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Erythropoiesis and Myocardial Energy Requirements Contribute to the Hypermetabolism of Childhood Sickle Cell Anemia

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Abstract

Objectives—We hypothesized that an elevated hemoglobin synthesis rate (SynHb) and myocardial oxygen consumption (MVO₂) contribute to the excess protein and energy metabolism reported in children with sickle cell anemia.

Patients and Methods—Twelve children (6–12 years old) with asymptomatic sickle cell and 9 healthy children matched for age and sex were studied. Measurements were whole-body protein turnover by [1-¹³C]leucine, SynHb by [¹⁵N]glycine, resting energy expenditure by indirect calorimetry and the systolic blood pressure–heart rate product used as an index of MVO₂. Protein energy cost was calculated from protein turnover. Statistical analysis included Spearman correlations and partial correlation analyses.

Results—Although body mass index was significantly lower for sickle cell versus controls ($P < 0.02$), children with asymptomatic sickle cell had 52% higher protein turnover ($P < 0.0005$). Proportional reticulocyte count, SynHb, MVO₂ and resting energy expenditure were also significantly higher in children with sickle cell ($P < 0.01$). Protein turnover correlated significantly with both SynHb ($r = 0.63$, $P < 0.01$) and reticulocyte percentage ($r = 0.83$, $P < 0.0001$). Partial correlation of these 3 variables showed reticulocyte percentage as the only variable to be significantly associated with protein turnover, even after adjusting for sickle cell anemia ($P = 0.03$). Partial correlation of log resting energy expenditure on MVO₂ was significant, controlling for protein energy cost, sex and age ($P = 0.03$).

Conclusion—These results indicate that metabolic demands of increased erythropoiesis and cardiac energy consumption account for much of the excess protein and energy metabolism in children with sickle cell anemia.

Keywords

Energy cost; Hemoglobin synthesis; Cardiac work; Protein turnover; Leucine kinetics

Homozygous sickle cell disease (HbSS) is characterized by severe chronic hemolytic anemia associated with increased rate of erythropoiesis (1). Resting energy expenditure (REE), whole-body protein turnover (Q) (2–6) and protein catabolism, via increased urea production rate (7,8), are concurrently elevated in patients with HbSS. These findings support the notion that patients with HbSS are hypermetabolic, requiring more dietary protein and energy, compared with healthy controls with normal hemoglobin (Hb) genotype (HbAA). Children with HbSS experience poor growth and development (9), often becoming short thin adults (10). This growth failure may be a direct consequence of inadequate dietary protein and energy.

Because protein turnover consumes energy, a direct link between increased REE and elevated erythropoiesis has been inferred; so far, no reports confirm this association (1). Cardiac output is estimated to account for 7% to 33% of REE in healthy adults (11–13), but its contribution to energy requirements of HbSS is not known. Buchowski et al. (14) showed that basal energy requirements were higher than normal for adolescents with HbSS and provided the following simple equation for predicting the REE based on body weight and Hb concentration:

$$\begin{aligned}\text{REE (kcal/d)} &= 1305 + 18.6 * \text{weight (kg)} - 55.7 * \text{Hb (g/dL)} \text{ for boys} \\ &= 1100 + 13.3 * \text{weight (kg)} - 30.2 * \text{Hb (g/dL)} \text{ for girls}\end{aligned}$$

The components of this equation suggest an important role for cardiac involvement, as a function of anemia, in the increased REE of HbSS. Salman et al. (4) reported increased REE associated with increased leucine turnover in 11-year-old children with HbSS compared with age-matched controls. In addition, cardiac assessments using ultrasound showed that stroke volume was 75 mL for HbSS compared with 49 mL for controls. This study highlighted the increased energy needs of children with HbSS, and the authors suggested that both increased Hb synthesis and cardiac workload may contribute to excess protein and energy use. However, Salman et al. (4) did not investigate the contributions of Hb synthesis or cardiac work to the protein and energy hypermetabolism in children with HbSS.

Healthy young children generally have higher REE per fat-free mass (FFM) than adolescents and adults, and faltering growth is an early indicator of energy deficiency at a young age (11). Because children with HbSS are particularly at risk for relative energy deficiency that can retard growth (15), a precise understanding of their energy expenditure is essential for developing more effective approaches for matching their energy needs. In the literature, there are no reports evaluating the contribution of cardiac energy expenditure to the altered REE of children with HbSS, nor has the rate of heme formation been directly measured in HbSS. We tested the hypothesis that energy expenditure from elevated cardiac metabolism contributes significantly to the hypermetabolism of children with HbSS. We measured Q converted to protein energy cost (Q_{equiv} , in kilocalories) and calculated

myocardial oxygen consumption (MVO_2). Using multivariate analysis, with log REE as the dependent variable, we estimated the contributions of Q and MVO_2 to the REE outcome.

