Inherited metabolic disease

G213 AETIOLOGY OF ACUTE HYPOGLYCAEMIA: RE-AUDIT OF PROCEDURES FOR DIAGNOSIS

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Introduction: A protocol exists for the collection of samples to investigate non-diabetic hypoglycaemia, termed the ''hypopack''. These packs are kept in A&E departments, most children wards and neonatal SCBUs throughout the region. A retrospective audit of 107 hypopacks received between July 2001 and December 2003 highlighted a numbers of problems: samples collected when patient was receiving dextrose, incomplete clinical history, insufficient and haemolysed samples and filing of reports in charts. These were addressed by redesigning the request form, updating the protocol and introducing a summative report. The new protocol was introduced in April 2006 and was supported by presentations to regional centres.
Methodology: A retrospective audit of 100 hypopacks received between April 2006 and May 2007 was performed to assess whether all samples were analysed and reported, and were taken when the patient was hypoglycaemic. Charts were reviewed to determine the cause of hypoglycaemia and to check reports were filed appropriately.
Results: 49% of patient were hypoglycaemic (<2.6 mmol/l) compared with 35% in the original audit. 64% of patients had samples taken before dextrose compared with 54% previously. Haemolysed insulin samples remained a problem with 21% of samples being rejected. In both audits 35% of laboratory reports were missing from patients charts. Intrauterine growth retardation was the most common problem in neonates and fasting due to gastroenteritis was the most common in children. In the re-audit period, one case of isolated adrenocorticotrophic hormone deficiency, three cases of hyperinsulinism of infancy (of which two were transient), one case of medium chain acyl-CoA dehydrogenase deficiency and one patient with a previous diagnosis of Morquio disease were identified.
Conclusions: The new hypopack protocol has increased the number of appropriately performed investigations but there is still scope for improvement. Provision of clinical history and information concerning dextrose infusion has assisted with the interpretation of the hypopack results.

G214 ERRORS IN EMERGENCY FEEDS IN INHERITED METABOLIC DISORDERS: A RANDOMISED CONTROLLED TRIAL OF THREE PREPARATION METHODS

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Background/objectives: Emergency feeds or drinks based on glucose polymer are used to prevent metabolic decompensation and encephalopathy during illness in many inherited metabolic disorders (IMD). In a randomised, prospective, crossover study, the aim was to investigate if a pre-measured sachet of glucose polymer, used in preparing age-appropriate solutions, decreased the number of carer errors when compared with two conventional methods of measuring glucose polymer (weighing using digital scales and measuring using scoops).
Subjects/methods: 48 carers (three male, 45 female) of 52 children/patients with IMD (25 girls; 27 boys; median age 5.8 years) were recruited. The carers made each emergency feed using the three techniques (weighing, scoops and sachets) to measure the powder amount under controlled (1 litre prepared) and home conditions (1 litre and 200 ml prepared). An aliquot of 100 ml of each feed, prepared by all three methods, was collected to analyse its carbohydrate concentration.
Results: Under controlled conditions when preparing 1 litre of feed, the median percentage of glucose polymer concentration closest to the final calculated amount was the pre-measured sachets (105%); closely followed by the weighing of powder using scales (107%); and finally scoops (118%) ($p<0.001$). Similarly, under home conditions, the closest method was the pre-measured sachets (111%), followed by weighing (112%), and finally scoops (118%) ($p<0.05$). When only 200 ml of emergency feed was produced at home, there were similar results observed for all three methods (sachets and scoops, 112% and 112% respectively; weighing, 113%). Common errors observed associated with feed production in the three methods were inability to measure water accurately (40% controlled and home conditions), failure to measure or count scoops correctly and difficulty with using digital scales.
Discussion: Accuracy and technique when preparing emergency feeds significantly deteriorated for all three methods of feed preparation (weighing, scoops and pre-measured sachets) between controlled and home conditions when preparing 1 litre of emergency feed. Pre-measured sachets was the most accurate method overall and the scoops was the least accurate method. Scoops are commonly used to prepare many specialist paediatric feeds and may be associated with wide error.

