

Ontogenetic Development of Energy-Supplying Enzymes in Rat and Guinea-Pig Heart

A. BASS¹, M. STEJSKALOVÁ², A. STIEGLEROVÁ¹, B. OŠŤÁDAL¹,
M. ŠAMÁNEK²

¹Institute of Physiology, Academy of Sciences of the Czech Republic, ²Kardiocentrum, University Hospital Motol, Prague, Czech Republic

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Summary

The purpose of the present study was to compare the ontogenetic development of the activity of myocardial energy-supplying enzymes in two mammalian species, differing significantly in their level of maturation at birth. The animals were investigated during the late prenatal period and 2, 7, 14, 21, 25, 30, 63, 120 and 730 days after birth in the rat and 2, 21, 84 and 175 days in the guinea-pig. The following enzymes were assayed in the right and left ventricular myocardium: lactate dehydrogenase (LDH, lactate uptake and/or formation), triose phosphate dehydrogenase (TPDH, carbohydrate metabolism), glycerol phosphate dehydrogenase (GPDH, glycerol-P shuttle), hexokinase (HK, glucose phosphorylation), malate dehydrogenase (MDH, tricarboxylic cycle), citrate synthase (CS, tricarboxylic cycle) and hydroxyacyl-CoA dehydrogenase (HOADH, fatty acid breakdown). The rat heart, highly immature at birth, exhibits three different developmental patterns of energy-supplying enzymes, identical in both ventricles: (i) two mitochondrial enzymes of aerobic metabolism (CS, HOADH) and GPDH have a relatively low activity at the end of prenatal life; thereafter their activity steadily increases, approaching the adult levels between the 3rd and 4th postnatal weeks. A significant decrease was observed between the 4th and 24th months. (ii) MDH and LDH: prenatal values were significantly higher as compared with the 2nd postnatal day; after this period the activities increased up to adulthood (4 months) and decreased during senescence. (iii) The activities of HK and TPDH are characterized by only moderate changes during development. HK differs from all other enzymes by the highest prenatal values, which exceed even adult values. In contradiction to the rat heart, the developmental differences in more mature guinea-pig heart were significantly less pronounced. The only ontogenetic differences observed were the lower activities of enzymes connected with aerobic metabolism at the end of the prenatal period. Our results point to possible differences in the development of adaptive metabolic pathways in animals with different levels of maturation at birth.

Key words

Heart • Ontogenetic development • Energy metabolism • Rat • Guinea-pig

Introduction

Cardiac metabolism of adult homeotherms is almost exclusively aerobic. The principal substrates

utilized by the ventricular myocardium include lipids, carbohydrates and amino acids, with long-chain fatty acids as the predominant source (Bing *et al.* 1954, Opie 1989, Bass *et al.* 1988, 1993). On the other hand, fetal

metabolism is primarily anaerobic and this adaptive property is retained during the neonatal period. The high stores of glycogen that characterize the fetal and newborn myocardium are essential for enhancing tolerance to hypoxia, but these decrease rapidly after birth (Ošťádalová *et al.* 1998, Ošťádal *et al.* 1999). Anaerobic production of energy may also be related to enhanced stores of amino acids that allow substrate level phosphorylation (Julia *et al.* 1990). The immature heart mainly depends on glycolysis because the capacity to use fatty acids is impaired either due to delayed maturation of enzymes associated with mitochondrial fatty acid transport and metabolism or due to a deficiency of carnitine (Breuer *et al.* 1967, Lopaschuk *et al.* 1992).

Experimental data on the ontogenetic development of cardiac metabolism are, unfortunately, not consistent (for review see Riva and Hearse 1991, Lopaschuk *et al.* 1992). Possibly, developmental studies often compare only two ontogenetic periods (e.g. neonatal and adult), and hence the changes characterizing the intermediate stages remain unknown. Furthermore, there are significant species differences in the development of myocardial energetics (e.g. rat – Walpurger 1967, Abo Zeit-Har and Drahota 1975, Andrés *et al.* 1984, rabbit – Stave 1964, Hoerter *et al.* 1991, guinea-pig – Barrie and Harris 1977, pig – Scholz *et al.* 1997, cattle – Marin-Garcia *et al.* 1994, human – Šamánek *et al.* 1989, Marin-Garcia *et al.* 1998), which may be related not only to cardiac efficiency (Loiselle 1987), but also to the degree of maturation. Finally, the spectrum of enzymes investigated in most of the studies covers only a very limited range of metabolic pathways.