PATIENTS AND METHODS

Subjects

Prepubertal children (9–12 years old) with HbSS, confirmed by Hb electrophoresis, were recruited from Georgia Comprehensive Sickle Cell Center in Atlanta. Controls with normal Hb (HbAA), confirmed by Hb testing, were matched for age, sex, Tanner stage 1 for sexual development (16) and ethnicity. The control children were recruited from Morehouse Medical Associates general pediatric practice in Atlanta. A medical evaluation, including a physical examination and screening blood tests, was conducted on each potential subject. Only clinically asymptomatic individuals were enrolled. Children were also excluded from the study for any medical reason that may alter protein and energy metabolism such as renal disease, abnormal liver function, chronic asthma or blood transfusion during the preceding 4 months. Children with HbSS were also excluded if they were receiving antisickling therapy such as hydroxyurea or taking pain medicine within 3 to 5 days before the study. Written informed assent and consent were obtained from the participants and their guardians after all of the procedures were explained. The institutional review boards of Morehouse School of Medicine and Emory University School of Medicine approved the study.

Study Design

The study was a cross-sectional comparison design. Participants were admitted by selection of people fitting the enrollment criteria, determined at the screening visit to Emory Hospital General Clinical Research Center (GCRC), Atlanta. Participants qualifying for the study were admitted to the GCRC for inpatient stay, during which several stable isotopic tracers were used to measure whole-body protein kinetics, with serial blood samples. Standard hematology, reticulocyte, REE and anthropometry were measured, and MVO_2 was estimated.

Experimental Protocol

The participants were admitted to the GCRC on the night before the procedures. The study began the following morning at approximately 8:00 AM. The procedures are outlined in Figure 1. During the isotope infusions, the children received their habitual daily 12-hour intake divided into 4 equal portions to allow even distribution of protein and energy consumption. Individual intakes were calculated by converting food weight to protein and energy content using dietary analysis software (Nutrition Data Systems, version 8A/2.6; University of Minnesota, Minneapolis, MN).

Whole-body leucine kinetics were measured by simultaneous administration of [$1-^{13}C$]leucine, [$2H_3$]leucine and $NaH^{13}CO_3$ (99.9%; Cambridge Isotopes Laboratories, Woburn, MA), using a modification of methods by Patterson et al. (17) and Jahoor et al. (18) Rate of incorporation of glycine nitrogen into heme, an index of Hb synthesis rate (SynHb), was measured with [^{15}N]glycine (99.9%) using a modification of our previous method (19). Two intravenous catheters were placed in opposite arms, one for infusion of

labeled compounds and the other for blood sampling. Isotopes for intravenous infusion were prepared in sterile solutions with normal saline (9 g/L NaCl). Baseline blood and breath samples were collected before the isotopes were administered. Primed-intermittent oral doses of [^{15}N]glycine (priming and intermittent dosage, 40 $\mu\text{mol/kg/h}$) were given at hourly intervals for 12 hours. A primed-intermittent oral administration of [$^2\text{H}_3$]leucine (priming dose, 5 $\mu\text{mol/kg}$; intermittent dosage, 5 $\mu\text{mol/kg/h}$) was given at 30-minute intervals for 6 hours, whereas a primed constant intravenous infusion of $\text{NaH}^{13}\text{CO}_3$ (priming dose, 4.5 $\mu\text{mol/kg}$; intravenous infusion, 6 $\mu\text{mol/kg/h}$) was maintained for 2 hours. This was immediately followed by a primed constant intravenous infusion of [$1\text{-}^{13}\text{C}$]leucine (same dosing as [$^2\text{H}_3$]leucine) for 4 hours. Blood samples for leucine measurements were collected at 4.0, 4.5, 5.0, 5.5 and 6.0 hours. Additional blood samples were collected at 8, 10, 12 and 24 hours for fractional heme synthesis and reticulocytes at 24 hours. Additional breath samples were collected at 15-minute intervals during the final hour of the labeled bicarbonate and leucine infusions. Systolic blood pressure (SBP), heart rate (HR) and REE were measured soon after awakening on day 2.

