

# Pressure Overload Selectively Increases n-3 PUFA in Myocardial Phospholipids During Early Postnatal Period

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## Summary

Increasing hemodynamic load during early postnatal development leads to rapid growth of the left ventricular (LV) myocardium, which is associated with membrane phospholipid (PL) remodeling characterized by n-3 polyunsaturated fatty acids (PUFA) accumulation. The aim of this study was to examine the influence of additional workload imposed early after birth when ventricular myocytes are still able to proliferate. Male Wistar rats were subjected to abdominal aortic constriction (AC) at postnatal day 2. Concentrations of PL and their fatty acid (FA) profiles in the LV were analyzed in AC, sham-operated (SO) and intact animals on postnatal days 2 (intact only), 5 and 10. AC resulted in LV enlargement by 22 % and 67 % at days 5 and 10, respectively, compared with age-matched SO littermates. Concentrations of phosphatidylcholine, cardiolipin, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and sphingomyelin decreased in AC myocardium, albeit with different time course and extent. The main effect of AC on FA remodeling consisted in the accumulation of n-3 PUFA in PL. The most striking effect of AC on FA composition was observed in phosphatidylinositol and cardiolipin. We conclude that excess workload imposed by AC inhibited the normal postnatal increase of PL concentration while further potentiating the accumulation of n-3 PUFA as an adaptive response of the developing myocardium to accelerated growth.

## Key words

Phospholipids • n-3 PUFA • Myocardium • Postnatal development  
• Pressure overload • Rat

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## Introduction

In early postnatal life, the developing mammalian heart must cope with increasing hemodynamic load, which is associated with rapid growth of the left ventricle (LV). While this period is one of continuing mitotic proliferation (hyperplasia), in most mammals, ventricular myocytes lose their capability for division shortly after birth and further growth of the heart is considered to occur due to their hypertrophy along with the hyperplasia and hypertrophy of other cell types (Clubb and Bishop 1984). It has been shown that the switch from hyperplastic to hypertrophic growth of rat ventricular myocytes occurs as early as after the third postnatal day (Li *et al.* 1996). Rapid heart growth during the first postnatal week is accompanied by metabolic, morphological and functional remodeling of myocardial tissue (Girard *et al.* 1992, Ošťádal *et al.* 1999). Membrane structures are among the main targets of this developmental process, including both their protein (Novotný *et al.* 1999, Hamplová *et al.* 2003) and lipid components (Novák *et al.* 2006). As the main membrane lipids, phospholipids (PL) create the environment for membrane proteins. The functions of these proteins depend on the proportions of individual PL species and/or on their fatty acid (FA) compositions.

Numerous experimental results obtained mostly from isolated cardiomyocytes indicate that hydrolytic

products derived from membrane PL by the action of phospholipases play an important role as second messengers in signal transduction involved in stimulating cell growth and proliferation (Dorn and Force 2005, Tappia 2007). In view of these observations, the remodeling of membrane PL induced by pathological load superimposed on physiological growth of the cardiomyocytes during early ontogeny may influence the postnatal structural and functional characteristics of the developing heart. In this study, we used a model of pressure overload induced by aortic constriction (AC) in neonatal rats at postnatal day 2 as described earlier (Kolář *et al.* 1998). The concentration of individual PL species and their FA profiles were analyzed at days 5 and 10. This model allowed us to follow the adaptive response of membrane lipid components in myocardium which was still in the proliferative phase of ventricular myocyte growth (Clubb and Bishop 1984, Sedmera *et al.* 2003).

## Methods

Newborn male Wistar rats were obtained from the animal care facility of the Institute of Physiology in Prague, Czech Republic. Their number per litter was kept at eight throughout the weaning period. Nursing mothers were fed a vitamin-enriched, low-fat (3.5 % by weight) standard diet (Velaz, Czech Republic). The diet contained the following FA (in mol %), 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. Pressure overload was induced by constriction of the abdominal aorta (AC) in randomly selected animals in each litter. This intervention was performed in 2-day-old rats under light ether anesthesia as described earlier (Kolář *et al.* 1998). The abdominal cavity was opened by a small incision from the left dorsolateral side, and the aorta was exposed in the subdiaphragmatic suprarenal region. A silk ligature was tied around the aorta and a hypodermic needle of 0.25 mm outer diameter. The needle served as a template, and after its subsequent removal the aorta remained constricted to the diameter of the template. The incision was sutured and smeared with 1 % iodocollodium. Two types of controls were used for this study: intact littermates (intact) and sham-operated pups (SO) of the same age for which the identical procedure was carried out but without placement of the ligature. The animals were sacrificed by decapitation on days 2

