Dexamethasone increases plasma amino acid concentrations in bronchopulmonary dysplasia

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Abstract
Nine ventilated low birthweight babies were treated with dexamethasone (0-6 mg/kg/day). Appreciable suppression of weight gain was accompanied by uraemia and significant increases in the concentration of all amino acids except phenylalanine, tyrosine, threonine, and glutamate. Ornithine, citrulline, alanine, glutamine, and cystine concentrations increased threefold or more. The findings could not be explained by changes in dietary intake and presumably reflect pronounced catabolism, though the effects of dexamethasone on intermediary metabolism and membrane transport could also play a part.

Dexamethasone is used increasingly to treat infants with bronchopulmonary dysplasia, principally to facilitate the withdrawal of mechanical respiratory support.1, 2 The precise mode of action of this drug in bronchopulmonary dysplasia is not clear, but lung compliance is known to increase, possibly through clearance of interstitial oedema. Treatment, which may be given for six weeks or more,2 is usually accompanied by suppression of weight gain and uraemia, indicating the presence of catabolism.3 The metabolic vulnerability of immature infants provided with protein in excess of growth requirements is well known,4, 7 but there are no data about the metabolic effects of high dose corticosteroid treatment in this group of patients. In this study we have measured the effect of dexamethasone on plasma amino acid concentrations.

Patients and methods
PATIENTS
The infants studied had been mechanically ventilated from birth. Table I gives the birth weight and gestational and postnatal age of each. Dexamethasone was used in the presence of radiographic abnormalities suggestive of chronic lung disease when conventional respirator weaning techniques had failed. The decision to treat was taken by the clinician responsible and no other experimental intervention to the standard nursery protocol was made. The dose of dexamethasone employed was 0·6 mg/kg/day in three divided doses given intravenously or orally.

FEEDING
The method of feeding was determined by each infant’s clinical state and not regulated for the purpose of this study. None of the infants was completely enterally fed and all were receiving commercial parenteral nutrition solutions (Vamin Infant, Kabi Pharmacia, 10% dextrose ±20% Intralipid, Kabi Pharmacia), as a part or the whole of their nutrient intake. The parental nutrition regimens used in our nursery provide between 167 and 502 kj/kg/day, depending on lipid tolerance, and amino acid intake is adjusted to constrain protein equivalent: energy ratio between 3·35 and 6·70 g/MJ. Enteraly fed infants received low birthweight formula (Cow and Gate) or human milk when available. Total nitrogen and energy intakes were calculated from nursing records and manufacturers’ compositional data. The nitrogen content of human milk was assumed to be 200 mg/100 ml, and the energy content 251 kj/100 ml or 293 kj/100 ml, depending on whether banked milk (a mixture of dripped and expressed milk) or mother’s own milk respectively was used.

ANTHROPOMETRY
Weight (g) was measured daily when clinical state permitted, using an averaging electronic scale with resolution of 1 g and precision of ±4 g. Weight gain was calculated as the linear regression coefficient (slope) of these measurements on time (days). This was divided by body weight at the commencement of treatment for comparative expression in units of g/kg/day. Although such ratio ‘correction’ can lead to erroneous conclusions when comparison is made between infants of differing size, we made comparison only within subjects between treatment periods.

BIOCHEMICAL ANALYSIS
Heparinised venous blood samples (0·5 ml) were drawn before the first dose of dexamethasone and on one occasion between two and seven days later when a clinical indication

Table 1 Clinical characteristics of the infants studied

<table>
<thead>
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<th>Patient No</th>
<th>Birth weight (g)</th>
<th>Gestation (weeks)</th>
<th>Postnatal age (days)</th>
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</tr>
<tr>
<td>Median</td>
<td>842</td>
<td>26</td>
<td>22</td>
</tr>
</tbody>
</table>
for routine sampling arose. Plasma was separated immediately and frozen to −20°C. A 200 µl aliquot was deproteinised with 20 µl of 35% (w/v) sulphosalicylic acid after the addition of 200 µl of 250 µmol/l l-norleucine, which was used as an internal standard. The mixture was vortexed for a few seconds before incubating at room temperature for 5 minutes and then centrifuging again for 5 minutes at 15 850 g. An aliquot of 350 µl of the deproteinised supernatant was mixed with an equal volume of Beckman lithium diluent (ph 2.2) and the sample either stored at −60°C or filtered through a 0.22 µm filter for immediate analysis.

