Protein turnover and energy expenditure increase during exogenous nutrient availability in sickle cell disease[1–3]

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ABSTRACT In the present study, energy expenditure (EE) and rates of whole-body protein, glucose, and lipid metabolism were assessed in 8 African American sickle cell disease (SCD) patients and in 6 healthy African American control subjects during the infusion of amino acids, glucose, and lipid. Whole-body protein, glucose, and lipid kinetics were estimated by using L-[1-^{13}C]leucine, D-[6,6-$^{2}H_2$]glucose, and [$^{2}H_5$]glycerol, respectively. After a 2-h tracer equilibration period and a 0.5-h basal period, nutrients were administered intravenously for 3 h with 16% of the energy as protein, 52% as carbohydrate, and 32% as fat. Breath and blood were collected during the last 30 min of nutrient infusion and EE was measured by indirect calorimetry. EE was 14% greater ($P \leq 0.05$) in SCD patients [145.0 ± 3.5 kJ · kg fat-free mass (FFM)$^{-1}$ · d^{-1}] than in control subjects (126.8 ± 3.8 kJ · kg FFM^{-1} · d^{-1}). Whole-body protein breakdown (4.4 ± 0.4 compared with 3.1 ± 0.1 mg · kg FFM^{-1} · min^{-1}, $P \leq 0.05$) and protein synthesis (4.6 ± 0.4 compared with 3.2 ± 0.1 g · kg FFM^{-1} · min^{-1}, $P \leq 0.05$) were 42% and 44% greater, respectively, in the SCD patients than in control subjects, but whole-body amino acid oxidation (0.90 ± 0.05 compared with 1.03 ± 0.09 mg · kg FFM^{-1} · min^{-1}) was not significantly different between the 2 groups. Whole-body glucose and lipid kinetics did not differ significantly between the groups. EE increased in SCD patients during exogenous nutrient availability, and the additional energy required for the accelerated rates of whole-body protein breakdown and synthesis made a significant contribution to the increase in EE. These metabolic aberrations may increase the dietary energy and protein requirements of SCD patients. *Am J Clin Nutr* 1998;68:607–14.

KEY WORDS Sickle cell disease, sickle cell anemia, energy expenditure, protein metabolism, carbohydrate metabolism, lipolysis, amino acids, nutrient availability, nutrient infusion, African Americans, adults

INTRODUCTION

Sickle cell disease (SCD) is a genetic disorder in which abnormal hemoglobin, known as sickle Hb or Hb S, is produced because of the substitution of valine for glutamic acid in position 6 of the β-chains of hemoglobin (1). The resulting structural change to red blood cells (RBCs) causes them to become sickled in shape and leads to a decrease in RBC life span from ≈120 to 10 d, causing chronic hemolytic anemia (2). Recently, we showed that basal metabolism in adult SCD patients is altered (3). Specifically, we found that rates of resting energy expenditure were accelerated, due, in part, to a dramatic increase in the energy consuming processes of basal whole-body protein breakdown and synthesis. These results support previous findings in children, adolescents, and adults with homozygous SCD (4–8). Because SCD patients have greater rates of resting energy expenditure and basal whole-body protein breakdown and synthesis than do healthy African Americans, the dietary energy and protein requirements of individuals with SCD may be greater. However, information regarding meal-related nutrient utilization in SCD patients is limited.

Although previous studies showed that digestive and absorptive function appear to be relatively normal in homozygous SCD patients (9–11), virtually nothing is known about the effect of SCD on nutrient metabolism when nutrient availability is increased during meal consumption. Thus, the purpose of the present study was to examine the effect of SCD on whole-body protein, carbohydrate, and lipid homeostasis and on energy expenditure during the increased availability of nutrients from an intravenous nutrient infusion. To accomplish this goal, we measured rates of whole-body protein synthesis and breakdown, whole-body glucose utilization and production, whole-body lipolysis, energy expenditure, and whole-body oxidation of individual nutrients (amino acids, carbohydrate, and lipid) during nutrient availability in a group of SCD patients and a group of control subjects with a normal hemoglobin phenotype.

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SUBJECTS AND METHODS

Subject selection

Eight African American SCD patients (6 men and 2 women) aged 18–50 y and within 25% of ideal body weight based on Metropolitan Life Insurance Company tables (12) were identified and screened for participation in the study at the Sickle Cell Clinic of the Comprehensive Sickle Cell Center at Meharry Medical College, Nashville, TN, and at the Vanderbilt University Medical Center, Nashville, TN. In addition, 6 African American subjects (3 men and 3 women) aged 18–50 y of age and within 25% of their ideal body weight and who did not carry the sickle cell gene (Hb S) were selected as control subjects for the study. Each subject's hemoglobin phenotype was determined with standard electrophoretic methods (13) to confirm the presence of *1*) homozygous SCD (Hb SS; $n = 2$), in which both genes coding for the β-chains of hemoglobin produce Hb S; *2*) sickle cell hemoglobin C disease (Hb SC; $n = 3$), in which one gene codes for Hb S and the other for HbC; *3*) sickle cell β thalassemia disease (Hb Sβ thal; $n = 3$), in which one gene codes for Hb S and the other for reduced or no production of normal hemoglobin (HbA); or *4*) normal hemoglobin (HbAA; $n = 6$), in which both genes code for HbA (2). Subjects eligible for participation in the study were provided with an explanation of the study, and informed consent was obtained for procedures to be performed at the Vanderbilt University General Clinical Research Center (GCRC). The procedures were approved by both Vanderbilt University Medical School and Meharry Medical College.

