e-Learning
Fatty Acid Oxidation Defects

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Fatty Acids

Fatty acids are a major source of energy and body fat is an energy dense material. They can be readily mobilised in the non-fed state with production of ketones by hepatic β-oxidation and give a high yield of ATP through fatty acid oxidation & oxidative phosphorylation. They are preferentially used by some tissues as a major energy source. Heart muscle derives 60% of its energy from long-chain fatty acid oxidation and skeletal muscle uses them at both rest but during extended aerobic muscle exercise. Most dietary fat consists of triglycerides containing long-chain fatty acids. The energy obtained from excess calorie intake is used for the biosynthesis of long chain fatty acids and storage as fat. Fat is stored in adipose tissue in the form of long-chain triglycerides.
In the non-fed state, at a certain point, fatty acids are mobilised from adipose tissue. In neonates and infants this may occur after only a few hours whilst in adults it occurs from 6 to 12 hours post feeding.

They are transported to the liver as free fatty acids (FFA) and undergo β-oxidation in the liver to produce ketone bodies. These ketone bodies cannot be used by the liver but are exported to extra-hepatic tissues to be used for energy production.

The ketones are used by extra-hepatic tissues during fasting, although the brain has only a limited capacity to use them for energy. In fact the use of ketones by peripheral tissues “spares glucose” for the brain which continues to use it as its major energy source.

Neonates and infants have a increased head/body ratio as compared to adults and therefore they have a relatively high cerebral glucose requirement. They also have a small liver and hence relatively low glycogen stores. Without the “glucose sparing” effect of ketones neonates and infants would rapidly develop hypoglycaemia.
Long-term Fasting

On long term fasting (eg starvation) the body adapts by increasing use of body fat & muscle and the brain slowly switches from using glucose as its main energy source to using ketone bodies.

Over 80% of energy requirements are met by fatty acids and muscle is slowly broken down to provide gluconeogenic substrates as some glucose is still needed.
Defects of Fatty Acid Oxidation

Fatty acid oxidation defects lead to a reduced or absent ability to produce ketones leading to hypoketotic hypoglycaemia, encephalopathy, coma and death. There are also adverse effects in tissues & organs that preferentially use fatty acids for energy. In skeletal muscle this can lead to muscle weakness, pain and rhabdomyolysis and in heart muscle cardiomyopathy and conduction defects. Liver function can also be compromised leading to abnormal liver function tests.
Fatty acid oxidation occurs in the mitochondria by a multi-step process.
1. Long chain fatty acids enter the mitochondria as fatty acyl-CoA derivatives via the carnitine shuttle
2. Short and medium chain fatty acids enter the mitochondria independently
3. During β-oxidation the fatty acyl-CoA’s are sequentially shortened by two carbon units for each turn of the cycle with the production of acetyl-CoA
4. Some of the reducing equivalents (NADH, FADH) produced during β-oxidation are fed directly into the electron transport chain ETC to produce ATP
5. Acetyl-CoA is also fed into the citric acid cycle to generate more reducing equivalents & subsequently ATP in the ETC.
6. In the liver - ketones are synthesised from acetyl-CoA for export to peripheral tissues for further oxidation & ATP production
Diagnosing Fatty Acid Oxidation Defects

A fatty acid oxidation defect should be considered in any child with unexplained hypoglycaemia. The fact that the child may be ketotic does not exclude this group of disorders.

For the metabolic laboratory the first line tests would be measurement of the ratio of plasma free fatty acids to beta-hydroxybutyrate, urine organic acid analysis and plasma acylcarnitine analysis by tandem mass spectrometry (TMS) and details of these are given in the slides following. It is very important to collect urine and plasma samples when the child is hypoglycaemic as in some cases the abnormalities may disappear once glucose concentrations have been normalised.

