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Glucose Homeostasis in Children with Falciparum Malaria: Precursor Supply Limits Gluconeogenesis and Glucose Production*

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ABSTRACT

To evaluate glucose kinetics in children with falciparum malaria, basal glucose production and gluconeogenesis and an estimate of the flux of the gluconeogenic precursors were measured in Kenyan children with uncomplicated falciparum malaria before ($n = 11$) and during infusion of alanine (1.5 mg/kg·min; $n = 6$). Glucose production was measured by [6,6- $^2\text{H}_2$]glucose, gluconeogenesis by mass isotope-pomer distribution analysis of glucose labeled by [2- ^{13}C]glycerol.

Basal plasma glucose concentration ranged from 2.1–5.5 mmol/L, and basal glucose production ranged from 3.3–7.3 mg/kg·min. Glucose production was largely derived from gluconeogenesis ($73 \pm 4\%$; range,

52–93%). During alanine infusion, plasma glucose increased by 0.4 mmol/L ($P = 0.03$), glucose production increased by 0.8 mg/kg·min ($P = 0.02$), and gluconeogenesis increased by 0.8 mg/kg·min ($P = 0.04$).

We conclude that glucose production in children with uncomplicated falciparum malaria is largely dependent on gluconeogenesis. However, gluconeogenesis is potentially limited by insufficient precursor supply. These data indicate that in children with falciparum malaria, gluconeogenesis fails to compensate in the presence of decreased glycogen flux to glucose, increasing the risk of hypoglycemia. (*J Clin Endocrinol Metab* 82: 2514–1521, 1997)

HYPOGLYCEMIA is a common complication in acute falciparum malaria, associated with a high morbidity and mortality (1–3). Although hypoglycemia is caused by an imbalance between the production and disposal of glucose, the pathophysiology of hypoglycemia in malaria is incompletely understood. In adults with complicated malaria and in pregnant patients with uncomplicated malaria (both with plasma glucose concentrations >3 mmol/L), average glucose production was increased during acute falciparum malaria compared to that during convalescence (4, 5). There are no data available on glucose production during falciparum malaria in children, although globally, children are by far the biggest at risk group for the development of hypoglycemia (1, 3).

Postabsorptive glucose production consists of two components: gluconeogenesis and glycogenolysis. Methods used in the past to measure gluconeogenesis with different tracers (6, 7) have required assumptions about the enrichment of the triose phosphate pool, the true precursor pool for gluconeogenesis. Recently, a new method was developed that gives

an accurate estimation of the triose phosphate pool enrichment using [2- ^{13}C]glycerol (8) and thus allows the measurement of gluconeogenesis and its regulation in more detail.

The first objective of this study, therefore, was to measure gluconeogenesis, basal glucose production, and flux of gluconeogenic precursors into the triose phosphate pool in children with acute falciparum malaria by infusion of two stable isotopes: [6,6- $^2\text{H}_2$]glucose and [2- ^{13}C]glycerol. The second objective of this study was to evaluate the effect of an extra precursor supply on glucose production, gluconeogenesis, and precursor flux into the triose phosphate pool by infusion of alanine (1.5 mg/kg·min) in these children.

Subjects and Methods

Patients

All pediatric patients admitted to Kilifi District Hospital with a primary diagnosis of malaria during the study period of 6 weeks were considered for inclusion in the study. Inclusion criteria were acute falciparum malaria, age between 2–10 yr, and a postabsorptive period of at least 4 h. Exclusion criteria were admission plasma glucose concentration below 2.2 mmol/L, complicated malaria according to WHO criteria (9) (because clinical practice dictates constant glucose infusion in all of these patients), treatment with quinine [quinine stimulates insulin secretion by the pancreas (10)], concomitant infectious disease, severe malnutrition, and severe chronic diarrhea [this may induce hypoglycemia in childhood (11)]. Fully informed consent was obtained from the accompanying parent or guardian. This study was approved by the Kenya National ethical committee. Performance of iv infusion studies in healthy Kenyan children (as controls) or repeat studies after full recovery were specifically prohibited by the ethical committee.

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Study design

Patients were recruited immediately after laboratory confirmation of the clinical diagnosis and after exclusion of quinine use by a quinine dipstick test (12). Each patient was weighed and treated with pyrimethamine/sulfadoxine (Fansidar, Roche, Basel, Switzerland), in some cases combined with chloroquine. An iv cannula was introduced in a forearm vein for stable isotope infusion. A second cannula for blood sampling was introduced into a suitable vein of the contralateral arm. The catheters were kept patent by a slow saline drip.