(intact only), 5 and 10 (i.e., 3 and 8 days after the surgery). The study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). The experimental protocol was approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

The hearts were dissected free of atrial tissue and large blood vessels and the right ventricular (RV) free wall and septum were separated from the LV. All parts were rinsed in cold (5 °C) saline, weighed separately, and the LV was frozen in liquid nitrogen and stored there until use. Frozen LV myocardium was pulverized and homogenized. PL were extracted in three consecutive steps according to the method of Folch *et al.* (1957). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipin (CL), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM) were separated by two-dimensional thin-layer chromatography on plates with Silica Gel H (0.5 mm), the individual PL spots were detected by iodine vapor, then scraped out and analyzed for phosphorus (Rouser *et al.* 1970). For FA analysis, the spots of individual PL were observed under UV light after staining with 0.005 % 2,7-dichlorofluorescein in methanol, scraped out and stored in nitrogen atmosphere at -20 °C until the next day, at which time methyl esters were prepared. For FA methyl ester preparation, sodium methanolate was added to tubes with silica gel and tubes were then incubated for 60 min at room temperature in darkness. Methyl esters were extracted with hexane; the extracts were evaporated under a stream of nitrogen and stored at -20 °C. FA methyl esters were separated by CP 438 A gas chromatograph (Chrompack, Middelburg, The Netherlands) using a CP WAX 52 CB middle polar column (25 m × 0.25 mm i.d.). The oven temperature was programmed from 145 to 230 °C at 2 °C/min. Hydrogen was used as carrier gas. FA were identified using a mixture of FA methyl ester standards (Tvrzická *et al.* 2002). All chemicals were purchased from Sigma unless otherwise indicated.

## Statistical analysis

All results are expressed as means ± S.E.M. The statistical significance of differences between groups was determined by one-way ANOVA and subsequent Newman-Keuls test. Differences were considered significant when  $P < 0.05$ .

**Table 1.** Effects of aortic constriction on body and heart weight parameters.

	Day 2		Day 5		Day 10		
	Intact	Intact	SO	AC	Intact	SO	AC
<i>BW (g)</i>	6.08 ± 0.15	13.30 ± 0.35	12.60 ± 0.36	12.80 ± 0.31	24.50 ± 0.70	24.90 ± 0.54	24.30 ± 0.83
<i>HW (mg)</i>	37.30 ± 0.87	64.30 ± 1.27	59.20 ± 1.30 <sup>†</sup>	69.10 ± 1.92 <sup>‡</sup>	99.10 ± 2.31	99.60 ± 2.53	160.90 ± 16.4 <sup>‡§</sup>
<i>LV (mg)</i>	19.90 ± 0.59	32.30 ± 0.90	29.10 ± 1.00 <sup>†</sup>	36.30 ± 1.24 <sup>‡</sup>	54.20 ± 1.59	53.30 ± 1.54	95.80 ± 9.98 <sup>‡§</sup>
<i>RV (mg)</i>	10.10 ± 0.29	16.60 ± 0.49	15.90 ± 0.46	16.30 ± 0.43	24.20 ± 0.71	24.20 ± 0.84	34.80 ± 3.47 <sup>‡§</sup>
<i>S (mg)</i>	7.30 ± 0.24	15.30 ± 0.45	14.30 ± 0.33 <sup>†</sup>	16.50 ± 0.72 <sup>‡</sup>	20.80 ± 0.74	22.10 ± 0.99	30.30 ± 3.14 <sup>‡§</sup>
<i>HW/BW (%)</i>	0.55 ± 0.01	0.49 ± 0.01	0.47 ± 0.01	0.54 ± 0.01 <sup>‡</sup>	0.41 ± 0.01	0.40 ± 0.01	0.60 ± 0.09 <sup>‡</sup>
<i>LV/BW (%)</i>	0.29 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.28 ± 0.01 <sup>‡</sup>	0.22 ± 0.01	0.21 ± 0.01	0.35 ± 0.05 <sup>‡§</sup>
<i>RV/BW (%)</i>	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.13 ± 0.02
<i>RV/LV</i>	0.53 ± 0.02	0.52 ± 0.02	0.56 ± 0.02	0.46 ± 0.02 <sup>‡</sup>	0.46 ± 0.02	0.46 ± 0.02	0.36 ± 0.01 <sup>‡§</sup>

SO – sham-operation; AC – aortic constriction; BW – body weight; HW – heart weight; LV – left ventricle; RV – right ventricle; S – septum. Values are mean ± S.E.M. from 20 experiments in each group. <sup>†</sup>P<0.05 vs. age-matched intact; <sup>‡</sup>P<0.05 vs. age-matched SO; <sup>§</sup>P<0.05 vs. AC at day 5.

