Published in final edited form as:

JPEN J Parenter Enteral Nutr. 2007 ; 31(4): 263–268.

Defects in Postabsorptive Plasma Homeostasis of Fatty Acids in Sickle Cell Disease

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Abstract

Background—The chronic hemolytic anemia experienced by sickle cell disease (SCD) patients leads to adverse effects on oxygen transport by the blood and to a decrease in oxygen availability for peripheral tissues. Limited tissue oxygen availability has the potential to modify events of intracellular metabolism and, thus, alter lipid homeostasis.

Methods—The impact of SCD on plasma fatty acid homeostasis was determined in 8 African American SCD patients and in 6 healthy African American control subjects under postabsorptive conditions and during a 3-hour IV infusion of a nutrient solution containing lipid, glucose, and amino acids.

Results—SCD patients had higher fasting levels of plasma nonesterified fatty acids (NEFA), triglycerides, and phospholipids than healthy controls. Similarly, SCD patients had higher fasting levels of fatty acids in plasma triglycerides and phospholipids than healthy controls. Infusion of nutrients resulted in equivalent plasma NEFA profiles, total NEFA, and triglycerides in SCD patients and controls. However, the plasma phospholipid concentrations and fatty acid composition of plasma triglycerides and phospholipids were significantly higher in SCD patients; in particular, plasma pools of oleic acid were consistently increased in SCD. Plasma free oleic acid levels were elevated basally, leading to increased oleic acid content in triglycerides and phospholipids both postabsorptively and during nutrient infusion.

Conclusions—There is an underlying defect in lipid metabolism associated with SCD best manifested during the fasting state. This abnormality in lipid homeostasis has the potential to alter red blood cell (RBC) membrane fluidity and function in SCD patients.

Sickle cell disease (SCD) is a genetic disorder characterized by the production of abnormal hemoglobin (Hb), referred to as sickle Hb or HbS.¹ Production of this abnormal Hb, which results from the replacement of glutamic acid with valine in position 6 of the β -chains of Hb,² causes red blood cells (RBCs) to become rigid and sickled in shape upon deoxygenation in

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peripheral tissues.³ As a result, SCD patients experience a reduced RBC lifespan, chronic hemolytic anemia, and painful vaso-occlusive events that damage bone, tissues, and organs.² The chronic anemia experienced by SCD patients adversely affects oxygen transport by the blood and leads to a decrease in oxygen availability for peripheral tissues.

Another characteristic associated with SCD is RBC rigidity and rheological abnormalities. Because the erythrocyte membrane is composed of approximately 50% lipid,⁴ alterations in lipid homeostasis could significantly alter RBC membrane fluidity. However, data defining the effect of SCD on plasma lipid homeostasis are limited. Oxidation, elongation, and desaturation of nonesterified fatty acids (NEFA) are aerobic oxygen-dependent metabolic processes, and decreased oxygen availability has been shown to limit lipid absorption, transport, and endogenous synthesis.⁵

Erythrocyte membranes are synthesized from plasma phospholipids through a process of constant interchange between erythrocyte membrane and phospholipids in the plasma.⁴ Furthermore, the pool of plasma NEFA serves as a precursor pool for the erythrocyte membrane and has the potential to significantly influence the structure and function of the RBCs.⁶ Hence, any abnormalities in these precursor pools could alter RBC membrane content and functions and could lead to significant worsening of SCD. In the current study, we examined whether SCD is associated with significant alterations in lipid homeostasis. Our studies are preliminary and examine the effect of SCD on plasma concentration of NEFA, triglycerides, and phospholipids and on the fatty acid composition of plasma triglycerides and phospholipids after an overnight fast and after exposure to IV nutrients for a period of 3 hours.