The purpose of the present study was, therefore, to compare the ontogenetic development of the activity of representative energy-liberating enzymes in two mammalian species, differing significantly in their level of maturation at birth, namely rat and guinea-pig. Since the data available are almost exclusively limited to the investigation of the left heart, particular attention was paid to the possible right-to-left differences.

Methods

The animals were investigated during the late prenatal period and 2, 7, 14, 21, 25, 30, 63, 120, 730 days after birth in the rat and 2, 21, 84, 175 days in the guinea-pig. All animals were killed by decapitation, the hearts were removed, and starting from day 7 in rats and in all groups of guinea-pigs dissected into the right and

left ventricles. Tissue samples weighing 15 to 30 mg were rapidly inserted into precooled homogenization tubes with 200 μ l of a 50 μ mol/l Na-K-phosphate buffer solution, containing 10 μ mol/l of EDTA and 1 ml/l Triton X-100 at pH 7.25. The samples were then made up to 20 times their mass with the same buffer solution, homogenized in the cold and centrifuged for 10 min at 15 000 \times g. The supernatant was then decanted, the pellet rehomogenized and centrifuged as above. In the combined supernatants, the activity of seven enzymes of energy supply was estimated photometrically (Bücher *et al.* 1964, Bass *et al.* 1968, Stieglerová *et al.* 1999). The assayed enzymes were: lactate dehydrogenase (LDH; lactate uptake and/or formation; EC 1.1.1.27), triose phosphate dehydrogenase (TPDH; carbohydrate metabolism; EC 1.2.1.12), glycerol-3-phosphate: NAD dehydrogenase (GPDH; glycerol-P-shuttle and metabolism; EC 1.1.1.8), hexokinase (HK; glucose phosphorylation; EC 2.7.1.1.), malate: NAD dehydrogenase (MDH; tricarboxylic cycle = TCC, reducing equivalent transport; EC 1.1.1.37), citrate synthase (CS; TCC; EC 4.1.3.7) and 3-hydroxyacyl-CoA dehydrogenase (HOADH; fatty acid breakdown; EC 1.1.1.35).

Statistical evaluation

All results are expressed as the means \pm S.E.M. Analysis of variance was used for the evaluation of ontogenetic differences among individual groups and paired t-test for the analysis of right-to-left differences. Values of $p < 0.05$ were considered statistically significant.

Results

Rat

The activities of cardiac enzymes of energy liberating metabolism (expressed as U/g wet weight) during ontogenetic development are shown in Table 1. After the 7th postnatal day the individual values for right and left ventricles were estimated separately.

In 7-day-old animals the activities of all enzymes with the exception of HOADH and CS were significantly higher in the left as compared with the right ventricular myocardium. During further development the right to left differences either appeared only exceptionally (MDH, CS, HOADH), became less pronounced (GPDH), or persisted practically during the whole investigated period (TPDH, LDH, HK).

Table 1. Ontogenetic development of enzyme activities in the rat heart.

