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Non-radioisotopic glucose turnover in children with falciparum malaria and enteric fever

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Abstract

To determine whether glucose turnover is increased in acute falciparum malaria compared to enteric fever in children, steady-state 6,6-D_2-glucose turnover was measured in 9 Malaysian children with uncomplicated malaria (6 males and 3 females; median age 10 years, body weight 22 kg) and in 12 with uncomplicated enteric fever (8 males and 4 females; median age 10 years, body weight 24 kg) in acute illness, after quinine (5 malaria patients) and in convalescence. Baseline plasma glucose concentrations in malaria and enteric fever were similar (all values are medians [ranges in brackets]) 5·6 [3·2–11·3] vs. 5·5 [4·2–8·0] mmol/L), as were serum insulin levels (5·6 [0·4–26·5] vs. 6·8 [1·1–22·5] milliunits/L; $P>0·4$). Glucose turnover in the malaria patients was higher than in patients with enteric fever (6·27 [2·71–6·87] vs. 5·20 [4·50–6·08] mg/kg.min; $P=0·02$) and in convalescence (4·74 [3·35–6·79] mg/kg.min; $P=0·05$ vs. acute malaria study), and fell after quinine together with a rise in serum insulin ($P=0·03$). Basal plasma lactate concentrations were higher in enteric fever than in malaria (3·4 [1·8–6·4] vs. 0·8 [0·3–3·8] mmol/L; $P<0·0001$) and correlated inversely with glucose turnover in this group ($r_s=-0·60$; $n=12$; $P=0·02$). These data suggest that glucose turnover is 20% greater in malaria than in enteric fever. This might reflect increased non-insulin-mediated glucose uptake in falciparum malaria and/or impaired gluconeogenesis in enteric fever, and may have implications for metabolic complications and their clinical management in both infections.

Keywords: malaria, *Plasmodium falciparum*, enteric fever, *Salmonella typhi*, glucose turnover, insulin, gluconeogenesis, quinine, children

Introduction

Hypoglycaemia is more than twice as likely to complicate falciparum malaria in children than in adults both before and during treatment (WHITE *et al.*, 1987; MARSH *et al.*, 1995; HIEN *et al.*, 1996; VAN-HENSBROEK *et al.*, 1996). However, there is still debate as to whether malaria is more commonly associated with hypoglycaemia than are other serious paediatric infections (KAWO *et al.*, 1990). A central issue is the comparability of the clinical severity of falciparum malaria and other infections in children (BREWSTER *et al.*, 1990; DAVIS, 1990; TAYLOR *et al.*, 1990). One view is that hypoglycaemia is related to the time of the last meal and is correctable when identified (KAWO *et al.*, 1990). By contrast, hypoglycaemia has been considered to be closely associated with specific complications of malaria such as coma, when it may prove refractory to treatment and signify a poor prognosis (TAYLOR *et al.*, 1990).

Glucose turnover is accelerated by approximately 50% in both untreated, non-pregnant adults with severe falciparum malaria (DAVIS *et al.*, 1993) and in pregnant women with uncomplicated *Plasmodium falciparum* or *P. vivax* malaria (DAVIS *et al.*, 1994). Although control studies in healthy children remain ethically problematic, available evidence suggests that glucose turnover is, by contrast, decreased in children with uncomplicated falciparum malaria (DEKKER *et al.*, 1996). This difference may reflect age-related changes in the balance between glucose production and peripheral utilization. A negative association between the plasma glucose concentration and its turnover in severely ill adults suggests that increased peripheral glucose uptake predominates (DAVIS *et al.*, 1993; BINH *et al.*, 1997). A positive correlation between plasma glucose and its turnover in children with uncomplicated malaria supports the view that hepatic glucose production is the main determinant of glycaemia in this age group (DEKKER *et al.*, 1996).

