CHAPTER 5

Acylcarnitine Profile Study in Fibroblasts from Patients with Respiratory Chain Defects

5.1 Introduction

The pathways of mitochondrial fatty acid β-oxidation (FAO) and respiratory chain (RC) oxidative phosphorylation are linked via the ubiquinone pool at two stages (Eaton et al 1996a), and also via the activity of the citric acid cycle. Inborn errors of mitochondrial fatty acid β-oxidation (Pollitt 1995; Roe and Ding 2001; Rinaldo et al 2002) and the respiratory chain (Christodoulou 2000; Shoffner and Wallace 2001) are clinically and biochemically heterogenous. Whereas FAO defects usually involve single enzymes, defects in the RC may occur as multiple defects of two or more of the five enzyme complexes. These two groups of disorders have some clinical and biochemical features in common including muscle weakness, cardiomyopathy, encephalopathy, liver dysfunction, metabolic decompensation during catabolic stress, hypoglycaemia and lactic acidosis. Patients with primary RC defects can exhibit organic acidurias mimicking FAO disorders (Christensen et al 1993; Bennett et al 1994b; Enns et al 2000). Functional enzyme analyses have revealed concomitantly reduced activities of some enzymes in both pathways (Reichmann et al 1992; Tyni et al 1996; Das et al 2000; Diogo et al 2000). Histopathological findings (Watmough et al 1990; Tyni et al 1996), and in vitro oxidation rate studies (Venizelos et al 1998) may not discriminate between defects of these two groups of disorders. It is apparent that impairment of one pathway may lead to secondary alterations in flux through the other.

The finding reported in Chapter 4 shows that in vitro acylcarnitine profiling is a useful tool for the investigation of patients suspected with FAO disorders when in
in vivo findings are not conclusive. However, it is not clear whether acylcarnitine profiling is entirely specific for FAO disorders, as fibroblasts from patients with RC defects have not previously been studied in depth. This chapter reports the findings of acylcarnitine profiles in fibroblasts from patients with RC defects.

5.2 Aim

Specificity study: To investigate the acylcarnitine profiles in fibroblasts from patients with respiratory chain defects.

5.3 Materials and Methods

5.3.1 Cell lines

Control: Normal control cultured skin fibroblasts were obtained from 14 healthy laboratory workers.

Respiratory chain defects: Cultured skin fibroblasts were available from 16 patients with RC defects confirmed by measurement of the activities of the enzyme complexes in one or more tissues including skeletal muscle, heart muscle, liver and cultured skin fibroblasts, with DNA mutation analysis performed in some cases (mostly by David Thorburn at The Murdoch Children’s Research Institute, Royal Children’s Hospital, Victoria). These cell lines were from patients with isolated complex I (n=5), complex II (n=1), complex IV (n=3), combined deficiencies of complexes I, III and IV (n=5), and combined complexes I, II + III, III and IV deficiencies (n=2). Cell lines between passages 4 and 19 were used for the assays. A summary of clinical phenotype, functional defects and gene defects (if identified) for each patient is presented in Table 5.1.
5.3.2 Case Reports of Some Respiratory Chain Deficient Patients

The case presentations reported below are from selected patients with RC defects whose acylcarnitine profiles in cultured skin fibroblasts resemble those from patients with various FAO disorders.

**Patient R4:** A female patient presented with skeletal myopathy. Complex I activity in muscle was reduced to 11% of normal control, and normal activity was found in fibroblasts. Molecular studies revealed mutation in mitochondrial tRNA\textsuperscript{Leu(UUR)} gene, and this patient is responsive to riboflavin therapy.