Age in days	n	LDH	TPDH	GPDH	HK	MDH	CS	HOADH	
Prenatal	WH	6	216.5 ± 4.7	121.7 ± 6.3	0.3 ± 0.1	7.1 ± 0.2	348.2 ± 24.0	14.6 ± 0.7	3.1 ± 0.4
	2	WH	12	123.0 ± 18.5	114.1 ± 4.2	0.6 ± 0.1	3.6 ± 0.3	116.6 ± 5.7	30.3 ± 0.9
7	RV	8	199.3 ± 7.4**	95.8 ± 4.2*	0.7 ± 0.1*	6.1 ± 0.2*	248.1 ± 9.8*	27.5 ± 1.1	13.4 ± 0.9
	LV	8	263.9 ± 14.5	119.1 ± 5.2	0.9 ± 0.1	6.7 ± 0.2	302.3 ± 11.6	31.6 ± 1.0	13.6 ± 0.7
14	RV	6	226.8 ± 24.6	100.2 ± 4.6	1.3 ± 0.1	5.2 ± 0.3	265.3 ± 42.9	36.0 ± 3.4	20.0 ± 1.3
	LV	6	258.0 ± 16.1	112.5 ± 7.1	1.4 ± 0.1	6.1 ± 0.4	322.5 ± 48.6	34.1 ± 4.4	22.5 ± 2.2
21	RV	6	239.8 ± 18.2	109.5 ± 9.3	2.1 ± 0.2	5.8 ± 0.8	361.5 ± 31.2	53.8 ± 1.7	44.5 ± 4.3
	LV	5	256.0 ± 10.3	126.4 ± 13.3	2.2 ± 0.2	6.0 ± 1.1	358.0 ± 26.8	54.0 ± 1.8	40.5 ± 1.8
30	RV	8	238.6 ± 17.8*	156.0 ± 7.4*	3.2 ± 0.3	7.2 ± 0.2*	990.8 ± 28.6**	68.5 ± 1.9*	22.7 ± 0.8**
	LV	9	352.8 ± 23.7	88.0 ± 22.2	2.7 ± 0.2	6.4 ± 0.5	492.7 ± 50.8	84.6 ± 3.2	45.8 ± 3.7
63	RV	6	323.7 ± 16.6**	149.3 ± 14.3**	3.3 ± 0.2*	3.3 ± 0.2	449.3 ± 37.9	87.6 ± 2.5*	63.9 ± 1.9
	LV	6	384.3 ± 20.5	175.5 ± 12.9	2.9 ± 0.1	3.6 ± 0.2	475.0 ± 41.4	94.0 ± 2.1	57.8 ± 1.8
120	RV	14	418.5 ± 27.9	202.4 ± 9.2	5.1 ± 0.3	4.3 ± 0.2*	1219.4 ± 104.7	90.8 ± 1.5	46.9 ± 2.3
	LV	14	471.4 ± 29.1	215.1 ± 11.7	5.3 ± 0.2	5.2 ± 0.3	1425.9 ± 120.8	92.2 ± 3.3	49.1 ± 4.0
730	RV	13	338.6 ± 17.4	159.5 ± 11.3	3.5 ± 0.3	4.2 ± 0.4	1025.0 ± 72.9	61.7 ± 3.4	26.2 ± 3.6
	LV	14	394.1 ± 20.6	167.8 ± 9.0	4.1 ± 0.2	5.6 ± 0.5	1132.1 ± 61.0	61.2 ± 4.0	28.9 ± 2.9

Enzyme activities in U/g wet weight. Data are means ± S.E.M. WH – whole heart, RV – right ventricle. LV – left ventricle, significant difference between RV and LV: * $p < 0.05$; ** $p < 0.01$.

The ontogenetic development of the enzyme activities exhibits three different patterns, identical in both ventricles (Fig. 1): (i) two mitochondrial enzymes (CS, HOADH) and GPDH exhibit a relatively low activity at the end of prenatal life; thereafter this value steadily increases approaching the adult activity between the 3rd and 4th postnatal week. A significant decrease was observed between the 4th and 24th month (Fig. 1a-c); (ii) the activities of MDH and LDH increase from the 2nd postnatal day up to adulthood (4 months); similarly as the enzymes in the previous group, these values decrease during senescence. There is, however, a difference concerning the prenatal values: they are considerably lower than on the 2nd postnatal day

(Fig. 1 d,e); (iii) the activities of enzymes in the last group (HK, TPDH) are characterized by only moderate changes during development. HK oscillates around the adult value during the whole period; it differs from all other enzymes by the highest prenatal value, which exceeds even the adult level. The activity of TPDH exhibits no developmental changes up to the 3rd postnatal week; thereafter it approaches the adult values (Fig. 1 f,g).

