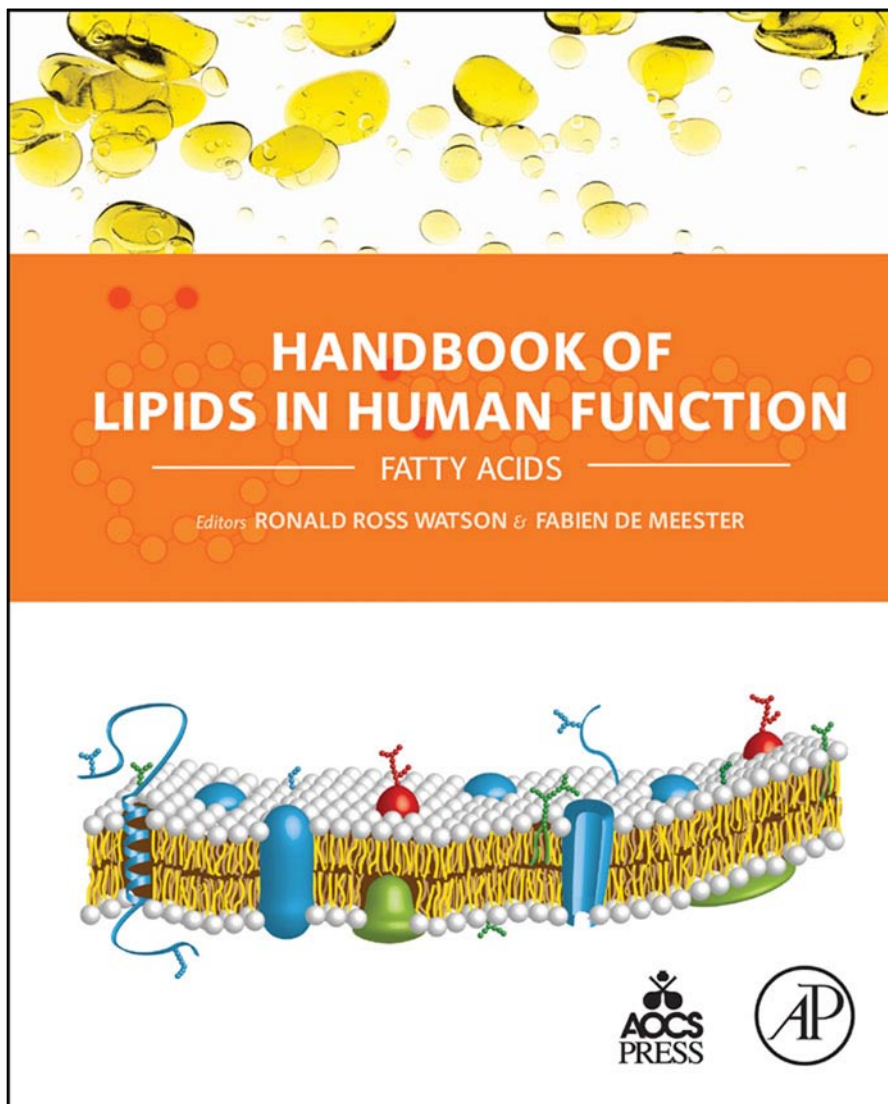**Provided for non-commercial research and educational use only.**
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HANDBOOK OF LIPIDS IN HUMAN FUNCTION

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Blood Cell Membrane Omega-3 (n-3) Fatty Acid Abnormality and Supplementation in Patients with Sickle Cell Anemia

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Sickle Cell Disease

Sickle cell disease (SCD) is a group of autosomal recessive genetic blood disorders caused by a mutation in the sixth codon of the β goblin gene that results in abnormal hemoglobin (Hemoglobin S, HbS) (Knight-Perry et al., 2009; Rees et al., 2010; Serjeant and Serjeant, 2001). The principal phenotypes are homozygous sickle cell (HbSS) disease, sickle cell-hemoglobin C , sickle cell-β^0 thalassemia, sickle cell-β^+ thalassemia, $HbSO_{Arab}$ and $HbSD_{Punjab}$ and $HbS_{Lepore\ Boston}$ SCD (Nagel et al., 2003; Serjeant and Serjeant, 2001). Deoxygenated HbS forms insoluble rigid polymers (sickle) under hypoxic conditions and reverts back to normal on re-oxygenation. However, with repeated cycles of sickling and unsickling, erythrocytes become irreversibly sickled and lose their biconcave shape and fluidity.

The primary pathological process in SCD, namely vasoocclusive crisis is a recurrent occlusion of blood vessels which causes ischemia, severe pain episodes (painful crisis), and damage to the brain, eyes, lungs, spleen, liver, and other vital organs (Ballas et al., 2010; Serjeant and Serjeant, 2001). Despite the apparent genetic simplicity, patients with SCD display a remarkable diversity in clinical manifestations and disease severity (Chui and Dover, 2001; Fertrin and Costa, 2010). The Cooperative Study of Sickle Cell Disease (Platt et al., 1991) found that 39% of 3578 patients with SCD did not have painful episodes,

whereas 1% had more than six per year. It appears that type and severity of the complication of the disease are modulated by genetic, environmental, and other factors (Sebastiani et al., 2005; Steinberg, 2005).