MATERIALS AND METHODS

Participant Selection

We identified 8 African American SCD patients (6 male and 2 female) and screened them for participation in the study at the Sickle Cell Center at both Meharry Medical College and Vanderbilt University Medical Center, Nashville, TN. Additionally, we selected 6 African Americans (3 male and 3 female) who did not carry the sickle cell (HbS) gene as control subjects. The patients and controls were between 18 and 50 years of age and were within 25% of ideal body weight according to Metropolitan Life Insurance tables.⁷ We determined each subject's Hb phenotype using standard electrophoretic methods⁸ to confirm the presence of either (1) homozygous SCD (HbSS; $n = 4$), in which both genes coding for the β chains of Hb produce HbS; (2) sickle cell HbC disease (HbSC; $n = 3$), in which 1 gene codes for HbS and the other for HbC; (3) sickle cell β thalassemia disease (HbS β thal; $n = 1$), in which 1 gene codes for HbS and the other for reduced or no production of normal Hb (HbA); or (4) normal control (HbAA; $n = 6$), in which both genes code for HbA.⁸ We pooled the results for the 3 Hb phenotypes in the SCD group because there were no differences among the subjects.

We provided all subjects eligible for participation in the study with an explanation of the study and obtained informed consent (approved by both Vanderbilt University School of Medicine and Meharry Medical College institutional review boards). We performed all the metabolic studies at the Vanderbilt University General Clinical Research Center (GCRC). Before participation in the study, subjects underwent a complete history and physical examination, including metabolic, hematologic, hepatic, and renal function tests. Female subjects were not pregnant, as determined by a pregnancy test, were premenopausal with regular menstrual cycles, and were studied between 1 and 14 days after the onset of menses (follicular phase) to reduce experimental variability.

Body Composition Measurements

We measured participants' body density by hydrostatic weighing⁹ and calculated percent body fat using an equation specific for African Americans.¹⁰ We calculated (a) fat mass by multiplying body weight and percent body fat; (b) fat-free mass by subtracting fat mass from body weight; and (c) body mass index (BMI) as the ratio of body weight (kg) to height (m²).

Experimental Protocol

On the day of the metabolic study, subjects reported to the GCRC after a 12-h overnight fast. We placed an indwelling catheter in a superficial hand vein for arterialized blood sampling and placed the catheterized hand in a heated Plexiglas box with the temperature automatically adjusted to 55°C for complete arterialization of venous blood samples.¹¹ In 2 subjects, we were unable to place blood-sampling catheters in a hand vein and collected mixed venous blood *via* already existing intravenous access (Port-A-Cath). Each study consisted of a 2-hour resting period, a 30-minute blood sampling (basal) period, and a 180-minute nutrient infusion period. We collected blood at 10 and 30 minutes during the basal postabsorptive period and at 150 and 180 minutes during the nutrient infusion period for determination of plasma individual and total NEFA concentrations, total triglycerides concentrations, total phospholipids concentrations, and the percent contribution of individual fatty acids to plasma triglycerides and phospholipids. At the conclusion of the study, we removed the catheter, and participants were fed and discharged from the GCRC.

Nutrient Infusion

The nutrient infusion period consisted of IV administration of a nutrient solution containing amino acids (10% Travasol; Baxter Healthcare Corporation, Deerfield, IL), glucose (10% dextrose; Avabe America Inc, Princeton, NJ), and lipid (Intralipid 20%; Clayton LP, Clayton, NC), as we and others have previously reported.^{12–15} The nutrient infusion was designed to approximate the composition of a normal diet, with 16% of the energy coming from protein, 52% from carbohydrate, and 32% from fat, and provided a steady-state availability of nutrients with an osmolarity (≤ 700 mOsm/L) acceptable for peripheral administration. Glucose infusion rate was 3.3 mg/kg/min and non-protein calorie to nitrogen ratio was 140. The Intralipid solution consisted of 20% soybean oil, 1.2% egg yolk phospholipids, and 2.25% glycerin. The soybean oil contained a mixture of neutral triglycerides, with predominantly unsaturated fatty acids. The major component fatty acids were 44%–62% linoleic acid, 19%–30% oleic acid, 7%–14% palmitic acid, 4%–11% linolenic acid, and 1.4–5.5% stearic acid.

We designed the infusion rates to deliver glucose at a rate of 3.3 mg/kg/min, with a nonprotein calorie to nitrogen ratio of 140. This design allowed the provision of energy to be adjusted for each subject's body weight and gender. According to the recommended dietary intakes,¹⁶ the average energy allowance for a reference man 25–50 years of age, weighing 79 kg, is 12,122 kJ/d (505 kJ/h), whereas that for a reference woman of the same age range, weighing 63 kg, is 9196 kJ/d (383 kJ/h). Thus, over the course of the 3-hour experimental period the energy provided was for SCD patients and controls, respectively, 1254 ± 100 and 1329 ± 121 kJ, protein 12 ± 1 vs 12 ± 1 g, carbohydrate 39 ± 3 vs 41 ± 4 g, and fat 11 ± 1 vs 12 ± 1 g.