The study design is shown in Fig. 1. After obtaining a baseline blood sample in all 11 patients for determination of background (natural) isotope abundance, plasma glucose, plasma cytokine, and basal hematological and biochemical tests, a primed (13.5 mg/kg), continuous (0.15 mg/kg-min) infusion of [6,6-²H₂]glucose (>99% enriched; Isotec, Miamisburg OH) dissolved in sterile isotonic saline and sterilized by passage of the solution through a Millipore filter (0.2 µm; Minisart, Sartorius, Gottingen, Germany) was administered by a motor-driven calibrated syringe pump (Perfusor Secura FT, Braun, Melsungen, Germany). The actual concentration of [6,6-²H₂]glucose was measured in the infusate. The time at the start of the infusion was set as time zero.

After a 90-min equilibration period of [6,6-²H₂]glucose infusion, 3 blood samples were collected at intervals of 15 min in 6 of the 11 patients for determination of plasma glucose concentration and [6,6-²H₂]glucose enrichment. In all 11 patients at 120 min, a primed (35 mg/kg), continuous (0.58 mg/kg-min) infusion of [2-¹³C]glycerol (>99% enriched; Isotec) dissolved in sterile isotonic saline was started concurrently. Starting at 330 min, after 210 min of [2-¹³C]glycerol infusion, 3 blood samples were collected at intervals of 15 min for determination of glucose concentration, [6,6-²H₂]glucose enrichment, and [¹³C]glucose and [¹³C]glycerol enrichments in plasma. Blood samples for measurement of plasma concentrations of alanine, lactate, insulin, and counterregulatory hormones were also collected at 360 min.

At 360 min, infusion of alanine at a rate of 1.5 mg/kg-min was started in seven patients, administered by a calibrated infusion pump during continuation of the [6,6-²H₂]glucose infusion. In addition, in five of these seven patients, [2-¹³C]glycerol was continued. After 90 min of alanine infusion, three blood samples were collected at intervals of 15 min for determination of glucose concentration and glucose and glycerol enrichments. At the end of the study, another blood sample was obtained for measurement of plasma alanine and lactate concentrations.

Blood samples for measurements of plasma glucose, glucose and glycerol enrichments, insulin, counterregulatory hormones, and cytokines were collected in prechilled heparinized tubes, and samples for alanine and lactate determinations were collected into fluoride tubes. All samples were kept on ice and centrifuged promptly. Aliquots of separated plasma were stored at less than -20 °C and were transported on dry ice before assay.

Assays

All measurements were performed in duplicate, and all samples from each subject were analyzed in the same run. The plasma glucose concentration was measured using the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Mijdrecht, The Netherlands).

Gas chromatography/mass spectrometry analyses were performed with an HP model 5971 instrument (Hewlett-Packard, Palo Alto, CA).

FIG. 1. Study design.

After isolation, glucose samples were divided into two portions. The first was oxidized to saccharic acid and then derivatized to tetra-acetyl dimethyl saccharate by a modification of the method of Mehlretter (13). Thirty-five microliters of concentrated nitric acid and 25 µL sodium nitrite catalyst (0.5 g/mL) were added to lyophilized glucose, loosely capped, and heated (to allow gases to escape) for 1 h at 60 °C. The samples were lyophilized. Lyophilized samples were resuspended in 400 µL 0.5 N methanolic HCl, capped tightly, and heated at 80 °C for 2 h. Samples were then dried under nitrogen, and 400 µL freshly prepared acetic anhydride-pyridine (2:1) were added. Samples were capped and heated at 60 °C, dried under nitrogen, and extracted twice with 200 µL ethyl acetate. Next, samples were filtered through glass wool in a Pasteur pipette, dried with nitrogen, resuspended with 100 µL ethyl acetate, and placed in a gas chromatography vial for analysis. The second portion of the glucose sample was converted to glucose penta-acetate with acetic anhydride in pyridine. Mass spectral analysis for both derivatives employed chemical ionization with methane. Selected ion monitoring of the [m-OAc]⁺ fragment was used to observe ions m/z 347–349 for tetra-acetyl dimethyl saccharate and m/z 331–333 for glucose penta-acetate. The triacetyl derivative of glycerol was analyzed by chemical ionization with methane gas (m/z 159–160) (8).

