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Arginine, nitric oxide, carbon monoxide, and endothelial function in severe malaria

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Abstract

Purpose of review—Parasitocidal therapy of severe falciparum malaria improves outcome, but up to 30% of these patients die despite best therapy. Nitric oxide is protective against severe disease, and both nitric oxide and arginine (the substrate for nitric oxide synthase) are low in clinical malaria. Parasitized red blood cell interactions with endothelium are important in the pathophysiology of malaria. This review describes new information regarding nitric oxide, arginine, carbon monoxide, and endothelial function in malaria.

Recent findings—Low arginine, low nitric oxide production, and endothelial dysfunction are common in severe malaria. The degree of hypoargininemia and endothelial dysfunction (measured by reactive hyperemia peripheral artery tonometry) is proportional to parasite burden and severity of illness. Plasma arginase (an enzyme that catabolizes arginine) is elevated in severe malaria. Administering arginine intravenously reverses hypoargininemia and endothelial dysfunction. The cause(s) of hypoargininemia in malaria is unknown. Carbon monoxide (which shares certain functional properties with nitric oxide) protects against cerebral malaria in mice.

Summary—Replenishment of arginine and restoration of nitric oxide production in clinical malaria should diminish parasitized red blood cells adherence to endothelium and reduce the sequelae of these interactions (e.g., cerebral malaria). Arginine therapy given in addition to conventional antimalaria treatment may prove to be beneficial in severe malaria.

Keywords

arginine; carbon monoxide; endothelium; malaria; nitric oxide

Introduction

Malaria causes more deaths worldwide than any other parasitic disease. *Plasmodium falciparum* is responsible for severe malaria and an estimated 1.5–2.7 million deaths occur from malaria each year [1]. The majority of deaths are in African children who are less than 5 years of age, with most of these deaths resulting from cerebral malaria, severe metabolic acidosis/respiratory distress, or severe anemia [2]. In contrast, severe malaria in adults is more commonly a multisystem disease characterized by cerebral malaria, metabolic acidosis, renal failure, pulmonary edema, anemia, hypoglycemia, shock, and jaundice. Host

protective responses and malaria disease phenotype are influenced by many factors including age, heterogeneity in parasite virulence, and intensity of malaria transmission. Host genetic polymorphisms (or mutations) are also important. Despite recent advances in malaria research (e.g., mapping of the *P. falciparum* genome and identification of novel vaccine candidates), we still do not fully understand the mechanism(s) of protection from clinical malaria.

Many processes contribute to the pathogenesis of severe malaria; interactions of parasitized red blood cells (pRBCs) with endothelium are especially important. pRBCs adhere to constitutive and cytokine-inducible receptors on microvascular endothelium resulting in sequestration and vascular obstruction, impaired perfusion, and tissue hypoxia in critical organs [3–5]. pRBCs bind by parasite proteins expressed on the surface of RBC to a variety of endothelial ligands; this causes alteration of endothelium biology and sequestration of pRBCs. Real time in-vivo imaging in severe falciparum malaria has demonstrated extensive microvascular obstruction proportional to the severity of disease [6•]. The major histopathological finding at autopsy in cerebral malaria is sequestration of pRBCs within the postcapillary venules, associated with widespread ‘activation’ of endothelial receptors [3,4].

Ten to 30% of patients with severe malaria die despite treatment with the best available antimalarial drugs and supportive care [7,8]. Additional management strategies such as adjunctive therapies are needed to reduce case-fatality rates in severe malaria.

Nitric oxide, nitric oxide synthase2, and arginine

Nitric oxide and the related species peroxynitrite and nitrosothiols have multiple important physiologic and pathologic functions. Among these are resistance to tumors and microbes, regulation of vascular tone and blood pressure, neurotransmission, learning, neurotoxicity, carcinogenesis, and control of cellular growth and differentiation [9]. Separate genes encode three forms of nitric oxide synthase (NOS). Inducible NOS (NOS2) is capable of high-level nitric oxide production. Levels of nitrate and nitrite (the stable oxidized metabolites – ‘NOx’) in plasma and urine are generally valid markers of nitric oxide production in rodents and humans in a variety of disease states, provided there is adequate control for the potential confounding effects of dietary nitrate ingestion and nitrate retention in renal impairment [10,11].

Arginine is the substrate for all forms of NOS and arginase (Fig. 1 Fig. 1). Arginine is considered an essential amino acid in newborns and infants. In adults, arginine is considered nonessential, except for conditions of stress such as inflammation and infection. This amino acid plays many important roles, serving as a critical precursor for synthesis of proteins, nitric oxide, creatine, proline, citrulline, polyamines, urea, agmatine, and glutamate [12]. *In vivo*, arginine derives from exogenous (diet) and endogenous (whole-body protein degradation and endogenous synthesis from citrulline) sources. In adults, de-novo arginine synthesis accounts for only 5–15% of endogenous arginine flux. Whole-body protein turnover probably contributes the most to endogenous arginine flux. The intestine is the net site of citrulline synthesis (glutamate → citrulline in enterocytes) from which the amino acid enters the circulation to be taken up by the kidneys. The kidneys are considered to be the main organ site for net de-novo arginine synthesis as citrulline is converted to arginine. Arginine is also synthesized in cells actively making nitric oxide via the citrulline-nitric oxide cycle. Enzymes that convert citrulline to arginine are coinduced by inflammatory mediators that induce NOS2 [12]. There are multiple routes of arginine entry into the circulation and multiple routes of arginine disposition and use (see Fig. 1 for details).

