Infectious disease-related differences in the adaptation of glucose metabolism to fasting in children and the effect of age
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Glucose production in response to glucagon is higher in Surinamese children with severe malaria compared to children with severe pneumonia

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\textit{Submitted}
SUMMARY

BACKGROUND & AIMS Fasting is an important risk factor for hypoglycemia in children with malaria or pneumonia. Young children are more at risk, because of impaired endogenous glucose production (EGP) presumably due to smaller liver glycogen stores. The aim was to measure the effect of a bolus glucagon on glucose kinetics, as an indicator of glycogen content, in fasted children with malaria and pneumonia.

METHODS After a 16-hour fast glucose concentration and EGP were measured in six children with severe malaria (1-5 years) and 12 with severe pneumonia (6 young (1-3 years), 6 older (3-5 years)) before and after a bolus glucagon.

RESULTS Basal glucose concentration and EGP were higher in children with malaria, p=0.034 and p=0.010 respectively. Glucose concentration and EGP increased after glucagon in both groups (p<0.001). The peak in glucose concentration and in EGP was higher in children with malaria, p=0.029 and p=0.023 respectively. There were no differences between young and older children with pneumonia.

CONCLUSIONS After a 16-hour fast, the increase in glucose concentration and EGP in response to glucagon are higher in children with malaria suggesting smaller glycogen stores in children with pneumonia; glycogen stores in young and older children with pneumonia are equally diminished.
INTRODUCTION

Hypoglycemia frequently occurs in young children with various illnesses of which malaria, pneumonia and diarrhea are the most common (1,2). Young sick children with hypoglycemia have a four to six time greater chance of dying compared to normoglycemic sick children (1,3). Hypoglycemia is not a specific feature of disease, but it is considered the consequence of prolonged fasting in severely ill children (2,4). Very young children below the age of 3 years are particularly at risk for hypoglycemia after a period of extended fasting (1,2,5,6). Reduced endogenous glucose production (EGP) due to lower liver glycogen content is considered the underlying mechanism for this increased risk (7). Glycogen content in children can be measured by liver biopsy or by nuclear magnetic resonance imaging combined with stable isotopes. 13C-NMR is a non-invasive alternative for quantification of the liver glycogen content in patients, but only a limited number of studies report on this technique in young children (8). The use of both liver biopsies and 13C-NMR for research purposes in children is limited for ethical and practical reasons. A more feasible non-invasive approach to test the ability to release glucose from glycogen stores in young children is to measure the response to a bolus glucagon, a method often used in clinical practice (9). In young growth hormone-deficient children (1.1-6.5 years) who developed hypoglycemia after a 24 hour fast, the majority (66%) had a normal response to glucagon suggesting sufficient hepatic glycogen (10). Similar results were found in children with acute lymphoblastic leukemia who were fasted for 16 hours: 7 out of 11 (64%) children (median age 4.5 years) who developed hypoglycemia had an adequate response to glucagon stimulation (11).

A limitation of these studies is that only the plasma glucose concentration was measured. To adequately quantify the increase in EGP after glucagon, stable isotopes are needed (12). Currently there are no data on the glucose or EGP response to glucagon stimulation in young children with malaria or pneumonia after prolonged fasting.

The aim of this study was to estimate possible differences in liver glycogen content between children with malaria and pneumonia after 16 hours of controlled fasting by measuring the change in plasma glucose concentration and EGP using [6,6-2H2]glucose in response to a bolus glucagon. We hypothesized that glucose concentration and EGP would increase in response to a bolus glucagon, suggesting that liver glycogen content is not exhausted after a 16-hour fast. Second, we hypothesized that the increase is not influenced by different infectious diseases, i.e. that the increase is comparable in children with malaria and pneumonia. Third, we hypothesized that the response to glucagon is lower in very young children than in older children because of lower liver glycogen stores.
MATERIALS AND METHODS

