Patients with Sickle Cell Disease have Reduced Blood Antioxidant Protection

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Abstract: In previous studies, we found that homozygous sickle cell (HbSS) patients, compared with their healthy (HbAA) counterparts, had reduced levels of the omega-3 fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, in red cells, platelets, and mononuclear cells. These differences were not due to lower intake of the two fatty acids. We have investigated whether reduced antioxidant status in the patients could help explain the observed phenomenon. Blood specimens previously obtained for fatty acid study from Nigerian (26 HbSS and 30 HbAA) and British (30 HbSS, 9 sickle cell-hemoglobin C/HbSC, and 15 HbAA) subjects were analyzed for antioxidant status. The Nigerian HbSS patients compared with the controls had lower plasma retinol, α-tocopherol, and β-carotene concentrations ($p < 0.005$) and reduced activity of red cell Cu/Zn-superoxide dismutase (Cu/Zn-SOD) ($p < 0.05$). Similarly, the British HbSS group had reduced concentrations of plasma α-tocopherol ($p < 0.005$), and activities of red cell Cu/Zn-superoxide dismutase ($p < 0.05$) and Se-glutathione peroxidase (Se-GPx) ($p < 0.005$) than the controls. In addition, the British patients in comparison with those who had HbSC, a mild form of the disease, had lower α-tocopherol than that of the HbAA controls ($p < 0.005$). In the British sickle cell patients, there was a positive correlation between red cell ethanolamine phosphoglyceride (EPG) DHA and Cu/Zn-SOD activity ($r = 0.700$, $p < 0.05$), choline phosphoglyceride (CPG) DHA and Se-GPx activity ($r = 0.605$, $p < 0.05$), and CPG EPA and Se-GPx activity ($r = 0.558$, $p > 0.05$). Similarly, the percent DHA in red cell EPG was positively related with the activity of Se-GPx in the patients with HbSC ($r = 0.674$, $p < 0.05$). These findings suggest that the lower levels of membrane EPA and DHA in blood cells of the HbSS patients could be due to peroxidation resulting from a compromised antioxidant competence.

Key words: Fatty acids, sickle cell disease, α-tocopherol, superoxide dismutase, glutathione peroxidase

Introduction

Red cells contain iron, which is a potent catalyst of the generation of hydroxyl radical [1–3], and a high level of polyunsaturated fatty acids [4] that are susceptible to oxidation. In addition, red cells are continuously exposed to high oxygen tension. Hence, the red cell environment is potentially conducive for the generation of reactive oxygen species, oxidative chain reactions, and injury. Iron-mediated oxidative damage is prevented by the hydrophobic globin pocket that tightly binds heme and consequently limits the interaction between the red cell membrane iron and unsaturated fatty acids [5].

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Reactive free radicals are neutralized by a network of antioxidants, including the plasma antioxidant vitamins A, E, and C, and β-carotene, and the antioxidant enzymes, Cu/Zn-superoxide dismutase (SOD), Fe-catalase, and Se-glutathione peroxidase (GPx). Several studies have assessed the antioxidant status of patients with sickle cell disease. However, the findings have been inconsistent. Some have reported a decrease in the activities of red cell Cu/Zn-SOD [6, 7], red cell Se-GPx [6–8], and whole blood Se-GPx [9], while others have observed an increase in red cell Cu/Zn-SOD [8, 10], red cell Se-GPx [10, 11], and whole blood Se-GPx [12]. Similarly, reduced vitamin A [13, 14], β-carotene [15, 16], and vitamin E [13–15 17–20], normal vitamin A [21] and vitamin E [21, 22], and increased β-carotene [23] concentrations have been reported. These contradictory findings are difficult to explain.

In previous studies, we found that the levels of the omega-3 long-chain polyunsaturated fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA), in red cells (Figure 1), platelets, and mononuclear cells, were reduced in HbSS patients as compared to healthy (HbAA) controls [24, 25]. Similarly, although the reduction was not as remarkable as in the HbSS patients, individuals with the compound heterozygous sickle cell-hemoglobin C (HbSC) disease (a generally less severe form of sickle cell disease) had decreased DHA levels in red blood cells [26].

In the current study, we have investigated whether the a) antioxidant status of these patients was compromised by the oxidative overload that is inherent in sickle cell disease; and b) whether reduced levels of EPA and DHA observed in the patients was associated with the reduction of blood antioxidant status.

Subjects and Methods

Subjects

Steady-state HbSS (n = 30, aged 19–65 years) and HbSC patients (n = 9, aged 26–45 years), and healthy HbAA controls (n = 15, aged 20–52 years), were enrolled from St. Thomas' Hospital in London, United Kingdom. Similarly, steady-state HbSS patients (n = 26, aged 11–43 years) and healthy HbAA controls (n = 30, aged 22–53 years) were recruited from the Haematology Clinics of the University of Nigeria Teaching Hospital in Enugu, Nigeria. None of the sickle cell disease (SCD) patients had received hydroxyurea therapy or had had blood transfusion in the 3 months prior to recruitment. Steady-state was defined as absence of sickle cell crisis or related acute illness from one month before, and up to 2 weeks after blood collection for the study.

The study was approved by the Research Ethics Committee of both Institutions, and informed consent was obtained from the participants.

Sample preparation

Whole blood (5 mL) was obtained by venipuncture into heparin tubes. Plasma and red cells were separated by cold centrifugation at 1200 g for 10 minutes. The red cells were washed three times with physiological saline (0.85% NaCl) and the plasma and red cells were stored at –70°C until analysis.

