**Infectious disease-related differences in the adaptation of glucose metabolism to fasting in children and the effect of age**

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Submitted
Chapter 7

The adaptation of glucose metabolism to fasting in young children with infectious diseases, a perspective
INTRODUCTION

Hypoglycemia is a frequently encountered complication in young children during fasting and severe illness and may result in permanent neurological damage or even death. Mortality rate in young children is increased four- to six-fold when severe infectious disease is complicated by hypoglycemia (1-9). There are certain well-recognized risk factors such as prolonged fasting, severity of disease and young age. Several epidemiological studies in children with various infectious diseases, of which malaria, pneumonia and diarrhea are particularly common, confirm the association between the occurrence of hypoglycemia and duration of fasting (1-13) with especially the younger children below the age of 3 years being at greater risk (7,9,13). Impaired endogenous glucose production (EGP) due to smaller liver glycogen stores in these younger children is presumed to be the reason for this increased risk (14,15).

Although hypoglycemia is a frequently experienced complication in clinical practice, very few studies on glucose kinetics in children under five years of age are performed in order to elucidate the underlying pathophysiological mechanism of this phenomenon. And yet the results of such studies may have significant implications for treatment of young children with severe illnesses. To explore this issue we have done studies in children with malaria and with pneumonia under five years of age during a prolonged period of controlled fasting.

Specifically, several aspects of glucose metabolism were addressed:
1. the effect of age as a risk factor for hypoglycemia in children with infectious disease (16,17).
2. the influence of duration of fasting as a determinant of glucose metabolism in children with infectious disease (17,18).
3. the possibility of differences in liver glycogen content after prolonged fasting in children with different infectious diseases (19).
4. the effect of the type of infection on different aspects of glucose metabolism (17-19).

The results of these studies are discussed and implications for clinical practice are suggested.

Feasibility, limitations and ethical considerations in studying glucose kinetics in young children

Research on glucose kinetics in young children requires special attention as to the feasibility, limitations and ethical considerations of the techniques used. EGP, gluconeogenesis and glycogenolysis can be accurately measured using stable isotope techniques. The use of stable isotope techniques in studying glucose metabolism is safe and is considered to be the golden standard. Measurements of glucose kinetics have changed the insight in the pathophysiology of glucose metabolism and have revealed differences in the regulation of glucose metabolism between adults and children.
Apart from the general restrictions that accompany research in children (20-22), an important restriction is the limited blood-sampling volume, and therefore the amount of data that can be obtained. In addition, the procedures that are applied for research purposes have to be minimally invasive in young children, precluding e.g. techniques involving catheterization of the splanchnic bed or liver biopsies. Another restriction is that for ethical reasons children cannot be exposed to long durations of fasting with the risk of hypoglycemia. Hypoglycemia requires prompt treatment thereby hindering proper study of glucose kinetics during hypoglycemia. Furthermore it is not allowed to study a matched healthy control group, as studies in humans incompetent to act for oneself are considered unethical especially as the healthy subjects involved do not directly (neither indirectly as a group) benefit from those studies.

Age as a risk factor for hypoglycemia in children with malaria and with pneumonia during fasting

Younger age is a well-recognized risk factor for hypoglycemia in healthy and in sick children. In healthy children fasting plasma glucose concentration increases progressively with age. Both in healthy children (23) and in children with ketotic hypoglycemia, who are considered to represent the lower tail of the Gaussian distribution of fasting tolerance in healthy children (15), hypoglycemia was found only in the youngest children after an overnight fast. Epidemiological studies performed on substantial groups of patients in different African countries made similar observations in children with various infectious diseases (3-7).

