An inadequate glycaemic response to glucagon is linked to insulin resistance in preterm infants?

L Jackson, A Burchell, A McGeechan, R Hume

Aims: To define clinical, metabolic, and hormonal characteristics of preterm infants relative to glucagon responsiveness.

Methods: Two phase study of 78 preterm infants (25–36 weeks gestation) on regular four hourly feeds anticipating discharge home at 36 weeks mean corrected gestation. In phase 1 infants were fasted until hypoglycaemic, or maximally for eight hours. Endocrine and metabolic profiles were obtained at completion. Phase 2 was performed the following day. A feed was omitted and replaced by a bolus dose of intravenous glucagon (100 µg/kg). Main outcome measures were measurements of blood glucose and lactate concentrations, taken immediately pre-glucagon, and thereafter every 15 minutes for 60 minutes. A rise in glucose concentration of >1 mmol/l (55 infants) was defined as an adequate response to glucagon. An inadequate glycaemic response was <1 mmol/l (23 infants).

Results: Several differences in fasting blood glucose and hormone concentrations were identified in infants with an inadequate glycaemic response to glucagon compared to those with an adequate response: relative fasting hyperglycaemia (mean 3.7 v 3.3 mmol/l, p = 0.008); fasting hyperinsulinaemia (mean 4.3 v 2.6 mU/l, p = 0.014); an increased insulin:glucagon ratio (0.19 v 0.11, p = 0.014), and a lower insulin sensitivity QUICKI index (0.19 v 0.22, p = 0.04). There was no distinctive phenotype to reliably predict response to glucagon.

Conclusion: Some preterm infants show an inadequate glycaemic response to glucagon and have features suggestive of insulin resistance. The potential long term implications of such insulin resistance may have appreciable public health consequences.

SUBJECTS AND METHODS

We recruited a consecutive series of preterm infants (78 infants, 25–36 weeks gestation) who were admitted to the Neonatal Intensive Care Unit, Ninewells Hospital, Dundee and who survived to discharge home at 36 weeks mean corrected gestation. There were no exclusion criteria (table 1). Infants were on no medications apart from iron and vitamin supplements, which are given routinely in this hospital. The study protocol was approved by the Tayside Medical Research Ethics Committee. Informed written parental consent was obtained.

Extensive epidemiological data were collected on each infant; these included maternal health, both generally and in pregnancy, as well as any prescribed drugs in pregnancy; disorders of pregnancy; details of delivery and resuscitation; neonatal morbidity; and any medications prescribed.

An extensive range of anthropometrical measurements was obtained at the time of the study. Birth weight ratios and study weight ratios were calculated for each infant using reference values attained from the Scottish Morbidity Record...
Glycaemic response to glucagon in preterm infants

**Table 1 Clinical characteristics of infants studied**

<table>
<thead>
<tr>
<th></th>
<th>Adequate glycaemic response group (n=55)</th>
<th>Inadequate glycaemic response group (n=23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.69 (0.05)</td>
<td>1.61 (0.09)</td>
<td>0.46</td>
</tr>
<tr>
<td>Birth weight ratio</td>
<td>0.88 (0.02)</td>
<td>0.93 (0.04)</td>
<td>0.19</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>32.6 (0.35)</td>
<td>31.6 (0.47)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>At study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected gestation</td>
<td>36.6 (0.26)</td>
<td>36.7 (0.35)</td>
<td>0.72</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>2.21 (0.04)</td>
<td>2.27 (0.05)</td>
<td>0.42</td>
</tr>
<tr>
<td>Study weight ratio</td>
<td>0.79 (0.01)</td>
<td>0.81 (0.02)</td>
<td>0.68</td>
</tr>
<tr>
<td>Occipito-frontal circumference (cm)</td>
<td>32.0 (0.56)</td>
<td>32.8 (0.22)</td>
<td>0.39</td>
</tr>
<tr>
<td>Crown-heel length (cm)</td>
<td>44.7 (0.25)</td>
<td>44.7 (0.39)</td>
<td>0.78</td>
</tr>
<tr>
<td>Sum of skinfold thickness (mm)</td>
<td>23.3 (0.62)</td>
<td>23.5 (1.02)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

An adequate glycaemic response to glucagon >1 mmol/l; inadequate response <1 mmol/l increment in blood glucose.

