Infectious disease-related differences in the adaptation of glucose metabolism to fasting in children and the effect of age
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Chapter 1

Introduction and outline
INTRODUCTION

Hypoglycemia in children is a common and serious condition that needs to be prevented because of the high risk of brain damage and mortality. There are several generally accepted risk factors for the occurrence of hypoglycemia in children among which young age, prolonged fasting and severe infectious disease are well recognized. Hypoglycemia is an important feature in children with infectious diseases (1-13), and it predicts mortality (13-16). The mortality rate increases four- to six-fold in children with infectious diseases complicated by hypoglycemia (3,5,7); particularly young children below the age of three years are at risk (2,6,11). Although hypoglycemia is a frequently occurring complication in clinical practice, the underlying pathophysiological mechanism (abnormalities in glucose production, glucose uptake or a combination) is not well explored. This thesis is aimed to unravel the derangements in glucose metabolism resulting in hypoglycemia in children with infectious diseases.

OUTLINE OF THE THESIS

Chapter 2
To stress the need for studies on glucose kinetics in children with hypoglycemia, in chapter 2 we summarized the scarce data on the effect of age and duration of fasting on glucose kinetics both in healthy children and in children with infectious diseases. Differences between adults and children are addressed, since glucose metabolism is partly regulated differently in children compared to adults.

Chapter 3
Age is an acknowledged risk factor for hypoglycemia. Studies in both healthy children (17-20) and in children with an illness (1,2,4,7,11) suggest a relationship between age and plasma glucose concentration. Several studies show that hypoglycemia is a major outcome predictor in children with falciparum malaria and it is particularly common in very young children below the age of 3 years (2,6,11,21). Even though hypoglycemia is a frequently encountered complication in clinical practice, few studies on glucose kinetics are performed in children under five years of age. Limited capacity to increase or maintain endogenous glucose production (EGP) could play a role in the pathophysiology of hypoglycemia in children with malaria. In a study of children with uncomplicated malaria and without hypoglycemia a positive correlation between EGP and plasma glucose concentration was found (22), however, other studies are lacking. Although hypoglycemia primarily occurs in children with severe malaria, the risk for hypoglycemia in young children with uncomplicated malaria may be increased in the presence of other risk factors that compromise EGP. This assumption results in the first research question:
is the risk for hypoglycemia in children with non-severe malaria increased in the presence of fasting and young age?

In chapter 3 glucose kinetics are measured in children under 3 years of age and compared with children 3-5 years of age with uncomplicated malaria after an 8 hour fast.

Chapter 4

Several studies suggest that fasting is an important factor in the occurrence of hypoglycemia in children and in adults since an association between the occurrence of hypoglycemia and the time since the last meal has been found (1,2,4,7,11,20,23,24). These studies indicate that young children probably have a higher risk for developing hypoglycemia when fasting compared to older children and adults. Since severity of infection in malaria is considered a determining risk factor for hypoglycemia (6), glucose kinetics in children with severe malaria may differ from children with non-severe malaria. The second research question therefore is: do glucose kinetics in children with severe malaria differ from children with non-severe malaria after an objectively controlled longer fasting period? In chapter 4 glucose kinetics are measured in children with severe malaria and compared with children with non-severe malaria during an objectively controlled 16-hour fasting period.

Chapter 5

Hypoglycemia does not only occur in children with malaria but is also seen in children with other infectious diseases, such as pneumonia and diarrhea (1,2,7,8,11,13,14). The mortality rate of children with other infectious diseases is similar to that of children with malaria (2-5,7,11,13). Therefore hypoglycemia may not be a disease-specific symptom but can be regarded as a serious metabolic complication in acute severe infections in young children (1,2,7,11,13,25-27). If prolonged fasting predisposes for hypoglycemia in young children with malaria, such an association may be present in young children with other critical infectious diseases such as severe pneumonia. This leads to the third research question: is prolonged fasting a risk factor for hypoglycemia in children with severe pneumonia and are very young children more at risk than older children? In chapter 5 glucose kinetics in children with severe pneumonia are studied during a controlled 16 hour fast. Children under 3 years of age are compared with children 3-5 years of age.

Chapter 6

In order to gain more insight in the underlying pathophysiological mechanism of hypoglycemia in young fasted children with infectious disease further study of EGP and its components is required. Impaired EGP caused by decreased glycogenolysis due to smaller liver glycogen stores in young children is presumed to be the reason for the increased risk of hypoglycemia (27,28). Glycogen content can be estimated by measuring the response
of EGP to a glucagon bolus (29,30), but studies evaluating such a response in children with infectious diseases are scarce. One single study reports on the response of plasma glucose concentration to a bolus glucagon in children with HIV infection, but stable isotopes were not used in this study (31). It is therefore unknown whether during fasting glycogen content is influenced by age and if there is an effect of the type of infectious disease. This leads to the fourth research question: does the response of plasma glucose concentration and EGP to a bolus glucagon differ between young and older children after a prolonged fast and is this response influenced by different infectious diseases?

In chapter 6 the responses of plasma glucose concentration and EGP to a bolus glucagon are measured in children with severe malaria and in children with severe pneumonia after a 16 hour fast. Within the group of children with pneumonia the responses are compared in children under three years of age and in children 3-5 years of age.

