# Extrapulmonary Effects of Inhaled Nitric Oxide
## Role of Reversible S-Nitrosylation of Erythrocytic Hemoglobin

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**Early applications of inhaled nitric oxide (iNO), typically in the treatment of diseases marked by acute pulmonary hypertension, were met by great enthusiasm regarding the purported specificity of iNO: vasodilation by iNO was specific to the lung (without a change in systemic vascular resistance), and within the lung, NO activity was said to be confined spatially and temporally by Hb within the vascular lumen. Underlying these claims were classical views of NO as a short-lived paracrine hormone that acts largely through the heme groups of soluble guanylate cyclase, and whose potential activity is terminated on encountering the hemes of red blood cell (RBC) Hb. These classical views are yielding to a broader paradigm, in which NO-related signaling is achieved through redox-related NO adducts that endow NO synthase products with the ability to act at a distance in space and time from NO synthase itself. Evidence supporting the biological importance of such stable NO adducts is probably strongest for S-nitrosothiols (SNOs), in which NO binds to critical cysteine residues in proteins or peptides. The circulating RBC is a major SNO reservoir, and RBC Hb releases SNO-related bioactivity peripherally on $O_2$ desaturation. These new paradigms describing NO transport also provide a plausible mechanistic understanding of the increasingly recognized peripheral effects of inhaled NO. An explanation for the peripheral actions of inhaled NO is discussed here, and the rationale and results of attempts to exploit the "NO delivery" function of the RBC are reviewed.**

**Keywords:** hemoglobin; nitric oxide; red blood cell; S-nitrosohemoglobin; S-nitrosothiol

Clinical and mechanistic reports on the therapeutic use of inhaled nitric oxide (iNO) in a variety of settings have uncovered a surprisingly wide array of changes outside the intended targets (Table 1). These findings contrast with the early reports and ideas about iNO. Specifically, iNO was touted early on for its perceived specificity: vasodilation by iNO was specific to the lung (without a change in systemic vascular resistance), and within the lung, NO activity was said to be confined spatially and temporally by virtue of dead-end reactions with red blood cell (RBC) Hb within the vascular space (1, 2). The increasingly recognized peripheral effects of iNO (Table 1) are typically dose-dependent and can take place in the absence of any change in systemic hemodynamics. Cell-specific effects go well beyond relaxation of vascular smooth muscle, and include inhibition of platelet aggregation, inhibition of leukocyte adhesion and ablumenal migration, and increases in renal glomerular filtration rate (Tables 1 and 2). Such peripheral actions of iNO are not well rationalized by classical paradigms of NO biology. In addition, early interpretations of iNO use highlighted the fundamental problem of how upstream resistance vessels in the lung could respond to a vasoactive gas that enters the body via the alveolar–capillary interface. Some investigators had suggested that iNO may bind to circulating targets and, in subsequent circulatory cycles, contribute to pulmonary vasodilation in patients with pulmonary hypertension. New insights into the biological significance and molecular workings of stable, circulating NO adducts and their interactions with RBCs have provided a new framework within which to reasonably reinterpret the literature showing peripheral effects in patients breathing NO and NO donors. A complete analysis of the extrapulmonary effects of iNO is beyond the scope of this review; herein, we focus on those effects that may be mediated by circulating erythrocytes (Table 1).

### STABILIZATION AND HARNESSING OF NO BIOACTIVITY BY REACTION WITH Hb: *S*-NITROSOHEMOGLOBIN

In 1996, Stamler and coworkers reported on the discovery of a novel activity of Hb: in its R (oxygenated) structure, Hb binds NO at its reactive and highly conserved Cys-β93 residues, forming S-nitrosohemoglobin (SNO-Hb) (3). In one biologically relevant and exemplary route to the formation of SNO-Hb *in vivo*, molecular $O_2$ serves not only to trigger the necessary allosteric transition to the R structure (by binding to vacant hemes) but also acts as the electron acceptor necessary for S-nitrosothiol (SNO) formation in Hb (Figure 1) (4). Under physiologic conditions, SNO-Hb is produced in NO–Hb interactions in quantities and on time scales that compete favorably with those of methemoglobin and heme-iron nitrosyl hemoglobin, which were classically viewed as the terminal and sole products of reactions between NO and Hb (4, 5). Interactions between NO and Hb are highly complex, and are governed not only by Hb $O_2$ saturation (as dictated by $O_2$ tension; Figure 2) but also by local redox conditions and other allosteric effectors. Thus, RBC Hb senses and transduces local allosteric and redox gradients into context-sensitive quenching or dispensing of NO bioactivity (reviewed in Reference 6).