Extensive, carefully done studies by Castillo *et al.* using infusion of heavy isotope-labeled amino acid substrates to calculate flux rates for arginine [13–17] have demonstrated that

arginine is conditionally essential in the acute inflammation noted in burnt and septic patients. In acute inflammation and infection (situations in which dietary supply of arginine may be low), hypoargininemia may occur because de-novo synthesis does not keep pace with increased arginine catabolism [13,14,17].

Arginine transport is important because this can regulate intracellular substrate availability, and control production of nitric oxide by NOS. The cationic amino acid transporters (CAT-1 and CAT-2; the system Y⁺ amino acid transporters) move arginine, lysine, and ornithine across membranes in human cells [12]. CAT expression is regulated transcriptionally; it is coinduced with NOS2 in response to inflammatory stimuli [18]. Cellular production of nitric oxide by NOS is dependent on extracellular concentrations of arginine and sufficient CAT activity to transport the arginine to the inside of the cell.

Arginine is a component of the normal human diet. Arginine therapy has been safely used in humans for decades [19]. Orally supplemented arginine is 30–70% absorbed. In normal fasting people, peak plasma concentrations appear approximately 2 hours after oral ingestion of arginine. Intravenous (i.v.) arginine is well tolerated. Arginine has been used routinely as a diagnostic test in children and adults at doses up to 30μg by i.v. bolus administration to induce growth hormone secretion (an nitric oxide-mediated process). Arginine has been well tolerated when given short-term (6–30μg i.v. or orally) and long-term (6–12μg/day orally) in healthy individuals and in those with cardiovascular disease. Studies of efficacy of arginine therapy in cardiovascular disease show mixed results, with some reports revealing no benefit or mild benefit in some, or possible harm in others [19–22].

Arginases

Arginases convert arginine to ornithine and urea and control availability of arginine for synthesis of nitric oxide, polyamines, agmatine, proline, and glutamate [12]. Arginase I, an inducible cytosolic enzyme, is very high in hepatocytes and a few other cell types including RBCs [23]. Arginase II is a mitochondrial enzyme expressed constitutively at low levels, primarily in macrophages, kidney, brain, and small intestine. Endothelial cells and macrophages may express both types I and II. Presence of arginase with consequent consumption of arginine can limit the production of nitric oxide even when high levels of NOS are present. Arginase expression is increased by IL-4, IL-10, or IL-13. ‘Alternately activated’ macrophages [24] expressing arginase produce less nitric oxide than do ‘classically activated’ macrophages. Arginase modulates resistance to parasitic diseases such as schistosomiasis, trypanosomiasis, and leishmaniasis [25]. This resistance may result in part from depletion of arginine and limitation of nitric oxide production and by increasing polyamines that enhance parasite growth and differentiation [25]. *P. falciparum* appears to express arginase [26], but the relationship of *P. falciparum*-encoded arginase to the hypoargininemia noted in clinical malaria [27,28•] is not known. Plasma arginase may be increased in patients with a variety of conditions associated with RBC hemolysis (e.g., sickle cell anemia and malaria), and this can be accompanied by low-arginine levels [28•, 29–31].

Nitric oxide and infections

Nitric oxide mediates host resistance to a wide variety of infectious organisms and kills *P. falciparum* *in vitro* [32–34]. Nitric oxide is anti-inflammatory and beneficial to host survival in many animal models of infection, including a number of rodent models of malaria. Although the mechanism of nitric oxide-mediated parasite toxicity remains uncertain [33], a number of mechanisms for its antidisease effects have been proposed. These include direct antiparasite effects, decrease in endothelial cell adhesion molecule expression, decrease in cytokine production, and decrease in adherence of pRBC adherence to endothelial cells.

Binding of pRBC to endothelium increases endothelial cell adhesion molecule expression and this in turn increases the RBC binding (Table 1 Table 1). Nitric oxide reduces endothelial expression of receptors/adhesion molecules [e.g., intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM)] used by pRBC to adhere to vascular endothelium [35], and reduces cytoadherence of pRBC to vascular endothelium [36]. Nitric oxide is also a potent inhibitor of tumor necrosis factor (TNF) production and other pro-inflammatory cytokines implicated in malaria immunopathology [37]. Although nitric oxide has direct antiparasite activity *in vitro* [34], this has not been noted *in vivo* in malaria [38,39].