G215 TEMPERATURE-SENSITIVE MUTANTS: MILD DEFECTS OF FATTY ACID OXIDATION ARE UNMASKED BY EXPOSURE TO INCREASED TEMPERATURE

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The diagnosis of fatty acid oxidation defects is customarily achieved through a combination of clinical evaluation and metabolite assays that may include blood acyl-carnitine analysis, urine organic acids and intermediary metabolites. Confirmation of a fatty acid oxidation defect can, where there is a common mutation, be readily achieved through mutation analysis, eg, medium chain acyl-CoA dehydrogenase deficiency (MCADD) and long chain 3-hydroxyacyl-CoA dehydrogenase deficiency. However, for many disorders of fatty acid oxidation, studies in fibroblasts still remain the best means of confirmation, particularly where mutation analysis shows only one mutation or if pathogenicity is uncertain. An additional means of unmasking milder defects is to grow and assay cells at both normal (37°C) and elevated temperature (41°C). We have used such assays to investigate myopathic carnitine palmitoyltransferase II (CPT2) and very long chain fatty acid dehydrogenase deficiency (VLCADD). Some CPT2-deficient patient fibroblasts can give normal fatty acid oxidation at 37°C but are reliably low at 41°C, percentage residual fatty acid oxidation was 96 ± 13 vs 20 ± 13 respectively ($n = 7$), mean CPT2 activity was $21 \pm 7\%$. We have diagnosed 15 patients that were CPT2-deficient, five symptomatic CPT2 carriers and 15 patients with myopathic VLCADD. Using similar techniques we have been able to further characterise some patients with MCADD detected either clinically or on newborn screening. The approximately 799 G>A MCAD mutation was detected in homozygous status by newborn screening in our region. Biochemical follow-up was consistent with MCADD. The heterozygous father had raised C8 on acylcarnitines. His fibroblasts oxidised myristate at 37°C and 41°C at 82% and 52% of controls respectively. Heterozygotes with the common MCAD

mutation have normal myristate oxidation at both temperatures. A patient with a single copy only of approximately 799 G>A presented at 4.5 years with severe acute metabolic decompensation and severe hypoglycaemia. Organic acideamias and acyl-carnitines were abnormal but not typical of MCADD. Fatty acid oxidation studies for myristate at 37°C and 41°C were 82% and 50% of controls respectively, indicating abnormal medium chain fatty acid oxidation. The patient was also shown to be riboflavin deficient, suggesting an interaction of diet with a dominant negative mutation. Such functional studies in intact fibroblasts at physiological substrate concentration, where all determinative and modifying cellular factors are present, can often provide valuable additional information that complements mutation analysis.

G216 A SYNDROME OF LIVER CIRRHOSIS, DYSTONIA, POLYCYTHAEMIA AND HYPERMANGANESAEMIA: NEW INSIGHTS

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We have identified a recessively inherited syndrome comprising cirrhosis, dystonia, polycythaemia and hypermanganesaemia (CDPH), which we propose is an inborn error of manganese (Mn) homeostasis. A 12-year-old girl born to consanguineous parents presented with the above symptoms and a blood Mn level of above 3000 nmol/l (normal range <320 nmol/l). Her brain MRI revealed signal abnormalities of the basal ganglia consistent with Mn deposition. An older brother with the same phenotype died at 18 years, suggesting a potentially lethal, autosomal recessive disease. This disorder is most probably caused by a defect of Mn metabolism with the accumulation of Mn in the liver and the basal ganglia similar to the copper accumulation in Wilson's disease. In order to assess the genetic basis of this syndrome we investigated two candidate genes: *ATP2C2* and *ATP2A3*, encoding the two Mn transporting calcium—ATPases, SPCA2 and SERCA3, respectively. Genotyping the patient and the family for microsatellite markers surrounding *ATP2C2* and *ATP2A3* excluded these genes. The patient was found to be heterozygous for both gene loci. A whole genome homozygosity mapping using a 10k snp chip provided a further candidate gene. V-ATPase subunit F is involved in acidification of endocytotic vesicles and is required to release Mn from the Mn—transferrin (Tf)—Tf receptor complex. Sequencing this gene did not reveal a mutation and excluded it to be disease causing. Further candidate genes found in the homozygosity mapping are currently under investigation. Despite the unknown pathophysiology, we were able to develop a successful treatment regimen. Chelation therapy with disodium calcium edetate combined with iron supplementation is the treatment of choice, lowering blood Mn levels significantly and improving clinical symptoms.

