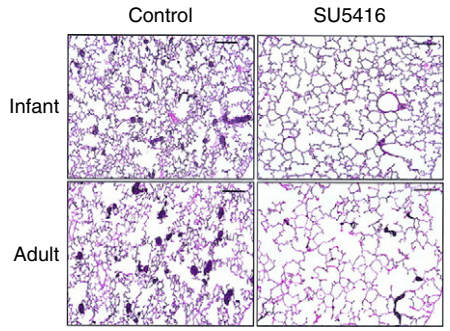

Figure 1. Effects of neonatal SU5416 treatment on lung histology in infant and adult rats. Lung histology of infant rats treated with a single dose of SU5416 on the first day of life showed alveolar simplification and decreased pulmonary arteries, which appear brown in color because of barium infusion. This pattern of reduced alveolar number and vessel density persisted into adulthood. Rats in this study were maintained in room air at Denver's altitude (5,280 ft above sea level) and not exposed to hypoxia. Scale bars, 100 μm. Reprinted by permission from Reference 6.

susceptibility for chronic lung disease, especially with a late secondary injury such as tobacco smoke, pollution, vaping, or other insult. ■

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Timothy D. Le Cras, Ph.D.*
University of Cincinnati School of Medicine
Cincinnati, Ohio
and
Cincinnati Children's Hospital
Cincinnati, Ohio

Steven H. Abman, M.D.
University of Colorado Anschutz Medical Center and Children's Hospital Colorado
Aurora, Colorado

*Corresponding author (e-mail: tim.lecras@cchmc.org).

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The Adult Sprague-Dawley Sugen-Hypoxia Rat Is Still "the One:" A Model of Group 1 Pulmonary Hypertension: Reply to Le Cras and Abman

From the Authors:

Kojonazarov and colleagues recently reported severe emphysema in the SU5416/hypoxia (SuHx) rat model of pulmonary hypertension (PH) (1). The authors found that adult male Wistar Kyoto (WKY) rats had an increased air-to-tissue ratio as judged by nongated *in vivo* microcomputed tomography, and an increased mean linear intercept (MLI) as a surrogate for emphysema (1, 2). Le Cras and Abman now respond to Kojonazarov and colleagues' report by underlining the "important role of the developmental timing of disrupted VEGF signaling." They cite earlier studies conducted on ovine fetuses that showed that VEGF inhibition caused vascular remodeling, a reduction in vascular and airway growth, and neonatal PH at birth (3).

Although the VEGFR inhibitor SU5416 is known to induce emphysema in rats housed in room air at Denver altitude (1,609 m altitude) (4, 5), we contended in our response letter (6) 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 rather to pulmonary arterial hypertension (PAH) due to severe angioproliferative remodeling (7, 8). A similar degree of PH without apparent alveolar simplification was seen when VEGF blockade was administered *in utero* to fetal or neonatal sheep (3). *In utero*, P_{O_2} is approximately 19 mm Hg in the fetal pulmonary artery and 34 mm Hg in the umbilical vein (maximum systemic oxygenation), which represent hypoxemic/hypoxic values for newborns after postnatal cardiopulmonary adaptation. Thus, it may not be surprising that VEGF blockade *in utero* causes

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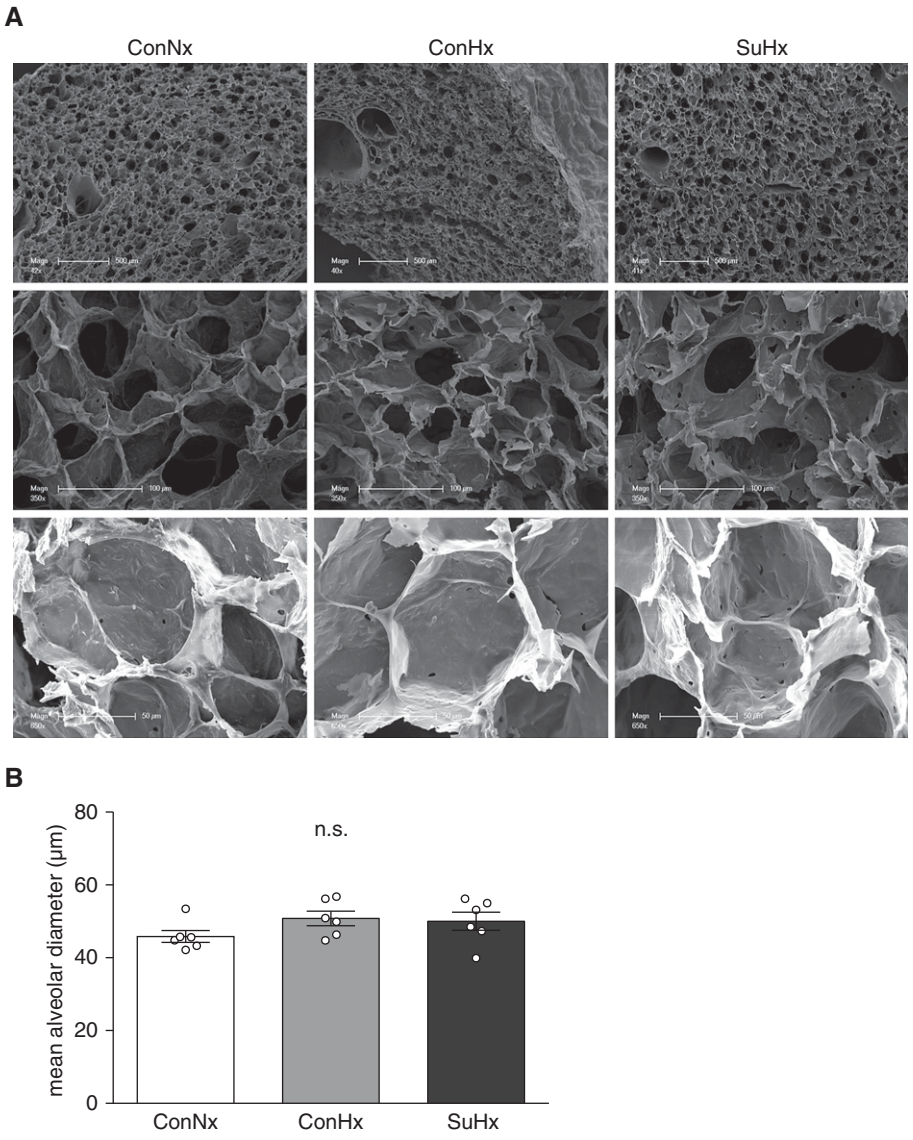