Guinea-pig

The enzyme activities in the right and left ventricles of developing guinea-pig hearts are summarized in Table 2. Right-to-left differences were observed only prenatally and during further development

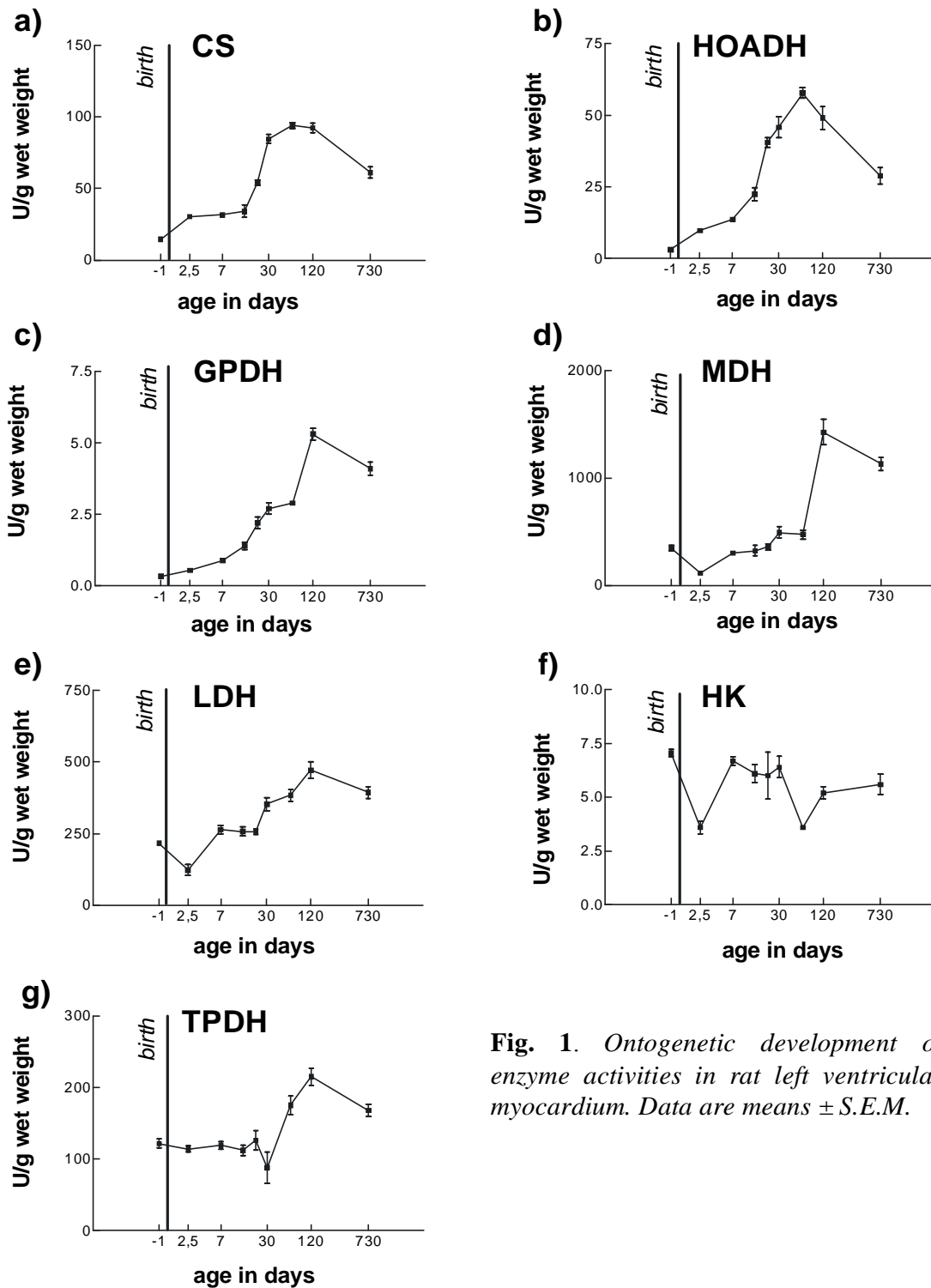


Fig. 1. Ontogenetic development of enzyme activities in rat left ventricular myocardium. Data are means \pm S.E.M.

