Studying Metabolic Pathways in Native Human Islets by ^{13}C NMR Spectroscopic Techniques Using a Low-temperature Microcoil Apparatus: Optimizing a Method

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Introduction

Diabetes exerts a significant health and economic toll on societies worldwide. Although there are a number of therapeutic approaches to this disease (e.g., insulin injection; pharmacologic intervention), these aim to mitigate consequences of the disease, rather than cure the disease outright. A therapeutic approach towards curing diabetes is to replace the destroyed insulin-secreting β-cells and restore optimum blood glucose control. Native human islets or β-cells are optimal; but a severe shortage of such tissue precludes clinical use of this approach, and places a premium on identifying appropriate surrogate insulin-secreting cell sources. However, we do not have a full understanding of mechanisms of insulin secretion in the islet. NMR spectroscopy and isotopomer analysis [1] of cell extracts can quantitatively assess metabolic pathways associated with the TCA cycle following metabolism of a ^{13}C-labeled substrate (e.g., glucose). We previously published the first ^{13}C spectra of porcine islets (100,000 islet equivalents on a 5 mm coil in a 500 MHz magnet), and have evidence that aspartate metabolism may play a key role in insulin secretion [2]. Because human islets are difficult to procure in numbers necessary for these techniques (i.e., 200,000 would be desirable), as well as expensive (10 cents/islet), we focus to exploit the enhanced sensitivity of a low-temperature microcoil apparatus on a 600 MHz magnet, thus significantly reduce the number of islets needed for such studies to characterize key metabolic pathways associated with insulin secretion in the native human islet.

Experimental

20,000 native human islets were exposed for 4 hours to media (CMRL) containing 15 mM uniformly-^{13}C- labeled glucose and then extracted [3]. The aqueous phase was lyophilized and resuspended in ~7 microliters of D_2O and examined by ^{13}C NMR spectroscopy on a 600 MHz vertical bore Bruker magnet using a low-temperature microcoil apparatus for ~53 h.

Results and Discussion

An extract of human islets was tested under conditions to maximize signal (low volume, wide bore capillary tube, and extended observation) and demonstrated that obtaining strong ^{13}C data from small islet populations is feasible [Fig.1]. Although the signal-to-noise (S/N) is still not sufficient for quantitative isotopomeric analysis, the technique is close to obtaining valuable information regarding metabolic pathways of the islets. Next steps to increase the S/N to levels permissive of isotopomeric analysis include increasing the exposure time to the label, increasing the number of islets to 30,000 (if possible), and increasing the NMR acquisition time. Studying islet populations under differing insulin-stimulating media conditions would be a great step toward obtaining fundamental information integral toward understanding the links between metabolic pathways associated with the TCA cycle and insulin secretion in the native human islet.

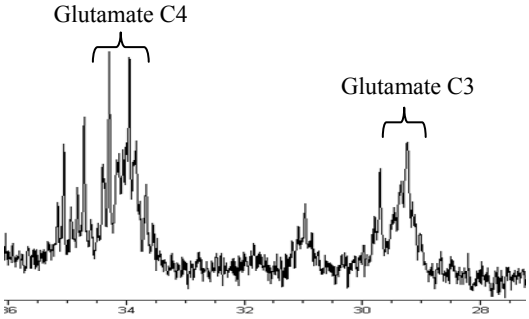

Fig. 1. Partial ^{13}C spectra of a human islet extract exposed to uniformly-labeled ^{13}C-glucose. Isotopomeric patterns of glutamate peaks (key for analysis) are discernible, though noise still precludes proper analysis.

Acknowledgements

This work was supported by pilot NHMFL funding through the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility at the University of Florida.

References

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