## Results

### Weight parameters

Body weight was significantly altered by neither SO nor AC in any age group. SO caused moderate heart and LV growth retardation at day 5, but these effects waned by day 10. AC led to the LV's enlargement, as evident from 22 % increase in the LV/BW ratio by day 5 and 67 % increase by day 10 compared with age-matched SO littermates. While AC slightly increased RV weight, the RV/BW ratio was not significantly influenced (Table 1).

### Concentration of phospholipids

Table 2 presents the developmental changes in the concentration of individual PL species in LV of intact animals between days 2 and 10 and the effects caused by SO and AC. The normal physiological increment of total PL in intact pups was 17 % between days 2 and 5. During the period, PC and PE increased by 17 %, CL by 33 % and PI by 20 %. There was no further significant increase in the concentration of any PL between days 5 and 10. The concentrations of PS and SM remained at unchanged levels during the entirety of the investigated period. The effect of SO on PL concentration was observed only at day 10, when total PL concentration increased by 10 % compared with intact animals due to slight increases in all PL (significant only in the case of PE). AC decreased the concentration of total PL by 12 % and 14 % at days 5 and 10, respectively, compared with age-matched SO littermates. This effect was due to decreases of PC, PI and CL by 11 %, 16 % and 17 %, respectively, at day 5 and of PC, PE, CL, PS and SM by 12 %, 15 %, 25 %, 24 % and

17 %, respectively, at day 10. No significant difference in the individual PL concentrations was observed between days 5 and 10, except for increase of PI by 11 % and decrease of PS by 18 % at day 10.

### Fatty acid composition of phospholipids

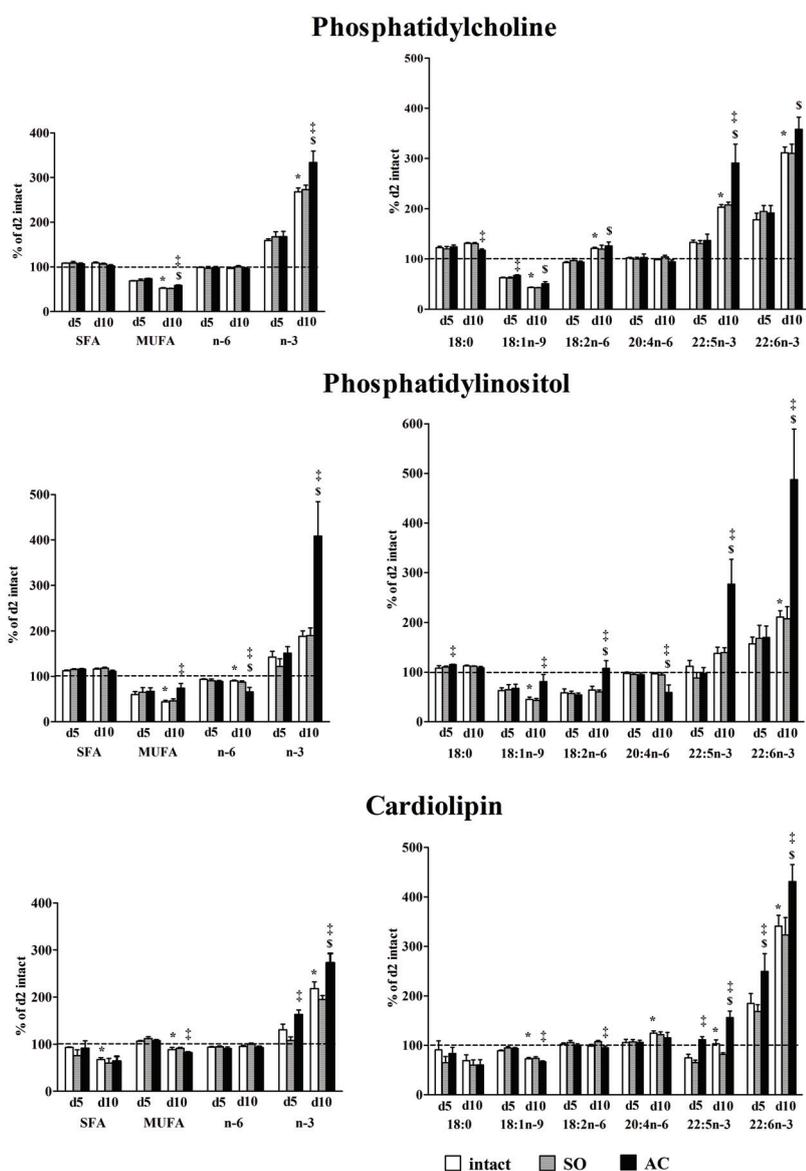
Figure 1 shows the proportions of FA classes in myocardial PL of intact, SO and AC animals. Saturated FA (SFA), monounsaturated FA (MUFA), n-6 and n-3 polyunsaturated FA (PUFA) in PC, PI, and CL at postnatal days 5 and 10 are expressed as the percentage of values for the intact 2-day-old group, which are presented in Table 3. FA that were mainly responsible for the changes induced by AC in PL are also shown in Figure 1. In intact hearts, the n-3 PUFA proportion in PC and CL gradually increased while that of MUFA declined. The increase of n-3 PUFA in PI was accompanied by the decrease in both MUFA and n-6 PUFA.