Direct and indirect effects of the metabolically active *P. falciparum* parasite in the microvasculature of organs such as the liver (MACPHERSON *et al.*, 1985) and treatment with quinine (DAVIS *et al.*, 1990) may influence glucose metabolism in malaria and increase the likelihood of hypoglycaemia relative to its occurrence in other paediatric infections. To investigate this hypothesis in a stable clinical situation in which valid metabolic data could be collected, we measured glucose turnover using a safe, minimally recycled, non-radioactive glucose isotope (6,6-D_2-glucose) in a group of Malaysian children with acute uncomplicated falciparum malaria. The same measurements were performed in a control group of children with enteric fever, a common bacterial infection in tropical countries. The results suggest that glucose production is relatively high in malaria due to increased non-insulin mediated glucose uptake in falciparum malaria and/or impaired gluconeogenic capacity in enteric fever.

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Patients and Methods

Patients

We studied 9 children with acute uncomplicated falciparum malaria (WARRELL *et al.*, 1990) and 12 with enteric fever (see Table 1). All had been admitted to Kuala Krai, Tanah Merah, Pasir Mas or Kota Bharu Hospitals, Kelantan State, Malaysia. All patients with malaria had blood films containing *P. falciparum* and those with enteric fever had blood or stool cultures with *Salmonella typhi*, and/or had a positive Widal test or dot-enzyme immunosorbent assay (ISMAIL *et al.*, 1991). None of the patients had other illnesses, including diarrhoeal disease, and none had clinical evidence of severe malnutrition or prolonged starvation before admission. Each patient's parent or guardian gave witnessed, informed consent to participation. The study protocol was approved by the ethics committee of the Ministry of Health, Malaysia.

Methods

Clinical procedures. Patients were studied within 48 h of hospital admission. Initial management comprised full clinical assessment, baseline blood samples for routine laboratory tests, oral or intravenous rehydration and commencement of antimicrobial therapy. The children with enteric fever received oral or parenteral chlor-

Table 1. Admission clinical and biochemical details of patients with falciparum malaria and enteric fever

	Malaria[a]	Enteric fever[a]
No. of patients	9	12
Age (years)	10 (8–14)	10 (7–12)
Gender (male)	67%	67%
Body weight (kg)	22·0 (17·0–51·0)	24·0 (20·0–29·5)
Duration of fever (d)	5 (1–30)	10 (5–30)[b]
Oral temperature (°C)	38·2 (37·0–39·5)	37·8 (37·0–39·5)
Pulse (per min)	104 (90–120)	91 (80–110)
Mean blood pressure (mmHg)	73 (70–87)	82 (70–87)
Parasitaemia (red blood cells infected)	1·2% (0·6–3·1)	–
Serum urea (mmol/L)	4·3 (2·5–12·4)	2·6 (2·0–4·9)[b]
Serum creatinine (μmol/L)	69 (51–147)	56 (38–75)[b]
Serum total bilirubin (μmol/L)	3 (3–4)	4 (1–12)
Serum aspartate transaminase (U/L)	10 (4–67)	51 (7–1464)[b]
Serum alkaline phosphatase (U/L)	83 (49–237)	102 (58–298)
Serum albumin (g/L)	29 (19–37)	28 (16–38)

[a]Data are medians (ranges in parentheses) unless otherwise indicated.
[b]$P<0·025$ vs. malaria group.

amphenicol (75 mg/kg body weight/d) given 4 times daily for at least 2 weeks. Those with malaria received either single-dose oral chloroquine and Fansidar® (sulfadoxine and pyrimethamine) or, if they could be studied promptly, quinine dihydrochloride (10 mg/kg) thrice daily for 7 d, with the first dose given as part of the glucose turnover protocol (see below). Monitoring of vital signs and urine output, and blood glucose estimations every 4 h, were also started.

Once patients were clinically stable and informed consent had been obtained, an intravenous cannula was inserted into a suitable forearm vein and non-dextrose-containing fluids were administered. Patients were fasted and kept supine over the next 4 h. Bedside blood glucose concentrations were checked at regular intervals. A second cannula was introduced retrogradely into a suitable vein on the dorsum of a warmed hand to enable repeated sampling of arterialized blood. This cannula was kept patent by flushing with small volumes of sterile heparinized saline solution.

