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Research Article

L-arginine enhances blood trace metals and reduces oxidative stress burden in sickle cell anaemia subjects in the steady state

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L-arginine, sickle cell anaemia, antioxidant enzymes, trace metals, malondialdehyde,

ABSTRACT

Background: It is not clear how arginine supplementation, which may be beneficial in the management of sickle cell anaemia, interplays with trace metals which are required as cofactors for antioxidant enzymes levels and activity. **Methods:** We compared the effect of oral, low-dose (1 g/day), 6-week supplementation with L-arginine on some trace metals and antioxidant enzymes levels in 33 HbAA and 28 HbSS subjects. Ten (10) milliliters of blood was withdrawn from an ante-cubital vein for the estimation of plasma arginine concentration ([R]), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels and serum concentrations of Zn^{++}, Mn^{++} and Cu^{++}. **Results:** HbAA subjects had higher levels of Zn^{++}, Mn^{++}, [R], CAT, SOD and GPx but lower [MDA] ($p < 0.05$ in each case) than HbSS subjects. In both groups, L-arginine supplementation increased [R], SOD, GPx, CAT, Zn^{++} and Cu^{++} ($p < 0.05$ in each case and group) but decreased [MDA] ($p < 0.001$ in each group). Mn^{++} level decreased in HbAA but, it increased in HbSS subjects ($p < 0.001$ in each case). The degree of change (%Δ) in all the measured parameters (except MDA) was higher in HbSS than in HbAA subjects. Correlation coefficients (r) calculated between changes (Δ) in trace metals levels and changes (Δ) in antioxidant levels were higher in HbSS subjects. Conclusion: Study showed that L-arginine boosted plasma arginine, serum trace metals and antioxidant enzymes but decreased malondialdehyde in HbSS subjects in the steady state. Associations between changes in antioxidant enzymes and changes in trace metal levels were higher in HbSS than in HbAA subjects.

INTRODUCTION

Sickle cell haemoglobinopathy which arises from a replacement on the beta chain of haemoglobin of glutamic acid by valine at the 6th position (Ingram, 1956) is common among black populations of the world. In Africa, the incidence of sickle cell anaemia (SCA) is between 5% and 40% (Diallo and Tchemia, 2012). The World Health Organisation (WHO, 1994) estimates that in Africa 120,000 to 200,000 babies are born each year with SCA while in Nigeria, the estimated annual number of HbSS neonates is about 85,000 (Piel *et al.,* 2013). In the USA, sickle cell anaemia (SCA) is considered the most common haemoglobinopathy and it is estimated that about 100,000 individuals suffer from the disease (Hassel, 2010).

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Recent evidence suggests that supplementation with arginine may be beneficial in the management of sickle cell anaemia (SCA). Arginine is a naturally occurring basic amino acid which is involved in many important biochemical reactions associated with normal physiology. It is found in proteinous foods like meat, poultry, nuts and fish and also in watermelon. Arginine level has been shown to be low in adults with SCA in the steady state (Enwonwu *et al.,* 1990; Morris *et al.,* 2000) and in patients with SCA in vaso-occlusive crises and acute chest syndrome (Morris *et al.,* 2000; Dasgupta *et al.,* 2006). Children suffering from the disease, however, have plasma levels that are similar to normal controls (Morris *et al.,* 2000).

Although earlier studies had suggested the arginine-NO pathway as the major pathway for arginine activity in sickle cell anaemia (SCA) subjects (Morris *et al.,* 2000; Styles *et al.,* 2007; Bakshi and Morris, 2016), there may be other pathways through which arginine may exert its