### VASOACTIVITY COUPLED TO RELEASE OF (S)NO FROM Hb

Conversely, and in accordance with principles of thermodynamic linkage, the NO group equivalent is released from Hb coincident with the allosteric transition to the T structure of Hb, as triggered by deoxygenation. Functionally, this is seen as vasodilation by SNO-Hb at low $Po_2$, in contrast to the vasoconstriction induced by unmodified Hb in a manner essentially independent of $Po_2$ (3, 7, 8).

### NOVEL NO-DEPENDENT FUNCTION OF THE RBC: BLOOD FLOW REGULATION

Given the allosterically controlled bioactivity of SNO-Hb, it was predicted that, in hypoxia, RBCs would be capable of eliciting

(*Received in original form July 13, 2005; accepted in final form September 7, 2005*)

Supported by the Institute for Medical Research, Inc. (Durham Veterans Affairs Medical Center) and the American Lung Association (RG-1326-N; T.J.M.); also supported by NIH/NIGMS 1K08GM069977 and UVa Children's Medical Center (A.D.).

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**Proc Am Thorac Soc Vol 3. pp 153–160, 2006**
**DOI: 10.1513/pats.200507-066BG**
**Internet address: www.atsjournals.org**

**TABLE 1. EXTRAPULMONARY EFFECTS OF INHALED NITRIC OXIDE**

| Organ | Effect of iNO | Species | Reference |
|---|---|---|---|
| CNS | ↑ Cerebral blood flow | Swine | 66, 67 |
| | CSF [NOx] ↑ proportionally with ↑ iNO dose | Swine | 67 |
| | Alters EEG | Human | 68 |
| | ↑ Neurodevelopmental outcome in premature infants | Human | 69 |
| Heart | Attenuates LV dysfunction during sepsis | Swine, rat | 70–73 |
| | ↑ Coronary artery patency after thrombolysis | Dog | 55, 74 |
| | ↓ Infarction size and LV dysfunction after ischemia–reperfusion injury | Mouse, rat | 57, 58 |
| | ↓ Myocardial injury during cardiopulmonary bypass | Human | 75 |
| Peripheral vasculature | Alters regional blood flow remote from lung | Human, sheep, dog, rat | 15, 17, 76–80 |
| Kidney | ↑ Renal blood flow, GFR, and urine volume alter tubular salt and water resorption, no effect on CCr | Swine | 81 |
| | | Human | 82 |
| Bowel | ↓ Leukocyte adhesion in microcirculation during sepsis and ischemia–reperfusion | Rat, cat | 54, 56, 59 |
| | ↑ Mesenteric blood flow after ischemia–reperfusion | Cat | 61 |
| Pancreas | ↓ Trypsinogen activation during pancreatitis | Rat | 83 |
| Liver | Improves hepatic tissue oxygenation | Human | 84 |
| | ↑ Hepatic nitrotyrosine | Rat | 85 |
| Coagulation system | ↓ Platelet aggregation | Human | 86–89 |
| Immune system | PMN respiratory burst attenuated | Human | 90 |
| | ↓ Pulmonary PMN sequestration during ECMO | Swine | 91 |
| | ↓ Platelet–leukocyte interactions in ARDS | Human | 88 |

*Definition of abbreviations*: ARDS = acute respiratory distress syndrome; CCr = creatinine clearance; CNS = central nervous system; CSF = cerebrospinal fluid; ECMO = extracorporeal membrane oxygenation; EEG = electroencephalogram; GFR = glomerular filtration rate; iNO = inhaled NO; LV = left ventricular; NOx = nitrite and nitrate; PMN = polymorphonuclear neutrophil.