Before participation in the study, subjects underwent a complete physical examination and gave a medical history. None of the control subjects or SCD patients had any apparent hepatic, renal, or metabolic dysfunction. Female subjects were not pregnant, as determined by a pregnancy test, and were premenopausal with regular menstrual cycles. They were studied between 1 and 14 d after the onset of menses (follicular phase) to reduce experimental variability. The SCD patients were studied in the steady state; ie, they were not experiencing a sickle cell crisis during the study nor had they experienced a crisis for ≥96 h before the study.

Body-composition measurements

Each subject's body density was measured by hydrostatic weighing (14), and the percentage body fat was calculated by using an equation specific for African Americans (15). Fat mass was calculated by multiplying body weight and percentage body fat, and fat-free mass (FFM) was calculated by subtracting fat mass from body weight. Body mass index (BMI) was calculated as the ratio of body weight (kg) to height (m) squared.

Experimental protocol

On the day of the metabolic study, subjects reported to the GCRC after a 12-h overnight fast so that they were studied in the postabsorptive state. Indwelling catheters were placed in a superficial hand vein for arterialized blood sampling and in an antecubital vein for the infusion of stable isotopic tracers and nutrients. The catheterized hand was placed in a heated thermoplastic box with the temperature adjusted automatically to 55°C for complete arterialization of venous blood samples (16). In 2 subjects, placement of the blood-sampling catheter in a hand vein was not possible; therefore, blood samples were collected via a Port-A-Cath (Sims Deltec, Inc, St Paul) providing mixed venous blood. In addition, catheter placement in the antecubital vein was not possible in one subject; thus, the external jugular vein was catheterized to allow for tracer and nutrient infusions. At the beginning of the metabolic study, each subject received a bolus infusion of $NaH^{13}CO_3$ (0.12 mg/kg), L-[1-^{13}C]leucine (7.2 μmol/kg), and D-[6,6-$^2H_2$]glucose (3.6 mg/kg) to prime the carbon dioxide, leucine, and glucose pools, respectively. A continuous infusion of the leucine (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and glucose (0.06 $mg \cdot kg^{-1} \cdot min^{-1}$) isotopes as well as of [$^2H_5$]glycerol (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was then started and continued throughout the remainder of the study (17–19). Each study consisted of a 2-h tracer equilibration period, a 0.5-h basal period, and a 3-h nutrient infusion period.

Blood samples were collected every 15 min during the basal period and during the last 30 min of the nutrient infusion period for determination of hormone, metabolite, and amino acid concentrations as well as isotopic enrichments. Simultaneous with the blood sampling, breath samples were collected from each subject with a Douglas bag with duplicate 20-mL samples placed into nonsiliconized evacuated glass tubes for measurement of breath $^{13}CO_2$ enrichment. In addition, steady state carbon dioxide production, oxygen consumption, and resting energy expenditure were measured for ≥30 min during the last hour of nutrient infusion by indirect calorimetry with a Sensormedics 2900 metabolic cart (Yorba Linda, CA). At the end of the study, the catheters were removed and subjects were fed a meal and discharged from the GCRC. Data from the basal period of study were presented elsewhere (3); data from the nutrient infusion period of the study are the focus of this article.

Nutrient infusion

During the nutrient infusion period, the increase in nutrient availability associated with consumption of a meal was simulated via intravenous administration of a nutrient solution containing amino acids (10% Travesol; Baxter Healthcare Corporation, Deerfield, IL), glucose (10% dextrose; Avebe America Inc, Princeton, NJ), and lipid (20% Intralipid; Kabi Pharmacia Inc, Clayton, NC). The nutrient infusion was designed to approximate the composition of a normal diet. The diet provided 16% of energy 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 (20). The exogenous glucose infused was derived from potato starch because it has a lower natural enrichment of ^{13}C than does glucose derived from cornstarch (21). Thus, breath $^{13}CO_2$ production reflected that of amino acid ([^{13}C]leucine) oxidation, with a negligible contribution of $^{13}CO_2$ from glucose oxidation (22). Preliminary hyperinsulinemic-euglycemic clamps conducted in our laboratory confirmed that there is negligible $^{13}CO_2$ produced from the infused potato glucose but that there is significant $^{13}CO_2$ produced from infusion of glucose derived from cornstarch.

The energy level provided by the nutrient infusion during the experimental period was adjusted for each subject based on body weight and sex. The average recommended dietary allowance of energy for a reference man 25–50 y of age weighing 79 kg is 12 122 kJ/d whereas that for a reference woman of the same age range weighing 63 kg is 9196 kJ/d (23). Over 24 h, this equals 505 kJ/h for the reference man and 383 kJ/h for the reference woman. Thus, over a 3-h experimental period, a man weighing 79 kg would receive 1515 kJ in the nutrient infusion and a

woman weighing 63 kg would receive 1149 kJ. On the basis of these reference values, the energy level provided was adjusted accordingly, depending on the weight and sex of the subject. The energy, protein, carbohydrate, and fat contents of the 3-h nutrient infusion were similar between the SCD patients and the control group and are presented in **Table 1**.