beneficial effects. Arginine may have antioxidant effects since co-administration with hydroxyurea increased the plasma level of glutathione peroxidase in SCA patients (Little *et al.,* 2009). In addition, arginine had been shown to increase total antioxidant enzymes (TAE) and decrease malondialdehyde (MDA) levels in SCA subjects (Kehinde *et al.,* 2015). Furthermore, Jaja *et al.,* (2016) showed that change in plasma arginine concentration (Δ[R]) correlated negatively with change in some liver enzymes (aspartate aminotransferase, (AST), alanine aminotransferase, (ALT), and alkaline phosphatase, (ALP)) and change in [MDA] suggesting a role for arginine-antioxidant activity. In sickle cell mouse model, arginine increased erythrocyte glutathione levels (Romero *et al.,* 2002) and protected against oxidative stress (Dasgupta *et al.,* 2006). The levels of antioxidant enzymes in sickle cell anaemia had been found to be variable in the steady state. While some studies showed an increase, others showed a decrease in plasma levels of these antioxidants (Das and Nair, 1980; Dasgupta *et al.,* 2006). It had been suggested that the increase in antioxidant enzymes may be protective; in order to scavenge hydrogen peroxide, while the decreased levels could be due to the overwhelming level of oxidative stress or reactive oxygen species (Chirico and Pialoux 2012).

Antioxidant enzymes which include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and heme-oxygenase-1 (HO-1) are present as metallo-enzymes or metallo-proteins and require trace metals as cofactors. Superoxide dismutase requires copper, zinc and manganese as cofactors, while catalase requires iron as a cofactor to catalyse the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen (Hyacinth *et al.,* 2010). Glutathione peroxidase requires selenium as a cofactor and catalyses the degradation of H_2O_2 and hydro-peroxides at the expense of reduced glutathione (GSH) (Hyacinth *et al*., 2010). Trace metals levels in blood had been reported as similar (Kehinde *et al.,* 2010), lower (Olaniyi and Ariola, 2010) or higher (Akenami *et al*,. 1999) in HbSS subjects in the steady state when compared to their HbAA counterparts. Also, Kehinde *et al,* (2010) reported that plasma levels of Cu^{++}, Zn^{++} or Mn^{++} in HbSS subjects in the acute phase or during pain crises were higher than those of HbAA subjects or HbSS subjects in the steady state.

We have designed this study to investigate the effect of an oral, low dose (1 g/day), 6-week supplementation with L-arginine (Kehinde *et al*., 2015) on some trace metals and antioxidant enzymes levels in HbSS subjects with HbAA subjects serving as controls. The study also examined the relationship between the change in the serum levels of the trace metals and the antioxidant enzymes in both groups of subjects.

MATERIALS AND METHODS

Sixty one male and female adult subjects were recruited for the study after their medical history had been taken. Thirty three (33) participants were subjects without sickle cell anaemia (HbAA). They served as control subjects and were students of some tertiary institutions in Lagos, Nigeria. They were non-smokers and non-alcoholics. Twenty eight (28) participants were sickle cell anaemia (HbSS) subjects. The red blood cell indices were done. The sickling test was performed using solubility (hard red band on top and colourless solution, using a freshly prepared buffer mixture and packed red cell from EDTA anticoagulation blood). The haemoglobin electrophoretic pattern was determined for confirmation (Dacie, 1991). Persons with the sickle cell trait (HbAS) were excluded from the study.

The HbSS subjects were patients attending the Sickle Cell Out-Patients' Clinic of The Lagos University Teaching Hospital, (LUTH), Idi-Araba, Lagos, Nigeria. They were in the steady state. None of the subjects had been admitted to the ward for pain crisis in the preceding six months. There was also no history of blood transfusion in the last twelve months.

Institutional approval was obtained from the Ethics and Experimentation Committee of the College of Medicine of University of Lagos, Lagos, Nigeria. Each subject gave an informed consent before commencement of the study.

In the laboratory, anthropometric data: age (years), height (meters) and weight (kilograms), were recorded. Ten millilitres (10 mL) of blood was withdrawn from an ante-cubital vein of each subject for the estimation of red blood cell indices, plasma arginine ([R]) and malondialdehyde ([MDA]) concentrations and also serum concentrations of catalase (CAT), superoxide dismutase (SOD) glutathione peroxidase (GPx), Zn^{++}, Cu^{++} and Mn^{++} . L-Arginine (Mason Vitamins, Inc. Miami Lakes, Florida, USA.) was then administered to each subject orally at a dose of 1 g/day for 6 weeks. After 6 weeks, the parameters were measured again.

Processing of all blood samples was done immediately after collection. Two millilitres (2 mL) of blood from each subject was put into a bottle containing ethylene-diamine-tetraacetic acid (EDTA) for the measurement of red blood cell indices. Four millilitres (4 mL) was put into heparinised bottles and centrifuged at 3000 rpm for 15 minutes to obtain plasma. The remaining 4 mL of blood was left standing in plain vacuum bottles to obtain serum. Plasma samples were collected using an ice

centrifuge machine. The plasma and serum samples were then stored within an hour at a temperature of – 20 ^{0}C in a biofreezer.

