CHAPTER 6

Summary and Future Studies

6.1 Summary

At least 21 different enzyme deficiencies of mitochondrial fatty acid β-oxidation (FAO) have been characterised and gene defects have been identified in 15 of these enzyme deficiencies. Investigation of suspected FAO disorders commonly begins with a search for marker metabolites in physiological fluids, followed by in

\textit{vitro} \ functional studies if the \textit{in vivo} findings are inconclusive, with confirmation by enzymology and molecular analyses. Several studies reported that incubating cells from patients with FAO disorders in media enriched with fatty acids and L-carnitine revealed disease-specific acylcarnitine profiles (Kler et al 1991; Pourfarzam et al 1994; Schaefer et al 1995; Nada et al 1995b; Schmidt-Sommerfeld et al 1998; Roe and Roe 1999; Ventura et al 1999). Thus \textit{in vitro} acylcarnitine profiling was shown to be a useful screening tool for the investigation of patients suspected of having one of the numerous enzyme defects of FAO. This project further investigated the analytical sensitivity, diagnostic specificity and prognostic potential of the \textit{in vitro} acylcarnitine profile assay using ESI-MS/MS.

The possibility of improving the analytical sensitivity of the \textit{in vitro} quantitative acylcarnitine profiling was explored by the comparison, linearity and time course studies reported in Chapter 3. In this project, only the acylcarnitines accumulating in the reaction media were analysed, in contrast to previous studies where cells plus media were used (Roe and Roe 1999; Ventura et al 1999). This approach has been validated, is less demanding technically, requires fewer fibroblasts, and probably better simulates the \textit{in vivo} acylcarnitine status. The possible improvement in the analytical sensitivity is indicated by the unique profile of CPT1A deficiency (Sim et al 2001), which has been previously reported to be
indistinguishable from controls (Roe and Roe 1999). Although there was only one such cell line available for investigation, the results were promising.

The diagnostic specificity and prognostic potential of the *in vitro* quantitative acylcarnitine profiling were assessed in fibroblasts from patients with FAO and respiratory chain (RC) defects. Using this approach, all the FAO deficient cell lines investigated showed abnormal profiles compared to normal control lines, and cell lines with the same defective enzymes exhibited disease-specific acylcarnitine profiles irrespective of the severity of clinical symptoms or of different mutations. Furthermore, initial studies in fibroblasts from patients with CPT2, LCHAD, VLCAD and MAD deficiencies with more severe clinical courses and poor outcomes accumulated higher concentrations of long-chain species. The results suggest that this test may be employed for the prediction of prognosis of long-chain FAO disorders, which would be especially useful for the asymptomatic / pre-symptomatic patients diagnosed by newborn screening. However, some cell lines from patients with RC defects showed profiles similar to those of controls, whereas others revealed abnormal profiles mimicking those found in FAO disorders. The acylcarnitine profiles of patients with RC enzyme defects were not predictable, and defects caused by mutations in either nuclear-encoded genome or mitochondrial DNA were associated with acylcarnitine abnormalities in some patients.

This project showed that the *in vitro* acylcarnitine profile assay is a useful screening tool for the detection of suspected FAO disorders, and perhaps can assist in the prediction of the long term outlook of patients with long-chain FAO disorders, facilitating therapeutic intervention and genetic counselling decisions. However, there are limitations: neither defects in plasma membrane cellular uptake of long-chain fatty acids (Odaib et al 1998) nor in ketogenesis not involving acyl-CoA intermediates would be detected. Tissue-specific enzymes that are not expressed in fibroblasts
would also be overlooked. Moreover, abnormal acylcarnitine profiles do not exclusively indicate FAO disorders, and primary defects of the RC remain a possibility.

6.2 Future Studies

Lymphocytes isolated from fresh whole blood are capable of oxidising fatty acids, as demonstrated by the tritium water release assay (Brivet et al 1995). If lymphocytes were a reliable resource for in vitro acylcarnitine profiling study, this would obviate the need for tissue culture, save time and cost, and may be able to differentiate FAO deficiencies from RC defects. A study of this nature is clearly required.

Riboflavin is the precursor of flavin adenine dinucleotide (FAD), the obligate coenzyme for acyl-CoA dehydrogenases, ETF and ETFDH. Some patients with MAD deficiency have been shown to respond clinically to pharmacological doses of riboflavin (Gregersen et al 1982; Green et al 1985; Rhead et al 1993). However, to objectively prove this clinically or metabolically is difficult. Investigation of patients with suspected defects associated with riboflavin-responsiveness could theoretically be achieved by incubating cells in media with known concentrations of riboflavin. Other inherited metabolic defects that result in accumulation of acyl-CoA esters could be investigated using precursors of the pathways.