vasodilation. We tested this prediction in isolated aortic rings exposed to "hypoxia" like that encountered normally in respiring tissues ($Po_2$, $\sim$ 7 mm Hg) and in the arterioles perfusing these tissues (7, 9). Thus, aortic rings serve here as an experimental model of hypoxic vasodilation, which under physiologic conditions is an activity of the resistance arterioles that regulate blood flow. These experiments revealed a novel activity of the RBC: the RBC regulates its own principal function—that is, $O_2$ delivery. Specifically, RBCs elicit graded vasorelaxation that is an inverse function of Hb $O_2$ saturation (Figure 2B) across a range of Hb $O_2$ saturations encountered normally. We emphasize the distinction of "RBC-derived relaxing factor" activity from that of endothelium-derived relaxing factor, or NO itself, in several regards. RBC-derived relaxing factor activity is independent of NO synthase (NOS) activity (7), and similarly, hypoxic vasodilation in humans is NOS independent (10). Vasodilation in hypoxia is a function of Hb $O_2$ saturation (Figure 2C), rather than $Po_2$ *per se*. Taken together, these properties of the physiologic adaptation known as hypoxic vasodilation are best rationalized as mediated by the RBC itself. Indeed, although hypoxic vasodilation can be demonstrated in RBC-free (buffer-bathed) vessel preparations, that response is potentiated in the presence of RBCs, and is qualitatively and temporally distinct from the hypoxic vasodilator response *in vivo*, which takes place on a second-to-second basis. Under some conditions, RBC-mediated vasorelaxation involves the activation of soluble guanylate cyclase (11).

## RELEVANCE TO CIRCULATING HUMAN BLOOD UNDER NORMAL CONDITIONS

Experiments were performed to test the hypothesis that changes in $Po_2$ (and hence in Hb $O_2$ saturation) within the normal human circulation are sufficient to shift the equilibrium between the binding of NO to either thiols or hemes as RBCs traversed either the arterial or venous limbs of the human circulation. A preponderance of SNO-Hb was found in oxygenated arterial blood from normal humans, whereas in venous blood, iron nitrosylhemoglobin (Hb[Fe]NO) predominated and SNO-Hb levels

**TABLE 2. COMPARISON OF THE EFFECTS OF INHALED NITRIC OXIDE WITH THOSE OF RED BLOOD CELL–DERIVED NITRIC OXIDE BIOACTIVITY AND ENDOTHELIAL NITRIC OXIDE SYNTHASE–DERIVED NITRIC OXIDE ON NORMAL PHYSIOLOGIC PARAMETERS**

| Biological Effect | RBC-SNO | iNO | eNOS-NO | Reference |
|---|---|---|---|---|
| Regulation of vascular tone | | | | |
| Shear stress | No | No | Yes | 92, 93 |
| Hypoxic (peripheral) vasodilation | Yes | Yes | No | 3, 6, 8, 10, 15, 17 |
| Hypoxic pulmonary vasoconstriction | Yes | Yes | No* | 12, 94, 95 |
| $O_2$ delivery to tissues | Yes | Yes | Yes | 7, 11, 40, 96, 97 |
| Modulation of leukocyte adhesion and migration | ? | Yes | Yes | 54, 56, 73, 88, 90, 98, 99 |
| Inhibition of platelet aggregation | Yes | Yes | Yes | 86–88, 90, 100, 101 |

*Definition of abbreviations*: eNOS-NO = endothelial nitric oxide synthase–derived nitric oxide; iNO = inhaled nitric oxide; RBC-SNO = red blood cell S-nitrosothiol.

* eNOS-NO *counteracts* hypoxic pulmonary vasoconstriction.

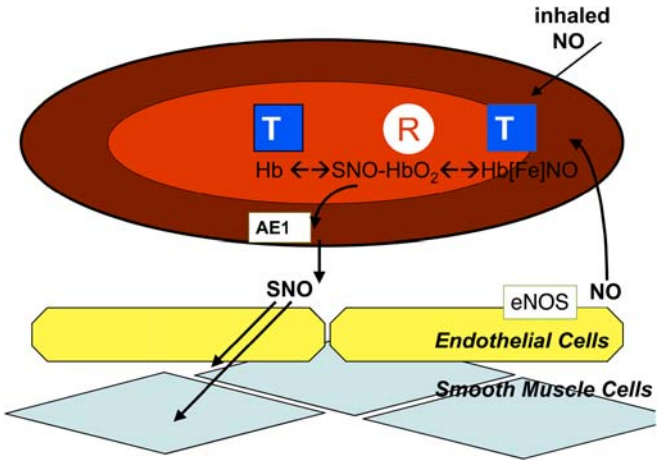


*Figure 1.* Schematic summary of NO–Hb interactions relevant to remote delivery of native (e.g., derived from NO synthase) or administered (e.g., inhaled) NO by the red blood cell (RBC). Within the RBC, S-nitrosohemoglobin (SNO-Hb) is formed by one of several possible reactions, including via intramolecular transfer of NO from heme to thiol within Hb, as shown here. Such S-nitrosylation of Hb is favored in the R (relaxed, oxygenated) conformation of Hb. Deoxygenation of Hb induces transition to the T structure, promoting the interaction of Hb with the RBC membrane protein anion exchanger-1 (AE1), which in turn accepts SNO groups in transfer from Hb (102). SNO-related bioactivity is then exported from the RBC membrane, but the precise identity of the exported species remains undetermined. In addition, the precise molecular targets in the vessel wall and tissues are unknown.