Determination of Plasma L-Arginine Concentration ([R])
Plasma L-arginine concentration ([R]) (µmol/L) was determined using a modification of the Sakaguchi reaction (Kehinde *et al.,* 2015). In order to determine the concentration of L-arginine in plasma, 1.0 mL of test plasma was used instead of 100% L-arginine. Absorbance was read on the Spectrophotometer and plasma L-arginine concentration (µmol/L) was calculated from the standard curve. L-Arginine concentration ([R], mg/L) was then converted to µmol/L.

Determination of Serum Trace Metals Concentration
Concentrations of Zn^{++}, Mn^{++} and Cu^{++} in serum were determined using 2000 Series Bulk Scientific Atomic Absorption Spectrometer with a hollow cathode lamp and fuelled acetylene air mixture. The serum samples were aspirated and mean signal responses with each trace metal at its respective wavelength (Doherty *et al.*, 2010).

Determination of Plasma Catalase Level
Serum catalase (CAT) activity was assayed as described by Rukkumani *et al*, (2004). 0.01 M phosphate buffer, serum sample and 2 M hydrogen peroxide were combined to form a reacting mixture. The assay was then read at 620 nm on a spectrophotometer (at zero, 1, 2 and 3 minutes). CAT activity was calculated and expressed as U/mg protein.

Determination of Plasma Superoxide Dismutase Level
Serum superoxide dismutase (SOD) level was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at a temperature of 30 ºC as described by Rukkumani *et al.,* (2004). Reagents used were 50 mM Na_2CO_3 buffer, plasma samples and epinephrine stock solution. Absorbance was read at 480 nm on a spectrophotometer. SOD activity was calculated and expressed as U/mg protein.

Determination of Plasma Glutathione Peroxidase Level
The serum glutathione peroxidase (GPx) level was measured as described by Ellman (1959), 0.4 M phosphate buffer (pH 7.0), 10 mM sodium azide, plasma samples, glutathione and H_2O_2 to form a reacting mixture. The supernatant was then treated with Ellman's reagent. The absorbance was then read at 412 nm and GPx activity was calculated and expressed as U/mg protein (Rukkumani et al., 2004).

Estimation of Plasma Malondialdehyde Concentration
Plasma malondialdehyde concentration ([MDA]) was determined using plasma, thiobarbituric acid-trichloroacetic acid- hydrochloric acid reagent to form a reacting mixture. The mixture was heated in a boiling water bath for 15 minutes. Flocculent precipitate was removed and absorbance was read at 535 nm. Plasma lipid peroxidation was then estimated as plasma [MDA] (Doherty *et al.,* 2010).

Data Analyses.
Data was analysed and expressed as mean ± SEM. Statistical comparison was made using Student Neuman-Keuls post-hoc ANOVA test. Significance was accepted when $p < 0.05$. Correlation coefficients (r) were also calculated between change (Δ) in [R] and Δ in serum concentrations of trace metals or antioxidant enzymes.

RESULTS

The anthropometric and haematologic characteristics of the subjects are shown in Table 1.

Table 1: Physical and Haematological Parameters of HbAA and HbSS Subjects

Parameters	HbAA Mean ± SEM	HbSS Mean ± SEM
Age (years)	23.7 ± 1.0	24.5 ± 1.2
Height (cm)	172.0 ± 3.0	169.0 ± 2.0
Weight (kg)	67.2 ± 1.0	61.7 ± 1.1**
BMI (kg/m^2)	23.7 ± 0.6	21.8 ± 0.6*
RBC (10^6/mm^3)	4.8 ± 0.2	3.0 ± 0.2***
Hb (g/dL)	13.7 ± 0.4	8.9 ± 0.3***
PCV (%)	39.3 ± 1.1	25.1 ± 1.1***

BMI = Body Mass Index; RBC = red blood cell; Hb = haemoglobin; PCV = packed cell volume; * = p <0.05; ** = p<0.01; *** = p <0.001