Analysis of plasma retinol, α-tocopherol, and β-carotene

Plasma (100 μL) was deproteinized with 2 mL absolute ethanol and vortexed thoroughly. Hexane (HPLC grade, 5 mL) was added to the mixture, vortexed, and centrifuged at 1200 g for 10 minutes at 4°C. The resulting top organic layer containing the vitamins was carefully removed and dried under a stream of nitrogen at 30°C. The residue was re-dissolved in 500 μL methanol containing 0.01% butylated hydroxytoluene (BHT) and 25 μL was taken for analysis. The vitamins were separated by high-performance liquid chromatography (HPLC) (Agilent Technologies, Waldbronn, Germany) on a 5-micron, 150 × 4.6 mm, C18 reverse-phase column (HiChrom Limited, UK) and detected with a diode-array detector (Agilent Technologies, Waldbronn, Germany). The vitamins were eluted with 100% methanol (HPLC grade) at a flow rate of 2 mL/minute. Retinol, α-tocopherol, and β-carotene were detected at 325 nm, 292 nm, and 453 nm, respectively. Their respective retention times were 1.5 minutes, 4.8 minutes, and 30.0 minutes. Concentrations were computed from standard curves with the use of the software ChemStation (Agilent Technologies, Germany).

Hemoglobin concentration and activities of antioxidant enzymes

Red blood cell hemolysate for the determination of hemoglobin concentration and activity of antioxidant enzymes was prepared by adding 600 μL distilled water to 200 μL of washed red cells (3/1, v/v) followed by a vigorous mixing.

Hemoglobin

Hemoglobin concentration was measured by the use of a kit (Cat. No: D5941) purchased from Sigma-Aldrich (UK). Drabkin's reagent, 5 mL, was added to 20 μL of the

red cell lysate to convert hemoglobin to the stable cyanomethemoglobin. The absorbance of the resulting cyanomethemoglobin was read at 540 nm with a Lambda 35 UV/Vis Spectrometer (Perkin Elmer Ltd., UK). Concentrations were determined from a calibration curve prepared from lyophilized hemoglobin standards.

Cu/Zn-Superoxide dismutase (SOD)

The activity of SOD was assayed with a Ransod kit (Cat. No. SD125, Co. Antrim, UK) based on the reaction of superoxide radical ($O_2^{\cdot-}$) generated from xanthine by the action of xanthine oxidase with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) to form a red formazan dye [27]. The inhibition of the reaction and formation of the red formazan dye due to the dismutation of $O_2^{\cdot-}$ by SOD was measured with a UV-VIS spectrophotometer (Lambda 35, PerkinElmer Ltd., UK) at 505 nm. A unit of activity of SOD is defined as the amount of enzyme that inhibits the formation of the formazan dye by 50%.

Se-Glutathione peroxidase (GPx)

GPx activity was measured by a modification of the coupled assay of Paglia and Valentine [28] with the use of Ransel Kits (Cat. No. RS506, Co. Antrim, UK). Glutathione is oxidized by cumene hydroperoxide by a Se-GPx-catalyzed reaction. In the presence of glutathione reductase and NADPH, the oxidized glutathione is reduced, and the NADPH is oxidized to $NADP^+$ concomitantly. The decrease in the absorbance of NADPH following oxidation, which is proportional to the amount of Se-GPx present in the hemolysate, was monitored at 340 nm with UV-VIS spectrophotometer (Lambda 35, PerkinElmer Ltd., UK).

Statistical Analyses

The data are expressed as means and standard deviations. One-way analysis of variance (ANOVA) was used to test for any significant difference in plasma and red cell antioxidant levels between the British sickle cell patients, British healthy controls, Nigerian sickle cell patients, and Nigerian healthy controls. ANOVA was also used to investigate differences in the levels of the antioxidants between the British HbSS and HbSC patients and healthy controls. When a significant difference was indicated among the groups, the Tukey's honestly significant difference (HSD) post hoc test was used to determine the means which differ. The strength of relationship between the concentrations of the vitamins and activities of the antioxidant enzymes, and red cell EPA or DHA, was assessed by Pearson two-tailed correlation analysis. All statistical analyses were conducted with the use of the statistical package, SPSS for Windows, version 10 (SPSS Ltd., Woking, Surrey, UK).

Results

Fatty acids

Percent EPA and DHA in red cell choline (CPG) and ethanolamine (EPG) phosphoglycerides of the Nigerian and British HbSS patients and matched HbAA controls are given in Figure 1. In comparison with the British HbAA controls, the British HbSS patients had reduced EPA ($p < 0.05$) and DHA ($p < 0.05$) in red cell CPG, and EPA ($p < 0.005$) in EPG. Similarly, the Nigerian HbSS patients had lower levels of EPA ($p < 0.005$) and DHA ($p < 0.005$) in red cell CPG, and EPA ($p < 0.005$) in EPG than in the Nigerian HbAA controls. There was a positive correlation between red cell EPG DHA and Cu/Zn-SOD activity (Figure 4), CPG DHA, and Se-GPx activity (Figure 5), and CPG EPA and Se-GPx activity in the British sickle cell patients (Figure 6). Similarly, although it did not reach the level of a statistical significance, both EPA and DHA in CPG and EPG were also positively associated with the activities of red cell Cu/Zn-SOD and Se-GPx in the Nigerian HbSS patients. Moreover, in the patients with HbSC disease, the DHA in red cell EPG was positively correlated with the activity of Se-GPx (Figure 7).