AC slightly increased the n-3 PUFA and MUFA proportions at day 10 in PC. The most pronounced effect of AC was observed in PI where the proportions of MUFA and n-3 PUFA increased while that of n-6 PUFA declined. In PI, docosapentaenoic acid (22:5n-3, DPA) and docosahexaenoic acid (22:6n-3, DHA) were responsible for the elevation of n-3 PUFA, oleic acid (18:1n-9, OA) was the main contributor to the increase of MUFA, and the decrease of n-6 PUFA was caused by arachidonic acid (20:4n-6, AA). Concerning the effect of AC on CL, the increase in n-3 PUFA was present already at day 5 and more pronounced at day 10, offset by a decrease in MUFA. The increases of 22:5n-3 and 22:6n-3 and the decreases of 18:1n-9 and linoleic acid (18:2n-6, LA) were mainly responsible for these effects.

**Table 2.** Effect of aortic constriction on myocardial concentration of phospholipids ( $\mu\text{mol P.g}^{-1}$  wet weight).

	Day 2		Day 5		Day 10		
	Intact	Intact	SO	AC	Intact	SO	AC
PC	8.47 ± 0.30	9.92 ± 0.14	10.56 ± 0.40	9.37 ± 0.30 <sup>‡</sup>	10.16 ± 0.38	11.03 ± 0.29	9.70 ± 0.37 <sup>‡</sup>
PE	6.07 ± 0.18	7.12 ± 0.15	7.51 ± 0.20	6.85 ± 0.21	7.45 ± 0.36	8.25 ± 0.11 <sup>†</sup>	7.04 ± 0.13 <sup>‡</sup>
CL	1.22 ± 0.09	1.62 ± 0.07	1.86 ± 0.11	1.54 ± 0.07 <sup>‡</sup>	1.82 ± 0.08	2.02 ± 0.10	1.52 ± 0.09 <sup>‡</sup>
PI	1.06 ± 0.03	1.27 ± 0.03	1.39 ± 0.03	1.17 ± 0.03 <sup>‡</sup>	1.18 ± 0.05	1.32 ± 0.04	1.30 ± 0.02 <sup>S</sup>
PS	0.89 ± 0.02	0.94 ± 0.02	1.03 ± 0.05	0.90 ± 0.04	0.90 ± 0.07	0.97 ± 0.05	0.74 ± 0.04 <sup>‡S</sup>
SM	0.80 ± 0.03	0.83 ± 0.03	0.83 ± 0.05	0.74 ± 0.04	0.86 ± 0.04	0.94 ± 0.02	0.78 ± 0.04 <sup>‡</sup>
$\Sigma\text{PL}$	18.57 ± 0.31	21.77 ± 0.34	23.23 ± 0.70	20.53 ± 0.66 <sup>‡</sup>	22.43 ± 0.71	24.57 ± 0.34 <sup>†</sup>	21.12 ± 0.56 <sup>‡</sup>

SO – sham-operation; AC – aortic constriction; PL – phospholipids; PC – phosphatidylcholine; PE – phosphatidylethanolamine; CL – cardiolipin; PI – phosphatidylinositol; PS – phosphatidylserine; SM – sphingomyelin. Values are mean ± S.E.M. from 6 experiments in each group. <sup>†</sup> P<0.05 vs. age-matched intact; <sup>‡</sup> P<0.05 vs. age-matched SO; <sup>S</sup> P<0.05 vs. AC at day 5.



