ORIGINAL ARTICLE

Glucose and leucine kinetics in idiopathic ketotic hypoglycaemia

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Aims: To investigate glucose and leucine kinetics in association with metabolic and endocrine investigations in children with ketotic hypoglycaemia (KH) in order to elucidate the underlying pathophysiology.
Methods: Prospective interventional study using stable isotope tracer in nine children (mean age 4.23 years, range 0.9–9.8 years; seven males) with KH and 11 controls (mean age 4.57 years, range 0.16–12.3 years; four males).
Results: Plasma insulin levels were significantly lower in KH compared to subjects in the non-KH group. Plasma ketone body levels were significantly higher in KH than in non-KH. Basal metabolic rate was significantly higher in subjects with KH (45.48 ± 7.41 *v* 31.81 ± 6.72 kcal/kg/day) but the respiratory quotients were similar in both groups (KH *v* non-KH, 0.84 ± 0.05 *v* 0.8 ± 0.04. Leucine oxidation rates were significantly lower in children with KH (12.25 ± 6.25 *v* 31.96 ± 8.59 μmol/kg/h). Hepatic glucose production rates were also significantly lower in KH (3.84 ± 0.46 *v* 6.6 ± 0.59 mg/kg/min).
Conclusions: KH is caused by a failure to sustain hepatic glucose production rather than by increased glucose oxidation rates. Energy demand is significantly increased, whereas leucine oxidation is reduced.

Symptomatic hypoglycaemia during ketosis in children, either induced by fasting or a ketotic diet, has been recognised since the 1920s.[1 2] Ketotic hypoglycaemia or idiopathic hypoglycaemia (KH) is the most common form of hypoglycaemia beyond infancy.[3] However, despite numerous efforts to elucidate the underlying pathophysiology, its aetiology remains unclear.[4–7] Typically, children with KH present between 18 months and 7 years of age with recurrent episodes of hypoglycaemia and ketonuria during an intercurrent illness. Seizures may occur, but neurological sequelae are rare. Hormonal counter-regulation remains intact, and diagnostic work-up, including work-up for defects of ketolysis, does not yield any significant abnormalities. Hypoglycaemia responds promptly to oral or intravenous glucose administration. KH improves with age and is rarely observed after puberty.

Ketone bodies are derived from oxidation of fatty acids or certain amino acids (leucine, lysine, and tryptophan) in the liver and transported to other organs such as the brain, heart, skeletal muscle, adrenal cortex, and kidneys.[8] During ketosis the two main ketone bodies, acetoacetate and 3-hydroxybutyrate, are present in increased concentrations physiologically following fasting, after prolonged exercise, and during consumption of a high fat diet.[9–11] Moderate ketosis may also be present during pregnancy, the neonatal period, and infancy as part of physiological adaptation.[11] In addition, ketosis may be found in association with decompensation in diabetes mellitus, corticosteroid or growth hormone deficiencies, intoxication with alcohol or salicylates, rare inborn errors of ketolysis, maple syrup urine disease, and organic acidopathies.[7 8 13–15]

Earlier studies of KH suggested that there was a functional defect of hepatic glucose production via gluconeogenesis or a lack of gluconeogenic substrates, including alanine.[6 7] As part of the counter-regulatory mechanism, glucagon causes a modest increase in blood glucose levels during hypoglycaemia and an additional decline in plasma alanine levels. Hypoalaninemia in KH, which may be secondary, is only restored by administration of alanine or prevented by administration of cortisone acetate.[6] In addition, some patients with KH are known to have increased concentrations of branched chain amino acids during hypoglycaemia, possibly secondary to hypoinsulinism.