Antioxidants

Patients versus controls (British and Nigerians)

Compared with their healthy HbAA counterparts, the British HbSS patients had lower mean α-tocopherol concentration ($p < 0.005$), and red cell Cu/Zn-SOD ($p < 0.05$) and Se-GPx ($p < 0.005$) activities. Similarly, the concentrations of plasma retinol, α-tocopherol, and β-carotene ($p < 0.005$) and the activity of red cell Cu/Zn-SOD ($p < 0.05$) were reduced in the Nigerian HbSS patients relative to their HbAA controls. The activity of red cell Se-GPx in the Nigerian patients and level of plasma β-carotene in the British patients were low but not significantly different from the controls. This latter observation may be attributed to the small number of cases.

Plasma α-tocopherol levels of the British HbSC patients were lower than those of their HbAA controls ($p < 0.005$) (Figures 1 and 2).

Table I: Concentrations of plasma retinol, α-tocopherol and β-carothene; and activities of red cell CU/Zn-superoxide dismutase (Cu/Zn-SOD) and Se-glutathione peroxidase (Se-GPx) in HbSS patients and HbAA healthy controls

	British Group		Nigerian Group	
	HbSS	HbAA	HbSS	HbAA
Retinol (μmol/L)	2.61 ± 0.53 (n = 30)$^{+++}$	2.79 ± 0.96 (n = 15)	2.04 ± 0.70 (n = 26)$^{\circ\circ\circ}$	2.98 ± 0.52 (n = 30)
α-tocopherol (μmol/L)	13.82 ± 3.7 (n = 30)*** $^{+++}$	22.54 ± 6.0 (n = 15)^^^	5.74 ± 2.8 (n = 26)$^{\circ\circ\circ}$	14.62 ± 4.5 (n = 30)
β-carotene (μmol/L)	0.20 ± 0.09 (n = 30)$^{+++}$	0.30 ± 0.12 (n = 15)^^^	0.76 ± 0.32 (n = 26)$^{\circ\circ\circ}$	1.08 ± 0.28 (n = 30)
Cu/Zn-SOD (U/g Hb)	1234.78 ± 259.0 (n = 13)*	1565.13 ± 251.1 (n = 13)	962.97 ± 274.0 (n = 15)$^{\circ}$	1315.74 ± 362.2 (n = 15)
Se-GPx (U/g Hb)	22.36 ± 12.5 (n = 13)***	41.27 ± 18.1 (n = 13)	18.06 ± 9.3 (n = 15)	28.78 ± 12.0 (n = 15)

British HbSS vs. British HbAA: *$p < 0.05$, ***$p < 0.005$;
Nigerian HbSS vs. Nigerian HbAA: $^{\circ}p < 0.05$, $^{\circ\circ\circ}p < 0.005$;
British HbSS vs. Nigerian HbSS: $^{+++}p < 0.005$;
British HbAA vs. Nigerian HbAA: ^^^$p < 0.005$.