More detailed testing would include measurement of the flux of fatty acid oxidation in cultured fibroblasts using radioisotopic methods, specific assays for individual enzymes, mutation analysis for common mutations and, in some cases, full mutation screening.
Techniques used in the investigation of a fatty acid oxidation defect

Hexanoyl glycine (MCADD)

Organic acids

Acylcarnitines by TMS

Tissue culture

Specific enzyme assays
Screening Tests for a Fatty Acid Oxidation defect

1. **Intermediary Metabolites**
   Free fatty acids/ 3-hydroxy butyrate. A ratio >2 is indicative of a fatty acid oxidation defect. Fatty acids are mobilised but not converted to ketones. This test is really only useful when the sample is taken during a period of hypoglycaemia or fasting.

2. **Organic Acid Profile (OA)**
   Urine organic acids are extracted and converted to their TMS esters and detected by GC-MS. Specific organic acids and glycine conjugates will be present in urine from patients with fatty acid oxidation defects. Note these abnormalities are often only seen during crisis.

3. **Acylcarnitine Profiles (AC)**
   Plasma and blood spot acylcarnitines are derivatised and detected by tandem mass spectrometry. Specific acylcarnitine species will be present or increased in some disorders of fatty acid oxidation. Not all disorders have abnormal acylcarnitine profiles and some only have subtle changes.
**Confirmatory tests**

1. **Fatty Acid Oxidation Flux Studies**
   Cultured fibroblasts are incubated in multi-well plates with labelled fatty acids in a buffer – usually [9,10-\(^3\)H]myristate, palmitate or oleate. The fatty acids are transported into the mitochondria & \(\beta\)-oxidised & the \(^3\)H converted to \(^3\)H\(_2\)O. The released labelled water equilibrates within the cell & in turn with the incubation buffer. When the buffer is removed after 2 hours incubation it contains the released \(^3\)H\(_2\)O as well as the unmetabolised fatty acid. The Released label (\(^3\)H\(_2\)O) is separated from unmetabolised fatty acid using an ion exchange column and counted in a scintillation counter. The amount of label released is expressed relative to the amount of fibroblast protein/well. This value will give a measure of the patient's ability to carry out fatty acid oxidation. Several substrates can be used which will require different chain-length specific enzymes to metabolise them.

2. **Specific Enzyme Studies**
   Sonicated fibroblasts are incubated with a specifically labelled compound. A product is formed by the enzyme that will incorporate the label. The old labelled compound is separated and discarded. The amount of new compound formed is counted in a scintillation counter. This value will give a measure of the specific enzyme activity for that patient as nmol product/mg fibroblast protein/min.

3. **Mutation Analysis**
   Has some limitations but useful when a common mutation exists. Some examples:
   - MCAD (common K304E)
   - VLCAD (no common mutation)
   - LCHAD (common E474Q (~85%))
     - Trifunctional Protein Deficiency presents in same way and has no common mutation
   - CPT I no common mutation
   - CPT II S113L accounts for ~50% of “mild myopathic” disease
# Fatty Acid Oxidation Defects

See appendix for definitions of the abbreviations

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**Abbreviations**
These are used both in this presentation and in many text books, literature or discussions about fatty acid oxidation defects

- FFA – Free Fatty Acids
- 3-HB - 3-Hydroxy Butyrate
- CTD- Carnitine Transporter Defect
- CPT1 – Carnitine Palmitoyl Transferase 1
- CPT2 – Carnitine Palmitoyl Transferase 2
- MCAD – Medium Chain Acyl CoA Dehydrogenase
- VLCAD – Very Long Chain Acyl CoA Dehydrogenase
- LCHAD – Long Chain 3-Hydroxy Acyl CoA Dehydrogenase
- HMG – Hydroxy Methyl Glutaryl
- ETF - Electron transfer flavoprotein
- ETF-DH Electron transfer flavoprotein dehydrogenase
- CAR – Carnitine
- AC – Acylcarnitines
- OA – Organic Acids
- MCT – Medium Chain Triglycerides
- Hex gly – Hexanoyl glycine
Medium Chain AcylCoA Dehydrogenase Deficiency

Clinical Presentation: The main feature is hypoketotic hypoglycaemia following intercurrent illness. This can cause sudden death or may progress to Reye’s Syndrome. Onset tends to be more commonly from 2 months to 4 years of age but may occur at any time even in adulthood. Muscle and liver problems are not a major feature of this disorder. Sometimes there is significant ketosis.