Many have worked to determine the role of nitric oxide in the pathogenesis of malaria in humans, by measuring urine and plasma NO_x and blood mononuclear cell NOS activity as guides to NOS activity and nitric oxide production *in vivo* [11,40–43]. Because ingestion of nitrate and nitrite can affect plasma and urine NO_x levels, investigators have controlled dietary intake of NO_x and corrected for renal insufficiency in their research. In general, there is an inverse correlation of malaria disease severity with nitric oxide production/NOS2 expression [11,40,42,44]. Gramaglia *et al.* [38] used a mouse model of experimental cerebral malaria (ECM) (*P. berghei* ANKA) to show similar results. They found low nitric oxide and nitric oxide bioavailability and low-plasma levels of arginine in ECM, and noted partial rescue/prevention of ECM manifestations by administration of a nitric oxide prodrug. They also demonstrated that hemoglobin released from RBC scavenged nitric oxide reduced its bioavailability. Despite marked differences in survival with ECM and low nitric oxide bioavailability, parasitemia levels did not differ [38]. Hobbs *et al.* [39] noted in African children that presence of a NOS2 promoter polymorphism was associated with increased nitric oxide production and protection from severe malarial anemia, but this polymorphism was not associated with altered levels of parasitemia.

Plasma arginine concentrations were inversely correlated with disease severity in Tanzanian children [27] and in Indonesian adults [28••] with malaria. Children with the lowest arginine levels were those most likely to die [27]. Arginine concentrations improved after antimalaria chemotherapy [28••]. Hypoargininemia has also been noted in a variety of severe bacterial infections [45–47,48•]. Some have noted that hypoargininemia correlates strongly with noninfectious inflammation [48•]. This suggests that infection-associated inflammation (rather than infection, per se) may be a major cause of hypoargininemia in infections.

Arginine, nitric oxide, and endothelial dysfunction in malaria

Patients with a variety of vascular diseases may have defective endothelial function [49]. Endothelial function (assessed by reactive vasodilation following ischemic stress) is largely a nitric oxide-mediated process. Yeo *et al.* [28••] recently reported studies of arginine, nitric oxide levels, and endothelial function in Indonesian adults with severe malaria (Fig. 2 Fig. 2). They measured endothelial function in patients with moderately severe (MSM) and severe falciparum malaria, and investigated the effects of arginine infusion on levels of exhaled nitric oxide and endothelial function in patients with MSM. The reactive hyperemia peripheral arterial tonometry (RH PAT) index in severe malaria was lower than in MSM, but there was no significant difference between healthy controls and MSM (Fig. 2). The proportion of patients with impaired endothelial function was significantly higher in severe malaria than in MSM. Endothelial dysfunction was associated with increased blood lactate and measures of hemolysis [plasma hemoglobin, Lactate dehydrogenase (LDH), and arginase]. Plasma levels of soluble ICAM-1, E-selectin, and parasite histidine-rich protein 2 (HRP2, an indicator of total parasite biomass) were significantly higher in patients with severe disease. Exhaled nitric oxide was significantly lower in severe malaria relative to MSM and controls. RH PAT was negatively correlated with parasite load (plasma HRP2),

lactate concentration, LDH, plasma arginase, and soluble ICAM-1. Arginine administration significantly increased RH PAT index and exhaled nitric oxide. The investigators concluded that endothelial dysfunction in malaria is near-universal in severe disease, is reversible with arginine administration, that hypoargininemia and hemolysis in severe malaria likely reduce nitric oxide bioavailability [28••], and that arginine infusion is safe in adults with MSM [50•]. The relative values of some of the variables noted in Indonesian adults with malaria are displayed in Figure 3 Figure 3.

Arginine levels in severe malaria are very low [27,28••] – below the K_m for arginine transport into cells by the CAT indicating that adequate amounts of arginine would not likely be available for nitric oxide synthesis by NOS intracellularly. When this occurs, NOS generates superoxide instead of nitric oxide [51], adding to the degree of oxidant stress. With arginine infusion, arginine levels rose to above normal levels after a single arginine infusion [28••]. The half-life of infused arginine was 15µmin in patients with MSM and severe malaria (T.W. Yeo, S.B. Duffull, D.A. Lampah, *et al.*, in preparation), considerably shorter than the 40–60µmin noted in healthy people [52]. After treatment with i.v. quinine or artesunate, arginine levels and endothelial function recovered [53]. Patients with high baseline arginase activity had slower recovery of arginine levels.

As noted above, patients with malaria have ‘activated endothelium’ as judged by increased adhesiveness and expression of cell adhesion molecules [3–5]. pRBCs can induce tissue factor expression in endothelial cells *in vitro*, and brain vessels from children who die with cerebral malaria have overexpression of tissue factor [54•,55] (for review). Tissue factor is a critical initiator of coagulation *in vivo*. With the pRBCs endothelial cell interaction, there is an increased tissue factor expression, assembly of an extrinsic factor Xase complex on cell surfaces, and initiation of the coagulation cascade [54•,55]. Both nitric oxide and carbon monoxide can inhibit expression of tissue factor and coagulation/thrombosis [56•,57–59].