TABLE 1
Composition of the 3-h nutrient infusion[1]

	Control group (n = 6)	Sickle cell disease patients (n = 8)
Energy (kJ)	1329 ± 121	1254 ± 100
Protein (g)	12 ± 1	12 ± 1
Carbohydrate (g)	41 ± 4	39 ± 3
Fat (g)	12 ± 1	11 ± 1

[1] $\bar{x}$ ± SEM.

Analytic procedures

Whole-blood hemoglobin concentrations were measured colorimetrically by using the cyanmethemoglobin method (Procedure no. 525; Sigma Chemical Company, St Louis). Blood samples were collected into separate Venoject tubes (Terumo Medical Corporation, Elkton, MD) containing 15 mg Na_2EDTA. A 1-mL aliquot of whole blood was deproteinized with 3 mL 4% perchloric acid for determination of whole-blood glycerol and lactate concentrations by using enzymatic methods (24). In addition, 3 mL blood was transferred to a tube containing EGTA and reduced glutathione with the plasma stored at −80°C for later measurement of plasma epinephrine and norepinephrine concentrations by HPLC (25). The remaining blood was spun in a Beckman Instruments (Fullerton, CA) desktop centrifuge at 2000 × g and 4°C for 10 min, and the plasma was collected. An aliquot of this plasma was analyzed enzymatically for plasma glucose concentrations with a model II glucose analyzer (Beckman Instruments).

The remaining plasma was stored at −80°C for later analysis. Immunoreactive insulin was determined with a double-antibody system (26). Plasma for glucagon analysis was collected in tubes containing 25 kallikrein-inhibitor units of aprotinin (Trasylol; FBA Pharmaceutical, New York) and was analyzed by radioimmunoassay with a double-antibody system modified from the method of Morgan and Lazarow (26) for insulin. Insulin and glucagon antisera and standards as well as [^{125}I]insulin and glucagon were obtained from RL Gingerich (Linco Research, St Louis). Clinical Assays Gammacoat radioimmunoassay kit (Travenol-Gentech, Cambridge, MA) was used to measure plasma cortisol concentrations. Plasma amino acid concentrations were determined by reversed-phase HPLC after derivatization with phenylisothiocyanate (27).

After deproteinization with barium hydroxide and zinc sulfate and elution over cation and anion resins, plasma [$^2H_2$]glucose enrichment was determined by gas chromatography–mass spectrometry (GC-MS) according to the method of Bier et al (17). Plasma enrichments of [^{13}C]leucine and [^{13}C]α-ketoisocaproate (KIC) were determined with GC-MS. Plasma was deproteinized with 4% perchloric acid, and the supernate was passed over a cation exchange resin to separate the keto and amino acids. The keto acids were further extracted with methylene chloride and 0.5 mol NH_4OH/L (28). After being dried under nitrogen gas, both the keto and amino acid fractions were derivatized (29) with *N*-methyl-*N*-(*t*-butyldimethylsilyl)-trifluoroacetamide containing 1% *t*-butyldimethylchlorosilane (MtBSTFA + 1% *t*-BDMCS; Regis Technologies, Inc, Morton Grove, IL). The derivatized samples were then analyzed with GC-MS (5890a GC and 5970 MS; Hewlett-Packard, San Fernando, CA) for plasma leucine and KIC enrichments. For determination of [$^2H_5$]glycerol enrichment, plasma was deproteinized with 4% perchloric acid, and the supernate was passed over cation and anion exchange resins. The eluate was dried overnight at 50°C, the glycerol fraction was derivatized with MtBSTFA + 1% *t*-BDMCS, and the derivatized samples were analyzed by GC-MS for determination of plasma glycerol enrichment. Breath $^{13}CO_2$ enrichment was measured by isotope-ratio MS (Metabolic Solutions, Merrimack, NH; 30).