We followed the analytic and experimental design guidelines that were previously proposed (8) for optimizing mass spectrometric accuracy and reliability of gluconeogenesis estimates. In brief, these include 1) frequent testing of natural abundance (baseline) samples as an index of instrument accuracy for each mass isotopomer (8, 14); 2) requiring that baseline fractional abundances for all mass isotopomers be within 0.0050 (0.50 MPE) of theoretical values for instrument performance to be considered acceptable (8, 14); 3) rejection of data if enrichments for any mass isotopomer were less than 0.0050 (0.50 mol percent excess), as being below the detection limit for reliable quantitation (8, 15); 4) preinjection of samples to establish concentrations present and reinjection to maintain ion abundances within a constant range for the baseline and all samples analyzed to avoid concentration effects on isotope ratios (15–17); and 5) administration of [¹³C]glycerol at doses necessary to maintain the triose phosphate pool enrichment (p) in the optimal range (8) between 0.10–0.20 (too low p results in inadequate M₊₂ enrichments, and too high p introduces a potential substrate effect on metabolism). When these analytic criteria are adhered to, reproducible isotopomer enrichments and appropriate values of gluconeogenesis are achieved (see below) (8, 15).

The plasma alanine concentration was determined by amino acid analyzer (Chromocon 500, Teqimenta AG, Rotkreuz, Switzerland), and plasma lactate was determined by enzymatic methods (Boehringer Mannheim, Almere, The Netherlands) on a Cobas Bio Centrifugal analyzer. The plasma insulin concentration was measured by commercial RIA (Pharmacia Diagnostics, Uppsala, Sweden), glucagon was determined by RIA (Daiichi Radioisotope Laboratories, Tokyo, Japan; glucagon antiserum elicited in guinea pigs against pancreatic specific glucagon; cross-reactivity with glucagon-like substances of intestinal origin, <1%), catecholamines were determined by high performance liquid chromatography and electrochemical detection after purification on Biorex 70 (Biorad Laboratories, Hercules, CA) and concentration by solvent extraction (18), and cortisol was measured by fluorescence polarization immunoassay on TDx (Abbott Laboratories, North Chicago, IL). For cytokine assays, tumor necrosis factor-α (TNFα) concentrations were measured by enzyme amplified sensitivity immunoassay (EASIA) (Medgenix, Amersfoort, The Netherlands) with a detection limit of 5 pg/mL. Soluble TNF receptor types I and II were measured by EASIA (Medgenix), with detection limits of 0.1 and 0.5 ng/mL, respectively. Plasma concentrations of interleukin-1 (IL-1) were measured by immunoradiometric assay (Medgenix; detection limit, 10 pg/mL), and IL-6 was determined by enzyme-linked immunosorbent assay (CLB, Amsterdam, The Netherlands; detection level, 2 pg/mL). Plasma concentrations of IL-10 were measured by enzyme-linked immunosorbent assay (Schering-Plough Research Institute, Kenilworth, NJ; detection limit, 20 pg/mL).

Calculations and statistics

Because plasma glucose concentrations and tracer/tracee ratios for [6,6-²H₂]glucose and [2-¹³C]glycerol were constant during each sam-

pling phase of the study, calculations for steady state kinetics were applied, adapted for the use of stable isotopes (19).

Fractional and absolute gluconeogenesis were measured by a combined infusion of [2-¹³C]glycerol and [6,6-²H₂]glucose. Fractional gluconeogenesis (f) was determined by the mass isotopomer distribution analysis (MIDA) technique performed on the saccharic acid derivative of glucose, as described in detail previously (8). This technique uses probability logic with mass spectrometric analysis to calculate biosynthetic parameters. Plasma glucose is considered to be a polymer of two triose subunits. The distribution of isotopically labeled molecular species (molecules containing zero, one, or two labeled triose subunits) reveals the triose phosphate enrichment (p) by application of the binomial expansion (8). From p, the fraction of glucose synthesized by the gluconeogenic pathway is calculated (14). Because saccharic acid is oxidized at positions C-1 and C-6, label from [6,6-²H₂]glucose is stripped off and does not contaminate the isotopic contribution from [¹³C]glycerol that is used for calculating fractional gluconeogenesis. The rate of appearance (Ra) of glucose is calculated by the dilution technique from infused [6,6-²H₂]glucose (19). Because some M₊₂ isotopic species in glucose are produced from [¹³C]glycerol, the M₊₂ in the glucose penta-acetate derivative (which contains label from [6,6-²H₂]glucose and [¹³C]glycerol) must be corrected to account for the M₊₂ in the saccharic acid derivative (which does not contain [6,6-²H₂] label; see above) to determine true [6,6-²H₂]glucose enrichment. For this correction, at first the contribution to the penta-acetate isotopomeric spectrum from [¹³C]glycerol incorporation is determined based on the saccharic acid analysis of the same sample. Then, a theoretical standard curve is generated in which various combinations of unlabeled (natural abundance) and [6,6-²H₂]glucose-labeled molecules are superimposed upon the isotopomeric distribution from the gluconeogenesis-derived molecules. The slope and intercept for M₊₂-glucose as a function of the proportion of [6,6-