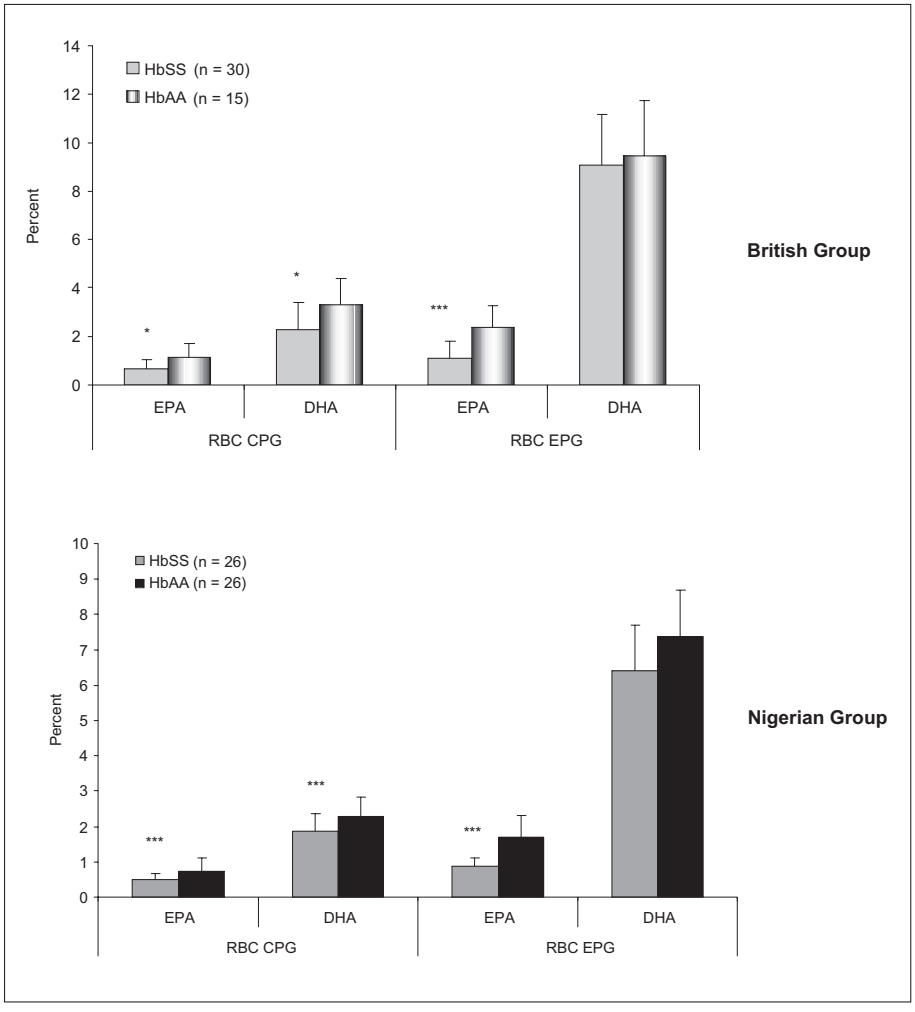

Figure 1: Elcosapentaenoic (EPA) and docosahexaenoic (DHA) acids in red cell choline (CPG) and ethanolamine (EPG) phosphoglycerides of the Nigerian and British HbSS patients and HbAA controls.

British versus Nigerian HbSS patients

The British HbSS patients had increased plasma retinol and α-tocopherol, but decreased β-carotene levels, compared with the Nigerian patients ($p < 0.005$).

British versus Nigerian HbAA controls

The Nigerian HbAA subjects had increased plasma α-tocopherol but decreased β-carotene levels, compared with their British counterparts ($p < 0.005$).

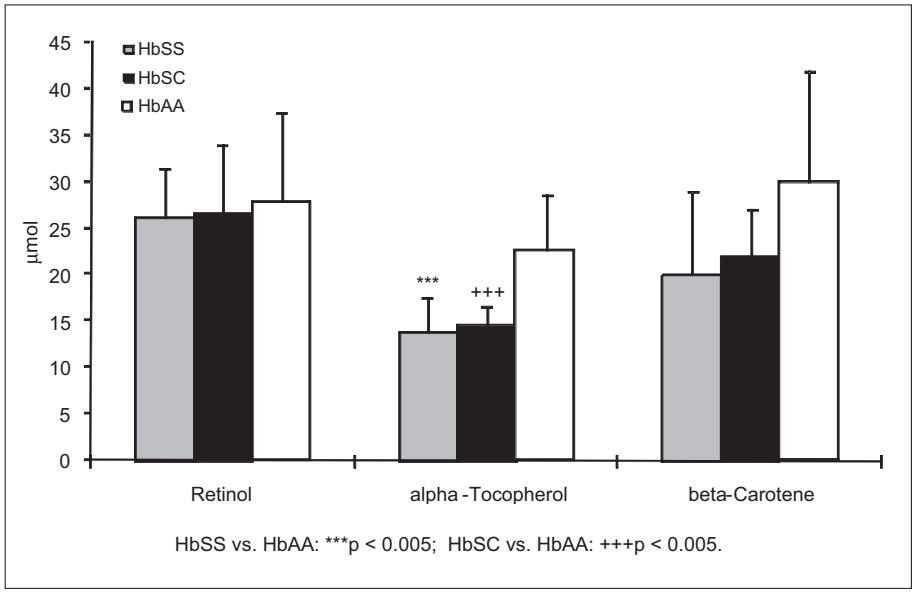

Figure 2: Plasma retinol (×10 µmol/l), α-tocopherol (µmol/l) β-carotene (×100 µmol/l levels in British HbSS (n = 30), HbSC (n = 9), and HbAA (n = 15) subjects.

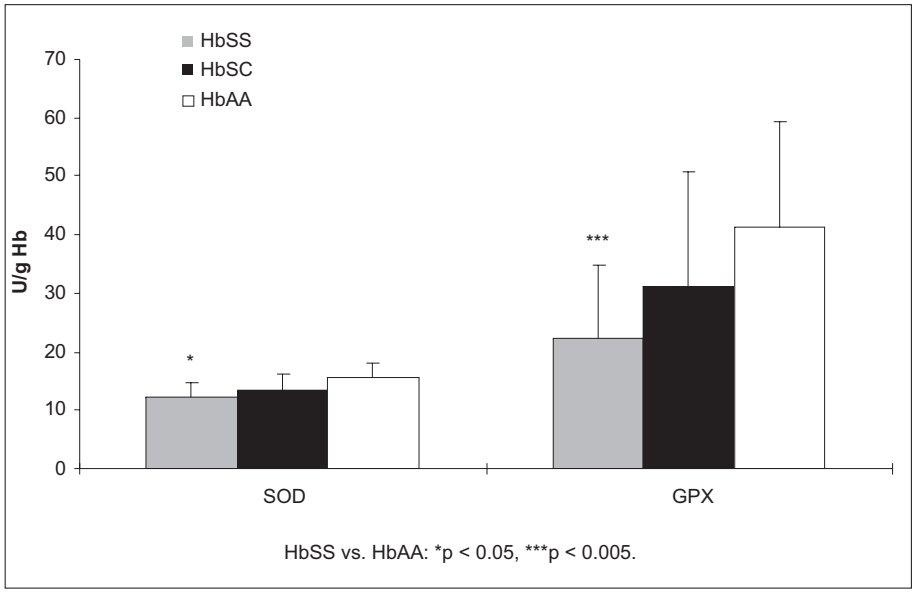

Figure 3: Red cell Cu/Zn-SOD ($\times 10^{-2}$ U/g Hb) and Se-GPx (U/g Hb) activities in British HbSS (n = 30), HbSC (n = 9), and HbAA (n = 15) groups.

