

Age-related features in systemic inflammatory response in male Wistar rats with different hypoxia tolerance

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Introduction

Organism's hypoxia tolerance depends on many factors, including age. High newborn organism's tolerance and high level of oxidative stress throughout aging were demonstrated by many studies. However, there is still lack of investigations, reflecting the intensity of systemic inflammatory response in different age organisms in correlation to hypoxia tolerance.

Aims: to determine the relationship between age-related tolerance to hypoxia, HIF-1 and PHD2 (prolyl hydroxylase domain protein) expression levels and the intensity of systemic inflammatory response in newborn, prepubertal and adult Wistar rats.

Methods

Newborn (age 2 days), prepubertal (age 10 days) and adult (age 2-3 month) male Wistar rats (n=18) were exposed in special decompression chamber to gradual hypobaric hypoxia, equal to 11 500 m elevation (Kirova et al., 2013; Tregub et al., 2013; Dzhaliilova et al., 2018, 2019). Hypoxic tolerance was determined by measuring the time taken for the onset of gasping (GT). Based on literature data, such characteristics reflects the organism's hypoxia tolerance (Jain et al., 2013; Kirova et al., 2013; Tregub et al., 2013).

The second group of rats (n=18) was euthanized by an overdose (15 mg/kg) of anesthetic Zoletil (Virbac Sante Animale). For the research of genes and proteins expression, was chosen the liver, which is a sensitive to hypoxia organ. Liver fragments were fixed for subsequent PCR in IntactRNA Reagent (Evrogen CJSC) and for Western Blot analysis in a liquid nitrogen.

Systemic inflammatory response was modeled by intraperitoneal injection 15 mg/kg lipopolysaccharide (LPS) *E.coli* O26:B6 (Sigma). In newborn and prepubertal rats, 15 mg/kg LPS induced progressing degenerative changes and necrosis. This dose is sublethal for adult Wistar rats. The control rats were intraperitoneally injected with physiological saline. Animals were euthanized by an overdose (15 mg/kg) of anesthetic Zoletil after 24 hours of LPS injection.

The histological sections of the liver were manufactured and stained by hematoxylin and eosin. The severity of pathological changes in the liver was evaluated morphometrically - the area of cells pathology and necrotic changes was estimated to the total area of the histological section. The serum collected was frozen at -70°C and was kept no longer than two months. ELISA was employed to assay proinflammatory markers neopterin (IBL) and C-reactive protein (eBioscience). The serum level of endotoxin was assessed with the LAL test. For the evaluation of the statistical significance of taken results were used the methods of nonparametric statistics (Mann-Whitney U test, Kruskal-Wallis method). The differences were considered significant when P<0.05.

Age-related differences of hypoxia tolerance

After the assessment of animals gasping time in a decompression chamber at 11,500 m, it was demonstrated that prepubertal rats are the least tolerant to hypoxia (Fig. 1). Not less important is the high variability of parameters in adult rats.

