{b}
n-6	**38.06 ± 0.73**	**30.77 ± 1.45**^{a}	**39.70 ± 0.98**	**38.86 ± 1.12**^{b}	**37.86** ± 1.49	**29.92 ± 2.17**^{a}	**36.77 ± 0.91**	**37.91 ± 1.53**^{b}
n-3	**10.08 ± 0.83**	**15.33 ± 1.12**^{a}	**11.82 ± 0.61**^{b}	**11.64 ± 0.63**^{b}	**11.44 ± 0.96**	**16.05 ± 0.91**^{a}	**12.95 ± 0.65**	**12.59 ± 1.21**
SFA/UFA	0.70 ± 0.02	0.81 ± 0.03^{a}	0.61 ± 0.01^{b}	0.62 ± 0.02^{b}	0.69 ± 0.03	0.84 ± 0.06	0.64 ± 0.02	0.62 ± 0.03^{b}
n-6/n-3	**3.93 ± 0.39**	**2.09 ± 0.23**^{a}	**3.42 ± 0.23**	**3.41 ± 0.28**^{b}	**3.47 ± 0.41**	**1.92 ± 0.22**^{a}	**2.89 ± 0.19**	**3.20 ± 0.51**
20:4/18:2	2.25 ± 0.12	3.63 ± 0.30^{a}	2.40 ± 0.06	2.37 ± 0.13^{b}	2.13 ± 0.13	3.51 ± 0.33^{a}	2.22 ± 0.11	2.35 ± 0.34
UI	197.48 ± 3.50	207.14 ± 4.92	214.55 ± 0.81^{b}	210.40 ± 2.24	202.82 ± 4.20	206.89 ± 5.04	210.44 ± 3.14	212.08 ± 5.67

For symbols, see Table III.

TABLE V

Fatty acid composition (in mole %) of diphosphatidylglycerol in left and right ventricles of rats adapted to intermittent high-altitude hypoxia and rats recovering from hypoxia in comparison with corresponding age-matched controls

