Relationship of Omega-3 Fatty Acids DHA and EPA with the Inflammatory Biomarker hs-CRP in Children with Sickle Cell Anemia

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Abstract

Background: Inflammation and vaso-occlusion play key roles in Sickle Cell Disease (SCD) pathophysiology. Lipoxygenase products of the omega-3 fatty acids (O3FAs), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids, are potent anti-inflammatory mediators modulating pain. O3FAs decrease episodes of vaso-occlusion in SCD.

Methods: We assessed erythrocyte fatty acid composition in two major cell membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, in children with SCD HbSS-disease (n=38) and age/race-matched HbAA-controls (n=18). Ratio of pro-inflammatory arachidonic acid (AA) to anti-inflammatory DHA and EPA (FA-Ratio), and its relationship to hs-CRP were evaluated.

Results: FA-Ratios were increased in both phosphatidylcholine and phosphatidylethanolamine in HbSS compared to controls. Correlations were noted in HbSS subjects between hs-CRP and FA-Ratios (p=0.011). FA-Ratios increased with age (p=0.0007) due to an increase in pro-inflammatory AA with a concomitant decrease in antiinflammatory DHA.

Conclusions: Findings demonstrate relative deficiencies in HbSS of the anti-inflammatory precursor fatty acids DHA and EPA, which correlates positively with hs-CRP.

Keywords

sickle cell anemia; polyunsaturated fatty acids; omega-3 fatty acids; docosahexaenoic acid; inflammatory biomarkers; C-reactive protein

1. Introduction

Sickle Cell Disease (SCD), the most common Hemoglobinopathy, is caused by a mutant hemoglobin, hemoglobin S (HbS). The most common and debilitating feature of the disease is the painful vaso-occlusive crisis (VOC) characterized by micro-vessel occlusion [1-5]. Evidence from both clinical and transgenic animal models strongly suggest that VOC is a chronic inflammatory pathology [6-10]. Using a transgenic mouse model, Kaul and Hebbel have linked the reperfusion injury paradigm to the VOC event [8]. Later studies by Frenette and his colleagues, and others have focused on activated leucocytes and their released inflammatory mediators as playing a critical role in the pathogenesis of VOC [10-12]. In subjects with SCD, studies have documented elevated levels of inflammatory cytokines [13-15] including interleukin-1ß (IL-1ß) and tumor necrosis factor-α (TNF-α) and lipid mediators including leukotriene B4 (LTB4) [16]. These mediators promote cell-cell adhesion, endothelial activation, up-regulation of endothelial adhesion molecules, activation of transcription factors including nuclear factor (NF) κB, and further leukocyte recruitment [9-12,17]. In addition, these inflammatory mediators sensitize nociceptors acting as agonists for the transient receptor potential (TRP) vanilloid subtype-1 (TRPV1) and ankyrin subtype-1 (TRPA1) which together with other TRP channels play a role in neural and nociceptive pain [18,19].

Polyunsaturated fatty acids (PUFAs) are structural and functional components of cell membranes. These fatty acids are present as acyl chains primarily esterified at the sn-2 position of glycerol in phospholipids (PLPs) including phosphatidylethanolamine (PE) and phosphatidylcholine (PC). PE and PC are the major PLPs of the inner and outer leaflet of the plasma membrane, respectively [20,21]. Based on the position of the terminal double bond, PUFAs are grouped into either omega-3 or omega-6 fatty acids. The omega-3 fatty acids (O3FAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the omega-6 fatty acid, arachidonic acid (AA), have been extensively evaluated by many investigators. AA is the precursor for the production of majority of pro-inflammatory lipid modulators [22-24]. This fatty acid can also serve as the substrate for the synthesis of lipoxins, a minor sub-group of eicosanoids with anti-inflammatory and pro-resolving properties [25]. EPA and DHA serve as the precursors for the synthesis of antiinflammatory and pro-resolving lipid mediators [25,26]. These fatty acids have profound effects on multiple biologic and pathologic processes including inflammation and pain [reviewed in 26-29]. In addition, studies have evaluated health benefits of O3FAs in cardiovascular disease, rheumatoid arthritis, diabetes, inflammatory bowel disease, asthma, neurologic disorders, and pain [reviewed in 26,30-34, and references 35,36]. While some studies have documented clinical benefits of O3FAs and their protective effects against inflammation, others have failed to demonstrate any health benefits.