Results expressed as mean (SEM).

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Commercial immunological assay kits were used to measure the following: cortisol (dl 5.5 mmol/l, cv 4.5%), Chiron; insulin (dl 1.0 mU/l, cv 5%), Abbott Laboratory; glucagon (dl 1.5 pmol/l, cv 10%), Diagnostic Products Corporation; human growth hormone (dl 0.01 mU/l, cv 6%), Diagnostic Products Corporation.

Fasting insulin (I) and glucose (G) values were fitted to the Quantitative Insulin Sensitivity Check Index (QUICKI) equation (QUICKI = 1/log(I1) + log(G1)) to assess insulin sensitivity in this cohort of preterm infants. Statistical analysis was performed using SPSS for Windows (release 10.0, SPSS Inc., Chicago, Illinois, USA). Normally distributed data were analysed using parametric tests. Non-normal data were analysed using non-parametric tests. Statistical significance was taken at p < 0.05.

**RESULTS**

Fifty five infants had an adequate glycaemic response to glucagon (>1 mmol/l), and 23 an inadequate response (<1 mmol/l) at initial testing at 36 weeks mean corrected gestation. The infants with an inadequate glycaemic response had lower blood glucose values and higher lactate concentrations at 30 minutes following glucagon stimulation compared to the infants with an adequate glycaemic response (table 2).

No factors relating to antenatal health, pregnancy, delivery, or postnatal morbidity influenced the infants' response to glucagon stimulation between the two groups. In particular, there was no relation between antenatal steroid exposure and response to glucagon. No differences were identified in anthropometric measurements, or in ponderal indices, placental:birth weight ratios, or in combined skin fold thicknesses (table 1).

Differences in blood glucose and hormone concentrations, obtained from the controlled fast in phase 1 of the study, were observed between infants with an inadequate and adequate glycaemic response to glucagon. Infants with an inadequate response compared to those with an adequate response had the following: relative fasting hyperglycaemia (3.7 v 3.3 mmol/l, p = 0.008); fasting hyperinsulinaemia (4.3 v 2.6 mU/l, p = 0.014); an increased insulin:glucagon ratio (0.19 v 0.11, p = 0.014), and a lower QUICKI index (0.19 v 0.22, p = 0.04) indicating reduced insulin sensitivity (table 3). Human growth hormone concentrations were higher in infants with an adequate glycaemic response but there were no differences in β-hydroxybutyrate, cortisol, or glucagon concentrations (table 3).

Retrospective review of case records showed an increased frequency of biochemical hypoglycaemia in the early postnatal period in the group with the inadequate response to glucagon (7.76 v 4.02 episodes, p = 0.017).

The glucagon stimulation test was repeated at 40 weeks gestation corrected age in the 23 infants who had an...
DISCUSSION

In one third of preterm infants, at a corrected gestation of 36 weeks, hepatic glucose production in response to exogenous glucagon was inadequate. Our arbitrary cut off for an adequate glycaemic response (>1 mmol/l) was defined early in the study in an attempt to identify infants clinically most at risk of hypoglycaemia post-discharge home. The response to glucagon in preterm infants is under explored and no previous work to determine population norms for this group was available to otherwise determine a grouping strategy. Previous studies have been limited in actual number, and confined predominantly to term infants, particularly those who were growth restricted. In a group of 11 infants with comparable gestational age to our group of infants at first test (36 weeks gestation) but at a median age of 24 hours postnatal age, a different pattern of response was evident to our testing protocol, or merely reflected variable development between infants. A similar conclusion can be made for the mechanisms underlying the induction of key gluconeogenic and glycogenolytic enzymes. All infants identified in our study with an inadequate response to glucagon eventually developed an adequate glycaemic response to glucagon. Previous studies in infants 0.5–12 months of age have shown a mean glycaemic response of 3 mmol/l in response to glucagon. We do not know whether these maturational patterns in our infants were influenced by the repeated doses of glucagon inherent in our testing protocol, or merely reflected variable development between infants. A similar conclusion can be made for the subgroup of infants initially identified as hypoglycaemic on fasting, where glycaemic control improved with time, as all infants had at least a single dose of prescribed glucagon.