Analytical Procedures

Whole-blood Hb concentrations were measured in the hospital laboratory using a standard methodology. Blood samples were collected into separate vacuum ethylenediaminetetraacetic acid (EDTA) tubes, spun at 3000 rpm and 4°C for 10 minutes, and the plasma was collected and stored at –80°C for later analysis.

Plasma NEFA were quantified by gas chromatography using a modification of the method described by Ko and Royer.¹⁷ NEFA were extracted from plasma using heptane/isopropanol (30:70) and were separated from other lipids by thin-layer chromatography on silica gel 60A plates (Whatman K6; Fisher Scientific, Atlanta, GA) using petroleum ether/ethyl ether/acetic acid (80: 20:1) as the developing solvent. The NEFA band was visualized using rhodamine 6G, scraped from the plate, and methylated with boron trifluoride (BF₃)/methanol (12 wt % BF₃; Supelco, Bellefonte, PA) without elution from the silica gel. Fatty acid methyl esters were analyzed using a Hewlett Packard 5890A gas chromatograph interfaced with a Hewlett Packard 3365 Chem Station and equipped with a 6-ft- $\times$ -5-2-mm ID glass column packed with 10% SP 2330 on 100/120 Chromosorb WAW (Supelco). Fatty acid methyl esters were identified by comparison of retention times to those of known standards, and total mass of NEFA was determined by inclusion of an internal standard (pentadecanoic acid) and a comparison of the areas of the sample to that of the standard.

Plasma triglycerides and phospholipids were quantified by gas chromatography¹⁸ after extraction from plasma by the method of Folch et al.¹⁹ Individual lipid classes were isolated by thin layer chromatography as described above. The triglycerides and phospholipids were methylated using boron trifluoride (BF₃)/methanol and fatty acid methyl esters analyzed as described above. The mass of lipid was determined using triecosenoin and dipentadecanoylphosphatidylcholine as internal standards added before the extraction process.

Statistical Analysis

The data were analyzed using a 2-way repeated measures ANOVA with disease status (SCD vs control) as the between-subject factor and nutrition status (post-absorptive vs nutrient availability) as the within-subject factor. Significant ($p \leq .05$) differences between means were determined using contrasts of the least square means (LS Means) for *a priori* comparisons of disease status by nutrition status. Statistical analyses were performed using the SPS statistical software version 13.0 (SPSS Inc, Chicago, IL). Values presented in the text and tables represent the mean $\pm$ SEM for each group.

RESULTS

Participant Characteristics

The SCD ($n = 8$; 6 male and 2 female) and control ($n = 6$; 3 male and 3 female) groups of subjects were similar with regard to age (30 ± 3 vs 26 ± 2 years), weight (68.3 ± 5.2 vs 72.8 ± 5.8 kg), height (173.7 ± 4.2 vs 175.9 ± 4.3 cm), BMI (22.4 ± 1.2 vs 23.4 ± 1.4 kg/m²), percent body fat ($21.2\% \pm 3.1\%$ vs $21.7\% \pm 4.1\%$), fat-free mass (53.5 ± 4.1 vs 56.1 ± 3.1 kg), and fat mass (14.8 ± 2.6 vs 16.6 ± 4.0 kg). Furthermore, as would be expected, the Hb concentration of the SCD group (9.9 ± 10 g/dL) was significantly lower than that of the control group (13.7 ± 7 g/dL).

Plasma Hormone Concentrations

The responses to nutrient infusion in SCD and controls did not differ significantly for insulin (192 ± 37 vs 292 ± 95 pmol/L), cortisol (258 ± 30 vs 214 ± 30 nmol/L), epinephrine (147 ± 27 vs 185 ± 47 pmol/L), and norepinephrine (0.81 ± 0.11 vs 0.79 ± 0.11 nmol/L). Plasma glucagon was higher in SCD than in controls (60 ± 5 vs 47 ± 3 ng/L, $p < .05$).