Arginine, nitric oxide, and endothelial dysfunction in sickle cell disease

Malaria and sickle cell disease have similar pathophysiologies. These include altered RBC undergoing hemolysis, and altered RBC binding to endothelial cells resulting in interference with blood flow. Hemolysis caused by a variety of conditions (e.g., sickle cell disease, paroxysmal nocturnal hemoglobinuria, and malaria) is associated with release of RBC cytoplasmic contents such as hemoglobin, lactate dehydrogenase, and arginase into the plasma [31]. Plasma free hemoglobin reduces nitric oxide bioavailability as it binds nitric oxide and quenches/scavenges its bioactivity [60]. Plasma arginase diminishes arginine levels, and thus may reduce production of nitric oxide [61]. In sickle cell disease, this reduction in nitric oxide bioavailability likely contributes to sickle cell disease-associated pulmonary hypertension, and the relative nitric oxide deficiency state may contribute to mortality [62]. Arginine therapy corrects the hypoargininemia and impaired nitric oxide production found in patients with sickle cell vaso occlusive syndrome [29], a syndrome with endothelial dysfunction, impaired nitric oxide production, and low plasma arginine levels similar to malaria [30,63•,64•]. Also, arginine reduces the pulmonary hypertension in patients with sickle cell disease [65]. Whether arginine is beneficial in other settings such as sepsis and multiorgan failure is still an open question [66,67].

Heme oxygenase, carbon monoxide, nitric oxide synthase, nitric oxide, and malaria

Hemolysis associated with malaria results in release of hemoglobin, and oxidation of the hemoglobin generates free heme. Pamplona *et al.* [68••] reported that heme oxygenase-1 prevents the development of experimental cerebral malaria in mice. Heme oxygenase

degrades heme to carbon monoxide, iron, and biliverdin (which is rapidly converted to bilirubin). Certain mice infected with *P. berghei* ANKA upregulated heme oxygenase-1 expression and activity and did not develop ECM. However, deletion of the heme oxygenase-1 gene or inhibition of heme oxygenase activity increased ECM incidence. Inducing increased expression of heme oxygenase-1 or administration of carbon monoxide protected against cerebral malaria. Heme oxygenase-1 expression induction or carbon monoxide administration prevented blood brain barrier disruption, brain microvasculature congestion, neuroinflammation, and reduced overall death, but it did not alter parasitemia levels. Carbon monoxide toxicity could limit the utility of its administration as a treatment. They concluded that carbon monoxide likely binds to ferrous iron in hemoglobin and prevents release of heme, a key molecule in cerebral malaria pathogenesis [68••].

Carbon monoxide has many biological actions similar to nitric oxide (Table 1). These include modulation of vessel and bronchial dilation, smooth muscle cell proliferation, neurotransmission, apoptosis, inflammation, platelet aggregation, tissue factor expression, and thrombosis [56•,57–59,69]. In addition to their individual abilities to protect against cerebral malaria, nitric oxide and carbon monoxide (and NOS and heme oxygenase) may interact to decrease malaria severity. Nitric oxide potently induces heme oxygenase-1 expression [70], and thus leads to generation of more carbon monoxide as heme is degraded. Carbon monoxide diminishes heme liberation from hemoglobin. Furthermore, carbon monoxide binding to hemoglobin reduces hemoglobin's nitric oxide quenching ability, thus increasing nitric oxide bioavailability.

Conclusion

Hypoargininemia is seen in almost all patients with severe malaria, but its precise cause is not known. Endothelial dysfunction is very common in severe malaria, and it likely contributes to the pathophysiology of malaria disease. We do not know if children with severe malaria have endothelial dysfunction comparable to that noted in adults, and we do not know if arginine administration would benefit adults or children with malaria. As many with severe malaria die despite appropriate treatment with parasitocidal therapy, there is a great need for new or adjunctive therapy. Treatments to correct endothelial dysfunction in severe malaria may prove to be beneficial. Further study of arginine therapy as a possible adjunct to treatment of severe malaria is needed.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. World Health Organization, communicable diseases cluster. Severe falciparum malaria. *Trans R Soc Trop Med Hyg.* 2000; 94 (Suppl 1):S1–90. [PubMed: 11103309]