The first phase of this study showed that infants with a hypoglycaemic tendency on fasting had lower birth weights, and study weights, in spite of a higher postnatal energy intake, than normoglycaemic infants. In infants where hypoglycaemia was severe and/or persistent, concentrations of cortisol, corticotropin, and blood lactate were higher than those in infants with transient hypoglycaemia, or normoglycaemia. Low birth weight is associated with increased urinary cortisol and metabolites in children, and in adult men with higher plasma cortisol, increased cardiovascular risk, and impaired glucose tolerance. The mechanisms underlying the inadequate response to the initial test. Eighteen infants had an adequate glycaemic response (>1 mmol/l). Five infants had an inadequate response (<1 mmol/l) to this second glucagon stimulation test, but had an adequate glycaemic response (>1 mmol/l) to a third glucagon stimulation test at 3 months corrected postnatal age.

Table 2 血糖和乳酸浓度在反应到促胰高血糖素

<table>
<thead>
<tr>
<th></th>
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<th>Inadequate glycaemic response group (n=23)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>Blood glucose (mmol/l)</td>
<td>Blood glucose (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.25 (0.07)</td>
<td>3.61 (0.12)</td>
<td>0.08</td>
</tr>
<tr>
<td>15</td>
<td>4.40 (0.10)</td>
<td>4.00 (0.11)</td>
<td>0.02</td>
</tr>
<tr>
<td>30</td>
<td>4.91 (0.10)</td>
<td>4.14 (0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>45</td>
<td>4.60 (0.12)</td>
<td>3.80 (0.12)</td>
<td>0.01</td>
</tr>
<tr>
<td>60</td>
<td>3.90 (0.11)</td>
<td>3.32 (0.14)</td>
<td>0.03</td>
</tr>
<tr>
<td>Time (min)</td>
<td>Blood lactate (mmol/l)</td>
<td>Blood lactate (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.15 (0.06)</td>
<td>1.32 (0.16)</td>
<td>0.09</td>
</tr>
<tr>
<td>15</td>
<td>1.29 (0.06)</td>
<td>1.55 (0.09)</td>
<td>0.012</td>
</tr>
<tr>
<td>30</td>
<td>1.10 (0.04)</td>
<td>1.45 (0.12)</td>
<td>0.01</td>
</tr>
<tr>
<td>45</td>
<td>1.19 (0.05)</td>
<td>1.31 (0.10)</td>
<td>0.015</td>
</tr>
<tr>
<td>60</td>
<td>1.03 (0.05)</td>
<td>1.17 (0.10)</td>
<td>0.08</td>
</tr>
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An adequate glycaemic response to glucagon >1 mmol/l; inadequate response <1 mmol/l increment in blood glucose.

Results expressed as mean (SEM).

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</thead>
<tbody>
<tr>
<td>Cortisol (nmol/l)</td>
<td>206 (20)</td>
<td>192 (32.9)</td>
<td>0.72</td>
</tr>
<tr>
<td>Glucagon (pmol/l)</td>
<td>29 (1.7)</td>
<td>25 (2.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Growth hormone (mU/l)</td>
<td>53 [3.9]</td>
<td>37 [4.7]</td>
<td>0.025</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>2.6 [0.29]</td>
<td>4.3 [0.76]</td>
<td>0.014</td>
</tr>
<tr>
<td>Insulin:glucagon ratio</td>
<td>0.11 (0.01)</td>
<td>0.19 (0.03)</td>
<td>0.014</td>
</tr>
<tr>
<td>QUICKI score</td>
<td>0.22 (0.007)</td>
<td>0.19 (0.009)</td>
<td>0.04</td>
</tr>
<tr>
<td>β-Hydroxybutyrate (mmol/l)</td>
<td>0.11 (0.012)</td>
<td>0.15 (0.019)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

An adequate glycaemic response to glucagon >1 mmol/l; inadequate response <1 mmol/l increment in blood glucose.

Results expressed as mean (SEM).

QUICKI score: Quantitative Insulin Sensitivity Check Index equation (QUICKI = 1/[log(Io) + log(Go)]).