Three small-volume baseline venous blood samples (at −10, −5 and zero min) were taken for determination of labelled and native plasma glucose, as well as for plasma lactate serum insulin concentrations. A 3·5 mg/kg body weight bolus injection of 6,6-D_2-glucose (Tracer Technologies, Massachussets, USA) in sterile solution was given after the zero min sample, followed immediately afterwards by a constant intravenous infusion of 35 μg/kg/min administered by motor-driven syringe pump (Perfusor®, Braun, Melsungen, Germany). Further sets of blood samples were taken at 80, 85 and 90 min.

After the 90 min sample, untreated patients were given quinine dihydrochloride (10 mg salt/kg) intavenously by constant infusion over 2 h and the labelled glucose infusion was continued in parallel. Further blood samples were drawn at 210, 215 and 220 min. In patients already treated with chloroquine and Fansidar®, the labelled glucose infusion was stopped at 90 min. A sample of the labelled glucose infusate was retained in each case, as well as additional plasma for quinine assay from samples drawn at zero min and, in those treated with quinine, at 210 min. Samples for plasma glucose and serum insulin assay were kept on ice and centrifuged promptly at 4°C. Separated plasma and serum were stored below −20°C and transported on dry ice before assay.

A parent or guardian of each patient was asked to give consent to restudy in convalescence. In the children with malaria, the convalescent study was performed at least 2 d after parasite clearance (when 2 consecutive blood films had been negative) or, when quinine had been given, >48 h after the last dose. Those with enteric fever were restudied after fever clearance. The protocol followed in convalescence was identical to that used up to the 90 min sample in the acute study.

Assay methods. Plasma glucose was measured by the hexokinase method (Cobas Mira® analyser, Roche, Australia). The interassay coefficient of variation was <8% over the range 4·7–16·6 mmol/L. Serum insulin was determined by two-site immunoenzymometric assay (Tosoh®, Tokyo, Japan) with coefficient of variation <14% over 4–40 milliunits/L. Serum total and unbound quinine were measured using high-performance liquid chromatography (DYER *et al.*, 1994) and other biochemical assays were performed using standard automated techniques.

Measurement of 6,6-D_2-glucose was by gas chromatography/mass spectrometry (WIECKO & SHERMAN, 1976; DAVIS *et al.*, 1994). In brief, 20–50 μL of plasma were deproteinized in 1·0 mL of absolute ethanol (BDH, Dorset, UK), centrifuged, and the supernatant was dried under oxygen-free nitrogen. After addition of 100 μL of butylboronic acid in pyridine (10 mg/mL; Sigma, St Louis, Missouri, USA), samples were derivatized at 95°C for 30 min, allowed to cool and mixed with 100 μL of acetic anhydride (BDH). The mixture was incubated for 1 h, dried under nitrogen at 40°C and resuspended in 200–500 μL of decane (Sigma). Derivatized samples were run on a Hewlett Packard 6170 gas chromatography/mass spectrometer using an HP1 non-polar capillary column with selected ion monitoring of mass ratio 297/299 at 200°C. The interassay coefficient of variation was 7·4%.

Data analysis. Glucose turnover was calculated from the formula $((IE_i/IE_p)-1)r$, where IE_i is infusate isotopic enrichment, IE_p is plasma isotopic enrichment at steady state and r is the rate of infusion (RIGGS, 1963; DAVIS *et al.*, 1994). Baseline (zero min), pre-quinine (90 min) and post-quinine (210 min) values for biochemical variables and turnover rates were taken as the median of the 3 levels determined during the 10 min period preceding each of these times. Statistical analysis was by non-parametric tests (SIEGEL & CASTELLAN, 1988). Two-sample comparisons were by Wilcoxon–Mann–Whitney tests, comparisons between multiple related samples by Friedman two-way analysis of variance, and associations between variables by Spearman's rank correlation coefficient. Data are reported as medians and ranges.

Results

Clinical course

The children with malaria had similar baseline clinical characteristics to those with enteric fever, except that the median pre-admission duration of fever in enteric fever was twice that in malaria ($P=0·025$; see Table 1). The patients with malaria had significantly higher serum urea and creatinine ($P<0·02$), and lower serum aspartate transaminase (AST) concentrations ($P= 0·002$) than the children with enteric fever (see Table 1). Serum total bilirubin, alkaline phosphatase and albumin concentrations were similar in the 2 groups ($P<0·07$). Despite between-group differences in hepato-renal function, most values were within laboratory reference ranges. Peripheral blood parasite counts in the malaria group were <3·1% of red cells infected (<150000 parasites/μL).

