L-arginine increases nitric oxide and attenuates pressor and heart rate responses to change in posture in sickle cell anemia subjects

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Summary: Pressor and heart rate changes following change in posture without or with L-arginine supplementation (1g/day for 6 weeks) were studied in 28 sickle cell anemia (SCA) and 32 non-sickle cell anemia (NSCA) subjects. Change in posture increased HR ($p<0.01$), RPP ($p<0.05$) in both groups of subjects, MABP ($p<0.05$) in SCAS but reduced MABP ($p<0.01$) in NSCAS and PP ($p<0.01$) in SCAS. L-Arginine supplementation increased plasma L-Arginine concentration ([R]) in both groups of subjects ($p<0.001$ in each group) and serum nitric oxide metabolites concentration ([NOx]) ($p<0.01$ in each group). Change (Δ) [R] correlated positively with Δ [NOx] in both groups (+ 0.7 in each group). L-Arginine supplementation caused greater reduction of MABP ($p<0.001$) in NSCAS than in SCAS. However, reduction in HR was greater ($p<0.001$) in SCAS than in NSCAS. After supplementation, MABP and PP responses to change in posture were attenuated in the two groups. However, while HR and RPP responses in SCAS were attenuated, the same responses were enhanced in NSCAS by change in posture after supplementation. In conclusion, study shows that oral, low dose, chronic supplementation with L-arginine increased NO availability and attenuated pressor and heart rate responses to change in posture in sickle cell anemia subjects.

Keywords*:* Sickle cell anemia, Change in posture, L-arginine, Pressor and heart rate changes, Nitric oxide

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INTRODUCTION

Sickle cell anemia (SCA) is a hemoglobinopathy characterized by chronic hemolytic anemia and periodic vaso-occlusive events (Raghupathy and Billet, 2009). The disease may lead to progressive microvascular damage in several organs including the kidneys, lungs and brain (Hebbel, 2009). Sufferers possess a blunted autonomic cardiovascular response to change in posture (Jaja et al., 2008) indicating an abnormal autonomic cardiovascular function that may result in sudden death (Romero-Mestre et al., 1997). Although there is no cure for this ailment, new, novel or promising therapies have been tested (Morris et al., 2003; Ataga, 2009; Raghupathy and Billet, 2009).

One of the novel and promising therapies is L-Arginine supplementation. Sickle cell disease subjects have a dysregulated arginine metabolism, leading to increased oxidative stress and depleted arginine levels. (Schnog et al., 2004; Dasgupta et al., 2006; Kaul et al., 2008) In sickle cell transgenic mice, L-Arginine normalizes red blood cell density and induces Gardos channel inhibition (Romero et al.,

2002), protects against oxidative stress (Dasgupta *et al..* 2006) and improves microvascular function (Kaul *et al..* 2008). In humans suffering from SCA, it increases NO bioavailability (Morris et al., 2000) and relieves pulmonary hypertension (Minter and Gladwin, 2001; Morris et al 2003). It also decreases BP, HR and improves cardiac performance in congestive heart failure, hypertensive and type II diabetic patients (Bocchi et al., 2000; Vasdev and Gill, 2008; Huynk and Tayek, 2002). In healthy humans, L-Arginine plays a role in the regulation of autonomic cardiovascular control in humans through NO synthesis (Chowdhary and Townend, 1999; Chowdhary et al., 2002).

We have studied the pressor and heart rate changes following a chronic, low dose, (1g/day for 6 weeks), oral supplementation of L-Arginine on NSCA and SCA subjects without or with postural change. In addition, we also investigated the effect of supplementation on the relationship between L-Arginine concentration [R] and concentration of nitric oxide metabolites [NO_x] in blood.

MATERIALS AND METHODS

Fifty (50) male and female subjects were recruited for the study. Thirty-two (32) participants were non-sickle cell anemia subjects (NSCAS) while twenty-eight (28) were sickle cell anemia subjects (SCAS). The NSCAS attended tertiary institutions in Lagos. They were of genotype AA and selected based on their medical records on admission into their institutions. They were non-smokers, non-alcoholics and were not on any prescription during the study. Each of the NSCAS had a measured blood pressure of less than 140/90.

The SCAS were patients attending the Out-Patients Sickle Cell Clinic of The Lagos University Teaching Hospital, (LUTH), Idi-Araba, Lagos. They were in the steady state. None of the subjects with SCA 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.