Calculations

Regression of the isotopic variables of interest with time during the last 30 min of the nutrient infusion period indicated that the slopes were not significantly different from zero, thereby showing that a metabolic steady state was achieved. Thus, isotopic steady state values are reported as the mean of all 3 time points. Steady state rates of whole-body glucose disappearance (R_d) were calculated by dividing the [$^2H_2$]glucose infusion rate by plasma [$^2H_2$]glucose enrichment (19, 31). Rates of endogenous glucose production were calculated by subtracting the rate of exogenous glucose infusion from the total glucose R_d. Rates of whole-body nonoxidative glucose utilization were calculated by subtracting whole-body carbohydrate oxidation from whole-body glucose R_d. The steady state rates of whole body leucine appear (R_a; an estimate of whole-body protein breakdown) were calculated by dividing the [^{13}C]leucine infusion rate by the plasma [^{13}C]KIC enrichment (19, 31). Plasma KIC provides a better estimate of intracellular leucine enrichment than does plasma leucine enrichment because KIC is derived only from intracellular leucine metabolism (32). Endogenous leucine R_a was calculated by subtracting the rate of exogenous leucine infusion from the total leucine R_a. Breath $^{13}CO_2$ production was determined by multiplying the total carbon dioxide production rate by breath $^{13}CO_2$ enrichment (19). The rate of whole-body leucine oxidation was calculated by dividing breath $^{13}CO_2$ production by 0.8 (correction factor for the retention of $^{13}CO_2$ in the bicarbonate pool; 33) and by the plasma KIC enrichment. Nonoxidative leucine R_d (an estimate of whole-body protein synthesis) was determined indirectly by subtracting leucine oxidation from the endogenous leucine R_a. Rates of whole-body protein breakdown, amino acid oxidation, and protein synthesis were calculated from the leucine R_a, the leucine oxidation rate, and the nonoxidative leucine R_d, respectively, assuming that 7.8% of whole-body protein is composed of leucine (34). Because glycerol released during lipolysis cannot be reincorporated into triacylglycerol in the adipose cell because of the lack of glycerol kinase activity, the R_a of endogenous glycerol multiplied by 3 was used to determine rates of whole-body lipolysis (35). The endogenous glycerol R_a was calculated by dividing the [$^2H_5$]glycerol infusion rate by the plasma glycerol enrichment and subtracting the exogenous glycerol infusion rate (19, 31).

Rates of whole-body amino acid, carbohydrate, and lipid oxidation were determined from indirect calorimetry in combination with the leucine oxidation data. The energy expended due to amino acid oxidation was subtracted from the total energy expenditure, and the net rates of carbohydrate and lipid oxidation were calculated based on the nonprotein respiratory quotient (36). The assumptions and limitations of calculating net sub-

strate oxidation based on indirect calorimetry measurements were reviewed previously (36). Net whole-body nutrient balances were calculated by subtracting the rates of whole-body nutrient oxidation from the rates of nutrient infusion. Nutrient energy stored was calculated by subtracting the energy expended during nutrient infusion from the nutrient energy infused. Nutrient-induced thermogenesis was calculated as the increase in energy expenditure during nutrient availability above resting energy expenditure. Energetic efficiency was defined as the nutrient energy stored during nutrient infusion expressed as a percentage of nutrient energy infused.

Statistical analysis

Comparisons between the SCD and control groups were made by using unpaired *t* tests, with differences significant at $P \leq 0.05$. Results are presented as means ± SEMs for each group.

RESULTS

Subject characteristics

The SCD ($n = 8$) and control ($n = 6$) groups did not differ significantly in age (30 ± 3 compared with 26 ± 2 y), weight (68.3 ± 5.2 compared with 72.8 ± 5.8 kg), or height (1.74 ± 0.04 compared with 1.76 ± 0.04 m), respectively. In addition, BMI (22.4 ± 1.2 compared with 23.4 ± 1.4), FFM (53.5 ± 4.1 compared with 56.1 ± 3.1 kg), fat mass (14.8 ± 2.6 compared with 16.6 ± 4.0 kg), and percentage body fat (21.2 ± 3.1 compared with 21.7 ± 4.1%) were not significantly different between the SCD and control groups, respectively. As expected, the hemoglobin concentration of the SCD group (99 ± 10 g/L) was significantly lower ($P \leq 0.05$) than that of the control group (137 ± 7 g/L).

SCD is a collective term for the various hemoglobin phenotypes, including Hb SS, Hb SC, and Hb Sβ thal, in which Hb S is produced, leading to the occurrence of sickled RBCs, hemolytic anemia, and vasoocclusive events (2). In the present study, mean hemoglobin concentrations were not significantly different between the 3 hemoglobin phenotypes. In addition, when compared with the control group, the responses of all 3 hemoglobin phenotypes in the SCD group were not significantly different; thus, they were pooled and reported as one mean.

Plasma hormone concentrations

The responses of plasma insulin, cortisol, epinephrine, and norepinephrine concentrations to nutrient infusion did not differ significantly between the SCD and control groups (**Table 2**). In contrast, plasma glucagon concentrations were 28% greater ($P \leq 0.05$) in the SCD patients than in the control subjects.

Plasma amino acid concentrations

The total plasma concentration of amino acids during nutrient infusion was not significantly different between the SCD patients and the control subjects (**Table 3**). Plasma 3-methylhistidine concentrations were 90% greater ($P \leq 0.05$) and plasma histidine, phenylalanine, and tyrosine concentrations were 26%, 25%, and 23% lower ($P \leq 0.05$), respectively, in the SCD patients than in the control subjects during nutrient infusion. In addition, there was a trend for plasma valine concentrations to be 25% lower ($P = 0.0514$) in the SCD patients than in the control subjects. In contrast, plasma concentrations of other individual amino acids and of total branched-chain, total gluconeogenic, and total nonessential amino acids were not significantly different between the SCD patients and the control subjects. However, there was a trend for the plasma concentration of total essential amino acids during nutrient infusion to be 20% lower

TABLE 2
Hormone and metabolite concentrations[1]