²H₂]glucose in the complete mixture are then calculated. This standard curve is applied to the measured M₊₂-glucose penta-acetate derivative to establish [6,6-²H₂]glucose enrichment. A unique standard curve is generated for each time point based on the gluconeogenic contribution observed in the concurrent saccharic acid derivative, as a unique contribution from gluconeogenic ¹³C needs to be accounted for in each sample. In a separate experiment this correction method was validated by adding [2-¹³C]glycerol to a preexisting infusion of [6,6-²H₂]glucose and [U-¹³C]glucose (15, 16). The Ra of glucose calculated using these corrections did not change from baseline [6,6-²H₂]glucose values and was identical to values calculated from the concurrent [U-¹³C]glucose infusion.

The glycogen contribution to total glucose production, which reflects the portion of glucose production not due to gluconeogenesis under postabsorptive conditions, was calculated from the difference between these two parameters.

It is important to note that the occurrence of renal gluconeogenesis (20) will not affect our estimates, since glycerol is actually taken up as a gluconeogenic precursor by the kidney (21).

The dilution of infused [¹³C]glycerol in the triose phosphate precursor pool for gluconeogenesis has been used previously (8) as an index of the turnover of this pool (Ra triose-p), based on the assumption that infused glycerol enters exclusively into gluconeogenic tissues. This assumption has recently been questioned (21a) (see below for discussion). The calculation of Ra triose-p was described previously (8) using steady state triose-p enrichments.

Data are reported as the mean ± SEM unless otherwise stated. Data within the experiment were analyzed by ANOVA for randomized block design and Fisher's least significant differences test when indicated. Statistical significance was set at *P* < 0.05.

Results

Clinical data

Eleven children (two girls and nine boys) with uncomplicated acute falciparum malaria were studied. Clinical and laboratory details are given in Table 1. Their illness had a median duration of 2 (range, 1–6) days, and they had not eaten for a median period of 15 (range, 4–24) h before the study (Table 2). The median axillary temperature was 39.0 (range, 37.1–40.0) °C. Albumin concentrations were normal, except for the concentration in one child (23 g/L), who was indiscernible from the other subjects in terms of height for

TABLE 1. Clinical and biochemical data of 11 Kenyan children with uncomplicated falciparum malaria

	Median	Range
Age (yr)	3.0	2.0–6.5
Height (cm)	89	79–132
Weight (kg)	12.4	9.4–22.0
Parasitemia (/μL)	160,000	828–664,000
Hemoglobin (g%)	10.0	5.4–12.7
Serum albumin (g/L)	35	23–42
SGOT (U/L)	33	15–50
SGPT (U/L)	14	9–29
Serum bilirubin (μmol/L)	9	3–39

TABLE 2. Parameters of glucose metabolism and glycerol production in 11 Kenyan children with uncomplicated falciparum malaria: basal and during alanine infusion

