**Purpose**
To determine the effects of repeated oxygen fluctuations on the regulation of variants of insulin-like growth factor-1 (IGF-1) in the rat 50/10 oxygen-induced retinopathy (OIR) model of retinopathy of prematurity (ROP) at a time point when physiologic retinal vascular development is delayed compared to room air.

**Introduction**
Retinopathy of prematurity (ROP) can lead to retinal detachment and is a major cause of severe visual deficits in premature infants. Its pathological progression can be divided into two stages: 1) delay in normal vascular development causing avascular retina and 2) abnormal neovascularization that grows into the vitreous.

Preterm babies born at very low birth weights are at the greatest risk of developing ROP. Low serum IGF-1 has been correlated with poor postnatal growth and delayed physiologic retinal vascular development in human infants and with more severe retinopathy in the mouse OIR model. IGF-1 is characterized by multiple promoters and splice variants (Figure 1), which regulate the expression and function of the IGF-1 gene.

The rat 50/10 OIR model recreates delayed physiologic retinal vascular development seen in human ROP. We wished to determine the expression of the IGF-1 variants in the 50/10 OIR model at the time when physiologic retinal vascular development was delayed (postnatal day 14) compared to room air.

**Methods**
1. **Animal model:** As reported, within 4 hours of birth, pups and their mothers were placed into an oxygen environment, which cycled between 50% and 10% every 24 hours for 14 days. Litter numbers were between 12 and 14 pups for each experiment to assure consistency in outcomes. Pups raised at room air were placed into an oxygen environment, which cycled between 50% and 10%.

2. **Retinal flat mount:** For each pup, one eye was prepared for retinal flat mounts, and another eye was analyzed for mRNA by real-time PCR or protein expression were decreased at P14 compared to RA.

3. **RNA extraction and Real-Time PCR:** Total RNA of retinas was extracted and cDNA was generated by using commercial kits. RT-PCR for igf-1a, igf-1b and igf-1r was performed using TaqMan probes. Expression levels for all genes were normalized to the mean value of internal control GAPDH.

4. **Protein Extraction and Western Blotting:** Dissected retinal samples were homogenized in modified radio immuno precipitation assay (RIPA) buffer containing protease cocktail inhibitors and orthovanadate. 50 µg of total protein for each sample was separated by NuPAGE® 4-12% Bis-Tris Gels, transferred to PVDF membranes, and incubated with primary antibodies to IGF-1, IGF-1R, and p-IGF-1R overnight at 4°C. All membranes were probed with β-actin.

5. **Results**
   - **Figure 1:** IGF-1 splicing variants. Igf-1 gene contains 6 exons (Ex), with Ex 3 and 4 encoding the IGF-1 mature peptide (pept). Alternative promoter usage occurs, with P1 transcripts starting from Ex1 and P2 from Ex2. Multiple splicing variants also occur, with igf-1a lacking Ex5 and IGF-1b containing Ex5.
   - **Figure 2:** Lectin staining in Retinal flatmount at P14. (A) Room Air; (B) 50/10 OIR model; (C) Percent avascular area.
   - **Figure 3:** mRNA levels in 50/10 OIR and RA at P14. Retinal igf-1a (A), igf-1b (B) and igf-1r (C) mRNA levels were decreased in 50/10 OIR compared to RA. All data are shown as means ±S.E.M. *p<0.05, **p<0.001 vs. RA (n=5).
   - **Figure 4:** Protein levels in 50/10 OIR and RA at P14. IGF-1 (A) was decreased in 50/10 OIR compared to RA, while IGF-1R (B) was increased. There was no difference in p-IGF-1R (C). All data are shown as means ±S.E.M. *p<0.05 vs. RA (n=5).

6. **Conclusions**
   - 1. In the rat 50/10 OIR model, igf-1a, igf-1b and igf-1r mRNAs and IGF-1 protein expression were decreased at P14 compared to RA.
   - 2. Decreased IGF-1 was associated with avascular retina at P14 in the rat 50/10 OIR model compared to fully vascularized retinas in room air pups.
   - 3. Even though IGF-1R expression was increased at P14, its activation was not, and this may reflect the persistence of avascular retina in the 50/10 OIR model.

**References**

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