Patients
All children included in this study were admitted to the Diakonessen Hospital in Paramaribo with a primary diagnosis of malaria or pneumonia. Inclusion criteria were: age between 1 and 5 years, severe malaria or severe pneumonia, necessity of intravenous infusion and plasma glucose concentration ≥ 3.0 mmol/l on admission.
Severe malaria was defined according to the WHO criteria (5): > 2% of erythrocytes infected, or malaria with presence of severe anemia, respiratory distress, impaired consciousness, multiple convulsions within the past 24 hours, or (other) signs of cerebral malaria (Blantyre coma score ≤ 2) not attributable to any other cause. All patients with suspected cerebral malaria had a lumbar puncture to exclude other causes of coma.
Severe pneumonia was defined according to WHO guidelines (13): cough or difficult breathing with respiratory distress (lower chest wall retractions or abnormally deep breathing), tachypnoe (≥ 40 breaths per minute) and chest auscultation signs (decreased breath sounds, bronchial breath sounds, crackles, abnormal vocal resonance, pleural rub).
Exclusion criteria were: glucose concentration < 3.0 mmol/l on admission, very severe pneumonia according to the WHO criteria (central cyanosis, inability to drink or profound vomiting, convulsions, lethargy or unconsciousness, severe respiratory distress, or X-ray signs of pleural effusion, empyema, pneumothorax, pneumatocele and interstitial pneumonia) (13), need for mechanical ventilation, severe anemia (hemoglobin concentration < 3.5 mmol/l (63 mg/dl)), severe acidosis (pH < 7.25), severe chronic diarrhea (which may induce hypoglycemia in childhood), documented endocrine diseases and concomitant infectious diseases.
Nutritional status was assessed by weight for length/height on the WHO Child Growth Standards for children less than five years of age (14).
Written informed consent was obtained from the accompanying parent or guardian. This study was approved by the Suriname National Ethical Committee.

Study design
All severely ill children admitted at the Diakonessenhuis Hospital were treated according to the guidelines of proper pediatric intensive care medicine. After admittance, patients were stabilized and recruited immediately after laboratory and radiodiagnostic confirmation of the clinical diagnosis and exclusion of quinine-use by a quinine dipstick.
Basal hematological and biochemical parameters were measured for clinical purposes. Patients with severe malaria were treated with intramuscular artemotil, patients with pneumonia were treated with intravenous ampicillin. The time the children had their last meal or drink either at home or in the hospital was recorded and considered as the start of the fasting period prior to the study. After inclusion the children were fasted for another 16 hours (controlled fasting period).
An intravenous catheter was introduced in a peripheral vein for clinical reasons (IV therapy) and also used for stable isotope infusion. A second catheter for blood sampling was introduced into a suitable vein in the contralateral arm or foot. Both catheters were introduced after Emla® cream application for local anesthesia. Blood sampling from the venous catheter proved to be technically possible and was tolerated well by all children. Whenever possible, blood samples for study purposes were tied with samples for clinical reasons. The catheters were kept patent by a slow isotonic saline drip.

The study design is shown in figure 1. After obtaining a baseline blood sample at t = -16 hr for plasma glucose concentration, the patients were fasted until the end of the study but were allowed to drink tap water ad libitum. In order to detect hypoglycemia without delay, glucose concentrations were checked regularly during the entire fasting period using a bedside point of care device (Precision Q•I•D, MediSense Inc., II, USA) in addition to the glucose measurements on admission and samples taken for the study.

At t = -10 hr a blood sample was drawn to determine background enrichment of [6,6-2H₂]glucose and plasma glucose concentration. Immediately thereafter a primed (5.4 mg/kg), continuous (3.6 mg/kg/h or 0.33 µmol/kg•min) infusion of [6,6-2H₂]glucose (Cambridge Isotope Laboratories, Andover MA, USA) dissolved in sterile isotonic saline and sterilized by passage of the solution through a millipore filter was administered by a motor-driven, calibrated syringe pump (Perfusor® Secura FT, B.Braun, Germany). At t = 0 (16 hours of controlled fasting, start of the study) a bolus glucagon was administered intravenously (0.1 mg/kg). Between t = 0 min and t = 60 min blood samples for plasma glucose concentration and enrichment of [6,6-2H₂]glucose were obtained every 15 minutes. Blood samples for determination of plasma concentration of insulin and glucagon were collected at t = 0 min and t = 15 min.
Assays

Blood for [6,6-2H2]glucose enrichment as well as hormones was collected in prechilled heparinized tubes. All samples were kept on ice and centrifuged immediately. Plasma was stored at −20°C and was transported on dry ice before assay in the Netherlands. Plasma samples for glucose enrichments of [6,6-2H2]glucose were deproteinized with methanol. The aldonitril penta-acetate derivative of glucose 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, d1 0.25 µm). Glucose concentrations were determined by gas chromatography using xylose as an internal standard. Glucose was monitored at m/z 187, 188 and 189. The enrichment of [6,6-2H2] glucose was determined by dividing the peak area of m/z 189 by the peak area of 187 and correcting for natural enrichments.