The Scope of the Problem

SCD is the commonest genetic blood disorders worldwide. About 5.2% of the world population (over 7% of pregnant women) carries a significant hemoglobin variant. HbS accounts for 40% of carriers but causes over 80% of the disorders because of the localized very high carrier prevalence (Modell and Darlison, 2008). The disease affects predominantly people of African, Mediterranean, Indian, and Middle Eastern lineage. There are approximately 100,000 people with SCD in the United States, 20,000 in United Kingdom, 1−2 million in Nigeria, and a lot more in the subcontinent of India. A recent estimate suggests more than 200,000 affected children, about 80% of the global total, are born in Africa every year (0.74% of birth in sub-Saharan Africa) (Modell and Darlison, 2008).

There is no reliable data in the literature on life expectancy and mortality rate of patients with SCD in Africa (Serjeant, 2005). However, the available scantly information from hospitals and sickle clinics reveal that life expectancy is as low as 20 years (Tshilolo et al., 2008). In the United States, because of the good clinical health care system and clinical management about 50% of patients live for more than 50 years of age (Platt et al., 1994) and the most frequent causes of death are infection (33−48%) and stroke (9.8%) (Manci et al., 2003).

Clinical Manifestation of SCD

Sickle cell pain

Sickle cell pain is the commonest manifestations of the disease in which episodic microvessel occlusion in one or more sites induces tissue damage accompanied by severe pain and inflammation (Ballas, 2005; Stuart and Nagel, 2004). The pain may be acute or chronic, somatic or visceral, unilateral or bilateral, localized or diffuse (Ballas, 1998). Acute painful episodes affect long bones and joints, particularly the lower back and pelvis (Ballas and Delengowski, 1993). Other region of the body, including the scalp, face, jaw, abdomen, and pelvis may also be affected be involved (Charache et al., 1995, 1996). The objective signs of a painful crisis, such as fever, leukocytosis, joint effusions, and tenderness occur in about 50% of patients at initial presentation (Ballas et al., 1988). Painful crisis affects nearly all patients often beginning in late infancy and recurring throughout life (Almeida and Roberts, 2005) and it is the major cause of hospital admissions (Brozovic et al., 1987). Moreover, adults who experience painful crises more than three times per year tend to have shorter life expectancies (Platt et al., 1991).

Bone and Joint Complications

Vasoocclusion can occur in any organ but it is particularly common in the bone marrow and it leads bone marrow infarction typically in the medullary cavity or epiphyses (Kim and Miller, 2002; Lonergan et al., 2001). Epiphyseal infarction has a predilection for the head of the femur (avascular necrosis), followed by the humorous, knee, and the small joints of the hands and feet (Jean-Baptiste and De Ceulaer, 2000; Lonergan et al., 2001). A significant number of HbSS patient develop epiphyseal osteonecrosis (Styles and Vichinsky, 1996; Ware et al., 1991), bone infection (Almeida and Roberts, 2005; Neonato et al., 2000), and transient red cell aplasia (abnormal decrease of reticulocytes) (Goldstein et al., 1987; Serjeant et al., 2001).

Cardiopulmonary Complications

Pulmonary complications account for 20–30% of mortality (Maitre et al., 2011) and significant morbidity. Moreover, acute chest syndrome (ACS), a form of acute lung injury which occurs frequently with variable severity in patients with the disease, is the second most common cause of hospital admission (Platt et al., 1994). Repeated episodes of ACS often predispose to chronic pulmonary scarring and high blood pressure in the arteries of the lungs (pulmonary hypertension) (Gladwin et al., 2004; Stuart and Setty, 2001a, 2001b; Vichinsky et al., 2000). SCD is associated with multiple morphological and functional cardiac anomalies (Ballas et al., 2010; Lester et al., 1990; Lindsay et al., 1974), such as dilated chambers, septal hypertrophy, and abnormal contractility (Covitz et al., 1995; Liem et al., 2009).

Neurological complications

Neurological and cranial complications occur in at least 25% of patients with SCD (Hebbel, 2005). These complications include transient ischemic attacks, overt and silent cerebral infarction, cerebral hemorrhage, posterior reversible encephalopathy syndrome, cerebral venous thrombosis and atrophy, and seizures (Alkan et al., 2009; Henderson et al., 2003; Liu et al., 1994; Yildirim et al., 2005). Cerebral infarction is the common cause of stroke in the first two decades of life and from the fourth decade onward; whereas, hemorrhagic stroke occurs commonly in the third decade (Ohene-Frempong et al., 1998). Clinical stroke with focal signs lasting more than 24 h is more common in children (Earley et al., 1998). Regardless of brain structural abnormalities, children with sickle disease with or without a history of overt stroke tend to have lower cognitive ability (Hogan et al., 2006; Noll et al., 2001; Watkins et al., 1998), and ocular (Elagouz et al., 2010; Nagpal et al., 1977) and ophthalmic (Babalola and Wambebe, 2005; To and Nadel, 1991) complications.

Renal abnormalities

Various functional and morphological renal abnormalities are manifested in SCD. Renal failure which occurs in 4–21% of adult HbSS patients is a significant contributor to

premature death (Guasch et al., 2006). The renal features of SCD include hematuria, proteinuria, tubular disturbances, acute kidney injury, and chronic kidney disease (Scheinman, 2009). A urinary concentration defect is the most common tubular abnormality and it can present as enuresis (Devereux and Knowles, 1985; Scheinman, 2009).