Table 3 表3 表2 摘要
association of type 2 diabetes, raised blood pressure, and dyslipidaemia (that is, the metabolic or insulin resistance syndrome) with their occurrence is not yet known. Epidemiological studies have shown that low birth weight predicts subsequent insulin resistance, glucose intolerance, hypertension, and cardiovascular disease. Events in early life may have long-term effects on the hypothalamic-pituitary-adrenal axis, and exposure of pregnant rats to adverse influences during gestation results in the birth of small offspring with hypertension, insulin resistance, and increased cortisol secretion. Although our infants had severe growth restriction, and raised cortisol concentrations, there was no evidence of insulin resistance and hyperglycaemia, but on the contrary hypoglycaemia on admission of a regular feed as the dominant clinical problem.

It was surprising in phase 2 of the study to find that infants selected on the basis of a glucagon stimulation test as showing an inadequate glycaemic response were no different in growth at birth, or at the time of study, or in plasma cortisol concentrations, from those infants with an adequate glucagon response. There was no distinctive phenotype, which could be used to reliably predict response to glucagon. In addition, infants with an inadequate glycaemic response had higher fasting blood glucose concentrations, insulin concentrations, and insulin:glycogen ratios, and lower QUICKI index values suggestive of insulin resistance. Current methods are limited to assess insulin sensitivity in preterm infants. The hyperinsulinemic euglycaemic clamp technique is the “gold standard” for quantifying insulin sensitivity in vivo because it directly measures the effects of insulin to promote glucose utilisation under steady state conditions. The glucose clamp technique is difficult to perform in preterm infants, as it is the alternative of a minimal model analysis of a frequently sampled intravenous glucose tolerance test (FSIVGTT). More recently a simpler method based on fasting glucose and insulin concentrations, the Quantitative Insulin Sensitivity Check Index (QUICKI), has been shown to correlate well with glucose clamp and minimal model analysis methods in adults and children. Infants with an inadequate glycaemic response compared to those with an adequate response had a lower QUICKI index (table 3) consistent with relative insulin resistance and in keeping with the relative fasting hyperglycaemia, fasting hyperinsulinaemia, and the increased insulin:glycogen ratio in these infants. As far as we are aware, this is the first application of the QUICKI index in preterm infants, but without direct comparison to established methods of assessing insulin resistance, interpretation of hormonal effects in infants must be made with caution. For example, we have recently shown a paradoxical association of increased amounts of prescribed insulin in preterm infants with increased hepatic glucose-6-phosphatase activity, which in normal adults and diabetics reduces enzyme activity, and reduces transcription of the human gene.

This study is limited by the lack of long term metabolic and hormonal data from these infants, but is suggestive that different hormonal dysfunctions are present in the low birth weight population, perhaps predisposing them in the long term towards different elements of the metabolic syndrome. Repeated episodes of early neonatal hypoglycaemia characterise groups of infants with fasting hypoglycaemia and raised cortisol concentrations, and as in this study, with an inadequate response to glucagon linked to parameters suggestive of insulin resistance. This suggests preterm infants have variable hormonal responses in an attempt to correct recurrent neonatal hypoglycaemia. This is perhaps not surprising as we have already shown variable postnatal hepatic expression of glucose-6-phosphatase in preterm infants, but also in term infants, and varied metabolic responses to perinatal factors, and to hypoglycaemia, particularly in the generation of alternative substrates such as lactate and ketone bodies. The lack of correlation between fasting blood glucose concentrations and glycaemic response to glucagon means that it is likely that these infants have multiple and varied failures in developmental regulation of hormonal responses and in the expression of key genes in the process of hepatic gluconeogenesis. These regulatory defects are variable and the infants who were identified as hypoglycaemic on fast were not exclusively those with the inadequate responses to glucagon.

We have identified, in the postnatal period, a subset of preterm infants who show an inadequate glycaemic response to glucagon and features of insulin resistance. The long term implications of the findings of this study are not yet known. For instance we are unsure of whether the metabolic differences found in our groups of infants are transient or persistent. Clearly such metabolic and hormonal studies also need to be extended to infants born at term, and pilot work suggests similar patterns to this preterm group.

ACKNOWLEDGEMENTS

The work carried out was supported by grants from the Scottish Executive (AB, RH), British Diabetic Association (AB), Wellcome Trust (AB, RH), Research Trust (AB, RH), Paediatric Diabetes cases in Children (AB), Paediatric Metabolic Research Trust (RH), and Anonymous Trust (AB, RH).

REFERENCES


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Arch Dis Child Fetal Neonatal Ed 2003 88: F62-F66
doi: 10.1136/fn.88.1.F62

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