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The adult Sprague Dawley Sugen-hypoxia rat is still "the one" – a model of group 1 pulmonary hypertension (PAH)

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To the editor:

Kojonazarov *et al.* recently reported severe emphysema in the SU5416/Hypoxia (SuHx) rat model of pulmonary hypertension (1). The authors found that adult male Wistar Kyoto (WKY) rats had increased air-to-tissue ratio as judged by non-gated *in vivo* micro-computed tomography (CT), and an increased mean linear intercept (MLI) as surrogate of emphysema (1, 2). Le Cras and Abman now responded to the Kojonazarov report by underlining the "important role of the developmental timing of disrupted VEGF signaling" (3). They cite earlier studies conducted on the ovine fetus showing that VEGF inhibition caused vascular remodeling, reduction in vascular/airway growth, and neonatal pulmonary hypertension at birth (4).

Although SU5416 is known to induce emphysema in rats housed in room air at Denver altitude (1609m altitude) (5, 6), we contended in our response letter (11) that, at least in male Sprague Dawley (SD) rats, the combination of VEGFR inhibition and hypoxia does not lead to any biologically relevant emphysema or other significant parenchymal lung disease (7) but to pulmonary arterial hypertension (PAH) due to severe angioproliferative remodeling (7, 8). A similar degree of pulmonary hypertension without apparent alveolar simplification was seen when VEGF-blockade is administered *in utero* to fetal or neonatal sheep (4). In utero, the PO_2 is approximately 19 mmHg in the fetal pulmonary artery and 34 mmHg in the umbilicial vein (maximum systemic oxygenation); such values represent hypoxemic/hypoxic values for newborns after postnatal cardiopulmonary adaptation. Thus, it may not be surprising that VEGF-blockade *in utero* causes severe pulmonary hypertension in fetal or neonatal sheep. The lack of significant alveolar simplification in this fetal model (with low systemic "arterial" PO_2) is consistent with the earlier observations in adult hypoxic rats when VEGFR blockade did not cause emphysema in the setting of hypoxia (7).

As the authors discussed, the WKY rat strain (Janvier Labs, Germany) (1) may be more prone to emphysema after SuHx exposure than other strains (9, 10) . In contrast, only a mild increase in MLI (+18%) was seen in adult male SuHx-Sprague Dawley (SD) rats obtained from Harlan (Indianapolis, USA) (10), whereas no emphysema was found in male

SD rats obtained from Charles River (Sulzfeld, Germany) (8, 10). Of note, even in WKY rats emphysema is not a universal finding after exposure to SuHx (11).

While differences in rat strains may account for some of the discrepancies related to the observed extent of emphysema, there is yet another explanation. Importantly, different methodologies were used to assess the degree of emphysema. Kojonazarov *et al. (2)* used non-gated *in vivo* chest micro CT scans – a method that is fundamentally different from *ex vivo* MLI measurements. To provide a quantitative three-dimensional analysis of the lung parenchyma in SD SuHx rats(8), we performed *scanning electron microscopy (SEM)*. Even this comprehensive, high resolution 3D imaging method did not reveal any significant emphysema in the adult SuHx SD rat model (**Figure 1**).

Whether or not adult SuHx-exposed SD rats develop biologically *relevant* emphysema is an important question since the presence of significant parenchymal lung disease would invalidate this as a model of human PAH (group 1 PH). Based on our literature search, and the analysis of our own SuHx rat studies (10), now including 3D SEM imaging (**Figure 1**), we conclude that there is little evidence of biologically relevant emphysema when SuHx is used to model PAH in adult male SD rats (5). At the most, there may be mild enlargement of distal intraalveolar spaces in this rat substrain, depending on the method of tissue fixation and timing of lung harvest.

Thus, we conclude that exposure of adult rats to Sugen-hypoxia still provides one of the best models to study PAH(8), and one that lacks a significant emphysema-like lung phenotype, at least in the Sprague Dawley strain (**Figure 1**; ref.(8)).

Conflicts of interest

D.J.S. is a founding scientist, consultant for and has equity interest in Northern Therapeutics Inc. G.H. holds a patent application (USPTO no. 1289344) and an investigational new drug application (IND no. 105,428) related to the use of PPARγ agonistic agents for the treatment of pulmonary hypertension.