Plasma NEFA, Triglyceride, and Phospholipid Concentrations (Table I)

During the postabsorptive period, plasma total NEFA, triglyceride, and phospholipid concentrations were 58%, 16%, and 8% higher, respectively, in the SCD patients than in controls. Nutrient infusion resulted in equivalent (~42%) increases in plasma total

triglycerides in both SCD patients and control subjects, whereas plasma total phospholipid concentrations remained unchanged in both groups. Mean plasma concentrations of individual NEFA, total triglycerides, and total NEFA were not different between the 2 groups during the nutrient infusion period; however, the individual NEFA components of various lipids varied between the 2 groups, as shown below. Plasma total phospholipids concentrations were 8% greater in the SCD subjects than in the controls during nutrient availability.

Relative Fatty Acid Composition of Plasma Triglycerides (Table II)

During the fasting period, the percent contribution of oleic acid to plasma triglycerides was 5% greater in the SCD patients than in the controls. Conversely, the contributions of myristic acid, palmitoleic acid, stearic acid, and arachidonic acid to plasma triglycerides were 46%, 11%, 16%, and 29% lower, respectively, in the SCD patients.

The infusion of nutrients led to a significant change in the relative composition of plasma triglycerides for many individual fatty acids in both the SCD and the control groups. The contributions of myristic and palmitoleic fatty acids to plasma triglycerides were 50% and 12% lower, respectively, in the SCD patients than in the controls during nutrient availability. In contrast, the contributions of palmitic and oleic fatty acids to plasma triglycerides were 7% and 9% greater, respectively, in the SCD patients. There were no significant differences between the groups with respect to the contributions of stearic, linolenic, and arachidonic fatty acids.

Relative Fatty Acid Composition of Plasma Phospholipids (Table III)

During the fasting period, the percent contributions of oleic and arachidonic fatty acids to plasma phospholipids were 21% and 10% greater, respectively, for the SCD patients than for the controls. In contrast, the contributions of palmitic, linoleic, docosapentaenoic, and docosahexaenoic fatty acids to plasma phospholipids were slightly (5%–13%) lower in the SCD patients.

During the nutrient availability period, the percent contributions of oleic, eicosatrienoic, and arachidonic fatty acids to plasma phospholipids during nutrient availability were 16%, 4%, and 12% greater, respectively, in the SCD patients than in the control subjects. Contributions of palmitic, linoleic, docosapentaenoic, and docosahexaenoic fatty acids to plasma phospholipids were slightly lower (3%–10%) in the SCD patients.

DISCUSSION

The present study found that patients with SCD, and without documented vaso-occlusive crises for at least 3 months, had elevated postabsorptive plasma concentrations of total and individual NEFA, triglycerides, and phospholipids when compared with control participants matched for age, weight, and body fat. These changes were much more pronounced in the fasting state. Infusion of a nutrient solution, with similar composition to an ingested meal, resulted in equivalent lipid profiles except for elevated plasma phospholipid concentration in the SCD patients. The reasons for the observed exaggerated changes during the fasting post-absorptive period remain unclear and deserve further investigation.

Previously published measurements of plasma lipid homeostasis are sparse, and large variation exists with regard to study subjects, their vaso-occlusive crisis state, nutrition status, and type of lipids measured. Furthermore, the majority of the published studies used children with SCD^{4,20} or a mixed population of children and adults.^{21–25} For example, plasma triglyceride concentrations were shown to increase in response to a painful vaso-occlusive crisis in homozygous SCD patients.²² Consequently, we designed the present

study to control for these variables. Our subjects did not have any documented crises for at least 3 months before study. In addition, we controlled for body fat content because differences in body fat could account for differences in lipid, and finally we studied our subjects after an overnight fast (postabsorptive state) and after the exogenous IV administration of a nutrient solution designed to simulate carbohydrate, protein, and fat composition of an ingested meal.

