

Dimethylarginines: Endogenous Inhibitors of Nitric Oxide Synthesis in Children With Falciparum Malaria

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Background. Nitric oxide (NO) bioavailability is impaired in children and adults with severe falciparum malaria (SM). Asymmetric-dimethylarginine (ADMA) limits NO production by inhibiting NO synthase and is increased in adult SM. The role of ADMA in the pathogenesis of childhood SM is unknown.

Methods. We studied Tanzanian children ages 4–8 years with malaria. Plasma levels of arginine, arginase, cell-free hemoglobin, ADMA, symmetric-dimethylarginine (SDMA), histidine-rich protein-2, and angiotensin-2 were measured.

Results. ADMA was low in children with SM relative to controls. Nevertheless, arginine and arginine:ADMA ratios were very low in SM. SDMA was high in children with SM. With treatment, arginine and the arginine:ADMA ratio normalized, but SDMA did not. Arginine:ADMA ratios, but not arginine, were significantly and independently inversely associated with lactate and angiotensin-2. Plasma arginase was not elevated in those with malaria, and plasma free hemoglobin was elevated only in patients with cerebral malaria.

Conclusions. In contrast to adults, plasma ADMA is reduced in SM in children, but hypoargininemia is more severe. Arginine bioavailability (reflected by low arginine:ADMA ratios) is therefore comparably low in SM in children as in adults. Therapies to increase NO bioavailability in malaria may be useful as adjunctive treatment of severe malaria in children.

Keywords. falciparum malaria; nitric oxide; arginine; asymmetric dimethylarginine; symmetric dimethylarginine; arginase; lactate; angiotensin 2.

Falciparum malaria remains a significant problem worldwide causing over 0.6 million deaths yearly, mostly in children [1, 2]. New insights into malaria pathophysiology and new treatments are needed. Nitric oxide (NO) is important in resistance to severe falciparum malaria, and there is markedly diminished NO bioavailability in falciparum malaria both adults and

children [3, 4]. We have identified several processes contributing to the NO insufficiency ([5] for review). Recently, we found Indonesian adults with severe falciparum malaria had elevated plasma levels of endogenous methylated arginines [asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)] and that ADMA levels correlated with decreased NO production, endothelial dysfunction, disease severity, and mortality [6].

Dimethylarginines are formed primarily as a result of protein breakdown (eg, in muscle or erythrocytes) and release of methylated arginine residues [7, 8]. High levels of ADMA and SDMA are predictive of poor outcomes in a variety of diseases including coronary artery disease, peripheral vascular disease, renal failure, sepsis, and malaria in Asian adults [7, 9, 10]. ADMA is a competitive inhibitor of NO synthases (NOS), and the ratio of plasma arginine to ADMA is a measure of

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arginine bioavailability to NOS. SDMA is not active as an inhibitor of NOS, but at supraphysiological concentrations it reduces arginine transport into cells by competitive inhibition of the cationic transporter (CAT2) [11] and enhances oxidative stress and inflammatory mediator release by macrophage-like cells [7, 12]. A recent study evaluating whole genome sequences of African children with falciparum malaria found polymorphisms of the enzyme dimethylarginine-dimethylaminohydrolase-1 (DDAH), which metabolizes ADMA to be associated with an increased risk of severe disease. However, no studies to our knowledge have measured concentrations of dimethylarginines in children with malaria to date, and the relationship with disease severity is not known.

The purpose of the current study was to determine whether, as in Asian adults, plasma dimethylarginines are also increased in African children with falciparum malaria in proportion to disease severity and whether impaired L-arginine bioavailability (reflected by the arginine:ADMA ratio) would be associated with markers of impaired perfusion and endothelial activation.

METHODS

Written informed consent for participation was obtained from parents or guardians of all subjects. Children between ages 4 and 8 years were enrolled from Amana or Mwananyamala District Hospitals, or the Hubert Kairuki Memorial University Hospital in Dar es Salaam, Tanzania. Healthy control (HC) subjects were without fever or clinical illness and had negative blood microscopy for malaria. Cerebral malaria (CM) was defined as *Plasmodium falciparum* parasitemia, Blantyre Coma Score ≤ 2 and absence of an alternative cause of coma. Noncerebral severe malaria (SM) was defined as *P. falciparum* parasitemia and at least 1 other modified World Health Organization (WHO) criterion of severity [1–4]. Moderately SM (MSM) was defined as fever within the preceding 48 hours, more than 1000 asexual parasites/ μL , no WHO warning signs or severe malaria criteria, and a requirement for in-patient parenteral therapy because of inability to tolerate oral treatment [4]. Heparinized blood was collected and processed rapidly, and plasma was stored at -70°C . Parasite counts were determined by microscopy. Hemoglobin, biochemistry, acid base parameters, and lactate were measured with a bedside analyzer (i-STAT Corp). Plasma levels of arginine, ADMA, and SDMA were quantitated by HPLC as described elsewhere [13]; histidine-rich protein-2, and angiopoietin-2 were quantified by enzyme-linked immunosorbent assay (ELISA) [6]; plasma arginase activity was measured using a radiometric assay we have described before [4]; and cell-free plasma hemoglobin was measured by ELISA [14]. Patients were treated with antimalarials including quinine or artemisinins and antibiotics using standard national protocols. In total, 97% of the MSM and SM patients were treated with parenteral quinine on admission, and 3% were given artemether.