**Patient R6:** A boy with intra-uterine growth retardation and mild dysmorphism with proximal limb shortening and large fontanelles was well until seven months of age when he developed severe liver dysfunction in conjunction with an intercurrent viral illness. Blood lactate and pyruvate concentrations were normal at the time. Following this episode, he had further similar episodes with intercurrent viral illnesses and marked deterioration with prostration, drowsiness, abnormal liver function tests and ketoacidosis. He died at the age of 14 months after a period of illness. A diagnosis of complex II deficiency was made by measurement of enzyme complexes in the liver (by D. Thorburn). Complex II activity in liver homogenate was reduced to 16% of control mean, while the activities of complex II, II+III, and IV were normal in fibroblasts. Fatty acid oxidation rate studies in intact fibroblasts using [9,10-\textsuperscript{3}H]myristate (M) and [9,10-\textsuperscript{3}H]palmitate (P) showed marginally reduced oxidation rates compared to intra batch controls, with ratio of P/M=1. As a consequence of the acylcarnitine findings in the fibroblasts, mutation analysis in the newborn screening dried blood sample of this patient revealed that he was heterozygous for the medium chain acyl-CoA dehydrogenase mutation, 985A>G.
**Patient R8:** This patient presented with facial dysmorphism, developmental delay, hypotonia and was found to have a lactic acidosis. Isolated complex IV deficiency was demonstrated in skeletal muscle, transformed lymphoblasts and fibroblasts (26% of control). She is homozygous for the 312del10, insAT mutation in exon 4 of the \textit{SURF-1} gene.

**Patient R10:** A female infant of consanguineous parents presented with liver disease and failure to thrive. Biochemical abnormalities included lactic acidosis, hypoglycemia and coagulopathy. Analyses of enzyme complex activities in liver homogenate showed that there were multiple deficiencies including complex I, III, and IV, with 15, 16, and 37% of activity of intra batch controls. Similar results were obtained in muscle homogenate but were less dramatic. However, the complex IV activity in fibroblasts was normal.

**Patient R15:** This patient had primary lactic acidosis, with multiple complex deficiencies of complex I, II+III, III, and IV, with activities of 45, 35, 65 and 39% of normal controls demonstrated in fibroblasts.

5.3.3 **Cell Culture**

This is described in Chapter 2, Section 2.5.

5.3.4 **In Vitro Acylcarnitine Profile Assay**

This is described in Chapter 2, Section 2.8, and Chapter 4, Section 4.3.4.

5.3.5 **Total Protein Determination**

This is described in Chapter 2, Section 2.9.
5.3.6 Data Analysis

Reference range of control cell lines (n=14, 72 observations) were determined as described in Chapter 4 Section 4.3.6.

All cell lines from patients with RC defects were analysed in duplicate in each batch and batches were repeated two to four times following successive subcultures. Fibroblast acylcarnitine levels of individual cell lines were calculated as the average of four to eight observations obtained from two to four separate assays (Table 5.1).

5.4 Results

Figures 5.1 to 5.5 show the acylcarnitine mass spectra from skin fibroblasts of some patients with RC defects and from patients with FAO deficiencies, for comparison of profiles between these two groups of disorders.

Quantitative fibroblast acylcarnitine profiles in individual patients with RC defects are shown in Table 5.1. Of the five cell lines from patients with isolated complex I deficiency, three showed a profile similar to those of controls, while two revealed increased concentration of $^2$H$_5$-C$_4$-acylcarnitine (R3 and R4), suggestive of SCAD dysfunction. The cell line from a patient with complex II deficiency demonstrated in liver but not skin fibroblasts (R6) exhibited elevated concentrations of $^2$H$_5$-labelled C$_6$-, C$_8$-, C$_{10}$- with C$_8$-acylcarnitine being the dominant species, strongly resembling a profile of MCAD deficiency. One of the three cell lines with isolated complex IV defect (R8) showed accumulation of a number of medium- and long-chain species suggestive of MAD deficiency. Of the five cell lines from patients with multiple deficiencies of complexes I, III and IV, profiles similar to controls were observed in two (R11 and R13), or mimicking those with CPT II/CACT deficiency.
were seen in two (R10 and R14) or LCHAD deficiency was seen in one (R12). One of
the two cell lines with combined deficiencies of complexes I, II + III, III and IV
revealed marked accumulation of $^{2}\text{H}_5\text{-C}_{16}$- and $^{2}\text{H}_5\text{-C}_4$-acylcarnitine (R15), a profile
suggestive of combined dysfunction of both short- and long-chain (but not
medium-chain) fatty acid oxidation, while the other had a normal profile.
**Figure 5.1a:** Acylcarnitine profile found in the reaction medium of fibroblasts from a patient with complex II deficiency demonstrated in liver but not in skin fibroblasts (R6, Table 5.2)