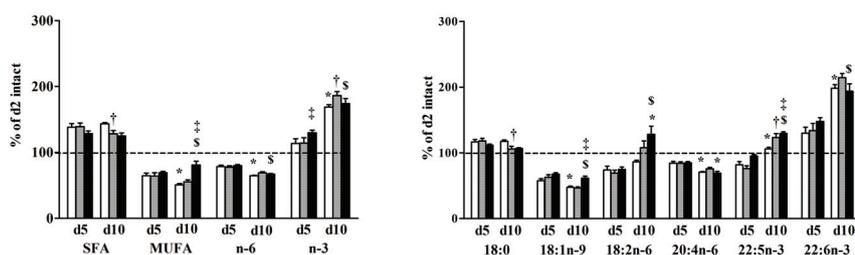
**Fig. 1.** Proportions of fatty acid (FA) classes and main FA in selected phospholipids of the left ventricle of intact, sham-operated (SO) and aorta-constricted (AC) rats at postnatal days 5 and 10 (i.e., 3 and 8 days after AC), expressed as percentages of values for 2-day-old intact controls. SFA – sum of saturated FA; MUFA – sum of monounsaturated FA; n-6 – sum of n-6 polyunsaturated FA (PUFA); n-3 – sum of n-3 PUFA; 18:0 – stearic acid; 18:1n-9 – oleic acid; 18:2n-6 – linoleic acid; 20:4n-6 – arachidonic acid; 22:5n-3 – docosapentaenoic acid; 22:6n-3 – docosahexaenoic acid. Values are means ± S.E.M. from 6 experiments in each group. <sup>\*</sup> P<0.05 vs. intact at day 5; <sup>‡</sup> P<0.05 vs. age-matched SO; <sup>S</sup> P<0.05 vs. AC at day 5. Shown are only those FA that were mainly responsible for AC-induced effects.

**Table 3.** Fatty acid (FA) composition (mol %) of myocardial phospholipids in 2-day-old intact rats.

FA	PC	PE	CL	PI	PS
<i>SFA</i>	45.78 ± 2.12	30.55 ± 0.99	9.00 ± 1.12	39.54 ± 0.60	52.34 ± 5.80
<i>18:0</i>	18.10 ± 0.66	23.51 ± 0.27	3.71 ± 0.66	34.16 ± 1.18	45.66 ± 5.20
<i>MUFA</i>	17.76 ± 0.45	8.73 ± 0.39	33.25 ± 0.87	9.88 ± 0.60	10.27 ± 1.01
<i>18:1n-9</i>	11.26 ± 0.32	5.83 ± 0.24	20.15 ± 0.42	6.50 ± 0.34	8.03 ± 1.60
<i>n-6</i>	33.15 ± 1.83	48.63 ± 0.96	50.89 ± 1.26	46.24 ± 0.67	22.28 ± 2.30
<i>18:2n-6</i>	3.17 ± 0.13	1.63 ± 0.13	29.59 ± 1.11	2.37 ± 0.14	1.85 ± 0.31
<i>20:4n-6</i>	26.95 ± 1.41	38.14 ± 0.78	10.41 ± 0.73	38.97 ± 1.19	9.21 ± 1.38
<i>n-3</i>	3.31 ± 0.48	12.08 ± 0.80	6.85 ± 0.57	4.40 ± 0.50	14.82 ± 1.99
<i>22:5n-3</i>	1.33 ± 0.19	3.99 ± 0.44	2.47 ± 0.47	1.88 ± 0.17	4.92 ± 0.81
<i>22:6n-3</i>	1.86 ± 0.34	7.87 ± 0.45	3.31 ± 0.17	2.53 ± 0.37	9.15 ± 1.18

SO – sham-operation; AC – aortic constriction; PC – phosphatidylcholine; PE – phosphatidylethanolamine; CL – cardiolipin; PI – phosphatidylinositol; PS – phosphatidylserine; SFA – sum of saturated FA; MUFA – sum of monounsaturated FA; n-6 – sum of n-6 polyunsaturated FA (PUFA); n-3 – sum of n-3 PUFA; 18:0 – stearic acid; 18:1n-9 – oleic acid; 18:2n-6 – linoleic acid; 20:4n-6 – arachidonic acid; 22:5n-3 – docosapentaenoic acid; 22:6n-3 – docosahexaenoic acid. Values are mean ± S.E.M. from 6 experiments in each group. Shown are only those FA that were mainly responsible for AC-induced effects.