Based on what is known about the pathophysiology of SCD, the demonstrated effects of the O3FAs on leukocytes, endothelium and inflammatory mediators, O3FAs may have great potential, by way of multiple mechanisms, to favorably impact the vaso-occlusive inflammatory and the painful complications of SCD. In fact, limited clinical and nutritional studies in subjects with SCD and sickle cell mouse models have shown that O3FA therapy provided protection against VOC-related pain, lowered NFκB gene expression, and down-regulated adhesion molecule expression [37-41]. While previous studies have shown that the levels of O3FAs are reduced under basal condition in subjects with SCD [42-48], no studies to date have assessed the relationship between the levels of O3FAs and steady state inflammation in SCD. The aim of our study was to assess whether the PUFA levels in SCD correlate with the levels of high sensitivity-C-reactive protein (hs-CRP), the well-characterized biomarker of both acute and chronic inflammatory processes [49-51].

2. Methods and Materials

2.1. Subjects

Individuals with SCD (n=38, age range 1.7 to 20 years) with homozygous hemoglobin S (HbSS) were at baseline steady state health. The individuals with SCD were recruited for the study between 2008 and 2012 when hydroxyurea (HU) therapy had not been established as a definitive and highly recommended therapy for individuals with SCD. These subjects were recruited for the study at St Christopher Hospital Children, Drexel University School of Medicine, Philadelphia, PA. Children receiving HU therapy or chronic transfusion, and subjects with any acute illness, hospitalization, or vaso-occlusive episode within 2 weeks pre- and post- blood sampling were excluded from the study. Age- and race- matched control subjects (n=18) were recruited at St Christopher's Hospital for Children and Thomas Jefferson University, Philadelphia, PA. Plasma and washed erythrocytes, prepared from blood samples collected in citrate anticoagulant, were stored frozen at −80°C until processed. The study was approved by the Institutional Review Committee for Human Subjects Protection at both study sites. In accordance with the Declaration of Helsinki, blood samples were obtained following informed consent. For minors, subject's assent was also obtained.

2.2. Materials

Lipid standards for thin layer chromatography (TLC) and reference fatty acid methyl ester (FAME) standards for gas chromatography (GC) analyses were obtained from Avanti Polar Lipids (Alabaster, AL) and Sigma-Aldrich (St Louis, MO). All chromatographic-grade solvents and reagents were obtained from Fisher Scientific (Wilmington, DE).

2.3. Fatty Acid Analysis

Red cell fatty acids were assayed by capillary GC as previously described [42,52]. In brief, total lipids from erythrocytes (10^6 cells) were extracted using a mixture of chloroform and methanol as described by Folch *et al* [53] in the presence of 0.01% butylated hydroxytoluene. Lipid extracts were further subjected to TLC to isolate PE and PC. FAMEs were prepared by transmethylating the isolated PLPs with anhydrous methanolic-HCl (0.5 N) [54], and analyzed using an Agilent 6850 Gas Chromatograph equipped with a

polyethyleneglycol capillary column (30m × 250μm × 0.25μm, Agilent INNO-wax) and flame ionization detector. Sample peaks were identified by comparing their retention times with those from authentic FAME standards. Peak areas were integrated using the Agilent ChemStation Software, and quantitated using a FAME standard curve. Recoveries of erythrocyte lipids, monitored using appropriate synthetic PLPs [1,2-dipentadecanoyl-sn-glycero-3-phosphoethanolamine (a synthetic PE), and 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine (a synthetic PC)], were found to be >80%.

2.4. Analysis of hs-CRP

Plasma hs-CRP levels were measured using an ELISA kit (ALPCO Immunoassays, Salem, NH) as previously described [49].

2.5. Analysis of LDH and Hematologic Parameters

Plasma LDH levels were measured using an LDH assay kit (TOX07, Sigma-Aldrich) as previously described [49]. Hematologic parameters (erythrocyte count, hemoglobin, WBC counts, neutrophil counts, and platelet counts) were obtained using a Coulter Counter (Model StK). Reticulocytes were enumerated in blood stained with methylene blue.

2.6. Data analysis

All blood samples were evaluated for red cell membrane lipid markers including fatty acids (AA, EPA and DHA), and the Fatty Acid Ratio (FA-Ratio). FA-Ratio was obtained by dividing AA (in nMol) by DHA plus EPA (in nMols), and was employed as a measure to assess the relative deficiency of O3FAs. Statistical evaluation including unpaired t-test, Mann-Whitney Rank-sum test, Pearson correlation and Spearman rank correlation tests were performed using SigmaPlot (Version 12, Systat Software, San Jose CA).