Hemolytic crisis

SCD is associated with variable degrees of anemia depending with genotype; with most severe being in HbSS. After the first 5 years of life, the hemoglobin (Hb) concentration remains constant in steady-state conditions. However, a significant drop in concentration occurs periodically due to hyperhemolysis, splenic sequestration, and aplastic crisis (Manci et al., 2003). Hyperhemolysis is diagnosed when the exacerbation of anemia occurs in the absence of splenic and hepatic sequestrations. Isolated hyperhemolysis in the absence of painful crisis is referred to as hemolytic crisis. Intravascular hemolysis (1/3 of SCD hemolysis) and extravascular hemolysis are driven by HbS polymerization and HbS instability respectively (Bensinger and Gillette, 1974; Hebbel, 2010). Recent evidence suggests that chronic intravascular hemolysis is associated with a state of progressive vasculopathy, characterized by reduced nitric oxide (NO) bioavailability, prooxidant and pro-inflammatory stress, coagulopathy, pulmonary hypertension, stroke, leg ulcers, and priapism (Gladwin and Kato, 2005; Gladwin et al., 2004; Kato et al., 2007; Morris et al., 2008; Reiter et al., 2002).

Splenic complications

Abnormal splenic function in HbSS patients is common by 6 months of age, and it affects more than 20% by 1 year and over 40% by 2 years (Serjeant and Serjeant, 2001). This abnormality is the result of trapping and subsequent destruction of sickled cells in the spleen. The relatively hypoxic and acidic splenic environment and slow blood flow provide a conductive milieu for sickling (Harrod et al., 2007). Enhanced sickling and repetitive infarctions lead to functional asplenia and ultimately splenic fibrosis and atrophy (Adekile et al., 2002; Pearson et al., 1969, 1985). This functional asplenia in turn results in increased susceptibility to sepsis, particularly from encapsulated bacteria (Ballas et al., 2010).

Pathophysiology of Vasoocclusion

The two major pathophysiological processes underpinning the complications associated with SCD are vasoocclusion with reperfusion injury and hemolytic anemia (Frenette, 2002; Rees et al., 2010). According to the classical paradigm, acute vasoocclustion was thought to be caused by entrapment of RBC containing the rope-like fiber of deoxygenated HbS. HbS polymerizes when deoxygenated, since valine which substituted glutamic acid in position six can interact hydrophobically with the complementary sites on adjacent globin chains (Hebbel et al., 2009; Stuart and Nagel, 2004). The polymerization of HbS is a nucleation-initiated reaction with a delay time, during which no polymer is detectable. At the end of the delay time, the critical nucleus is formed and exponential polymer formation

follows (Eaton and Hofrichter, 1990; Ferrone, 2004). Although HbS polymerization and red cell sickling are central to the pathophysiology of the disease, the primary events in vasoocclusion involve interactions of complex factors (Embury, 2004; Kaul et al., 2009). First, studies on polymerization kinetics have shown that the range of the transit times of RBC in the microcirculation is short relative to the range of delay times of HbS, and consequently most of HbS under physiological conditions fails to polymerize unless the delay times are lengthened, such as inflammation and enhanced adhesion of sickle cell and vascular endothelium (Mozzarelli et al., 1987; Turhan et al., 2002; Hebbel et al., 2009). Second, there no correlation between painful events and the number of sickled cells. Moreover, there is evidence that white blood cells of sickle cell patients, which seem to have a higher propensity to adhere to vascular endothelium, play a critical role vasoocclusion (Canalli et al., 2008; Frenette, 2002, 2004). Leukocytes adhesion to vascular endothelium is mediated by the interaction of leukocyte adhesion molecules L-selectin (CD62L), αMβ2 integrine (CD11b/CD18) and LFA-1 (CD11A/CD18) with endothelial adhesion molecules including, intercellular adhesion molecule-1, vascular adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin (Johnson and Telen, 2008; Okpala, 2002; Turhan et al., 2002). The fact that leukocytes are far larger, stiffer, and stickier than red cells, they are more effective in slowing microvascular blood flow, and ultimately, the initiation and propagation of vasoocclusion (Chiang and Frenette, 2005; Hebbel et al., 2009). The prothrombotic activity (Ataga and Orringer, 2003; Stuart and Setty, 2001a, 2001b) and elevated levels of markers of platelet activation, such as plasma soluble P-selectin (CD62P) and CD40L (Inwald et al., 2000; Tomer et al., 2001a; Wun et al., 1997), that are manifested in steady state patients are thought to contribute significantly to vasoocclusive events.

Management of SCD

A matched allogeneic hemopoietic cell transplantation (HSCT) is the only curative treatment for SCD (Johnson et al., 1984; Pinto and Roberts, 2008). Most groups reported event-free survival rates of around 80−90% (Bernaudin et al., 2007; Panepinto et al., 2007). However, the HSCT is restricted by the availability of matched related donors and associated risks of graft rejection, graft-versus-host-disease, recurrent infections, infertility, organ damage, and mortality (Fitzhugh et al., 2008; Pinto and Roberts, 2008). Similarly, gene therapy has been shown to have a curative potential in mouse models of sickle cell anemia (Perumbeti and Malik, 2010). But, there are several technical and safety challenges to overcome before it could be translated into viable clinical applications (Olowoyeye and Okwundu, 2010). Regardless, bone marrow and gene therapies are unlikely to be readily accessible to most sickle patient in sub-Saharan Africa because of the health care costs. Hence, in spite of their limitations, the current pathophysiology-based supportive therapies are likely to remain the cornerstone of symptomatic management for the disease in the foreseeable future (Sankaran, 2011).