### Phosphatidylethanolamine



**Fig. 2.** Proportions of fatty acid (FA) classes and main FA in aminophospholipids of left ventricle of intact, sham-operated (SO) and aorta-constricted (AC) rats at postnatal days 5 and 10 (i.e., 3 and 8 days after AC), expressed as a percentage of 2-day-old intact controls. † P<0.05 vs. age-matched intact. See Figure 1 for further legend.

### Phosphatidylserine

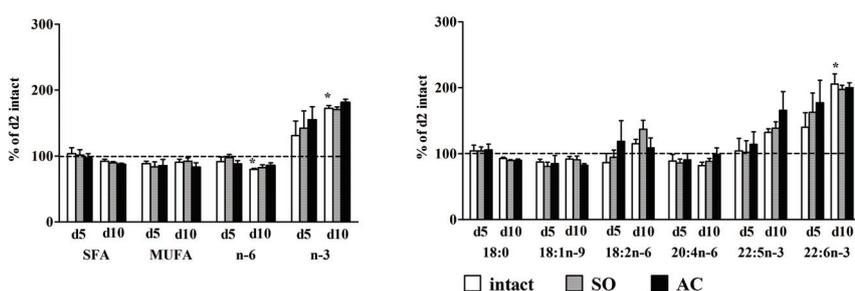


Figure 2 shows the relative proportions of FA classes in myocardial amino-phospholipids (PE and PS) in intact, SO and AC groups. Gradual developmental increase in n-3 PUFA in PE was accompanied by decrease of both MUFA and n-6 PUFA. Slight increase in n-3 PUFA and decrease in n-6 PUFA were observed in PS at day 10. PE was the only PL affected by SO, resulting in a slight increase in n-3 PUFA and decrease in

SFA at day 10. Concerning the effects of AC, increase in n-3 PUFA (due to 22:5n-3) was seen already at day 5, waning at day 10, whereas a decreased proportion of MUFA was maintained until day 10. No effect of AC on FA composition was observed in PS.

Changes in the n-6/n-3 PUFA, 20:4n-6/18:2n-6 and 20:4n-6/22:6n-3 ratios induced by AC are shown in Table 4. AC did not affect these ratios in any PL at day 5,

**Table 4.** Effects of aortic constriction on 20:4/18:2, 20:4/22:6 and n-6/n-3 PUFA ratios in myocardial phospholipids.

	Day 2		Day 5		Day 10		
	Intact	Intact	SO	AC	Intact	SO	AC
<i>PC</i>							
20:4/18:2	8.50±0.51	9.44±0.68	8.75±0.45	9.31±0.53	6.92±0.15*	7.52±0.55	6.47±0.52
20:4/22:6	14.49±0.84	8.29±0.49	7.42±0.93	7.77±0.63	4.61±0.12*	4.92±0.32	3.86±0.23 <sup>‡§</sup>
n-6/n-3	10.02±1.25	6.12±0.53	5.78±0.49	5.86±0.61	3.62±0.07*	3.69±0.15	2.91±0.17 <sup>‡§</sup>
<i>PE</i>							
20:4/18:2	23.40±3.51	26.77±2.65	28.35±2.82	26.59±1.90	19.26±0.62*	17.04±1.37	13.5±1.97 <sup>§</sup>
20:4/22:6	4.85±0.41	3.16±0.18	3.04±0.21	2.79±0.17	1.73±0.05*	1.72±0.07	1.75±0.11
n-6/n-3	4.03±0.38	2.78±0.16	2.74±0.18	2.48±0.15	1.54±0.04*	1.49±0.06	1.56±0.56 <sup>§</sup>
<i>PS</i>							
20:4/18:2	4.98±0.51	5.11±0.65	4.53±0.82	3.80±0.90	3.54±0.62	3.20±0.37	4.52±0.97
20:4/22:6	1.01±0.41	0.64±0.18	0.53±0.21	0.51±0.17	0.40±0.05	0.45±0.07	0.50±0.11
n-6/n-3	1.50±0.18	0.82±0.16	0.82±0.18	0.66±0.15	0.69±0.04	0.73±0.06	0.71±0.06
<i>PI</i>							
20:4/18:2	16.44±0.56	27.54±0.68	27.50±0.65	28.87±0.53	26.18±2.87	26.56±2.70	10.06±3.08 <sup>‡§</sup>
20:4/22:6	15.40±0.87	9.55±0.49	8.74±0.53	8.59±0.63	7.19±0.58	7.47±1.03	2.59±1.07 <sup>‡§</sup>
n-6/n-3	10.51±0.81	6.89±0.50	6.73±0.49	6.17±0.61	5.12±0.41	5.01±0.56	2.11±0.67 <sup>‡§</sup>
<i>CL</i>							
20:4/18:2	0.35±0.03	0.36±0.04	0.33±0.05	0.38±0.06	0.45±0.07*	0.40±0.04	0.43±0.05
20:4/22:6	3.15±0.34	1.80±0.22	1.80±0.21	1.34±0.13	1.15±0.11*	1.18±0.09	0.85±0.08 <sup>‡§</sup>
n-6/n-3	7.43±0.45	5.32±0.53	6.49±0.49	4.14±0.31 <sup>‡</sup>	3.24±0.14*	3.80±0.30	2.58±0.24 <sup>‡§</sup>