2. World Health Organization. Guidelines for the treatment of malaria. Geneva: WHO; 2006.
3. Taylor TE, Fu WJ, Carr RA, et al. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med.* 2004; 10:143–145. [PubMed: 14745442]
4. Turner GD, Ly VC, Nguyen TH, et al. Systemic endothelial activation occurs in both mild and severe malaria. Correlating dermal microvascular endothelial cell phenotype and soluble cell adhesion molecules with disease severity. *Am J Pathol.* 1998; 152:1477–1487. [PubMed: 9626052]
5. Pongponratn E, Turner GD, Day NP, et al. An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. *Am J Trop Med Hyg.* 2003; 69:345–359. [PubMed: 14640492]
6. Dondorp AM, Ince C, Charunwatthana P, et al. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. *J Infect Dis.* 2008; 197:79–84. Using orthogonal polarization spectral direct imaging of rectal mucosal microcirculation, the authors noted that patients with severe falciparum malaria had extensive microvascular obstruction proportional to the severity of illness. The abnormalities disappeared after recovery. Normal individuals had no disturbances, and those with sepsis had only hyperdynamic flow. [PubMed: 18171289]
7. Dondorp A, Nosten F, Stepniewska K, et al. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet.* 2005; 366:717–725. [PubMed: 16125588]
8. Day N, Dondorp AM. The management of patients with severe malaria. *Am J Trop Med Hyg.* 2007; 77:29–35. [PubMed: 18165472]
9. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med.* 1993; 329:2002–2012. [PubMed: 7504210]
10. Granger, DL.; Miller, WC.; Hibbs, JB, Jr. Methods of analyzing nitric oxide production in the immune response. In: Feelisch, M.; Stamler, JS., editors. *Methods in nitric oxide research.* John Wiley & Sons Ltd; 1996. p. 603–617.
11. Anstey NM, Weinberg JB, Hassanali M, et al. Nitric oxide in Tanzanian children with malaria. Inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J Exp Med.* 1996; 184:557–567. [PubMed: 8760809]
12. Morris SMJ Jr. Arginine metabolism: boundaries of our knowledge. *J Nutr.* 2007; 137:1602S–1609S. [PubMed: 17513435]
13. Yu YM, Ryan CM, Castillo L, et al. Arginine and ornithine kinetics in severely burned patients: increased rate of arginine disposal. *Am J Physiol Endocrinol Metab.* 2001; 280:E509–E517. [PubMed: 11171607]
14. Argaman Z, Young VR, Noviski N, et al. Arginine and nitric oxide metabolism in critically ill septic pediatric patients. *Crit Care Med.* 2003; 31:591–597. [PubMed: 12576971]
15. Castillo L, Sanchez M, Vogt J, et al. Plasma arginine, citrulline, and ornithine kinetics in adults, with observations on nitric oxide synthesis. *Am J Physiol.* 1995; 268:E360–E367. [PubMed: 7864114]
16. Castillo L, deRojas TC, Chapman TE, et al. Splanchnic metabolism of dietary arginine in relation to nitric oxide synthesis in normal adult man. *Proc Natl Acad Sci U S A.* 1993; 90:193–197. [PubMed: 8419922]
17. Yu YM, Young VR, Castillo L, et al. Plasma arginine and leucine kinetics and urea production rates in burn patients. *Metabolism.* 1995; 44:659–666. [PubMed: 7752916]
18. Hammermann R, Dreij MDM, Dreissig MDM, et al. Nuclear factor-kappa B mediates simultaneous induction of inducible nitric-oxide synthase and up-regulation of the cationic amino acid transporter CAT-2B in rat alveolar macrophages. *Mol Pharmacol.* 2000; 58:1294–1302. [PubMed: 11093766]
19. Boger RH, Bode-Boger SM. The clinical pharmacology of L-arginine. *Annu Rev Pharmacol Toxicol.* 2001; 41:79–99. [PubMed: 11264451]
20. Boger RH. L-Arginine therapy in cardiovascular pathologies: beneficial or dangerous? *Curr Opin Clin Nutr Metab Care.* 2008; 11:55–61. [PubMed: 18090660]
21. Schulman SP, Becker LC, Kass DA, et al. L-arginine therapy in acute myocardial infarction: the vascular interaction with age in myocardial infarction (VINTAGE MI) randomized clinical trial. *JAMA.* 2006; 295:58–64. [PubMed: 16391217]
22. Wilson AM, Harada R, Nair N, et al. L-arginine supplementation in peripheral arterial disease: no benefit and possible harm. *Circulation.* 2007; 116:188–195. [PubMed: 17592080]