**Figure 5.1b:** Acylcarnitine profile found in the reaction medium of fibroblasts from a patient with medium-chain acyl-CoA dehydrogenase deficiency (F35, Chapter 4, Table 4.1)
Figure 5.2a: Acylcarnitine profile seen in the reaction medium of fibroblasts from a patient with complex IV deficiency demonstrated in skeletal muscle and cultured skin fibroblasts (R8, Table 5.2)

Figure 5.2b: Acylcarnitine profile seen in the reaction medium of fibroblasts from a patient with multiple acyl-CoA dehydrogenase deficiency (F26, Chapter 4, Table 4.1, Figure 4.9)
Figure 5.3a: Acylcarnitine profile seen in the reaction medium of fibroblasts from a patient with multiple deficiencies of complex I, III and IV in liver (R14, Table 5.2).

Figure 5.3b: Acylcarnitine profile seen in the reaction medium of fibroblasts from a patient with carnitine palmitoyltransferase II deficiency (F2, Chapter 4, Table 4.1)
Figure 5.4a: Acylcarnitine profile found in the reaction medium of fibroblasts from a patient with multiple deficiencies of complex I, III and IV in skeletal muscle with mitochondrial tRNA\textsuperscript{Lys} mutation (R12, Table 5.2).

Figure 5.4b: Acylcarnitine profile found in the reaction medium of fibroblasts from a patient with long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency (F19, Chapter 4, Table 4.1)
Figure 5.5: Acylcarnitine profile found in the reaction medium of fibroblasts from a patient with multiple deficiencies of complex I, II+III, III and IV in skin fibroblasts (R15, Table 5.2).

Peak identities are: \( m/z \) 260, acetylcarnitine; 274, propionylcarnitine; 277.2, \( \text{d}_3 \)-propionylcarnitine (IS); 288.2, butyrylcarnitine; 291.2, \( \text{d}_3 \)-butyrylcarnitine (IS); 293, \( \text{d}_5 \)-butyrylcarnitine; 302, isovaleryl carnitine and 2-methylbutyrylcarnitine; 311, \( \text{d}_9 \)-isovaleryl carnitine (IS); 316.2, hexanoylcarnitine; 321.2, \( \text{d}_5 \)-hexanoylcarnitine; 344.2, octanoylcarnitine; 347.2, \( \text{d}_3 \)-octanoylcarnitine (IS); 349.2, \( \text{d}_5 \)-octanoylcarnitine; 372.2, decanoylcarnitine; 377.2, \( \text{d}_5 \)-decanoylcarnitine; 400.3, dodecanoylcarnitine; 405.3, \( \text{d}_5 \)-dodecanoylcarnitine; 428.4, myristoylcarnitine; 433.6, \( \text{d}_3 \)-myristoylcarnitine; 437.6, \( \text{d}_9 \)-myristoylcarnitine (IS); 456.6, palmitoylcarnitine; 459.6, \( \text{d}_3 \)-palmitoylcarnitine (IS); 461, \( \text{d}_5 \)-palmitoylcarnitine; 472.6, hydroxypalmitoylcarnitine, 477.6, \( \text{d}_5 \)-hydroxypalmitoylcarnitine.
Table 5.1: Fibroblast acylcarnitine results from patients with respiratory chain defects after incubation with [15,15,16,16,16-2H5]hexadecanoic acid and L-carnitine for 72 hours