The Table shows that the HbSS subjects were smaller in body weight and body mass index (BMI) than the HbAA subjects. Also the measured haematological parameters in the HbSS subjects were significantly less than in the HbAA subjects. Table 2 (a) Vs (b) shows that before supplementation with L-arginine, plasma [R], serum [Zn^{++}], [Mn^{++}], [CAT], [SOD] and [GPx] were significantly higher in HbAA than in the HbSS group ($p < 0.001$ in each case except Zn ($p < 0.05$)). However, [MDA] was significantly higher in HbSS group than in the HbAA group ($p < 0.001$). Supplementation with L-

Table 2: Effect of L-Arginine Supplementation on Concentrations of Trace Metals and Antioxidant Enzymes in both Groups of Subjects

	Pre Supplementation		Post Supplementation		* P Level			
Parameters	HbAA (a)	HbSS (b)	HbAA (c)	HbSS (d)	a Vs b	a Vs c	b Vs d	c Vs d
Zn^{++} (µg/L)	29.0 ± 3.0	20.0 ± 3.0	38.0 ± 3.0	34.0 ± 4.0	< 0.05	< 0.05	< 0.01	NS
Mn^{++} (µg/L)	146.0 ± 16.0	43.0 ±11.0	71.0 ± 19.0	93.0 ± 18.0	< 0.001	< 0.001	< 0.01	NS
Cu^{++} (µg/L)	19.0 ± 3.0	20.0 ± 3.0	34.0 ± 5.0	34.0 ± 3.0	NS	< 0.05	< 0.05	NS
CAT (U/mg prot)	91.3 ± 1.6	41.0 ± 0.6	96.0 ± 1.7	97.2 ± 6.1	< 0.001	< 0.05	< 0.001	NS
SOD (U/mg prot)	4880.0 ± 110	3390 ± 40.0	5820.0 ± 40.0	5410.0 ± 19.0	< 0.001	< 0.001	< 0.001	< 0.05
GPx (U/mg prot)	5.3 ± 0.07	3.7 ± 0.03	5.7 ± 0.03	5.8 ± 0.2	< 0.001	< 0.001	< 0.001	NS
[R] (µmol/L)	71.0 ± 3.0	48.0 ± 4.0	108.0 ± 3.0	87.0 ± 3.0	< 0.001	< 0.001	< 0.01	<0.05
[MDA] (mM/mg prot)	33.0 ± 2.0	41.4 ± 0.4	14.2 ± 0.7	14.9 ± 1.1	< 0.001	< 0.001	< 0.001	NS

HbAA = control subjects; HbSS = sickle cell anaemia subjects; CAT = catalase; SOD = superoxide dismutase; GPx = glutathione peroxidase; R = L-arginine; MDA = malondialdehyde; NS = not Significant; *P = Student Neuman-Keuls post-hoc ANOVA test

Table 3: Correlation Coefficients (r) between Measured Parameters after L-Arginine Supplementation in both Groups of Subjects

	Correlation Coefficient (r)	
Parameters	HbAA	HbSS
Δ[R] Vs Δ[Zn^{++}]	0.5*	0.6**
Δ[R] Vs Δ[Mn^{++}]	0.1	0.7***
Δ[R] Vs Δ[Cu^{++}]	0.6*	0.5*
Δ[R] Vs ΔCAT	- 0.02	0.7***
Δ[R] Vs ΔSOD	0.4*	0.5*
Δ[R] Vs ΔGPx	-0.01	0.5**
ΔCAT Vs Δ[Zn^{++}]	0.2	0.5*
ΔCAT Vs Δ[Mn^{++}]	0.2	0.5*
ΔCAT Vs Δ[Cu^{++}]	0.02	0.3
ΔSOD Vs Δ[Zn^{++}]	0.3	0.6**
ΔSOD Vs Δ[Mn^{++}]	0.3	0.5*
ΔSOD Vs Δ[Cu^{++}]	0.5*	0.5*
ΔGPx Vs Δ[Zn^{++}]	0.2	0.3
ΔGPx Vs Δ[Mn^{++}]	0.03	0.5*
ΔGPx Vs Δ[Cu^{++}]	0.2	0.6**