they almost disappeared. In good agreement with the relatively high level of maturation at birth, the developmental changes were significantly less expressed as compared with the highly immature rat (Fig. 2). The only ontogenetic differences observed were the lower activities of enzymes connected with aerobic metabolism (HOADH, CS, MDH, Fig. 3 a,b,d) at the end of the

prenatal period and transient increase of HK in newborns (Fig. 3 f).

Discussion

Our results clearly demonstrate significant differences in the ontogenetic development of enzyme activities between the rat and guinea-pig heart. The rat

Table 2. Ontogenetic development of enzyme activities in the guinea-pig heart.

Age in days	n	LDH	TPDH	GPDH	HK	MDH	CS	HOADH	
Prenatal	RV	8	270.2 ± 21.0**	134.4 ± 13.0**	0.6 ± 0.1*	14.8 ± 0.6	492.1 ± 29.4	35.3 ± 2.0	13.0 ± 1.8
	LV		242.6 ± 22.6	121.4 ± 10.0	1.1 ± 0.4	15.0 ± 0.9	467.1 ± 34.8	35.3 ± 2.0	13.0 ± 1.8
2	RV	6	249.1 ± 21.9	146.8 ± 9.3	1.7 ± 0.1	21.9 ± 1.2	801.3 ± 57.1	60.8 ± 2.9	28.3 ± 3.9
	LV		226.3 ± 12.5	134.8 ± 11.4	2.0 ± 0.1	22.0 ± 1.2	839.0 ± 76.0	57.2 ± 2.0	30.1 ± 2.9
21	RV	6	209.0 ± 19.9	107.3 ± 12.1	1.6 ± 0.2**	14.3 ± 1.0	821.8 ± 86.8	53.13 ± 3.9	21.2 ± 1.9
	LV		209.0 ± 16.4	116.1 ± 12.7	1.7 ± 0.2	16.0 ± 1.4	1014.5 ± 94.7	58.05 ± 3.2	26.6 ± 2.5
84	RV	3	290.0 ± 22.3	132.1 ± 8.1	2.5 ± 0.2	13.4 ± 1.1*	956.0 ± 12.5	70.5 ± 5.9	23.8 ± 2.4
	LV		237.9 ± 44.4	136.7 ± 23.4	2.5 ± 0.6	13.4 ± 1.1	1107.5 ± 155.7	72.6 ± 16.2	29.9 ± 4.3
175	RV	2	172.7 ± 6.4	130.9 ± 3.8	2.1 ± 0.1*	13.6 ± 0.2	996.5 ± 13.8	61.1 ± 7.8	24.1 ± 1.8
	LV		228.8 ± 17.0	134.4 ± 22.8	2.3 ± 0.2	13.5 ± 0.5	1118.5 ± 97.1	65.6 ± 5.5	30.1 ± 1.4

Enzyme activities in U/g wet weight. Data are means ± S.E.M. RV – right ventricle. LV – left ventricle; significant difference between RV and LV: * $p < 0.05$; ** $p < 0.01$.

heart, highly immature at birth, exhibits three different developmental patterns of energy-supplying enzymes: (i) the mitochondrial enzymes of aerobic metabolism (CS, HOADH) and cytoplasmic proton transporting GPDH increase steadily from the end of prenatal life up to adulthood and decrease during senescence; (ii) LDH and MDH decrease during the late prenatal life and then increase similarly as the former group; (iii) HK (bringing glucose into metabolism) and TPDH (lower part of glycolysis) oscillate about the adult value without significant changes during the whole investigated period. Similar developmental trends for CS, MDH and HK were also described by Andrés *et al.* (1984) in the rat heart and for CS by Marin-Garcia *et al.* (1994) in the bovine myocardium. Moreover, the activity of cytoplasmic creatine kinase studied by Andrés *et al.* (1984) and Hoerter *et al.* (1994) in rats as well as by Hoerter *et al.* (1991) in rabbits exhibits an analogous ontogenetic pattern as our group (i).