3. Results

3.1. Hematologic Characteristics of individuals with SCD

Hematologic parameters from individuals with SCD (n=38) enrolled in the study are presented in Table-1. Hematologic indices from SCD were compared to those from the age- and race-matched controls (n=18). Compared to HbAA controls, children with SCD demonstrated decreased levels of hemoglobin and red cell count (p<0.001), and increased levels of reticulocytes, platelets, total white blood cells and neutrophils (p=0.008). Plasma levels of LDH were also increased in children with SCD (p<0.001).

3.2. Composition of red cell membrane AA, EPA and DHA

Composition of erythrocyte membrane AA, EPA and DHA from the PC and PE fractions and the FA-Ratio are shown in Table-2. Consistent with published reports [42-48], DHA levels in subjects with SCD were significantly decreased in both PC (p=0.047) and PE (p=0.042) fractions when compared to those from control children. While EPA levels were similar in the PC fraction, they were significantly decreased in the PE fraction from individuals with SCD (p<0.001). In contrast to DHA and EPA, AA levels were significantly increased in the PC fraction from subjects with SCD (p=0.039). No differences were noted

in the PE fraction. The FA-Ratios in children with SCD were significantly increased in both PC (p=0.003) and PE (p=0.035) fractions when compared to those from control children. In the PC fraction, the observed increase in fatty acid ratio was due to a significant decrease in DHA with a concomitant elevation in AA content. In the PE fraction, the abnormal ratio was due to decreases in both DHA and EPA content.

3.3. Association of Fatty Acid Ratios with age

While we have noted significant positive correlations between age and the FA-Ratios from both PC (r=0.53, p=0.0007, n=38) and PE (r=0.51, p=0.001, n=38) fractions from SCD erythrocytes, no significant associations were noted in the control group (r=0.39, p=0.101 with PC and r=0.36, p=0.142 with PE, n=18, Figure-1). Age correlated negatively with levels of DHA in the red cell PC fraction (r=−0.42, p=0.009, n=38) in the subjects with SCD. In additional regression analysis, we assessed the correlation between age and hs-CRP and found no age-related association with hs-CRP in either the SCD (r=0.21, p=0.20, n=38) or the control subjects (r=0.03, p=0.89, n=18).

3.4. Association of Fatty Acid Ratios with hs-CRP

Consistent with previous reports, plasma hs-CRP levels were significantly elevated in children with SCD (p<0.001) when compared to those from the age- and race-matched controls (Table-1). In the SCD group, hs-CRP demonstrated significant positive correlation with the FA-Ratios from the erythrocyte PC fraction (r=0.41, p=0.011, n=38). While a positive relationship was also noted between hs-CRP and the FA-Ratios from the PE fraction, the correlation did not reach statistical significance (r=0.29, p=0.08, n=38). In the control (n=18), hs-CRP did not correlate with the FA-Ratios from either the PC or PE fractions (r=−0.04, p=0.882 with PC and r=−0.09, p=0.723 with PE). The scatter plots of the association between hs-CRP and the FA-Ratios from the erythrocyte PC (panel A) and PE (panel C) fractions from SCD, and the PC (panel B) and PE (panel D) fractions from control erythrocytes are shown in Figure-2.

4. Discussion

The polyunsaturated fatty acids AA, EPA and DHA have been evaluated by several investigators to assess clinical abnormalities and nutritional status of O3FAs. AA, an omega-6 fatty acid, is the substrate for the synthesis of majority of pro-inflammatory eiocosanoids including prostaglandins, thromboxane and leukotrienes [22-24]. In contrast, DHA and EPA, the major omega-3 fatty acids, are the precursors for the synthesis of potent anti-inflammatory, analgesic and pro-resolving mediators including resolvins, protectins and maresins [25,26]. The balance between omega-3 and omega-6 fatty acids and the relative levels of lipid mediators generated from these fatty acids at the site of inflammation, therefore, may play a role in the pathogenesis of inflammation and its resolution. Studies have shown that O3FA levels in SCD are decreased in multiple lipid fractions from several blood components with marked changes noted in the red cell PC and PE fractions [42-48]. Choline and ethanolamine containing phospholipids are the major inner and outer plasma membrane phospholipids [20,21], respectively. In the present study, we evaluated the levels of AA, DHA and EPA in the red cell PC and PE fractions from children with SCD, and

demonstrate that the levels of DHA and EPA are decreased with concomitant increases in AA resulting an increase in the FA-Ratios. Our findings are in agreement with those reported previously in subjects with SCD from Sudan and Nigeria [42-47]. In addition, we demonstrate that the O3FA deficiency in SCD increases with subject's age and correlates with an increase in plasma levels of the inflammatory bio-marker hs-CRP.