were low (7). To further test whether this oxygen-dependent distribution of NO disposition within human Hb was allosterically governed, we measured SNO-Hb and Hb[Fe]NO in blood from humans alternately exposed to hypobaric hypoxia or hyperbaric hyperoxia, respectively. The results indicated that when the R structure was promoted in Hb (via experimental hyperoxia) SNO-Hb predominated in not only arterial but also central venous blood, whereas under conditions (hypobaric hypoxia) that promoted the T structure in both arterial and central venous blood, Hb[Fe]NO was prominent in blood from both sites (7). Other laboratories have also independently documented oxygen-dependent circulatory gradients in SNO-Hb (11–13). Funai and coworkers confirmed this principle in the human placentofetal circulation (14), where umbilical venous blood (flowing toward the fetus) is oxygenated and rich in SNO-Hb, whereas umbilical arterial blood (flowing away from the fetus and toward the mother) is relatively deoxygenated and lower in SNO-Hb content (14).

## SNO-Hb VERSUS OTHER CANDIDATE CARRIERS IN THE PERIPHERAL EFFECTS OF iNO

Direct evidence for the formation of SNO-Hb during inhalation of NO has been problematic and difficult to interpret. Gladwin and coworkers used inhaled NO as an investigative tool designed to test the concept that peripheral delivery of NO equivalents can take place, and to identify the mechanism. They administered iNO at 80 ppm to normal human volunteers and measured changes in arterial and venous blood Hb[Fe]NO, SNO-Hb, and nitrite (15–17). Increases in SNO-Hb levels were shown but did not reach statistical significance (18). Unfortunately, the chemical reduction techniques used to provide those measurements have more recently been shown to be insensitive to SNOs in the