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FIGURE LEGENDS

Figure 1. ***Scanning electron microscopy does not reveal any significant emphysema in the adult Sprague Dawley Sugen-Hypoxia (SuHx) rat model of pulmonary arterial hypertension (PAH)..***

(**A**) Scanning electron micrographs illustrate the alveolar architecture of adults rats subdivided in 3 experimental groups. Study design as described in reference (8): Six to eight week old male Sprague Dawley rats weighing ≈180-200 g were purchased from Charles River (Sulzfeld, Germany) and divided into 3 age-matched groups, according to the experimental design: (1) control normoxia (ConNx), (2) control hypoxia (ConHx), i.e. rats injected once subcutaneously with vehicle (DMSO; vol./vol.), then exposed to chronic hypoxia (FiO2 0.1, CO2 < 10.000 ppm) for 3 weeks, followed by a 6 week period in room air (FiO2 0.21), (3) Sugen hypoxia (SuHx), i.e. rats injected with the VEGFR2 inhibitor SU5416 (Sigma), 20mg/kg/dose subcutaneously dissolved in DMSO, and subsequently exposed to chronic hypoxia (3 weeks), followed by 6 weeks of room air. The rat lungs were perfused in vivo by injecting a total of 50 ml normal saline into the beating RV. After perfusion, heart and lungs were taken out “en bloc”; the left lung lobe was ligated and snap-frozen in liquid nitrogen, while the right lobes were tracheally inflated with 10% formalin at a standardized pressure of 25 cm H_2O for at least 5 min., and fixed. The lungs were freeze-dried and sputtered with gold in an argon atmosphere and examined using a Philips ESEM XL-30 scanning electron microscope at 15 keV and 21 µA (Philips, Eindhoven, Netherlands).

(**B**) Morphometric analysis showed mean alveolar diameters to be not significantly different between the three groups: ConNx (45.8±1.6µm), ConHx (50.8±2.0µm), and in Sugen-hypoxia-exposed (SuHx) animals (50.0±2.5µm), in both non-parametric (Kruskal-Wallis/Benjamini-Krieger-Yekutieli) and parametric statistical tests (ANOVA/Bonferoni posthoc) and multiple comparisons. Mean alveolar diameters were determined by morphometric image analysis (Scandium, OLYMPUS Soft Imaging Solutions) of SEM micrographs of different groups (n=6 rats per group), equaling more than 100-150 measured data points per animal. Mean ± SEM. N = 6 adult male Sprague Dawley rats per group. All animal experiments were conducted under the approval of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES, Lower Saxony, Germany; #15/2022, #13/1328).