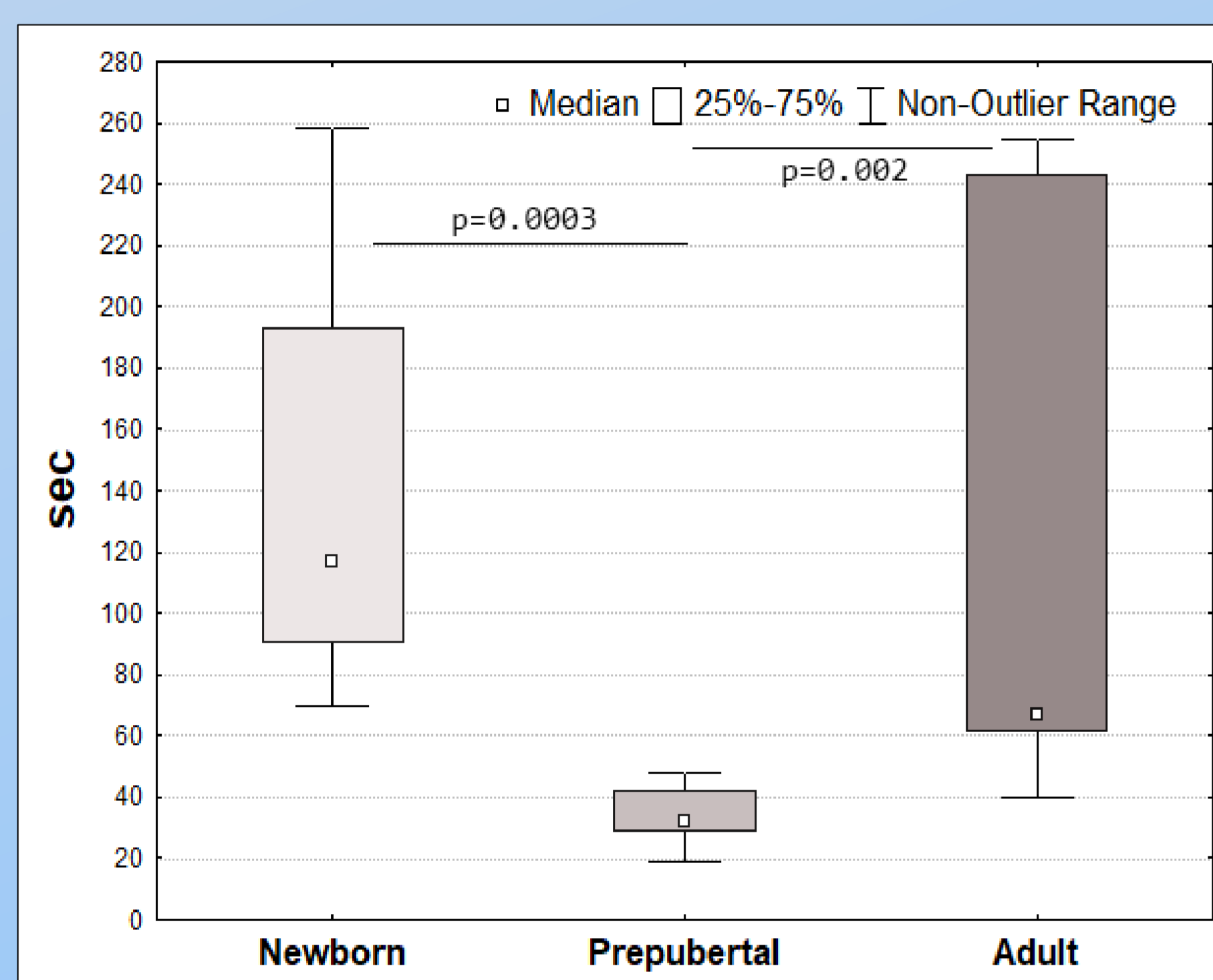


Fig. 1. Evaluation of gasping time for newborn, prepubertal and adult Wistar rats in the decompression chamber (Me; 25%-75%). p – statistically significant differences, Kruskal-Wallis method.

Age-related differences of SIRS

Morphological study of the liver of rats revealed pathological changes for SIRS. In the liver in experimental groups there were cells degeneration, which were more spread among prepubertal rats. According to the results of the morphometric evaluation of the area of cells degeneration and necrosis it turned out that necrosis area were significantly more pronounced among prepubertal animals (Table 1). The lowest tolerance of the prepubertal males to hypoxia correlated with the greatest manifestations of hepatic inflammation and elevated endotoxin, neopterin, and C-reactive protein levels in LPS-induced systemic inflammatory response.

Table 1. Effect of LPS on hepatic necrosis area and serum endotoxin, neopterin and C-reactive protein content in newborn, prepubertal and adult Wistar rats in 24 h after injection, Me (25%-75%)

Age period	Group	Necrosis area, 10 ³ mkm ²	Endotoxin, U/ml	Neopterin, nM/l	C-reactive protein, mg/ml
Newborn	Control	-	1.9 (0.5-3.3) ¹	9.12 (7.12-10.37) ¹	3.2 (3.1-3.3) ¹
	LPS	1.6 (1.4-4.3) ¹	57.0 (47.3-90.7) ²	7.33 (6.02-8.71) ²	2.6 (2.5-3.2) ²
Prepubertal	Control	-	2.6 (1.3-4.8) ³	2.74 (2.26-3.91) ³	3.0 (2.9-4.2) ³
	LPS	24.5 (7.9-99.5) ²	430 (417.8-451.3) ⁴	4.62 (3.98-5.76) ⁴	4.6 (3.2-4.7) ⁴
Adult	Control	-	0.7 (0.5-0.8) ⁵	0.84 (0.17-1.14) ⁵	3.0 (2.8-3.2) ⁵
	LPS	1.5 (0.6-2.3) ³	22.1 (3.6-100.0) ⁶	1.53 (1.39-1.94) ⁶	3.0 (2.9-3.1) ⁶
Statistically significant differences, p<0.05			0.00003 ¹⁻² 0.00002 ³⁻⁴ 0.00015 ⁵⁻⁶ 0.02 ¹⁻⁵ 0.004 ²⁻⁴ 0.00012 ⁵⁻⁶ 0.000014 ⁴⁻⁶	0.018 ³⁻⁴ 0.04 ⁵⁻⁶ 0.00011 ¹⁻³ 0.000005 ¹⁻⁵ 0.03 ³⁻⁵ 0.02 ²⁻⁴ 0.012 ⁵⁻⁶ 0.014 ⁴⁻⁶	0.04 ¹⁻² 0.01 ³⁻⁴ 0.04 ²⁻⁴

Results

Age-related differences of HIF-1α and PHD-2 expression levels in the liver

Newborn rats are characterized by high mRNA HIF-1α expression level in the liver (Fig. 2), accompanied by low content of HIF-1 protein and high level of PHD2 (Fig. 3).