The malaria patients remained uncomplicated and cleared parasitaemia and fever in a median of 7 d. Although the children with enteric fever were uncomplicated when first studied, 3 subsequently developed bronchitis or pneumonia and one developed anaemia requiring transfusion. Fever clearance time was longer in the enteric fever group than in the malaria group, as was the duration of stay in hospital (20 vs. 13 d; $P<0·003$). As a result, the period between acute and convalescent studies tended to be longer for the children with enteric fever (median 15 vs. 12 d; $P=0·08$).

Plasma glucose

No subject developed hypoglycaemia (plasma glucose <2·2 mmol/L) at any stage of either study. There was no significant difference between the basal plasma glucose concentration in the malaria patients at the time of the acute and convalescent studies ($P>0·4$) and this was also the case for the children with enteric fever ($P>0·2$; see Table 2). The children with malaria had similar plasma glucose profiles to those of the enteric fever group during both acute and convalescent turnover studies (see Fig. 1, A).

Serum insulin

In neither the malaria patients nor in those with enteric fever was there a significant difference between the basal serum insulin in acute and convalescent studies ($P>0·4$). Concentrations over the first 90 min of the studies in acute illness and convalescence were also similar in the 2 patient groups (see Fig. 1, B). The serum insulin increased when quinine was administered in all 5 malaria patients ($P=0·03$; see Fig. 1, B).

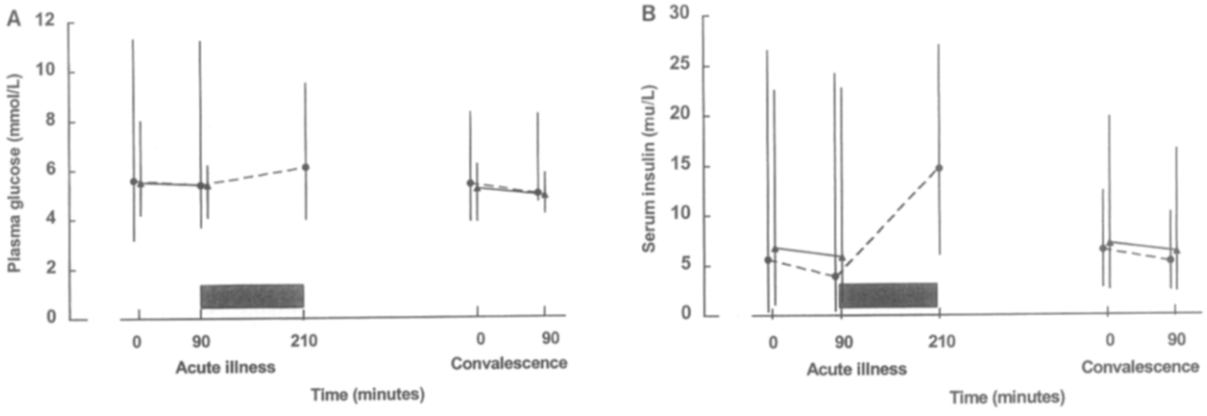

Fig. 1. A, plasma glucose and B, serum insulin concentrations in 9 patients with acute falciparum malaria (●- - ●) and in 12 patients with enteric fever (▲——▲) during glucose turnover studies in acute illness and in convalescence. Intravenous quinine was given to 5 patients with falciparum malaria between 90 and 210 min. during the acute phase (shaded bar) (medians and ranges; mu=milliunits).

