Quantitative Fibroblast Acylcarnitine Profiling

In

The Diagnostic and Prognostic Assessment of Mitochondrial Fatty Acid ß-Oxidation Disorders

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A thesis submitted in fulfilment of the requirements for the degree of Master of Science in Medicine

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Mitochondrial fatty acid β-oxidation disorders are a group of clinically and biochemically heterogeneous defects mainly associated with intolerance to catabolic stress. The diseases are potentially fatal, but treatable and the prognosis for most diagnosed disorders is generally favourable. Early diagnosis is thus important to prevent morbidity and mortality.

This project describes an improved and validated quantitative fibroblast acylcarnitine profile assay for the investigation of suspected fatty acid β-oxidation disorders. Intact cells were incubated with deuterium-labelled hexadecanoate and L-carnitine, and the accumulated acylcarnitines in the medium analysed using electrospray tandem mass spectrometry. This modified procedure is less demanding technically, requires fewer cells and better reflects the in vivo acylcarnitine status than previously published methods.

Mitochondrial fatty acid β-oxidation is coupled to the respiratory chain. Functional defects of one pathway may lead to secondary alterations in flux through the other. The diagnostic specificity and the prognostic potential of the in vitro acylcarnitine profile assay were investigated in fibroblasts from 14 normal controls, 38 patients with eight enzyme deficiencies of fatty acid β-oxidation presenting with various phenotypes, and 16 patients with primary respiratory chain defects including both isolated and multiple enzyme complex defects. All fatty acid β-oxidation deficient cell lines revealed disease-specific acylcarnitine profiles related to the sites of defects irrespective of the severity of symptoms or of different mutation. Preliminary studies suggested a
correlation between severity of symptoms and higher concentrations of long-chain acylcarnitine species. However, the fibroblast acylcarnitine profiles from some patients with respiratory chain defects were similar to those of controls, whereas others had abnormal profiles resembling those found in fatty acid β-oxidation disorders.

*In vitro* acylcarnitine profiling is useful for the detection of fatty acid β-oxidation deficiencies, and perhaps the prediction of disease severity and prognostic evaluation facilitating decisions of therapeutic intervention and genetic counselling. However, abnormal profiles do not exclusively indicate these disorders, and primary defects of the respiratory chain remain a possibility. Awareness of this diagnostic pitfall will aid in the selection of subsequent confirmatory tests and therapeutic options.
DECLARATION

The experimental work described in this thesis was carried out in the Department of NSW Biochemical Genetics Service, The Children’s Hospital at Westmead, Sydney, during the period from January 2000 to December 2001. All experimental work and data presented is a result of my own work, except where explicit reference to the work of others is given in the text or acknowledgements.

None of the material presented herein has been submitted to any other university or institute for the purpose of obtaining a higher degree.

Keow Giak Sim

Date
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Publications Arising From This Thesis

Original Manuscripts


Abstracts


Conference. The University of Sydney, College of Health Sciences. Leura, New South Wales, Australia.