In practice, high-risk patients, particularly children, in the developed countries are treated with periodic blood transfusion. This therapy reduces recurrent (Pegelow et al., 1995) and

initial stroke by over 80% (Gebreyohanns and Adams, 2004). Unfortunately, it is associated with a high rate of complications—transmission of infective agents, alloimmunization and transfusion reactions, and severe iron overload (Wang and Dwan, 2013). Hydroxyurea (HU), which is the only FDA approved pharmacological agent, is efficacious in reducing vasoocclusive crisis, blood transfusion requirement, ACS, organ damage and mortality in children and adults. However it remains a vastly underutilized drug due to continuous concerns about short and long-term side effects (Hankins and Aygun, 2009), which include myelosuppression (Lanzkron et al., 2008), malignancy (Zumberg et al., 2005), irreversible male subfertility, and teratogenicity (Ballas et al., 2009; Berthaut et al., 2008). Other factor that complicates HU usage are that it undergoes renal clearance, therefore dose adjustment and close monitoring of myelotoxicity must be implemented in individual with renal impairment (Yan et al., 2005). In addition, a large number of patents with SCD do not respond to treatment with HU (Stuart and Nagel, 2004). Other antiplatelet, anticoagulation, and anti-inflammatory experimental treatments have been studied extensively; nevertheless, the reported outcomes either controversial in term of safety or disappointing in efficacy (Ataga and Key, 2007; Hebbel et al., 2004; Strouse et al., 2006). The failure to develop safe and effective therapy is due to the multifactorial nature of SCD and its numerous and diverse clinical features including, chronic inflammation (Hebbel et al., 2004), blood cell membrane defect (Hebbel, 1991; Ren et al., 2005), chronic hemolysis (Kato et al., 2007), and high oxidative stress (Wood and Granger, 2007). Therefore, a single multifunctional or combination treatment is necessary to help ameliorate the varied abnormalities associated with the disease.

Cell Membrane Defect in SCD

Abnormal red blood cell membrane transport, (Gibson and Ellory, 2002), phospholipid organization (Barber et al., 2009; Kuypers, 2007), phospholipid fatty acid composition (Connor et al., 1997; Ren et al., 2006), and enhanced red cell membrane lipid peroxidation (Browne et al., 1998; Repka and Hebbel, 1991; Sugihara et al., 1992) have been reported in SCD. Moreover, there is evidence that platelets and mononuclear cells of patients with the disease have defective phospholipid fatty acid composition (Ren et al., 2005b). These abnormalities are thought to play a significant role in the pathophysiology and clinical severity of the disease (Hebbel, 1991).

Blood Cell Membrane Fatty Acid Composition

We have investigated fatty acid composition of (i) red blood cells of Nigerian HbSS patients and matched health controls, (ii) red blood cells, platelets and mononuclear cells of British HbSS patients and healthy controls (Ren et al., 2005a, 2005b), and (iii) red blood cells of Sudanese HbSS patients and controls (Daak et al., 2011). The red cells of the patients from the three countries with contrasting environmental and nutritional background
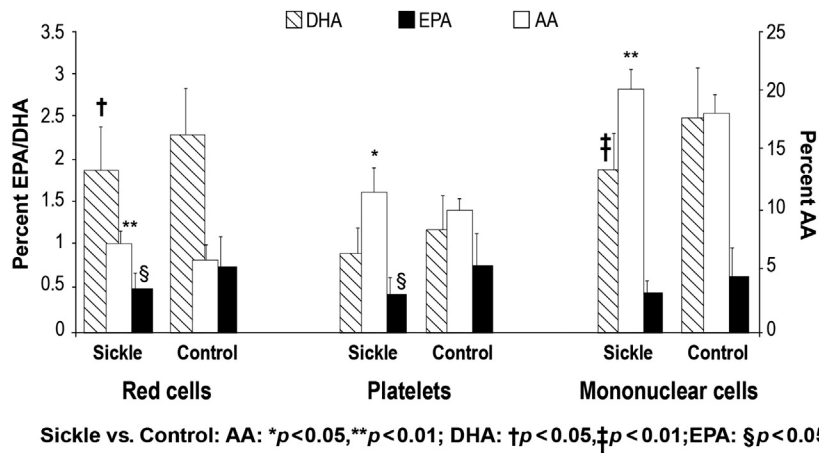
Figure 27.1

Arachidonic (AA, 20:4*n*-6), eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3) fatty acid composition of red cells, platelets and mononuclear cells of patients with SCD and healthy controls.

had abnormal fatty acid composition. This abnormality, which was evident in the inner and outer leaflets of the lipid bilayer, was manifested primarily by an increase in arachidonic acid (AA, 20:4*n*-6) and a concomitant decrease in the omega-3 fatty acids, eicosapentaenoic (EPA, 20:5*n*-3), and docosahexaenoic (DHA, 22:6*n*-3). Perhaps, more surprisingly, the mononuclear cells and platelets of the patients, although not affect directly by the genetic defect, had the same fatty acid abnormality as the red blood cells (Figure 27.1).Therefore, it appears that the high omega-6 and low omega-3 fatty acids of blood cells is a peculiar biochemical feature of the disease and unlikely to be a reflection of nutritional intake (Connor et al., 1997; Daak et al., 2011; Manodori et al., 2000; Ren et al., 2005b, 2006).