Patient no.	Fasting (h)	Plasma glucose (mmol/L)		Ra glucose (mg/kg · min)		Gluconeogenesis [mg/kg · min (%)]		Ra Triose-p (mg/kg · min)		Ra glycerol (mg/kg · min)	
		Basal	During alanine	Basal	During alanine	Basal	During alanine	Basal	During alanine	Basal	During alanine
1	6	5.5	5.4	5.4	6.2	4.0 (75)	—	4.5	—	2.3	—
2	18	3.3	4.6	4.1	4.7	3.6 (89)	—	3.6	—	2.6	—
3	4	3.4	3.9	3.3	4.2	2.3 (69)	3.4 (82)	3.0	4.1	1.9	1.8
4	24	4.2	4.4	5.8	5.9	3.9 (67)	4.3 (73)	4.2	4.7	2.2	2.0
5	8	4.6	4.8	7.3	7.7	5.2 (72)	5.8 (76)	4.7	5.1	2.7	2.3
6	15	2.8	3.4	4.4	6.3	3.6 (82)	5.1 (81)	3.5	4.3	1.5	1.9
7	13	2.8	3.2	3.5	4.0	2.8 (80)	3.3 (82)	2.9	3.3	2.0	2.2
8	24	4.3	—	4.3	—	2.7 (63)	—	3.2	—	2.4	—
9	18	4.5	—	4.4	—	2.3 (52)	—	3.8	—	2.2	—
10	18	2.1	—	4.5	—	4.1 (93)	—	4.2	—	2.9	—
11	14	3.3	—	5.2	—	3.0 (58)	—	3.4	—	1.8	—
Mean ± SE (n = 11)	14 ± 2	3.7 ± 0.3	—	4.7 ± 0.3	—	3.4 ± 0.2 (73 ± 4)	—	3.7 ± 0.2	—	2.2 ± 0.1	—
Mean ± SE (n = 7)	13 ± 3	3.8 ± 0.4	4.2 ± 0.3	4.8 ± 0.5	5.6 ± 0.5	3.6 ± 0.4 (76 ± 3)	—	3.8 ± 0.3	—	2.2 ± 0.2	—
Mean ± SE (n = 5)	13 ± 4	3.6 ± 0.4	3.9 ± 0.3	4.9 ± 0.8	5.6 ± 0.7	3.6 ± 0.5 (74 ± 3)	4.4 ± 0.5 (79 ± 2)	3.7 ± 0.4	4.3 ± 0.3	2.1 ± 0.2	2.1 ± 0.1

age and weight for height by American standards (National Center of Health Statistics) (22). For these standards, eight children were below the 10th percentile of height for age, and two were below the 10th percentile of weight for height. All patients responded quickly to therapy and made uneventful recoveries.

Basal glucose kinetics

Plasma glucose concentrations decreased from 4.1 ± 0.3 to 3.5 ± 0.3 mmol/L between 120 and 360 min ($P = 0.028$; $n = 6$). This was associated with a decrease in glucose production from 5.5 ± 0.7 to 4.9 ± 0.6 mg/kg·min ($P = 0.08$), respectively. Glucose clearance (total glucose production/plasma glucose concentration) did not change significantly during this interval (0.75 ± 0.05 vs. 0.77 ± 0.05 mL/kg·min).

The values obtained in 11 patients after 6 h of infusion of $[6,6\text{-}^2\text{H}_2]$ glucose and 4 h of infusion of $[2\text{-}^{13}\text{C}]$ glycerol are provided in Table 2. Glucose production was largely derived from gluconeogenesis ($73 \pm 4\%$). There was a positive correlation between glucose production and absolute gluconeogenesis and between glucose production and glycogenolytic contribution to glucose (Fig. 2). The subjects with the lowest plasma glucose concentrations had the highest fractional contribution of gluconeogenesis ($P = 0.08$), or, in other words, the lowest fractional contribution of glycogen (Fig. 3).

FIG. 2. Correlation between gluconeogenesis and glucose production and between glycogen contribution to glucose and glucose production in 11 Kenyan children with uncomplicated falciparum malaria.

The mean glycogen contribution to glucose production was 1.3 ± 0.2 mg/kg·min, representing $27 \pm 3\%$ of the total glucose production. The ratio of hepatic triose-phosphate enrichment to plasma glycerol enrichment was $60 \pm 2\%$. Thus, only $40 \pm 2\%$ of triose phosphates were derived from other gluconeogenic precursors. Ra triose-p, or the estimated flux into the gluconeogenic precursor pool, correlated closely with gluconeogenesis ($r = 0.83$; $P = 0.002$; Fig. 4) and total glucose production ($r = 0.83$; $P = 0.002$). Values of gluconeogenesis were $91 \pm 3\%$ of the calculated Ra triose-p values.

There was no correlation between any of the measured parameters of glucose metabolism and body temperature, duration of fasting, or duration of illness.

Glucose kinetics during alanine infusion

Plasma glucose concentrations increased during alanine infusion from 3.8 ± 0.4 to 4.2 ± 0.3 mmol/L, or by about 11% ($P = 0.03$; Table 2). This was associated with an increase in glucose production from 4.8 ± 0.5 to 5.6 ± 0.5 mg/kg·min, or by about 17% ($P = 0.02$; $n = 7$; Fig. 5).