Omega-3 fatty acids modulate many cellular functions including those involved in the pathogenesis of inflammation. Some of the anti-inflammatory effects mediated via EPA and DHA include decreased leukocyte chemotaxis, decreased production of chemo-attractants (such as LTB4), suppressed production of inflammatory cytokines, decreased transcription and expression of adhesion molecules (VCAM-1, ICAM-1 and E-selectin), decreased leukocyte-endothelial interaction, decreased production of inflammatory eicosanoids, and increased production of anti-inflammatory and proresolving mediators, among others [26-29,55-60]. Many of these inflammatory processes have been documented to occur in individuals with SCD [8,11-17]. Since O3FAs can modulate these cellular functions [26-29], they could serve as potential therapeutic agents in the management of VOC pain in SCD. In this regard, it is interesting to note that several clinical studies in SCD have evaluated the beneficial effects of either fish oil (the major source of O3FAs) or O3FA supplements, and have demonstrated a reduction in VOC pain frequency or clinical indicators of pain [37-40]. In addition, studies have also shown that O3FAs reduced oxidant production [61], and the expression of adhesion molecules [41] and NFκB gene in SCD [41]. Since NFκB is a transcription factor which plays a key role in the up-regulation of inflammatory cytokines and adhesion molecules [62,63], this study provides further evidence that O3FA supplementation may have a beneficial effect on the inflammatory processes in SCD.

In mouse models of SCD, mice treated with an O3FA-enriched diet showed a reduction in vascular activation, inflammatory response and SCD-related end organ damage [64]. DHA supplementation was shown to improve RBC flexibility, decrease irreversibly sickled cells, and reduce cold hypersensitivity [65,66]. In addition, in a most recent study, Matte et al [67] have demonstrated that orally administered 17R-RvD1, a DHA derived SPM, provided protection in SCD mice against hypoxia/reoxygenation-induced systemic/local inflammation, organ damage, and vascular dysfunction in the lung and kidney by modulating multiple biological processes involved in inflammatory and resolution pathology [67]. These findings further highlight the beneficial effects in SCD of DHA, and SPMs derived from this fatty acid.

In this study, we demonstrate that the relative deficiency of DHA and EPA (assayed as the FA-Ratio) increases with age (Figure-1, panels A and C). Clinical complications in subjects with SCD commence in the first year of life, and the disease severity increases with age [68]. While the formation of hemoglobin polymers is the initiating event, multiple pathobiologic processes including inflammation, blood cell adhesion to endothelium, and oxidative stress also play a role in disease pathology [4]. Studies have shown that O3FAs provide protection against these SCD-related pathophysiologic processes [26-29,61,69]. Our results taken together with the documented beneficial effects of O3FAs against inflammation, oxidant stress, and blood cell-endothelial adhesion provide additional

complementary evidence to support the initiation of O3FA therapy at an early age in children with SCD.

In our study cohort, we demonstrate that O3FA deficiency is significantly associated with increased levels of plasma hs-CRP. CRP is a well-documented stable plasma biomarker of low-grade, chronic systemic inflammation and it is produced in the liver in response to a host of pro-inflammatory cytokines [50,51]. A previous study from our laboratory has demonstrated that hs-CRP was the most significant correlate of hospitalization for VOC pain in SCD [49]. We have therefore selected hs-CRP as a cardinal inflammatory biomarker to assess the relationship between inflammation and relative O3FA deficiency in children with SCD. We demonstrate that an increased FA-Ratio (a measure of relative O3FA deficiency) in the PC fraction is significantly associated with increased levels of plasma hs-CRP in children with SCD (Figure-2, panel A). While a positive trend was also noted between O3FA deficiency in the PE fraction and hs-CRP, the association did not reach statistical significance (Figure-2, panel C). In contrast to PE, which appears to be relatively stable [70], erythrocyte PC is actively involved in fatty acid turnover, can rapidly incorporate PUFAs [71,72], readily exchange its acyl groups with plasma fatty acids [73], and transfer to other erythrocyte phospholipids including PE [74]. The dynamic nature of red cell PC and the turnover of fatty acids documented in this phospholipid may therefore account for the observed fatty acid abnormalities, and the significant association noted with hs-CRP in SCD. Other studies in patients with coronary artery disease [75] and peripheral artery disease [76] have shown significant inverse correlations between levels of CRP and red cell membrane O3FA content. In a previous study, CRP levels in a cohort of SCD subjects on O3FA supplements were compared to those from an untreated cohort [41]. While the CRP levels were lower in the O3FA-treated group, the changes were not statistically significant probably due to a small sample size (n=18) and a large sample variability, and therefore require confirmation using a larger SCD cohort.