In the laboratory, each subject's age (years), height (meters), weight (kilograms), were measured and recorded. The subject then lay supine on a couch 80 cm high for about 30 minutes. Arterial blood pressure (BP) and heart rate (HR) were measured simultaneously in this position. Arterial BP was measured on the right brachial artery in each subject using the Omron automated sphygmomanometer (Healthcare Co. Ltd. Kyoto, Japan).

Heart rate was measured in each subject using a 9-lead electrocardiographic (ECG) machine (GE Medical Systems, Freiburg, Germany). The electrodes were placed as recommended by the American Heart Association. The machine was set to show a deflection of 10 mm/mV. The paper speed was 25 mm/sec.

Responses to change in posture were measured as earlier described by Jaja et al (2008). Briefly, from the lying position the subjects were asked to rise to the standing position and measurements made immediately. Arterial blood pressure (BP) and ECG measurements were made within 30 seconds of taking the upright position. Two milliliters of blood were withdrawn from the ante-cubital vein of each subject for the estimation of plasma arginine concentration ([R]) and concentration of nitric oxide metabolites ([NOx]). L-Arginine (Mason Vitamins, Inc. Miami Lakes, Florida, USA.) was administered to each subject at a dose of 1g/day for 6 weeks. At the end of the 6-week period, arterial BP and HR measurements were made while [R] and [NOx] were estimated again.

Institutional approval was obtained and each subject gave informed consent.

Determination of Serum concentration of nitric oxide metabolites ([NOx])
The formation of [NOx] was measured by determination of its stable end products in serum, i.e.

nitrite (NO_2^-) and nitrate (NO_3^-) in mmol/l, as previously described (Sun et al, 2003).

Determination of Plasma L-Arginine level ([R])
L-Arginine concentration ([R]) in plasma (µM/L) was determined as earlier described by Li et al., (2008).

Statistical analysis
One of us, (SIO), read all the ECG tracings. Heart rate was calculated from the R-R interval and converted to beat/min. Mean arterial blood pressure (MABP, mmHg) was calculated as one-third of pulse pressure plus diastolic pressure while pulse pressure was the difference between systolic and diastolic blood pressures. Rate pressure product (RPP, arbitrary units) was the product of systolic blood pressure and heart rate. Body mass index (BMI) was calculated as weight/height^2.

Statistical comparisons were made using Student Neuman-Keuls post-hoc ANOVA test and results expressed as Mean ± SEM. Significance was accepted when $p < 0.05$. Correlation coefficients, (r), were calculated between change (Δ) in [R] and Δ in [NOx] in each group.

RESULTS

Table 1 shows a summary of the physical parameters in both groups of subjects.

Effect of change in posture.
Table 2 shows that resting MABP was greater in NSCAS than in SCAS ($p<0.01$) while resting PP and HR were higher in SCAS than in NSCAS ($p<0.05$ in each case). Change in posture decreased MABP in NSCAS ($p<0.01$) but increased HR in both groups ($p<0.001$ in each case) and RPP in both groups ($p<0.05$ in each group). In SCAS, change in posture decreased PP ($p<0.01$). (See Table 2)

Effect of L-Arginine supplementation.
Table 3 shows that L-Arginine supplementation decreased MABP ($p<0.01$) and RPP ($p<0.05$) in NSCAS. In SCAS, PP, HR and RPP fell ($p<0.05$ in each case). It however increased plasma [R] ($p<0.001$ in each group) and [NOx] ($p<0.01$ in each group). (See Table 3). In each group, plasma [R] correlated positively with serum [NOx] (+0.7 in each case).

Table 1. Summary of the Physical Parameters in Non-Sickle ans Sickle cell Anemia Subjects.

Variables	NSCAS	SCAS†
Age (yrs)	23.7 ± 1.0	24.5 ± 1.2
Height (m)	170.0 ± 2.0	168.0 ± 2.0
Weight (kg)	67.2 ± 2.0	61.7 ± 1.1**
BMI (kg/m2)	23.7 ± 0.6	21.8 ± 0.6*

NSCAS = Non-Sickle Cell Anemia Subjects; SCAS = Sickle Cell Anemia Subjects, † = Unpaired Student t-test, * = P < 0.05; ** = P < 0.01

Table 2. Effect of Change in posture on measured parameters in Non-Sickle Cell and Sickle Cell Anemia Subjects before L-Arginine Supplementation.