Absolute gluconeogenesis increased during alanine infusion by 0.8 mg/kg·min from 3.6 ± 0.5 to 4.4 ± 0.5 mg/kg·min, or by about 22% ($P = 0.04$). The increase in glucose production was accounted for entirely by the increase in gluconeogenesis. In accordance, the contribution of glycogen to glu-

FIG. 3. Correlation between fractional gluconeogenesis and plasma glucose concentration in 11 Kenyan children with uncomplicated falciparum malaria.

FIG. 4. Correlation between the rate of appearance of triosephosphates (Ra triose-p) and gluconeogenesis in 11 Kenyan children with uncomplicated falciparum malaria.

FIG. 5. Effect of alanine infusion on glucose production ($n = 7$), gluconeogenesis ($n = 5$), and glycogen contribution to glucose ($n = 5$) in Kenyan children with uncomplicated falciparum malaria.

TABLE 3. Plasma concentrations of insulin and counterregulatory hormones in 11 Kenyan children with uncomplicated falciparum malaria

	Patients	Normal postabsorptive range
Insulin (mU/L)	5 (3–25)	5–25
Cortisol (μ mol/L)	0.56 (0.14–2.27)	0.22–0.65
Glucagon (ng/L)	208 (117–340)	40–140
Noradrenaline (nmol/L)	0.79 (0.45–1.75)	<3.25
Adrenaline (nmol/L)	0.45 (0.05–1.65)	<0.55

Data are presented as the median (range).

cose did not change during alanine infusion (1.3 ± 0.3 vs. 1.2 ± 0.2 mg/kg·min; $P = \text{NS}$). The relative contribution of gluconeogenesis to total glucose production increased from $74 \pm 3\%$ to $79 \pm 2\%$ ($P = 0.08$), and conversely, the relative contribution of glycogenolysis decreased from $26 \pm 3\%$ to $21 \pm 2\%$ ($P = 0.08$). Thus, the increases in glucose production and gluconeogenesis both represented about 53% of the alanine infused (1.5 mg/kg·min) in those children. The Ra of glycerol did not change during alanine infusion (2.1 ± 0.2 vs. 2.0 ± 0.1 mg/kg·min). The Ra of triose-p increased by 0.6 mg/kg·min (from 3.7 ± 0.4 to 4.3 ± 0.3 mg/kg·min) and continued to correlate closely with gluconeogenesis during alanine infusion. The absolute change in the Ra of triose-p was similar to the change in gluconeogenesis and glucose production.

Precursors

The mean plasma alanine concentration at 360 min (before alanine infusion) was slightly low at 135 ± 13 (normal postabsorptive range, 158 – 314 μ mol/L). During alanine infusion, plasma alanine increased to 499 ± 21 μ mol/L at 480 min ($P = 0.02$).

The mean plasma lactate concentration did not change from 360 min (1.21 ± 0.13 mmol/L) to 480 min (0.96 ± 0.05 mmol/L; normal postabsorptive range, 0.6 – 2.0 mmol/L).

Hormones and cytokines

Plasma concentrations of insulin and counterregulatory hormones are presented in Table 3, indicating that plasma insulin concentrations are appropriately low and plasma concentrations of the counterregulatory hormones are appropriately high in all children, with two exceptions. One child had a plasma insulin concentration of 25 mU/L. The glucose production in this child was 5.4 mg/kg·min. Another child had a low plasma cortisol concentration (0.14 μ mol/L); the glucose production rate in this child was 4.5 mg/kg·min. These values of glucose production are both intermediate in our study group. The plasma concentrations of the other glucoregulatory hormones in these two children were appropriate.

Plasma concentrations of the cytokine $\text{TNF}\alpha$, soluble TNF receptor types I and II, IL-1, IL-6, and IL-10 are presented in Table 4. The concentrations of all cytokines and soluble receptors, except for IL-1, were elevated in the majority of the patients, as found previously in patients with falciparum malaria (23–26).

TABLE 4. Plasma cytokine concentrations in 11 Kenyan children with uncomplicated falciparum malaria

	Patients	Normal range
TNF α (pg/mL)	76 (34–468)	<20
sTNF-RI (ng/mL)	4.7 (2.1–10.5)	0.3–2.9
sTNF-RII (ng/mL)	30.4 (12.6–79.9)	1.9–8.5
IL-1 (pg/mL)	<10 (<10)	<10
IL-6 (pg/mL)	30 (4–134)	<10
IL-10 (pg/mL)	355 (49–1150)	<20

sTNF-RI and sTNF-RII denote soluble TNF receptor types I and II, respectively. Data are presented as the median (range).