In this connection the question arises whether this spontaneous clustering is of metabolic significance. It is obvious that there is no difference between groups (i) and (ii) as far as the postnatal development is concerned. This generally means that utilization of fatty acids and overall aerobic metabolism increase during postnatal

ontogeny (Lopaschuk *et al.* 1992). On the other hand, the ability to use carbohydrates (group iii) is maximal already during late prenatal life (Warshaw 1972, Andrés *et al.* 1984, Riva and Hearse 1991).

In contradiction to the rat heart, the developmental differences in more mature guinea-pig heart were significantly less pronounced. The lower activities of enzymes connected with aerobic metabolism during the last days of prenatal life described above, as well as in the paper of Barrie and Harris (1977), suggest similar developmental trends as in the rat heart but shifted towards the prenatal ontogeny. More detailed information on the metabolic changes during fetal life would, therefore, be desirable.

The comparison of absolute values of the enzyme activities in the rat and guinea-pig hearts has revealed that during the early phases of ontogeny (up to the 2nd day of postnatal life) the activities of all enzymes studied in the rat myocardium were significantly lower than in the guinea-pig. During further development, this ratio changes steadily so that all enzymes in adult animals, except HK, were significantly higher in the rat heart (Bass *et al.* 1993). This difference is obviously due to the fact that animals of small species by virtue of their reduced dimensions pay a disproportionately high energy