The increased risk for hypoglycemia in the youngest children, below the age of 3 years, was confirmed in our glucose kinetic studies of children with uncomplicated falciparum malaria (16) and with severe pneumonia (17): plasma glucose concentrations were similar in both groups at the start of the study but decreased faster during the first 8 hours of controlled fasting in children below 3 years of age than in children of 3-5 years (table 1). However, during prolongation of the fast (16 hours) the change over time was not different for age groups, indicating that after longer duration of fasting the risk of hypoglycemia concerns the older children as well. The observation that age-related differences in the rate of decline of plasma glucose exist only in the early 8 hours of fasting indicates that glucose metabolism in children younger than 3 years of age with malaria and with pneumonia adapts adequately albeit slower to fasting than in older children and is an until now not recognized phenomenon.

Since EGP was similar in both groups (table 1) it can be hypothesized that the older children, in order to maintain the plasma glucose concentration, were initially better capable of compensating for the decrease in EGP by reducing glucose utilization. Glucoregulatory hormonal responses in the young and older children differed between 8 and 16 hours of fasting: insulin concentrations decreased and norepinephrine concentrations increased in the young children whereas glucagon concentrations increased in the older children. Cortisol and epinephrine concentrations did not change in either group, concentrations
of free fatty acids (FFAs) increased in both groups. Insulin suppresses hepatic glucose production and in high concentrations stimulates peripheral glucose uptake (24), while glucagon stimulates EGP (25).

These differences in hormonal responses during fasting between young and older children can explain (at least partly) their differences in glucose kinetics. After an overnight fast, insulin concentrations decrease to basal levels resulting in a decrease in glucose uptake by insulin-dependent tissues such as resting muscle and adipose tissue, which can use FFAs for their energy supply instead of glucose (26). In order to maintain plasma glucose concentrations between 8 and 16 hours of fasting the young children decreased insulin levels while older children increased glucagon levels to prevent EGP from further decline. Furthermore, the decrease in insulin levels may have resulted in reduced peripheral glucose uptake in the young children, however, no clamp studies were performed to confirm this hypothesis. Catecholamines stimulate both glycogenolysis and gluconeogenesis, but the concentrations of norepinephrine in the young children were far below the threshold to exert an effect on EGP (27).

In conclusion, in children under five years of age there are age-related differences in the rate of decline of plasma glucose in the early 8 hours of fasting, but during prolongation of the fast this risk concerns all children. Since EGP is not different, this decline is probably explained by differences in peripheral glucose uptake. Furthermore, there are age-related differences in hormonal responses during fasting and infectious diseases which in part could be responsible for the observed differences in the early metabolic adaptation to fasting. The pathophysiology of these age-related differences in hormonal responses during fasting is an unexplored area of research.

### The influence of fasting on glucose production in children with malaria and with pneumonia

Fasting is a well-known and important risk factor for hypoglycemia in healthy children. Healthy pre-pubertal children develop hypoglycemia (plasma glucose < 3.0 mmol/l) within 24-30 hours of fasting (28,29). Numerous studies suggest that fasting significantly

### Table 1 Glucose concentration and glucose production in 40 Surinamese children 1-5 years of age with malaria and with pneumonia at the start of the study, after 8 hours and after 16 hours of controlled fasting

<table>
<thead>
<tr>
<th></th>
<th>&lt; 3 years (n = 23)</th>
<th>3-5 years (n = 17)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hr fast</td>
<td>5.7 ± 1.3</td>
<td>5.7 ± 1.4</td>
<td>0.889</td>
</tr>
<tr>
<td>8 hr fast</td>
<td>4.4 ± 0.7</td>
<td>5.1 ± 1.0</td>
<td>0.022</td>
</tr>
<tr>
<td>16 hr fast</td>
<td>3.8 ± 0.9</td>
<td>4.0 ± 1.1</td>
<td>0.546</td>
</tr>
<tr>
<td>Glucose production (µmol/kg•min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 hr fast</td>
<td>32.2 ± 8.1</td>
<td>34.1 ± 8.0</td>
<td>0.454</td>
</tr>
<tr>
<td>16 hr fast</td>
<td>27.9 ± 9.3</td>
<td>27.9 ± 8.7</td>
<td>0.980</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.
contributes to the occurrence of hypoglycemia in sick young children with the implicit suggestion that limitations in EGP are its cause. However, this suggestion is primarily based on observational studies on plasma glucose concentrations. Only a few studies measuring glucose kinetics in children with infectious disease were published: one in Ghanaian children 11 months to 10 years of age with severe malaria and three in Kenyan children aged 2-10 years with uncomplicated malaria (30-33). The period of controlled fasting in these studies varied from 2 (33) to maximum 8 (32) hours, which may be relatively short for adequate measurement of the influence of fasting on glucose metabolism.

