HYPOXIA-AUGMENTED GLOMERULOSCLEROSIS IN SPONTANEOUSLY DIABETIC db/db MICE

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Summary

We tried to produce advanced glomerulosclerosis in spontaneously diabetic mice by chronic hypoxic exposure. The db/db mce were bred in a normobaric hypoxic chamber (12 %O_2) at 8 w.o. for up to 16 weeks. The biological and pathological findings were compared with age-matched db/db mice bred in room air.

The hypoxic mice, with no hypertension nor serum creatinine increase, showed significantly higher urinary podocalyxin (PCX) levels as well as urinary albumin at 1-4 weeks of hypoxia ($P<0.05$ to $P<0.001$), which thereafter reduced to normoxic mouse levels discordant with persistent albuminuria. The hypoxic mice generally showed mesangiolytic glomerulosclerosis with microaneurysms, occasional nodular fibrosis and rare insudative lesions, in contrast to mild to moderate mesangial sclerosis in normoxic mice. The hypoxic mice showed decreases of CD34+ endothelial cell number and WT-1+ podocyte density with increased macrophage infiltration. Glomerular mRNA analysis disclosed enhanced expression of MCP-1 from 4 weeks and angiogenic (eNOS, Ang1, Ang2) and fibrogenic (TGFß1, CTGF) cytokines at 16 weeks of hypoxia, with no significant changes of VEGF, VEGFR-1 and VEGFR-2. Ultracentrifugation analysis on PCX in hypoxic urine samples suggested the podocyte shedding of PCX as microcellular particulates.

In conclusion, chronic hypoxic exposure of db/db mice induced advanced glomerulosclerosis based on mesangiolysis with macrophage infiltration and podocyte injury, resembling advanced human diabetic glomerulopathy.

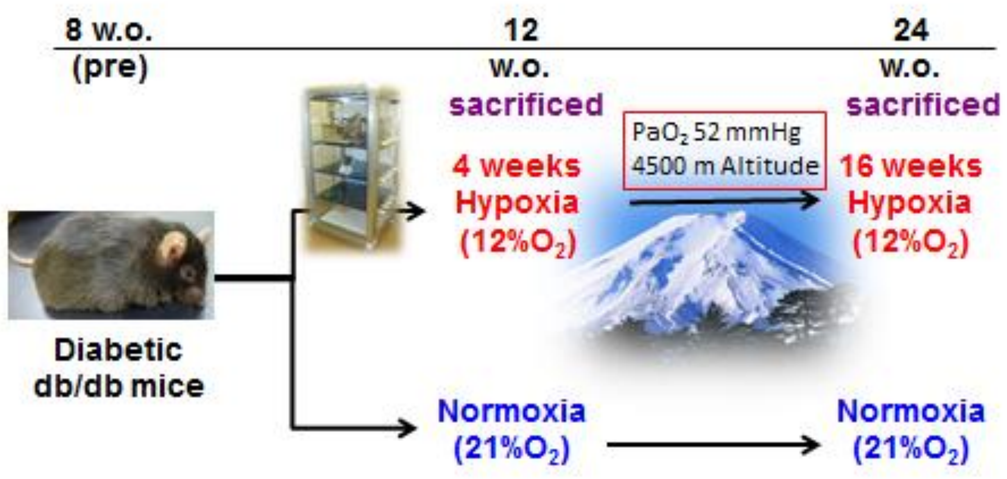


Fig. 1 Experimental design

Male db/db mice at 8 week-old (w.o.) were put in a normobaric hypoxic chamber of 12%O_2, and bred for 4 weeks (n=11) or 16 weeks (n=11), (hypoxic group). The normoxic mice were bred in room air until sacrificed at 12 w.o. (n=12) and 24 w.o (n=12).

Table. Comparison of physiological and laboratory findings between nomoxia and hypoxia groups of db/db mice.

	8 w.o.	12 w.o.		24 w.o.	
		Normoxia	4w Hypoxia	Normoxia	16wHypoxia
N*	12	12	11	12	10
Hct (%)	52.9±2.5	51.8±2.4	73.6±2.7[a]	52.1±7.8	75.5±4.4[a]
BW (g)	36.3±2.1	45.8±2.9	42.8±3.3	48.2±7.5	49.3±5.0
KW/BW (mg/g)	5.3±0.3	5.8±0.7	4.8±0.4[a]	7.2±1.0	5.4±1.0[a]
Glu (mg/dL)	783±70	878±85	637±123[a]	900±261	469±218[a]
SBP (mmHg)	114±9.5(7)	130±9.2(7)	121±9.0(6)	118±11(9)	109±10(7)
Cr (mg/dL)	0.17±0.03	0.21±0.05	0.18±0.04	0.18±0.03	0.18±0.03

*N, number of mice, except for SBP specified in the parenthesis.
Data is shown as mean ± SD. [a]$P<0.001$, compared with normoxia group

Fig 2. Comparison between Normoxic and Hypoxic db/db mice at 24 w.o.