The literature is full of contradictory results regarding lipid profiles in SCD patients. Our finding of elevated postabsorptive plasma triglyceride concentrations was in agreement with that of Erasmus et al,²⁰ who reported that plasma triglyceride concentrations in overnight fasted subjects were 22% greater in Nigerian children with homozygous SCD compared with matched controls. Similarly, el-Hazmi et al²¹ observed that male and female adults and children with SCD had a nonsignificant 35% increase in plasma triglycerides, and Muskiet and Muskiet²⁴ and Sasaki et al⁴ reported increases in plasma oleic acid in patients with SCD. In contrast, other studies^{20–23,26} have reported no change in plasma triglyceride or even lower postabsorptive plasma phospholipid concentrations in adults and children with homozygous SCD.^{4,26,27} Finally, a large number of other studies focused primarily on measurements of total plasma lipid content and reported the concentrations to be significantly lower in homozygous SCD patients than in normal controls.^{26–28} Although we found increases in plasma concentrations of total NEFA triglycerides and phospholipids, we did not measure plasma cholesterol, which could contribute between 30% and 60% of the total plasma lipid mass.²⁹ In previous reports, patients with SCD had decreased concentration of plasma total cholesterol,^{4,21–25,27,28} which could account for the observed hypolipidemia in SCD patients.

An additional difference between studies pertains to the method of presenting data for fatty acids levels. For example, although Muskiet and Muskiet²⁴ reported fatty acid data as plasma concentrations of individual NEFA and percent contributions of individual fatty acids to total fatty acids, Sasaki et al⁴ reported their data as percent mass of total fatty acids. It is interesting to note, however, that our results are in agreement with all previously published observations^{4,20–28} when the data are reported per number, mass, or surface area of erythrocytes. Taken together, all these findings point to increased availability of lipids per RBC affected by SCD, thus resulting in altered RBC membrane lipid composition. Although we did not measure membrane lipid changes in our study, others have reported higher levels of RBC total lipids,^{4,24,26,27} total fatty acids,^{24,25} phospholipids,^{4,25–27} and cholesterol^{4,24–26} concentration in RBC of patients with SCD.

The present study examined the impact of SCD on plasma NEFA homeostasis during a constant infusion of a mixture of amino acids, glucose, and fat emulsion. Accordingly, plasma triglyceride levels increased, whereas plasma phospholipid concentrations remained unchanged for both patients with SCD and controls. The percent share of linoleic acid in plasma triglycerides increased in both groups to more closely correspond to its percent share (44%–62%) in exogenous lipid (Intralipid) infused. In contrast, plasma NEFA concentrations decreased in both study groups. Although our study did not address the mechanism of the changes in lipid profiles and content, it is quite plausible that SCD could alter lipid metabolism *via* multiple pathways. SCD could interfere with normal lipid metabolism *via* oxygen-dependent processes, such as fatty acid elongation, desaturation, and β -oxidation. In addition, the lack of oxygen availability in SCD (exaggerated by the presence of anemia) promotes increased potential for free-radical-mediated oxidative damage to RBC fatty acids,²⁸ resulting in reduced RBC lifespan. Consequently, it is likely that resultant abnormalities in lipid metabolism in SCD patients could alter membrane fluidity and function of RBC.

The nutrient infusion entered the circulation at a constant rate, and the processes of digestion and absorption were not involved. The major component fatty acids (linoleic, [44%–62%]; oleic, [19%–30%]; palmitic, [7%–14%]; linolenic, [4%–11%]; and stearic, [1.4%–5.5%]) were in the form of triglycerides. Accordingly, during the infusion of nutrients, plasma triglyceride concentrations were increased, whereas plasma phospholipid concentrations remained unchanged for both patients with SCD and controls. Furthermore, the percent share of linoleic acid in plasma triglycerides increased in both groups to more closely correspond to its percent share (44%–62%) in exogenous lipid (Intralipid) infused. Simultaneously with these changes, the levels of plasma NEFA decreased in both study groups. The differences that had existed between SCD patients' and controls' plasma concentrations of individual NEFA and total NEFA significantly decreased. In contrast, the magnitude of the difference (~8%) in plasma phospholipid concentrations between the 2 groups during nutrient availability remained significant and similar to the postabsorptive differences seen in patients with SCD. Further research is required to determine the specific mechanisms involved, but it is possible that SCD may interfere with normal lipid metabolism *via* oxygen-dependent processes, such as fatty acid elongation, desaturation, and β -oxidation. In addition, the lack of oxygen availability in SCD promotes increased potential for free-radical-mediated oxidative damage to RBC fatty acids,²⁸ resulting in reduced RBC lifespan. Ultimately, the SCD abnormality in lipid homeostasis has great potential to alter the membrane fluidity and function of RBCs in SCD patients. The impact of anemia on observed changes in plasma lipid profile cannot be explained from our data, and these need to be examined in future studies, including a control group of patients with β +-thalassemia intermedia who are anemic but do not have SCD. Similarly, the study cannot answer questions related to RBC rigidity, but these questions could be answered in our future studies that will examine influence of macronutrients on RBC lipid membrane in SCD, especially sickle cell anemia.