The long-chain omega-6 and -3 polyunsaturated fatty acids (LCPUFA) are vital structural and functional components of cells and organelles, and the balance between the two fatty acid families is a determinant of blood cell aggregation and coagulation, adhesion, deformability, and inflammatory response (Mills et al., 1993; Mukherjee et al., 2004; Nishiyama et al., 2000; Saito and Kubo, 2003). Hence, it is postulated that the imbalance of membrane *n*-6/*n*-3 LCPUFA is the antecedent of the loss of membrane asymmetry, blood cell adhesion and aggregation, and vasoocclusion in SCD (Ren et al., 2005).

## HU Treatment and Red Cell Membrane Fatty Acids

HU, which is commonly used as an effective therapy for SCD (Hoppe et al., 2000; Segal et al., 2008; Strouse et al., 2008; Stuart and Nagel, 2004), is a chemotherapeutic agent that inhibits ribonucleotide reductase and interferes with the S-phase of the cell cycle

(Trompeter and Roberts, 2009). The myelosuppressive and cytotoxic effects of HU induce RBC regeneration and the recruitment of earlier progenitors programmed to produce higher levels of HbF (Dover et al., 1986; Fathallah and Atweh, 2006). There is evidence that one of the mechanisms by which HU increases HbF levels is mediated through a NO-dependant activation of soluble quanylyl cyclase in erythroid cells (Cokic et al., 2003; Lou et al., 2009). It was thought that HU mediates its action solely through induction of fetal hemoglobin (HbF) and subsequent inhibition of polymerization of deoxyhemoglobin S (Steinberg et al., 1997). However, clinical improvements do occur prior to a significant rise in levels of HbF (Charache et al., 1996) suggesting that HU may modulate the pathophysiology of the disease through other additional mechanisms. Indeed, emerging evidence reveals that the mechanisms of action of HU involve a reduction of leukocytes, reticulocyte and platelet counts (Ballas et al., 1999), myeloperoxidase activity, blood cell adhesion (Johnson and Telen, 2008), the externalization of the proaggregatory aminophospholipid, serine (Covas et al., 2004), and an increased production of NO (Nahavandi et al., 2002).

The myriad of effects elicited by HU in SCD seem to involve plasma membrane of blood and endothelial cells. Consequently, we have investigated whether or not the compound has any effect on red blood cell membrane phospholipid fatty acids. Sudanese HbSS patients at steady-state, HU-treated ($n = 19$) and -untreated ($n = 17$), and healthy (HbAA) controls ($n = 20$) matched for ethnicity and economic background were recruited from Abnaof Pediatric Hospital, Khartoum, Sudan. The two main findings of this study were:

1. The HU-treated patients compared with their untreated counterparts had lower AA, docosatetraenoic (DTA, 22:4n-6), docosapentaenoic (22:5n-6) acids in red blood cell ethanolamine (EPG) and serine (SPG) phosphoglycerides which are primarily located in the inner leaflet of membrane lipid bilayer (Daak et al., 2011) (Figure 27.2). There were no such reductions in the aforementioned fatty acids in choline phosphoglycerides and sphingomyelin, which are found predominantly in the outer leaflet of membrane lipid bilayer. This finding leads us to suggest that HU releases selectively AA from the inner membrane phospholgycerides (Daak et al., 2011). Our suggestion is consistent with reports that: (i) Prostaglandin E2, the vasodilator metabolite of AA, induces the synthesis of HbF in erythroid colonies derived from peripheral blood cells (Datta, 1985) and the synthesis is obviated by aspirin, the potent inhibitor of cyclooxygenase, COX (Datta et al., 1991); (ii) HU generates NO *in vivo* (Glover et al., 1999; King, 2004; Nahavandi et al., 2002) and that NO activates cytostolic phospholipase A2 alpha (cPLA2α) and COX2 (Fitzpatrick and Soberman, 2001; Kim et al., 2005; Xu et al., 2008). cPLA2 α has a high selectivity for liberating AA from membrane phosphoglycerides.