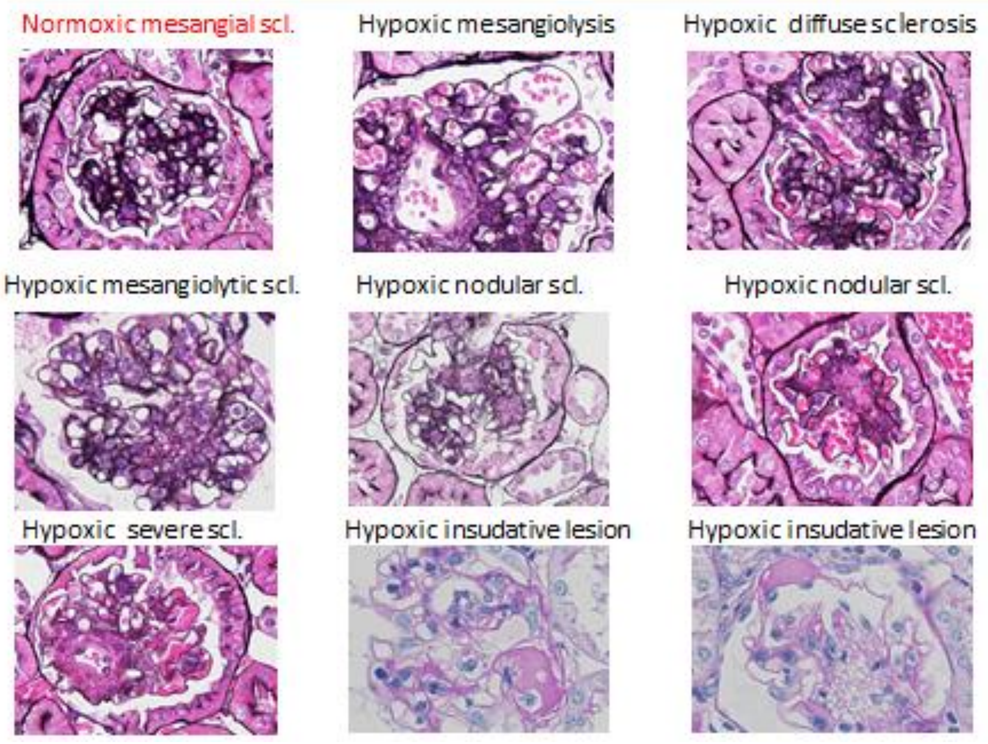


Fig 3. Hypoxic db/db/mice (■) showed significantly higher urinary (u) albumin & podocalyxin (PCX) than normoxic mice (□), with no such changes in u-VEGF.

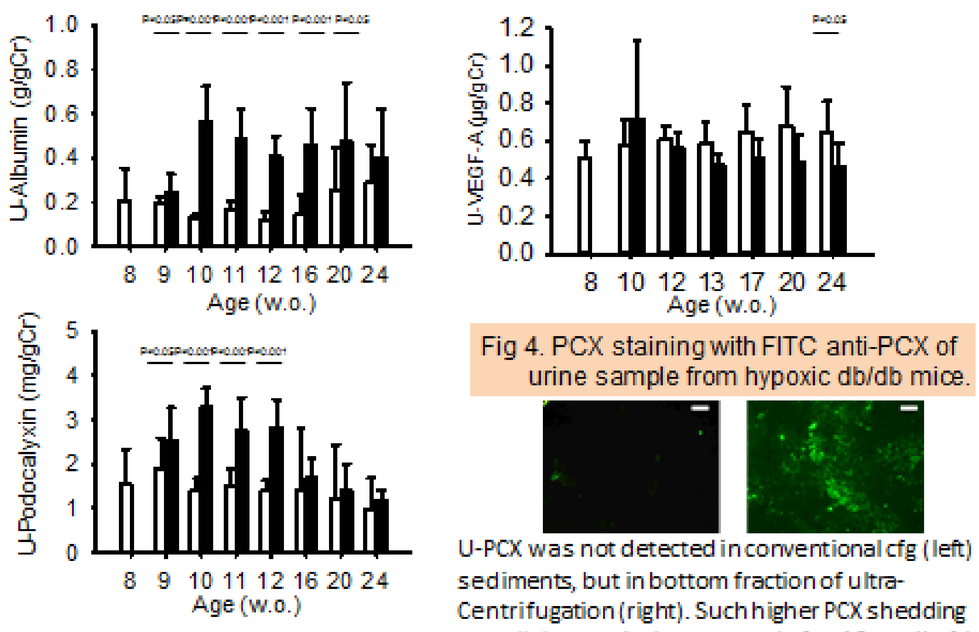


Fig 4. PCX staining with FITC anti-PCX of urine sample from hypoxic db/db mice.