CONCLUSIONS

In summary, our study showed that SCD patients have significant alterations in plasma NEFA profiles during the postabsorptive state. Patients with SCD had increased levels of oleic and decreased levels of linoleic acid in plasma phospholipids. These differences, however, virtually disappeared after a 3-hour infusion of nutrients aimed at simulating a postprandial state. These observations suggest the existence of an underlying defect in lipid metabolism that could alter the membrane structure, function, and rigidity of RBCs in this patient population. Further studies are needed to explore the mechanisms of these changes and to address the impact of these changes on the overall function of the RBC in sickle cell patients.

Acknowledgments

This research was supported by National Institutes of Health grants DK26657 (Clinical Nutrition Research Unit, Vanderbilt University Medical Center), GCRC Program RR00095 (General Clinical Research Center, Vanderbilt University Medical Center), and DK20593 (Diabetes Research and Training Center, Vanderbilt University Medical Center). M.S.B. was supported by HL677715. We thank Myfanwy J. Borel, PhD, RD, and Whitney Robinson, BS, for their help with conducting the study. We also thank the subjects for their participation in the study, the nursing staff of the GCRC at Vanderbilt University for their help with this project, and Carla Harris for her technical assistance in completing this research.

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Table I

Plasma lipid profiles for sickle cell disease (SCD) and control groups during the basal and nutrient availability periods (mean $\pm$ SEM)

Nonesterified fatty acids (mg/dL)	Basal		Nutrient availability	
	SCD (n = 8)	Control (n = 6)	SCD (n = 8)	Control (n = 6)
Myristic (14:0) [‡]	0.14 $\pm$ 0.01	0.12 $\pm$ 0.03	0.04 $\pm$ 0.01 [†]	0.03 $\pm$ 0.00 [†]
Palmitic (16:0)	2.70 $\pm$ 0.28 [*]	1.84 $\pm$ 0.50	1.03 $\pm$ 0.21 [†]	0.88 $\pm$ 0.27 [†]
Palmitoleic (16:1)	0.37 $\pm$ 0.06	0.27 $\pm$ 0.07	0.12 $\pm$ 0.04 [†]	0.07 $\pm$ 0.00 [†]
Stearic (18:0)	1.18 $\pm$ 0.10 [*]	0.80 $\pm$ 0.18	0.53 $\pm$ 0.08 [†]	0.59 $\pm$ 0.21
Oleic (18:1)	5.17 $\pm$ 0.52 [*]	2.98 $\pm$ 0.76	1.83 $\pm$ 0.39 [†]	1.28 $\pm$ 0.33 [†]
Linoleic (18:2)	2.18 $\pm$ 0.16 [*]	1.40 $\pm$ 0.36	1.78 $\pm$ 0.33	1.53 $\pm$ 0.38
Total nonesterified fatty acids (mg/dL)	11.9 $\pm$ 1.1 [*]	7.52 $\pm$ 1.93	5.49 $\pm$ 1.08 [†]	4.49 $\pm$ 1.21 [†]
Total triglycerides (mg/dL)	60.2 $\pm$ 2.8 [*]	50.9 $\pm$ 5.8	81.0 $\pm$ 3.9 [†]	73.8 $\pm$ 5.9 [†]
Total phospholipids (mg/dL)	134 $\pm$ 3 [*]	123 $\pm$ 6	133 $\pm$ 2 [*]	123 $\pm$ 5

* $p \leq .05$ between the SCD and control groups during the basal or nutrient availability periods.

[†] $p \leq .05$ between the basal and nutrient availability periods within the SCD or control groups.

[‡] First number denotes number of carbons in fatty acid chain. Second number denotes number of *cis* double bonds.