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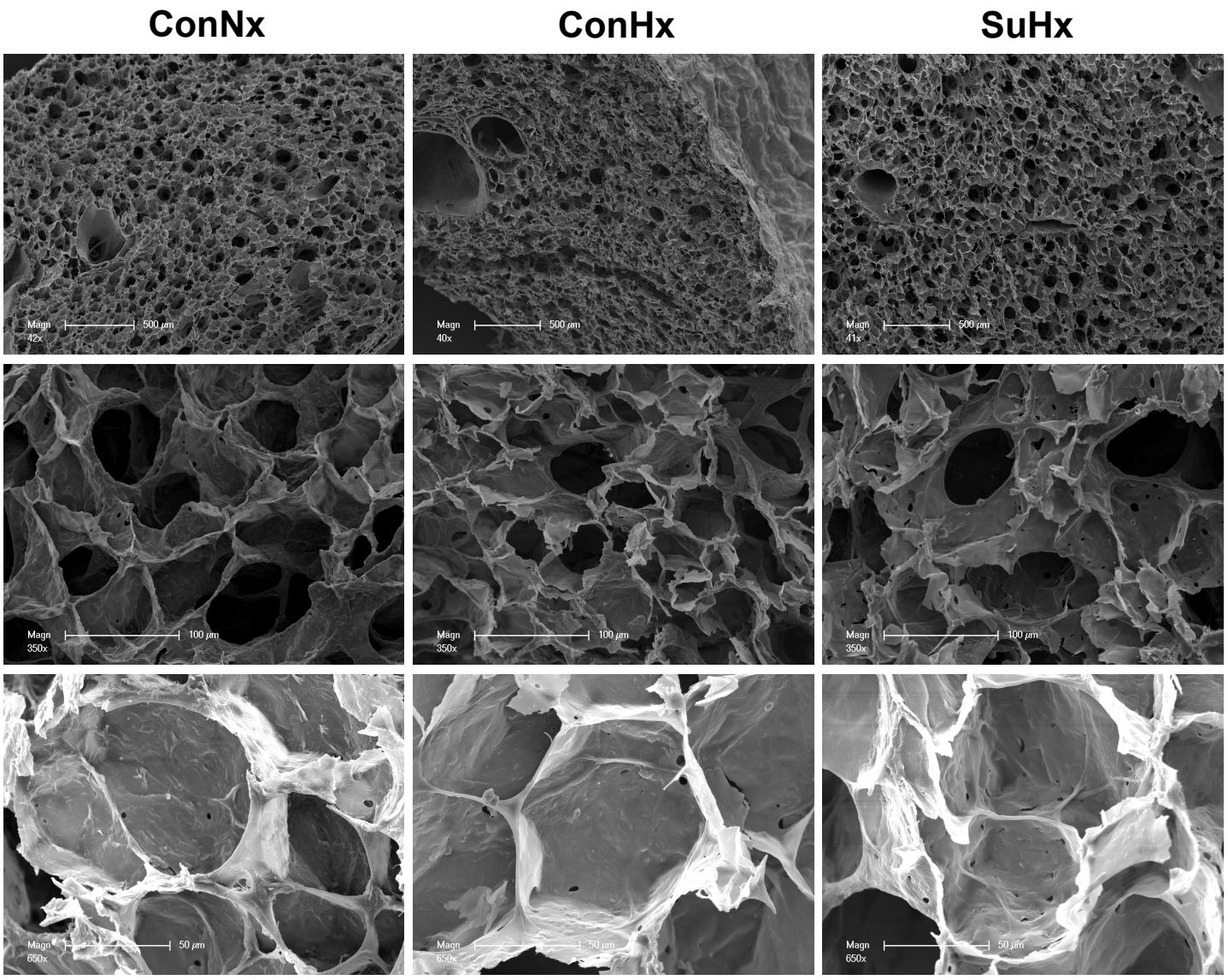
A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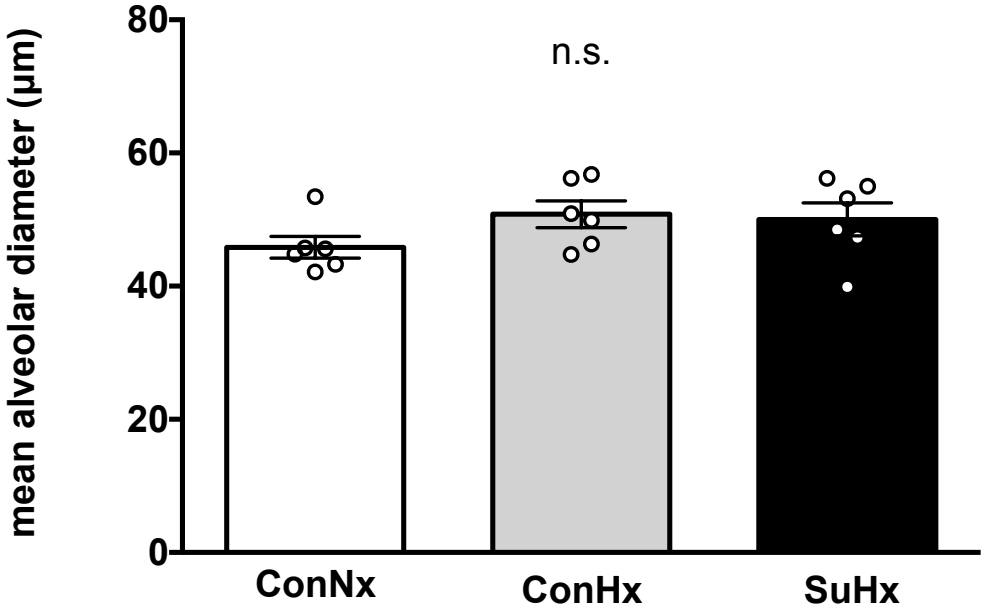
mean alveolar diameter (µm)
80
60
40
20
0
n.s.
ConNx
ConHx
SuHx