Table 2. Details of glucose turnover studies in patients with falciparum malaria and enteric fever in acute illness, after initial quinine treatment and in convalescence

	Malaria[a]	Enteric fever[a]
No. of patients	9	12
Acute basal plasma glucose (mmol/L)	5·6 (3·2–11·3)	5·5 (4·2–8·0)
Acute basal serum insulin (milliunits/L)	5·6 (0·4–26·5)	6·8 (1·1–22·5)
Acute basal plasma lactate (mmol/L)	0·8 (0·3–3·8)	3·5 (1·8–6·4)[b]
Acute glucose turnover (mg/kg.min)	6·27 (2·71–6·87)[c]	5·20 (4·50–6·08)[b]
Post-quinine glucose turnover (mg/kg.min)[d]	5·65 (4·55–7·22)	–
Convalescent basal plasma glucose (mmol/L)	5·4 (3·9–8·3)	5·2 (3·9–6·2)
Convalescent basal serum insulin (milliunits/L)	6·5 (2·8–12·4)	7·1 (2·6–19·8)
Convalescent glucose turnover (mg/kg.min)	4·74 (3·35–6·79)	5·10 (4·05–6·45)

[a]Data (except no. of patients) are medians (ranges in parentheses).
[b]$P<0·05$ vs. malaria group.
[c]$P<0·05$ vs. convalescent group.
[d]Five patients.

Glucose turnover

Glucose turnover in acute illness was significantly greater in the children with malaria than in those with enteric fever ($P=0·023$; see Table 2 and Fig. 2). There was no association between acute glucose turnover and plasma glucose when the data from the 21 patients were pooled ($r_s=-0·08$; $P=0·36$), or when the malaria and enteric fever groups were considered separately ($r_s=0·08$ and $-0·36$; $P=0·42$ and $0·12$, respectively). There was no significant difference between the initial glucose turnover rates in the 4 children with malaria who had been treated with chloroquine and Fansidar® (median 6·01 mg/kg.min, range 2·71–6·87) and in the 5 who were subsequently treated with quinine (6·74 mg/kg.min, range 4·67–6·70; $P>0·3$). In 4 of the 5 quinine-treated children, the glucose turnover rate fell during quinine infusion but this was not a statistically significant change ($P=0·15$; see Fig. 2). There was, however, a significant difference between the pre-quinine turnover and that in convalescence in the malaria group as a whole (see Table 2 and Fig. 2; $P=0·05$). The turnover rates in the enteric fever patients were similar in acute illness and convalescence ($P=0·36$).

Plasma lactate

Basal plasma lactate concentrations in the children with enteric fever were significantly greater than those of the malaria patients ($P=0·0003$; see Table 2). There was a significant inverse association between acute glucose turnover and plasma lactate when data from the 21 patients were pooled ($r_s=-0·59$; $P=0·003$; see Fig. 3). When the malaria and enteric fever groups were considered separately, the association was evident only in the latter group ($r_s=-0·02$ and $-0·60$; $P=0·48$ and $0·02$, respectively). Plasma lactate did not correlate with biochemical indices of liver function including serum bilirubin, AST and albumin in the whole series of patients or in the sub-group with enteric fever ($P>0·2$).

Plasma quinine

The 5 quinine-treated patients had a median total serum quinine concentration of 10·5 mg/L (range 9·9–17·6) at the end of the infusion and free drug comprised a median of 8% of the total. There was no association between the end-infusion total or free serum quinine concentration and the change in serum insulin between 90 and 120 min ($P>0·3$).

Discussion

We found that steady-state glucose turnover in Malaysian children with uncomplicated malaria was a median of 20% higher than that both in convalescence and in a demographically and clinically comparable group of children with enteric fever. The 2 patient groups had similar plasma glucose and serum insulin concentrations in acute illness, which suggests that insulin-independent glucose disposal was relatively high in the children with malaria. In the enteric fever group,

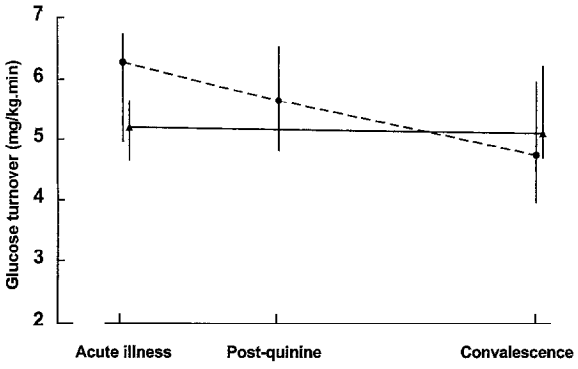

Fig. 2. Glucose turnover in 9 patients with falciparum malaria (●- - ●) and in 12 patients with enteric fever (▲——▲) before treatment, immediately after quinine treatment of malaria patients, and in convalescence (medians and ranges).