Sample Analyses

Protein Turnover Measurements—Plasma amino acids were extracted and converted to the *n*-propyl ester heptafluorobutyramide derivative, as described elsewhere (17). Isotope enrichments of plasma leucine and α -ketoisocaproic acid were measured by chemical ionization gas chromatography–mass spectrometry and breath $^{13}\text{CO}_2$ by isotope ratio mass spectrometry (Metabolic Solutions, Inc, Nashua, NH). Protein kinetics were calculated from enrichments of leucine and α -ketoisocaproic acid using standard equations (18) and 590 μmol of leucine equivalent to 1 g of body protein.

Hb Measurements—Glycine was isolated, and derivative enrichment was measured (Metabolic Solutions, Inc) as described previously (19). Heme was extracted from hemolyzed red cells by a previously described method (20). Enrichment of the glycine incorporated into heme was measured by combustion/continuous flow isotope ratio mass spectrometry (Metabolic Solutions, Inc). Fractional SynHb was calculated from enrichment of isotopic glycine in heme and the red blood cell (RBC)–free glycine pool as previously described (19). Absolute SynHb was calculated as the product of total circulating Hb (g) and the fractional SynHb. Hemoglobin synthesis rate is expressed as gram per kilogram body weight per day.

Body Composition

Body weight, height and fat mass were measured, as previously described (21). Body mass index (kg/m^2) was calculated from weight and height. Percent body fat was calculated from the sum of 2 skinfold measurements using equations for children (22). The FFM was calculated from body weight and fat mass.

Hemodynamic and REE Measurements

The mean of duplicate measurements for HR by pulse rate and blood pressure using a pressure cuff were recorded after the participant was awake and resting in bed. The REE (kilocalories per day) was measured for 30 minutes by indirect calorimetry using a

metabolic cart (DeltaTrac; SensorMedics, Yorba Linda, CA) with hood after 12 hours of overnight fast with water (21). Energy expenditure was calculated by the data acquisition system of the metabolic cart using the Weir formula (23).

Myocardial Oxygen Consumption

The product of SBP and HR was used as an index of MVO₂. Nelson et al. (24) and other researchers reported the product of HR and SBP as the best hemodynamic predictor of MVO₂ in healthy adults. Cardiologists use the pressure-rate product as a standard noninvasive method, which has been applied to pediatric patients (25). This approach was used to estimate MVO₂ (ie, myocardial energy requirement).

Energy Cost of Protein Turnover

Energy cost per gram of protein turnover derived from a stoichiometric calculation by Waterlow and Millward (26,27) was used to estimate energy cost of the protein turnover measurements. Briefly, the calculation is based on the premise that peptide bond synthesis requires at least 4 mol of ATP + GTP (5 ATP) per mole peptide (26,28). This gives a value of 0.9 kcal/g (3.78 kJ/g) protein as the theoretical minimum energy requirement. Waterlow (27) added theoretical allowances for the ATP costs of amino acid transport and 1 round of messenger RNA transcription, increasing the estimated costs to 2.2 kcal/g (9.24 kJ/g) protein. We used the 2.2 kcal/g (9.24 kJ/g) protein estimate to determine the energy cost of protein turnover by the following equation:

$$\text{Protein energy (Q}_{\text{equiv}}, \text{ kcal/kg/day}) = \text{Protein turnover (Q, g/kg/day)} \times 2.2 \text{ (kcal/g)}$$

Statistical Analysis

Descriptive statistics for continuous variables are presented as means $\pm$ SD and ranges, and descriptive statistics for categorical variables are presented as frequencies (%). Intergroup comparisons of continuous variables were made using the Wilcoxon rank sum test. The Spearman correlation test was used to assess associations among variables. Partial correlations (29) were generated from multivariate analyses to determine the strength of associations. Data for REE, an important outcome measure, were slightly skewed requiring log transformation before multivariate analysis. The ratio of REE to FFM was compared between the study groups. The appropriateness of this ratio was confirmed by comparing the results for regression model ($\text{REE/FFM} = (\text{slope} + \text{intercept} \times \text{FFM})/\text{FFM}$) with values for ratio method. Statistical interactions among disease and control groups were determined using the likelihood ratio test (29). Statistical significance was assumed at $P < 0.05$ for all analyses. The data were analyzed using STATA 8.2 (StataCorp, College Station, TX) for Windows data analysis package.