FA composition	Right ventricle				Left ventricle			
	control	hypoxic	control recovery I	hypoxic recovery I	control	hypoxic	control recovery I	hypoxic recovery I
14:0	0.78 ± 0.10	0.91 ± 0.10	0.63 ± 0.08	0.48 ± 0.05[b]	0.58 ± 0.06	0.66 ± 0.09	0.67 ± 0.08	0.59 ± 0.07
16:0	**6.40 ± 1.01**	**8.41 ± 1.59**	**2.87± 0.29**[b]	**2.57 ± 0.31**[b]	**5.05 ± 1.02**	**4.84 ± 0.41**	**2.97 ± 0.15**	**3.04 ± 0.29**[b]
16:1	2.45 ± 0.21	2.61 ± 0.19	2.13 ± 0.29	1.85 ± 0.06[b]	1.74 ± 0.22	2.31 ± 0.33	2.34 ± 0.29	2.37 ± 0.14
18:0	**5.61 ± 1.31**	**7.33 ± 0.89**	**1.87 ± 0.18**[b]	**2.11 ± 0.27**[b]	**5.35 ± 0.78**	**4.25 ± 0.86**	**2.52 ± 0.25**[b]	**2.42 ± 0.29**
18:1n-9	5.31 ± 0.83	5.65 ± 0.74	3.11 ± 0.23[b]	3.17 ± 0.28	4.24 ± 0.32	4.01 ± 0.36	2.89 ± 0.22[b]	3.09 ± 0.16
18:1n-7	3.23 ± 0.21	3.80 ± 0.25	2.29 ± 0.14[b]	2.24 ± 0.13[b]	3.85 ± 0.38	4.10 ± 0.35	2.45 ± 0.13[b]	2.61 ± 0.13[b]
18:2n-6	**70.17 ± 2.57**	**62.69 ± 3.27**	**83.67 ± 0.87**[b]	**83.29 ± 0.97**[b]	**71.27 ± 1.61**	**70.91 ± 1.82**	**80.85 ± 0.63**[b]	**80.11 ± 0.82**[b]
20:3n-6	1.16 ± 0.10	1.52 ± 0.07	0.62 ± 0.05[b]	0.62 ± 0.07[b]	1.33 ± 0.19	1.78 ± 0.08[a]	0.63 ± 0.04[b]	0.67 ± 0.05[b]
20:4n-6	**2.01 ± 0.29**	**2.88 ± 0.53**	**1.11 ± 0.07**[b]	**1.44 ± 0.28**[b]	**3.49 ± 0.58**	**2.82 ± 0.19**	**2.22 ± 0.23**	**2.73 ± 0.58**
20:5n-3	ND	ND	ND	ND	ND	ND	ND	ND
22:5n-3	**0.63 ± 0.06**	**0.86 ± 0.04**	**0.52 ± 0.07**	**0.44 ± 0.03**[b]	**0.78 ± 0.09**	**0.66 ± 0.05**	**0.49 ± 0.03**	**0.41 ± 0.07**
22:6n-3	**2.33 ± 0.21**	**3.33 ± 0.36**[a]	**1.66 ± 0.09**[b]	**1.78 ± 0.14**[b]	**2.32 ± 0.18**	**3.07 ± 0.22**	**1.86 ± 0.24**	**1.83 ± 0.25**[b]
SFA	12.79 ± 2.16	16.64 ± 2.49	5.37 ± 0.53[b]	5.16 ± 0.55[b]	10.98 ± 1.62	9.75 ± 1.22	6.16 ± 0.42	6.05 ± 0.49[b]
MUFA	11.00 ± 0.85	12.06 ± 0.93	7.53 ± 0.53[b]	7.26 ± 0.30[b]	9.82 ± 0.54	10.41 ± 0.53	7.67 ± 0.39[b]	8.07 ± 0.19[b]
n-6	**73.34 ± 2.65**	**67.09 ± 3.44**	**85.40 ± 0.90**[b]	**85.35 ± 0.81**[b]	**76.09 ± 1.46**	**75.50 ± 1.68**	**83.69 ± 0.79**[b]	**83.51 ± 0.49**[b]
n-3	**2.96 ± 0.23**	**4.19 ± 0.39**[a]	**2.18 ± 0.11**[b]	**2.22 ± 0.15**[b]	**3.10 ± 0.21**	**3.73 ± 0.23**	**2.35 ± 0.24**	**2.24 ± 0.30**[b]
SFA/UFA	0.15 ± 0.03	0.21 ± 0.04	0.06 ± 0.01[b]	0.05 ± 0.01[b]	0.13 ± 0.02	0.11 ± 0.02	0.07 ± 0.01	0.06 ± 0.01[b]
n-6/n-3	**25.83 ± 2.76**	**16.78 ± 1.72**[a]	**39.66 ± 2.31**[b]	**39.46 ± 2.95**[b]	**24.97 ± 1.18**	**20.66 ± 1.48**	**37.67 ± 4.35**[b]	**39.92 ± 4.23**[b]
20:4/18:2	0.03 ± 0.01	0.05 ± 0.01	0.01 ± 0.01[b]	0.02 ± 0.01[b]	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01[b]	0.03 ± 0.01
UI	179.98 ± 4.56	177.8 ± 5.84	193.73 ± 1.23[b]	194.34 ± 1.28[b]	188.14 ± 3.85	190.58 ± 2.94	193.76 ± 2.03	194.25 ± 2.66

For symbols, see Table III. ND, not determined.

The adaptation to IHA hypoxia led to an increase in 18:0 and substantial decrease in 18:2n-6 proportions, which were compensated by an increase in 20:4n-6, 22:5n-3 and 22:6n-3 FA proportions in both PC and PE. These changes decreased the n-6/n-3 PUFA ratio and increased the 20:4n-6/18:2n-6 PUFA ratio. In DPG, IHA hypoxia caused a significant decrease in the n-6/n-3 PUFA ratio due to the increase in 22:6n-3 FA in the RV with a similar trend in LV. No left-right ventricle difference in FA composition of PC, PE and DPG was found in any experimental group of rats. A similar effect in FA remodelling of phospholipids was also observed in hearts of adult rats that were exposed to IHA hypoxia under equal conditions by Ježková et al.[12]