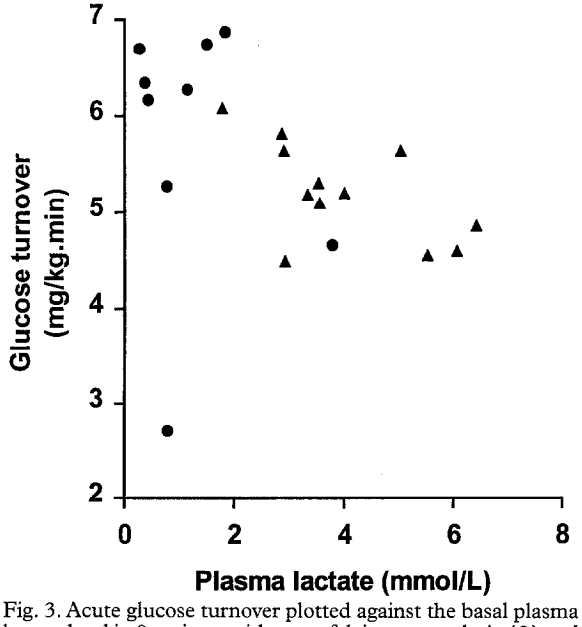

Fig. 3. Acute glucose turnover plotted against the basal plasma lactate level in 9 patients with acute falciparum malaria (●) and in 12 patients with enteric fever (▲).

the plasma lactate level was significantly increased and inversely associated with the glucose turnover, consistent with impaired utilization of gluconeogenic substrates by the liver. These preliminary data provide evidence that factors determining glucose metabolism in children with infection, and thus the risk of hypoglycaemia, may be influenced by the type of infecting organism.

In the only other published study of glucose production in malaria in children, DEKKER *et al.* (1996) used similar methods to those of the present study to measure steady-state glucose turnover in African children aged 2 to 10 years with uncomplicated *P. falciparum* infections. The median and range of turnover values reported by these authors (5·0 and 4·1–8·4 mg/kg.min), which were consistent with those in our patients (6·3 and 2·7–6·9 mg/kg.min), were compared to limited data from normal Caucasian children (BIER *et al.*, 1977; HAYMOND *et al.*, 1978). It was concluded that glucose production was an average of 30% lower in malaria (DEKKER *et al.*, 1996). By contrast, acute glucose turnover rates were significantly higher in acute malaria than in convalescence in our patients. In addition, our second group of children with enteric fever lived in the same rural area of Malaysia and had a similar age, body weight, gender distribution and vital signs to those with malaria. Despite close matching for such factors which might influence glucose metabolism, glucose turnover was significantly higher in our children with malaria.

The basal plasma glucose and serum insulin levels were similar in our 2 patient groups, suggesting that pancreatic β cell function and tissue insulin sensitivity were also similar (MATTHEWS *et al.*, 1985). One explanation for the relatively high glucose turnover in the children with malaria is, therefore, that non-insulin mediated glucose disposal was increased. This suggestion contradicts the hypothesis that the glucose production rate is the main determinant of the plasma glucose in children with acute malaria but may explain why, in contrast to the findings of DEKKER *et al.* (1996), we found no association between acute glucose turnover and the basal plasma glucose level.

Evidence for accelerated non-insulin mediated glucose disposal has been found recently in adult patients with severe malaria using minimal model analysis (BINH *et al.*, 1997). It has been hypothesized that the underlying mechanism is increased cellular glucose transporter expression, perhaps mediated by cytokines such as tumour necrosis factor α (TNFα) or through an as yet unidentified lipopolysaccharide-like parasite product (MIZOCK, 1995; BINH *et al.*, 1997). DEKKER *et al.* (1996) found that plasma concentrations of TNFα and its soluble receptors, and interleukins IL-1, IL-6 and IL-10, were not associated with glucose production, but measurement of cytokines was beyond the scope of the present study. Nevertheless, it is of interest that cytokine production is inhibited in the acute phase of enteric fever (KEUTER *et al.*, 1994), which might contribute to a relatively low non-insulin-mediated glucose uptake in this infection.

