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Gluconeogenesis and fasting in cerebral malaria

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ABSTRACT

Background: In healthy subjects after an overnight fast, glucose production is for ~50% derived from glycogenolysis. If the fast is prolonged, glucose production decreases due to a decline in glycogenolysis, while gluconeogenesis remains stable. In cerebral malaria, glucose production is completely derived from gluconeogenesis after an overnight fast. It is not known if glucose production also decreases during fasting when its only source is gluconeogenesis.

Design: Glucose production was measured by infusion of [6,6-$^2H_2$]glucose in seven patients with cerebral malaria after prolonging a fast from 20.30 to 00.30 hours.

Results: Glucose production decreased by ~10% (27.4 ± 2.1 to 24.7 ± 1.6 μmol/kg/min, p=0.05), without changes in the plasma concentrations of glucoregulatory hormones, FFA or precursors.

Conclusions: In the patients with cerebral malaria, glucose production decreases during fasting due to a decrease in the rate of gluconeogenesis. These data suggest that the decrease in the rate of glucose production during short-term fasting is actively regulated and not simply due to shrinkage of glycogen content, as in the absence of glycogenolysis, glucose production decreases at the same rate as normally seen in healthy subjects whose glucose production is for ~50% derived from glycogen and in whom gluconeogenesis is stable.

INTRODUCTION

The adaptive response to starvation in healthy subjects involves a series of metabolic alterations. These include a decrease in the rate of glucose production by about 10 to 20% in the first 24 hours of fasting.[1,2] Glucose production has two components: gluconeogenesis and glycogenolysis. After an overnight fast both contribute more or less equally to total glucose production.[3-7] With progression of the fast the percentage contribution of gluconeogenesis to glucose production increases; the absolute rate of gluconeogenesis, however, neither increases or decreases, but remains stable for periods of up to 64 hours at the level obtained after an overnight fast.[4-9] The decrease in total glucose production with a *constant* rate of gluconeogenesis over time during fasting is not only found in healthy subjects, but also in the few studies in patients with an increased rate of gluconeogenesis.[7-9]

The change in glucose production during fasting seems merely to be a diminution in the rate of glycogenolysis, simply ascribed to a decline in glycogen content. However, in the last decades it has become clear that regulation of the rate of glucose production involves a network of regulatory systems (the classical hormones, liver glycogen content, paracrine mediators and the autonomic nervous system). Such a network suggests active regulation of glucose production during fasting and not simple dependence on glycogen content.[10] This notion is supported by studies showing that the liver is able to autoregulate between gluconeogenesis and glycogenolysis in order to fix glucose output at a set level only dependent on the duration of the fast.[11]

If this is true, it can be expected that even when glucose production is completely derived from gluconeogenesis,

glucose production during short-term fasting will diminish at the same rate as in healthy subjects, whose glucose production is for ~50% derived from glycogen. Recently we showed in cerebral malaria patients that after an overnight fast glucose production is for 100% derived from gluconeogenesis.[12] We hypothesised that if during fasting, glucose output is actively set at a level *only* dependent on the duration of the fast, glucose production will also decrease during fasting in patients whose glucose production after an overnight fast is completely dependent on gluconeogenesis.

We therefore studied glucose kinetics during fasting, using [6,6-$^2H_2$]glucose to measure glucose production in seven patients with cerebral malaria, consecutively admitted to Bao Loc General Hospital in Vietnam. As the greatest changes in glucose metabolism induced by starvation occur in the first 24 hours[1] and withholding food for prolonged periods in critically ill patients is unethical, we measured glucose kinetics during the last four hours of a ~24 hour fast.

MATERIAL AND METHODS

Subjects

Seven nonpregnant patients with cerebral malaria consecutively admitted to the intensive care unit of Bao Loc General Hospital were recruited. The inclusion criteria were based on the definition of the WHO for cerebral malaria.[13] Exclusion criteria were treatment with quinine,[14] severe anaemia (Hct <15%) and concomitant infectious disease. The study was approved by the local health authorities and by the Medical Ethics Committee of the Academic Medical Centre, Amsterdam, the Netherlands.

Study design

Patients were recruited on the day of admission after quinine use was excluded by quinine dipstick.[15] Patients were treated with artesunate intravenously according to the standard regimen of the hospital, as previously described,[12] right after laboratory confirmation of the diagnosis. After receiving informed consent signed by a first-degree relative, the patient was given a standard meal of approximately 400 to 450 ml of soup (of rice and pork meat) through a gastric tube (standard regimen for comatose patients in this hospital), followed by a fast until completion of the study. Plasma glucose concentration was measured at the bedside two hourly or hourly if a previous value was low or whenever there was suspicion of hypoglycaemia.