The individuals with SCD were recruited for the study between 2008 and 2012, and all evaluations were made using a naive group of individuals untreated with HU. HU [77,78], and more recently L-glutamine [79,80], are the only FDA approved pharmacologic agents used in the preventive treatment of SCD-related complications. The beneficial effect of HU in SCD is attributed to an increased production of fetal hemoglobin, and a decreased production of leukocytes and reticulocytes [77]. Other pharmacological effects including modulation of erythrocyte adhesion to endothelium, and vaso-dilatory and anti-inflammatory effects through the release of nitric oxide directly from the HU molecule may also contribute to the beneficial effects of HU in this disease entity [81]. Treatment with HU is a standard therapy at the present time for all individuals with HbSS including children and infants as young as 9 months of age [82]. At the time of patient recruitment for our study, parents and caregivers were reluctant to agree to HU therapy for their children due to concern as to the long-term safety of the drug. Thus, the majority of children with SCD were not taking HU at the time of our study. Since HU can also modulate inflammation [81], the blood samples collected at that time of our investigation provided a unique opportunity to assess the relationship between O3FA deficiency and inflammation under baseline steady state condition in the absence of any disease-specific pharmacologic interference. In a cohort of modest sample size, we were able to demonstrate a significant positive correlation between

O3FA deficiency in the red cell membrane phospholipid fractions and plasma inflammatory biomarker hs-CRP. Study limitations include a modest sample size and the lack of comparison of plasma fatty acids between healthy controls and individuals with SCD. However, a previous study has shown that O3FA abnormalities similar to those observed in erythrocytes were also noted in plasma [42]. An additional limitation of our study is the lack of repeat measurements to assess either the short-term or long-term intra-individual variability of PUFA levels.

In summary, in children with HbSS we have identified an additional potential cause for the increase in inflammatory tone. This observation is supported by the findings that the membrane levels of EPA and DHA, the precursor fatty acids for the synthesis of anti-inflammatory and pro-resolving protectins, resolvins, and maresins, are decreased in red cells. In contrast, the membrane levels of AA, the precursor fatty acid for the synthesis of majority of pro-inflammatory eicosanoids prostaglandins, leukotriene and thromboxane, is increased, and in addition, the ratio of pro- to anti-inflammatory precursor fatty acids was also increased. We also document that the FA-Ratios in HbSS correlated positively with the inflammatory bio-marker hs-CRP. Our current results taken together with published findings provide evidence that DHA and EPA deficiency plays a component role in the pathogenesis of inflammation in SCD.

Acknowledgments

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HIGHLIGHTS

- Chronic inflammation is one of the hallmarks of sickle cell disease (SCD). We have previously demonstrated that the inflammatory biomarker hs-CRP positively correlated with vaso-occlusive pain in SCD.

- In children with SCD, ratios of pro-inflammatory arachidonic acid (AA) to anti-inflammatory docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids are increased in red cell phospholipids due to a relative decrease in omega-3 fatty acids.

- A relative deficiency of DHA and EPA correlates positively with hs-CRP in children with SCD.