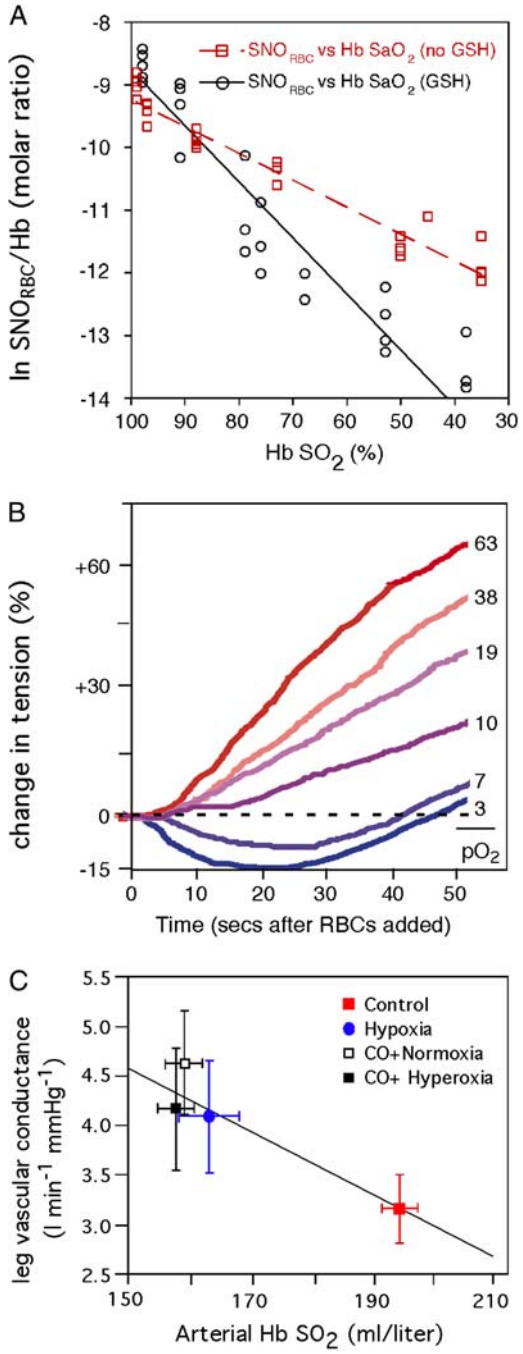


*Figure 2.* RBC-SNO content (*A*, $SNO_{RBC}$), RBC bioactivity (*B*), and human peripheral blood flow (*C*) are governed by Hb $O_2$ saturation. (*A*) RBCs from normal humans with (*black*) or without (*red*) extracellular glutathione (GSH) were steadily deoxygenated under inert gas (argon). The natural logarithm of the ratio of $SNO_{RBC}$ to Hb was linearly dependent on Hb $O_2$ saturation. Extraerythrocytic GSH was included as a covariate, and the two lines generated demonstrate the relative acceleration of $SNO_{RBC}$ decay when GSH is present (12). (*B*) RBCs induce graded relaxation inversely related to Hb $O_2$ saturation, recapitulating hypoxic vasodilation (7). Tissue bath $P_{O_2}$ (shown at *right*) was varied across the physiologic range, and vascular responses to RBCs were studied; the resulting saturations are represented by the *tracing colors*, and span the visible spectrum from *red* (oxy, > 90% Hb $SO_2$) to *purple* to *blue* (deoxy, < 40% Hb $SO_2$). (*C*) Leg vascular conductance (reflecting regional blood flow) increases as blood $O_2$ content falls (hypoxic vasodilation). Hb $O_2$ saturation and thus arterial blood $O_2$ content were manipulated by CO exposure ± varying $F_{I_{O_2}}$ (hypoxia and hyperoxia). Neither vascular conductance nor blood flow correlated with blood $P_{O_2}$ *per se*. Reprinted by permission from Reference 10.

presence of significant background heme concentrations (such as those present in the samples studied) (19). Although the authors argued that the arterial–venous gradients seen in iron nitrosylhemoglobin (Hb[Fe]NO) supported the claim that NO is transported to tissues by Hb[Fe]NO, this species does not in fact produce vasodilation because it cannot release NO (20). This limitation applies likewise to more recent claims (21, 22) that the "nitrite reductase" activity of Hb may endow RBC Hb with NO-related bioactivity: the Hb[Fe]NO produced in this reaction cannot and does not release NO radical. Indeed, work modeling the behavior of NO generated within the RBC, via the nitrite reductase function of Hb, was interpreted to indicate that the product could not escape the RBC as NO radical (23). Rather, the species exported by the RBC in hypoxia may be a nitrosonium equivalent or SNO, and Luchsinger and colleagues demonstrated that SNO-Hb is formed as a product of nitrite reduction by Hb, with Hb[Fe]NO serving as a chemical intermediate (24). The relative importance of this particular route to SNO-Hb formation remains to be determined.

Finally, other laboratories have demonstrated that the intravascular delivery of NO solutions results in the transport and delivery of NO as SNOs along the vascular tree (25–27). All of these groups confirm alterations in regional blood flow remote from the site of NO administration.

## CONTROVERSY OVER BLOOD SNO-Hb LEVELS: METHODOLOGIC ISSUES

We and others have investigated the basis for the wide variation in reported differences in circulating levels and dynamic disposition of SNO-Hb. Reported concentrations have ranged from undetectable to as high as micromolar, expressed in terms of the concentration in whole human blood. In large part, these differences can be traced to a lack of sensitivity and specificity in certain techniques when applied to biological molecules and matrices (6, 12, 19, 28). We emphasize the need to replicate key findings using multiple, complementary methods. In one such instance, our group reported agreement on the levels of SNO-Hb in paired samples from normal human blood, using the fluorogenic NO scavenger 4,5-diaminofluorescein in an assay in which inorganic mercury is used to generate signal, and the photolysis–chemiluminescence method, in which a mercury-induced loss of signal is identified with the SNO moiety. These issues are reviewed in detail elsewhere (6, 19, 29).

## AUTOCAPTURE OF NO BY HEMES OF Hb IN CHEMICAL REDUCTION METHODS

In one widely used technique, triiodide-based chemical reduction is coupled with chemiluminescence detection of evolved NO. However, until recently it was not recognized that autocapture of NO by the hemes in Hb markedly depresses the quantum yield of NO recovery from SNOs when using this technique. Interestingly, the depressed recovery due to autocapture is particularly pronounced for SNO-Hb, likely owing to the intramolecular proximity of hemes to NO leaving the reactive thiol groups. Rogers and coworkers demonstrated that the efficiency of NO-equivalent recovery was inversely proportional to heme concentration in the reaction mixture, and that a modification of the mixture that blocked the hemes (ferricyanide pretreatment) preserved NO sensitivity (19). We provided evidence for a similar depression of NO recovery by another chemical reduction method, the cysteine–cuprous chloride method. Recovery was restored when the assay system included excess carbon monoxide, overwhelming the hemes so as to negate their ability to recapture released NO (12). By exploiting this technique to study SNO content in intact RBCs, a natural logarithmic relationship between RBC-SNO content and Hb $O_2$ saturation was revealed. This relationship was a function of the abundance of extracellular thiol capable of sustaining trans-S-nitrosylation (Figure 2A), supporting the premise that (S)NO may be exported from RBCs and reach specific cellular targets via serial transnitrosation reactions.