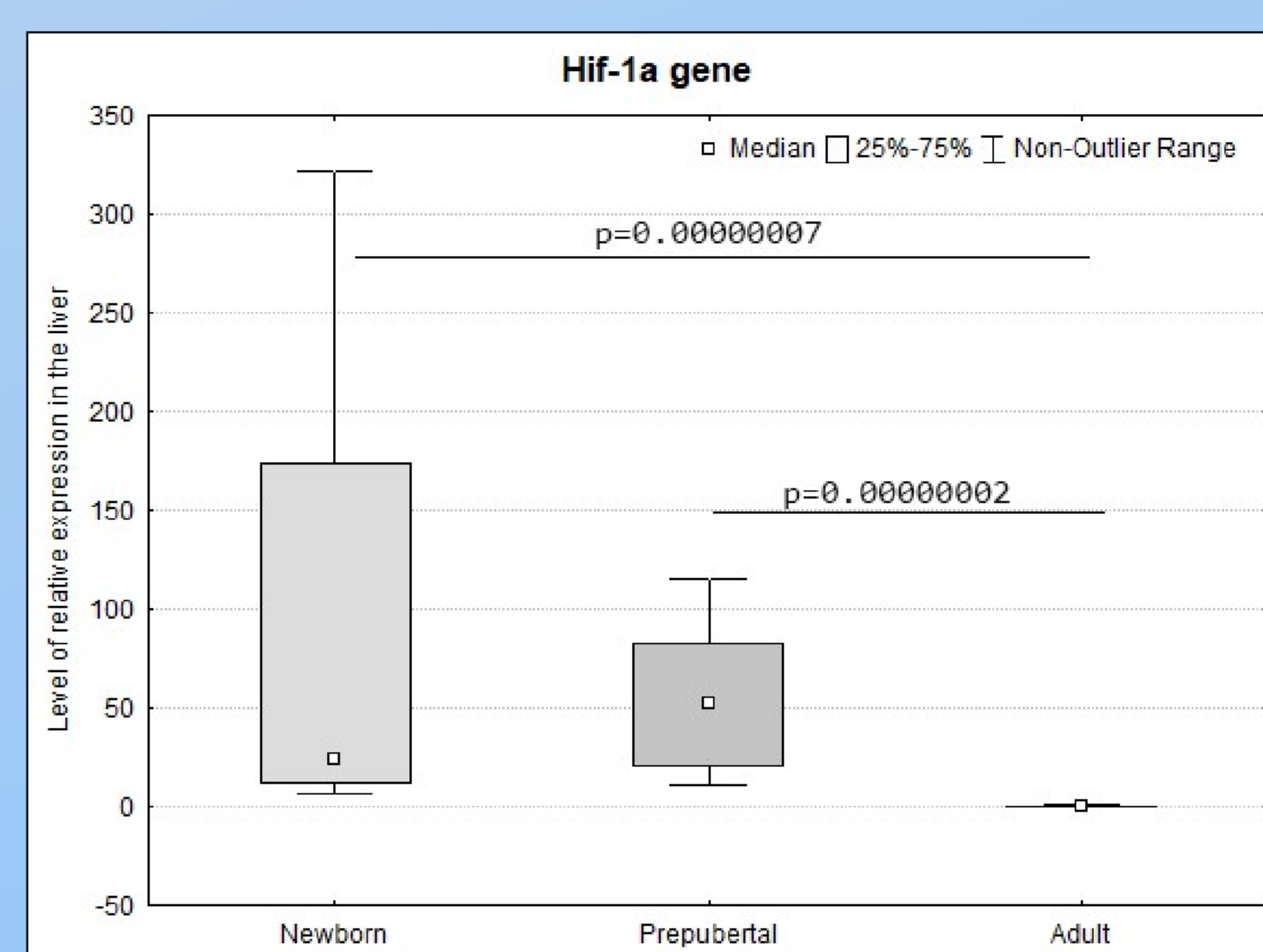


Fig. 2. The expression level of mRNA *Hif-1α* in the liver of newborn, prepubertal and adult Wistar rats. Me; 25%-75%, p – statistically significant differences, Kruskal-Wallis method.

Prepubertal animals are the least hypoxia tolerant and their HIF-1α mRNA expression level was higher than in adult animals. The PHD2 activity in prepubertal animals was significantly reduced in comparison to newborn rats, and the HIF-1α protein level was not changed.