Insulin was measured in an Immulite 2000 system (Diagnostic Products Corporation, Los Angeles, USA) with a chemiluminiscent immunometric assay (intra-assay variation 47 pmol/L 6%, 609 pmol/L 3%; inter-assay variation 91 pmol/L 4%, 120 pmol/L 6%; detection limit 15 pmol/L). Glucagon was determined by RIA (Linco Research, St. Charles, MO, USA), intra-assay c.v.: 3--5 %, inter-assay c.v.: 9--13 %, detection limit: 15 ng/L.

Calculations

After glucagon administration the glucose rate of appearance (Ra) was calculated by the isotope dilution from the [6,6-2H2] enrichment of glucose in plasma, using non steady state equations as described by Steele (15):

\[
Ra = \frac{F - pV \times \left[\frac{(C_2 + C_1)}{2}\right] \times \left[\frac{(E_2 - E_1)}{(t_2 - t_1)}\right]}{(E_2 + E_1) / 2}
\]

where Ra = rate of appearance of glucose (in µmol/kg•min), F = [6,6-2H2]glucose infusion rate (in µmol/kg•min), E = percent of glucose molecules enriched with 2H (in absolute values), C = plasma glucose concentration (in mmol/L), t = time at the sampling points (in min) and pV = effective distribution volume of glucose, assumed to be 75% of the extracellular water volume. The volume of extracellular water was calculated by normogram from body weight and height.

Statistics

To investigate the response of EGP and plasma glucose concentration to glucagon, mixed models analysis with repeated measurements analysis of variance (SPSS version 16.0) was used with time as a categorical variable. Since some data of glucose kinetics were not normally distributed, ranks of valuables were used. For each of the models, the residuals were normally distributed (Wilk-Shapiro’s W > 0.90), but since they did not show constant variance nonparametric mixed models analysis was performed. Differences between the groups in clinical and laboratory data and glucose kinetics at t = 0 were analyzed by the
t-test for independent variables, the paired data of hormones by the paired samples t-test. Data are presented as medians and ranges unless stated otherwise. Statistical significance was set at p < 0.05.

RESULTS

Clinical data
Clinical and laboratory details are given in table 1. Eighteen Surinamese children under the age of five were studied: 6 children had severe malaria and 12 children had severe pneumonia. The children with severe pneumonia were divided in a group of 6 young (<3 years) and 6 older (3-5 years of age) children. In the group of children with malaria, 3 children were younger than 3 years and 3 children were between 3-5 years of age. There

Table 1. Clinical and biochemical characteristics on admission. Data are presented as median and range.