KH may be regarded as a disorder of glucose homoeostasis in which glucose utilisation exceeds glucose production. In this context ketosis may represent a physiological response to hypoglycaemia rather than a pathological event.[4 5] However, in addition, ketones do not appear to be utilised normally, particularly by the brain. Data on glucose and leucine kinetics are, to our knowledge, not yet available. Consequently we devised a study in a group of children with KH following a fast, using stable isotopes to measure glucose and leucine kinetics. In addition, basal metabolic rate (BMR) and respiratory quotient (RQ) were measured; all results were compared to another group of children having a diagnostic fast. The hypothesis is that there is a failure of both ketone utilisation and of the increase in glucose production during illness and fasting

METHODS

Subjects

Nine children with KH were studied. All had a past medical history of at least two episodes of symptomatic documented hypoglycaemia following a period of fasting or during intercurrent illness. During hypoglycaemia, ketonuria of 3+ on dipstick was documented; hypoglycaemia resolved rapidly and completely with glucose administration. In addition, all

Abbreviations: BMI, body mass index; BMR, basal metabolic rate; GC-MS, gas chromatography-mass spectrometry; KH, ketotic hypoglycaemia; KIC, ketoisocaproic acid; NEFA, non-esterified fatty acids; NHS, National Health Service; RQ, respiratory quotient; MAT, mitochondrial acetoacetyl CoA thiolase; MSUD, maple syrup urine disease; SCOT, succinyl CoA oxoacid transferase; SD, standard deviation

Table 1 Anthropometric measurements

Subjects	Age (mean (range))	Gender	Weight (kg)	Height (m)	BMI
KH (n = 9)	4.23 (0.9–9.8 y)	7 M/2F	17.8 ± 5.5	0.99 ± 0.13	16.42 ± 1.42
Controls (n = 11)	4.57 (0.16–12.3 y)	4 M/7F	18.9 ± 12.1	1.1 ± 0.28	17.1 ± 1.76

Weight and height are expressed as mean ± SD. BMI (body mass index) was calculated as weight/height^2.

children with KH had had an unremarkable diagnostic work-up in the past that included urinary organic acid, plasma acylcarnitine, intermediate metabolites, and hormone analysis, without identifiable pathology.

Table 1 summarises age, gender, and anthropometric data of the children. There was no statistically significant difference in age, height, weight, or body mass index (BMI) between those with KH and a group of 11 children who were referred to Great Ormond Street Hospital for a diagnostic fast. Indications included recurrent episodes of documented or suspected hypoglycaemia without fulfilling the diagnostic criteria for ketotic hypoglycaemia (n = 9), recurrent ketoacidosis (n = 1), or suspicion of fatty acid oxidation defect (n = 1). There were more males in the KH group than in the non-KH group (7/9 versus 4/11).

Protocol

All children were admitted to the programmed investigation unit at Great Ormond Street Hospital NHS Trust and fasted according to previously published guidelines.[16] In particular the maximum fasting period was defined prior to the start of the fasting period.[16] Two cannulas were inserted, one in the cubital fossa for tracer infusion (see below), the other in a contralateral superficial hand vein for blood sampling. Blood for intermediate metabolites, hormones, amino acids, acylcarnitine profile, and carnitine concentrations was drawn at specified times.[16] Blood glucose was measured at hourly intervals or more frequently where appropriate. The fast was stopped when symptoms of hypoglycaemia occurred, when the blood glucose dropped below 2.6 mmol/l, or when the fast exceeded the specified duration.[16]

Organic acids were analysed in a urine sample at the end of the fast by gas chromatography-mass spectrometry (GC-MS). Plasma NEFA levels were measured using a kit (Wako Chemicals 99475409). Glucose concentrations were measured using a Kodak Ektachem analyser. Lactate and 3-hydroxybutyrate concentrations were measured using kits (Sigma 826B; Randox Laboratories 123530 respectively). Plasma pyruvate and acetoacetate concentrations were analysed by a centrifugal analyser with a fluorimetric attachment as reported previously.[17] Plasma cortisol and insulin concentrations were measured using kits (Abbott 9116-60 and 2410-20). Growth hormone was measured by an immunoradiometric assay (NETRIA). Total and free carnitine levels were measured by a radiochemical enzymatic assay.[17] Acylcarnitine profile was analysed using tandem mass spectrometry. Plasma amino acids were analysed by a LKB Biochrom 4400 automated amino acid analyser (Milton Keynes, UK).

