Guidelines for the investigation of rhabdomyolysis for inherited metabolic disorders

Definition of rhabdomyolysis

Rhabdomyolysis is defined as an acute increase in serum concentrations of creatine kinase to more than five times the upper normal limit of normal when myocardial infarction has been excluded as a cause (Troponin I < 0.03ng/ml / Troponin T <0.03ng/ml). Rhabdomyolysis results from injury to the sarcolemma (membrane) of skeletal muscle cells causing leakage of cellular components into the blood or urine. CK peaks in serum at 30-50 hours following muscle injury. Myoglobin peaks in serum much sooner than CK at around 17 hours post injury and visible myoglobinuria (tea or cola coloured urine) occurs when urinary myoglobin exceeds 300-1000μg/ml (normal <10 ng/ml). However the term myoglobinuria is insufficiently defined to be of use in the definition of rhabdomyolysis.

Guidelines

Patients with unexplained rhabdomyolysis (i.e. after exclusion of acquired causes -for extensive list see Beetham R 2000) should be investigated for possible metabolic causes. Fig 1 is a suggested outline for investigation of patients with no specific clues to the diagnosis. These will include clinical assessment and first line blood and urine biochemical testing. Non-invasive non-biochemical testing may also have been undertaken at this stage (appendix 1). It may be useful to use exercise as a tool to facilitate evaluation (appendix 2). Such tests will include cycle ergometers or treadmills that will provide information on cardiac output during exercise, oxygen extraction per unit of blood, ventilation relative to oxygen uptake and lactate production. The forearm non-ischaemic exercise test is potentially very useful when undertaken by experienced clinicians for evaluating the probability of some GSD's or myoadenylate deaminase deficiency in adolescents and adults (see protocol in appendix 2). An in depth description of the metabolic myopathies is described elsewhere. More specialist biochemical testing is only indicated on muscle or skin fibroblasts after this preliminary assessment and investigations have been completed and evaluated (Fig 2). The specialised laboratory investigations are detailed in appendix 3.
Presentation with Rhabdomyolysis

?Family History of Disease
Note: - there may be variable phenotype expression of the same disease among family members
Physical Examination

Exclude non-metabolic cause:
- inflammatory myopathies
- toxicological
- infection

Clinical Investigation
Neurological
Cardiac
*Gastrointestinal
*Ophthalmology
*Audiology

1st line Biochemical Investigations

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<th>Plasma</th>
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<th>CSF*</th>
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<td>Amino acids</td>
<td>Lactate</td>
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<td>Acylcarnitine profile</td>
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Consider Additional Testing (non invasive)
Exercise testing (e.g. treadmill studies)
** Forearm exercise test
EMG
ECG
31P-MRS
*Particularly important in the investigation of possible respiratory chain disease
** For uses and protocol see appendix 3
EMG – Electromyography, ECG – Electrocardiography, $^{31}$P-MRS – Phosphorus magnetic resonance spectroscopy

Figure 2

**Suspicion of metabolic myopathy**

**Glycogenoses**
- Early exercise intolerance (e.g. cramps / pain / myoglobinuria on climbing stairs / heavy lifting)
- CK usually chronically raised
- Other first line tests usually normal
- Respiratory muscles may be involved e.g. late onset Pompe disease (GSD II)

**Fatty acid oxidation defects**
- Exercise intolerance – pain / stiffness / myoglobinuria – typically on prolonged sustained aerobic exercise, exacerbated by poor food intake / cold / heat
- There may be myalgia with intercurrent infection
- Peripheral neuropathy and episodic rhabdomyolysis in mild TFP deficiency
- CK usually normal between episodes
- Plasma acylcarnitines may be abnormal e.g. $C_{14:1}^\dagger$ in VLCAD, $C_{16}^\dagger - C_{18:1} / C_2$ ratio in CPT2
- Mild increase in plasma long-chain hydroxyacylcarnitines in mild TFP deficiency.
- Urine organic acids – DCA / (OH) DCA

**Purine cycle defect**
- Exercise intolerance – cramps / pain
- CK usually normal between episodes
- Other first-line tests normal

**Muscle biopsy**
Histology and enzyme assay where indicated by initial results
**EDTA blood**
*Mutation analysis of PYGM (GSDV)

**Skin biopsy**
Fatty acid oxidation flux assay
Specific CPT2 enzyme assay
**EDTA blood**
*Common p.S113L mutation in CPT2

**EDTA blood**
*Mutation analysis for myoadenylate deaminase AMPD1**
**Muscle biopsy**
If there is a high suspicion of a relatively common disorder where a common mutation(s) is known (e.g., McArdle’s disease, GSD type V, PYGM gene) or myoadenylate deaminase deficiency AMPD1, then mutation analysis may be indicated at this stage. The "common" p.S113L mutation of CPT2 deficiency (accounting for ~55% of disease) may be worth screening for if CPT2 is strongly suspected. However, negative results on mutation testing do not of course completely exclude CPT2 deficiency, only specific CPT2 assay on fibroblasts will do this.