In the recent studies performed by our group on glucose kinetics in children 1-5 years of age with severe and non-severe malaria and with severe pneumonia, the subjects were fasted for a longer controlled period of 16 hours (17,18). The predominant influence of prolonged fasting on plasma glucose concentration in children with infectious disease was stressed by combining our data on glucose kinetics of 40 children 1-5 years of age with infectious disease, (28 children with severe and non-severe malaria (18) and 12 children with severe pneumonia (17)), after a 16 hour controlled fast. Plasma glucose concentration decreased significantly over time (fig. 1) as did endogenous glucose production (EGP) (fig. 2). Plasma glucose concentration and EGP were strongly correlated (fig. 3) indicating that the implicit suggestion made in literature was correct as EGP proved to be the important determinant of the plasma glucose concentration in fasted young children with infectious disease.

These glucose kinetic studies are the first in sick children to identify EGP to be decreased after prolonged fasting in the presence of an infectious disease, which predisposes for hypoglycemia. These studies suggest that limiting EGP may be a potential therapeutic strategy for preventing hypoglycemia in sick children with infectious disease.
hypoglycemia. EGP rates were 20% lower than of age-matched children with ketotic hypoglycemia after a 16 hour fast (15). The decline in EGP during fasting is also observed in healthy adults (34) and in children with ketotic hypoglycemia (15), but does not occur

**Figure 2.** Change over time of endogenous glucose production in 40 children 1-5 years of age with malaria and with pneumonia between 8 and 16 hours of controlled fasting with an average decrease of 0.76 µmol/kg/hour, p < 0.0001. Data are means ± SEM.

**Figure 3.** The relationship between plasma glucose concentration and endogenous glucose production between 8 and 16 hours of controlled fasting in 40 children 1-5 years of age with malaria and with pneumonia, R-Square 0.542, p < 0.0001.
within the first hours of fasting, as observed in these sick children. In healthy adults, EGP declines by approximately 20% between 16 and 22 hours of fasting, with a minimal change in glucose concentration, indicating that the mechanism of preventing hypoglycemia is in adults via a reduction in peripheral utilization (34). A similar rate of decline in EGP (18%) was found in normoglycemic older children (4.2-11.5 years) with ketotic hypoglycemia while EGP declined by 32% in the youngest children (2.5-3.9 years) who all developed hypoglycemia (plasma glucose < 3 mmol/l) between 16 and 22 hours of fasting (15). This indicates that hypoglycemia in fasted young children is induced by impaired EGP and that EGP is further compromised in the presence of infectious disease.

In the young children with malaria and with pneumonia, hypoglycemia is therefore primarily induced by impaired EGP accompanied by a relative inability to compensate by restricting peripheral glucose utilization (17,18). In adults with severe malaria facilitated peripheral uptake rather than decreased production seems the most important determinant for glucose concentration since an inverse correlation between plasma glucose concentration and EGP was found (35). This mechanism was also observed in several other studies in fasted adults with malaria: in adults with uncomplicated malaria, EGP was approximately 20% higher than in healthy controls while plasma glucose concentrations were higher, but within the normoglycemic range (36,37). In adults with cerebral malaria EGP even doubled whereas plasma glucose concentrations were 40% higher than in healthy controls (38). These findings indicate that peripheral glucose uptake is sometimes facilitated in adult patients with certain infectious diseases.