2. There was no reduction in n-3 fatty acids in sphingomyelin, choline phosphoglycerides or serine phosphoglycerides in the HU treated group. As membrane n-3 fatty acid abnormality is one of the biochemical features of SCD, it appears this was ameliorated
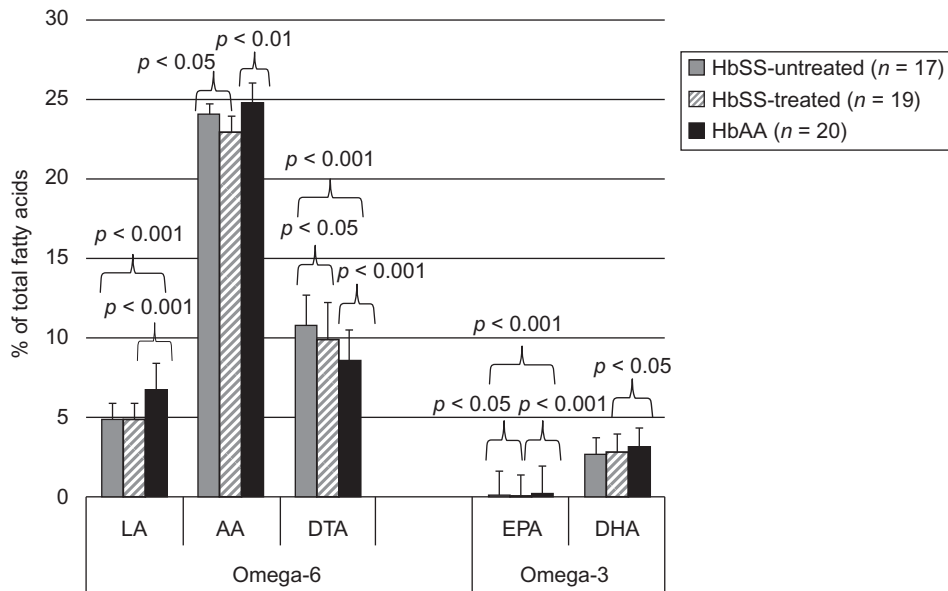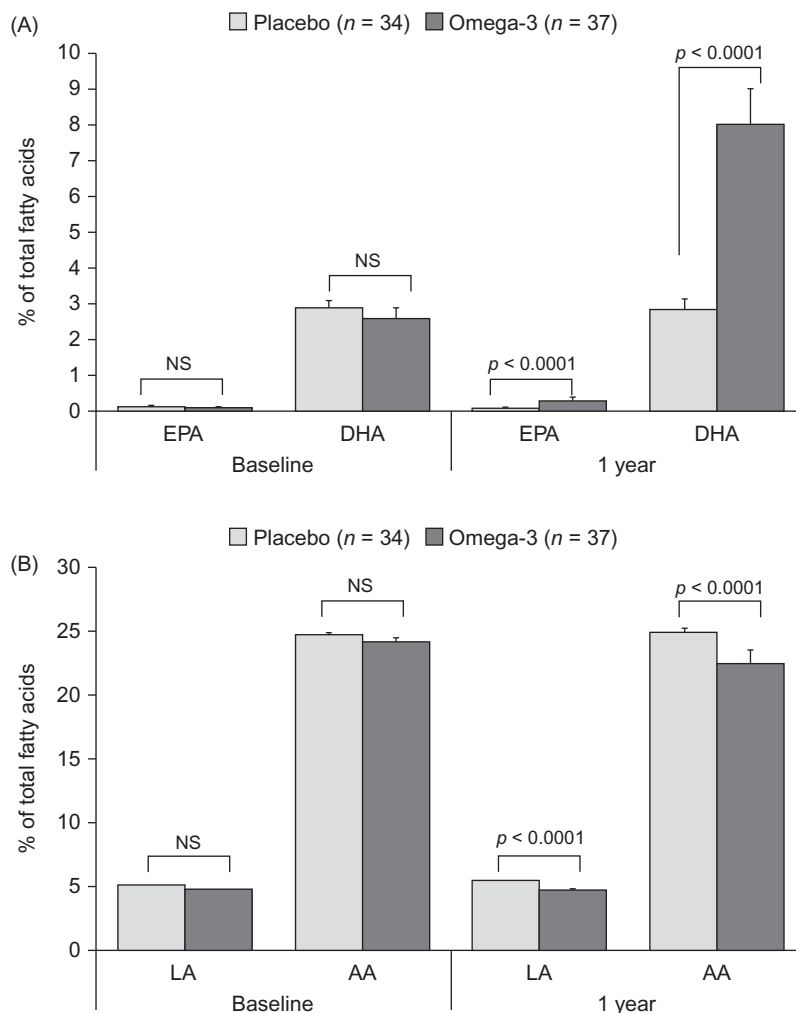
Figure 27.2
Linoleic (LA, 18:2*n*-6), arachidonic (AA, 20:4*n*-6), docosatetraenoic (DTA, 22:4*n*-6),
eicosapentaenoic (EPA, 20:5*n*-3), and docosahexaenoic (DHA, 22:6*n*-3) acid composition of red
blood cell ethanolamine phosphoglycerides of hydoxyurea-untreated and -treated sickle cell
patients (HbSS) and healthy controls (HbAA).

by HU therapy. This modulation of membrane fatty acid composition would be
expected to help enhance transmembrane ion flux, cell hydration, rheology, and
deformability (Djemli-Shipkolye et al., 2003; Ho et al., 1999; Poschl et al., 1996),
factors which are known to improve in HU-treated sickle cell patients (Adragna et al.,
1994; Athanassiou et al., 2006; Ballas et al., 1989).