Discussion

This is the first study of glucose kinetics in children with falciparum malaria. During short term fasting, plasma glucose concentration decreased in these children with malaria. This was caused by a decrease in glucose production, not by an increase in glucose consumption. Glucose production was largely derived from gluconeogenesis, and the children with the lowest plasma glucose concentrations had the lowest rate of gluconeogenesis and the lowest rate of absolute and relative contributions of glycogen to glucose production. Therefore, basal gluconeogenesis failed to compensate when glycogen breakdown was low, resulting in low glucose production. This failure might be due either to an impaired capacity for gluconeogenesis or a lack of substrates. There was no impairment in gluconeogenesis *per se*, since infusion of alanine increased absolute gluconeogenesis by about 0.8 mg/kg·min in association with a nearly identical increase in glucose production flux. Therefore, these children with malaria tended to develop decreased plasma glucose concentrations during short term fasting when glycogen decreased because of an impaired flux of gluconeogenic precursors into the triose phosphate pool.

The factors not related to malaria that could theoretically also explain these results are malnutrition, starvation, and disturbances in glucoregulatory hormone secretion. None of these seems to be applicable to our patients. The clinical data indicated that the Kenyan children evaluated in this study had a weight for height and height for age around the 10th percentile of the U.S. standards of weight for height and height for age (22). Nonetheless, physical examination indicated that these children were well proportioned and, therefore, not malnourished. The data for plasma albumin concentrations supported this finding. Hence, stunted growth in the past is the most probable explanation for the low anthropometric values. We were not permitted to study healthy Kenyan children as controls, but compared to healthy American children of similar body weight (27), basal hepatic glucose production was, on the average, approximately 20% lower in the Kenyan children in our study. Taylor *et al.* reported that in Malawian children with cerebral malaria who had fasted for an average of 25 h, there was no relation between plasma glucose concentrations and the duration of fasting (28), whereas in Tanzanian children with falciparum malaria and a starvation period of up to 90 h, the occurrence of hypoglycemia was significantly correlated with the time since the last meal (29). These data taken together suggest that only prolonged, not short term, fasting, as was present in our study, is a factor that contributes to hypoglycemia.

Finally, the interpretation of the data within our study was not affected by differences in the duration of the postabsorptive state between the subjects. Therefore, it is very unlikely that our data are merely explained by the duration of fasting.

As pointed out by Krebs *et al.* (30), measurement of gluconeogenesis with carbon-labeled precursors has been complicated by the fact that the metabolic pathway from pyruvate proceeds through mitochondrial oxalacetate, which is exposed to numerous metabolic sources of carbon dilution (6). This dilution of tracer is a problem because it is necessary to establish the isotope enrichment of the true biosynthetic precursor (triose phosphates) to interpret incorporation from labeled gluconeogenic substrates. Recently, the MIDA method was developed by our laboratory (8) and validated by others (31,32) as a technique for accurate estimation of the triose phosphate pool enrichment using [2-¹³C]glycerol, [1-¹³C]lactate, or [3-¹³C]lactate.

Previs *et al.* (33) and Landau *et al.* (34) recently criticized the use of MIDA with labeled glycerol for measuring gluconeogenesis. They reported that *f* was much less than 100% (*i.e.* systematically underestimated) using MIDA with [U-¹³C]glycerol in perfused livers, intact rats and monkeys, and humans after prolonged fasts. Although the researchers made no measurements of pools in the liver (33, 34), they proposed that the liver exhibits functional heterogeneity, such that labeled glycerol fails to have access to all hepatocytes across the lobule. They concluded that labeled glycerol is inappropriate for measuring gluconeogenesis by MIDA because it results in an underestimation. Their critique is not relevant to the use of [2-¹³C]glycerol. First, Previs *et al.* (33) and Landau *et al.* (34) reported low values of *f* from labeled glycerol only when [U-¹³C]glycerol (triple labeled glycerol) was used. When they used [2-¹³C]glycerol, as we originally described (8), their values of *f* were appropriately high and similar to reports from other laboratories using [2-¹³C]glycerol and MIDA (8, 31, 33). In contrast, [U-¹³C]glycerol gave variable values of *f* in perfused livers from fasted rats (ranging between 75–92%), and [U-¹³C]lactate gave even more unexpected results (*f* as low as 36% and maximally 64%) (33). Thus, study by Previs *et al.* (33) clearly indicates that the problem is with triple labeled precursors, not with single labeled glycerol or the MIDA model *per se*. Second, there are established analytic reasons why [U-¹³C]glycerol or [U-¹³C]lactate might give very different results than single labeled glycerol. The use of triple labeled precursors requires a different metabolic model than that of single labeled precursors. This invokes a complex model involving analysis of and fitting to seven isotopomers (M_0 – M_{+6}) instead of three (M_0 – M_{+2}) and fitting to a recombinatorial rather than a simple combinatorial model. Moreover, the use of triple labeled precursors brings to light problems relating to mass spectrometric accuracy. The most important analytic factor that leads to variations in mass spectrometric accuracy among different isotopomers is the concentration effect (16, 17), namely that relative abundances or isotope ratios change with the increasing total amount of material injected. Concentration effects can be due to overloading of the most abundant ion with spillover into adjacent masses, detector nonlinearity, ion chemistry in the source, or other causes (16,