LIST OF ABBREVIATIONS

\(^2\text{H}\) deuterium

\(^2\text{H}_2\)-C\(_{18}:1\) [9,10-\(^2\text{H}_2\)]oleic acid

\(^2\text{H}_3\)-C\(_{16}\) [16,16,16-\(^2\text{H}_3\)]palmitic acid

\(^2\text{H}_4\)-C\(_{18}:2\) 9,12-[17,17,18,18-\(^2\text{H}_4\)]linoleic acid

\(^2\text{H}_5\)-palmitate [15,15,16,16,16-\(^2\text{H}_5\)]hexadecanoic acid

\(^3\text{H}\) tritium

L litre

µmol micromole

µg microgram

µL microlitres

mg milligram

mL millilitre

nmol nanomole

pmol picomole

C\(_0\) free carnitine

C\(_2\)- acetylcarnitine

C\(_3\)- propionylcarnitine

C\(_4\)- butyryl- or isobutyrylcarnitine

C\(_5\)- isovaleryl or 2-methyl-butyrylcarnitine

C\(_6\)- hexanoylcarnitine

C\(_8\)- octanoylcarnitine

C\(_{10}\)- decanoylcarnitine

C\(_{12}\)- dodecanoylcarnitine
C$_{14}$- tetradecanoylcarnitine
C$_{14:1}$- tetradecenoylcarnitine
C$_{16}$- hexadecanoylcarnitine or palmitoylcarnitine
C$_{16:1}$- hexadecenoylcarnitine
C$_{18}$- octadecanoylcarnitine
C$_{18:1}$- octadecenoylcarnitine
C$_{4}$-OH- hydroxy-butyrylcarnitine
C$_{5}$-OH- 3-hydroxyisovalerylcarnitine or 2-methyl-3-hydroxy-butyrylcarnitine
C$_{6}$-OH- hydroxy-hexanoylcarnitine
C$_{8}$-OH- hydroxy-octanoylcarnitine
C$_{14}$-OH- hydroxy-tetradecanoylcarnitine
C$_{16}$-OH- hydroxy-hexadecanoylcarnitine
C$_{18}$-OH- hydroxy-octadecanoylcarnitine
ACD acyl-CoA dehydrogenases
ACS acyl-CoA synthetase
AFLP acute fatty liver of pregnancy
Alb-BP albumin-binding protein
ATP adenosine triphosphate
CACT carnitine acylcarnitine translocase
CoA coenzyme A
CID collision-induced dissociation
CPT1 carnitine palmitoyltransferase I
CPT1A hepatic carnitine palmitoyltransferase I
CPT1B muscle carnitine palmitoyltransferase I
CPT2 carnitine palmitoyltransferase II
CT  plasma membrane carnitine transporter
CV  coefficient of variation
DBS whole blood dried on filter paper sample
DNA  deoxyribonucleic acid
DECR1 2,4-dienoyl-CoA reductase
ECH enoyl-CoA hydratases
ESI-MS/MS electrospray ionisation tandem mass spectrometry
ETF electron transfer flavoprotein
ETFDH electron transfer flavoprotein dehydrogenase
FAB-MS/MS fast atom bombardment tandem mass spectrometry
FABPpm membrane-associated plasmalemmal fatty acid-binding protein
FABPc cytoplasmic fatty acid-binding proteins
FAD flavin adenine dinucleotide
FADH$_2$ reduced form of FAD
FAO mitochondrial fatty acid β-oxidation
FAT fatty acid translocase
FATP long-chain fatty acid transporter protein
GC-CI-MS gas chromatography chemical ionisation mass spectrometry
HAD hydroxyacyl-CoA dehydrogenases
HELLP haemolysis, elevated liver enzymes and low platelet counts
HMGCL 3-hydroxy-3-methyl-glutaryl-CoA lyase
HMGCS2 3-hydroxy-3-methyl-glutaryl-CoA synthetase
KAT ketoacyl-CoA thiolases
LACS long chain acyl-CoA synthetase
LCAD long chain acyl-CoA dehydrogenase
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<td>LCEH</td>
<td>long-chain 2-enoyl-CoA hydratase</td>
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<tr>
<td>LCFA</td>
<td>long-chain fatty acid</td>
</tr>
<tr>
<td>LCFA-CoA</td>
<td>long-chain fatty acyl-CoA ester</td>
</tr>
<tr>
<td>LCHAD</td>
<td>long-chain 3-hydroxyacyl-CoA dehydrogenase</td>
</tr>
<tr>
<td>LCKAT</td>
<td>long-chain 3-ketoacyl-CoA thiolase</td>
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<td>LSI-MS/MS</td>
<td>liquid secondary ionisation tandem mass spectrometry</td>
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<td>M</td>
<td>[9,10-³H]myristate</td>
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<td>MAD</td>
<td>multiple acyl-CoA dehydrogenase</td>
</tr>
<tr>
<td>MCA</td>
<td>multi-channel acquisition</td>
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<td>medium-chain acyl CoA dehydrogenase</td>
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<td>MCKAT</td>
<td>medium chain 3-ketoacyl-CoA thiolase</td>
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<tr>
<td>MELAS</td>
<td>mitochondrial encephalomyopathy, lactic acidosis and stroke like episodes</td>
</tr>
<tr>
<td>MERRF</td>
<td>myoclonic epilepsy ragged red fibre syndrome</td>
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<tr>
<td>M/SCHAD</td>
<td>medium/short chain 3-hydroxyacyl-CoA dehydrogenase</td>
</tr>
<tr>
<td>MTP</td>
<td>mitochondrial trifunctional protein</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>reduced form of NAD</td>
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<tr>
<td>O</td>
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<td>radio-HPLC</td>
<td>radio-high-pressure liquid chromatography</td>
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<tr>
<td>SCAD</td>
<td>short-chain acyl CoA dehydrogenase</td>
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SCEH    short chain 2-enoyl-CoA hydratase
SCFA    short-chain fatty acid
SCFA-CoA short-chain fatty acyl-CoA ester
SCHAD   short-chain 3-hydroxyacyl-CoA dehydrogenase
SD      standard deviation
SIDS    Sudden infant death syndrome
VLCAD   very-long-chain acyl CoA dehydrogenase