Omega-3 Fatty Acid Supplementation of Sickle Cell Patients

The effect fish oil supplementation, a source of EPA and DHA, was investigated in
African-American HbSS patients ($n = 6$) by Tomer et al. (2001a). The patients were given
menhaden fish oil (0.25 g/kg/day) containing 12% EPA and 18% DHA, or placebo (olive
oil, 0.25 g/kg/day) for 1 year. Subsequent to supplementation there was a remarkable
reduction in the frequency of pain episodes requiring hospital presentation (from 7.8 to 3.8
per year) and plasma levels of thrombolytic products (D-dimer; prothrombin fragment 1.2,
F1.2; plasmin:antiplasmin complex) in the fish oil group. Similarly, Okpala et al. (2011)
have demonstrated a significant decrease in the number of crisis and steady-state hemolysis
in 16 Nigerian HbSS patients treated with Cod liver oil containing EPA and DHA. Golfetto
I (unpublished data) observed clinically significant improvement and catch-up growth in
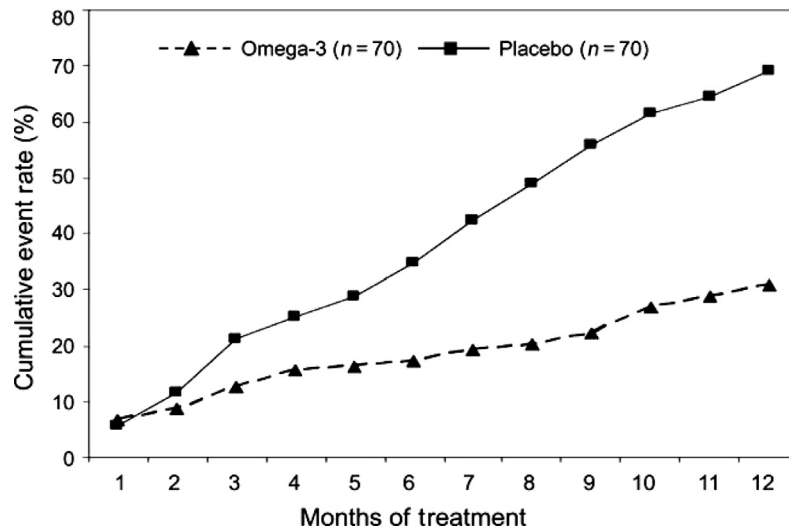
Figure 27.3

Mean percentage eicosapentaenoic (EPA), docosahexaenoic (DHA) (A) and linoleic (LA)
and arachidonic (AA) (B) acid composition of red blood cell membrane ethanolamine
phosphoglycerides at baseline and after 1 year supplementation. *Reproduced from the* American
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Venezuelan teenagers with SCD treated with fish oil. The potential clinical benefit of fish
oil-derived omega-3 fatty acids for patients with SCD was not fully appreciated because the
above studies were either underpowered or did not use placebo controls.

We investigated the therapeutic potential of omega-3 fatty acids for patients with
homozygous SCD in a randomized, placebo-controlled, double-blind trial (Daak et al.,
2013a), One hundred forty patients recruited from a single center in Sudan were randomly
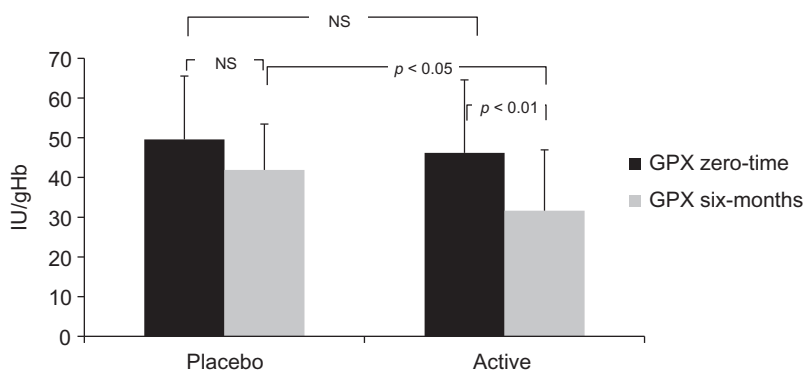
Figure 27.4

Cumulative event rate of clinical vasoocclusive crises in the omega-3 supplemented and unsupplemented (placebo) patients. *Reproduced from the* American Journal of Clinical Nutrition *(Daak et al., 2013a) with permission from the American Society of Nutrition.*

assigned and received, daily omega-3 capsules containing 277.8 mg DHA and 39.0 mg EPA or placebo for 1 year. One hundred twenty-eight patients were followed up and data obtained for intention-to-treat analysis. The primary and secondary endpoints were: rate of clinically overt vasoocclusive events, degree of hemolysis, blood transfusion rate, school attendance and blood cell counts. Supplementation for 12 months increased the levels of EPA and DHA threefold, and decreased the levels of linolenic acid (LA) and AA (Figure 27.3) in red blood cell choline and ethanolamine phosphoglycerides compared with their placebo counterparts. Clinically, omega-3 fatty acid treatment reduced the median rate of clinical vasoocclusive events (0 compared with 1.0 per year, $p < 0.0001$) (Figure 27.4), severe anemia (3.2% compared with 16.4%; $p < 0.05$), blood transfusion (4.5% compared with 16.4%; $p < 0.05$), white blood cell count (14.4 ± 3.3 compared with $15.6 \pm 4.0 \times 10$ (3)/μL; $p < 0.05$), and the OR of the inability to attend school at least once during the study period because of illness related to the disease to 0.4 (95% CI: 0.2, 0.9; $p < 0.05$). The evidence of this randomized study (Daak et al., 2013a) and the two pilot investigations (Tomer et al., 2001b) provide robust evidence that omega-3 fatty acids can be an effective, safe, and affordable treatment for patients with the disease.