	NSCAS	SCAS	NSCAS	SCAS	P level ‡			
Variables	Supine(a)	Supine(b)	Erect (c)	Erect (d)	(a)Vs(b)	(a)Vs (c)	(b)Vs(d)	(c)Vs(d)
MABP(mmHg)	86.2± 1.5	78.2± 1.9	78.2± 1.7	81.8± 2.1	< 0.01	<0.01	NS	NS
PP (mmHg)	52.3± 1.6	57.6± 1.3	51.2± 2.0	45.6±2.7	< 0.05	NS	<0.01	NS
HR (b/min)	69.8± 2.6	75.6± 1.8	83.3± 2.1	88.1± 2.2	< 0.05	<0.001	<0.01	NS
RPP (AU)	8454± 322	8819±229	9399±247	9881±325	NS	<0.05	<0.05	NS

NSCAS = Non-Sickle Cell Anemia Subjects; SCAS = Sickle Cell Anemia Subjects, MABP =Mean arterial blood pressure; PP = Pulse Pressure; HR = Heart Rate; RPP = Rate Pressure Product, AU = Arbitrary Units; ‡ = Student Neuman-Keul Post-Hoc ANOVA test; NS = Not Significant

Table 3. Effect of L-Arginine Supplementation on Blood Pressure Parameters, Plasma L-Arginine and Nitric Oxide Metabolites' Concentrations in both Groups of Subjects

	Before Supplementation		After Supplementation		P level ‡			
Variables	NSCAS (a)	SCAS (b)	NSCAS (c)	SCAS (d)	(a)Vs(b)	(a)Vs(c)	(b)Vs(d)	(c)Vs(d)
MABP(mmHg)	86.2± 1.5	78.2± 1.9	78.9± 1.3	77.7± 2.6	< 0.01	<0.01	NS	NS
PP (mmHg)	52.3± 1.6	57.6± 1.3	47.0± 2.3	50.7±2.1	< 0.05	NS	<0.01	NS
HR (b/min)	69.8± 2.6	75.6± 1.8	67.4± 1.3	70.6± 1.6	< 0.05	<0.001	<0.01	NS
RPP (AU)	8454± 322	8819±229	7406±246	7770±378	NS	<0.05	<0.05	NS
[R] (mg/L)	124±5.0	83.0±7.0	188.0±6.0	152.0±4.0	<0.001	<0.001	<0.001	<0.001
[NO$_x$]	100.0	80.0	130.6	110.0	< 0.05	< 0.01	< 0.01	< 0.05

NSCAS = Non-Sickle Cell Anemia Subjects; SCAS = Sickle Cell Anemia Subjects, MABP =Mean arterial blood pressure; PP = Pulse Pressure; HR = Heart Rate; RPP = Rate Pressure Product; AU = Arbitrary Units; [R] = Plasma L-Arginine Concentration; [NO$_x$] = Serum Concentration of Nitric oxide metabolites; ‡ = Student Neuman-Keul Post-Hoc ANOVA test; NS = Not Significant
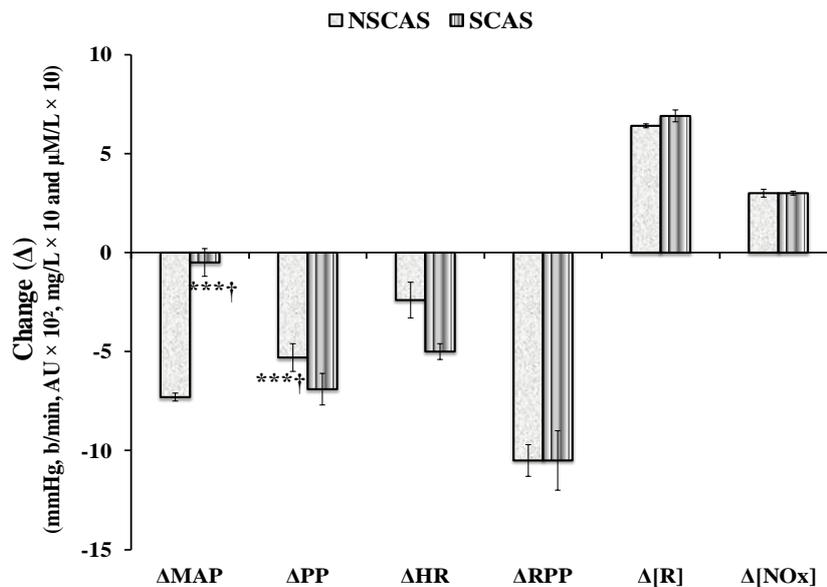

Fig. 1: Comparison of the effect of L-Arginine supplementation on measured parameters in the two groups of subjects. *** = P < 0.001; † = unpaired t-test

Figure 1 shows that the effect of supplementation on MABP was greater in NSCA than in SCAS (p<0.001). However, on HR the effect was greater in SCAS (p<0.001).