* = p <0.05; ** = p <0.01; *** = p <0.001

HbAA = control subject; HbSS = sickle cell anaemia subjects; Δ[R] = change in L-arginine concentration; Δ[Zn^{++}] = change in Zinc concentration; Δ[Mn^{++}] = change in Manganese concentration; Δ[Cu^{++}] = change in copper concentration; Δ[CAT] = change in catalase concentration; Δ[GPx] = change in glutathione peroxide concentration; Δ[SOD] = change in superoxide dismutase concentration; Δ[MDA] = change in malondialdehyde concentration.

arginine increased all the measured parameters in both groups except [MDA] that significantly decrease in the two groups (Table 2 (a) Vs (c) and (b) Vs (d)).

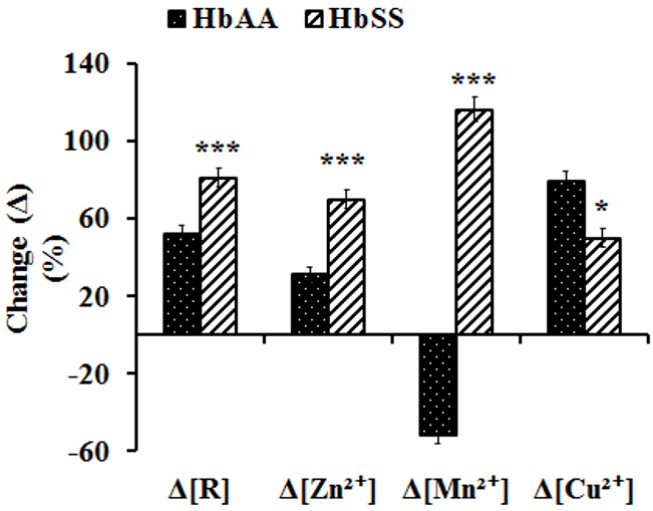

Fig.1: Changes in plasma L-arginine and serum trace metals concentrations in HbAA and HbSS subjects following L-arginine supplementation; HbAA = control subject ; HbSS = sickle cell; anaemia subject; Δ[R] = change in L-arginine concentration; Δ[Zn^{2+}] = change in Zinc concentration, Δ[Mn^{2+}] = change in Manganese concentration; Δ[Cu^{2+}] = change in copper concentration; * = p < 0.05, *** = p < 0.001 = significant .

Figure 1 shows that supplementation caused significantly greater percent change (% Δ) in [R] (p < 0.001), [Zn^{++}] (p < 0.001), [Mn^{++}] (p < 0.001) in HbSS

than in the HbAA group. Figure 2 shows that significantly greater increases (% Δ) occurred in [CAT] ($p < 0.001$), [SOD] ($p < 0.001$) and [GPx] ($p < 0.001$) in the HbSS subjects than in the HbAA subjects. Changes (% decrease) in [MDA] in the 2 groups were similar.

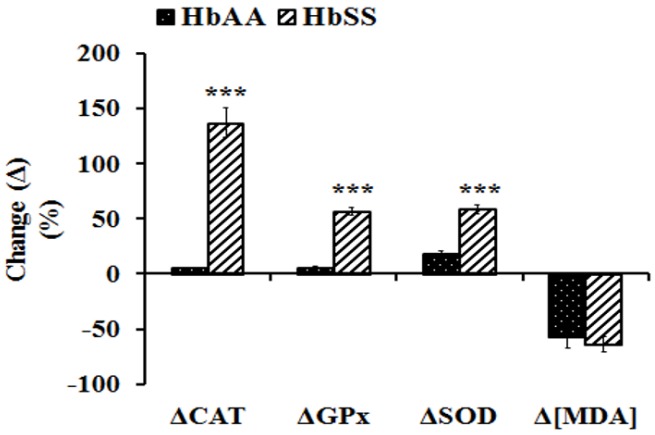

Fig. 2: Changes in plasma antioxidant enzymes and malondialdehyde concentrations in HbAA and HbSS subjects following L-arginine supplementation; HbAA = control subject; HbSS = sickle cell anaemia subject, Δ[CAT] = change in catalase concentration; Δ[GPx] = change in glutathione peroxide concentration; Δ[SOD] = change in superoxide dismutase concentration; Δ[MDA] = change in malondialdehyde concentration; *** = $p < 0.001$ = significant

Table 3 shows that in almost all cases the calculated correlation coefficients (r) between Δ [R] and Δ trace metals or antioxidant enzymes or between Δ in antioxidants and trace metals were higher in the HbSS than in the HbAA group.