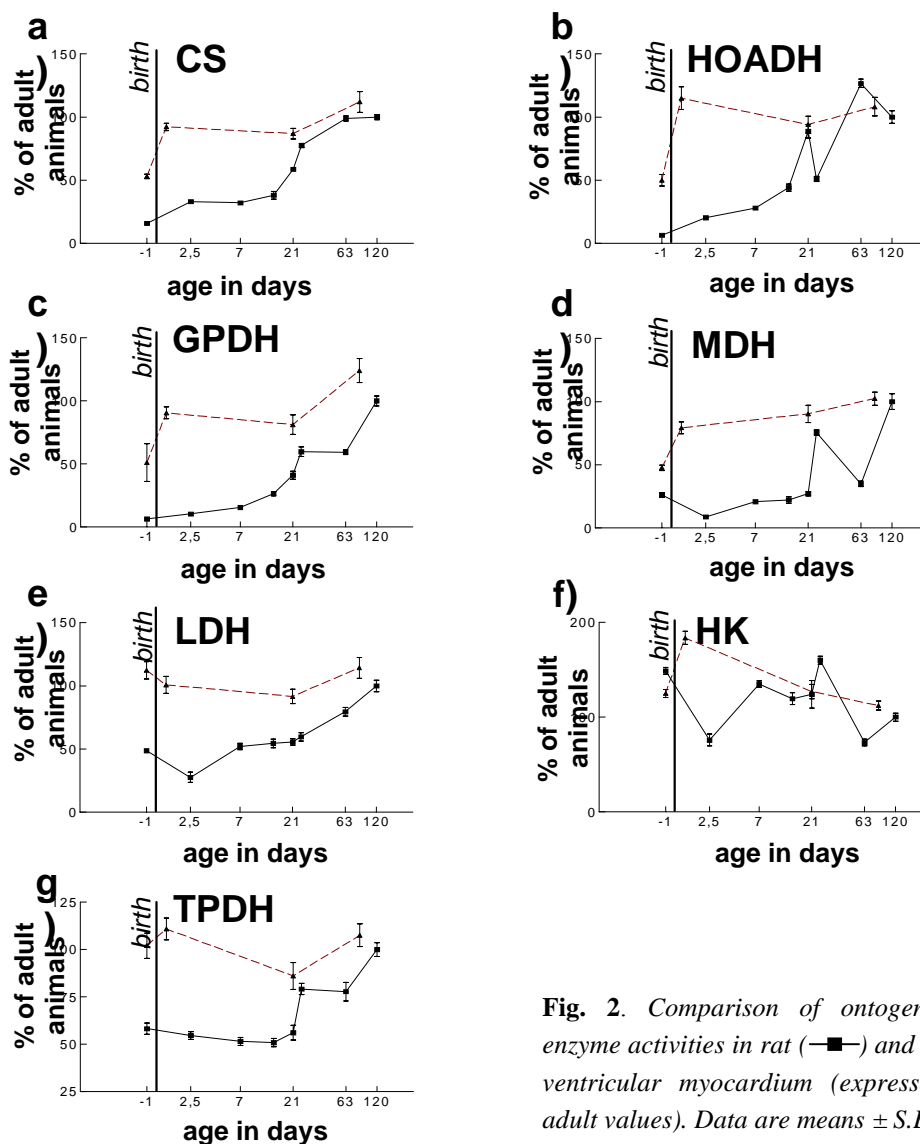


Fig. 2. Comparison of ontogenetic development of enzyme activities in rat (—■—) and guinea-pig (---▲---) left ventricular myocardium (expressed as percentage of adult values). Data are means \pm S.E.M.

expenditure for cardiac performance (Grande and Taylor 1965); it seems, however, that cardiac energy expenditure across species is not directly proportional to the heart rate (Gibbs and Loiselle 1978). In addition, our results suggest that the guinea-pig heart is more dependent on glucose phosphorylation than the rat myocardium.

Marked interspecies differences were also observed in the right-to-left ratios of enzyme activities. We did not observe significant right-to-left differences in

the guinea-pig hearts during postnatal development. The only difference in the study of Barrie and Harris (1977) concerned higher activity of LDH in the right ventricles of adults. On the other hand, the activities of all enzymes studied, except CS and HOADH, were significantly higher in the left ventricular wall of 7-day-old rats. This difference slowly disappears during further development.