<table>
<thead>
<tr>
<th>Functional Defect</th>
<th>Tissue Defect(s)</th>
<th>Gene Defect</th>
<th>Clinical Phenotypes</th>
<th>Acylcarnitine (nmol/mg protein) in media at 72h</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C4-</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
</tr>
<tr>
<td>Complex I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1 M, L, F NK</td>
<td></td>
<td></td>
<td>Dysmorphic, LA</td>
<td>2.0</td>
</tr>
<tr>
<td>R2 H NK</td>
<td></td>
<td></td>
<td>Cardiomyopathy</td>
<td>1.7</td>
</tr>
<tr>
<td>R3 M mt tRNA&lt;sub&gt;Leu(UUR)&lt;/sub&gt;</td>
<td>M</td>
<td>MELAS</td>
<td>2.8</td>
<td>0.8</td>
</tr>
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<td>R4 M mt tRNA&lt;sub&gt;Leu(UUR)&lt;/sub&gt;</td>
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<td>Skeletal myopathy</td>
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<td>1.1</td>
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<td>R5 M, F NK</td>
<td></td>
<td></td>
<td>Leigh disease</td>
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<tr>
<td>Complex II</td>
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<td></td>
<td>Hepatic failure</td>
<td>1.5</td>
</tr>
<tr>
<td>R6 L NK</td>
<td></td>
<td></td>
<td></td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Controls (n=14)</td>
<td></td>
<td></td>
<td>Mean of 72 observations</td>
<td>0.3-2.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Reference range (Mean ± 1.96 SD)</td>
<td>0.3-2.2</td>
</tr>
<tr>
<td>Functional Defect</td>
<td>Tissue Defect(s)</td>
<td>Gene Defect</td>
<td>Clinical Phenotypes</td>
<td>Acylcarnitine (nmol/mg protein) in media at 72h</td>
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<td></td>
<td></td>
<td></td>
<td>$C_{4^-}$</td>
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<tr>
<td>Complex IV</td>
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<td></td>
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<tr>
<td>R7</td>
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<td>NK</td>
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<td>Neonatal LA</td>
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<td>Complex I, III and IV</td>
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<tr>
<td>R10</td>
<td>L</td>
<td>NK</td>
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<td>R11</td>
<td>F</td>
<td>NK</td>
<td>LA</td>
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<tr>
<td>R12</td>
<td>M</td>
<td>mt tRNA$^{1\text{lys}}$</td>
<td>MERRF</td>
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<td>NK</td>
<td>Hypoglycaemia, LA</td>
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<td>R14</td>
<td>L</td>
<td>NK</td>
<td>Hepatic failure</td>
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<td>Controls (n=14)</td>
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<td>Mean of 72 observations</td>
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<td>Reference range (Mean ± 1.96 SD)</td>
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Table 5.1 continued
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<tr>
<th>Functional Defect(s)</th>
<th>Tissue</th>
<th>Gene Defect</th>
<th>Clinical Phenotypes</th>
<th>Acylcarnitine (nmol/mg protein) in media at 72h</th>
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<tr>
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<td>Patients</td>
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<td>(C_4^-)</td>
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<td>Complex I, II+III, III &amp; IV</td>
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<td>R15 F NK LA</td>
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<td></td>
<td>3.1</td>
</tr>
<tr>
<td>R16 F NK LA</td>
<td></td>
<td></td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>Controls (n=14)</td>
<td></td>
<td></td>
<td></td>
<td>0.9(^a)</td>
</tr>
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</table>

Table 5.1 continued:

Patient results are the average of 4 to 8 observations obtained in 2 to 4 separate assays.

The data shown are the butyl esters of \(^2\text{H}_5\)-labelled acylcarnitines, except for \(C_5^-\), which is the unlabelled species.

\(^a\) Logarithmic transformation of data to determine normal distribution

\(^b\) Median and upper limit of observed range

M: skeletal muscle, L: liver, H: heart, F: cultured skin fibroblasts, NK: not known, LA: Lactic acidosis

MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke like episodes

MERRF: Myoclonic Epilepsy Ragged Red Fibre syndrome
### Table 5.2: Fibroblast acylcarnitine results from a patient with mutations in the nuclear encoded genome

| Days
cultured | Passage number | Acylcarnitine (nmol/mg protein) in media at 72h | C_4^- | C_5^- | C_6^- | C_8^- | C_10^- | C_12^- | C_14^- | C_16^- | C_16-OH |
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</thead>
<tbody>
<tr>
<td>21</td>
<td>5</td>
<td>1.0</td>
<td>1.1</td>
<td>1.9</td>
<td>3.6</td>
<td>4.1</td>
<td>2.3</td>
<td>1.1</td>
<td>2.8</td>
<td>0.1</td>
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<tr>
<td>28</td>
<td>6</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>1.5</td>
<td>2.0</td>
<td>0.7</td>
<td>0.5</td>
<td>1.2</td>
<td>0.1</td>
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<tr>
<td>42</td>
<td>7</td>
<td>0.5</td>
<td>1.0</td>
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<td>2.5</td>
<td>1.4</td>
<td>0.6</td>
<td>1.5</td>
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<tr>
<td>49</td>
<td>8</td>
<td>0.3</td>
<td>0.6</td>
<td>0.8</td>
<td>1.5</td>
<td>1.9</td>
<td>0.7</td>
<td>0.4</td>
<td>1.1</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Average</td>
<td>0.6</td>
<td>0.9</td>
<td>1.2</td>
<td>2.2</td>
<td>2.6</td>
<td>1.3</td>
<td>0.6</td>
<td>1.6</td>
<td>0.1</td>
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</table>

(Observed lower-upper limits)

<table>
<thead>
<tr>
<th>Controls (n=14)</th>
<th>Mean of 72 observations</th>
<th>Reference range (Mean ± 1.96 SD)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.9</td>
<td>0.3-2.2</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>0.5-1.7</td>
</tr>
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<td></td>
<td>0.5</td>
<td>0.2-1.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.1-1.1</td>
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<tr>
<td></td>
<td>0.8</td>
<td>0.1-1.6</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>0.4-2.2</td>
</tr>
<tr>
<td></td>
<td>&lt;0.1</td>
<td>0.0-0.1</td>
</tr>
</tbody>
</table>

Skin fibroblasts were from a patient with complex IV deficiency who was homozygous for the 312del10, insAT mutation in exon 4 of the *SURF-1* gene. Cells were retrieved from liquid nitrogen and cultured as described in section 2.5, over a period of 7 weeks representing 5 to 8 passages following successive subcultures. Acylcarnitines accumulating in the reaction mixture were analysed as described in section 2.8 in 4 separate batches.

Data of each passage number represent average of duplicate determination.

All acylcarnitines with the corresponding chain length are the \(^2\text{H}_5\)-labelled butyl esters, except for C_5^-, which is the unlabelled species.

# : total number of days culturing in medium without uridine supplement before in vitro acylcarnitine profile assay.
### Table 5.3: Fibroblast acylcarnitine results from a patient with mitochondrial DNA mutations

<table>
<thead>
<tr>
<th>Days # cultured</th>
<th>Passage number</th>
<th>Acylcarnitine (nmol/mg protein) in media at 72h</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>C4-</td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>50</td>
<td>14</td>
<td>0.9</td>
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<tr>
<td>Controls (n=14)</td>
<td>mean of 72 observations</td>
<td>0.9</td>
</tr>
<tr>
<td>Reference range (Mean ± 1.96 SD)</td>
<td>0.3-2.2</td>
<td>0.5-1.7</td>
</tr>
</tbody>
</table>

Skin fibroblasts were from a patient with combined complex I, III and IV deficiency with mt tRNA\textsuperscript{Lys} mutation. Cells were retrieved from the liquid nitrogen store of the Metabolic Research Lab, which were previously grown in medium supplemented with uridine, and cultured as described in Chapter 2, section 2.5 (in medium without uridine supplement). \textit{In vitro} acylcarnitine profiling was performed when a 100% confluent cell line was established after 21 days, the results obtained correspond to passage number 12. The unused cells of this cell line were further cultured for 9 days before freezing down in liquid nitrogen, which were retrieved at a later date and cultured for 20 days, followed by \textit{in vitro} acylcarnitine profile assay (section 2.8). This results obtained correspond to passage number 14.

Data of each passage number represent average of duplicate determination.