DISCUSSION

Our study shows that a six-week, oral, low dose arginine supplementation may impact oxidative stress by elevating antioxidant enzymes and trace metals levels but decreasing malondialdehyde (MDA) level. These findings are intriguing owing to failed oral arginine clinical trials in the past (Styles *et al*., 2007) which demonstrated no clinical effect with prophylactic doses considered to be sub-therapeutic in treating adults and children at steady-state. Kehinde *et al.*, (2015) had highlighted different studies in which different high doses of L-arginine were used for varying durations of time. Those studies had employed higher doses over shorter periods of time. On the other hand, much lower dose (1 g/day) for a duration of six weeks had been shown to raise plasma arginine, reduced oxidative stress burden, and percent irreversibly sickle cell count and shifted the osmotic fragility curve to the right (Kehinde *et al.*, 2015). Also, Jaja *et al*, (2016) had shown that using a similar protocol, arginine decreased MDA, total bilirubin and liver enzymes (ALT, AST and ALP) but elevated serum nitric oxide metabolite concentration ($[NO_X]$). The chronic (6 weeks) nature of this study may have contributed to recovery of arginine stores especially in the HbSS subjects. The magnitude of change (Δ) in all the measured parameters was higher in HbSS subjects than in the HbAA control group, supporting earlier studies (Morris *et al*., 2000; Lopez *et al*., 2003) which showed that arginine metabolism was different between HbSS subjects and HbAA subjects.

Plasma L-arginine was estimated using the modified Sakaguchi reaction method (Kehinde *et al*., 2015). No significant difference between measurements using the HPLC amino acid analyser and the modified Sakaguchi method had been reported (Li *et al*., 2008; Kehinde *et al*., 2015). Values for [R] obtained and reported in this study are comparable to those reported by earlier authors that estimated plasma arginine using other methods (Schnog *et al*., 2004; Luneburg *et al*., 2011). Two (2) capsules of L-arginine (Mason Vitamins Inc. Miami Lakes, Florida, USA) contained L-arginine HCl (1000 mg) and dicalcium phosphate (120 mg). The concentrations of the other constituents, gelatine (bovine), magnesium stearate, stearic acid and silica were not stated by the manufacturer and their contributions to the results are unknown.

The lower plasma arginine concentration ([R]) seen in HbSS subjects agrees with earlier studies (Morris *et al*., 2000; Scavella *et al*., 2010). However, arginine levels in children suffering from SCD had been found to be similar to those of normal children but low in children suffering from vaso-occlusive crises (VOC) (Morris *et al*., 2000). Low arginine bioavailability had been associated with early mortality in adults with sickle cell anaemia (SCA) (Morris *et al*., 2005). In this study, supplementation with L-arginine elevated serum arginine levels in both groups of subjects. This result agrees with earlier studies in humans (Morris *et al*., 2000; Scavella *et al*., 2010) and mouse model of sickle cell disease (Romero *et al*., 2002).

Our results agree with earlier studies which showed that serum concentrations of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were lower in SCA subjects in the steady state than in normal subjects (Dasgupta *et al*., 2006) but contradicts the results of Das and Nair,(1980) which showed the opposite. Also, the higher resting MDA level seen in the SCA group in this study agrees with earlier studies (Nur *et al*., 2011). The higher MDA level when combined with the lower level of the antioxidant enzymes is an indication of high oxidative stress burden in the SCA group. Factors that contribute to high oxidative stress