- A relative deficiency of DHA and EPA increases with age in children with SCD
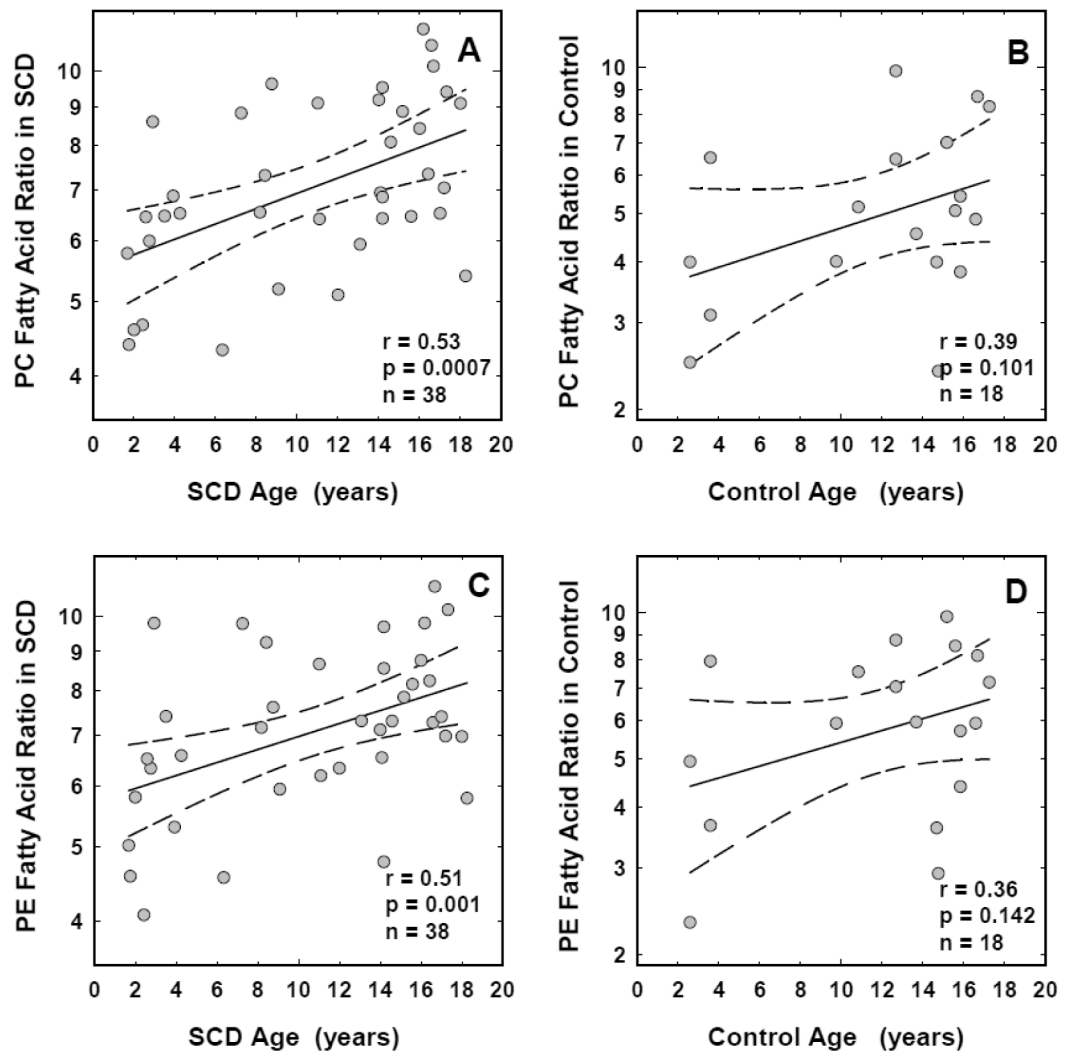
FIGURE 1: Association between age and fatty acid ratios in red cell phospholipids from children with sickle cell disease and race-matched controls.