17). Concentration effects are worst when the dynamic range of abundances across mass isotopomers is greatest (15, 16), *i.e.* very low abundance ions cannot be accurately compared to much higher abundance ions in the same analysis. Accordingly, the use of [U-¹³C]glycerol and [U-¹³C]lactate leads to predictable problems due to the enormous dynamic range for abundances of M_{+6} compared to M_0 , M_{+1} , *etc.* For example, the ratio of $M_{+6}:M_0$ with [U-¹³C]glycerol or [U-¹³C]lactate is greater than 1000:1 (33), whereas we found a significant concentration effect even at a ratio of 5:1. The problem is compounded if precursor pool enrichments are allowed to be too low, as was the case in studies by Previs *et al.* (33) and Landau *et al.* (34). In contrast, results from fasted animals (8, 31), perfused livers from fasted rats (31), and fasted human subjects (16) have achieved *f* values greater than 90% when [2-¹³C]glycerol is used with MIDA.

A remarkable finding in our study is the marked dependency of glucose production in children with malaria on the supply of alanine as a gluconeogenic precursor. During alanine infusion, plasma glucose concentrations increased by about 11%, total glucose production increased by about 17%, and gluconeogenesis increased by about 22%. In adults, the gluconeogenic substrate supply does not play a major role in the regulation of overall glucose output in the postabsorptive state unless its supply diminishes below a certain level (35, 36). In normal adults, infusions of glycerol or lactate do not increase glucose production, presumably because of hepatic autoregulatory adaptations (35, 36). Therefore, the increase in glucose production during alanine infusion in the present study in children is in marked contrast to the findings in adults. There are no data published on this subject in healthy children. Our results suggest that in children with uncomplicated falciparum malaria, there is an unexplained inability to provide sufficient (nonglycerol) gluconeogenic precursors, increasing the risk of a low blood sugar when liver glycogen becomes depleted. Gluconeogenesis was highly efficient and responded well to exogenous infusion of alanine in the children with low plasma glucose concentrations. Taken with our finding that only 50% of infused alanine entered the gluconeogenic precursor pool, it may be speculated that increased clearance of gluconeogenic metabolites by extrahepatic tissues plays a role in the low delivery of this precursor to the liver. The technique that we used to estimate total flux of gluconeogenic precursors (Ra triose-p) may lead to an overestimation if labeled glycerol enters nongluconeogenic tissues, as recently concluded by Landau *et al.* (21a). Under the conditions studied here, however, Ra triose-p was only 10% greater than absolute gluconeogenesis rates, so any overestimation could not be substantial. Moreover, infusion of alanine increased gluconeogenesis, glucose production, and triose-p flux by nearly identical values (0.6–0.8 mg/kg-min), again suggesting that Ra triose-p was not greatly overestimated. The explanation for this apparent lack of labeled glycerol uptake by nongluconeogenic tissues in these children compared to that in 60-h fasted adults (21a) is not clear, but may be related to the extremely high contribution (60 ± 2%) of plasma glycerol to the triose-p pool in these children.

Although we did not study patients with severe malaria, our data have potential implications for the pathophysiology

of hypoglycemia in this condition. Our data indicate that after a short period of fasting, glucose production is largely dependent on gluconeogenesis, which is highly efficient but is potentially limited due to insufficient precursor supply. The explanation for this unique primary metabolic abnormality of inadequate delivery of (nonglycerol) gluconeogenic precursors in uncomplicated malaria remains uncertain, but therapeutic interventions to maintain precursor delivery would be expected to prevent hypoglycemia when these children are exposed to periods of fasting.

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