A primed continuous infusion of 1-^{13}C leucine (0.5 mg/kg/h) and (6,6)-D_2 glucose (3 mg/kg/h) was started for 4 hours at the beginning of the final 4 hours during the fast; 0.5 mg/kg of 1-^{13}C bicarbonate, 0.5 mg/kg of 1-^{13}C leucine, and 3 mg/kg of (6,6)-D_2 glucose were given as a prime dose just prior to the start of the infusion. Leucine kinetics were measured in 9/9 children with KH and 6/11 controls. Glucose kinetics were measured in 7/9 children with KH and 9/11 controls as previously reported.[18 19] Blood and breath samples for isotopic enrichment were taken at baseline and every 15 minutes throughout the final 2 hours of the infusion. Blood was transferred to heparinised tubes, spun immediately, and the plasma stored at −70°C until analysis. Analysis for plasma isotopic enrichment of glucose and ketoisocaproic acid (KIC) was done by GC-MS as previously reported.[19 20] Isotopic enrichment of CO_2 was measured by isotope ratio mass spectrometry. Leucine and glucose kinetics were calculated using plasma isotopic enrichment of 1-^{13}C leucine and (6,6)-D_2 glucose and carbon dioxide production as described in more detail elsewhere.[18 19]

Indirect calorimetry (Deltatrac I, Datex, Finland) was used to measure basal metabolic rate and respiratory quotient under standardised conditions as described previously.[20]

Statistical significance was assumed at a level of $p < 0.05$ using Student's *t* test. All data were analysed using a standard software package (Microsoft Office 2000) run on a Hewlett Packard N5340 laptop computer.

The study was approved by the Joint Research Ethics Committee, and parents of all participants gave written informed consent. Where appropriate, the participants consented as well.

RESULTS

The length of the fast was 16 ± 2.28 hours (mean ± SD) in children with KH and 14.47 ± 5.58 hours in control subjects (not significant). Three children with KH became hypoglycaemic (glucose ⩽2.6 mmol/l) during the fast, while only one child from the control group became hypoglycaemic. Blood glucose levels at the beginning of the fast were 4.01 ± 0.43 in KH versus 4.54 ± 0.34 mmol/l in controls ($p < 0.05$) compared to 3.08 ± 0.40 in KH versus 3.60 ± 0.69 mmol/l in controls at the end of the fast. Plasma ketone body, non-esterified fatty acid (NEFA), insulin, cortisol, and growth hormone levels at the end of the fast are shown in table 2.

Plasma total and free carnitine levels as well as acylcarnitine species in both groups were within normal limits (data not shown). Plasma alanine concentrations were below our normal range in three subjects with KH, but normal in controls (data not shown). Branched chain amino acid levels were elevated in four subjects with KH but normal in controls (data not shown). Organic acid analysis was unremarkable, with an appropriate ketone response in all but one patient who showed excretion of keto acids.

Table 2 Plasma levels of glucose, metabolites, and hormones in children with KH compared to control subjects at the end of the fast

	End of fast	
	KH	Control
Glucose (mmol/l)	3.08 ± 0.40	3.60 ± 0.69
Insulin (mU/l)	0.875 ± 0.25*	3.55 ± 3.04*
Lactate (mmol/l)	0.85 ± 0.18	0.87 ± 0.55
Pyruvate (mmol/l)	0.05 ± 0.004	0.047 ± 0.023
Acetoacetate (mmol/l)	0.60 ± 0.14**	0.20 ± 0.15**
3-hydroxyutyrate (mmol/l)	2.14 ± 1.01*	0.82 ± 0.49*
NEFA (mmol/l)	1.40 ± 0.40	1.50 ± 0.95
Cortisol (nmol/l)	542.25 ± 208.9	410.50 ± 421.2
GH (mU/l)	11.83 ± 4.72	16.33 ± 9.98

*$p < 0.05$, **$p < 0.006$.
NEFA, non-esterified fatty acids; GH, growth hormone.

