Regulators of hepatic glucose and glycogen metabolism
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Citation for published version (APA):
Sprangers, F. (2001). Regulators of hepatic glucose and glycogen metabolism
Subnormal response of plasma glucose concentration to glucagon despite adequate glycogenolysis: the importance of kinetic measurements


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Abstract

The plasma glucose concentration response to a glucagon bolus is considered an important diagnostic tool in hypoglycemia of unknown origin. The response of plasma glucose concentration to glucagon can however also be misleading in the differential diagnosis. In a three weeks old male infant suffering recurrent severe preprandial hypoglycemia and dependent of continuous i.v. glucose infusion, extensive diagnostic screening including a liver biopsy, did not lead to a diagnosis. Based on an insufficient glycemic response (twice) to a glucagon bolus, a disorder of glycogenolysis was suspected. Glucose production and gluconeogenesis were measured (glycogenolysis calculated) during diminishing i.v. glucose infusion and after a glucagon bolus. Reducing glucose infusion resulted in a steep increase in glycogenolysis and gluconeogenesis maintaining total glucose turnover (production plus infusion) constant at ± 9 mg/kg/min (±60% gluconeogenesis, ±40% glycogenolysis). Plasma glucose concentration however decreased from 4.9 mmol/l to 3.4 mmol/l. Glucagon increased glucose production by 50% but resulted in only a minor increase in glucose concentration.

Conclusion. As glucose concentration depends on the balance between glucose production and utilization (uptake), facilitated glucose uptake rather than impaired glycogenolysis explains the hypoglycemic episodes in this patient. A subnormal response of plasma glucose to glucagon therefore does not necessarily imply a disturbance in glycogenolysis. In cases of hypoglycemia of unknown origin, measurement of glucose kinetics with stable isotopes is indicated.
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Introduction

Hypoglycemia is due to a imbalance between the endogenous and exogenous supply (or production and intake) and utilization (uptake) of glucose by tissues. Since the introduction of stable (non-radioactive) isotopes, kinetic measurements of glucose metabolism have been possible in a safe and reliable way in children, and recently techniques have been developed to measure intrahepatic pathways of glucose metabolism in vivo (7,10). For ethical reasons, however, not many studies have been done in infants and children. There is no good insight into the regulation of glucose production under fasting conditions in children. In the studies that have been done, the different regimes of glucose infusion and/or feeding that were used seriously hamper the interpretation and comparability of the results: glucose production was reported to be to 2.7 mg/kg/min in one year old fasted healthy infants, 6 mg/kg/min in five year old fasted Kenyan children with uncomplicated falciparum malaria and around 2 mg/kg/min in healthy postabsorptive adults (3,5,8). In other studies glucose production was measured during variable glucose administration in different age groups; in general, suppression of endogenous glucose production occurred at glucose infusion rates above ± 7 mg/kg/min (1,9,14).

In the differential diagnosis of non-insulin induced hypoglycemia, the rise in plasma glucose concentration during a glucagon stimulation test is considered a valuable diagnostic test for evaluating the glycogenolytic pathway in the liver. A brisk glycemic response to glucagon of at least 2 mmol/l is considered to indicate intact glycogenolytic mechanisms (12). The case report below shows that a subnormal plasma glucose reaction to a glucagon bolus is not necessarily indicative of a defect in the glycogenolytic pathway in the liver.

Case report

Three weeks after a normal pregnancy and delivery a male infant developed episodes of severe and recurrent preprandial hypoglycemia (1.0 mmol/l) despite being fed every 4 h. This made him dependent on continuous glucose infusion. Physical examination was normal and extensive endocrine and metabolic screening including a liver biopsy did not provide a specific diagnosis. On two separate occasions, a glucagon stimulation test, performed during hypoglycemia, resulted in
only a minimal increase in plasma glucose concentration, suggesting a disorder in glycogenolysis. Glucose production, gluconeogenesis and glycogenolysis were measured at the age of 2 months (as shown below).

In the follow-up, the hypoglycemic tendency gradually and spontaneously disappeared and at the age of 6 months the infant was clinically and biochemically normal.

Methods and study design

The study design is shown in Fig 1. To stimulate (endogenous) glucose production and prevent hypoglycemia, glucose turnover, gluconeogenesis and glucose concentration were measured while the i.v. glucose infusion rate was reduced in a stepwise manner. Glucose production and glucose concentration, but (for technical reasons) not gluconeogenesis were also measured after a glucagon bolus.

Fig 1. Study design. Glucose turnover was measured with [6,6-2H2]-glucose from t=3 h until t=13 h and gluconeogenesis with [2-13C]-glycerol at t=6 hrs and t=9 hrs, while the glucose infusion rate was being reduced from 8 to 2 mg/kg/min in steps of 2 mg/kg/min. At t=12 hrs, glucagon (0.2 mg/kg i.v) was injected. Bloodsamples at 5 min interval (arrows).
Informed consent was obtained from both parents. At 8 p.m on the day before the study, oral feeding was withdrawn (until the end of the study), i.v. cannulas were introduced in suitable veins of the forearms (one for glucose and stable isotope infusion and one for blood sampling), and glucose infusion was started at 8 mg/kg/min. During the study, from t=0 h (8.00 a.m.) to t=13 h (9.00 p.m.), the rate of glucose infusion was diminished in steps of 2 mg/kg/min as shown in Fig. 1.

Plasma background isotopic abundance was determined at t=0 h before stable isotope infusion was started. To measure glucose turnover, a primed (3.2 mg/kg), continuous (0.04 mg/kg/min) infusion of [6,6-^2H_2]-glucose (99% enriched, Isotec Inc, Miamisburg OH) was given from t=0 h to t=13 h (4,15).