Although chloroquine treatment has no significant effect on glucose metabolism in healthy adults (SMITH *et al.*, 1987), the drug may accelerate peripheral glucose uptake in non-insulin-dependent diabetic patients (POWRIE *et al.*, 1991). In our children with acute malaria, there was no difference in glucose turnover between those who had received chloroquine and those who were to receive quinine. This suggests that chloroquine had no effect on non-insulin-mediated glucose uptake. Furthermore, chloroquine also increases insulin secretion and reduces insulin clearance in diabetes (POWRIE *et al.*, 1991), but hyperinsulinaemia was not present in our chloroquine-treated children.

The raised plasma lactate concentrations in our children with enteric fever, none of whom was hypoxic, hypotensive or hypoglycaemic, were unexpected. Hyperlactaemia has not been reported previously as a feature of enteric fever. Four patients (33% of our group) has basal concentrations ≥4 mmol/L, levels associated with a poor prognosis in severe illness regardless of the underlying disease (CADY *et al.*, 1973) and typical of the most severe paediatric infections including meningitis, septicaemia and pneumonia (ENGLISH *et al.*, 1996). Given that plasma lactate concentrations are significantly greater in malaria than in other community-acquired infections (ENGLISH *et al.*, 1996; AGBENYEGA *et al.*, 1997), our data provide some evidence that marked hyperlactaemia (and perhaps also hypoglycaemia) may be a hitherto unrecognized feature of complicated enteric fever.

Gluconeogenesis and turnover by the liver account for half of the basal lactate utilization (BUCHALTER *et al.*, 1989). This observation and the significant inverse association between plasma lactate level and glucose production in our children with enteric fever suggest that they had a specific defect in hepatic lactate delivery or uptake. This defect is unlikely to be a result of cytokines as the production of inflammatory mediators which inhibit gluconeogenesis is attenuated in enteric fever (KEUTER *et al.*, 1994). It is also unlikely to reflect hepatocellular damage as there were only mild elevations of serum AST in our patients which did not correlate with the plasma lactate. In addition, gluconeogenic

capacity in adults with more severe infections is unimpaired (PUKRITTAYAKAMEE *et al.*, 1992, 1994). Reduced hepatic blood flow is associated with raised plasma lactate in severe malaria in adults (PUKRITTAYAKAMEE *et al.*, 1992). It is possible that, despite the similar pulse and mean arterial blood pressure in our 2 patient groups, enteric fever was associated with lower splanchnic blood flow and thus lactate delivery to the liver. Our data do not suggest that enhanced peripheral glucose uptake contributed to hyperlactaemia as it does in other stress states (MIZOCK, 1995).

During initial quinine therapy in our patients with falciparum malaria, glucose turnover tended to fall and the serum insulin level rose. This pattern is consistent with the known effect of quinine on pancreatic β cells and previous reports of changes in glucose turnover in quinine-treated adults with severe malaria (DAVIS *et al.*, 1993, 1994). Reduced parasite glucose consumption might contribute to reduced host glucose utilization after the first dose of quinine, while the increases in plasma insulin could predominantly suppress hepatic glucose output rather than increase peripheral glucose uptake. Rehydration and reduced neurological (including autonomic) stimulation may also attenuate hepatic glucose production during initial treatment.

Consistent with the data of DEKKER *et al.* (1996), our results suggest that a glucose infusion rate of approximately 6 mg/kg.min is sufficient to cover hepatic glucose production in children with uncomplicated malaria. This rate may need to be increased if the patients have severe malaria and/or when high-dose quinine is used as treatment. Lower infusion rates (around 5 mg/kg.min) appear sufficient in enteric fever but cannot be assumed, without further studies, to be applicable to other bacterial infections. The results of the present preliminary study indicate that further evaluation of glucose metabolism in children with well characterized acute infections is needed, especially in relation to lactate production and utilization.