G217 DISCRIMINATING PRIMARY FROM SECONDARY TRIMETHYLAMINURIA BY MOLECULAR ANALYSIS OF THE FLAVIN CONTAINING MONOOXYGENASE 3 GENE

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Background: Trimethylamine (TMA) exhibits the odour of rotting fish and is formed by intestinal bacteria from various foods (those rich in choline and carnitine as well as seafood). It is absorbed across the gut wall and oxidised by the hepatic enzyme flavin containing monooxygenase 3 (FMO3) to odourless trimethylamine oxide (TMAO). FMO3 deficiency results in TMA accumulation and offensive odour in sweat and breath.

Case report: A 2-year-old girl was noted to have an intermittent "fishy" body odour after certain foods, especially beans. A urine sample showed elevated TMA 122.5 μmol/mmol creatinine (2.5–10.9) with elevated TMAO 2457.4 μmol/mmol creatinine (17–147). The ratio TMA:TMAO was normal 0.05 (0.05–0.21), suggesting TMAU2, which was due to enterobacterial overgrowth. A repeat showed a reduction in TMA (49.9 μmol/mmol creatinine) and TMAO (80.4 μmol/mmol creatinine) but with an elevated ratio (0.62) suggesting TMAU1 or urinary tract infection (UTI). A third sample following antibiotic therapy for UTI showed increased TMA (22.1) but a normal ratio (0.16). Sequencing of the *FMO3* gene showed compound heterozygosity for the disease-associated mutations p.[Pro153Leu]+[Ile441Thr] and thus she was affected with TMAU1. Her treatment is with diet low in choline and avoiding marine fish, pH 5.5 soaps and antibiotics for exacerbations.
Laboratory experience: TMAU may present in childhood but diagnosis may be difficult even using choline or dietary loading tests. Of 190 urine samples referred to our laboratory this year for TMA evaluation 7 were from children under 5 years. All had elevated TMA; TMA:TMAO ratios were elevated in three, one normalising on repeat. Molecular analysis on 28 samples, 24 from adults and four from children revealed bialleleic pathogenic mutations in three adults and three children.
Conclusions: Suspicion of TMAU because of odour and biochemical abnormality is becoming more common, but genetic confirmation of TMAU1 is rarely used. Our results suggest children are rarely investigated for TMAU. Molecular analysis provides an efficient and acceptable method of distinguishing primary from secondary TMAU.

G218 ENZYME REPLACEMENT THERAPY IN INFANTILE POMPE DISEASE: A ROLE FOR IMMUNOMODULATION?

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Background: Infantile Pompe disease is a rapidly progressive lysosomal storage disease with fatality before 18 months. A deficiency of lysosomal α-glucosidase, results in glycogen storage within many cells, most importantly skeletal and cardiac myocytes. The initial trials of enzyme replacement therapy (ERT; *Myozyme*, Genzyme Corp.) have shown a reduction in the risk of death of 99% and a reduction in risk of death and any ventilation by 88%. While clinical experience and work from the mouse model has emphasised the importance of early initiation of therapy; it is also becoming clear this is not the only determinant of successful treatment. The CRIM (cross-reactive immunological material) status may be an important determinant in the production of enzyme-specific antibodies. CRIM−ve patients do not produce any detectable glucosidase protein. When these infants are subsequently exposed to ERT, they develop an antibody response that may decrease efficacy. Recent work in the mouse model has suggested that this effect can be ameliorated by the addition of methotrexate during the initiation of therapy.
Methods: Three infants are studied: patient 1 was CRIM−ve and diagnosed at 4 months; patient 2 was CRIM+ve diagnosed at 7 months; and patient 3 was CRIM indeterminate that was diagnosed at 4 months. We recorded the clinical progression, antibody status and echocardiographic findings during their treatment.
Results: Patient 1 who was CRIM−ve showed no improvement with ERT, clinically or echocardiographically, and showed high titres of neutralising antibodies. Even with late attempts at immunosuppression with rituximab, the continuing decline in cardiac function ultimately proved fatal. Patient 2 (CRIM+ve), progressed well, putting on weight and showed improvement in left ventricular mass. She was discharged back to her referring hospital at 1 year of age. Patient 3 (CRIM indeterminate) was treated with a combination of

rituximab and methotrexate and has never had an infusion reaction or shown any antibody-mediated effects. He also progressed the quickest in terms of echocardiographic and clinical improvement, eg, development of antigravity movement and weight gain.
Conclusions: The addition of immunodulation may help reduce antibody response and certainly in the patients that were CRIM−ve may be an important adjunct to future ERT in patients with Pompe disease.