23. Iyer R, Jenkinson CP, Vockley JG, et al. The human arginases and arginase deficiency. *J Inherit Metab Dis.* 1998; 21 (Suppl 1):86–100. [PubMed: 9686347]
24. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol.* 2003; 3:23–35. [PubMed: 12511873]
25. Vincendeau P, Gobert AP, Daulouede S, et al. Arginases in parasitic diseases. *Trends Parasitol.* 2003; 19:9–12. [PubMed: 12488215]
26. Muller IB, Walter RD, Wrenger C. Structural metal dependency of the arginase from the human malaria parasite *Plasmodium falciparum*. *Biol Chem.* 2005; 386:117–126. [PubMed: 15843155]
27. Lopansri BK, Anstey NM, Weinberg JB, et al. Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet.* 2003; 361:676–678. [PubMed: 12606182]
- 28••. Yeo TW, Lampah DA, Gitawati R, et al. Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. *J Exp Med.* 2007; 204:2693–2704. The authors demonstrated that adult Indonesians with severe malaria have low plasma arginine levels, low exhaled nitric oxide, and abnormal endothelial function. These abnormalities are reversed by a single infusion of arginine. They found that endothelial dysfunction in malaria is nearly universal in severe disease, is reversible with arginine administration, and that endothelial dysfunction likely contributes to the pathogenesis of severe malaria. [PubMed: 17954570]
29. Morris CR, Kuypers FA, Larkin S, et al. Arginine therapy: a novel strategy to induce nitric oxide production in sickle cell disease. *Br J Haematol.* 2000; 111:498–500. [PubMed: 11122090]
30. Morris CR, Kuypers FA, Larkin S, et al. Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. *J Pediatr Hematol Oncol.* 2000; 22:515–520. [PubMed: 11132219]
31. Rother RP, Bell L, Hillmen P, et al. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA.* 2005; 293:1653–1662. [PubMed: 15811985]
32. Fang FC. Mechanisms of nitric oxide-related antimicrobial activity. *J Clin Invest.* 1997; 100:S 43–S 50.
33. Anstey, NM.; Weinberg, JB.; Granger, DL. CT Nitric oxide and nitric oxide synthase type 2 in severe malaria. In: Fang, F., editor. *EBT Nitric oxide and infection*. New York: Kluwer Academic/Plenum Publishing D; 1999. p. 311–341.
34. Rockett KA, Awburn MM, Cowden WB, et al. Killing of *Plasmodium falciparum* in vitro by nitric oxide derivatives. *Infect Immun.* 1991; 59:3280–3283. [PubMed: 1879941]
35. Decaterina R, Libby P, Peng HB, et al. Nitric oxide decreases cytokine-induced endothelial activation: nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest.* 1995; 96:60–68. [PubMed: 7542286]
36. Serirom S, Raharjo WH, Chotivanich K, et al. Antiadhesive effect of nitric oxide on *Plasmodium falciparum* cytoadherence under flow. *Am J Pathol.* 2003; 162:1651–1660. [PubMed: 12707049]
37. Iuvone T, Dacquist F, Carnuccio R, et al. Nitric oxide inhibits LPS-induced tumor necrosis factor synthesis in vitro and in vivo. *Life Sci.* 1996; 59:L207–L211.
38. Gramaglia I, Sobolewski P, Meays D, et al. Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria. *Nat Med.* 2006; 12:1417–1422. [PubMed: 17099710]
39. Hobbs MR, Udhayakumar V, Levesque MC, et al. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. *Lancet.* 2002; 360:1468–1475. [PubMed: 12433515]
40. Kun JF, Mordmuller B, Perkins DJ, et al. Nitric oxide synthase 2(Lambarene) (G-954C), increased nitric oxide production, and protection against malaria. *J Infect Dis.* 2001; 184:330–336. [PubMed: 11443559]
41. Chiwakata CB, Hemmer CJ, Dietrich M. High levels of inducible nitric oxide synthase mRNA are associated with increased monocyte counts in blood and have a beneficial role in *Plasmodium falciparum* malaria. *Infect Immun.* 2000; 68:394–399. [PubMed: 10603415]