Table II

Relative fatty acid composition of plasma triglycerides for sickle cell disease (SCD) and control groups during the postabsorptive and nutrient availability periods (mean $\pm$ SEM)

Fatty acid composition (%)	Postabsorptive		Nutrient availability	
	SCD (n = 8)	Control (n = 6)	SCD (n = 8)	Control (n = 6)
Myristic (14:0) [§]	1.20 $\pm$ 0.04	1.49 $\pm$ 0.26	0.72 $\pm$ 0.02 ^{*†}	1.44 $\pm$ 0.28
Palmitic (16:0)	24.6 $\pm$ 0.2	24.1 $\pm$ 0.3	21.4 $\pm$ 0.1 ^{*†}	20.1 $\pm$ 0.2 [†]
Palmitoleic (16:1)	4.15 $\pm$ 0.07 [*]	4.73 $\pm$ 0.17	3.00 $\pm$ 0.07 ^{*†}	3.42 $\pm$ 0.15 [†]
Stearic (18:0)	3.78 $\pm$ 0.08 [*]	4.54 $\pm$ 0.27	3.93 $\pm$ 0.07	4.06 $\pm$ 0.14 [†]
Oleic (18:1)	40.8 $\pm$ 0.6 [*]	37.2 $\pm$ 0.8	35.9 $\pm$ 0.4 ^{*†}	33.1 $\pm$ 0.5 [†]
Linoleic (18:2)	23.6 $\pm$ 0.6	23.8 $\pm$ 0.9	33.8 $\pm$ 0.5 ^{*†}	36.3 $\pm$ 0.6 [†]
Arachidonic (20:4)	1.82 $\pm$ 0.06 [*]	2.52 $\pm$ 0.14 [‡]	1.22 $\pm$ 0.08 ^{*†}	1.81 $\pm$ 0.06 ^{†‡}

^{*} $p \leq .05$ between the SCD and control groups during the basal or nutrient availability periods.

[†] $p \leq .05$ between the basal and nutrient availability periods within the SCD or control groups.

[‡] Five measurements.

[§] First number denotes number of carbons in fatty acid chain. Second number denotes number of *cis* double bonds.

Table III

Relative fatty acid composition of plasma phospholipids for sickle cell disease (SCD) and control groups during the basal and nutrient availability periods (mean $\pm$ SEM)

Fatty acid composition (%)	Basal		Nutrient availability	
	SCD (n = 8)	Control (n = 6)	SCD (n = 8)	Control (n = 6)
Palmitic (16:0) [‡]	27.0 $\pm$ 0.2 [*]	28.9 $\pm$ 0.4	27.3 $\pm$ 0.2 [*]	29.3 $\pm$ 0.4
Stearic (18:0)	13.8 $\pm$ 0.1 [*]	14.3 $\pm$ 0.2	13.7 $\pm$ 0.1 [*]	14.2 $\pm$ 0.3
Oleic (18:1)	14.9 $\pm$ 0.3 [*]	12.5 $\pm$ 0.2	15.5 $\pm$ 0.3 ^{*‡}	13.4 $\pm$ 0.2 [‡]
Linoleic (18:2)	22.2 $\pm$ 0.3 [*]	23.1 $\pm$ 0.3	21.2 $\pm$ 0.3 ^{*‡}	22.0 $\pm$ 0.2 [‡]
Eicosatrienoic (20:3)	3.16 $\pm$ 0.09	3.07 $\pm$ 0.14	3.09 $\pm$ 0.0	3.00 $\pm$ 0.15
Arachidonic (20:4)	15.1 $\pm$ 0.2 [*]	13.9 $\pm$ 0.4	15.1 $\pm$ 0.2 [*]	13.6 $\pm$ 0.4
Docosapentaenoic (22:5)	0.91 $\pm$ 0.05 [*]	1.21 $\pm$ 0.04	0.89 $\pm$ 0.05 [*]	1.14 $\pm$ 0.04
Docosahexaenoic (22:6)	2.88 $\pm$ 0.08 [*]	3.08 $\pm$ 0.09	3.17 $\pm$ 0.08 ^{*‡}	3.43 $\pm$ 0.10 [‡]

^{*} $p \leq .05$ between the SCD and control groups during the basal or nutrient availability periods.

[‡] $p \leq .05$ between the basal and nutrient availability periods within the SCD or control groups.

[‡] First number denotes number of carbons in fatty acid chain. Second number denotes number of *cis* double bonds.