The time of the last meal was set at T=0. Twelve hours after administration of the meal, an intravenous cannula was introduced into a forearm vein for blood sampling. The catheter was kept patent by a slow isotonic saline drip. A blood sample for background enrichment of [6,6-$^2H_2$]glucose was drawn at T=18.30. Then a primed (3.2 mg/kg), continuous (2.4 mg/kg/h) infusion of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Andover MA, USA), dissolved in sterile isotonic saline, was administered by a motor-driven, calibrated syringe pump (Perfusor® Secura FT, B.Braun, Germany) through a millipore filter (size 0.2 μm; Minisart, Sartorius, Germany). Three blood samples were collected at intervals of ten minutes at T=20.20, 20.30 and 20.40 for determination of plasma glucose concentration and [6,6-$^2H_2$]glucose enrichment. Blood samples for the measurement of plasma concentration of insulin, counterregulatory hormones, alanine, lactate, FFA, glycerol and [6,6-$^2H_2$]glucose enrichment were collected at T=20.30 and 00.30. The study ended at T=00.30.

Blood for [6,6-$^2H_2$]glucose enrichment as well as hormones was collected in pre-chilled heparinised tubes and for lactate and alanine in fluoride tubes. All samples were kept on ice and centrifuged immediately. Plasma and urine were stored at below -20°C and were transported on dry ice before assay in the Netherlands.

Assays

Plasma samples for glucose enrichments of [6,6-$^2H_2$]glucose were deproteinised with methanol.[16] The aldonitril penta-acetate derivative of glucose[17] was injected into a gas chromatograph/mass spectrometer system. Separation was achieved on a J&W (J&W Scientific, FOL, CA, USA) DB17 column (30 m x 0.25 mm, d_f 0.25 μm). Glucose concentrations were determined by gaschromatography using xylose as an internal standard. Glucose was monitored at m/z 187, 188 and 189. The enrichment of glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for natural enrichments. The isotopic enrichments were measured on a gaschromatograph mass spectrometer (model 6890 gaschromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionisation mode, Hewlett-Packard, Palo Alto, CA) (coefficient of variation (CV) intra-assay 2%, inter-assay CV 4%).

Plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden), intra-assay CV was 3 to 5%, inter-assay CV 6 to 9%, detection limit 15 pmol/l; cortisol by enzyme immunoassay on an Immulite analyser (DPC, Los Angeles, CA), intra-assay CV was 2 to 4%, inter-assay CV 3 to 7%, detection limit 50 nmol/l; glucagon by RIA (Linco Research, St. Charles, MO, USA), intra-assay CV was 3 to 5%, inter-assay CV 9 to 13%, detection limit 15 ng/l; norepinephrine and epinephrine by an in-house HPLC method; norepinephrine intra-assay CV was 6 to 8%, inter-assay CV 7 to 10%, detection limit 0.05 nmol/l, epinephrine intra-assay CV was 6 to 8%, inter-assay CV 7

to 12%, detection limit 0.05 nmol/l; free fatty acids by an enzymatic method (NEFAC; Wako chemicals GmbH, Neuss, Germany), intra-assay CV was 2 to 4%, inter-assay CV 3 to 6%, detection limit 0.02 mmol/l.

Calculations and statistics

Because plasma glucose concentrations and tracer/tracee ratios for [6,6-$^2H_2$]glucose were varied (albeit only slightly) at each sampling phase of the study, calculations for non-steady-state kinetics were applied, adapted for the use of stable isotopes.[18]

Two sample comparisons were made using the Mann-Whitney test. Time points within either study were compared using a paired sample t-test. A p value ≤0.05 was considered statistically significant. SPSS statistical software programme was used for analysis.

Data are presented as means ± SEM, unless otherwise stated.

RESULTS

Table 1 shows the clinical and biochemical characteristics with cerebral malaria. All patients were admitted to the intensive care unit in a comatose state with a Glasgow coma scale less than 11. Their duration of illness was 4 ± 1 days. The current standard regimen for treatment of cerebral malaria in Vietnam (artesunate intravenously) was applied immediately after admission to the ICU. The data on glucose production in the extended fast after 20.30 hours in these patients have been published before.[12]

Glucose metabolism, precursors, free fatty acid and glucoregulatory hormones are shown in *tables 2* and *3*. Glucose production decreased over time by 10% in patients (p=0.05), without any change in the plasma concentrations of the glucoregulatory parameters.

Table 1

Clinical and biochemical characteristics of seven patients with cerebral malaria

CHARACTERISTICS CEREBRAL MALARIA PATIENTS	
Age (years)	32 ± 5
Sex (male/female)	6/1
BMI	19.3 ± 0.7
Temperature (°C)	38.2 ± 0.6
Parasitaemia (per µl)	99,849 ± 60,742
Glasgow coma score	6 ± 1
Haemoglobin (mmol/l)	7.8 ± 0.6
Serum AST (U/l)	183 ± 65
Serum ALT (U/l)	123 ± 28
Creatinine (µmol/l)	124 ± 18

Data are means ± SEM.

Table 2

Glucose concentration and glucose production in seven patients with cerebral malaria

TIME OF FASTING	20.30 H	00.30 H	CHANGE OVER TIME
Glucose concentration (mmol/l)	6.68 ± 0.31	6.57 ± 0.42	ns
Glucose production (µmol/kg/min)	27.4 ± 2.1	24.7 ± 1.6	p=0.05

Values are means ± SEM, ns= not significant.