Discussion

The HbSS patients, compared with their respective controls, had reduced antioxidant competence as assessed by concentrations of the plasma antioxidant vitamins and activities of membrane-bound antioxidant enzymes. The HbSC group had similar but a less pronounced reduction in antioxidant competence. This reduced antioxidant status in the sickle cell patients could be due to low intake, impaired absorption, and/or enhanced utilization.

Sufficient intake of the co-factors copper and zinc, and selenium, are vital for optimal activity of Cu/Zn-SOD and Se-GPx, respectively. Likewise, adequate consumption of vitamins A and E, and β-carotene is imperative for the maintenance of optimal blood levels of these antioxidant nutrients. The patients and their respective controls were of the same ethnicity, often relatives or neighbors, and of similar economic backgrounds. Hence, the observed difference in antioxidant protection between the two groups (patients and controls) could not have been a reflection of disparity in intake.

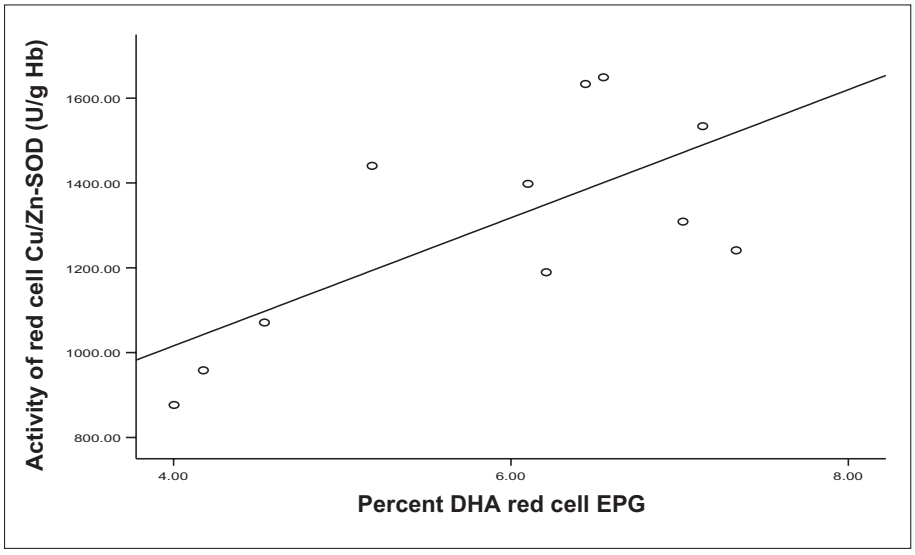

Figure 4: Relationship between the activity of Cu/Zn-SOD and percent DHA in red cell EPG of HbSS patients ($r = 0.700$, $p < 0.05$).

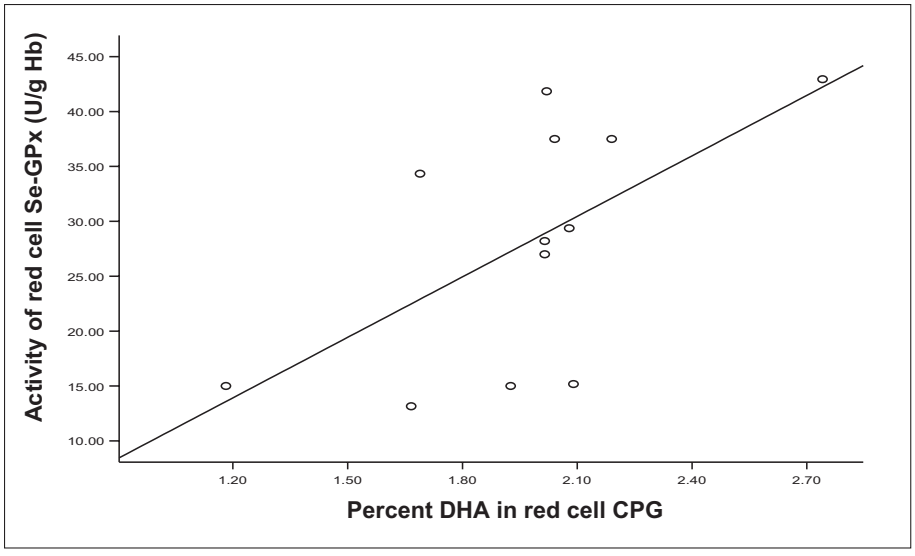

Figure 5: Relationship between the activity of Se-GPx and percent DHA in red cell CPG of HbSS patients ($r = 0.605$, $p < 0.05$).

It has been postulated that the oxidative damage of membranes and tissues in sickle cell disease is the consequence of iron decompartmentalization [29]. This proposition is sustained by the presence of abnormal deposits of denatured hemoglobin and non-heme iron [30–33]. Consistent with the proposition of iron decompartmentalization, sickle red cell membrane has been shown to produce a higher amount of hydroxyl radical generated from hydrogen peroxide (H_2O_2) through a superoxide-driven, iron-catalyzed Haber-Weiss reaction compared with normal red cells [34, 35].

The accelerated generation of free radicals would be expected to lead to an imbalance between oxidants and antioxidants and subsequently to enhanced peroxidation of labile structural components of plasma membranes, such as unsaturated fatty acids. EPA and DHA, which have five and six double-bond indices, respectively, are highly susceptible to peroxidation. It is likely that the reduced level of the two fatty acids in blood cell membranes of the two groups of sickle patients was caused by peroxidation due to low antioxidant competence. Indeed, the positive correlation between DHA of red cell EPG and Cu/Zn-SOD activity (Figure 4), DHA of red cell CPG and Se-GPx activity (Figure 5), and EPA of red cell CPG and Se-GPx activity (Figure 6) in the HbSS, and DHA of red cell EPG and activity of Se-GPx in the HbSC (Figure 7), appears to support our proposition.