	Control group ($n = 6$)	Sickle cell disease patients ($n = 8$)
Plasma insulin (pmol/L)	292 ± 95	192 ± 37
Plasma glucagon (ng/L)	47 ± 3	60 ± 5[2]
Plasma cortisol (nmol/L)	214 ± 30	258 ± 30
Plasma epinephrine (pmol/L)	185 ± 47	147 ± 27
Plasma norepinephrine (nmol/L)	0.79 ± 0.11	0.81 ± 0.11
Plasma glucose (mmol/L)	7.0 ± 0.4	6.4 ± 0.2
Whole-blood lactate (mmol/L)	0.7 ± 0.1	0.7 ± 0.2
Whole-blood glycerol (mmol/L)	0.08 ± 0.01	0.07 ± 0.01

[1] $\bar{x}$ ± SEM.
[2] Significantly different from control group, $P \leq 0.05$.

TABLE 3
Plasma amino acid concentrations[1]

	Control group ($n = 6$)	Sickle cell disease patients ($n = 8$)
1-Methylhistidine	9.1 ± 3.2	4.6 ± 2.1
3-Methylhistidine	4.6 ± 0.5	8.7 ± 1.0[2]
Alanine	458.2 ± 29.2	370.6 ± 40.0
Arginine	162.3 ± 15.5	117.2 ± 14.3
Asparagine	67.6 ± 6.2	53.2 ± 4.2
Aspartate	5.0 ± 0.7	9.5 ± 3.1
Citrulline	30.5 ± 1.9	29.0 ± 2.0
Glutamate	73.2 ± 8.2	88.9 ± 13.8
Glycine	361.8 ± 17.8	333.7 ± 37.3
Histidine	88.5 ± 8.4	65.7 ± 6.1[2]
Hydroxyproline	12.3 ± 4.5	11.1 ± 2.0
Isoleucine	89.8 ± 8.2	74.6 ± 7.6
Leucine	167.2 ± 17.2	142.4 ± 13.4
Lysine	201.7 ± 24.4	165.4 ± 16.1
Methionine	55.0 ± 5.1	43.4 ± 3.3
Ornithine	59.2 ± 5.6	71.0 ± 5.2
Phenylalanine	96.1 ± 8.7	71.7 ± 4.1[2]
Proline	259.2 ± 28.4	285.8 ± 26.2
Serine	148.3 ± 7.4	136.9 ± 16.9
Taurine	86.5 ± 7.7	76.9 ± 13.6
Threonine	152.1 ± 15.3	138.8 ± 16.8
Tryptophan	52.3 ± 3.4	45.2 ± 3.2
Tyrosine	52.2 ± 5.2	39.9 ± 2.4[2]
Valine	237.7 ± 24.0	177.3 ± 16.2
Branched-chain AAs[3]	494.7 ± 48.4	394.3 ± 34.1
Gluconeogenic AAs[4]	1120.5 ± 60.6	980.1 ± 73.9
Essential AAs[5]	1302.6 ± 98.1	1041.6 ± 74.5
Nonessential AAs[6]	1627.8 ± 52.8	1519.9 ± 97.4
Total AAs[7]	2930.4 ± 129.3	2561.5 ± 149.5

[1] $\bar{x}$ ± SEM; AAs, amino acids.
[2] Significantly different from control group, $P \leq 0.05$.
[3] The sum of leucine, isoleucine, and valine.
[4] The sum of alanine, glycine, serine, and threonine.
[5] The sum of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.
[6] The difference between total AAs and essential AAs.
[7] The sum of all AAs.

(P = 0.0521) in the SCD patients than in the control subjects.

Energy metabolism

During nutrient infusion, energy expenditure normalized to FFM was 14% greater in the SCD patients than in the control subjects (145.0 ± 3.5 compared with 126.8 ± 3.8 kJ·kg $FFM^{-1}\cdot d^{-1}$, $P \leq 0.05$; **Figure 1**, top). There was a trend for energetic efficiency (P = 0.0723) to be 26% lower in the SCD patients (25.1 ± 3.0%) than in the control subjects (34.1 ± 3.3%). The percentage of energy expenditure derived from carbohydrate (41 ± 6% compared with 36 ± 8%), protein (16 ± 1% compared with 20 ± 1%), and fat (43 ± 6% compared with 44 ± 8%) was not significantly different between the SCD and control groups, respectively.