Statistical analysis was performed using STATA 11 software. Intergroup differences among malaria (MSM and SM) and controls were compared by ANOVA or Kruskal–Wallis test, where appropriate. An a priori pairwise comparison, using the Sidak method, compared the various disease groups (eg, SM with MSM, and SM with HC). Pearson or Spearman correlation coefficients were determined depending on normality of distributions. Multivariable linear regression was used to adjust for confounding variables. We assessed longitudinal associations by mixed effects modeling using generalized estimating equations. A 2-sided value of $P < .05$ was considered significant.

RESULTS

Subjects

We enrolled 211 subjects—75 HC, 69 MSM, 31 severe malaria (SM) without cerebral malaria, and 36 with SM with CM (total of 67 SM). In the 67 children with SM (with or without CM) in whom we had the measurements, 9/66 (13.5%) had severe anemia (hemoglobin [Hb] $< 5 \text{ gm/dL}$), 26/67 (38.8%) had parasite counts $> 250\,000/\mu\text{L}$, 13/66 (19.7%) had pathologic deep breathing, 1 of 46 (2.2%) had plasma creatinine $> 2 \text{ mg/dL}$, 11/57 (19.3%) had lactic acidosis with blood lactate $> 5 \text{ mg/dL}$, 26/67 (38.8%) had coma, and 33/66 had seizures (50%). None of the patients died. In the malaria patients, blood samples were drawn immediately on presentation (day 0) and then on days 1, 2, 3, and 7 (and up to day 17 as long as they were hospitalized). Not all tests could be performed in every patient. Detailed clinical and laboratory characteristics of the participants at time of presentation are displayed in Table 1.

Laboratory Results

Hb and platelet counts were significantly lower in those with malaria than in HC children, but white blood cells (WBCs) were not different (Table 1). Blood lactate was significantly elevated in those with malaria (more so in CM than in MSM and SM). Parasite density was higher in SM without cerebral malaria than compared to CM and MSM, but HRP2 levels were not significantly elevated in SM compared to CM and MSM. Angiopoietin-2 was higher in SM and CM than in MSM, which was higher than in HC children. Creatinine levels were significantly higher in CM than in HC individuals. Plasma cell-free hemoglobin was significantly higher in patients with CM but not other malaria groups, compared to HC subjects. Plasma arginase was not significantly higher in any of the malaria groups compared to the HC individuals (Table 1).

Levels of Plasma Arginine, ADMA, and SDMA

Plasma arginine levels were significantly lower in those with malaria compared to HC subjects at the time of presentation (day 0; Table 1 and Figure 1). Among those with malaria (MSM, SM, and CM), levels of arginine, ADMA, and SDMA

Table 1. Baseline Characteristics and Laboratory Data According to Clinical Status

Characteristic	Healthy Control	Moderately Severe Malaria	Severe Malaria Without Cerebral Malaria	Cerebral Malaria	P Values ^a
Total number	75	69	31	36	
Age—years ^{b,c}	8 (7–8) (n = 73)	5 (4–6) (n = 68)	5 (4–8) (n = 31)	4 (4–5) (n = 36)	HC vs MSM—<0.0001 HC vs SM—0.0004 HC vs CM—<0.0001 MSM vs SM—NS ^d MSM vs CM—NS SM vs CM—NS
Males (% of total)	34 (45.3)	39 (56.5)	18 (58.1)	24 (66.7%)	
Weight ^c (Kg)	22.8 (20.0–25.5) (n = 75)	17 (15.0–20.0) (n = 68)	17 (15.0–20.3) (n = 31)	15 (14.3–19) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Temperature ^c (°C)	36.6 (36.5–36.8) (n = 75)	37.2 (36.5–38.5) (n = 68)	37.7 (36.7–38.5) (n = 31)	38.0 (37.1–39.0) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—0.038 SM vs CM—NS
Heart rate ^c (beats/min)	91 (86–98) (n = 75)	110 (101–120) (n = 68)	108 (100–123) (n = 31)	127 (108–142) (n = 46)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—<0.0001 SM vs CM—0.0002
Respiratory rate ^c (breaths/min)	24 (23–26) (n = 71)	29 (26–34) (n = 66)	30 (28–36) (n = 31)	40 (30–50) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—<0.0001 SM vs CM—0.004
Systolic blood pressure ^d (mm Hg)	90 (90–96) (n = 73)	90 (90–96) (n = 62)	90 (89–99) (n = 29)	90 (88–98) (n = 34)	HC vs MSM—NS HC vs SM—NS HC vs CM—NS MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Diastolic blood pressure ^c (mm Hg)	60 (60–60) (n = 73)	60 (55–60) (n = 62)	55 (51–60) (n = 29)	56 (50–60) (n = 25)	HC vs MSM—0.007 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—0.003 SM vs CM—NS
Hemoglobin ^c (gm/dL)	12.2 (11.4–13.3) (n = 75)	8.8 (7.1–10.3) (n = 67)	8.8 (6.7–10.4) (n = 31)	7.5 (5.2–8.7) (n = 33)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—0.002 SM vs CM—0.006
WBC ^d (10 ⁹ /L)	6.8 (5.7–8.8) (n = 74)	7.1 (6.0–9.6) (n = 67)	7.7 (5.2–13.0) (n = 31)	7.7 (6.8–9.5) (n = 36)	HC vs MSM—NS HC vs SM—NS HC vs CM—NS MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Platelets ^c (10 ⁹ /L)	320 (221–373) (n = 75)	105 (60–152) (n = 67)	80 (51–116) (n = 31)	70 (53–104) (n = 33)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—0.007 SM vs CM—NS