Relative to their Nigerian counterparts, the British pa-

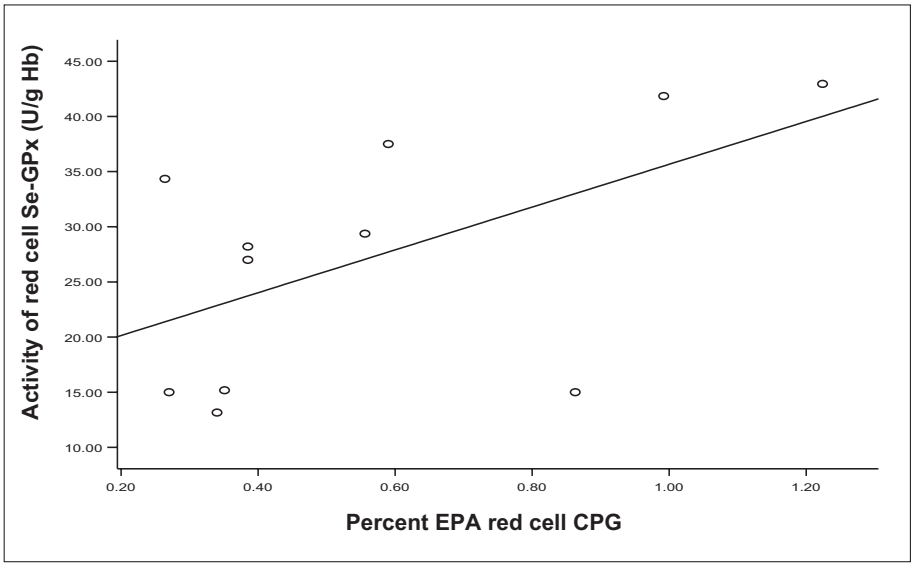

Figure 6: Relationship between the activity of Se-GPx and percent EPA in red cell CPG of HbSS patients ($r = 0.558$, $p > 0.05$).

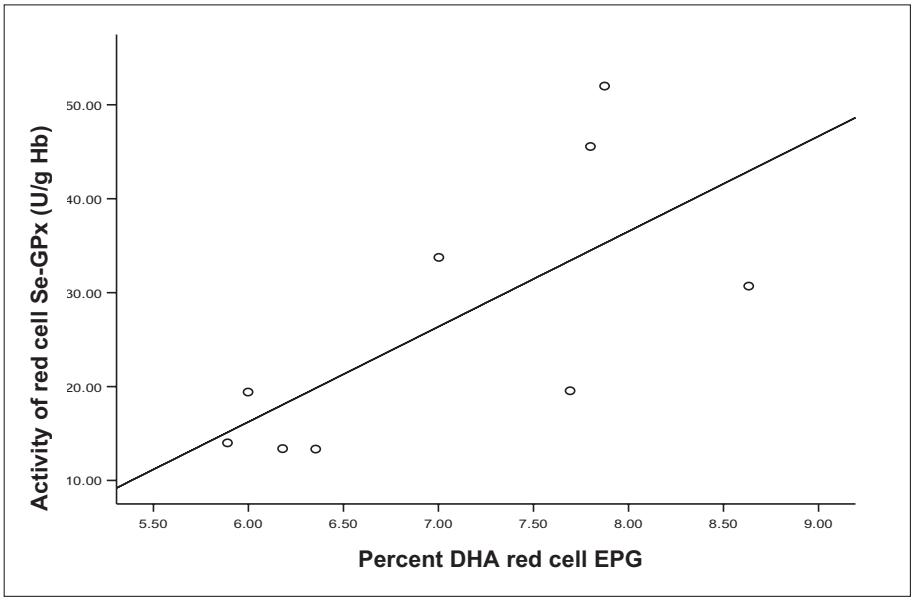

Figure 7: Relationship between the activity of Se-GPx and percent DHA in red cell EPG of HbSC patients ($r = 0.674$, $p < 0.05$).

tients had a higher level of α-tocopherol and retinol, and lower β-carotene. Similar contrasting patterns in the plasma levels of these vitamins were also evident in the control groups from the two countries. These findings are consistent with the dietary habits of the Nigerian and British populations. The consumption of fats and oils (particularly sunflower seed oil), eggs, meat, milk, and vitamin-fortified foods (cereals and juice), which contain appreciable amounts of vitamins E [36, 37] and A, is high in the British population. In contrast, the Nigerian patients had increased plasma β-carotene concentration mainly due to the high consumption of palm oil, which is rich in β-carotene [38, 39]. The contrasts in the profiles of the plasma antioxidant vitamins between the Nigerian and British patients suggest that the status of these nutrients in patients with the disease could be enhanced by supplementation. Consistent with this suggestion, it has been reported that supplementation with vitamin E increased plasma and red cell α-tocopherol [40–42], packed cell volume, hemoglobin concentration, fetal hemoglobin, and blood flow [42, 43] and decreased the number of irreversible sickle red cells [40, 41]. Similarly, dietary supplementation with the antioxidant nutrient, zinc increased the levels of lymphocytes and granulocytes [44] and reduced the

number of irreversible sickle red cells [41, 45], vaso-occlusive crises, and infections [44].