Whole-body protein metabolism

Rates of whole-body protein breakdown (4.4 ± 0.4 compared with 3.1 ± 0.1 mg·kg $FFM^{-1}\cdot min^{-1}$, $P \leq 0.05$) and synthesis (4.6 ± 0.4 compared with 3.2 ± 0.1 mg·kg $FFM^{-1}\cdot min^{-1}$, $P \leq 0.05$) were 42% and 44% greater, respectively, in the SCD patients than in the control subjects during nutrient infusion (**Figure 2**). However, rates of whole-body amino acid oxidation did not differ significantly between the SCD (0.90 ± 0.05 mg·kg $FFM^{-1}\cdot min^{-1}$) and control (1.03 ± 0.09 mg·kg $FFM^{-1}\cdot min^{-1}$) groups. The contribution of leucine oxidation to total leucine R_d was significantly lower ($P \leq 0.05$) in the SCD patients (17 ± 1%) than in the control subjects (24 ± 1%). Net whole-body amino acid balance, calculated as the difference between amino acids infused and oxidized, was not significantly different between the SCD (0.31 ± 0.05 mg·kg $FFM^{21}\cdot min^{21}$) and control (0.19 ± 0.06 mg·kg $FFM^{21}\cdot min^{21}$) groups. Furthermore, net whole-body protein storage, calculated as the difference between whole-body protein breakdown and synthesis, did not differ significantly between the SCD (0.23 ± 0.05 mg·kg $FFM^{21}\cdot min^{21}$) and control (0.11 ± 0.06 mg·kg $FFM^{-1}\cdot min^{-1}$) groups. In contrast, whole-body protein synthesis, expressed as a percentage of amino acids infused, was significantly greater ($P \leq 0.05$) in the SCD patients (380 ± 32%) than in the control subjects (266 ± 16%).

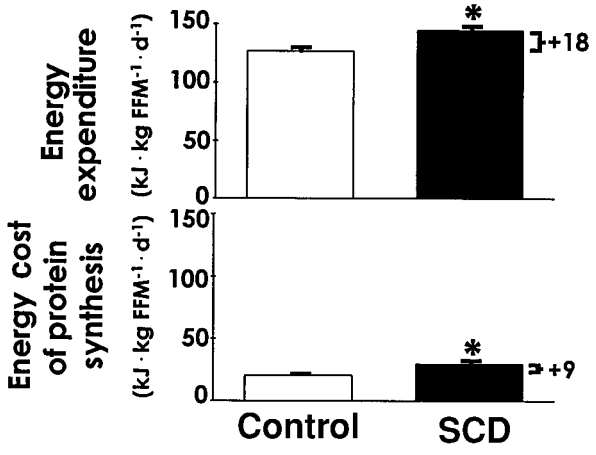

FIGURE 1. Energy expenditure during nutrient availability and the estimated energy cost of protein synthesis during nutrient availability for the 6 control subjects and the 8 sickle cell disease (SCD) patients. *Significantly different from the control group, $P \leq 0.05$. The greater energy cost of protein synthesis in the SCD patients [9 kJ·kg fat-free mass $(FFM)^{-1}\cdot d^{-1}$ above the control mean] accounted for ≈50% of their enhanced resting energy expenditure (18 kJ·kg $FFM^{-1}\cdot d^{-1}$ above the control mean).

Whole-body glucose metabolism

During nutrient infusion, plasma glucose and whole-blood lactate concentrations were not significantly different between the SCD and control groups (Table 2). Likewise, rates of whole-body glucose utilization (3.6 ± 0.2 compared with 3.6 ± 0.1 mg·kg body $wt^{-1}\cdot min^{-1}$), endogenous glucose production (0.5 ± 0.2 compared with 0.4 ± 0.1 mg·kg body $wt^{-1}\cdot min^{-1}$), and nonoxidative glucose utilization (1.8 ± 0.3 compared with 2.3 ± 0.3 mg·kg body $wt^{-1}\cdot min^{-1}$) did not differ significantly between the SCD and control groups, respectively. In addition, whole-body carbohydrate oxidation (1.8 ± 0.3 compared with 1.5 ± 0.3 mg·kg body $wt^{-1}\cdot min^{-1}$) and net whole-body carbohydrate retention (1.6 ± 0.3 compared with 1.9 ± 0.4 mg·kg body $wt^{-1}\cdot min^{-1}$) were not significantly different between the SCD and control groups, respectively.

Whole-body lipid metabolism

Whole-blood glycerol concentrations were not significantly different between the SCD patients and the control subjects during nutrient infusion (Table 2). Similarly, rates of whole-body lipolysis did not differ significantly between the SCD patients (23.6 ± 6.0 μmol·kg $fat^{-1}\cdot min^{-1}$) and the control subjects (21.0 ± 8.3 μmol·kg $fat^{-1}\cdot min^{-1}$). In addition, whole-body lipid oxidation (4.6 ± 1.0 compared with 4.2 ± 0.8 mg·kg $fat^{-1}\cdot min^{-1}$) and net lipid balance (0.12 ± 0.79 compared with 0.92 ± 1.06 mg·kg $fat^{-1}\cdot min^{-1}$) were not significantly different between the SCD and control groups, respectively.

DISCUSSION

The objective of this research was to examine the effect of SCD on meal-related nutrient and energy metabolism in a comprehensive manner by identifying the consequences of SCD on whole-body protein, carbohydrate, and lipid homeostasis as well as energy expenditure during intravenous nutrient availability. We found that SCD did not alter whole-body glucose and lipid