Where a homozygous mutation in the myoadenylate deaminase gene is confirmed, further studies should continue to establish whether or not a second defect is present, as most patients with isolated myoadenylate deaminase deficiency are asymptomatic and the possibility of another contributary defect should therefore be considered.5, 6
Appendix 1

Specialised non-invasive non-biochemical testing

Specialised non-invasive testing as outlined below is not universally available in the UK, but may in certain specialist centres provide additional information that can be used to indicate the nature of the underlying defect e.g. respiratory chain defect. It can be particularly useful to undertake this testing after exercising (see appendix 3).

Electromyography (EMG) may show myopathic features such as fibrillations, positive sharp waves, and myotonic discharges. Electrocardiography (ECG) shows left ventricular or biventricular hypertrophy in some patients with myopathy, whereas radiographic evidence for cardiomegaly is uncommon.

Phosphorus – magnetic resonance imaging (P-MRS) can be particularly useful in myopathies associated with impaired energy metabolism. MR spectroscopy is used non-invasively for the direct and continuous assessment of tissue metabolites and is particularly useful in repeat monitoring of muscle bioenergetics during rest, exercise and recovery. Not all compounds that contain phosphorus produce visible signals on MRS. In human muscle, only unbound metabolites present in millimolar concentrations give rise to distinct peaks. Five major peaks are seen in muscle and these include three from the ATP molecule, one from phosphocreatine (PCr), and one from inorganic phosphates (Pi). MRS can provide diagnostic information on the different cellular levels of high-energy phosphates.
Appendix 2

Forearm Non-ischaemic Exercise Test

Purpose
Test for muscle phosphorylase deficiency (McArdles disease) or other glycolytic defects, and myoadenylate deaminase deficiency.

Method
Fast patient from midnight and avoid exercise for 30 minutes before test starts. Insert an arterial cannula into the antecubital vein then rest the patient for a further 10 minutes. Keep the line patent with heparin.

Take a baseline resting sample for lactate, ammonia, urate, phosphate, carnitine, CK and renal profile and any other tests required for general investigation.

Exercise the forearm by squeezing a sphygmomanometer bulb to exhaustion (usually for 1-2 minutes). Pain may be experienced which is normal; despite this determined exercise should be encouraged to avoid equivocal results.

At the end of exercise sample for lactate and ammonia at 1, 2, 4, 6, 8 and 10 minutes. Each sample must be processed immediately.

Also at end of exercise clinically examine the forearm for contracture and the fingers for flexure. A rigid forearm or inability to extend the fingers may be an indication of metabolic muscle disease.

Interpretation of Results
Normal response is a significant rise, usually at least 3-5 fold, in lactate and ammonia. No increase in lactate indicates a defect in the glycolytic pathway. No increase in ammonia suggests myoadenylate deaminase deficiency. A generally poor response in all parameters may reflect inadequate exercise.³

Aerobic Exercise Test

Purpose
For the investigation of aerobic exercise capacity in patients with possible mitochondrial respiratory chain disease.

Patient
Fast overnight with water only unless the patient is diabetic on insulin when individual arrangements will be made with clinician concerned.
Samples
2 ml EDTA blood for ammonia.
5 ml heparinised blood for urate, phosphate, potassium and other tests that may be required.
Blood collected into a fluoride tube for lactate.

Method
Stress patient with bicycle ergometer. The level of exercise is set to achieve an output of 50 watts.

Exercise for 15 minutes taking samples at 0, 5, 10 and 15 minutes
With the patient at rest take further samples at 30, 40 and 60 minutes.

Interpretation of Results
Provided the patient does not become anoxic there should be little, or no, change in metabolite levels.

Appendix 3

Specialised Laboratory Investigations (muscle biopsy - histology, enzyme analysis & mutation analysis on blood)

The specialist tests are listed below:-

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<td><strong>Mutation analysis</strong></td>
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<td>(Common mutations)</td>
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<tr>
<td>AMPD1 c.34C&gt;T (p.Q12X)</td>
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<tr>
<td>PYGM c.158C&gt;T (p.R50X) in exon 1</td>
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<tr>
<td>PYGM c.613G&gt;A (p.G205S) in exon 5</td>
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<tr>
<td>CPT2 c.338C&gt;T (p.S113L) in exon 3</td>
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**Muscle Biopsy**

When taking a muscle biopsy several factors need to be considered as these may impact on the biochemical findings (e.g. anaesthetic influence, structural defects in muscle, lack of standardised handling and processing) and other additional environmental influences to the patient (e.g. genetic background, existing therapies at the time of biopsy, environmental triggers).