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Short Report

Trypanosoma brucei ssp. and *T. congolense*: mixed human infection in Côte d'Ivoire

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It is generally agreed that human African trypanosomiasis (HAT) in West and Central Africa is caused by *Trypanosoma brucei gambiense*. However, recent studies have found patients harbouring other *T. brucei* subspecies identified mainly by isoenzyme analysis, such as the bouaflé strain group (STEVENS & GODFREY, 1992). Furthermore, TRUC *et al.* (1997) have shown that the disease in these patients followed a more rapid and severe course; characterization of the parasite isolates was confirmed by isoenzyme electrophoresis using cellulose acetate (TRUC *et al.*, 1991).

Due to the toxicity of the available drugs to treat HAT, the presence of parasites must be confirmed before therapy is started. In the field, active case detection commonly involves establishing a suspicion of infection by serology, followed by the microscopical examination of blood or lymph of seropositive cases. The card agglutination trypanosomiasis test (CATT) is commonly used, on serum or plasma (MAGNUS *et al.*, 1978), followed by one or more parasitological tests, such as the capillary centrifugation test, the mini-anion exchange centrifugation technique (m-AECT) (LUMSDEN *et al.*, 1979) or the quantitative buffy coat (QBC®) technique.

In the Aboisso HAT focus in south-eastern Côte d'Ivoire, a 50 years old woman gave a weakly positive CATT result using whole blood but a negative result with plasma; she also gave a negative result in the latex agglutination test (BUSCHER *et al.*, 1991), using whole blood. Parasites were detected by the m-AECT using heparinized whole blood collected by venepuncture, as recently recommended by TRUC *et al.* (1998), over 100 trypanosomes being seen in the eluate. The morphology of these trypanosomes was different from that of *T. brucei* spp.; they were shorter, without a free flagellum, and were only weakly motile. No *T. brucei* slender form was observed and the trypanosomes died in the m-AECT collector tube in less than 20 min, unlike *T. brucei* ssp., which survive for several hours.

The extremely bad physical condition of this patient indicated that she was in the advanced stage of the disease. However, no trypanosome was found in her cerebrospinal fluid by double centrifugation, and the cell count was only 1/mm^3. No malaria parasite was found in a blood film, and no intestinal parasite was detected by faecal examination.

The blood collected for the m-AECT was divided into aliquots and deep frozen in liquid nitrogen, inoculated into a kit for the *in vitro* isolation of trypanosomes (KIVI) (AERTS *et al.*, 1992), and inoculated intraperitoneally to a white mouse. Both attempts to isolate trypanosomes *in vitro* and *in vivo* failed after 60 d follow-up.

Deoxyribonucleic acid (DNA) was isolated from the whole trypanosomes (PENCHENIER *et al.*, 1996) and amplified by the polymerase chain reaction (PCR) (MASIGA *et al.*, 1992) using specific primers for *T. brucei* spp. and *T. congolense* (Kilifi, forest and savannah groups). DNA amplification, done in 2 different laboratories, gave clearly positive results when using primers for the *T. congolense* savannah group (MAJIWA & OTIENO, 1990) and for *T. brucei* ssp. (MOSER *et al.*, 1989). Positive and negative controls confirmed the specificity of the PCR and the absence of contamination by parasite DNA.

The initial microscopical observation of the trypanosomes suggested that they might be *T. congolense* and the failure to isolate parasites *in vitro* or *in vivo* confirmed our suspicion that they were not *T. b. gambiense*. Furthermore, the weak CATT reaction and the negative results in the other serological tests corroborated this. The patient was seronegative for human immunodeficiency virus infection in the Murex® 200A Ice Pack enzyme-linked immunosorbent assay and the Innogenetics® 1036 line immunoassay.

Resistance of *T. congolense* to human serum has been suggested previously (JOSHUA, 1989). In a previous study, 3 stocks of *T. congolense* isolated from goats and sheep in Nigeria were resistant to human serum in the blood incubation infectivity test (RICKMAN & ROBSON, 1970).

The fact that this patient was treated successfully with pentamidine (7 intramuscular injections over 14 d), with no parasite being seen on follow-up examination immediately after treatment and one month later and her general health improving within 2 weeks after starting the treatment, suggests that she might have had a mixed infection. However, the presence of *T. brucei* was indicated only by the weak CATT reaction and the PCR result.

Mixed infections in humans involving allegedly non-infective trypanosomes, confirmed by DNA identification, have never, to our knowledge, been described.

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