The main limitation of the observed relationship between the levels of DHA and EPA and the activities of the antioxidant enzymes was the small number of subjects. Nevertheless, this study of two groups of sickle cell patients with different background diets and residing in different geographical environments reveals that the disease compromises blood antioxidant protection and consequently the blood levels of the omega-3 fatty acids, eicosapentaenoic and docosahexaenoic acids. Hence, there is a need to optimize antioxidant status of affected individuals with a judicious and tailored supplementation program.

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Reference

1. Graf, E., Mahoney, J.R., Bryant, R.G. and Eaton, J.W. (1984) Iron-catalyzed hydroxyl radical formation. Stringent requirement for free iron coordination site. J. Biol. Chem. 259(6), 3620–3624.
2. Kehrer, J.P. (2000) The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 149(1), 43–50.
3. Minotti, G. and Aust, S.D. (1989) The role of iron in oxygen radical mediated lipid peroxidation. Chem. Biol. Interact. 71(1), 1–19.
4. Emmelot, P. and Van Hoeven, R.P. (1975) Phospholipid unsaturation and plasma membrane organization. Chem. Phys. Lipids. 14(3), 236–246.
5. Marengo-Rowe, A.J. (2006) Structure-function relations of human hemoglobins. Proc. (Baylor Univ. Med. Cent.) 19(3), 239–245.
6. Schacter, L.P., DelVillano, B.C., Gordon, E.M. and Klein, B.L. (1985) Red cell superoxide dismutase and sickle cell anemia symptom severity. Am. J. Hematol. 19(2), 137–144.
7. Schacter, L., Warth, J.A., Gordon, E.M., Prasad, A. and Klein, B.L. (1988) Altered amount and activity of superoxide dismutase in sickle cell anemia. FASEB. J. 2(3), 237–243.
8. Das, S.K. and Nair, R.C. (1980) Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. Br. J. Haematol. 44(1), 87–92.
9. Natta, C.L., Chen, L.C. and Chow, C.K. (1990) Selenium and glutathione peroxidase levels in sickle cell anemia. Acta Haematol. 83(3), 130–132.
10. Beretta, L., Gerli, G.C., Ferraresi, R., Agostoni, A., Gualandri, V. and Orsini, G.B. (1983) Antioxidant system in sickle red cells. Acta Haematol. 70(3),194–197.
11. Chiu, D. and Lubin, B. (1979) Abnormal vitamin E and glutathione peroxidase levels in sickle cell anemia: evidence for increased susceptibility to lipid peroxidation *in vivo*. J. Lab. Clin. Med. 94(4), 542–548.
12. Zimmerman, C.P. and Natta, C. (1981) Glutathione peroxidase activity in whole blood of patients with sickle cell anaemia. Scand. J. Haematol. 26(3), 177–181.
13. Sess, D., Carbonneau, M.A., Thomas, M.J., Dumon, M.F., Peuchant, E., Perromat, A., Le Bras, M. and Clerc, M. (1992) First observations on the main plasma parameters of oxidative stress in homozygous sickle cell disease. Bull. Soc. Pathol. Exot. 85(2), 174–179.
14. Hasanato, R.M. (2006) Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anemia. Ann. Saudi Med. 26(1), 17–21.
15. Adelekan, D.A., Thurnham, D.I. and Adekile, A.D. (1989) Reduced antioxidant capacity in paediatric patients with homozygous sickle cell disease. Eur. J. Clin. Nutr. 43(9), 609–614.
16. Natta, C., Stacewicz-Sapuntzakis, M., Bhagavan, H. and Bowen, P. (1988) Low serum levels of carotenoids in sickle cell anemia. Eur. J. Haematol. 41(2), 131–135.
17. Natta, C. and Machlin, L. (1979) Plasma levels of tocopherol in sickle cell anemia subjects. Am. J. Clin. Nutr. 32(7), 1359–1362.
18. Chiu, D., Vichinsky, E., Yee, M., Kleman, K. and Lubin, B. (1982) Peroxidation, vitamin E, and sickle-cell anemia. Ann. N.Y. Acad. Sci. 393, 323–335.
19. Phillips, G. and Tangney, C.C. (1992) Relationship of plasma alpha tocopherol to index of clinical severity in individuals with sickle cell anemia. Am. J. Hematol. 41(4), 227–231.
20. Essien, E.U. (1995) Plasma levels of retinol, ascorbic acid and alpha-tocopherol in sickle cell anaemia. Cent. Afr. J. Med. 41(2), 48–50.
21. Tatum, V.L. and Chow, C.K. (1996) Antioxidant status and susceptibility of sickle erythrocytes to oxidative and osmotic stress. Free Radic. Res. 25(2), 133–139.
22. Muskiet, F.D. and Muskiet, F.A. (1984) Lipids, fatty acids and trace elements in plasma and erythrocytes of pediatric patients with homozygous sickle cell disease. Clin. Chim. Acta 142(1), 1–10.
23. Sess, D., Carbonneau, M.A., Thomas, M.J., Dumon, M.F., Peuchant, E., Perromat, A., Le Bras, M. and Clerc, M. (1992) First observations on the main plasma parameters of oxidative stress in homozygous sickle cell disease. Bull. Soc. Pathol. Exot. 85(2), 174–179.
24. Ren, H., Obike, I., Okpala, I., Ghebremeskel, K., Ugochukwu, C. and Crawford, M. (2005) Steady-state haemoglobin level in sickle cell anaemia increases with an increase in erythrocyte membrane n-3 fatty acids. Prostaglandins Leukot. Essent. Fatty Acids 72(6), 415–421.
25. Ren, H., Okpala, I., Ghebremeskel, K., Ugochukwu, C.C., Ibegbulam, O. and Crawford, M. (2005) Blood mononuclear cells and platelets have abnormal fatty acid composition in homozygous sickle cell disease. Ann. Hematol. 84(9), 578–583.
26. Ren, H., Ghebremeskel, K., Okpala, I., Ugochukwu, C.C.,