To measure gluconeogenesis a primed (35 mg/kg), continuous (0.58 mg/kg/min) infusion of [2-^13C]-glycerol (99% enriched, Isotec Inc Miamisburg, OH) was given from t=6 h to t=12 h (4,11). Both isotopes were dissolved in sterile isotonic saline, sterilized by passage through a Millipore filter (0.2 μm; Minisart, Sartorius, Göttingen, Germany), and administered by a motor-driven calibrated syringe pump (Perfusor Secura FT, Braun, Melsungen, Germany).

Three samples for determination of plasma glucose concentration, [6,6-^2H_2]-glucose enrichment, plasma insulin and glucagon concentrations were obtained at 5 min intervals at the end of each 3 h infusion period; at t=9 h and t=12 h additional blood samples enrichment were obtained for [2-^13C]-glucose. At t=12 h glucagon (Novo Nordisk Farma B.V., Alphen a/d Rijn, The Netherlands) was given (0.2 mg/kg i.v. bolus). Blood samples for plasma glucose concentration and [6,6-^2H_2]-glucose enrichment were taken during 1 h at 15 min intervals. The total volume of blood withdrawn was 20 ml.

Assays were done as previously described (4). As a steady state was reached at the end of each glucose infusion period, calculations for steady state kinetics were applied, adapted for the use of stable isotopes (4). After glucagon injection the non-steady state equations of Steele, in their derivative form, were applied (13). Endogenous glucose production was calculated as the difference between glucose turnover and glucose infusion, and glycogenolysis as the difference between endogenous glucose production and gluconeogenesis.
Results

Results are shown in Fig 2. Reducing glucose infusion from 8 to 2 mg/kg/min resulted in a steep increase in glycogenolysis and gluconeogenesis, maintaining total glucose turnover (endogenous production plus glucose infusion) constant at ± 9 mg/kg/min. Gluconeogenesis and glycogenolysis contributed ± 60% and ± 40% respectively to endogenous glucose production. Despite this constant glucose supply, plasma glucose concentration decreased steadily from 4.9 mmol/l (t=3 h) to 3.4 mmol/l (t=12 h). Between t=3 h and t=12 h glucose clearance rates increased from 10.3 to 13.3 ml/kg/min.

Between t=3 h and t=12 h, plasma insulin concentration remained low (from 36 to 14 pmol/l), plasma glucagon concentration increased (from 62 to 108 ng/l), and plasma free fatty acids rose (from 0.24 to 0.96 mmol/l). After 0.2 mg/kg i.v. glucagon bolus, endogenous glucose production increased 53% (from 6.1 to 9.4 mg/kg/min) within 30 min, and plasma glucose concentration increased by 0.3 mmol/l.

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**Fig 2. Glucose turnover, gluconeogenesis and glycogenolysis, and plasma glucose concentration before and after glucagon administration.** Glucose turnover (glucose infusion + glucose production) (black bars), glucose production (glycogenolysis + gluconeogenesis) (white bars), gluconeogenesis (triped bars), in mg/kg/min. The glucose infusion rate (mg/kg/min) is indicated in white in the bars. Plasma glucose concentrations is indicated in mmol/l above the bars. Glucagon bolus, 0.2 mg/kg i.v. (arrow).
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Discussion

The data from this two months old infant clearly show that despite glucose turnover being kept constant at 9 mg/kg/min (with normal contribution of glycogenolysis and gluconeogenesis), plasma glucose concentration decreased from 4.9 mmol/l to 3.4 mmol/l; after glucagon, glucose production increased by 53% but glucose concentration rose only 0.3 mmol/l. With no arguments in favour of an impairment in glucose production and since we know that glucose concentration results from the balance between production and uptake, the explanation for the hypoglycemia in this infant must lie in facilitated glucose uptake.

In normal infants aged 0.5-12 months, an i.v. glucagon bolus of 0.1 mg/kg increased glucose concentration by 3.3 mmol/l within 30 min (2). In 23 hypoglycemic neonates, i.v. glucagon (0.2 mg/kg) increased glucose production by 2.6 mg/kg/min and glucose concentration by 1.6 mmol/l (6). In our patient, the subnormal increase of only 0.3 mmol/l in glucose concentration 30 min. after glucagon is suggestive of a disorder in glycogen release. However, the steep increase in glycogenolysis that compensated for the diminishing glucose infusion, and the substantial rise in glucose production after glucagon, both seriously rebut this assumption.

Data in neonates and adults show that an acute increase of 50-60% in glucose production (as in our patient) will normally increase plasma glucose concentrations by at least 30-55% (3,6). As glucose concentration decreased despite a constant glucose turnover rate and increased only slightly after glucagon, we believe that the explanation for the hypoglycemia in this infant lies in facilitated glucose uptake rather than in impaired glucose production. This imbalance as a cause of childhood hypoglycemia has been described before (2).

The cause of this facilitated glucose uptake remains unclear. Glucose uptake is known to be facilitated by high insulin concentrations, or by lack of growth hormone and cortisol, neither of which is the case in this patient. The fact that the hypoglycemic tendency had spontaneously disappeared by the time the infant was 6 months old does not provide us with any additional pathophysiological insight into hypoglycemia in this infant based on current knowledge.
In conclusion, a subnormal increase in plasma glucose concentration in response to glucagon does not necessarily imply a disturbance in glycogenolysis. Measuring glucose concentration without measuring glucose kinetics may lead to misleading conclusions concerning hypoglycemia of unknown origin.

Acknowledgments

We are indebted to An Ruiter and the other technicians of the Endocrinology Laboratory for their assistance and pleasant collaboration.

References

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Chapter 6