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INVESTIGATION INTO NAD BIOSYNTHESIS FROM THE KYNURENINE PATHWAY USING ISOTOPICALLY LABELLED PRECURSORS AND IT-LC-MS/MS

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The project aim is to investigate the biochemical synthesis of nicotine adenine dinucleotide (NAD) in selected cells of the central nervous system via the kynurenine pathway, from either tryptophan or niacin precursors, under:

- a) physiological conditions and
- b) during immune mediated activity (i.e. IFN- γ activation).

Secondly, we aim to investigate the role of increased tryptophan catabolism during T-helper cell mediated (i.e. IFN- γ mediated) inflammation on NAD metabolism.

Knowing how cells in the brain produce and maintain their NAD concentrations will assist in the application of novel therapies in inflammatory diseases where NAD turnover is increased leading to niacin precursor depletion, cell dysfunction and death (e.g. Alzheimer's disease, ischaemic stroke, meningitis).

To date LC-MS and LC-MS/MS methods have been developed using a Finnigan LCQ Deca XP Plus ion trap, enabling us to detect NAD and the other kynurenine pathway metabolites at physiological concentrations. The NAD synthetic pathway had been followed using the addition of isotopically labelled tryptophan (13-C and 15-N total label) to the cell growth media. The mass spectrometer can then be used to detect the labelled precursor and products.

Tracking the incorporation of 13-C and 15-N into kynurenine pathway metabolites has allowed us to demonstrate, for the first time, the biosynthesis of NAD from tryptophan in both primary foetal human neurons and primary foetal human astrocytes. Ongoing work is focussing on the quantitative aspect of this assay, which will allow us to look at changes in the relative amounts of labelled and unlabelled kynurenine pathway metabolites during both precursor loading and cytokine (IFN- γ activation).