Figure 1. Scanning electron microscopy does not reveal any significant emphysema in the adult Sprague-Dawley Sugen-hypoxia (SuHx) rat model of pulmonary arterial hypertension. (*A*) Scanning electron micrographs illustrate the alveolar architecture of adult rats subdivided into three experimental groups. The study design is described in Reference 8. Sprague-Dawley rats (6–8 wk old, ∼180–200 g) were purchased from Charles River and divided into three 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] and then exposed to chronic hypoxia [$F_{IO_2} = 0.1$, $CO_2 < 10.000$ ppm] for 3 wk, followed by a 6-wk period in room air [$F_{IO_2} = 0.21$]); and *3*) SuHx (i.e., rats injected with the VEGFR inhibitor SU5416 [Sigma, 20 mg/kg/dose subcutaneously dissolved in DMSO] and subsequently exposed to chronic hypoxia [3 wk], followed by 6 wk of room air). The rat lungs were perfused *in vivo* by injecting a total of 50 ml of normal saline into the beating right ventricle. After perfusion, the heart and lungs were taken out en bloc. The left lung lobe was ligated and snap-frozen in liquid nitrogen, and the right lobes were tracheally inflated with 10% formalin at a standardized pressure of 25 cm H_2O for at least 5 minutes 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. Scale bars: top, 500μm; middle, 100μm; bottom, 50μm. (*B*) A morphometric analysis showed that the mean alveolar diameters did not differ significantly among the three groups (ConNx [45.8 ± 1.6 μm], ConHx [50.8 ± 2.0 μm], and SuHx [50.0 ± 2.5 μm] animals), in both nonparametric (Kruskal-Wallis/Benjamini-Krieger-Yekutieli) and parametric (ANOVA/Bonferroni *post hoc*) statistical tests and multiple comparisons. Mean alveolar diameters were determined by morphometric image analysis (Scandium, Olympus Soft Imaging Solutions) of scanning electron micrographs of different groups ($n = 6$ rats per group), with 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 with the approval of the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (#15/2022, #13/1328; LAVES). n.s. = not significant.

severe PH in fetal or neonatal sheep. The lack of significant alveolar simplification in this fetal model (with low systemic arterial P_{O_2}) is consistent with earlier observations in adult hypoxic rats when VEGFR blockade did not cause emphysema in the setting of hypoxia (7).

As discussed by the authors, the WKY rat strain (Janvier Labs) (1, 2) may be more prone to emphysema after SuHx exposure than other strains (6, 9). In contrast, only a mild increase in MLI (+18%) was seen in adult male SuHx-SD rats obtained from Harlan (6), and no emphysema was found in male SD rats obtained from Charles River (6, 8). Of note, even in WKY rats, emphysema is not a universal finding after exposure to SuHx (10).

Although differences among 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 and colleagues (1, 2) used nongated *in vivo* chest micro–computed tomography scans—a method that is fundamentally different from *ex vivo* MLI measurements. To perform a quantitative three-dimensional (3D) analysis of the lung parenchyma in SuHx-SD rats (8), we used scanning electron microscopy. 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 because 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 analysis of our own SuHx rat studies (6), now including 3D scanning electron microscopy (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 (4). At most, there may be mild enlargement of distal intraalveolar spaces in this rat substrain, depending on the method used for tissue fixation and the timing of the lung harvest.