Table 1 continued.

Characteristic	Healthy Control	Moderately Severe Malaria	Severe Malaria Without Cerebral Malaria	Cerebral Malaria	P Values ^a
Creatinine ^d (mg/dL)	0.4 (0.4–0.5) (n = 45)	0.4 (0.4–0.6) (n = 39)	0.4 (0.3–0.7) (n = 22)	0.7 (0.4–0.08) (n = 24)	HC vs MSM—NS HC vs SM—NS HC vs CM—0.0003 MSM vs SM—NS MSM vs CM—0.001 SM vs CM—0.002
Blood lactate ^c (mM)	1.8 (1.4–2.5) (n = 49)	2.8 (1.8–3.6) (n = 47)	2.8 (2.0–3.7) (n = 28)	3.4 (2.0–5.2) (n = 29)	HC vs MSM—0.0003 HC vs SM—0.001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Parasite density ^c (per μ L)	0 (0–0) (n = 54)	92 560 (43 600– 145 600) (n = 67)	251 280 (133 880– 376 000) (n = 31)	88 604 (31 138– 268 110) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—<0.0001 MSM vs CM—NS SM vs CM—0.002
Histidine rich protein-2 ^c (ng/mL)	0.3 (0.3–0.3) (n = 38)	357.2 (163.4– 1522.7) (n = 43)	1337.3 (234.8– 6377.4) (n = 9)	757.1 (397.2– 1978.2) (n = 26)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Angiopietin 2 ^c (pg/mL)	1058 (502– 1448) (n = 43)	2046 (1074–2798) (n = 63)	2250 (1343–3298) (n = 22)	3651 (2470–6134) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—0.0001 SM vs CM—0.01
Arginine ^c (μ M)	85.8 (72.0– 111.4) (n = 59)	45.8 (34.6–60.1) (n = 64)	49.8 (37.8–62.8) (n = 23)	41.1 (30.6–52.3) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Plasma arginase ^d (μ mole/mL/hr)	0.128 (0.072– 0.239) (n = 48)	0.122 (0.84–0.239) (n = 51)	0.157 (0.098–0.328) (n = 15)	0.169 (0.099–0.386) (n = 27)	HC vs MSM—NS HC vs SM—NS HC vs CM—NS MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Plasma cell-free Hb ^c (μ M)	0.82 (0.41– 1.57) (n = 59)	0.90 (0.49–1.38) (n = 64)	1.04 (0.58–1.31) (n = 23)	1.22 (0.79–1.81) (n = 36)	HC vs MSM—NS HC vs SM—NS HC vs CM—0.049 MSM vs SM—NS MSM vs CM—0.046 SM vs CM—NS
Asymmetrical dimethylarginine ^c (ADMA) (μ M)	0.65 (0.59– 0.75) (n = 59)	0.56 (0.48–0.69) (n = 64)	0.59 (0.47–0.73) (n = 23)	0.54 (0.46–0.68) (n = 36)	HC vs MSM—0.001 HC vs SM—NS HC vs CM—0.002 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
Symmetrical dimethylarginine ^c (SDMA) (μ M)	0.43 (0.37– 0.49) (n = 59)	0.54 (0.42–0.67) (n = 64)	0.58 (0.43–0.66) (n = 23)	0.57 (0.41–0.51) (n = 36)	HC vs MSM—0.0001 HC vs SM—0.0007 HC vs CM—0.0004 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS

Table 1 continued.