Effect of change in posture after supplementation.

Table 4 shows that after supplementation, change in posture decreased PP (p<0.01 in both groups), but increased HR (p<0.001 in NSCAS and p<0.05 in SCAS) and RPP (p<0.01 in NSCAS).

Figure 2 compares the changes in the cardio-vascular parameters brought about by change in posture without or with arginine supplementation. The fall (in NSCAS) or the increase (in SCAS) in MABP caused by change in posture was attenuated following change in posture after supplementation. Also, in NSCAS, the change (fall) in PP after change

Table 4. Effect of Change in posture on measured parameters in Non-Sickle Cell and Sickle Cell Anemia Subjects after L-Arginine Supplementation.

	NSCAS	SCAS	NSCAS	SCAS	P level ‡			
Variables	Supine(a)	Supine(b)	Erect (c)	Erect (d)	(a)Vs(b)	(a)Vs c)	(b)Vs(d)	(c)Vs(d)
MABP(mmHg)	78.9± 1.3	77.7± 2.6	77.5± 1.2	74.0± 1.6	NS	NS	NS	NS
PP (mmHg)	47.0± 2.3	50.7± 2.1	34.9± 2.4	38.0±3.1	NS	<0.01	<0.01	NS
HR (b/min)	67.4± 2.3	70.6± 2.6	84.9± 2.2	80.1± 2.0	NS	<0.001	<0.05	NS
RPP (AU)	7406±246	7770±378	8511±312	7958±286	NS	<0.01	NS	NS

NSCAS = Non-Sickle Cell Anemia Subjects; SCAS = Sickle Cell Anemia Subjects, MABP =Mean arterial blood pressure; PP = Pulse Pressure; HR = Heart Rate; RPP = Rate Pressure Product, AU = Arbitrary Units; ‡ = Student Neuman-Keul Post-Hoc ANOVA test, NS = Not Significant
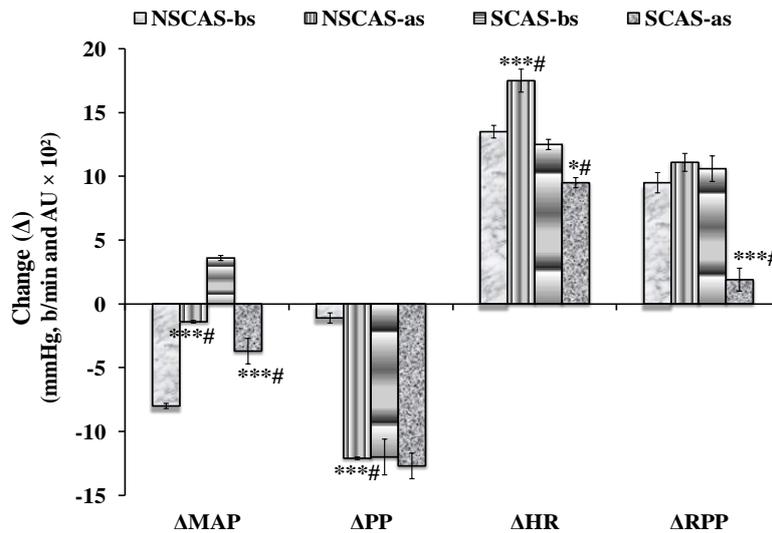

Fig. 2: Effect of change in posture on BP parameters without or with L-Arginine supplementation. *** = P < 0.001; ♯ = paired Student t-test

in posture was increased (p<0.001) following change in posture after supplementation. Supplementation equilibrated the changes in PP responses to change in posture in the two groups. (See Figure 2 please). In NSCAS, the change in HR response following change in posture after supplementation was greater than without supplementation (18 beat/min Vs 13 beat/min; p<0.001). In SCAS, the change in HR response following change in posture was less following supplementation than without supplementation (11 beat/min Vs 8 beat/min; p<0.01). While supplementation increased RPP responses in NSCAS (p<0.001), it decreased the responses in SCAS (p<0.001). These effects were significantly different (p<0.001). See Figure 2, please.