The decrease in the n-6/n-3 PUFA and the increase in the 20:4n-6/18:2n-6 PUFA ratio are the main features in remodelling of the FA proportion in heart PL of young rats induced by IHA hypoxia. During long-term exposure to hypoxia, numerous metabolic pathways can participate in the remodelling of FA composition in membrane phospholipids; deacylation-reacylation cycle where phospholipases A_2 and acyltransferases cooperate, desaturation-elongation processes and enzymes of phospholipid "de novo" synthesis belongs to those reactions[24]. The increase in the 20:4n-6/18:2n-6 ratio might be caused by activation of desaturation-elongation pathway of linoleic acid. The presence of both Δ-6 desaturase and elongase in cardiac myocytes was published[25]. In this study we have shown that the decreased content of 18:2n-6 was compensated by elevation of 22:6n-3, which is a poorer substrate for phospholipase A_2 than n-6 PUFA are[26]. Moreover, acyl-CoA synthetase with preferential affinity to 22:6n-3 was found in cardiac tissue[27]. Kawaguchi et al. reported[28] that hypoxia led to phospholipid breakdown due to the activation of phospholipase A_2. We speculate that increased oxidative stress could play an important role in the process of membrane phospholipid remodelling observed in chronically hypoxic heart. Chronic hypoxia is associated with increased oxidative stress as was evidenced by marked lipid peroxidation[29]. It was shown that phospholipase A_2 preferentially hydrolyses peroxid fatty acid esters in phospholipids thus protecting membranes from oxidative injury[30].

The most important observation in our study was the increase in proportion of n-3 PUFA to the detriment of n-6 PUFA following adaptation to hypoxia. Phospholipids, representing structural component of membranes, create fluid environment for membrane receptors, transporters and enzymes. Moreover, they also serve as a source of the signal molecules participating in signal transduction pathways, e.g., phosphoinositides, diacylglycerols (DAGs), free fatty acids, eicosanoids etc.[31] There are many pieces

of evidence that the changes in FA composition of cardiac membranes lead to alterations of lipid signal molecules and thus to functional changes. Dietary n-3 PUFA are lipids with very potent cardioprotective effect[13]. The beneficial role of n-3 PUFA in increased resistance of heart tissue to ischemia-reperfusion injury has been supported by numerous studies showing that dietary supplementation with fish oils increased the content of n-3 PUFA in rat heart and attenuated the incidence of life-threatening arrhytmias and myocardial infarction[32]. The prevention of sudden cardiac death by n-3 PUFA was verified by the numerous clinical trials[33]. On the other hand, independently on diet, some stress conditions such as high doses of catecholamines[34], hyperthyroidism[20] or pressure overload[35] increased n-3 PUFA content in heart membrane phospholipids. The mechanism of this fatty acid remodelling induced by stress is not clear yet. However, it was demonstrated that chronic high altitude hypoxia increases the tolerance of the heart to all major endpoints of acute ischemia-reperfusion injury[36]. Among many factors involved in this mechanism the activation of mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channels has been proposed[4]. Activity of these channels is regulated by protein kinase C[37] (PKC) which is activated by DAGs, the products of phospholipid breakdown. It has been reported that the activation of PKC in cardiomyocytes is dependent on certain DAG species[38].

The functional significance of changes in the level of n-3 and n-6 PUFA of phospholipids induced by chronic hypoxia may relate to the tolerance of heart to stress conditions. The effect of PUFA may be beneficial or detrimental depending on their proportion and antioxidative capacity of heart tissue[13,34].

CONCLUSIONS

The most important effect of adaptation of heart to chronic hypoxia observed in our study was the increase in proportion of n-3 PUFA to the detriment of n-6 PUFA in phospholipids. The observed remodeling of membrane phospholipids could have several consequences: changes in membrane fluidity, susceptibility to oxidative stress, quality of eicosanoids, quality of lipid signal molecules, relating activation of protein kinase C isoforms and mitoK_{ATP} channels. At present, we do not know how important role the shift in PUFA proportion in phospholipids plays in stress tolerance. However, the beneficial influence of diet supplemented with fish oils enriched in n-3 PUFA was proved in many clinical studies, and antiarrhytmic effect of n-3 PUFA was well documented in experiments in vitro. We be-

lieve that n-3 PUFA shift observed in this study may participate in enhanced tolerance of chronically hypoxic heart to ischemic injury. However, it must be taken into account that the observed changes in FA composition were reversible within thirty days after recovery to normoxic conditions and thus could not contribute to later protective effect.

We conclude that changes in FA composition of membrane phospholipids suggest the presence of general adaptation reaction of heart tissue as an answer to stress stimuli. Besides, these changes may lead to a better preservation of membrane integrity and thereby contribute to improved ischemic tolerance of chronically hypoxic heart.

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