RESULTS

Subjects' Characteristics

Nine subjects with HbAA (3 boys, 6 girls) and 12 subjects with HbSS (5 boys, 7 girls) were studied. Average dietary protein (HbAA, 1.57 ± 0.26 g/kg body weight per day; HbSS, 1.79

± 0.38 g/kg body weight per day) and energy (HbAA, 51 ± 13 kcal/kg body weight per day; HbSS, 60 ± 16 kcal/kg body weight per day) intakes were not different between the groups. Physical characteristics and hematology for the participants are shown in Table 1 and are described in detail elsewhere (21). There was no significant age difference between the HbSS and HbAA groups. No differences between the groups were found for FFM, weight and height, although these variables were slightly lower in the HbSS group. However, mean body mass index was significantly lower for the HbSS group compared with the HbAA group ($P < 0.05$). Hemoglobin concentrations, hematocrit and RBC counts were below the reference range for the HbSS group and significantly lower than in the HbAA ($P < 0.001$). At the same time, the HbSS had significantly higher proportional reticulocyte counts (reticulocyte percentage) compared with the HbAA ($P < 0.001$), consistent with more rapid RBC production rate to compensate for the hemolysis of sickle cell disease.

Relationship of SynHb and Reticulocyte Count to Q—All protein kinetic measurements (ie, protein turnover, protein synthesis, protein breakdown and heme synthesis rates) were significantly higher for the HbSS group compared with the HbAA group ($P < 0.002$; Table 2). The SynHb was significantly correlated with protein turnover ($r = 0.76$, $P < 0.05$) among the HbAA group (Fig. 2A) but not among the HbSS group, although a significant but weaker correlation was measured with combined groups ($r = 0.63$, $P < 0.01$). Reticulocyte percentage was associated with SynHb ($r = 0.73$) and protein turnover ($r = 0.83$, $P < 0.001$ [P value for both SynHb and protein turnover]) with combined groups (Fig. 2B,C). Adjusting for reticulocyte percentage by multivariate analysis diminished the strength of association between protein turnover (dependent variable) and SynHb and revealed instead a significant association of reticulocyte percentage with protein turnover ($r = 0.73$, $P < 0.005$), which was maintained after further adjustment for sickle cell anemia ($r = 0.55$, $P < 0.05$; Table 4).

Relationship of MVO₂ and Q_{equiv} to REE—Myocardial oxygen consumption, Q_{equiv} and REE were significantly elevated in HbSS group compared with HbAA group ($P < 0.01$; Table 3). The REE was significantly associated with MVO₂ ($r = 0.78$) and Q_{equiv} ($r = 0.53$) across the groups ($P < 0.05$; Fig. 2). Partial correlation of log REE (dependent variable) on MVO₂, adjusting for Q_{equiv}, sex and age showed maintenance of the significant association between MVO₂ and log REE ($r = 0.51$, $P = 0.03$; Table 4).

Hb Concentration—Hemoglobin concentration was inversely associated with protein turnover ($r = -0.82$), protein synthesis ($r = -0.76$), MVO₂ ($r = -0.72$) and reticulocyte count ($r = -0.85$, $P < 0.001$; Fig. 2).

DISCUSSION

Badaloo et al. (2) first reported elevated protein turnover and energy expenditure in HbSS adults, which have been confirmed in children with HbSS (4,30). However, not all determinants for this hypermetabolism are known, particularly regarding the REE. The prevailing consideration is that increased Q, an energy-consuming process in healthy individuals (31), contributes to the excess energy metabolism in HbSS via chronic hemolysis and compensatory increase of erythropoiesis. This notion is supported by studies reporting

concomitant reduction of protein turnover and energy expenditure after intervention. For example, splenectomy for hypersplenism, a subtle method to decrease red blood cell turnover, was associated with significant reduction of protein turnover and REE (by 30% and 15%, respectively) in HbSS (30). However, energy cost of protein turnover is not the only contributor to elevated energy expenditure in HbSS. We therefore investigated whether energy cost of myocardial work contributes significantly to increased REE in children with HbSS.