<table>
<thead>
<tr>
<th></th>
<th>malaria (n = 6)</th>
<th>pneumonia (n = 12)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>2.6 (1.1 ; 4.8)</td>
<td>2.8 (1.1 ; 4.9)</td>
<td>0.968</td>
</tr>
<tr>
<td></td>
<td>1.5 (1.1 ; 2.3)</td>
<td>4.2 (3.3 ; 4.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting prior to study (hr)</td>
<td>9 (2 ; 18)</td>
<td>7 (1 ; 20)</td>
<td>0.835</td>
</tr>
<tr>
<td></td>
<td>7 (1 ; 20)</td>
<td>6 (3 ; 20)</td>
<td>0.783</td>
</tr>
<tr>
<td>Weight for length/height (SD)</td>
<td>-1.7 (3.3 ; -0.2)</td>
<td>-1.3 (-2.5 ; +1)</td>
<td>0.320</td>
</tr>
<tr>
<td></td>
<td>-1.4 (-2.3 ; -0.3)</td>
<td>-1.2 (-2.5 ; +1)</td>
<td>0.447</td>
</tr>
<tr>
<td>Temperature (0C)</td>
<td>38.9 (36.5 ; 39.4)</td>
<td>39.0 (37.3 ; 40.4)</td>
<td>0.437</td>
</tr>
<tr>
<td></td>
<td>38.6 (37.3 ; 40.1)</td>
<td>39.0 (38.1 ; 40.4)</td>
<td>0.536</td>
</tr>
<tr>
<td>Haemoglobin after blood transfusion (mmol/L)</td>
<td>5.8 (3.8 ; 6.6)</td>
<td>6.6 (3.7 ; 8.0)</td>
<td>0.314</td>
</tr>
<tr>
<td></td>
<td>6.6 (4.9 ; 7.1)</td>
<td>6.1 (3.7 ; 8.0)</td>
<td>0.786</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>146 (18 ; 333)</td>
<td>146 (12 ; 366)</td>
<td>0.716</td>
</tr>
<tr>
<td></td>
<td>154 (14 ; 210)</td>
<td>90 (12 ; 366)</td>
<td>0.990</td>
</tr>
<tr>
<td>Serum AST (U/L)</td>
<td>106 (34 ; 410)</td>
<td>43 (17 ; 164)</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>29 (17 ; 67)</td>
<td>50 (31 ; 164)</td>
<td>0.118</td>
</tr>
<tr>
<td>Serum ALT (U/L)</td>
<td>26 (13 ; 52)</td>
<td>25 (13 ; 129)</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td>22 (13 ; 35)</td>
<td>28 (14 ; 129)</td>
<td>0.234</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>33 (19 ; 39)</td>
<td>38 (24 ; 69)</td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td>32 (28 ; 48)</td>
<td>40 (24 ; 69)</td>
<td>0.317</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>0/6</td>
<td>6/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/2</td>
<td>2/4</td>
<td></td>
</tr>
</tbody>
</table>
were no differences in any of the clinical and biochemical characteristics between the groups and values of biochemical characteristics were consistent with severe disease. One young child and one older child had cerebral malaria. The young child with cerebral malaria and another older child with severe malaria had a blood transfusion on admission because of severe anemia. Their haemoglobin values were above 5.0 mmol/l (90 mg/dl) after blood transfusion before the start of the controlled fasting period. None of the children had a decline in Hb greater than 0.5 mmol/l after the study. All patients responded well to therapy and made uneventful recoveries.

Basal glucose kinetics before glucagon administration

*Children with malaria versus pneumonia*

Data are shown in table 2. The basal plasma glucose concentration and EGP, after 16 hours of controlled fasting, were significantly higher in the children with malaria than in the children with pneumonia (p = 0.034 and p = 0.010 respectively).

<table>
<thead>
<tr>
<th></th>
<th>malaria (n = 6)</th>
<th>pneumonia (n = 12)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 3 years (n = 6)</td>
<td>3-5 years (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Glucose concentration (mmol/l)</td>
<td>4.2 (3.0 ; 4.7)</td>
<td>3.1 (2.1 ; 3.8)</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>2.8 (2.1 ; 3.5)</td>
<td>3.3 (2.1 ; 3.8)</td>
<td>0.224</td>
</tr>
<tr>
<td>Glucose production (µmol/kg•min)</td>
<td>25.3 (22.6 ; 36.4)</td>
<td>18.8 (14.1 ; 31.8)</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>19.2 (14.1 ; 23.3)</td>
<td>18.2 (14.5 ; 31.8)</td>
<td>0.725</td>
</tr>
</tbody>
</table>

*Young versus older children with pneumonia*

There were no differences in plasma glucose concentration (p = 0.224) or in EGP (p = 0.725) between the young and older children with pneumonia after the 16 hour controlled fast. Two young children and one older child had plasma glucose concentrations of 2.1, 2.3 and 2.1 mmol/l after 16 hours of fasting without clinical signs of hypoglycemia. They immediately received a bolus glucagon resulting in an increase of the plasma glucose concentrations to 2.5, 3.6 and 2.7 mmol/l respectively. Thereafter they remained normoglycemic.