burden in SCA are recurrent ischemia-reperfusion injury (Kaul and Hebbel, 2000), elevated cell-free haemoglobin (Reiter *et al*., 2002) and higher auto-oxidation of sickle haemoglobin (Akohoue *et al*., 2007). The consequences of high oxidative stress burden in SCA include increased haemolysis (Gladwin *et al*., 2010), endothelial damage (Hebbel *et al*., 2004) and reduced NO_X activity (Nur *et al*., 2011), resulting in vaso-occlusion and organ damage (Hebbel *et al*., 2009). Supplementation with L- arginine elevated blood antioxidant enzymes (CAT, SOD and GPx) levels but reduced MDA levels in both groups of subjects. This agrees with earlier results in humans (Little *et al*., 2009; Jaja *et al*., 2016) and transgenic sickle mice (Dasgupta *et al*., 2006). When taken together our results suggest that low dose chronic supplementation with arginine may result in reduced oxidative stress burden. The level of enhancement of the antioxidant enzymes was greater in the HbSS group than in the HbAA group. This result is further corroborated by the higher positive correlations seen between change in serum arginine (Δ[R]) and ΔCAT, ΔSOD or ΔGPx in HbSS group than in the control group (Table 3). Other authors had demonstrated in transgenic knockout sickle mice that arginine therapy reduced haemolysis and oxidative stress, improved nitric oxide metabolites (NO_X) bioavailability and micro-vascular function (Kaul *et al*., 2008). Morris *et al*, (2000) showed that arginine increased NO_X in normal subjects and dose-dependently in SCA patients with vaso-occlusive crisis but decreased it in SCA patients in the steady state. Furthermore, it had also been shown in SCA subjects that arginine supplementation increased serum NO_X but decreased MDA, total bilirubin (TB) and liver enzymes (ALT, AST and ALP) levels (Jaja *et al*., 2016).

Serum concentrations of Zn^{++}, Mn^{++} and Cu^{++} were lower in HbSS subjects than in the HbAA group. This is similar to earlier studies (Olaniyi and Ariola, 2010; Idonije *et al*., 2011) but different from those that observed higher values in HbSS subjects (Rukkumani *et al*., 2004). However, Kehinde *et al*, (2010) had reported that some trace metals concentrations were higher in SCA subjects in the acute (vaso-occlusive) phase than in healthy subjects. In sickle cell anaemia sufferers, deficient trace metals levels had been linked to deficient immune function, retarded growth hypogonadism in males, and delayed wound healing (Prassad, 2002; Silva and Marcos, 2005). Arginine supplementation caused increases in serum trace metals levels (except Mn^{++} in the HbSS group) in both groups of subjects. Greater increases were seen in the HbSS than in the HbAA group (except for Cu^{++}). It is not clear how arginine supplementation increased the serum levels of the trace metals. However, since the sources of the trace metals are dietary it is likely that arginine might have enhanced intestinal absorption of these trace metals from food.

Significant and positive correlations seen in this study between change in plasma arginine (Δ[R]) and change in the various trace metals levels and also between Δ[R] and change in the various antioxidant enzymes levels corroborate our results that increased arginine levels resulted in elevated levels of trace metals and antioxidant enzymes. In addition, the significant and positive correlation between change in each of the antioxidant enzymes levels and change in each of the trace metals levels suggest a positive association between them. The correlation coefficients (r) between change in measured antioxidant enzymes and change in trace metal levels (Table 3) were higher in HbSS than in their HbAA counterparts. Trace metals are integral parts and are required as co-factors by antioxidant enzymes to function properly (Harris *et al*., 1992; Prassad 2002; Farell, 2010). Superoxide dismutase requires copper, zinc and manganese as cofactors (Farewell, 2010; Chirico and Pialoux, 2012). Glutathione peroxidase also requires manganese as a cofactor (Olaniyi and Ariola, 2010) and catalyses the degradation of hydrogen peroxide (H_2O_2) and hydro-peroxides at the expense of reduced glutathione (GSH) (Chirico and Pialoux, 2010) while catalase requires iron as a cofactor to catalyse the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen (Hyacinth *et al*., 2010). The study therefore showed a relationship between serum trace metals concentrations and plasma antioxidant enzymes levels in the HbSS subjects. This could be another mechanism by which arginine exerts its antioxidant effects. Arginine is less expensive and had been shown to be a safe and efficacious intervention with narcotic-sparing effects in the management of vaso-occlusive pain episodes (Morris et al., 2013; Bakshi and Morris, 2016).\

In conclusion, this study demonstrated that chronic, low-dose, oral, arginine supplementation enhanced the recovery of arginine stores, elevated trace metals and antioxidant enzymes levels in the body. Elevation of trace metals levels may have contributed to the integrity and proper functioning of the antioxidant enzymes.

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