All acylcarnitines with the corresponding chain length are the $^2$H\textsubscript{5}-labelled butyl esters, except for C\textsubscript{5}-, which is the unlabelled species.

# : total number of days culturing in medium without uridine supplement before \textit{in vitro} acylcarnitine assay.
5.5 Discussion

It has been shown that there may be a progressive recovery of respiratory chain activities during in vitro proliferation of RC deficient skin fibroblasts (due to loss of the deleted mitochondrial DNA molecules), and that the presence of uridine in culture medium could preserve the expression of RC deficiencies in cultured fibroblasts (Bourgeron et al 1993). In this study, skin fibroblasts were cultured in Ham F10 nutrient mixture without uridine supplements. The possibility that passage number (age of cell lines) and long term culturing in media without uridine supplement could affect functional expressions was investigated in fibroblasts from two RC deficient patients, one with mutations in the nuclear encoded genome (R8) and one with mutations in the mitochondrial DNA (R12).

Patient R8 had complex IV deficiency demonstrated in skeletal muscle, transformed lymphoblasts and fibroblasts, who was homozygous for the 312del10, insAT mutation in exon 4 of the SURF-1 gene. Retrieved fibroblasts were cultured over a period of seven weeks representing five to eight passages following successive subcultures, and assays were performed in duplicate in four separate batches. The results showed that the variations in acylcarnitine concentrations between different passages for a particular analyte could be up to three fold. This degree of variation is more likely to be due to analytical error rather than differences in passage number because up to 30% variation was seen in the within-run reproducibility study (Chapter 3, 3.5). The $^{2}$H$_{5}$-labelled C$_{8}$-, C$_{10}$-, C$_{12}$-, C$_{14}$-acylcarnitines were consistently elevated above the reference range for every passage assayed (Observed lower-upper limits >1.96 SD of control mean, data in bold type, Table 5.2).

Patient R12 was affected with multiple complex I, III and IV deficiencies, who had mitochondrial tRNA$^{Lys}$ mutation. The hydroxy-C$_{16}$-acylcarnitine remained elevated after growing the cells in culture medium without uridine supplement for 50 days (Table 5.3).
These results indicate that passage number and culturing RC deficient cells in medium without uridine supplement under the existing conditions did not alter the functional expression of these two cell lines and there is no evidence of recovery of respiratory chain activity in the case of R12 which has mitochondrial DNA mutations.

Eight of the 16 cell lines from patients with RC defects (Table 5.1) showed acylcarnitine profiles similar to controls, while the other eight exhibited abnormal profiles mimicking several enzyme defects of FAO. There was no clear association of a characteristic acylcarnitine profile with a specific respiratory chain defect (R10 to R14). Acylcarnitine abnormalities were seen in some cell lines from patients with defects caused by mutations in the nuclear encoded genome (R8) and in some patients with mitochondrial DNA mutations (R3, R4, and R12). There was in fact no association between the expression of the RC defects in skin fibroblasts and a normal or abnormal acylcarnitine profile, as indicated by the demonstrable complex IV deficiency in the fibroblasts of patients of R7, R8 and R9.

The principal function of FAO is generation of acetyl-CoA, and that of the RC is the production of ATP. Functional defects in RC should not be associated with acylcarnitine abnormalities theoretically, as acyl-CoA esters are not intermediate substrates. The observation of abnormal acylcarnitine profiles could be related to the linkages of L-3-hydroxyacyl-CoA dehydrogenases to complex I via NADH, and the acyl-CoA dehydrogenases to ubiquinone via electron transfer flavoprotein (ETF) and ETF dehydrogenase, and the reversible reactions between them. A primary functional defect of the RC resulting in dysfunction of these dehydrogenases in FAO is evident by the accumulation of long-chain acyl-CoA esters, their L-3-hydroxyacyl derivatives and acylcarnitines, as has been demonstrated in rat heart mitochondria, although high levels were not reached (Eaton et al 1996b). However, the abnormal metabolites thus accumulated could further impair oxidative phosphorylation (Ventura et al 1996), and
exacerbate the FAO dysfunction by the inhibitory effects of acylcarnitines on CACT (Baillet et al 2000), and L-3-hydroxyacyl-CoA on enoyl-CoA hydratases (He et al 1992). The latter reaction could lead to accumulation of 2-enoyl-CoA, which has been shown to inhibit the acyl-CoA dehydrogenases (Powell et al 1987; Eaton et al 1996a). These and other genetic factors may explain the variable and unpredictable acylcarnitine profiles observed in fibroblasts from patients with primary RC defects.