Omega Fatty Acid Supplementation and Antioxidant Status in SCD

Despite the evident beneficial effects of *n*-3 fatty supplementation for patients with SCD, there was a lingering concern that the fatty acids, because of their high double bond index

and susceptibility to peroxidation (Hashimoto et al., 1999), might exacerbate the inherent oxidative stress associated with the disease. We have investigated antioxidant status of omega-3 fatty acid supplemented and unsupplemented sickle cell patients. Eighty ($n = 80$) steady-state patients with homozygous SCD, aged 2−14 years, who attend regular follow-up visits in the SCD Referral Clinic, Ibn-Aoaf Pediatrics, and Khartoum Teaching Hospitals, Sudan were recruited. After recruitment, the subjects were randomized and given 277.8 mg DHA and 39.0 mg EPA (active group) or a high oleic acid (41%) sunflower seed oil blend (placebo group) for 1 year. Vitamin E, $1 \cdot 5$ mg/capsule, was incorporated in both types of capsules to help prevent peroxidation.
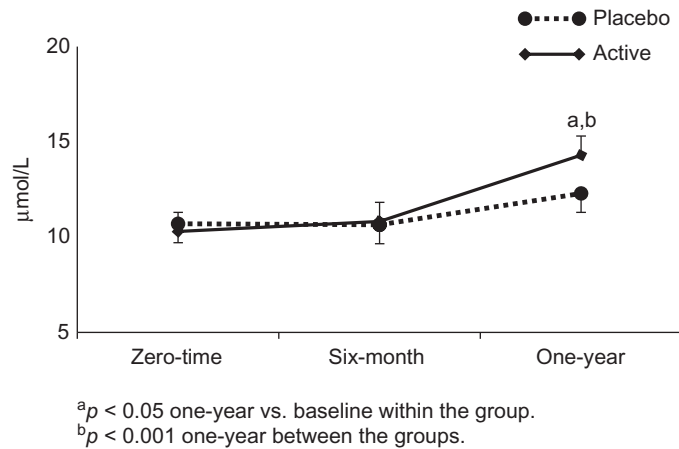

Figure 27.5

Activity of red blood cell cytosolic gluthatione peroxidase (GPx-1) of omega-3 fatty acid supplemented (active) and unsupplemented (placebo) HbSS sickle cell patients.
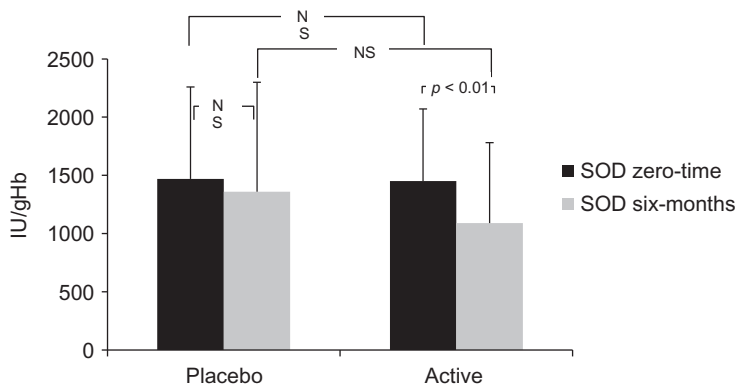
Figure 27.6

Activity of red blood cell Cu/Zn superoxide dismutase of omega-3 fatty acid supplemented (active) and unsupplemented (placebo) HbSS sickle cell patients.

Figure 27.7

Plasma α-tocopherol concentration of omega-3 fatty acid supplmented (active) and unsupplemented (placebo) HbSS sickle cell patients.

Plasma α -tocopherol concentration and the activities of cytosolic glutathione peroxidase (GPX-1) and Cu/Zn-superoxide dismutase (Cu/Zn-SOD) were used to assess the level of antioxidant protection. The omega-3 fatty acid supplemented patients compared with the placebo group had significantly lower GPX-1 (Figure 27.5) and Cu/Zn-SOD (Figure 27.6) activity and higher plasma alpha-tocopherol concentration (Figure 27.7) (Daak et al., 2013b) demonstrating that omega-3 fatty acid supplementation does not exacerbate oxidative stress in sickle cell patients. Indeed, perhaps paradoxically, it seems to bestow oxidative protection.

Conclusions

It is evident that patients with SCD have red blood cell, mononuclear cell, and platelet membrane fatty acid perturbation which is primarily manifested by lower LA, EPA, and DHA and higher AA, DTA, 22:4n-6, and docosapentaenoic (22:5n-6) acid levels. Moreover, studies conducted by us and others demonstrate that EPA and DHA supplementation ameliorates the membrane fatty acid abnormality and reduces the frequency and severity of vasoocclusive crisis and anemia without exacerbating the inherent oxidative stress. If these findings are reproduced in a large multicenter trial, EPA and DHA could be an effective, safe, and affordable therapy for the disease.

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Further Reading

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