Characteristic	Healthy Control	Moderately Severe Malaria	Severe Malaria Without Cerebral Malaria	Cerebral Malaria	P Values ^a
Arginine:ADMA ratio ^c	141.4 (111.7–172.0) (n = 59)	79.6 (66.4–93.1) (n = 64)	81.0 (66.4–99.6) (n = 23)	75.5 (61.0–90.1) (n = 36)	HC vs MSM—<0.0001 HC vs SM—<0.0001 HC vs CM—<0.0001 MSM vs SM—NS MSM vs CM—NS SM vs CM—NS
ADMA/Cr ratio ^c	1.58 (1.17–1.87) (n = 30)	1.29 (0.93–2.09) (n = 36)	1.52 (0.88–2.28) (n = 14)	0.92 (0.52–1.30) (n = 24)	HC vs MSM—NS HC vs SM—NS HC vs CM—0.0002 MSM vs SM—NS MSM vs CM—0.005 SM vs CM—0.03
SDMA/Cr ratio ^d	1.02 (0.81–1.16) (n = 30)	1.09 (0.93–1.87) (n = 36)	1.26 (0.91–1.71) (n = 14)	1.04 (0.66–1.28) (n = 24)	HC vs MSM—NS HC vs SM—NS HC vs CM—NS MSM vs SM—NS MSM vs CM—NS SM vs CM—NS

Abbreviations: ADMA, asymmetric dimethylarginine; ANOVA, analysis of variance; CM, cerebral malaria; MSM, moderately severe malaria; NS, not significant; SDMA, symmetric dimethylarginine; SM, severe malaria.

^a Sidak pairwise comparison comparing disease groups.

^b Median (interquartile range).

^c $P < 0.05$ (on ANOVA or Kruskal–Wallis comparing severe malaria, cerebral malaria, moderately severe malaria, and healthy controls).

^d Not significant.

did not differ. Arginine levels in those with MSM, SM, and CM recovered as treatment was given and the children recovered (Figures 1 and 2). ADMA plasma levels in the Tanzanian children with CM and SM at presentation were lower (rather than higher) than HC levels at day 0.

SDMA levels were elevated in each of the malaria groups at day 0. To control for retention of SDMA in renal impairment, we expressed SDMA as SDMA/creatinine ratio. Median SDMA/creatinine ratios were not different among the groups (Table 1). Arginine:ADMA ratios were significantly lower in MSM, CM, and SM compared to HC ($P < .001$), and the arginine:creatinine ratios were not different (Table 1 and Figure 2).

Correlations of Plasma Arginine, ADMA, SDMA, and Arginine:ADMA Ratios With Markers of Malaria Severity

We tested relationships of arginine, ADMA, SDMA, and the arginine:ADMA ratios in malaria patients to several variables related to malaria severity. Table 2 displays the several significant relationships. In the combined group of children with cerebral and noncerebral severe malaria, there was an inverse relationship between lactate and the arginine:ADMA ratio ($P = .017$) and a direct relationship with ADMA ($P = .037$), but not with arginine or SDMA alone (Table 2A). There was an inverse relationship between angiotensin-2 and the arginine:ADMA ratio ($P < .0001$), and direct relationships with SDMA ($P < .0001$) and ADMA ($P = .002$), but not with arginine alone (Table 2B). Also, there was a direct significant correlation between the arginine:

ADMA ratio and blood hemoglobin ($P = .018$) when considering all malaria patients (Table 2B) but no correlation with plasma cell-free hemoglobin. While plasma arginase activity was not elevated in patients with malaria compared to HC children and not correlated with ADMA or plasma cell-free hemoglobin, it did inversely correlate with the arginine:ADMA ratio in malaria patients ($P = .005$). The relationships between the arginine:ADMA ratio and lactate and angiotensin 2 remained significant after adjusting for disease severity, gender, age, and parasite count.

Longitudinal Course of ADMA, SDMA, and Arginine:ADMA Ratio

In children with cerebral malaria there was a significant increase in arginine ($P < .001$) and the arginine:ADMA ratio ($P = .001$) with clinical recovery but not in ADMA or SDMA concentrations. In those with severe noncerebral malaria, there was a significant increase in arginine ($P < .001$), ADMA ($P < .001$), and the arginine:ADMA ratio ($P = .04$) but not SDMA (Figure 2A–2H).

DISCUSSION

In adults with severe falciparum malaria, we have previously shown that ADMA is increased and associated with decreased exhaled NO and NO-dependent endothelial dysfunction [6]. In addition, we found that ADMA is an independent predictor of