The profile of the complex IV deficient cell line (R8) indicating general dysfunction of numerous enzymes acting on medium- and long-chain acyl-CoA is probably not surprising. However, it is not clear how a deficiency in complex I may be related to elevation of $^{2}$H$_{5}$-C$_{4}$-acylcarnitine (R3, R4), instead of the hydroxy-acylcarnitines.

Complex II performs a key step in the citric acid cycle, in which succinate is dehydrogenated to fumarate. In turn, the citric acid cycle is linked to FAO via acetyl-CoA, the end product of thiolytic cleavage. The predominant accumulation of C$_{8}$-acylcarnitine suggesting a specific inhibition of MCAD in the fibroblasts of this complex II affected patient (R6) was unexpected. The overall acylcarnitine profile and elevation of disease-specific species are similar to that seen in fibroblasts of patient F35 (Chapter 4, Table 4.1), who is a compound MCAD heterozygote. Subsequent investigation of DNA mutational analysis in the newborn screening dried blood spot sample of R6 revealed that he was heterozygous for the MCAD 985A>G mutation. The fatty acid oxidation rate study in fibroblasts of this patient showed a ratio of P/M = 1, which is not typical of a MCAD homozygous or compound heterozygosity. However, the possibility of the presence of another MCAD mutation cannot be ruled out until all 12 exons have been sequenced. The complex II activity was normal in skin fibroblasts, but deficient in liver. It is not clear whether reduced complex II activity detected in the liver of R6 is secondary to MCAD dysfunction, as has been
reported in other FAO defects (Reichmann et al 1992; Das et al 2000). It is possible that patient R6 harbours two genetic defects, with synergistic heterozygosity resulting from multiple partial defects in one or more metabolic pathways (Vockley et al 2000). This may explain the resemblance of fibroblast acylcarnitine profile of R6 (presumed MCAD heterozygote) with those affected with homozygous or compound heterozygote MCAD deficiency. It is interesting to note that the sibling of this patient died with a similar clinical phenotype, in whom the CPT II activity was partially reduced (50% of controls) but complex II activity in liver homogenate and acylcarnitine profiling in fibroblasts were normal.

Mitochondrial β-oxidation disorders described to date show autosomal recessive inheritance (nuclear encoded), and may be potentially fatal if undetected. However, in most diagnosed cases the prognosis is generally favourable (Pons and De Vivo 2001), even in patients with two null mutations (Touma et al 2001). Moreover, treatment is relatively simple, involving avoidance of fasting together with dietary therapy and L-carnitine in some disorders. In contrast, defects in RC can be caused by mutations in nuclear or mitochondrial DNA, and the inheritance patterns may be autosomal (recessive or dominant), or maternal (mitochondrial). Treatment in most individuals has generally been ineffective (Pons and De Vivo 2001; Walker and Byrne 1995) and the prognosis especially in early onset defects is poor, making prenatal diagnosis and genetic counselling particularly important.

In summary, *in vitro* acylcarnitine profiling in fibroblasts is a useful test for screening patients suspected with FAO disorders. However, fibroblast acylcarnitine profiles from patients with RC defects can resemble those from patients with FAO deficiencies. Abnormal profiles do not exclusively indicate FAO disorders and primary defects of the RC remain a possibility. Whereas the clinical and biochemical abnormalities of FAO and RC defects may be similar, patient management, prognosis
and genetic counselling are vastly different. Awareness of this diagnostic pitfall enables the appropriate selection of follow up confirmatory tests and assists in targeted therapeutic strategies. Integrated evaluation of clinical information, in vivo and in vitro findings, histopathology, enzymology and molecular studies are required for accurate diagnosis.