U-PCX was not detected in conventional cfg (left) sediments, but in bottom fraction of ultra-Centrifugation (right). Such higher PCX shedding as cellular particulates ceased after 16 w.o (Left).

Fig 5. Hypoxic db/db mice (■) showed decreased stainings of CD34+ endothelial cell number and WT-1+podocyte density, and increased F4/80+ macrophages with no change of VEGF staining, in compariason with normoxic mice (□).

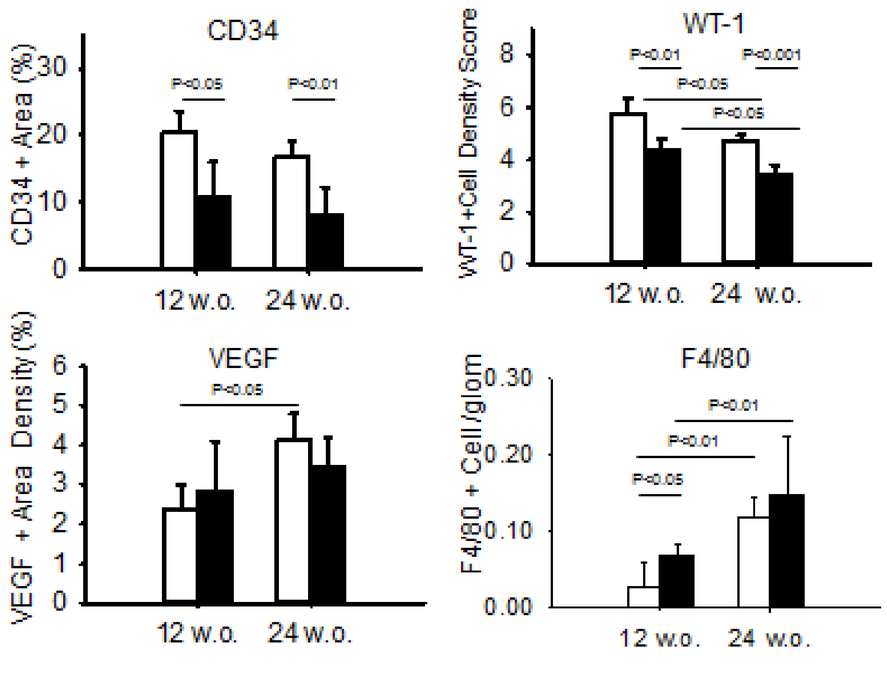
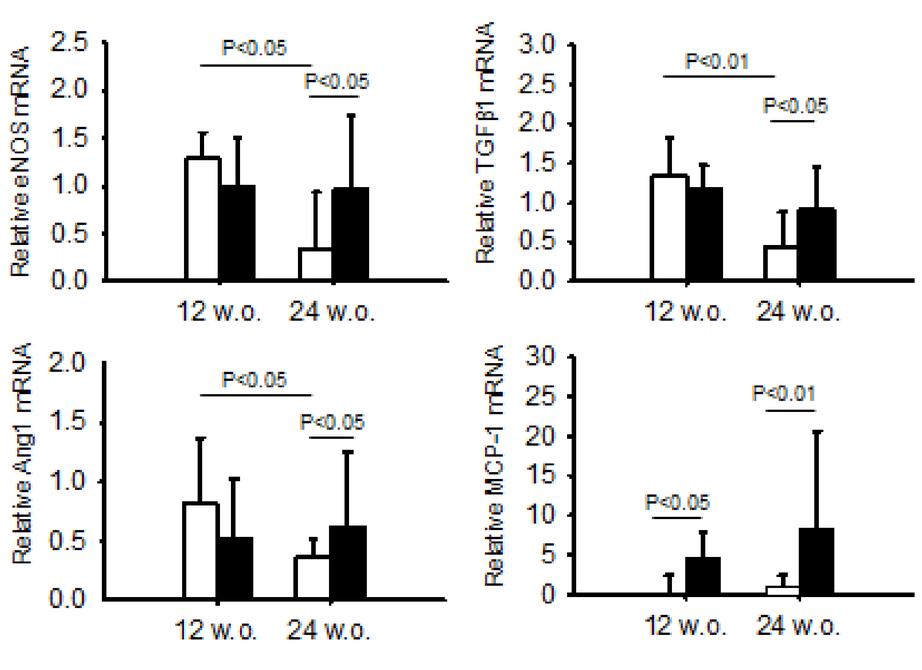


Fig 6. Hypoxic db/db mice (■) showed increased glomerular expression of mRNA of eNOS, Ang1, TGFß1 and MCP-1, as compared with normixic mice (□).



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Diabetes -Basic science
Haruyoshi Yoshida
DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
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