In conclusion: fasting is an important risk factor for hypoglycemia in children under five years of age. Hypoglycemia in these children is induced by impaired EGP. Infectious disease is a risk factor that further compromises EGP. This is in contrast with adults with infectious disease because adults are better capable of maintaining plasma glucose concentration within normal limits. Differences in peripheral uptake is in adults the denominator for differences in glucose concentration.

The effect of the type of infectious disease on glucose metabolism in children during fasting

Comparing data on glucose kinetics of children less than five years of age with malaria (18) or pneumonia (17) shows a number of disease-related differences in the adaptation of glucose metabolism. We found that during a 16 hour fast, plasma glucose concentration and EGP differed significantly between disease groups (fig. 4 and 5): glucose concentration and EGP were higher in children with malaria than in children with pneumonia. Plasma glucose concentration was largely determined by EGP (55-60%) in both groups. The difference in EGP can be explained by differences in the concentration of the glucoregulatory hormones insulin and glucagon. In children with malaria insulin concentrations decreased and glucagon concentrations increased between 8 and 16 hours of fasting while these concentrations remained stable in the children with pneumonia. Insulin inhibits both gluconeogenesis and glycogenolysis, however, as a
Figure 4. Plasma glucose concentrations in 28 children with severe and non-severe malaria (closed symbols, mean: black line) and 12 children with severe pneumonia (open symbols, mean: dashed line) 1-5 years of age between 8 and 16 hours of controlled fasting. In the children with malaria the plasma glucose concentration was on average 1.27 mmol/l higher than in the children with pneumonia (p < 0.0001). The decrease over time was not different between diseases (p interaction = 0.795). Data are means ± SEM.

Figure 5. Endogenous glucose production in 28 children with severe and non-severe malaria (closed symbols, mean: black line) and 12 children with severe pneumonia (open symbols, mean: dashed line) 1-5 years of age between 8 and 16 hours of controlled fasting. EGP was on average 12.4 µmol/kg•min higher in the children with malaria than in the children with pneumonia (p < 0.0001). The decrease over time was not different between diseases (p interaction = 0.708). Data are means ± SEM.
result of the fall in insulin levels after fasting the insulin-induced inhibition of hepatic glucose production is less prominent (39,40). Glucagon stimulates both gluconeogenesis and glycogenolysis although the stimulation of gluconeogenesis by glucagon is delayed (41-43). Concentrations of cortisol, another potent and effective regulator of glucose production, did not differ between groups. This observation suggests the existence of infectious disease-related differences in hormonal response during fasting in young children. Whether children with critical illness acutely reduce insulin production or increase insulin clearance as an adaptive response or whether this occurs as a direct effect of a specific infectious illness such as seen in meningococcal sepsis (44,45) remains to be investigated (46).

The decrease in EGP over time was similar in the children with malaria and with pneumonia (fig. 5). EGP consists of gluconeogenesis and glycogenolysis. Consequently EGP may decrease because of insufficient gluconeogenesis due to limited precursor supply or because of dysfunctional glycogenolysis due to diminished liver glycogen stores, or both. Absolute gluconeogenesis remained unchanged in both the children with malaria and with pneumonia. The contribution of gluconeogenesis to EGP (fractional gluconeogenesis) however significantly increased in the children with malaria whereas in the children with pneumonia fractional gluconeogenesis remained unchanged. Since absolute gluconeogenesis remained unchanged, the impairment of EGP must be due to decreased glycogenolysis. This can be attributed to less available glycogen, i.e. smaller liver glycogen stores (14).