Amino acid concentrations were measured using a cation exchange high performance liquid chromatography (HPLC) system (Beckman System Gold) with postcolumn ninhydrin derivatisation, detecting primary amino acids and related compounds at 570 nm wavelength. The system was calibrated using a 250 µmol/l Beckman physiological standard. For each infant, specimens collected before and during treatment were analysed sequentially to ensure compatible measurement conditions. The precision (coefficient of variation) of the assay procedure was established by replicate assay (n=8) of the same sample of adult blood. It lay between 1·5 and 5·2% for all amino acids except citrulline (10·7%).

Plasma urea was determined by the urease method on a random access analyser in the hospital routine laboratory.

STATISTICAL ANALYSIS
The number of patients studied was small (n=9) and most variables did not appear normally distributed. Consequently the Wilcoxon signed rank test has been employed to make comparisons before and during treatment within infants. Two tailed probability estimates (p) are quoted throughout; values of <0·05 were deemed significant.

Results
NITROGEN AND ENERGY INTAKE DURING STUDY
Table 2 shows the average daily nitrogen and energy intake of each baby during the seven days before and the seven days after dexamethasone was commenced. These, and protein:energy ratio, did not differ significantly between the two periods (Wilcoxon signed rank test). There was a small difference in the ratio of enteral to parenteral intake between treatment periods: the median proportion of total fluid intake as milk before treatment was 26% (range 0–91%) and 19% (range 0–66%) during treatment. This difference was not significant (Wilcoxon signed rank test, p>0·1) and was attributable to interruption of enteral feeding on endotracheal extubation.

WEIGHT GAIN
Figure 1 shows tied values for average daily weight gain or loss during the week before and the week after commencing dexamethasone treatment. In one case insufficient weight data were obtained during the second week to calculate rate of change. In six of the remaining eight cases there was a net weight loss on treatment. These changes were significant (Wilcoxon signed rank test, p<0·05).

PLASMA UREA CONCENTRATIONS
Figure 2 shows the distribution of plasma urea values with time. There was a clear increase in

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*Denotes insufficient data to calculate velocity.
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Figure 2 Plasma urea values (mmol/l) during the week before commencing dexamethasone and during the first week of treatment.

PLASMA AMINO ACID CONCENTRATIONS
Table 3 shows the median concentration (with range) for each amino acid before and after treatment. Significant increases were observed in all except glutamic acid, threonine, phenylalanine, and tyrosine concentrations (Wilcoxon signed rank test; p values given in table 3). The uniformity of the changes across infants was striking; in the case of most of the amino acids an increase was observed in every infant studied, notable exceptions being threonine, glutamic acid, phenylalanine, and tyrosine. The most appreciable increases were noted in ornithine, citrulline, cystine, methionine, glutamate, and alanine concentrations (fig 3).