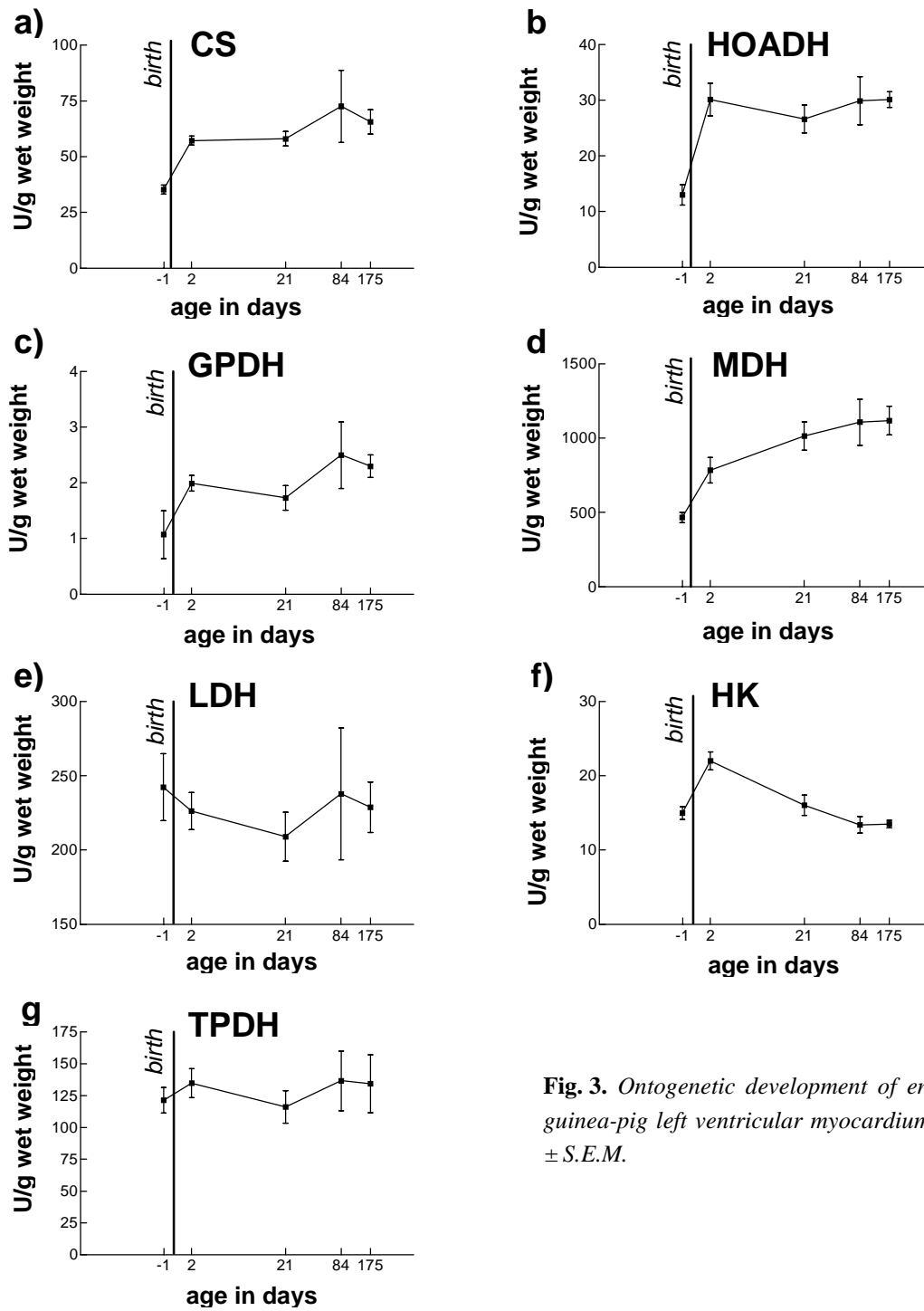


Fig. 3. Ontogenetic development of enzyme activities in guinea-pig left ventricular myocardium. Data are means \pm S.E.M.

Such an age-dependent heterogeneity may be – at least partly – the result of the steeply increased workload, selectively imposed on the left ventricle after birth. It is obvious from the right ventricle/left ventricle weight ratio, which steadily decreases and in rats becomes stabilized only after the weaning period (Ošťádal *et al.* 1967). The same trend is exhibited by the maturation of myocardial ultrastructure (Anversa *et al.* 1981) and blood

supply (Ošťádal *et al.* 1999). However, similar postnatal adaptive changes also occur in the guinea-pig where the development of the metabolic pattern in the right and left ventricular myocardium is almost the same. Our results thus point to the possible differences in the development of adaptive metabolic pathways in animals with a different level of maturation at birth.

In conclusion, cardiac metabolism changes in response to oxygen and substrate availability during ontogenetic development. The immature heart is relatively more dependent on anaerobic glycolysis, using glucose as its main energy substrate, whereas the mature heart is almost exclusively aerobic, with nonesterified fatty acids as the predominant source. The mechanisms of the higher resistance of the immature heart to oxygen deprivation have not yet been satisfactorily clarified (Ošťádal *et al.* 1999). In this connection, it may be speculated that this phenomenon can be at least partly explained by the lower energy demand, greater anaerobic glycolytic capacity, and higher glycogen reserves of the developing heart.

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List of abbreviations

- CS – citrate synthase
 GPDH – glycerol-3-phosphate: NAD dehydrogenase
 HK – hexokinase
 HOADH – 3-hydroxyacyl-CoA dehydrogenase
 LDH – lactate dehydrogenase
 MDH – malate:NAD dehydrogenase
 TPDH – triose phosphate dehydrogenase

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Reprint requests

Prof. Dr. B. Ošťádal, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, fax: 420 2 475 2125, e-mail: ostadal@biomed.cas.cz