Association between age and the fatty acid ratios in red cell PC (panel A) and PE (panel C) fractions from children with sickle cell disease, and the PC (panel B) and PE (panel D) fractions from race-matched controls are shown. The solid and the dotted lines represent the linear regression fit to the data, and the 95% confidence interval curves, respectively. The correlation coefficient (r) and the significance (p) values shown for each correlation were obtained using the Pearson correlation test with logtransformed data. Similar results were observed when the data was analyzed using the Spearman rank correlation test.
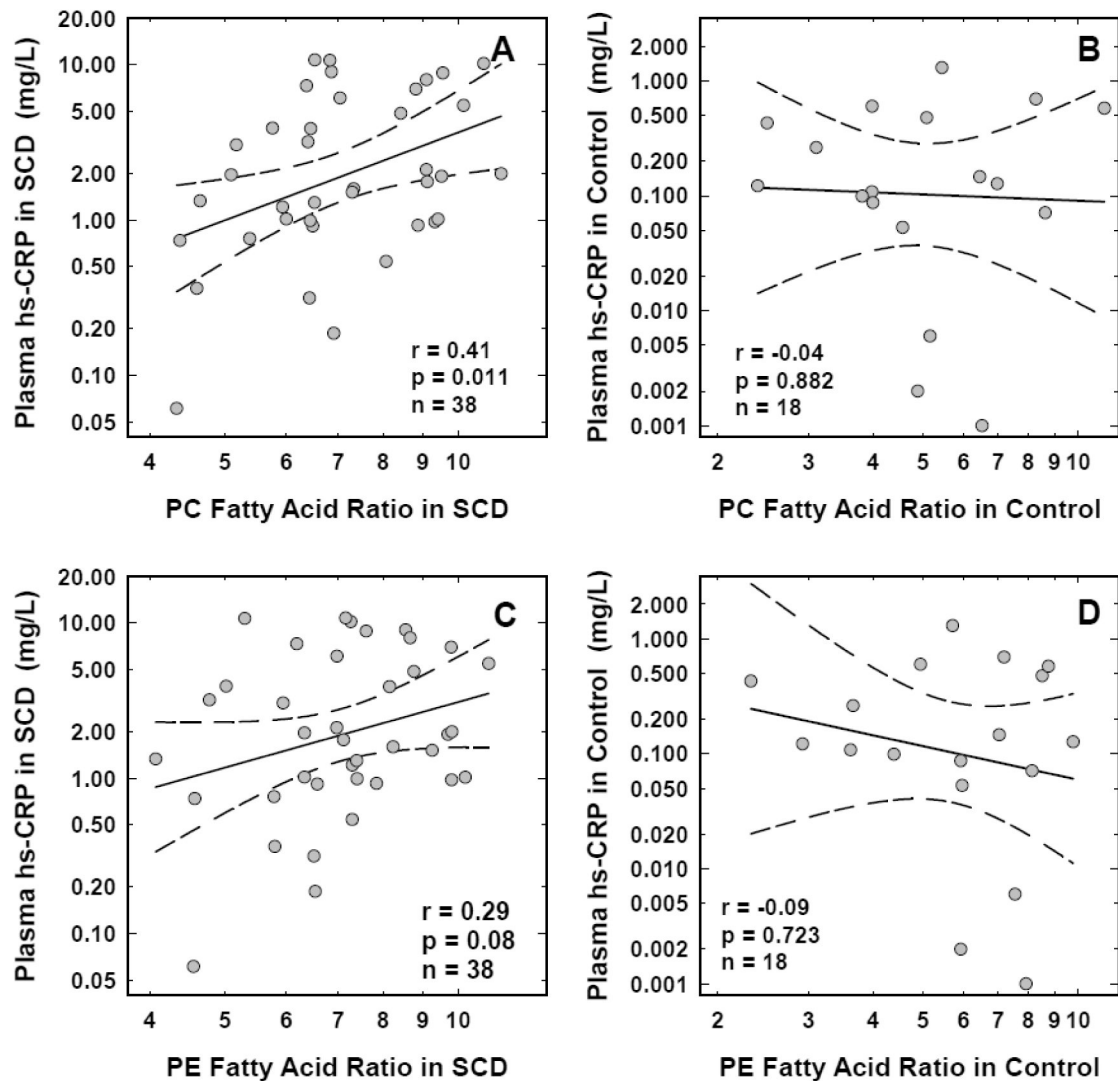
FIGURE 2: Association between plasma hs-CRP levels and fatty acid ratios in red cell phospholipids from children with sickle cell disease and age- and racematched controls.
Association between the levels of plasma hs-CRP and the fatty acid ratios in red cell PC (panel A) and PE (panel C) fractions from children with sickle cell disease, and the PC (panel B) and PE (panel D) fractions from age- and race-matched controls are shown. The solid and the dotted lines represent the linear regression fit to the data, and the 95% confidence interval curves, respectively. The correlation coefficient (r) and the significance (p) values shown for each correlation were obtained using the Pearson correlation test with log-transformed data. Similar results were observed when the data was analyzed using the Spearman rank correlation test.