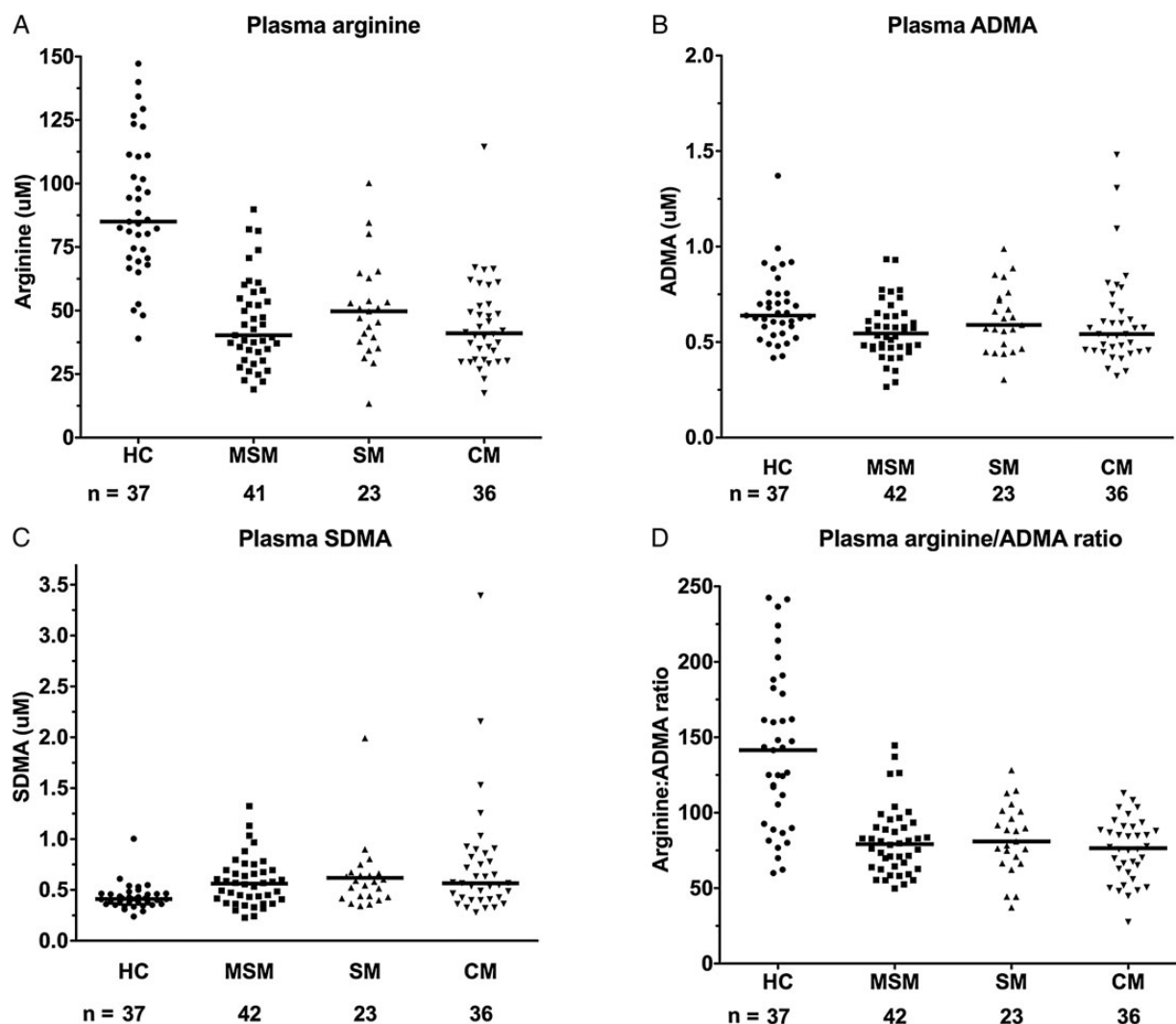


Figure 1. Plasma arginine, ADMA, and SDMA in children with malaria. *A*, Plasma arginine; *B*, plasma ADMA; *C*, plasma SDMA; and *D*, plasma arginine:ADMA ratio on presentation to the hospital (day 0). The horizontal bars represent the median values. The bottom row of numbers in A–D displays the numbers of subjects for each time point. Statistical analyses (Sidak pairwise comparisons) show that: *A*, For plasma **arginine**, MSM, SM, and CM are significantly lower than HC ($P = .0001$), and SM is not significantly different than CM. *B*, For plasma **ADMA**, HC is significantly higher than in MSM ($P = .001$), SM ($P = .045$), and CM ($P = .008$), but SM is not significantly different than MSM. *C*, For plasma **SDMA**, HC is significantly lower than MSM ($P = .0001$), SM ($P = .002$), and CM ($P = .0001$), but SM is not significantly different than MSM. *D*, For the plasma **arginine:ADMA ratio**, MSM, SM, and CM are all significantly different than HC ($P < .0001$), but SM is not significantly different than CM. Abbreviations: ADMA, asymmetric dimethylarginine; CM, cerebral malaria; HC, healthy control; MSM, moderately severe malaria; SDMA, symmetric dimethylarginine; SM, severe malaria.

disease severity and death in adults. Here we show that in contrast to adults, ADMA is reduced in severe falciparum malaria in children at the time of presentation. In children with falciparum malaria, the arginine:ADMA ratio was reduced in severe malaria and inversely associated with measures of perfusion and endothelial activation. This suggests that low arginine and not increased ADMA is the major contributor to impaired arginine bioavailability to NOS in children with severe malaria.

Although plasma ADMA has been shown to be an independent prognostic indicator for poor clinical outcomes in coronary artery disease, peripheral vascular disease, kidney failure

[7, 9], and acute critical illnesses such as sepsis and severe malaria in adults [[6] for review], these studies have been confined to adults. It is notable that ADMA is increased and associated with risk of death in adult sepsis but is decreased in pediatric sepsis [10, 15]. The pediatric sepsis study and our results in severe malaria suggest a difference in ADMA production or clearance in critically ill children with acute infection compared with that in adults.