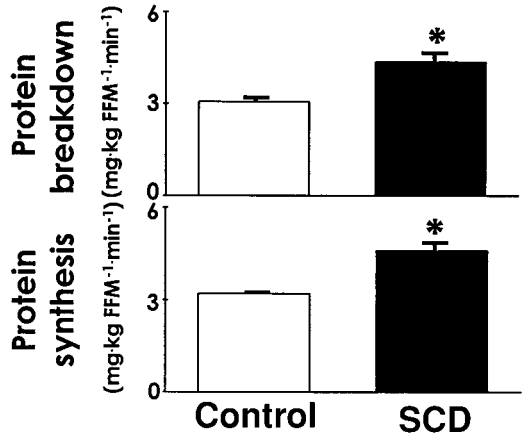

FIGURE 2. Rates of whole-body protein breakdown and protein synthesis during nutrient availability for the 6 control subjects and the 8 sickle cell disease (SCD) patients. *Significantly different from the control group, $P \leq 0.05$. FFM, fat-free mass.

metabolism during intravenous infusion of a nutrient solution. In contrast, energy expenditure increased by 14% and rates of whole-body protein breakdown and protein synthesis were 42% and 44% greater, respectively, in the SCD patients than in the control subjects during nutrient infusion. Thus, SCD creates aberrations in energy and protein homeostasis during exogenous nutrient availability such that dietary energy and protein requirements of SCD patients may be increased.

Previously, we (3) and others (4–8) showed clearly that basal rates of whole-body protein breakdown and synthesis as well as resting energy expenditure are higher in African American children, adolescents, and adults with SCD than in healthy African Americans. The present study extends these findings by showing that the rates of these processes are also significantly increased during exogenous nutrient availability in SCD patients. The enhanced energy expenditure of the SCD patients during nutrient infusion suggests an impaired efficiency of energy utilization. Indeed, there was a trend for energetic efficiency to be 26% lower in the SCD patients than in the control subjects.

Although the mechanisms responsible for the greater energy expenditure of SCD patients than of control subjects during nutrient infusion cannot be completely determined from the present study, several potential mechanisms can be hypothesized. For example, the increased rates of whole-body protein breakdown and synthesis, which are energy-consuming processes (37), likely contributed to the increased energy expenditure of the SCD patients during nutrient availability. In support of this hypothesis, the estimated energy cost of protein synthesis can be calculated based on the fact that the synthesis of each peptide bond requires the energy equivalent of ≈5 molecules of ATP (37) and that the energy cost of protein synthesis has been estimated to be 4.5 kJ/g protein (38). Thus, in the present study, the estimated energy cost of protein synthesis during nutrient availability was significantly greater ($P \leq 0.05$) in the SCD patients (29.7 ± 2.4 kJ · kg $FFM^{-1} \cdot d^{-1}$) than in the control subjects (20.6 ± 0.5 kJ · kg $FFM^{-1} \cdot d^{-1}$; Figure 1, bottom). These estimates of the energy cost of protein synthesis accounted for 20.4% and 16.3% of total energy expenditure in the SCD patients and the control subjects, respectively ($P \leq 0.05$). Furthermore, ≈50% of the observed increase in energy expenditure (18 kJ · kg $FFM^{-1} \cdot d^{-1}$) in the SCD patients could be accounted for by the enhanced energy cost of protein synthesis (9 kJ · kg $FFM^{-1} \cdot d^{-1}$; Figure 1). Thus, during exogenous nutrient availability, the energy required for the greater rates of whole-body protein breakdown and synthesis makes a significant contribution to the increased energy expenditure of the SCD patients.

Because the rates of both energy expenditure and protein turnover are accelerated with SCD, the fate of the amino acids made available during nutrient infusion must be considered. The ratio of whole-body protein synthesis to exogenous amino acids infused was significantly greater in the SCD patients than in the control subjects. In addition, the proportion of amino acids used for protein synthesis compared with that for oxidative processes was significantly increased in the SCD patients. Thus, the infused amino acids were not directed to meet the additional energy needs of the SCD patients but, rather, were diverted to support the accelerated rates of whole-body protein synthesis. These findings suggest that SCD patients may have a metabolic adaptation by which exogenous amino acids are used to preserve body protein stores.

Energy expenditure was ≈14–15% greater in the SCD patients than in the control subjects, both basally (3) and during exogenous nutrient availability. However, when the increase in energy expenditure above resting due to nutrient infusion was calculated, it was not significantly different between the 2 groups: 16.8 ± 2.8 compared with 16.0 ± 4.7 kJ · kg $FFM^{-1} \cdot d^{-1}$ for the SCD and control groups, respectively. Likewise, the decrease in whole-body protein breakdown and the increase in whole-body protein synthesis above basal values due to exogenous nutrient availability did not differ significantly between the SCD patients (breakdown: 0.64 ± 0.25 mg · kg $FFM^{-1} \cdot min^{-1}$; synthesis: 0.17 ± 0.25 mg · kg $FFM^{-1} \cdot min^{-1}$) and the control group (breakdown: 0.77 ± 0.13 mg · kg $FFM^{-1} \cdot min^{-1}$; synthesis: 0.00 ± 0.15 mg · kg $FFM^{-1} \cdot min^{-1}$). Thus, because nutrient-induced changes in thermogenesis and in whole-body protein turnover were not significantly different between the 2 groups, we speculated that the greater energy expenditure of the SCD patients during nutrient availability was due to the effect of the disease on basal metabolic processes rather than to defects in assimilating the infused nutrients. Furthermore, the addition of exogenous nutrients did not ameliorate the aberrations in basal metabolism.