Quantification of glycogen stores can be done by liver biopsy and by using $^{13}$C- nuclear magnetic resonance spectroscopy (NMR) but the use of both techniques in children for research purposes is limited for practical and ethical reasons (47,48). 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. The response of EGP to a glucagon bolus is considered an indicator of glycogen content (49,50). We measured the change in plasma glucose concentration and EGP in response to a bolus glucagon in the children with malaria and pneumonia after a 16 hour controlled fast (19). In both the children with malaria and pneumonia the response of EGP to glucagon in the children was comparatively low. The type of disease had a significant effect since the increase in glucose concentration and EGP in response to glucagon was higher in children with malaria than in children with pneumonia. This suggests that hepatic glycogen stores in children with pneumonia were smaller (but not depleted) than those in children with malaria after a 16 hour fast. In the same study the influence of age on glycogen content was measured within the group of children with pneumonia and showed no difference in response of glucose concentration and EGP to glucagon between the children under 3 years of age and the children 3-5 years of age after such a prolonged fast (19).

These observations indicate that during prolonged fasting the type of disease has effect on glycogen content, however an influence of age within the age-group 1-5 years could not be demonstrated. The effect of type of disease may partially be explained by
differences in alterations of glucoregulatory hormone concentrations. Since EGP declined comparably in both groups and fractional gluconeogenesis increased only in the children with malaria, consequently the contribution of glycogenolysis to EGP was higher in the children with pneumonia resulting in earlier depletion of glycogen stores whereas the glycogen content in the children with malaria was relatively preserved. Glycogenolysis and liver glycogen content are correlated during fasting (51), however the potential role of glycogen content on glycogen breakdown is limited in infectious disease as it was shown that the regulation of glycogenolysis in adults with malaria is not dictated by glycogen content, but is driven by the necessity to maintain euglycemia (52). Regulation of glycogenolysis is also dictated at the level of enzymes or transcription factors (53) whereas in vitro direct cytokine effects on glycogen metabolism during sepsis are demonstrated (54), indicating once more that alterations in glycogen content are not solely due to changes in circulating levels of glucoregulatory hormones.

In conclusion, studies on glucose metabolism in young fasting children with different infectious diseases reveal disease-specific differences in the adaptation of glucose metabolism. Several assumptions can be made as to the underlying cause of these differences. First, it can be explained by disease-related differences in the hormonal response to fasting leading to changes in the contribution of gluconeogenesis and glycogenolysis to EGP. Second, differences in hepatic glycogen content may lead to earlier depletion of glycogen stores in children with certain infectious diseases thereby compromising glycogenolysis and hence EGP. Finally, the potential influence of enzymes, transcription factors and cytokines on glucose metabolism in young children with infectious disease needs to be investigated in future studies.

Implications for clinical practice

Measurements of glucose kinetics in children confirm the observations made in epidemiological studies that prolonged fasting is an important risk factor for the occurrence of hypoglycemia in young children with infectious diseases. During the first 8 hours of fasting children under 3 years of age are at greater risk for hypoglycemia, but during longer duration of the fast this risk concerns the children 3-5 years of age as well. A novel finding is that, in contrast to adults, hypoglycemia in these children is caused by limited glucose production due to restricted glycogenolysis. Unique is the observation that there are disease-related differences in the adaptation of glucose metabolism during fasting in children.

The results of these studies have implications for the approach in clinical practice towards young children with infectious diseases. Infectious diseases are often characterized by starvation due to disease-induced anorexia as well as by cultural customs and traditional habits in disease (55-57). It is therefore imperative that in an early stage of disease adequate nutritional regimes are implemented in these children in order to prevent prolonged fasting periods and thereby hypoglycemia. In practice this means that, in case enteral nutrition at home is not guaranteed, mothers and caretakers must be urged to
seek help for their child in an early stage of disease. These recommendations especially concern children under three years of age with infectious illnesses, but they can readily be applied to older children as well. Whether the prevention of hypoglycemia by early interventions will result in better outcome remains to be established.

The discussed studies on glucose kinetic are the first to reveal the existence of infectious disease-related differences in the adaptation of glucose metabolism during fasting in children. This phenomenon has not been described before although hypoglycemia is a frequently encountered complication in children and adults with various severe illnesses. Further studies on glucose kinetics in both children and adults with different infectious diseases during an objectively recorded extended fasting period are recommended.

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