## BINDING AND REMOTE DELIVERY OF NO BY Hb IN DISEASE STATES

Given the allosteric governance of binding and peripheral delivery of NO equivalents by Hb, it may be predicted that allosterically modified Hb would differ from unmodified human Hb in its NO-dispensing functions. In fact, studies confirm that when Hb is either glycosylated (as in diabetes mellitus) or mutated genetically (e.g., in sickle cell hemoglobinopathy), both the concentrations of SNO-Hb and the functional correlate of these levels, namely RBC bioactivity, are altered under these conditions. In the case of diabetic Hb, nitrosylation is favored because glycosylation promotes the R structure in Hb (the equivalent of a leftward shift in the Hb $O_2$–binding curve for Hb) and, for similar reasons, offloading of (S)NO from Hb is disfavored, translating into reduced RBC bioactivity in hypoxia (11, 30). Furthermore, in patients with congestive heart failure, transpulmonary gradients of Hb-bound NO are evident and, interestingly, are inversely dependent on cardiac index. This suggests that as $O_2$ extraction increases in tandem with poor perfusion, Hb allostery–mediated transport and release of NO bioactivity by Hb would aid the resolution of perfusion insufficiency (13). Alterations in sickle cell Hb nitrosylation and denitrosylation are substantially more complex. Pawloski and coworkers discovered that the sickle cell erythrocyte is (*1*) defective in basal SNO-Hb concentrations, reflecting a disorder in the ability of Hb to use the molecular mechanisms that normally serve in SNO-Hb formation; (*2*) defective in the ability of RBC membrane constituents, such as the anion-exchange protein AE1 to sustain S-nitrosylation from SNO-Hb; and, consequently, (*3*) impaired in its ability to mediate hypoxic vasodilation (31).

## THE EXTREME CASE OF CIRCULATING SNOs: SEPSIS

Sepsis is characterized by nitrosative stress, reflecting overproduction of NO by the inducible form of NOS (32–34). Elevations in sepsis of circulating SNO-proteins (35, 36), including RBC SNO-Hb (12, 34, 37, 38), as well as elevated Hb[Fe]NO (39), have now been reported by multiple laboratories. Interestingly, in the case of Hb-NO accumulation in the RBC, these findings point to a protective function, rather than an exaggerated delivery function. Specifically, the Hb[Fe]NO that accumulates is predominantly in a form, the 5-coordinate α-heme-NO, from which NO release or transfer to the reactive Cys-β93 residue is essentially impossible. Oxygen dissociation from the 5-coordinate α-heme-NO is, however, favored (the $O_2$ dissociation curve is shifted rightward), so that $O_2$ delivery needs may be met without excessive vasodilation. Thus, interactions of excess NO with Hb lead to products that divert NO from producing toxicity.

## POTENTIAL CLINICAL APPLICATIONS OF THE PERIPHERAL EFFECTS OF iNO

### Implications of NO–Hb Interactions: Approaches to Disorders Involving Deficient NO Bioactivity

The revised model of the human respiratory cycle (Figure 3), involving remote delivery of NO equivalents, suggests novel approaches to the treatment of a variety of diseases. The unifying principle in such therapeutic development would be the harnessing