Our results confirm a significantly higher Q (1.5 times) in children with HbSS compared with children in the HbAA group. Considered separately, protein synthesis was increased 1.8 times and protein breakdown was increased 2 times in the HbSS group. Furthermore, heme synthesis rate (SynHb) was increased 20 times, and reticulocyte percentage was increased 16 times in the HbSS group. Heme synthesis was measured endogenously (19) to determine whether increased metabolism of this single protein could make a significant contribution to Q. Although there was a significant independent correlation of Hb synthesis with protein turnover, partial correlation analysis adjusting for reticulocyte percentage and disease status demonstrated that in the HbSS group, reticulocyte percentage was making the most significant contribution to the elevated protein kinetics (Table 4). Therefore, in children with HbSS, high protein turnover could be reflecting degradation and recycling of hemolyzed cells more than Hb production. This finding agrees with previous notions, derived from theoretical estimates, of low Hb contributions to increased Q (2,4).

Converting protein turnover into energy estimates demonstrated that the energy cost increased by 1.5 times in the HbSS group, whereas myocardial work doubled as a compensation for anemia (Table 3). Furthermore, MVO_2 showed the strongest association with REE in multivariate analysis adjusted for Q_{equiv} , sex and age (Table 4). These findings suggest that myocardial metabolism makes a significant contribution to the increased REE. Although outside the scope of this study, the ability to directly measure the magnitude of the myocardial energy cost compared with Q_{equiv} would allow the determination of which factor makes the larger quantitative contribution to the REE.

Our results demonstrate significant inverse relationships between Hb concentration and protein turnover, protein synthesis, MVO_2 and reticulocyte count ($P < 0.001$; Fig. 2F–I), emphasizing that most of the hypermetabolism measured in HbSS may result from compensation for the hemolytic anemia. Our data concur with speculation that erythropoiesis and/or anemia may have specific roles in increasing Q and ultimately, REE (3,6). This observation is consistent with the report from Badaloo et al. (30) in which splenectomy normalized both elevated protein turnover and REE in children (HbSS group) with hypersplenism, a condition that further increases the hemolytic rate in children with HbSS. Furthermore, Vaisman et al. (32) reported that blood transfusion corrected anemia and reduced HR, reticulocytes and REE in adults with thalassemia major, a hematologic disorder also characterized by erythropoiesis, hemolysis and anemia. Harmatz et al. (1), however, reported that in 5 adolescents with HbSS, REE was more elevated after blood transfusion, despite an increased Hb concentration and decreased erythropoiesis; their data showed no direct relationship between erythropoiesis and increased REE in HbSS subjects. However, our data demonstrate a strong association of proportional reticulocyte count with

protein turnover ($r = 0.83$, $P < 0.001$) but not with REE ($r = 0.32$, $P = 0.15$), suggesting an indirect relationship of erythropoiesis with REE via protein turnover.

It is likely that other metabolic control factors play a significant role in the abnormally high REE of HbSS subjects. Recent understanding of HbSS pathology includes baseline inflammation, which so far is defined mainly by marked leukocytosis linked with disease severity (33). We have shown that in patients with HbSS, C-reactive protein, a sensitive indicator of inflammation, is an important predictor of REE adjusted for FFM (21). It is therefore possible that consideration of inflammatory factors could further increase the precision of predictive equations for REE in HbSS subjects (14).

There is some limitation in our ability to fully interpret these results. Myocardial oxygen consumption was measured indirectly by using the product of SBP and HR (24) as a standard noninvasive method. Cardiologists have validated the HR-blood pressure product as a fairly reliable predictor of MVO_2 (24,34,35) in health and disease. Although ventricular volume and myocardial contractility are not assessed by this index, these may not be significant contributors in young children with HbSS (36,37). Indeed, using this approach, the difference between the study groups fits the expected patterns proposed by previous investigators (4,36,37). The in vivo measurement for heme synthesis rate is challenging because of the relatively low isotope enrichment that can be achieved within a suitable experimental period. Restricting the isotope infusion time to 12 hours returns an underestimate for heme synthesis rate. However, the relative rates of heme synthesis between groups can be measured accurately. The strong correlation of heme synthesis with reticulocyte percentage ($P < 0.001$), even with oral administration of the [^{15}N]glycine, strengthens the use of this method as an index of relative rates of erythropoiesis.

In summary, these results show that most of the hypermetabolism of children with HbSS is compensation for the hemolytic anemia. The increased protein turnover is directly linked to reticulocytosis, and increased MVO_2 is compensation mainly for degree of anemia. Multivariate analyses suggest that the high REE is determined primarily by energy needs for cardiac compensation and increased protein metabolism. Residual energy for other metabolic work is expected to be significantly lower for the HbSS group compared with controls, consistent with direct evidence of a relative energy shortage (15) that may disproportionately retard the growth and development of children with HbSS (9). Together, these data suggest that reducing hemolysis and erythropoietic protein turnover and increasing Hb concentration to reduce cardiac work may achieve improved growth. Antisickling therapy such as hydroxyurea (38), bone marrow transplant (39) and splenectomy (30) achieve both results and are reported to improve growth in children with HbSS.