Discussion
Dexamethasone treatment was associated with suppression of weight gain and striking increases in the plasma concentrations of most amino acids and urea. These changes could not be attributed to differences in nitrogen and energy intake between treatment periods. Our assumptions about the composition of human milk are unlikely to have influenced comparative nutrient intakes. This is firstly because the quantities employed were small and, secondly, because only within subject, between period comparisons were made. The small, statistically non-significant changes in the disposition of nutrient intake between parenteral and enteral routes are also unlikely to have been important, particularly as the protein:energy ratio was constrained during parenteral nutrition. Moreover, the pattern of hyperaminoacidaemia we describe differs from that previously observed by Raihā et al in dietary protein excess.7 Concentrations of alanine, glycine, ornithine, lysine, histidine, serine, and threonine measured during dexamethasone treatment in our study were greater than those observed by Raihā et al in low birthweight infants fed unadjusted cows’ milk protein at 4.5 g/kg/day. Conversely, the concentrations of leucine, isoleucine, valine, phenylalanine, tyrosine, citrulline, glutamate, and alanine were lower.
cystine which we measured were lower, and those of methionine, proline, glutamine, and taurine similar to those described by Raihia et al. The low cystine concentration measured in our study is particularly noteworthy and probably reflects the relatively high requirement for parental nutrition and low intake of human milk.

It seems unlikely that the plasma amino acid concentrations attained were intrinsically harmful as none approached levels characteristic of metabolic disease. Indeed, neonatal tyrosinaemia observed in one infant of 24 weeks' gestation improved after 48 hours of dexamethasone treatment, perhaps as a result of tyrosine aminotransferase induction. The complexity of the metabolic response to steroid treatment makes it difficult, however, to be certain of the mechanisms underlying the changes that we have observed. The most likely is to be a change in the balance between rate of protein synthesis and breakdown, but corticosteroid induced changes in active transport of amino acids across membranes could also affect cellular uptake. For example, corticosteroids reduce amino acid uptake by skeletal muscle and lymphoid tissue but increase uptake by hepatocytes. The effects of corticosteroids on intracellular metabolism appear similarly complex—for example, hydrocortisone treatment both increased carbamoyl phosphate synthetase-I activity and decreased ornithine transcarbamoylase activity in fetal rat liver. It is not clear to what extent the changes we observed in the urea cycle intermediates ornithine and citrulline were due to an overall increase in protein oxidation or to what extent any inhibition of ornithine transcarbamoylase might have been responsible.

Although the changes induced by glucocorticoids are often described as 'catabolic', protein turnover studies have suggested that nitrogen wasting results both from a decrease in protein synthesis and a rise in degradation rates, though the latter may fall in some tissues. Skeletal muscle has been considered to show the most appreciable effects. In the rat, those muscles with the highest proportion of fast fibres (for example, extensor digitorum longus) show the most noticeable atrophy and reduction in protein synthesis, whereas those with a greater proportion of slow fibres (for example, soleus) tend to be spared. The diaphragm, which contains a mixture of fast and slow fibres, appears to behave in an intermediate manner. At present, there are no data on the effect of corticosteroids on diaphragmatic structure and function in the immature human infant.

The effect of corticosteroids on skeletal muscle has also been extensively studied in mature dogs. Muhlbacher et al showed a significant efflux of glutamine from skeletal muscle in animals given 0.44 mg/kg/day of dexamethasone. However, the changes which they observed in plasma amino acid concentrations differ from those which we have noted in immature infants. Most notably, ornithine, glutamine, and cystine concentrations fell while alanine increased by a factor of only 1.5. In contrast, rats treated with 6 mg/kg/day of dexamethasone showed a rise in plasma glutamine concentration accompanied by efflux of glutamine and alanine from the lung. This is of interest because the human and the rat, unlike the dog and other species studied, appear to release glutamine from the lung. A recent observation that dexamethasone treatment appreciably suppresses lung growth and protein synthesis in the adolescent rat raises the possibility that the increased plasma alanine and glutamine concentrations we describe here could be attributable as much to suppression of protein synthesis in the lung as in skeletal muscle. By analogy with the effects of dietary protein restriction in the rat this might be expected to increase lung compliance and produce short term clinical improvement, but also to slow the rate of repair.

In summary, we observed striking increases in the plasma concentrations of most amino acids in immature infants treated with dexamethasone. These changes may reflect suppression of protein synthesis in both the lung and the respiratory musculature, but the relative contributions of effects of membrane transport and intermediary metabolism remain to be determined.
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