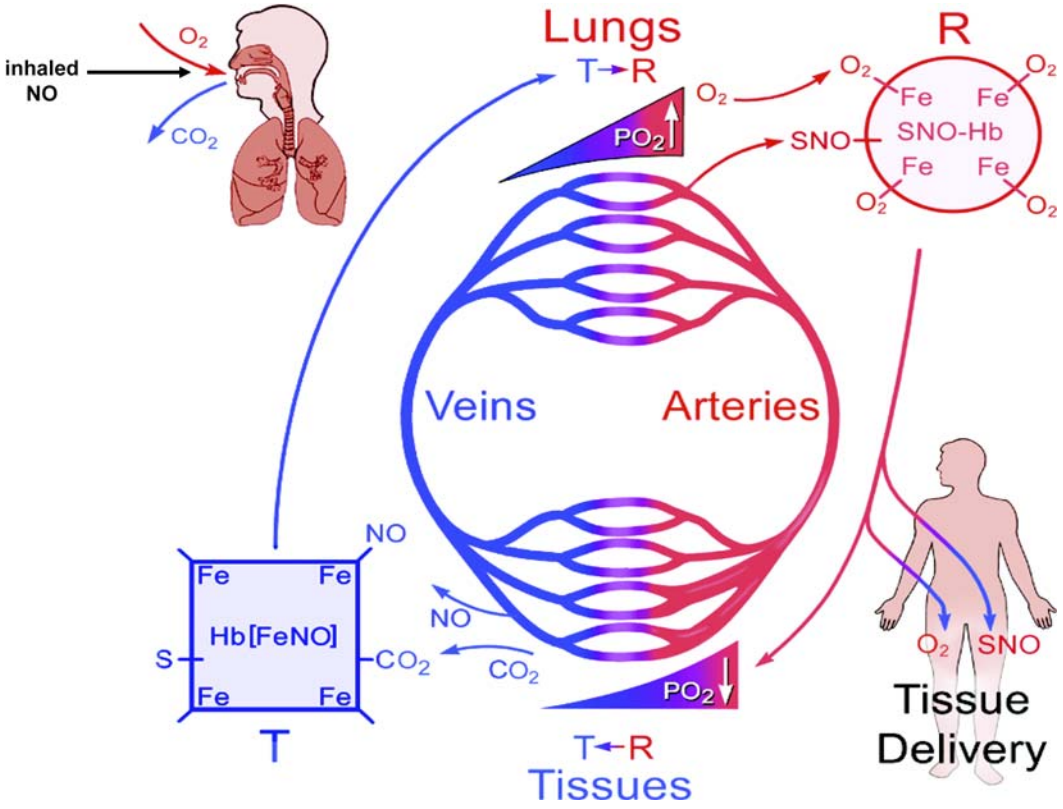


*Figure 3.* NO in the human respiratory cycle. RBC Hb alternates between R (oxygenated) and T (deoxy) states depending on $P_{O_2}$ (and other allosteric effectors, including $CO_2$). Blood oxygenation in the lung induces the R state, which in turns promotes Hb S-nitrosylation, forming SNO-Hb. The likely natural source of the NO group in SNO-Hb is endothelial NO synthase. RBCs taking up $O_2$ at the alveolar–capillary interface may take up inhaled NO (iNO) and process it in a similar manner to enable peripheral effects. In peripheral microvessels, RBCs offload $O_2$ as Hb senses falling $P_{O_2}$. This Hb $O_2$ desaturation also triggers the release of vasodilator SNO, increasing blood flow commensurate with metabolic demand (hypoxic vasodilation). Reprinted by permission from Reference 7.

of allosterically driven NO–Hb interactions that promote both SNO-Hb formation and the peripheral delivery of SNO bioactivity from the RBC. Importantly, given the potential remote activities of iNO, it is rational to postulate that NO-repleting strategies might successfully address pathologic lesions at sites distant from the lung (40).

Efforts are underway to take advantage of the NO transport functions of erythrocytic human Hb in the design of new treatments, including those for lesions at sites distant from the lung itself. Additional mechanistic and translational investigation is also needed, however, to develop a better understanding of the molecular approach to exploiting the NO delivery function of RBC Hb in the safest and most efficient manner. Specifically, a more defined picture of the NO/SNO deficiency states justifying repletion strategies by inhaled and other routes must be informed in each case by the redox environment signature for the disease under study. Such approaches should also be informed by new disease-specific knowledge of the functional state of key enzymes that increasingly appear critical to the expression of RBC-SNO bioactivities, which are discussed elsewhere (34, 41).

In addition, the ability to measure, in real time, changes in RBC-SNO content, flux, distribution (membrane vs. cytosol) and RBC function (e.g., vasoactivity), to predict outcomes or to serve as a surrogate for key clinical goals, may inform the therapeutic approach, both in real time in individual patients and in clinical trials of novel therapies. The development of widely applicable and reliable methods for the measurement of RBC-SNO (7, 12, 19) represents an important and promising opportunity in this regard.

### iNO in the Acute Chest Syndrome of Sickle Cell Disease

Intense interest has developed in the use of iNO to treat the complications of sickle cell hemoglobinopathy. Early reports that iNO raised the $O_2$ affinity of sickle cell Hb *in vivo* (42) in patients with sickle cell disease were later refuted (43). However, there is encouraging evidence, from animal models of sickle cell disease (44, 45) and preliminary human trials, that iNO therapy may be of benefit in sickle cell disease (16, 46–48). S-nitrosylation of either normal or sickle Hb clearly raises $O_2$ affinity, as predicted from consideration of thermodynamic linkage principles that govern the behavior of allosteric effectors. (If oxygenation favors S-nitrosylation, then S-nitrosylation should promote oxygenation [49, 50]). But the low incremental fraction of total RBC Hb that becomes S-nitrosylated with NO inhalation is too small to produce a significant change in Hb $O_2$ affinity.