DISCUSSION

Subjects in this study were of similar age and height. However, SCAS weighed less than the NSCAS. This resulted in a significantly lower BMI in the SCAS since BMI is a derivative of height and weight. Mean arterial blood pressure was significantly lower and PP and HR were significantly higher in SCAS. The

elevated HR is probably a compensatory response to reduced blood pressure. Similar results had earlier been reported (Francis and Johnson, 1991; Jaja et al, 2000).

Effect of change in posture
Change in posture produced different MABP responses in the subjects. While it significantly decreased MABP in NSCAS it increased MABP slightly in SCAS. The marked decrease in PP seen in SCAS, in contrast to the slight decrease in NSCAS suggests an abnormal autonomic cardiovascular function in SCD (Romero- Mestre et al, 1997; Jaja et al, 2008). However, similar HR and RPP responses obtained with change in posture in the two groups agree with earlier observations (Cooke et al., 2002; Jaja et al., 2008).

Effect of L-arginine supplementation
In this study, we administered L-arginine orally at a dose of 1g/day for 6 weeks. Different authors have used different doses and routes to administer L-Arginine. Boger (2007) reported the use of 500mg/day to 2g/day in oral supplementation studies.

Chowdhary et al, (2002) made an acute infusion of 1gm/min for 30 minutes in normal human subjects. In their studies, Wolf et al, (1997) administered 8.4g/day to hypercholesterolemic humans while Huynk and Tayek (2006) administered 3g/hr for 10hrs to type II diabetic patients.

At the dose used in this study, supplementation increased plasma L-arginine concentration ([R]) and serum NO_x. In addition, there was a positive and high correlation (+0.7 in each group) between change in [R] and change in [NO_x]. These results are in agreement with earlier studies (Chowdhary et al, 2002; Kaul et al 2008; Ataga 2009; Little et al, 2009) but in contrast to the study of Morris et al., (2000) which showed that L-arginine supplementation decreased NOx in sickle cell patients in the steady state.

L-arginine supplementation caused a fall in all resting cardiovascular parameters measured in this study. The fall in blood pressure and heart rate in the two groups of subjects is similar to results obtained in normal subjects (Chowdhary et al, 2002) or type II diabetic patients (Huynh and Tayek, 2002) following chronic administration of L-Arginine and attributed to the L-arginine-nitric oxide pathway (Chowdhary et al, 2002).

Effect of change in posture after L-arginine supplementation

This study shows that L-arginine supplementation attenuated MABP, HR and RPP responses to change in posture in SCAS . Pulse pressure in these subjects remained unchanged. (See Figure 2). Attenuated pressor responses to change in posture after supplementation are indices of improved cardiovascular response in our SCAS. Improved and thus protective cardiovascular response to change in posture may be important to sickle cell disease sufferers who possess an abnormal autonomic cardiovascular function (Romero-Vecchione et al, 1995; Romero-Mestre et al, 1997; Jaja et al, 2008). Abnormal autonomic cardiovascular function causes sudden death in sickle cell disease sufferers (Romero-Mestre et al, 1997).

The attenuation of pressor and heart rate responses to change in posture by L-arginine in SCAS is attributable to increased NO availability and activity (Chowdhary et al, 2002; Vasdev and Gill, 2008) as suggested by results of this study. As earlier shown, supplementation increased NOx in both groups of subjects. Nitric oxide plays an important role in the regulation of human cardiovascular autonomic control (Chowdhary and Townend 1999; Chowdhary et al, 2002; Cooke et al, 2002; Herman et al, 2006; Huynk and Tayek, 2006). Nitric oxide activates the guanylate cyclase in the smooth muscle cells raising the level of intracellular messenger cGMP. This rise causes smooth muscle relaxation presumably by a decrease in intracellular Ca^{2+} concentration (Moncada and Higgs, 1993; Jerca et al, 2002).

It is not clear why different HR and RPP responses to change in posture following L-Arginine supplementation were seen in the two groups of subjects. Following supplementation, change in posture increased RPP in NSCAS while the opposite was the case in SCAS. An increase in RPP is attributable to increase in HR (since RPP is a product of HR and SBP). A possibility is that the increased NO bioavailability generated by increased [R] as seen in this study exerted a tonic, direct opposite chronotropic effect on the SA node (Musialet et al, 1997; Chowdhary et al, 2000; Chowdhary et al, 2002).

In conclusion, we have shown that oral, low dose, chronic supplementation with L-arginine increased NO availability and activity. Supplementation also attenuated pressor and heart rate responses to change in posture especially in sickle cell anemia subjects.

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