For quantitative biochemical studies, it is important to take the biopsy from a clinically affected muscle but not from an area that represents end stage disease. Furthermore, if a local anaesthetic is used, it is important to avoid taking the biopsy from the site where the anaesthetic is administered as this may affect the result. It is important to note if the patient is on any co-factor / vitamin / carnitine therapy or IV glucose at the time of the biopsy as these therapies can up-regulate respiratory chain function or lead to high levels of stored muscle glycogen. Correct orientation of muscle fibres is vital for full evaluation. The muscle specimen should be placed in a dry pot (for example a deep 22mm diameter petri dish with lid) which is the put in the fridge if not handled immediately. A specimen in a dry pot in the fridge (or on ice) is good for upto 2 hours for histochemistry and most "fresh" biochemistry but is not so good for EM. An aliquot of the biopsy should be snap frozen at the bedside in liquid nitrogen and stored at -80°C for homogenate studies i.e. respiratory chain complexes and enzyme studies. An aliquot of tissue should also be processed for paraffin histology and electron microscopy. If molecular studies are pursued, tissue from any of the above can be used for DNA analysis.

The laboratory analysing muscle biopsies **MUST** be contacted prior to undertaking a biopsy and they will supply their local information.

**NB** Muscle which has been in contact with OCT (histochemistry mounting medium) is NOT suitable for assay of glycogen or glycolysis enzymes. OCT is a complex polysaccharide and interferes with these assays.

Experience suggests that using saline soaked gauze to keep a muscle biopsy fresh in a petri dish renders the biopsy fairly useless; washing out some of the enzymes; causing ice crystal and EM artefacts.
# MUSCLE BIOPSY ANALYSIS

## BIOCHEMISTRY

**FROZEN (homogenate)**
- Glycogen quantitation
- Glycogen structure
- RES complexes I, II/III, IV, ubiquinone
- GSD enzymes e.g. Types V, type VII, PbK
- Glycolytic enzymes e.g. PGK, PGM, LDH

**FRESH (intact mitochondria)**
- RES complexes I, II/III, IV
- Polarography – oxygen consumption with various substrates / inhibitors
- ATP synthesis (complex V)

- SDS electrophoresis / Western blotting for membrane associated abnormalities

## HISTOLOGY

- Abnormal enzyme staining (SDH, COX, COX+SDH, NADH, PPL, PFK, AMPD)
- Abnormal storage - lipid, glycogen
- Abnormal Gomori trichrome staining - RRF
- Also ragged blue fibres on SDH staining
- Abnormal fibre architecture e.g. myopathy / dystrophy

### Electron microscopy

- Abnormal mitochondrial structure and / or numbers
- Glycogen pooling, lipid accumulation

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AMPD - myoadenylate deasminase, PPL - (myo) phosphorylase - McArdle's disease (GSD type V), PFK - muscle phosphofructokinase (GSD type VII), PbK phosphorylase b kinase, PGK phosphoglycerate kinase, PGM phosphoglycerate mutase, LDH lactate dehydrogenase, RES - mitochondrial respiratory chain enzymes, GSD - glycogen storage disease, SDS sodium dodecylsulphate polyacrylamide gel electrophoresis, SDH - succinate dehydrogenase, COX - cytochrome oxidase (complex IV)

### Enzyme analysis on cultured fibroblasts

A skin biopsy is recommended for the investigation of fatty acid oxidation defects as enzyme analysis in muscle is generally much less reliable. Flux assays in fibroblasts using [9,10-\(^3\)H]myristate, [9,10-\(^3\)H]palmitate and [9,10-\(^3\)H]oleate have the potential to pick up "mild" VLCAD, CPT2 deficiency & mild mitochondrial trifunctional protein deficiency (TFP). Mild TFP deficiency can present with isolated peripheral neuropathy and episodic rhabdomyolysis in both children and adults. However, CPT2 activity should be assessed by specific enzyme assay, particularly as partial deficiencies of this enzyme can lead to clinical disease but may not be detected by flux assay.
References


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Disclaimer

These are laboratory guidelines reflecting current best practice in specialist metabolic laboratories across the UK. They are not evidence based but reflect expert opinion.
The Network cannot accept any responsibility for any errors/omissions, and users must take responsibility for use.

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