Glucose kinetics in response to glucagon bolus

*Children with malaria versus pneumonia (figures 2 and 3)*

The plasma glucose concentration increased significantly in both groups: mean increase of 2.1 ± 1.2 mmol/l (p < 0.001) in children with malaria, and of 1.0 ± 0.7 mmol/l (p < 0.001) in children with pneumonia. The increase was significantly larger in the children with malaria (p = 0.002). The peak plasma glucose concentration was higher in the children with malaria: 6.0 ± 1.7 mmol/l (increase of 52 ± 26 %) than in children with pneumonia: 3.9 ± 1.1 mmol/l (increase of 31 ± 23 %) (p = 0.029 for difference between the groups).
EGP increased significantly in both groups: increase of 32.0 ± 17.4 µmol/kg•min (p < 0.001) in children with malaria, and of 12.8 ± 9.3 µmol/kg•min (p < 0.001) in children with pneumonia. The increase was significantly larger in the children with malaria (p = 0.001). The peak EGP was higher in the children with malaria: 60.9 ± 22.4 µmol/kg•min

**Fig. 2.** Response of plasma glucose concentration to glucagon after 16 hours of controlled fasting in children with severe malaria (open symbols, mean: dashed line) and severe pneumonia (closed symbols, mean: black line). Individual data are shown. Plasma glucose concentration increased significantly in both groups (p < 0.001), but the increase was greater in the malaria children (p = 0.002).

**Fig. 3.** Response of endogenous glucose production to glucagon after 16 hours of controlled fasting in children with severe malaria (open symbols, mean: dashed line) and severe pneumonia (closed symbols, mean: black line). Individual data are shown. EGP increased significantly in both groups (p < 0.001), but the increase was greater in the malaria children (p = 0.001).

EGP increased significantly in both groups: increase of 32.0 ± 17.4 µmol/kg•min (p < 0.001) in children with malaria, and of 12.8 ± 9.3 µmol/kg•min (p < 0.001) in children with pneumonia. The increase was significantly larger in the children with malaria (p = 0.001). The peak EGP was higher in the children with malaria: 60.9 ± 22.4 µmol/kg•min
(increase of 106 ± 42 %) than in children with pneumonia: 31.9 ± 11.2 µmol/kg•min (increase of 70 ± 52 %) (p = 0.023 for difference between groups).

Young versus older children with pneumonia (figures 4 and 5)
Plasma glucose concentration increased significantly in both groups: + 0.8 ± 0.8 mmol/l (p < 0.001) in young children and + 1.1 ± 0.7 mmol/l (p < 0.001) in older children (p = 0.479 for difference between groups). The peak plasma glucose concentration after glucagon was similar in both groups: 3.6 ± 1.1 mmol/l (increase of 30 ± 27 %) in the young and 4.3 ± 1.1 mmol/l (increase of 33 ± 21 %) in the older children (p = 0.292). EGP increased significantly in both the young and older children with pneumonia: + 10.8 ± 7.5 µmol/kg•min (p < 0.001) in young children and + 14.8 ± 11.1 µmol/kg•min (p < 0.001) in older children (p = 0.960 for difference between groups). The peak EGP after glucagon was similar in both groups: 29.4 ± 6.2 µmol/kg•min (increase of 65 ± 56 %) in the young children and 34.4 ± 15.0 µmol/kg•min (increase of 74 ± 53 %) in the older children (p = 0.470).

Plasma glucagon and insulin in response to glucagon bolus
Children with malaria versus pneumonia
The plasma glucagon and insulin concentrations at t = 0 were not different between children with malaria and children with pneumonia (p = 0.981 and p = 0.371 respectively) (table 3).

Fig. 4. Response of plasma glucose concentration to glucagon after 16 hours of controlled fasting in children with severe pneumonia. The young children (< 3 years) are represented by closed symbols (mean: black line), the older children (3-5 years) are represented by open symbols (mean: dashed line). Plasma glucose concentration increased significantly in both groups (p < 0.001), no differences between the groups (p = 0.479).
Fifteen minutes after glucagon administration the plasma glucagon concentration increased above 1400 ng/l in all children with malaria and pneumonia (p < 0.001). There were no differences between the groups (p = 1.0). Fifteen minutes after glucagon administration the plasma insulin concentration increased in the children with malaria (p = 0.038) but not significantly in the children with pneumonia (p = 0.058). There were no differences between the groups (p = 0.326).

Fifteen minutes after glucagon administration the plasma glucagon concentration increased above 1400 ng/l in all children with malaria and pneumonia (p < 0.001). There were no differences between the groups (p = 1.0). Fifteen minutes after glucagon administration the plasma insulin concentration increased in the children with malaria (p = 0.038) but not significantly in the children with pneumonia (p = 0.058). There were no differences between the groups (p = 0.326).