What is already known on this topic

- KH is the most common form of hypoglycaemia in infancy
- KH is probably a disorder of glucose homoeostasis in which glucose utilisation exceeds glucose production and secondary increases in ketone body concentrations

What this study adds

- The first in vivo data on leucine and glucose kinetics in KH is presented
- KH is caused by a failure to sustain hepatic glucose production rather than by increased glucose oxidation rates; energy demand is significantly increased, whereas leucine oxidation is reduced

Plasma insulin levels were significantly lower at the end of the fast ($p < 0.05$) in KH compared to control subjects. Plasma ketone body levels were significantly higher in KH (acetoacetate, $p < 0.006$; 3-hydroxybutyrate, $p < 0.05$) than in controls (table 2).

Basal metabolic rate was significantly higher in subjects with KH compared with control subjects (45.48 ± 7.41 *v* 31.81 ± 6.72 kcal/kg/day, $p < 0.02$), although the respiratory quotients were similar in both groups (0.84 ± 0.05 *v* 0.8 ± 0.04). Hepatic glucose production rates were significantly lower in KH than controls (3.84 ± 0.46 *v* 6.6 ± 0.59 mg/kg/min, $p < 0.0005$). Leucine oxidation rates were significantly lower in children with KH compared to controls (12.25 ± 6.25 *v* 31.96 ± 8.59 μmol/kg/h, $p < 0.05$).

DISCUSSION

Ketosis is part of the physiological response to fasting or pregnancy, or a result of a high fat (ketogenic) diet.[9–12] It may also occur during decompensation of diabetes mellitus, panhypopituitarism with secondary growth hormone and corticosteroid deficiencies, intoxication with alcohol or salicylates, or in children with KH.[3 9 14–16] Rare causes include maple syrup urine disease and inborn errors of ketolysis, such as succinyl CoA:3oxoacid CoA transferase (SCOT) and mitochondrial acetoacetyl CoA thiolase (MAT) deficiencies.[9]

KH is the most common form of hypoglycaemia during infancy and childhood.[3] The typical patient is male and between the ages of 2 and 7 years. This age distribution is also reflected in the patient population which was studied here. These children usually present with hypoglycaemia associated with ketonuria during an intercurrent illness associated with fasting.[4] Hypoglycaemia responds promptly to glucose administration. Seizures secondary to hypoglycaemia may occur, but neurological sequelae are very rare. In addition, hypoglycaemia may reoccur and cataracts may develop in some patients.[6] KH typically improves with age and is usually not observed after children reach puberty. Diagnostic work-up excludes other causes of ketosis as outlined above.

The pathophysiology of KH is largely unknown. Some evidence suggested a disorder of hepatic glucose production, specifically a functional defect of gluconeogenesis secondary to a lack of gluconeogenic substrates, particularly alanine.[7 8] However, only three of our subjects with KH had low plasma alanine levels. Other studies indicated a failure of the adrenergic response during episodes of KH.[21] Based on a simple model, KH may be regarded as an imbalance of energy supply and demand during episodes of increased metabolic stress. Surprisingly, few data are available for glucose and leucine kinetics or basal metabolic rate. We have studied a group of children with KH and compared the data to a group of children of similar age, height, weight, and BMI using stable isotopes.[22–24]

Hepatic glucose production rates were significantly lower in KH, reflecting a failure of glucose supply as the main cause of ensuing hypoglycaemia. Obviously, this may be secondary to depletion of glycogen stores and/or failure of gluconeogenesis. As all tracer studies were done in steady state, glucose production equalled glucose utilisation rates excluding increased glucose oxidation rates as the cause of hypoglycaemia. However, there was little evidence to suggest the failure of gluconeogenic substrate supply as plasma alanine levels were only lower in three subjects with KH.