TABLE 1:

Hematological parameters in Children with SCD and Age- and Race- matched HbAA Controls

Hematological parameters	HbAA Control (n=18)	SCD HbSS (n=38)	p-value
Age (years)	11.90 ± 5.24 ‡**14.17 (8.21, 15.83)**	10.76 ± 5.62 **11.54 (5.81, 16.23)**	ns
Erythrocytes (M/μl)	4.68 ± 0.39 ‡**4.67 (4.51, 4.91)**	2.91 ± 0.63 **2.90 (2.52, 3.26)**	p<0.001
Hemoglobin (g/dl)	*12.21 ± 1.01 12.20 (11.78, 12.83)	**8.81 ± 1.19** 8.80 (8.05, 9.70)	p<0.001
Reticulocytes (K/μl)	31 ± 21 ‡**29 (11, 46)**	216 ± 85 **198 (153, 282)**	p<0.001
Platelets (K/μl)	310 ± 110 ‡**278 (238, 374)**	426 ± 138 **398 (338, 505)**	p=0.001
WBCs (K/μl)	*8.01 ± 3.37 6.85 (5.50, 10.30)	**11.07 ± 4.03** 10.60 (8.00, 14.40)	p=0.008
Neutrophils (K/μl)	3.30 ± 2.24 ‡**2.45 (1.70, 4.60)**	5.08 ± 2.47 **4.87 (3.29, 6.17)**	p<0.001
LDH (mU/ml)	265 ± 71 ‡**252 (223, 294)**	675 ± 217 **661 (459, 869)**	p<0.001
hs-CRP (ng/ml)	287 ± 339 ‡**125 (67, 502)**	3361 ± 3318 **1837 (961, 5629)**	p<0.001

Results presented are the mean ± SD values (first line), and the median and (1st, and 3rd quartile) levels (second line). Hematologic parameters were compared between groups using * unpaired t-test if the data set passed both normality and equal variance tests. ‡Mann-Whitney Rank Sum test was employed if the data set failed normality and/or equal variance tests. Only partial data was available for comparison from children with SCD for erythrocytes (n=37), hemoglobin (n=37), reticulocytes (n=33), platelets (n=36), WBC (n=35), neutrophils (n=35) and LDH (n=32). Only data from 16 controls was available for reticulocyte comparison. Abbreviations used in the Table: WBCs= white blood cells, LDH= lactate dehydrogenase, hs-CRP= high sensitivity-C-reactive protein

TABLE 2:

Red Cell Membrane Phospholipid Fatty Acids in Children with SCD and Age- and Race- matched HbAA Controls

Phospholipid Fraction	Fatty Acid	HbAA Control (n=18)	SCD HbSS (n=38)	p-value
PC Fraction	**FA-Ratio**	5.39 ± 2.30 ‡**4.99 (3.94, 6.63)**	7.35 ± 2.52 **6.88 (5.15, 9.10)**	0.003
	AA (nMol/10^9 RBCs)	15.59 ± 3.73 ‡**14.06 (13.00, 18.52)**	18.00 ± 3.59 **17.29 (15.81, 20.57)**	0.039
	DHA (nMol/10^9 RBCs)	*__2.72 ± 0.90__ 2.33 (1.98, 3.75)	**2.22 ± 0.82** 2.19 (1.61, 2.53)	0.047
	EPA (nMol/10^9 RBCs)	0.53 ± 0.44 ‡**0.60 (0.01, 0.69)**	0.48 ± 0.48 **0.45 (0.01, 0.76)**	ns
PE Fraction	**FA-Ratio**	*__6.13 ± 2.16__ 5.94 (4.21, 7.98)	**7.36 ± 1.94** 7.21 (5.91, 8.58)	0.035
	AA (nMol/10^9 RBCs)	33.50 ± 16.89 ‡**26.42 (21.30, 50.83)**	27.74 ± 6.51 **26.57 (24.82, 30.77)**	ns
	DHA (nMol/10^9 RBCs)	4.69 ± 2.25 ‡**4.29 (2.87, 5.95)**	3.47 ± 1.30 **3.50 (2.25, 4.12)**	0.042
	EPA (nMol/10^9 RBCs)	1.00 ± 0.54 ‡**1.13 (0.68, 1.32)**	0.54 ± 0.31 **0.58 (0.34, 0.73)**	<0.001

Results presented are the mean ± SD values (first line), and the median and (1st, and 3rd quartile) levels (second line). Biomarker levels were compared between groups using *unpaired t-test if the data set passed both normality and equal variance tests. ‡Mann-Whitney Rank Sum test was employed if the data set failed normality and/or equal variance tests. Abbreviations used in the Table: AA= arachidonic acid, DHA= docosahexaenoic acid, EPA= eicosapentaenoic acid, PC= phosphatidylcholine, PE= phosphatidylethanolamine, FA-Ratio= fatty acid ratio (ratio of AA to DHA plus EPA), ns= not significant