Table 3

Precursors, glucoregulatory hormones and free fatty acid

TIME OF FASTING	20.30 H	00.30 H	P VALUE
Precursors			
Alanine (µmol/l)	286 ± 29	343 ± 94	ns
Glycerol (µmol/l)	88 ± 6	93 ± 11	ns
Lactate (mmol/l)	2.55 ± 0.82	2.32 ± 0.77	ns
Hormones			
Insulin (pmol/l)	49 ± 11	41 ± 9	ns
Glucagon (ng/l)	132 ± 33	144 ± 36	ns
Cortisol (nmol/l)	1080 ± 163	949 ± 149	ns
Norepinephrine (nmol/l)	4.8 ± 3.5	4.3 ± 3.0	ns
Epinephrine (nmol/l)	0.27 ± 0.13	0.25 ± 0.14	ns
FFA (mmol/l)	0.80 ± 0.06	0.81 ± 0.05	ns

ns= not significant.

DISCUSSION

Our data clearly show that glucose production declined significantly over time in patients with cerebral malaria when the fast was extended from 20.30 to 00.30 hours. We have previously shown that in the patients with cerebral malaria, gluconeogenesis (GNG) accounted for 100% of glucose production at T=20.30.[12] Therefore the decline in glucose production during the prolongation of the fast was due to a decline in the rate of gluconeogenesis. This finding is not remarkable for the decrease in glucose production, as numerous studies have shown that in healthy subjects glucose production decreases by ~10% between 16 and 22 hours of fasting, due to a decrease in the rate of glycogenolysis.[1,2] The finding is remarkable for the decrease in the rate of gluconeogenesis.

One could postulate that in the case of 100% gluconeogenesis, glucose molecules formed during gluconeogenesis could lead to an underestimation of the glucose production because of the exchange of H on the C6 position with

deuterium from the water. However, during gluconeogenesis not only the H-atoms on the 6th position but also the ones on the C5 and the C2 will be labelled, thus leading to a glucose molecule with m+3 or m+4. In our [6,6-$^2H_2$] glucose analysis for measurement of glucose production only the fragment of m+2 with all six C-atoms is measured making this confounding variable unlikely.

The decrease in the rate of gluconeogenesis over time in our patients with cerebral malaria is remarkable. The gold standard for measurement of gluconeogenesis in humans *in vivo* is either deuterated water or NMR.[19] All existing data indicate that gluconeogenesis, measured with these techniques, is *constant* during fasting for periods up to 64 hours even in diabetic patients with an increased rate of gluconeogenesis.[3-7,9] Gluconeogenesis is dependent on precursor supply and the glucoregulatory hormones.[9,12,20] The plasma concentrations of the precursors and the glucoregulatory hormones did not differ between T=20.30 and T=00.30 and therefore do not explain the decrease in glucose production over time in our patients. A potential explanation for this decrease in production in our patients could be *active* regulation of the rate of total glucose production (independent of its source, either glycogen or gluconeogenesis) in relation to fasting time instead of a decrease in glucose production simply caused by shrinkage of glycogen content. This hypothesis of active regulation is supported by recent data from our group.[21] In that study we measured glucose production twice in healthy humans, fasting from 16 to 22 hours, once during infusion with a low dose of insulin in an amount that increased plasma insulin slightly above the basal level and another time without infusion of insulin (control study). In the control study, glucose production declined by ~18%. Within the first hour of insulin infusion a significant decline in glucose production was found compared with the control study. Subsequently a rebound increase in glucose production was found. From the third hour of insulin infusion onwards glucose production was no longer different between both study conditions. These changes in glucose production during low-dose insulin occurred without changes in the concentrations of the counter-regulatory hormones. This observation and the observation that the liver is able to autoregulate between gluconeogenesis and glycogenolysis suggest that during short-term fasting glucose production is set at a certain level, a level that changes (diminishes) over time with progression of the fast.[11] Data in healthy subjects suggest that this regulation is primarily directed at glycogenolysis.[6] Our data in patients with cerebral malaria suggest that when direction at this primary target is impossible, this regulatory process will be targeted at the rate of gluconeogenesis.

The nature of this regulatory process can not be inferred from this study. Glucoregulatory hormones and free fatty acids do not seem to play a role. A role for the autonomic nervous system is also less likely, although the influence of the sympathetic nervous system during a prolongation of the fast to 24 hours has not been studied.[22,23] Therefore, the available data point to a paracrine network in the liver itself that exerts a potent glucoregulatory role during fasting.[10]

We conclude that in cerebral malaria, the decline in glucose production during short-term fasting is due to a decrease in the rate of gluconeogenesis. We hypothesise that in humans the decrease in rate of glucose production during short-term fasting is actively regulated, only dependent on the duration of the fast, and not simply due to shrinkage of glycogen content, as in the absence of glycogenolysis, glucose production decreases at the same rate as normally seen in healthy subjects, whose glucose production is for ~50% derived from glycogen and in whom gluconeogenesis is stable.

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