Chapter 7

This chapter provides an overview of current literature and the findings of this thesis on the adaptation of glucose metabolism to fasting in young children with infectious diseases. Several aspects of glucose metabolism are addressed: first, the effect of age as a risk factor for hypoglycemia in children with infectious disease. Second, the influence of prolonged fasting as a determinant of glucose metabolism in children with infectious disease. Third, differences in liver glycogen content after prolonged fasting in children with different infectious diseases. And fourth, the effect of the type of disease on glucose metabolism. Recommendations are given for the approach towards all young children with severe infectious illnesses in clinical practice in order to prevent hypoglycemia.

QUANTIFYING GLUCOSE KINETICS IN CHILDREN

Stable isotope techniques

Glucose turnover or the glucose appearance rate is the sum of endogenous glucose production and exogenous glucose supply. During fasting, in the absence of enteral or parenteral glucose supply, the rate of appearance of glucose equals endogenous glucose production. Endogenous glucose production is the sum of gluconeogenesis and glycogenolysis. By combining measurements of endogenous glucose production and either gluconeogenesis or glycogenolysis the other pathway can be calculated. Endogenous glucose production and gluconeogenesis can be measured using stable isotope techniques. Measurements of glucose kinetics have changed the insight in the pathophysiology of glucose metabolism and e.g. have revealed differences in the regulation of glucose metabolism between adults and children. Stable isotope techniques are safe and considered highly sensitive, which makes it possible to pick up even small differences between subjects.
Methods to measure endogenous glucose production

Glucose production can be measured using [6,6-\(^2\)H\(_2\)]-glucose which is a glucose molecule carrying an extra neutron on each of the 2 hydrogens on position C6 of glucose. The procedure involves a priming dose (bolus), followed by a continuous infusion of the isotope for 2-3 hours to reach a steady state. The purpose of the priming dose is to label the whole glucose pool and instantaneously reach the desired plasma ratio of labeled versus unlabeled isotope. The continuous infusion is necessary to maintain the tracer/tracee ratio for the duration of the study allowing a technically reliable measurement of glucose production. The dilution of the glucose isotope in the pool of unlabeled glucose reflects the influx of glucose i.e. the rate of appearance (32).

When steady state is reached the rate of appearance of glucose can be calculated by using steady state equations as described by Steele (33):

\[
Ra = (E_i - E_p) \times I
\]

where \(Ra\) = rate of appearance of glucose (in µmol/kg•min), \(E_i\) and \(E_p\) are the [6,6-\(^2\)H\(_2\)] enrichments of the infusate and plasma respectively, and \(I\) is the [6,6-\(^2\)H\(_2\)]glucose infusion rate (in µmol/kg•min).

When steady state is not achieved, the equation has to be modified (34):

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

where \(Ra\) = rate of appearance of glucose (in µmol/kg•min), \(F\) = [6,6-\(^2\)H\(_2\)]glucose infusion rate (in µmol/kg•min), \(E\) = enrichment of glucose with \(^2\)H (in absolute values), \(C\) = plasma glucose concentration (in mmol/l), \(t\) = time (in min) and \(pV\) = effective plasma volume of glucose which in healthy adults is estimated to be 40 ml/kg (35). In children the volume of extracellular water is calculated by normogram from body weight and height (36).

Methods to measure gluconeogenesis

Quantifying the contribution of gluconeogenesis to total glucose production is possible with techniques using stable isotopes (37). Different stable isotope methods are developed to measure the contribution of gluconeogenesis to endogenous glucose production. In the studies presented in this thesis the deuterated water (\(^2\)H\(_2\)O) method is used, a technique introduced and described in detail by Landau (38,39). This method is based on the fact that body water is the source of the hydrogens that are attached to carbon 5 and 6 of glucose in the process of gluconeogenesis (fig. 1). In addition, hydrogen from body water is added to carbon 2 in both gluconeogenesis and glycogenolysis. The ratio of the two provides the fraction of glucose derived from gluconeogenesis. After ingestion of \(^2\)H\(_2\)O, the fifth carbon of glucose (C5) is labeled with deuterium at the level of the triose phosphate isomerase reaction during gluconeogenesis and the second carbon of glucose (C2) is labeled with deuterium during both glycogenolysis and gluconeogenesis (39). Therefore the plasma C5/C2 glucose ratio is used to estimate the fraction of glucose
Figure 1. The deuterated water method to quantify fractional gluconeogenesis using incorporation of deuterium (2H) at carbon 2, 5, and 6 of glucose.

derived from gluconeogenesis. If deuterated water is ingested at least 5 hours prior to blood sampling, deuterium enrichment in plasma will equal deuterium enrichment at C2 (40) and fractional gluconeogenesis can then be calculated from the ratio between deuterium enrichment at C5 over deuterium enrichment in water. The C3/C2 ratio can
also be used to assess gluconeogenesis because the third carbon of glucose (C3) and C5 are concurrently labeled during the triose isomerase reaction (41,42).

Methods to measure glycogenolysis

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 (43,44). 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. This method is often used in clinical practice and the response of EGP to a glucagon bolus is considered an indicator of glycogen content (29,30,45,46). Glucagon stimulates glycogenolysis by activation of glycogen phosphorylase, the rate-limiting enzyme for glycogenolysis in the liver (47). The increase in glycogenolysis occurs rapidly and is strongest within 15 minutes after a selective increase in plasma glucagon concentration (48) however, this effect is transient and is not regulated by changes in plasma glucose concentration (49).
REFERENCES