The dimethylarginines originate primarily from degradation of proteins from sources such as muscle and erythrocytes and the actions of protein methyl transferases [7, 9, 12, 16]. Others

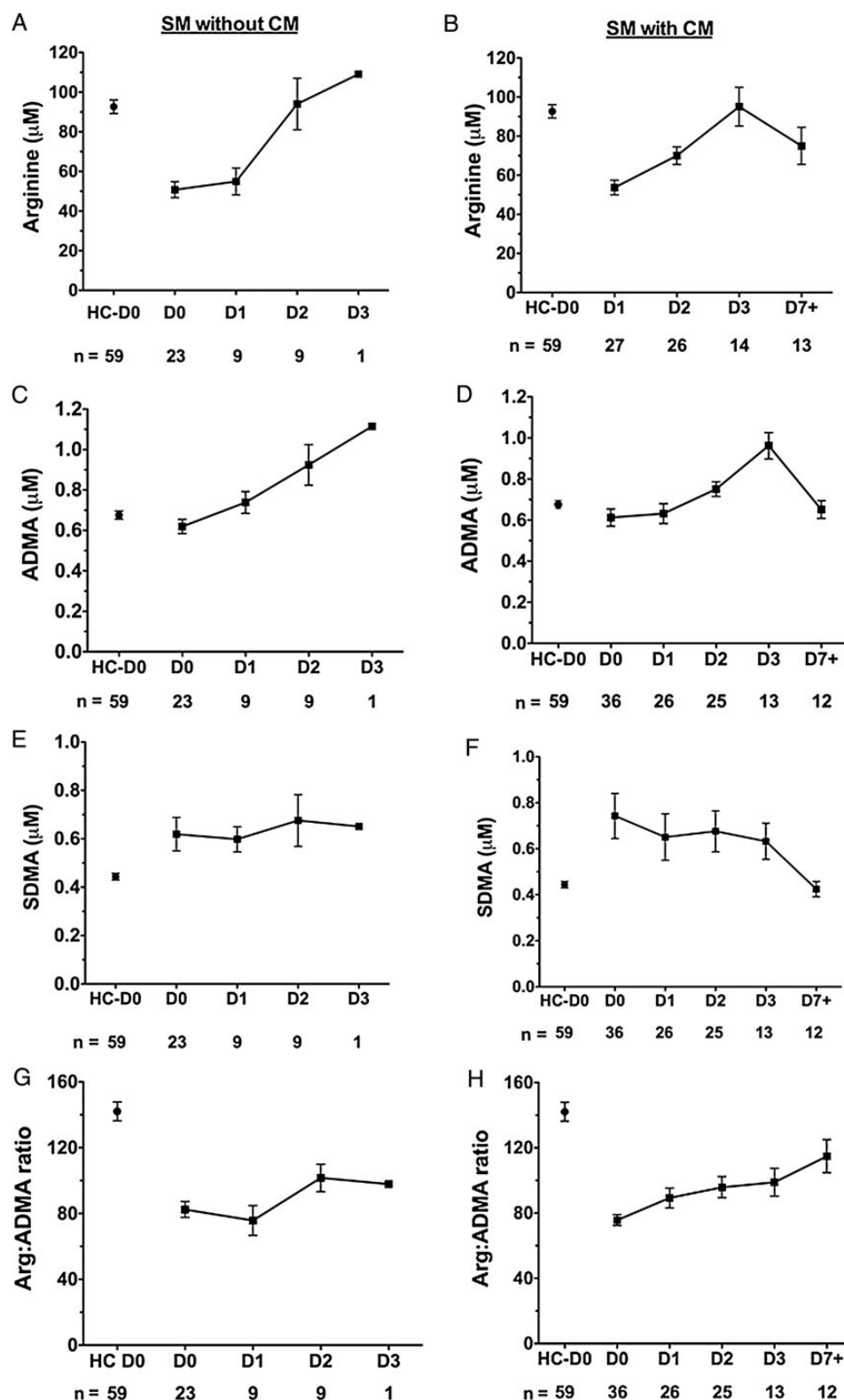


Figure 2. Time courses for plasma arginine, ADMA, SDMA, and arginine:ADMA ratios in children with severe malaria without cerebral malaria, and in children with severe malaria with cerebral malaria. *A, C, E, and G* display time courses (day 0 through D3) of plasma arginine (*A*); plasma ADMA (*C*); plasma SDMA (*E*); and plasma arginine:ADMA ratios (*G*). *B, D, F, and H* display time courses (day 0 through D7+, with D7+ representing values during the period from day 7 through day 17) of plasma arginine (*B*); plasma ADMA (*D*); plasma SDMA (*F*); and plasma arginine:ADMA ratios (*H*). Severe malaria without cerebral (“SM without CM”) is shown in *A, C, E, and G*, and severe malaria with cerebral malaria (“SM with CM”) is shown in *B, D, F, and H*. The bottom row of numbers in *A–H* displays the numbers of subjects for each time point. “HC-D0” is from healthy control children on day 0. The values represent the mean $\pm$ the standard error of the mean. Linear mixed effects modeling show that: *A*, For plasma arginine in SM without CM, there was a 23.3 $\mu\text{mol/L}$