SO – sham-operation; AC – aortic constriction; PC – phosphatidylcholine; PE – phosphatidylethanolamine; CL – cardiolipin; PI – phosphatidylinositol; PS – phosphatidylserine; n-6 – sum of n-6 polyunsaturated fatty acids (PUFA); n-3 – sum of n-3 PUFA; 18:2n-6 – linoleic acid; 20:4n-6 – arachidonic acid; 22:6n-3 – docosahexaenoic acid. Values are mean ± S.E.M.

except for decline in the n-6/n-3 PUFA ratio in CL. Significant decrease of n-6/n-3 PUFA and 20:4n-6/22:6n-3 ratios occurred after AC at day 10 in PC, PI and CL. A decreased 20:4n-6/18:2n-6 ratio due to AC was observed also in PI at day 10. Unlike these PL, PS was resistant to AC-induced changes.

## Discussion

The major finding of the present study is that LV enlargement due to the excess workload imposed by AC early after birth was accompanied by decrease of membrane PL concentration compared with that in SO littermates. Moreover, AC further potentiated the normal developmental accumulation of n-3 PUFA in PL. Importantly, the individual PL FA profiles were selectively affected by AC.

Concerning the physiological development of PL composition, we confirmed our previous observation of marked increase in total PL concentration during the

early postnatal period (by 32 % between days 2 and 10). This suggests rapid proliferation of membrane structures – predominantly mitochondrial – as indicated by 49 % increase in CL (Novák *et al.* 2006). Here, we show that the normal postnatal development of membranes, reflected by the increasing PL concentration, was inhibited by pressure overload selectively for each PL species. While the developmental increments of PC, PI and CL were attenuated by AC already at day 5, the decline of PE, PS and SM was observed only at day 10. The 31 % decrease of CL between days 2 and 5 suggests that mitochondria were the main membrane structures affected by AC just in the most critical and stressful period characterized by rapid growth of the LV due to increasing hemodynamic load (Ošťádal *et al.* 1999). Impaired development of mitochondria in neonatal AC hearts had been previously documented by quantitative electron microscopy (Page and McCallister 1973).

After AC, the PI concentration declined at day 5, but, in contrast with other PL, it returned to the control

level at day 10. This suggests a difference in regulation of the PI biosynthesis/degradation process that probably is due to its specific role in signal transduction pathways. PI is the most abundant phosphoinositide of the ventricular myocardium. It serves as a precursor for more phosphorylated phosphoinositides, generating inositol phosphate and diacylglycerols after phospholipase C activation. PC also plays an important role in signaling as a precursor of diacylglycerols and AA *via* the action of phospholipases D and A2, respectively. In these pathways, diacylglycerols activate protein kinase C, a signaling molecule activating the expression of genes that are upregulated during myocardial hypertrophy (Dorn and Force 2005, Tappia 2007). Changes in PI are in agreement with our recent observation of a transient upregulation of PKC $\alpha$ , PKC $\delta$  and PKC $\epsilon$  isoforms between postnatal days 2 and 5 in the same model of accelerated cardiac growth induced by AC (Hamplová *et al.* 2010). Furthermore, under the same conditions, Oliviero *et al.* (2000) have shown the transient increase of serum angiotensin II level already 2 h after AC, followed by upregulation of both AT1 and AT2 receptors in the myocardium. Moreover, AT2 transcript levels closely correlated with the left ventricular mass in the AC group. It is well known that angiotensin II-mediated signal transduction is coupled with PL signaling molecules produced by stimulation of phospholipases (Dinh *et al.* 2001). The increase in angiotensin II level preceding the increase in its binding capacity and PKC activation/upregulation might suggest that this peptide is one of the triggers implicated in the phosphoinositides/PKC-mediated early response of neonatal myocardium to pressure overload. Nevertheless, other factors might also contribute to accelerated neonatal cardiac growth in response to pressure overload (Novák *et al.* 2009).