The findings from the present study indicate that whole-body glucose and lipid metabolism were not dramatically altered by SCD during exogenous nutrient availability, a finding similar to our observations under basal conditions (3). Thus, rates of whole-body glucose utilization, endogenous glucose production, nonoxidative glucose utilization (glycogen storage and lactate production), and lipolysis during nutrient availability did not differ significantly between the SCD patients and the control subjects. Furthermore, rates of whole-body carbohydrate and lipid oxidation and net balance were not significantly different between the groups. During nutrient infusion, the increase in whole-body glucose utilization above basal values was not significantly different between the groups (1.2 ± 0.1 compared with 1.2 ± 0.1 mg · kg $FFM^{-1} \cdot min^{-1}$ for the SCD and control groups, respectively; $P > 0.1$), as was the decrease below basal values in whole-body lipolysis (12.6 ± 6.6 compared with 2.4 ± 5.0 μmol · kg $fat^{-1} \cdot min^{-1}$ for the SCD and control groups, respectively). Finally, SCD did not appear to affect the net deposition of the infused carbohydrate and lipid. Thus, SCD appears to have limited, if any, effects on whole-body carbohydrate and lipid metabolism during intravenous nutrient availability.

The requirements for specific individual amino acids during SCD have not been established. Although we and others have observed differences in the plasma concentrations of some individual amino acids between SCD patients and control subjects under basal conditions (3, 6, 39), nothing is known about the effect of SCD on plasma amino acid concentrations during nutrient availability. The total plasma concentration of amino acids during exogenous nutrient infusion was not significantly different between the SCD patients and the control subjects. In contrast, there was a trend for plasma total essential amino acid concentrations to be lower in the SCD patients. Plasma concentrations of individual amino acids during nutrient availability did not differ significantly between the SCD and control groups, except for greater 3-methylhistidine concentrations in the SCD group, potentially reflecting increased skeletal muscle proteolysis (40), and lower concentrations of histidine, phenylalanine, and tyrosine in the SCD patients. Thus, on the basis of the plasma amino acid profile, the ability of the SCD patients to handle an intravenous amino acid load did not appear to be

affected greatly.

Information regarding the hormonal milieu in patients with SCD is limited to the basal state. Thus, the present study provides insight regarding the effect of SCD on the hormonal response to intravenous nutrient availability. Plasma concentrations of insulin, cortisol, norepinephrine, and epinephrine during nutrient availability did not differ significantly between the SCD patients and the control subjects. Thus, circulating plasma concentrations of these hormones did not appear to play an important role in the metabolic profile observed in our SCD patients during intravenous nutrient availability. In contrast, plasma glucagon concentrations were 28% greater in the SCD patients than in the control subjects. However, it is unlikely that the greater rates of energy expenditure and whole-body protein turnover observed in the SCD patients was explained by the greater plasma glucagon concentrations. We showed previously that increased plasma glucagon concentrations do not promote whole-body protein breakdown, protein synthesis, amino acid oxidation, or energy expenditure but, rather, enhance hepatic amino acid uptake (41).

One of the uncertainties associated with the study of SCD is the role of hemoglobin concentrations in the regulation of metabolic events. Previous studies showed alterations in insulin-mediated glucose metabolism in iron-deficient anemic rats (42, 43). For the SCD patients in the present study, there were no significant correlations between hemoglobin concentrations and the variables of whole-body glucose utilization, lipolysis, amino acid oxidation, carbohydrate oxidation, and lipid oxidation ($r = -0.29$, 0.08, 0.34, 0.17, and -0.52, respectively; $P > 0.05$). More data are required, however, to conclusively show that such a relation does not exist, particularly for lipid oxidation. It is interesting to note that there were significant inverse relations between hemoglobin concentrations and the variables of whole-body protein breakdown, protein synthesis, and energy expenditure ($r = -0.68$, -0.76, and -0.79, respectively; $P \leq 0.05$). Hence, these data provide a basis to speculate that circulating hemoglobin concentrations may contribute to the mechanisms responsible for accelerated protein breakdown and synthesis during SCD. However, this speculation is limited because of the small number of SCD patients ($n = 8$) studied and by the fact that there are no previously published data to either support or refute this hypothesis. Thus, specifying the role of circulating hemoglobin in the regulation of whole-body protein turnover requires further investigation.

In conclusion, data from the present study clearly showed that energy expenditure during nutrient infusion increased with SCD. Furthermore, whereas whole-body glucose and lipid metabolism remain unaltered, rates of whole-body protein breakdown and protein synthesis are significantly increased in SCD patients, thereby suggesting that the additional energy required for the greater rates of whole-body protein breakdown and synthesis makes a significant contribution to the increase in energy expenditure. In addition, our data suggest that the greater energy expenditure of the SCD patients during exogenous nutrient availability was due to the effect of the disease on basal metabolic processes rather than to defects in assimilating the infused nutrients. Thus, SCD creates aberrations in energy expenditure and protein turnover during nutrient availability such that the dietary energy and protein requirements of SCD patients may be increased. Further research is required to quantify the dietary energy and protein needs of SCD patients so that optimal nutritional regimens can be designed with the goal of improving the health of these patients.

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