G219 11-YEAR FOLLOW-UP OF BILIOPANCREATIC DIVERSION FOR THE PREVENTION OF PANCREATITIS IN A PATIENT WITH LIPOPROTEIN LIPASE DEFICIENCY

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Aim: To report 11-year follow-up data after novel biliopancreatic diversion to prevent recurrent pancreatitis in a child with lipoprotein lipase deficiency.[1]
Methods: Case note review.
Results: A Pakistani infant presented with vomiting and hepatomegaly aged 7 months. Investigations showed marked hypertriglyceridaemia (19.2 mmol/l); lipoprotein lipase activity was less than 1 mmol/l per h (reference range 5–9) and apolipoprotein C-II was present (qualitative assay). She was maintained on a low fat, medium chain triglyceride supplemented diet, but compliance with fat restriction was thought to be poor, and hospital admissions with abdominal pain were frequent. Plasma triglyceride concentrations were often grossly elevated, resulting in eight admissions with pancreatitis between the ages of 9 and 11 years. She then underwent a biliopancreatic diversion (without partial gastrectomy) as an alternative strategy for reducing blood lipid concentrations by inducing fat malabsorption. In the 11 years following surgery she has remained well with no recurrence of pancreatitis and has fulfilled her growth potential. She opens her bowels just twice a day, but has needed to be supplemented with fat-soluble vitamins. Both plasma triglyceride and cholesterol concentrations have fallen.
Conclusions: This novel approach has prevented recurrent episodes of pancreatitis without surgical morbidity, while preserving the potential to restore normal gastrointestinal continuity. Ongoing management includes attention to fat-soluble vitamin status and bone mineralisation; the potential for neonatal fat-soluble vitamin deficiency in future offspring is recognised.

1. **Hodge D,** Stringer MD, Puntis JWL. Lipoprotein lipase deficiency: benefits and limitations of a novel surgical approach. *J Pediatr Gastroenterol Nutr* 2001;**32**:593–5.

G220 DIURNAL VARIATION OF PHENYLALANINE CONCENTRATIONS IN TYROSINAEMIA TYPE 1: SHOULD WE BE CONCERNED?

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Introduction: Tyrosinaemia type 1 (HT1) is treated with a tyrosine (tyr)/phenylalanine (phe) restricted diet; tyr/phe-free amino acids supplements; and nitisinone. Treatment guidelines for plasma tyr are 200–400 μmol/l and phe 30–100 μmol/l. Low phe concentrations (<30 μmol/l) may be associated with poor growth and developmental delay. There is little information on diurnal variation in phe or tyr levels in HT1 or recommendations on blood sampling times.
Aim: To document diurnal variation in phe and tyr levels in children with HT1.
Methods: Median phe and tyr plasma concentrations were retrospectively reviewed over a 3-year period in 11 subjects with HT1 (eight boys and three girls, median age 4 years (range: 3–11 years)). All subjects had more than 90 blood samples available: median 140 (range 93–145). Subjects routinely collected morning fasting tyr/phe blood samples but afternoon non-fasting samples were taken in clinics (<10% of samples). The median total protein equivalent intake (natural and tyr/phe free amino acid supplement) was 2.6 g/kg per day (range 1.5–3). Three subjects were taking phe supplements (100–200 mg/daily). Height and weight z scores were calculated.
Results: All median morning plasma phe concentrations were in the upper target range (median 70 μmol/l, range 65–90), but the median for afternoon phe was <30 μmol/l in all subjects ($p = 0.003$). 66% of all low blood phe concentrations levels were afternoon samples, but other factors associated with low blood phe were stabilisation of plasma tyr/phe post-diagnosis ($n = 2$) and overnight feeding ($n = 1$). Similarly, there was a decrease in tyrosine concentrations between morning (median 340; range 270–450) and afternoon (median 300; range 205–365) ($p = 0.004$). Almost all children were shorter than average (median height z score was −1.56 (range 1.01 to −3.26) and weight was −0.31 (range 3.16 to −0.83)).
Summary: Phe and tyr concentrations are consistently lower in the afternoon. In particular, afternoon phe concentrations are often below target ranges.
Conclusions: Further detailed study to examine the 24 h diurnal variation of plasma phe in HT1 is indicated. Taking blood samples at variable time points may lead to a different interpretation of dietary control. It is possible that very low phe concentrations for a substantive time within 24 h may impact on growth.