Table 2. Correlation of Arginine, ADMA, SDMA, and the Arginine: ADMA Ratio With Physiological Measures and Biomarkers of Malaria Severity^a

	Arginine			ADMA			SDMA			Arg/ADMA		
	R	P Value	df	R	P Value	df	R	P Value	df	R	P Value	df
A: For all severe malaria patients (severe malaria without cerebral malaria, and severe malaria with cerebral malaria)												
Temperature	−0.224	.049	58	−0.240	.037	58	...	NS	...	...	NS	...
Systolic BP	...	NS	...	...	NS	...	−0.240	.042	54	−0.241	.031	54
WBC	...	NS	...	+0.298	.012	58	...	NS	...	...	NS	...
Glucose	...	NS	...	−0.341	.024	35	...	NS	...	...	NS	...
Bicarbonate	...	NS	...	...	NS	...	−0.243	.046	51	...	NS	...
Creatinine	...	NS	...	...	NS	...	+0.863	<.0001	37	...	NS	...
Lactate	...	NS	...	+0.265	.037	48	...	NS	...	−0.311	.017	48
Angio 2	...	NS	...	+0.374	.002	57	+0.365	.042	55	−0.520	<0.0001	55
Arginase	...	NS	...	...	NS	...	...	NS	...	−0.363	.010	42
B: For all malaria patients (moderately severe malaria, severe malaria without cerebral malaria, and cerebral malaria)												
Temperature	+0.303	.0004	121	−0.265	.002	121	...	NS	...	...	NS	...
Systolic BP	...	NS	...	...	NS	...	...	NS	...	+0.184	.029	112
WBC	...	NS	...	...	NS	...	...	NS	...	−0.210	.011	121
Hb	...	NS	...	...	NS	...	...	NS	...	+0.195	.018	121
Glucose	...	NS	...	−0.319	.004	71	...	NS	...	...	NS	...
Bicarbonate	+0.277	.003	98	...	NS	...	−0.202	.020	98	...	NS	...
Creatinine	...	NS	...	...	NS	...	+0.818	<.0001	73	...	NS	...
Lactate	...	NS	...	...	NS	...	...	NS	...	−0.270	.005	93
Angio 2	...	NS	...	+0.263	.002	120	+0.411	<.0001	120	−0.394	<.0001	120
Arginase	−0.187	.002	93	...	NS	...	...	NS	...	−0.268	.005	93

Abbreviations: ADMA, asymmetric dimethylarginine; BP, blood pressure; df, degrees of freedom; Hb, hemoglobin; NS, not significant; R, correlation coefficient; SDMA, symmetric dimethylarginine; WBC, white blood cells.

^a The following parameters were examined: temperature, heart rate, respiratory rate, systolic BP, diastolic BP, parasitemia, WBC, Hb, platelet count, blood bicarbonate, blood glucose, creatinine, lactate, HRP2, angiopoietin 2, plasma arginase, plasma free hemoglobin. The table displays results of only those that had *P* values < .05.

have noted increased levels of ADMA in patients with sickle hemoglobin disorders [8, 16] and have speculated that the ADMA derives from hemoglobin degradation after hemolytic destruction of arginase-containing erythrocytes during disease-associated hemolysis. However, we did not find strong evidence for this in pediatric falciparum malaria. It is notable that we found no significant differences in plasma arginase enzyme activity in children with malaria compared to healthy children nor an association between plasma arginase and ADMA. Moreover, plasma cell-free hemoglobin was elevated only in those with CM and then only modestly so. Both findings contrast with our prior observations in adults with falciparum malaria where plasma arginase and cell-free hemoglobin were elevated to a greater extent in severe malaria [4]. It is possible

that intravascular hemolysis may contribute less to the impaired NO bioavailability of severe malaria in children than in adults.

Dimethylarginines are taken up by the liver where ADMA is acted upon by dimethylarginine-dimethylaminohydrolase-1 (DDAH), an enzyme that degrades ADMA (but not SDMA) to dimethylamine and citrulline [9]. Liver blood flow is increased in uncomplicated malaria and sepsis but reduced in severe malaria and sepsis in adults [17, 18]. This has been proposed as an explanation of the U-shaped relationship between plasma ADMA and disease severity in both illnesses in adults [6]. Because ADMA is low on admission in both moderately severe and severe malaria in children, it is possible that hepatic blood flow and/or DDAH-1 activity is increased in severe

Figure 2 continued. increase per day from D0 to D3 (*P* < .001). For the group with SM including CM, there was a 6.8 μmol/L increase per day from D0 to D7 (*P* < .001). B, For plasma ADMA in SM without CM there was a significant increase of 0.17 μmol/L per day (*P* < .001). For those with SM including CM, there was significant increase of 0.027 μmol/L per day (*P* < .014), but not for cerebral malaria alone (*P* < .06). C, For plasma SDMA in SM without CM and SM with CM, there was no significant change over D0 to D7 (*P* < .17). Abbreviations: ADMA, asymmetric dimethylarginine; CM, cerebral malaria; SDMA, symmetric dimethylarginine; SM, severe malaria.