It should be mentioned that myocardium during this early developmental period responds to pressure overload differently than in the case of adult heart. When AC was performed at day 2, the increase of LV mass resulted first from the stimulated cellular hyperplasia followed later by enlargement of cardiomyocytes (Sedmera *et al.* 2003). In this experimental model, cardiomegaly was associated with capillary proliferation in proportion to the increase of ventricular mass and exhibited neither increased rates of apoptosis nor myocardial fibrosis characteristic for adult pressure-overloaded hearts (Kolář *et al.* 1998, Oliviero *et al.* 2000, Sedmera *et al.* 2003). In contrast to neonatal

myocardium, rats subjected to AC in adulthood exhibited no decrease in total PL concentration in LV (Reibel *et al.* 1986). Nevertheless, the proportions of PC and PI increased at the expense of CL due to AC. It should be taken into account that the biosynthesis of protein and PL components predominates over their degradation during membrane maturation in the early postnatal period, whereas both processes are more in a dynamic equilibrium in the adult heart under physiological conditions (Girard *et al.* 1992, Nováková *et al.* 1994).

As demonstrated in our recent developmental study, each of the individual PL species has a characteristic profile of FA acyl chains that changes from birth until adulthood. On the other hand, all major PL exhibit some common features such as relatively higher proportions of OA and AA and lower proportions of LA and DHA in newborn rats than in adults. Another feature characteristic of the early postnatal period is the substantially increasing proportion of n-3 PUFA in all PL between delivery and the suckling-weaning transition (Novák *et al.* 2006). Several stress factors play a role in these developmental changes of the cardiac PL FA profiles, including nutritional, metabolic and hormonal changes as well as the increasing hemodynamic load (for review, see Novák *et al.* 2009).

In this study, we show for the first time the effect of pressure overload imposed on the rat heart just after birth, indicating additional n-3 PUFA increase superimposed upon that induced by normal postnatal growth. It has been reported that adult rat hearts subjected to pressure overload exhibit an increased proportion of DHA mainly in PE, counterbalanced by decrease of LA. Concerning PI, the proportion of DHA remained unchanged (Reibel *et al.* 1986). In our AC model, however, it was only PI in which the accumulation of DHA and DPA was the most pronounced at the expense of AA. It appears, moreover, that the effect on FA profile is more pronounced and selective when AC is imposed early after birth than in adulthood (Reibel *et al.* 1986).

Increased accumulation of n-3 PUFA in myocardial PL as a general adaptive response to various stressful conditions has been shown in several studies, for instance in rats exposed to chronic hypoxia (Ježková *et al.* 2002, Novák *et al.* 2004) and in animals chronically treated with catecholamines (Benediktsdóttir *et al.* 1995, Gudbjarnason and Benediktsdóttir 1996) or thyroid hormones (Hamplová *et al.* 2003). Noteworthy is that maturation of the  $\beta$ -adrenergic system and the increasing adrenergic responsiveness of the developing heart are

under the control of thyroid hormones (Novotný *et al.* 1999). Hence, it seems conceivable that the hormonal changes mentioned above can contribute to the increased myocardial accumulation of n-3 PUFA associated with normal development and to the further potentiation of this effect by pressure overload.

In conclusion, accelerated cardiac growth stimulated by pressure overload imposed by AC soon after birth is not followed by proportional changes of membrane PL components. The attenuation of the PL increment after AC is accompanied by significant remodeling of the FA profile resulting in the selective accumulation of n-3 PUFA. These changes in the PL

composition of membranes may affect the quality of lipid signaling molecules and properties of membrane proteins under stress conditions.

### Conflict of Interest

There is no conflict of interest.

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