42. Perkins DJ, Kremsner PG, Schmid D, et al. Blood mononuclear cell nitric oxide production and plasma cytokine levels in healthy Gabonese children with prior mild or severe malaria. *Infect Immun.* 1999; 67:4977–4981. [PubMed: 10456963]
43. Anstey NM, Granger DL, Hassanali MY, et al. Nitric oxide, malaria, and anemia: inverse relationship between nitric oxide production and hemoglobin concentration in asymptomatic, malaria-exposed children. *Am J Trop Med Hyg.* 1999; 61:249–252. [PubMed: 10463675]
44. Anstey NM, Weinberg JB, Wang Z, et al. Effects of age and parasitemia on nitric oxide production/leukocyte nitric oxide synthase type 2 expression in asymptomatic, malaria-exposed children. *Am J Trop Med Hyg.* 1999; 61:253–258. [PubMed: 10463676]
45. Becker RM, Wu G, Galanko JA, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. *J Pediatr.* 2000; 137:785–793. [PubMed: 11113834]
46. Freund H, Atamian S, Holroyde J, et al. Plasma amino acids as predictors of the severity and outcome of sepsis. *Ann Surg.* 1979; 190:571–576. [PubMed: 389183]
47. Freund HR, Ryan JAJ Jr, Fischer JE. Amino acid derangements in patients with sepsis: treatment with branched chain amino acid rich infusions. *Ann Surg.* 1978; 188:423–430. [PubMed: 99098]
- 48•. van Waardenburg DA, de Betue CT, Luiking YC, et al. Plasma arginine and citrulline concentrations in critically ill children: strong relation with inflammation. *Am J Clin Nutr.* 2007; 86:1438–1444. The authors demonstrated that plasma arginine and citrulline levels are low during the acute phase of critical illness (sepsis or injury) in children and normalize again during recovery. Plasma arginine and citrulline are strongly related to the severity of inflammation as assessed by plasma C-reactive protein concentrations. [PubMed: 17991657]
49. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation.* 2007; 115:1285–1295. [PubMed: 17353456]
- 50•. Yeo TW, Lampah DA, Gitawati R, et al. Safety profile of L-arginine infusion in moderately severe falciparum malaria. *PLoS ONE.* 2008; 3:e2347. The authors noted that intravenous infusion of up to 12μg of L-arginine hydrochloride over 30μmin is well tolerated in adults with moderately severe malaria, with no clinically important changes in hemodynamic or biochemical status. [PubMed: 18545693]
51. Xia Y, Roman LJ, Masters BS, et al. Inducible nitric-oxide synthase generates superoxide from the reductase domain. *J Biol Chem.* 1998; 273:22635–22639. [PubMed: 9712892]
52. Bode-Boger SM, Boger RH, Galland A, et al. L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. *Br J Clin Pharmacol.* 1998; 46:489–497. [PubMed: 9833603]
53. Yeo TW, Lampah DA, Gitawati R, et al. Recovery of endothelial function in severe falciparum malaria is related to improvement in plasma L-arginine and blood lactate concentrations. *J Infect Dis.* 2008:198.
- 54•. Francischetti IM, Seydel KB, Monteiro RQ, et al. Plasmodium falciparum-infected erythrocytes induce tissue factor expression in endothelial cells and support the assembly of multimolecular coagulation complexes. *J Thromb Haemost.* 2007; 5:155–165. The authors demonstrated that RBC with *P. falciparum* (late trophozoites and schizonts) induce tissue factor expression in endothelial cells *in vitro*, and that brains from children who die with cerebral malaria express tissue factor in brain endothelial cells. [PubMed: 17002660]
55. Francischetti IM. Does activation of the blood coagulation cascade have a role in malaria pathogenesis? *Trends Parasitol.* 2008; 24:258–263. [PubMed: 18467176]
- 56•. True AL, Olive M, Boehm M, et al. Heme oxygenase-1 deficiency accelerates formation of arterial thrombosis through oxidative damage to the endothelium, which is rescued by inhaled carbon monoxide. *Circ Res.* 2007; 101:893–901. The authors demonstrated that mice with disrupted heme oxygenase-1 gene have accelerated thrombosis and increased tissue factor expression. They also showed that administration of heme oxygenase degradation products of heme (carbon monoxide or biliverdin) decrease tissue factor expression and thrombosis. [PubMed: 17885218]
57. Perez-Ruiz A, Montes R, Velasco F, et al. Regulation by nitric oxide of endotoxin-induced tissue factor and plasminogen activator inhibitor-1 in endothelial cells. *Thromb Haemost.* 2002; 88:1060–1065. [PubMed: 12529760]

58. Snyder SH, Baranano DE. Heme oxygenase: a font of multiple messengers. *Neuropsychopharmacology*. 2001; 25:294–298. [PubMed: 11522457]
59. Morse D, Choi AM. Heme oxygenase-1: from bench to bedside. *Am J Respir Crit Care Med*. 2005; 172:660–670. [PubMed: 15901614]
60. Reiter CD, Wang X, Tanus-Santos JE, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. *Nat Med*. 2002; 8:1383–1389. [PubMed: 12426562]
61. Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA*. 2005; 294:81–90. [PubMed: 15998894]
62. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*. 2006; 107:2279–2285. [PubMed: 16291595]
- 63•. Wood KC, Hsu LL, Gladwin MT. Sickle cell disease vasculopathy: a state of nitric oxide resistance. *Free Radic Biol Med*. 2008; 44:1506–1528. The authors discuss the importance of hemolysis with increased plasma hemoglobin, increased reactive oxygen species, and reduced nitric oxide bioavailability in sickle cell disease and its vascular complications. [PubMed: 18261470]
- 64•. Aslan M, Freeman BA. Redox-dependent impairment of vascular function in sickle cell disease. *Free Radic Biol Med*. 2007; 43:1469–1483. The authors discuss the importance of RBC and leukocyte interactions with endothelium, coagulation, nitric oxide, and oxidant stress in generation of vascular injury and occlusion in sickle cell disease. [PubMed: 17964418]
65. Morris CR, Morris SMJ Jr, Hagar W, et al. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med*. 2003; 168:63–69. [PubMed: 12626350]
66. Kalil AC, Danner RL. L-Arginine supplementation in sepsis: beneficial or harmful? *Curr Opin Crit Care*. 2006; 12:303–308. [PubMed: 16810039]
67. Luiking YC, Deutz NE. Exogenous arginine in sepsis. *Crit Care Med*. 2007; 35:S557–S563. [PubMed: 17713409]
- 68••. Pamplona A, Ferreira A, Balla J, et al. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat Med*. 2007; 13:703–710. The authors demonstrated in an experimental model of mouse cerebral malaria that heme oxygenase-1 mRNA is increased, and that disruption of the heme oxygenase-1 gene or inhibition of heme oxygenase-1 increases severity of cerebral malaria. Heme oxygenase-1 expression induction or carbon monoxide administration prevents blood brain disruption, brain microvasculature congestion and neuroinflammation, and reduces overall death. However, heme oxygenase-1 expression induction or carbon monoxide administration does not alter parasitemia levels. [PubMed: 17496899]
69. Chung HT, Choi BM, Kwon YG, et al. Interactive relations between nitric oxide (NO) and carbon monoxide (CO): heme oxygenase-1/co pathway is a key modulator in no-mediated antiapoptosis and antiinflammation. *Methods Enzymol*. 2008; 441:329–338. [PubMed: 18554543]
70. Bouton C, Demple B. Nitric oxide-inducible expression of heme oxygenase-1 in human cells. Translation-independent stabilization of the mRNA and evidence for direct action of nitric oxide. *J Biol Chem*. 2000; 275:32688–32693. [PubMed: 11032845]

Figure 1.