**Table 3.** Plasma glucagon and insulin concentration in response to glucagon administration. Data are presented as median and range.

<table>
<thead>
<tr>
<th></th>
<th>malaria (n = 6)</th>
<th>pneumonia (n = 12)</th>
<th>p-Value</th>
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<tbody>
<tr>
<td></td>
<td>&lt; 3 years (n = 6)</td>
<td>3-5 years (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Glucagon concentration (ng/l) before glucagon bolus</td>
<td>89 (63 ; 143)</td>
<td>98 (54 ; 157)</td>
<td>0.981</td>
</tr>
<tr>
<td>Glucagon concentration (ng/l) after glucagon bolus</td>
<td>1400 †</td>
<td>1400 †</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin concentration (pmol/l) before glucagon bolus</td>
<td>7.5 (7.5 ; 19)</td>
<td>7.5 (7.5 ; 98)</td>
<td>0.371</td>
</tr>
<tr>
<td>Insulin concentration (pmol/l) after glucagon bolus</td>
<td>36 (19 ; 93) ††</td>
<td>23 (7.5 ; 312)</td>
<td>0.326</td>
</tr>
</tbody>
</table>

† p < 0.001 for comparison with glucagon concentration before glucagon bolus, †† p = 0.038 for comparison with insulin concentration before glucagon bolus
Young versus older children with pneumonia
The plasma glucagon concentration and the plasma insulin concentration at $t = 0$ were comparable in the young children and in the older children with pneumonia ($p = 0.884$ and $p = 0.270$ respectively).

The plasma glucagon concentration increased significantly 15 minutes after glucagon administration above 1400 ng/l in the young and older children with pneumonia ($p < 0.001$), no differences between the groups ($p = 1.0$). The plasma insulin concentration did not increase 15 minutes after glucagon administration in the young ($p = 0.186$) or in the older children ($p = 0.183$), no differences between the groups ($p = 0.230$).

DISCUSSION

This study shows that endogenous glucose production and plasma glucose concentration increase in response to a glucagon bolus after a 16 hour period of controlled fasting in children with severe malaria and in children with severe pneumonia. Adding the fasting period prior to the study (9 hours for the children with malaria and 7 hours for the children with pneumonia) implies that both in the children with malaria and in the children with pneumonia, hepatic glycogen stores are not completely depleted after a 23-25 hour fast. However the response was significantly larger in children with severe malaria, suggesting that after a prolonged fast, glycogen stores are larger in children with malaria than in children with pneumonia.

Second we show that the increase in EGP and plasma glucose concentration in children with severe pneumonia is not influenced by age since it is similar in the young and older children. These findings do not support our hypothesis that liver glycogen content in young children is lower than in older children.

An increase in plasma glucose of at least 50% 30 minutes after a glucagon bolus in healthy children, aged 2-6 years, following a 24 hour fast is considered normal (16). Most of the children with malaria had a normal response of plasma glucose concentration to glucagon (52%) whereas the response of the children with pneumonia was suboptimal (31%), both in the young (30%) and older (33%) children. Whether this inadequate response is specific for pneumonia is difficult to establish since only a few studies report on glucose responses to glucagon in children. Growth hormone deficient and growth retarded children showed an adequate glycemic response to glucagon after different durations of fasting (10,17). One study reports on children with infectious disease: 14 pre-pubertal HIV-infected children age 5-11 years (12 with asymptomatic HIV infection or mild disease and 2 with severe HIV infection) had a lower glycemic response to glucagon (4%) than age-matched healthy controls (91%) after a 15 hour fast (18). The present study is the first measuring both glucose concentration and production in response to
Glucagon in children 1-5 years of age with malaria and pneumonia after a period of prolonged fasting.

EGP is the sum of glycogenolysis and gluconeogenesis and glycogen stores are regulated by glycogen turnover and gluconeogenic flux into glycogen (19). Administration of glucagon leads to an increase in glucose production by stimulating both glycogenolysis and gluconeogenesis, although the initial response in hepatic glucose production is caused primarily by an increase in glycogenolysis (20). It can therefore be expected that the initial increase in glucose production we measured in the children in our study was mainly due to increased glycogenolysis.