as well as uncomplicated malaria in children, with ADMA being more efficiently cleared in children than in adults with critical illness. We speculate that the increase in ADMA seen in children with severe malaria after 2–3 days of treatment may reflect either a delayed increase in cellular breakdown or more likely, a diminution of ADMA clearance as increased hepatic blood flow returns to normal. In addition, because arginine can regulate ADMA metabolism by DDAH [19], arginine levels per se could influence levels of ADMA. Although *P. falciparum* parasites contain protein arginine methyltransferases and can generate methylated arginines [20], parasitemias are at least as high in children as in adults with severe malaria [6], and differences in parasite load are thus unlikely to explain the differences in ADMA levels between age groups.

We have confirmed our previous finding of marked hypoargininemia in severe pediatric malaria [4, 21] and now show that in children (as in adults) arginine levels recover to normal within 3 days [22]. At presentation, plasma arginine concentrations are lower in severe pediatric malaria compared to levels we have reported in adult severe malaria [3, 4]. In children, hypoargininemia is the major contributor to the diminished arginine:ADMA ratio, a measure of arginine bioavailability to cells, whereas in adults the higher ADMA concentrations contribute more to the reduction in the arginine:ADMA ratio [6]. Although systemic ADMA levels are reduced in children with both severe sepsis [15] and severe malaria at presentation (our current study), the degree of reduction likely still influences arginine and hence NO bioavailability in severe disease. In support of this, the arginine:ADMA ratio, but not arginine, is inversely associated with levels of angiopoietin-2, an autocrine mediator of endothelial activation that is both NO-dependent and an independent predictor of mortality in severe malaria [23, 24]. Further evidence for the importance of ADMA levels in pediatric severe malaria is that the arginine:ADMA ratio, but not arginine, is independently and inversely associated with blood lactate, a measure of organ perfusion and also a predictor of clinical outcome in malaria. Moreover, genome-wide association studies have shown that a DDAH-1 polymorphism is associated with an increased likelihood of severe malaria in children [25], and DDAH-2 polymorphisms have been linked to cold shock in pediatric sepsis [26] and increased severity of organ failure and early septic shock in adults [27].

In contrast to ADMA, we show here that in patients at presentation SDMA is significantly elevated in uncomplicated and severe pediatric malaria, a finding similar to our earlier finding in Papuan adults with malaria in which both ADMA and SDMA were increased at the time of presentation [6]. SDMA is renally excreted, and it is known to be elevated in critical illnesses with renal impairment [28]. Although not as marked as that seen in adult *falciparum* malaria, renal impairment does occur in severe pediatric malaria [3, 4]. When plasma SDMA concentrations were adjusted for renal dysfunction and

expressed as SDMA/creatinine ratios, the differences among disease groups disappeared, making it likely that impaired renal clearance of SDMA contributes to the elevated SDMA concentrations in pediatric severe malaria, as was shown before in adults. The return of SDMA concentrations to normal by day 7 and later likely reflects the resolution of renal impairment in these children following treatment.

Similar to ADMA, SDMA is associated with poor clinical outcomes including death in coronary heart disease, peripheral artery disease, and renal failure [7, 12]. SDMA is not an inhibitor of NOS activity, but at very high supraphysiological concentrations it competes with arginine for the cationic amino acid transporter and could potentially create an intracellular arginine deficiency state and limit NO production [11]. Although this is possible, this is very unlikely with the plasma concentrations we report here. SDMA augments proinflammatory processes in macrophage-like cells by increasing cytokine (interleukin 6 [IL-6] and tumor necrosis factor [TNF]) elaboration and oxidative stress (superoxide production), which in turn could quench NO and reduce its bioavailability [12, 29]. Although renal SDMA retention may contribute to the high SDMA concentrations noted in severe malaria, these elevated SDMA levels are nevertheless biologically active and may exacerbate NO deficiency.

Low NO bioavailability is central to the pathophysiology of both pediatric and adult severe malaria [3–6, 21, 30], with low bioavailability of arginine (reflected in low arginine:ADMA ratios) a major contributor in both age groups. In children with severe malaria, this low arginine:ADMA ratio is due more to severe hypoargininemia rather than to elevated ADMA. High SDMA may also add to low NO bioavailability through increased oxidative stress, and a worse clinical outcome. Strategies to increase NO bioavailability in severe malaria could prove to be useful as adjunctive malaria treatment in both children and adults.

Notes

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Potential conflicts of interest. N. M. A., D. L. G., and J. B. W. are named as inventors in a US patent for the use of L-arginine as treatment for severe malaria but have transferred all of their rights to their respective institutional

malaria research collaborations. This patent was issued for US rights only, and no rights are being sought in other countries. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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