Although iNO has been considered an attractive approach for treatment of the acute chest syndrome of sickle cell disease (51), this approach now deserves reappraisal. Specifically, data in humans with sickle cell disease show defects in the erythrocytic machinery that normally serves to transform NO, via Hb, into SNO-related bioactivity that is exported from the RBC to the vascular wall (31). Thus, in this disorder it may be most rational to aim to replete SNOs directly rather than to provide free NO. Further complicating this issue, hemolysis-generated circulating free Hb in patients with sickle cell disease may interfere with NO traffic between intact RBCs and cellular targets (31, 52, 53).

### iNO in Ischemia–Reperfusion Injury

There are encouraging preliminary animal data suggesting that iNO therapy may have salutary effects in the setting of ischemia–reperfusion injury (lung, heart, and bowel studied thus far) (54–62); these data may have important implications for the fields of transplantation medicine, trauma, and plastic surgery as well as for the care of specific conditions characterized by severe injury after vasoocclusion, such as in acute myocardial infarction, stroke, and acute tubular necrosis.

### Therapeutic Strategies Designed Specifically to Replete RBC SNO-Hb

Although hemoglobin clearly functions to promote S-nitrosylation from substrate NO, an agent that directly repletes tissue and circulating stores of SNOs might serve as a more physiologic NO donor for use when peripheral or pulmonary effects are an objective. Indeed, stable, bioactive SNOs constitute a large portion of the products of NOS activity within the lung and blood, and likewise recapitulate a broad spectrum of bioactivities classically attributed to NOS signaling (52). Furthermore, in those settings in which functional deficiencies of NO and SNOs are critical, a common coexisting problem is pulmonary dysfunction such as acute lung injury, leading to requirements for high concentrations of inspired oxygen. Here it would be advantageous to deliver an "NO donor" that is minimally reactive with molecular $O_2$ to avoid reactions with NO itself and thus to avoid the generation of injurious $NO/O_2$ reaction products such as peroxynitrite (63).

### SNO Repletion with Inhaled Ethyl Nitrite

Reasoning that direct repletion of SNOs in the lung and blood would reproduce local and distant benefits while avoiding the toxicity related to $NO/O_2$ reactions, Moya and coworkers administered gaseous ethyl nitrite (ENO) by inhalation in a porcine model of acute lung injury. Inhaled ENO (but not iNO) efficiently repleted lung SNOs, and lowered pulmonary vascular resistance and improved oxygenation dose-dependently (63). Interestingly, ENO also protected against a decline in cardiac output in these experiments. In human newborns with persistent pulmonary hypertension of the newborn, ENO inhalation likewise led to improvements in oxygenation and hemodynamics (64).

## CONCLUSIONS: LOADING AND DRIVING THE Hb CHARIOT OF NO BIOACTIVITY

The numerous extrapulmonary effects of iNO involve multiple cell types and organs. Stabilization of NO by erythrocytic Hb through the reversible S-nitrosylation reaction represents a likely mechanism for these effects. Therapeutic strategies that exploit this natural mechanism for remote, regulated delivery of NO bioactivity are rational and under active investigation. In the development of therapies exploiting the NO delivery function of RBC Hb (65), attention must be given to the molecular basis for disease-specific defects in NO bioactivity. For example, NO itself might be reasonably administered by inhalation, when the specific disorder is genuinely characterized by NO deficiency. But in disease settings in which there is derangement in the mechanisms that normally serve to channel NO into other, more stable bioactive adducts of NO such as SNOs, a strategy with the specific objective of direct SNO repletion may be preferable.

***Conflict of Interest Statement***: T.J.M. has served as a consultant to Nitrox, LLC, a company developing NO-based therapeutics. He also received research support ($106,000 total in 2004 and 2005) from Nitrox. A.D. received $1,500 in 2005 for serving on an advisory panel to INO Therapeutics in 2005 and received $20,000 from INO Therapeutics (as research support) for participating in clinical trials in 2005. He received $2,400 for consulting services to Nitrox, LLC, in 2004 and 2005, and received $98,000 in 2005 as research support from Nitrox, LLC for assay development.

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