

MetBioNet Intermediary Metabolites Meeting 2009

The Intermediary Metabolites meeting held at Great Ormond Street Hospital was a very interesting and thought-provoking meeting, providing detailed information on the commonly measured laboratory metabolites.

The meeting began with a presentation entitled “Metabolism and Regulation of Intermediary Metabolites”. Mick Henderson outlined the different control mechanisms utilised by the body, grouping them into three main areas:

- 1) Long-term control eg. Compartmentalisation (Glucose-6-phosphatase expressed in liver but not muscle)
- 2) Short-medium control eg. Haem regulates transcription of genes involved in the haem biosynthetic pathway
- 3) Rapid control eg. Competitive inhibition of acetyl CoA acetyl transferase by its product acetoacetyl CoA in ketone synthesis.

The second presentation by Stuart Moat looked specifically at the laboratory analysed metabolites and gave information regarding sample stability and the currently available methods for glucose, lactate, Non-Esterified Fatty Acids (NEFA) and 3-OH butyrate. The issue regarding the inaccuracy of glucose measurements in critically unwell patients was addressed. The audience was informed that glucose oxidase methods which rely on adequate pO_2 (a situation not found in critically unstable patients) are not accurate in these situations but that more accurate measurements are attained using pyrroloquinoline quinone (PQQ) glucose dehydrogenase methods. However, the alternative method is not as specific for glucose as glucose dehydrogenase also metabolises lactose, maltose and xylose and so hypoglycaemic patients could present with “normal” glucose measurements. This example was a reminder to us all that interpretation of “basic” metabolites such as glucose must be done with an understanding of the laboratory method used.

The issues regarding NEFA and 3-OH butyrate measurements were also discussed with Dr Moat highlighting the absence of an EQA scheme for NEFA and a need to validate the recently available Randox NEFA kit. Dr Moat suggested that MetBioNet guidelines should be written for Intermediary Metabolites in order to highlight the effects of analytical factors on metabolite measurement.

The next presentation by Dr Khalid Hussain discussed the investigation and treatment of hyperinsulinism and focussed specifically on SCHAD. SCHAD is typically identified by increased 3OH butyrate, 3OH glutarate and 3,4-dihydroxybutyrate levels in urine organic acid analysis and raised hydroxybutyrylcarnitine in acylcarnitine analysis. It was suggested that SCHAD is a negative regulator of insulin secretion, hence upon its suppression insulin levels increase leading to unregulated insulin secretion.

Dr Steve Krywawych then discussed the use of intermediary metabolites as biochemical markers in the diagnosis of hypoglycaemia. The measurement of FFA and 3-OH butyrate was shown to be useful as reduced levels of both analytes are commonly associated with hyperinsulinism and panhypopituitarism, whereas increased levels of FFA and reduced levels of 3OH butyrate are seen in fatty acid oxidation and carnitine transporter defects. An interesting case of HMG CoA synthase deficiency was discussed as in these patients there is no rise in 3OH butyrate during fasting and acylcarnitines are initially normal, although should be abnormal at the end of a fast. Mutation analysis was suggested as the optimum diagnostic tool in these patients.

Lactate homeostasis and the causes of dysregulation was discussed by Dr Jim Bonham. Acquired causes of lactic acidemia were highlighted including hypoxia, systemic disease eg. diabetes mellitus and liver failure, drugs and toxins eg. salicylate, ethylene glycol, ethanol and nutritional deficiency eg. thiamine deficiency. The causes of primary lactic acidemias were highlighted including: GSD Type 0, 1, 3 and 6, Organic acidemias (MMA, PA, HMG CoA Lyase deficiency, Fatty acid oxidation disorders (VLCAD, LCHAD) and urea cycle disorders (OTC deficiency and Citrullinaemia).

The investigations required in the investigation of lactic acidemias were discussed and the audience were reminded that these were highlighted in the current MetBioNet Lactic Acidemia guidelines.

The clinical presentations of the rarer causes of lactic acidemias were discussed by Dr Shamima Rhaman, including pyruvate carboxylase and biotinidase deficiencies. The day ended with an excellent presentation by Dr Simon Heales who considered the laboratory investigation of lactic acidemias. He particularly discussed the required sample handling of muscle biopsies required for the investigation of respiratory chain defects, asking for laboratories to send 50-100mg of flash-frozen skeletal muscle on dry-ice to the laboratory. The potential pitfalls of respiratory chain complex investigations were discussed. Heteroplasmy, Km mutants and secondary effects of anti-retroviral drugs on mitochondrial copy number were all factors that could potentially affect interpretation of respiratory chain investigations. Dr Heales suggested that in cases with strong clinical suspicion of respiratory chain defect and normal complex I, III and IV activities, complex V should be investigated. Complex V is currently studied using Blue Native gel electrophoresis and its catalytic activity investigated by “feeding” substrate into the gel. This method has been shown to be very sensitive to complex II defects, but has additionally detected defects in complex III.

Overall the meeting provided an excellent discussion of the value of intermediary metabolite measurement in the diagnosis of a large range of metabolic conditions and highlighted the potential pitfalls in the interpretation of these analytes.