Arginine production, destruction, and utilization in health. gr1

Arginine is provided by oral intake, synthesis from citrulline, and protein degradation. Disposition of arginine is through the urea cycle and certain anabolic pathways, as well as by actions of arginases and nitric oxide synthases (NOS). Arginases convert arginine to ornithine and urea, whereas NOS convert arginine to nitric oxide (NO) and citrulline. Only 1% of arginine is converted to NO. Under homeostatic conditions in the fed state, arginine flux is approximately 65 $\mu\text{mol/kg/h}$. In patients with acute inflammation, flux studies show that total arginine flux nearly doubles. Dietary sources become markedly reduced, and de-novo synthesis falls slightly. Arginine utilization for the urea cycle remains essentially unchanged, and the rate of arginine oxidation increases significantly. There is much demand for arginine via anabolic pathways for tissue repair (e.g., new protein synthesis and proline for collagen synthesis). Thus, maintenance of plasma arginine levels in the normal range requires a large influx of arginine from protein breakdown. If this supply route cannot match catabolic and anabolic consumption, plasma arginine levels fall. Low plasma arginine concentration may limit nitric oxide synthesis. The relatively high K_m ($\sim 110 \mu\text{mol/l}$) for enzyme (cationic amino acid transporter) transport of arginine into cells indicates that the low extracellular arginine levels noted in malaria would result in intracellular arginine levels too low for efficient nitric oxide production in NOS-bearing cells. Accordingly, studies on arginine balance in adults and children with acute inflammation show that though arginine may be a dispensable amino acid in health during fasting, it becomes an indispensable amino acid during disease. Thus, arginine supplementation may be necessary in certain disease states such as acute inflammation or infection.

A. Endothelial function

B. L-arginine levels

C. Exhaled NO levels

Figure 2.

Malaria and (a) reactive hyperemia peripheral artery tonometry index (b) plasma arginine, and (c) exhaled nitric oxide

(a) Reactive hyperemia peripheral artery tonometry (RH PAT) is different among Indonesian normal adults (HC), those with moderately severe malaria (MSM), and those with severe malaria ($P<0.0001$). Over 50% of RH PAT is determined by endothelial nitric oxide (NO) production. The horizontal line at 1.67 is the lowest value considered normal. RH PAT is a measure of endothelial function. (b) Plasma arginine is different among the groups ($P<0.0001$). (c) Exhaled NO at enrollment is different among the groups ($P=0.049$). The boxes with horizontal line represent the interquartile range and the median, and the whiskers show the full range. These figures are from work of Yeo *et al.* [28••].

Figure 3.

Relationships of malaria disease severity to nitric oxide, endothelial function, and clinical parameters in Indonesian adults

Indonesian adults were evaluated for parasitemia, plasma arginine, plasma arginase, plasma lactate, reactive hyperemia peripheral artery tonometry (RH PAT), and exhaled nitric oxide (NO) levels on presentation to the hospital. Healthy control adults (HC), those with moderately severe malaria (MSM), and those with severe malaria (SM) were assessed. The units of measure in this figure were normalized for purposes of presentation clarity; the measures do not represent the actual absolute values for the different parameters. With increasing malaria disease severity, there were progressive increases in parasitemia, arginase, and lactate (left panel) and progressive decreases in plasma arginine, RH PAT, and exhaled NO (right panel). This figure was constructed from data from Yeo *et al.* [28].

Table 1

Nitric oxide and carbon monoxide functions relevant to malaria

Stimulus or agent	Action
Parasitized RBC binding to endothelial cells:	↑ EC CAM expression ↑ Pro-inflammatory cytokine expression ↑ EC procoagulant expression
Nitric oxide:	↓ EC CAM expression ↓ Pro-inflammatory cytokine expression ↓ EC binding of parasitized RBC ↑ Vasodilation ↓ EC procoagulant expression ↓ Platelet aggregation ↓ Thrombosis ↓ Apoptosis
Carbon monoxide:	↓ EC CAM expression ↓ Pro-inflammatory cytokine expression ↑ Vasodilation ↓ EC procoagulant expression ↓ Platelet aggregation ↓ Thrombosis ↓ Heme release from hemoglobin ↓ NO binding by CO-hemoglobin ↓ NO bioavailability ↓ Apoptosis

The up arrow indicates 'increases' or 'increased,' and the down arrow indicates 'decreases' or 'decreased'.

CAM, cell adhesion molecule; CO, carbon monoxide; EC, endothelial cell; NO, nitric oxide; RBC, red blood cells