While the BMR was significantly elevated in subjects with KH by about 25% compared to controls, the respiratory quotient was comparable in both groups. The increase of BMR is not due to differences in BMI between the two groups but may be secondary to differences in intermediary metabolism. One explanation might be enhanced lipolysis for ketogenesis in KH which in part is reflected by increased ketone body concentrations in KH. However, this hypothesis is not supported by the respiratory gas exchange measurements as the RQs were similar in both groups. Both were oxidising similar substrates and glucose oxidation still exceeds fatty acid oxidation in both groups. This is also consistent with our hypothesis that children with KH fail to utilise ketone bodies.

Surprisingly leucine oxidation rates were significantly lower in KH compared to controls. The rates were comparable to those previously reported in children with intermediate maple syrup urine disease (MSUD).[24] This might explain the observation of raised concentrations of branched chain amino acids in KH during hypoglycaemia, but the corresponding ketoacids are not found as would be expected in maple syrup urine disease. The explanation for these findings is yet unknown.

In summary, we have shown that KH is caused by a failure to sustain sufficient hepatic glucose production rather than by increased glucose oxidation. Ketone utilisation does not explain the difference and hence the neurological symptoms. Surprisingly, leucine oxidation is reduced while energy expenditure is significantly increased. This may be of pathophysiological relevance and warrants further investigation.

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ARCHIVIST

Epidemiology of SIDS: then and now

Since the ''Back to Sleep'' campaign in the UK fifteen years ago, in which parents were advised to put their babies down to sleep on their backs, the number of deaths from sudden infant death syndrome (SIDS) in England and Wales has fallen by 75%. A consequence of this seems to have been that socioeconomic deprivation, which has always been a factor increasing the risk of SIDS, has become even more prominent. A study in the county of Avon (Peter S Blair and colleagues. *Lancet* 2006;**367**:314–9; see also Comment, ibid: 277–8) has illustrated this change and pointed also to the danger of sleeping with an infant on a sofa.

Since 1984 paediatricians in Avon have been notified of all sudden unexpected deaths in infancy (SUDI); and have interviewed the family soon after the death. An autopsy has been performed by a paediatric pathologist and the cause of death classified by a multidisciplinary group. Over a period of 20 years data were collected about 369 cases of SUDI, of which 300 were unexplained (SIDS). There were 158 cases of SIDS in 1984–88 and 36 in 1999–2003. The proportion of SIDS families in social classes IV or V or unemployed was 47% in 1984–88 and 74% in 1999–2003. In a control population in 1993–96 this proportion was 17%. Before 1991 28% of SIDS families had no income from employment; after 1991 this figure rose to 48%. The proportion living in the lowest 10% of areas for social deprivation rose from 23% in 1984–88 to 48% in 1999–2003. The prevalence in SIDS families of factors associated with social deprivation also rose: single mothers from 15% to 40%, mothers under 20 years old from 7% to 16%, maternal smoking in pregnancy from 57% to 86%. The proportion born preterm rose from 12% to 34%, multiple births from 2% to 8%, families of more than three children from 14% to 36%, and first children from 18% to 33%. The proportion of mothers who had tried to breastfeed fell from 50% to 26%.

The proportion of infants who had been in bed with their parents at the time of death rose from 11% to 39%. The absolute numbers of infants dying in these circumstances has, however, fallen so the increased proportion may, at least in part, be due to the fall in SIDS rate among infants sleeping alone. Between 1984 and 1991 two infants had died whilst sleeping with a parent on a sofa; between 1992 and 2003 nine infants died in these circumstances (four of 36 SIDS deaths in 1999–2003).

SIDS is now even more strongly associated with social deprivation. Parents should be warned against sleeping with infants in their bed and particularly against sleeping with an infant on a sofa.