Although glycogen stores were not depleted after the 23-25 hour fast, the response of glucose production to glucagon in the children was comparatively low. The amount of glucose released in the first hour after glucagon was 273 µmol/kg in the children with pneumonia and 539 µmol/kg in the children with malaria. Assuming a glucose utilization rate between 27 and 43 µmol/kg•min, which is the normal rate in 1 month to 6 year old healthy children (21), these amounts would be sufficient for only 6-10 minutes in the children with pneumonia and 13-20 minutes in the children with malaria. This is comparable to preterm infants receiving intravenous glucose at a low rate (25 minutes) (22), but much lower than in adults (150 min) (23). Since the liver weight/body weight ratio decreases with age (24) from approximately 5 % in preterm infants to 2.5% in adults (25) the amount of glucose released per liver mass unit in the children in this study was only about 10-20% of the amount released by healthy adults. This is also reflected by the relatively small increase in EGP: in the children with pneumonia EGP increased 1.7 fold and in the children with malaria EGP doubled after glucagon administration compared to a 2.5 fold increase in hypoglycemic patients with glycogen storage disease (26), a 4.5 increase in preterm infants (22) and a 9-fold increase in healthy adults after a 10 hour fast (23). However, the response of EGP to glucagon is influenced by the extent of the fasting period: when healthy adults are fasted for 60-72 hours, glucose production only increases one third in response to glucagon as a result of glycogen depletion (19). We therefore presume that the suboptimal response of EGP to glucagon after the 23-25 hour fast in the children with malaria and pneumonia is the result of an advanced stage of liver glycogen depletion. Since the response to glucagon is higher in the children with malaria and equal in young and older children with pneumonia, we conclude that glycogen content in the children with malaria is relatively preserved whereas it is lower in the children with pneumonia with no difference between the age groups.

Several factors could have influenced our results. Differences in regulatory hormones could alter glucose production response. There were no differences in basal plasma glucagon and insulin concentrations between the groups. Cortisol and growth hormone were not measured in this study, however we do not think these regulatory hormones will have been of influence. In contrast to the stimulating effects of glucagon and catecholamines, the stimulating effect of cortisol on hepatic glucose production takes several hours to occur (27). Growth hormone is a potent insulin-antagonist, but it takes approximately
three hours for the anti-insulin actions of growth hormone to become active (28). Second, there could be differences in the activity of glycogenolytic enzymes between the groups. This remains uncertain as we did not find any studies comparing the activity of enzymes involved in glycogenolysis between children or adults with malaria and pneumonia. The difference in response between the children with malaria and pneumonia could not be attributed to a difference in fasting duration or to a difference in the duration of illness prior to the study as these were similar in both groups. Possible differences in glucoregulatory hormone concentrations as an explanation seems unlikely for the above mentioned reasons. One explanation could be that the lower glucose production and concentration prior to glucagon administration in children with pneumonia is because of higher energy expenditure due to more intensive work of breathing and/or hypoxemia eventually resulting in a more rapid depletion of glycogen stores. Studies in critically ill children show a wide variation in individual energy requirements in different diseases and a wide range in the ratio of measured to predicted energy expenditure (29). A more likely possibility is that different infectious diseases exert different effects on the regulation of glucose metabolism at the level of enzymes or transcription factors. In a study in adults with uncomplicated malaria who were fasted for 22 hours the decrease in the rate of decline of glycogenolysis was slower than in healthy controls, despite a much lower rate of glycogenolysis in the malaria patients, indicating that the regulation of glycogenolysis in malaria is not dictated by glycogen content, but is driven by the necessity to maintain euglycemia (30).

Unfortunately we could not measure the influence of age in the children with malaria due to the small amount of subjects (three children in each group).

In conclusion, plasma glucose concentration and endogenous glucose production in response to glucagon after a 16 hour fast are higher in Surinamese children with severe malaria than in children with severe pneumonia, but there are no differences between the young and older children with pneumonia. Assuming that the response to a glucagon bolus is an indicator of glycogen content, this indicates that hepatic glycogen stores in children with pneumonia are smaller than those in children with malaria after a prolonged fast and that glycogen stores in young and older children with pneumonia are equally diminished.

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