Crawford, M. and Ibegbulam, O. (2006) Abnormality of erythrocyte membrane n-3 long chain polyunsaturated fatty acids in sickle cell haemoglobin C (HbSC) disease is not as remarkable as in sickle cell anaemia (HbSS). Prostaglandins Leukot. Essent. Fatty Acids 74(1), 1–6.
27. Jones, D.G. and Suttle, N.F. (1981) Some effects of copper deficiency on leukocyte function in sheep and cattle. Res. Vet. Sci. 31, 151–156.
28. Paglia, D.E. and Valentine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70(1), 158–169.
29. Hebbel, R.P. (1990) The sickle erythrocyte in double jeopardy: autoxidation and iron decompartmentalization. Semin. Hematol. 27(1), 51–69.
30. Campwala, H.Q. and Desforges, J.F. (1982) Membrane-bound hemichrome in density-separated cohorts of normal (AA) and sickled (SS) cells. J. Lab. Clin. Med. 99(1), 25–28.
31. Sears, D.A. and Luthra, M.G. (1983) Membrane-bound hemoglobin in the erythrocytes of sickle cell anemia. J. Lab. Clin. Med. 102(5), 694–698.
32. Kuross, S.A., Rank, B.H. and Hebbel, R.P. (1988) Excess heme in sickle erythrocyte inside-out membranes: possible role in thiol oxidation. Blood 71(4), 876–882.
33. Kuross, S.A. and Hebbel, R.P. (1988) Nonheme iron in sickle erythrocyte membranes: association with phospholipids and potential role in lipid peroxidation. Blood 72(4), 1278–1285.
34. Hebbel, R.P., Eaton, J.W., Balasingam, M. and Steinberg, M.H. (1982) Spontaneous oxygen radical generation by sickle erythrocytes. J. Clin. Invest. 70(6), 1253–1259.
35. Repka, T. and Hebbel, R.P. (1991) Hydroxyl radical formation by sickle erythrocyte membranes: role of pathologic iron deposits and cytoplasmic reducing agents. Blood 78(10), 2753–2758.
36. Davey, G.K., Spencer, E.A., Appleby, P.N., Allen, N.E., Knox, K.H. and Key, T.J. (2003) EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33,883 meat-eaters and 31 546 nonmeat-eaters in the UK. Public Health Nutr. 6(3), 259–269.
37. Glew, R.H., Williams, M., Conn, C.A., Cadena, S.M., Crossey, M., Okolo, S.N. and VanderJagt, D.J. (2001) Cardiovascular disease risk factors and diet of Fulani pastoralists of northern Nigeria. Am. J. Clin. Nutr. 2001; 74(6), 730–736.
38. Adams-Campbell, L.L., Agurs, T.D. and Ukoli, F.A. (1993) Dietary assessment in Nigerian women: a pilot study. Ethn. Dis. 3 Suppl: S62–66.
39. Ajose, O.A., Adelekan, D.A. and Ajewole, E.O. (2004) Vitamin A status of pregnant Nigerian women: relationship to dietary habits and morbidity. Nutr. Health 17(4), 325–333.
40. Natta, C.L., Machlin, L.J. and Brin, M. (1980) A decrease in irreversibly sickled erythrocytes in sickle cell anemia patients given vitamin E. Am. J. Clin. Nutr. 33(5), 968–971.
41. Muskiet, F.A., Muskiet, F.D., Meiborg, G. and Schermer, J.G. (1991) Supplementation of patients with homozygous sickle cell disease with zinc, alpha-tocopherol, vitamin C, soybean oil, and fish oil. Am. J. Clin. Nutr. 54(4), 736–744.
42. Gbenebitse, S., Jaja, S.I. and Kehinde, M.O. (2005) Effect of changes in plasma vitamin E level of vascular responses and lipid peroxidation in sickle cell anaemia subjects. Niger. Postgrad. Med. J. 12(2), 81–84.
43. Jaja, S.I., Aigbe, P.E., Gbenebitse, S. and Temiye, E.O. (2005) Changes in erythrocytes following supplementation with alpha-tocopherol in children suffering from sickle cell anaemia. Niger. Postgrad. Med. J. 12(2), 110–114.
44. Prasad, A.S., Beck, F.W., Kaplan, J., Chandrasekar, P.H., Ortega, J., Fitzgerald, J.T. and Swerdlow, P. (1999) Effect of zinc supplementation on incidence of infections and hospital admissions in sickle cell disease (SCD). Am. J. Hematol. 61(3), 194–202.
45. Brewer, G.J., Brewer, L.F. and Prasad, A.S. (1977) Suppression of irreversibly sickled erythrocytes by zinc therapy in sickle cell anemia. J. Lab. Clin. Med. 90(3), 549–554.

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