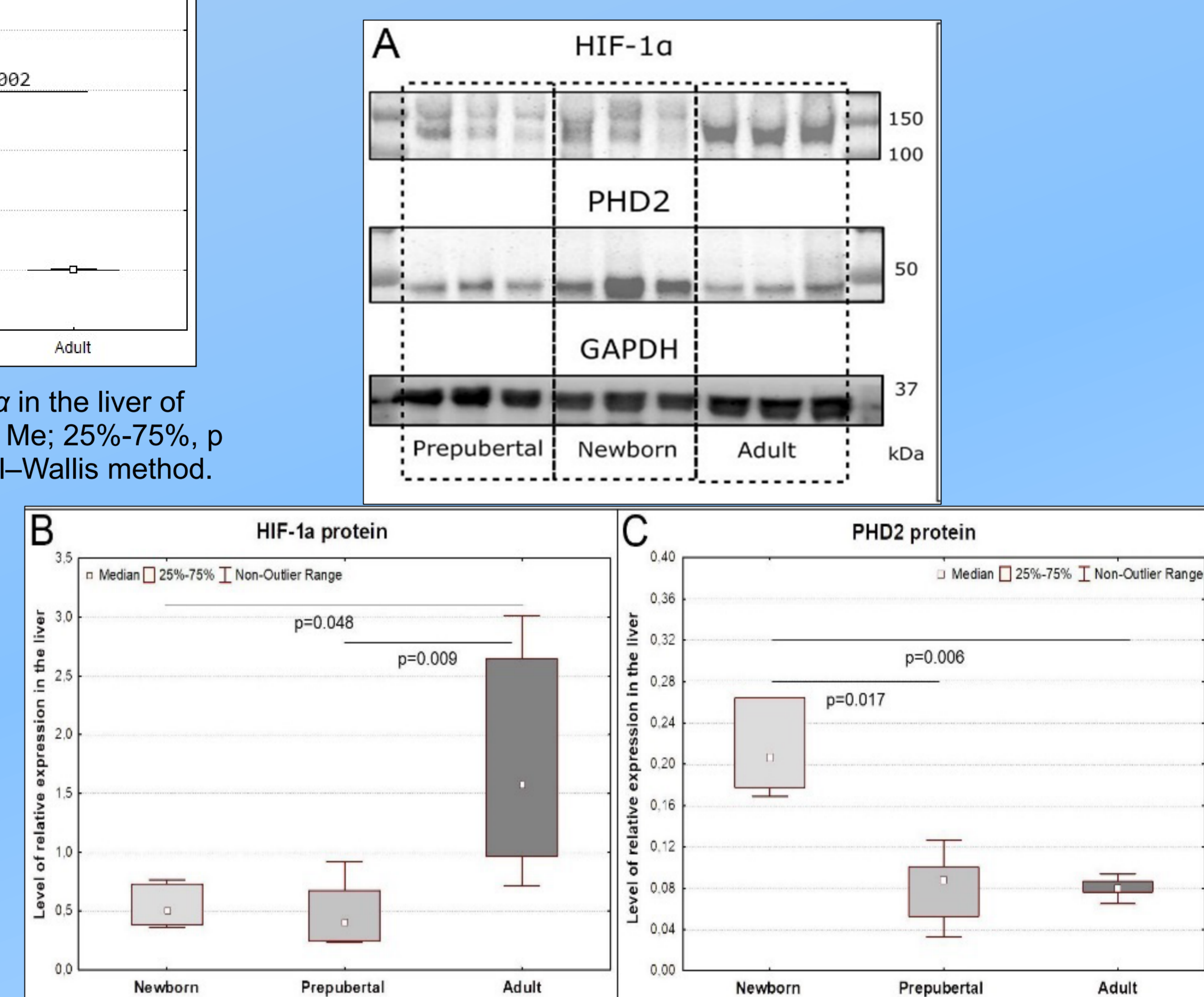


Fig. 3. (a) – Relative protein levels of HIF-1α and PHD2 in the liver of newborn, prepubertal and adult Wistar rats, normalized on GAPDH level, representative Western Blot images are shown. (b, c) – The expression level of HIF-1α (b) and PHD2 (c) proteins in the liver of newborn, prepubertal and adult Wistar rats. Me; 25%-75%, p – statistically significant differences, Kruskal-Wallis method.

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Conclusion

Prepubertal animals were the least hypoxia tolerant and their HIF-1α mRNA expression level was higher than in adult animals. The lowest tolerance of the prepubertal males to hypoxia correlated with the greatest manifestations of hepatic inflammation and elevated endotoxin, neopterin, and C-reactive protein levels in LPS-induced systemic inflammatory response. The obtained data should be taken into account during the development of therapeutic strategy for prepubertal children with infectious and inflammatory diseases.