Outcome: If untreated there is a high morbidity and mortality associated with the hypoglycaemia. Treatment is by avoidance of fasting and is usually very successful.

Enzymatic defect: Medium chain acyl-CoA dehydrogenase

Diagnosis: Patients show increased ratios of free fatty acids/3-hydroxybutyrate, and increased urine medium chain acylglycines (hexanoyl and suberylglycine) on organic acid analysis. Plasma medium chain acylcarnitines are elevated and fibroblast fatty acid oxidation of [9,10]-3Hmyristate is reduced. In the Caucasian MCADD population there is a common mutation (K304E).

Treatment: No specific dietary treatment required apart from the avoidance of fasting and an emergency regimen for times of illness.

Screening: MCADD is estimated to have a frequency of 1/10000 in the UK population and currently is now tested for as part of the National Newborn Screening Programme. However patients may still be missed and any patients born outside the UK or before full National Screening was implemented may not have been tested.
Long Chain 3-hydroxyacyl-CoA Dehydrogenase Deficiency/Trifunctional Protein Defect

Clinical Presentation: In its severest form this disorder presents with collapse and death in the neonatal period with acidosis and heart and liver disease. Slightly less severe forms may show failure to thrive from an early age with repeated attacks of lactic acidosis and hypoglycaemia often triggered by intercurrent infections and much milder adult onset forms with muscle or neurological problems have been reported.

Outcome: If untreated there is a very high morbidity and mortality. With treated there is a variable outcome but it can sometimes be very good.

Enzymatic defect: The Trifunctional Protein complex consists of three enzymes - long chain 3-hydroxyacyl-CoA dehydrogenase, long chain enoyl-CoA hydratase and long chain 3-keto acyl-CoA thiolase. In TFP deficiency all enzymes are affected. In the more common LCHAD deficiency only the long chain 3-hydroxyacyl-CoA dehydrogenase is defective.

Diagnosis: The plasma free fatty acid/3-hydroxybutyrate ratio is increased and urine organic acid analysis shows increased excretion of hydroxydicarboxylic acids. Plasma acylcarnitines show increased long chain hydroxy acylcarnitines. There is common mutation (E474Q) causing the isolated LCHAD deficiency. Fatty acid flux studies in fibroblasts show reduced long chain fatty acid oxidation.

Treatment: The severe hypoglycaemia is treated with high carbohydrate feeds often delivered overnight by nasogastric tube. To provide energy medium chain triglycerides are used as these bypass the enzyme block and provide energy predominantly through ketone body production in the liver. It is important to avoid a catabolic state with the consequential production of toxic metabolites.
Carnitine Palmitoyl Transferase Deficiency Type 2 (CPT2)

Clinical Presentation: The severe neonatal form is often fatal and presents with hypoketotic hypoglycaemia, cardiomyopathy, hypotonia and congenital abnormalities. There is a milder form often presenting in older children or adults with recurrent attacks of rhabdomyolysis triggered by prolonged aerobic exercise or sometimes catabolic stress.

Outcome: In the neonatal form there is high morbidity and mortality. In the adult form with repeated rhabdomyolysis attacks on exercise there is a risk of renal failure secondary to the rhabdomyolysis.

Enzymatic defect: **Carnitine Palmitoyltransferase type 2**

Diagnosis: In the severe form the plasma acylcarnitines show increased long chain acylcarnitines with free carnitine depletion and fatty acid flux studies show reduced long chain fatty acid oxidation. In the mild adult forms acylcarnitines and fatty acid flux studies may be normal. However specific enzyme assay of CPT2 will diagnose all forms.

Treatment: In the neonatal/infantile form treatment is by a low long-chain fat / high carbohydrate diet supplemented with medium chain triglycerides. It is important to avoid fasting and the patient needs an emergency regime during times of catabolism/infection/stress. In the adult forms medium chain triglyceride rich meals and maintenance of glycogen stores prior to exercise may help.
Questions

- Name four biochemical processes involved in energy production from fat
- Name four defects of fatty acid oxidation
- Which tests would you do as a basic screen for a fatty acid oxidation defect?
- How would you go about confirming a fatty acid oxidation defect?