Thus, we conclude that exposure of adult rats to SuHx 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 SD strain (Figure 1) (8). ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Harm J. Bogaard, M.D., Ph.D.*
Amsterdam UMC
Amsterdam, the Netherlands

Ekaterina Legchenko, Ph.D.*
Hannover Medical School
Hannover, Germany

Maximilian Ackermann, M.D.*
Johannes Gutenberg University Mainz
Mainz, Germany
and
University Clinics of the Heinrich-Heine-University Düsseldorf
Düsseldorf, Germany

Mark P. Kühnel, Ph.D.
Danny D. Jonigk, M.D.
Hannover Medical School
Hannover, Germany

Ketul R. Chaudhary, Ph.D.
Ottawa Hospital Research Institute
Ottawa, Canada
and
University of Ottawa
Ottawa, Canada

Xiaoqing Sun, Ph.D.
Amsterdam UMC
Amsterdam, the Netherlands

Duncan J. Stewart, M.D.
Ottawa Hospital Research Institute
Ottawa, Canada
and
University of Ottawa
Ottawa, Canada

Georg Hansmann, M.D., Ph.D.‡
Hannover Medical School
Hannover, Germany

ORCID IDs: 0000-0001-5371-0346 (H.J.B.); 0000-0001-7949-2973 (E.L.); 0000-0001-9996-2477 (M.A.); 0000-0003-3558-2576 (M.P.K.); 0000-0002-5251-2281 (D.D.J.); 0000-0003-1725-7438 (K.R.C.); 0000-0003-1914-1500 (X.S.); 0000-0002-9113-8691 (D.J.S.); 0000-0003-0709-3935 (G.H.).

*H.J.M., E.L., and M.A. contributed equally as first authors.
‡Corresponding author (e-mail: georg.hansmann@gmail.com).

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Limited Assessment of Respiratory Muscle Response to Nusinersen Treatment in Infants with Spinal Muscular Atrophy

To the Editor:

Optoelectric plethysmography is a novel and interesting method of assessing breathing patterns and thoracoabdominal paradox in children with spinal muscular atrophy (SMA). LoMauro and colleagues should be commended for their efforts to quantify breathing parameters in this group of children (1). Respiratory assessment of infants with SMA is important and necessary to better understand the effects of treatments on respiratory muscle strength and respiratory function (2).

Although LoMauro and colleagues did show a reduction in thoracoabdominal paradox in patients with SMA type 1C treated with nusinersen compared with untreated patients with type 1C, the patient groups they compared may not be equivalent. The treated group had higher SMN (survival motor neuron) copy numbers, which is associated with better outcomes and a milder clinical course. Patients in the treated group were significantly older than those in the untreated group, with treated patients with type 1C being the oldest of all. It is difficult to distinguish the effects of disease treatment from those of growth and development in the absence of appropriate reference data, especially considering that chest wall compliance and thoracoabdominal paradox decrease with age. Despite these differences between the patient groups, there is little doubt about the potential value of optoelectric plethysmography for providing objective outcome measures in clinical or epidemiological research studies involving infants with SMA. However, it is generally agreed that no single lung function test will ever provide the answer and that a combination of tests is required. Other respiratory assessment tools are available for children in this age group, including the widely available thoracic and abdominal effort respiratory impedance plethysmography bands, which are frequently incorporated into polysomnography recordings and record respiratory rate and thoracoabdominal paradox. It would be very interesting to compare these two methods of assessment in young children with SMA for use as clinical trial outcomes. In addition, oscillometry in children with SMA has been shown to provide valuable information and may be useful for assessing response to therapeutic interventions such as disease-modifying agents, including nusinersen (3).

One of the dangers of publishing incomplete data on respiratory outcomes in young patients with SMA is the potential risk that medical insurance companies or funding bodies will use these data to deny reimbursement for or access to treatment with a potentially life-saving medication. Thus, although LoMauro and colleagues have added valuable information regarding the respiratory effects of nusinersen in infants with SMA, we caution against concluding that types 1A and 1B did not show a treatment response based on optoelectric plethysmography alone. Major gaps in our knowledge and understanding of treatment response in SMA remain. ■

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Archana Chacko, M.B. B.S.*
Queensland Children's Hospital
Brisbane, Australia
and
University of Queensland
Brisbane, Australia

Peter D. Sly, M.B. B.S., M.D., D.Sc.
University of Queensland
Brisbane, Australia

Leanne Gauld, M.B. B.S., Ph.D.
Queensland Children's Hospital
Brisbane, Australia
and
University of Queensland
Brisbane, Australia

*Corresponding author (e-mail: archana.chacko@health.qld.gov.au).

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Reply to Chacko *et al.*

From the Authors:

We thank Chacko and colleagues for their interest in our study (1). We agree that multiple factors must be considered when discussing

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