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FIG. 1.
Diagram of the inpatient study procedures.

FIG. 2.

Significant correlations observed among the participants. Strong positive associations were observed between Q and reticulocyte count and between REE and MVO₂. Hemoglobin concentration was inversely correlated with Q, protein synthesis, MVO₂ and proportional reticulocyte count. HbAA = controls.

TABLE 1

Age, body composition and hematology for HbAA and HbSS subjects

Characteristics	HbAA (n =9)		HbSS (n=12)		P
	Mean ± SD	Range	Mean ± SD	Range	
Age (yr)	9 ± 2	6–12	9 ± 2	6–12	0.466
FFM (kg)	29 ± 9	20–49	24 ± 6	16–33	0.227
Weight (kg)	35 ± 10	22–57	28 ± 7	19–38	0.177
Height (cm)	139 ± 18	117–174	133 ± 12	113–150	0.522
BMI (kg/m ²)	18 ± 2	16–20	16 ± 1	13–19	0.019
Hb (g/L) [110–174]	124 ± 8	112–137	84 ± 13	66–111	<0.001
HCT (%) [33–52]	37 ± 2	33–40	24 ± 4	18–32	<0.001
RBC (×10 ¹² /L) [3.6–5.8 × 10 ¹²]	4.4 ± 0.3	4.0–4.7	2.8 ± 0.5	2.0–3.7	<0.001
Reticulocyte (%) [0.5–2.0]	0.7 ± 0.3	0.2–1.4	11.5 ± 4.0	3.2–16.4	<0.001

Values within brackets are reference ranges for hematology measurements; P values by Wilcoxon rank sum test.

BMI, Hb, HCT and RBC were significantly lower in the HbSS group, whereas reticulocyte percentage was significantly higher in the HbSS group compared with the HbAA group.

BMI = body mass index; HCT = hematocrit.

TABLE 2

Protein metabolism in HbAA and HbSS subjects

Subject	SynHb (g/kg/d)	Protein synthesis (g/kg/d)	Protein breakdown (g/kg/d)	Q
HbAA (n = 9)	0.01 ± 0.01	4.3 ± 0.7	3.6 ± 0.8	6.3 ± 0.6
Range	0.001–0.03	2.8–5.2	2.4–4.7	5.1–7.1
HbSS (n = 12)	0.2 ± 0.1	7.7 ± 2.0	7.1 ± 1.5	9.6 ± 1.9
Range	0.01–0.39	4.5–12.7	4.4–10.8	6.9–14.5
<i>P</i>	0.0018	0.0004	0.0002	0.0002

Values are mean ± SD; *P* values by Wilcoxon rank sum test.

All protein metabolism measurements were significantly elevated for HbSS subjects compared with HbAA subjects.

TABLE 3

Protein energy cost, myocardial oxygen use and REE in HbAA and HbSS subjects

Subject	Q_{equiv} (kcal/kg/d)	MVO_2 (mmHg · bpm/kg)	REE (kcal/kg FFM/d)
HbAA (n = 9)	13.8 ± 1.3	228 ± 72	43 ± 12
Range	11.2–15.7	98–359	28–71
HbSS (n = 12)	21.2 ± 4.1	431 ± 105	51 ± 6
Range	15.2–31.9	227–582	42–62
<i>P</i>	0.0002	0.0004	0.0069

Values are mean $\pm$ SD; *P* values by Wilcoxon rank sum test.

Q_{equiv} , MVO_2 and REE were significantly elevated in HbSS group compared with HbAA group.

TABLE 4

Partial correlation model results

Test	Partial correlation coefficient	<i>P</i>
Q		
SynHb	0.04	0.87
Retic %	0.55	0.03
Disease status	0.11	0.69
Log REE		
MVO ₂	0.51	0.03
Q _{equiv}	0.02	0.93
Sex	0.38	0.12
Age	-0.33	0.18

The REE data were slightly skewed, requiring log transformation.

Reticulocyte count showed significant association with Q. The MVO₂ maintained significant association with log REE after controlling for Q_{equiv}, sex and age.

Retic % = proportional reticulocyte count.