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SNO content (the bioactive species in RBCs) and levels of fetal and embryonic hemoglobins (which may contain enough compensatory βCys93-SNO to confound interpretation of the mouse model). Also missing are assessments of NOS isoform expression in the microcirculation and in RBCs (which may also complicate interpretation of the results), levels of *S*-nitrosoglutathione (GSNO) reductase (which determine the amounts of low-molecular-weight SNO in RBCs that are reportedly elevated in the βCys93-mutant mouse and which influence systemic hemodynamics), repeated assessments of hemodynamics (which may change as transgenic mice age) and, more generally, comparisons with responses in non-humanized mice (no wild-type mice or RBCs were studied), a crucial control for all experiments. Notably, increases observed in both hemoglobin concentration and the amount of low-molecular-weight SNO in the βCys93-mutant mouse suggest a deficit in tissue O_2 delivery, as these are established mechanisms by which mammals adapt to hypoxia[12,13].

βCys93 is highly conserved in all mammals and birds, and as Perutz[14] has noted, this invariance, like that of the proximal histidine required for O_2 binding, implies it has an essential function. Moreover, vasodilation is not the only function that has been ascribed to SNO-βCys93: SNO-Hb seems to be involved in the central respiratory drive and in the chronic response to hypoxia[2,5,12]—collectively reflecting a central role for SNOs in the respiratory cycle. Unlike the mice in this study, humans evidently cannot survive homozygous βCys93 mutation. Moreover, defects in SNO-Hb are implicated in a range of cardiovascular and pulmonary disorders, including in sickle cell disease, heart failure, diabetes and hypoxic pulmonary hypertension and in transfusion-related morbidity. The work of Isbell *et al.*[1] neither has an impact upon these clinical findings nor supports the statement that "…allosteric regulation of SNO-Hb bioactivity is not an essential component of the physiologic mechanisms that control pulmonary blood flow" or the claim that "SNO-Hb is not essential for the physiological coupling of erythrocyte deoxygenation with increased NO bioactivity *in vivo*, [nor is it involved] in systemic or pulmonary hemodynamics." Specific and rigorous experiments are needed to properly investigate the role of hemoglobin and RBCs in blood flow and of SNO-Hb in O_2-regulated dispensing of NO bioactivity, and it is not clear that the humanized mice will be suitable for such investigations.

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To the Editor:

Isbell *et al.*[1] studied hypoxic blood pressure responses in an elegant model in which the mouse hemoglobin β-chain was replaced with human hemoglobin β-chain with or without an alanine substitution for the cysteine at position 93 (βCys93). However, the role of a nitric oxide (NO)-thiol (*S*-nitrosothiol, SNO) modification of βCys93 in signaling oxyhemoglobin desaturation was not studied in these mice. NO transfer between thiols during erythrocytic oxyhemoglobin desaturation signals altered vascular structure and gene expression in chronic hypoxia[2], increased minute ventilation (physiological ventilatory response) in acute hypoxia[3] and matching of acute hemoglobin desaturation to local blood flow[4]. None of these responses was studied; instead, acute blood pressure changes were measured in normoxic mice. These changes do not reflect control of regional blood flow and would not be predicted to change in the βCys93-mutant mouse. Hence, the experiments reported provide no *in vivo* information about hypoxic responses mediated by βCys93.

Moreover, assuming that human β-chain hemoglobin behaves in the mouse as it does in the human, the presence of <1% prenatal mouse hemoglobin could normalize responses: the normal erythrocytic ratio of SNO to hemoglobin is less than 1:1,000 (ref. 4), so if 10% of fetal hemoglobin were SNO modified, the mutant mouse could have SNO-βCys93–mediated responses. Furthermore, concerns about the validity of the iodine-based assay employed in this paper remain, despite attempts at its validation[5,6]. Finally, the results shown in the vascular ring studies suggests that the control erythrocytes with an unmutated human hemoglobin β-chain were SNO depleted. Specifically, both control and βCys93-mutant erythrocytes caused minimal responses compared to those in previously published experiments, either because of the rapid *ex vivo* decay of the deoxyhemoglobin βCys93-SNO bond[2,7,8] or because the rings were not healthy.

The importance of SNO-modified βCys93 in signaling oxyhemoglobin desaturation in human blood is well established[2–5,7,8]. All of the oxyhemoglobin desaturation–signaled responses that would be predicted to be abnormal in the βCys93-mutant mouse were left untested.

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Patel and Townes reply:

In response to our recent paper[1], Stamler *et al.* state that "an extensive body of evidence from our own and other laboratories supports a major role for SNO-Hb [*S*-nitrosohemoglobin] (in which a nitric oxide (NO) group is covalently coupled to Cys93 within the β-chain [βCys93]) in the physiological response of hypoxic vasodilation…"