Studying Metabolic Pathways in Native Human Islets by ^{13}C NMR Spectroscopic Techniques Using a Microcoil Apparatus

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Introduction

Diabetes exerts a significant health and economic toll on society. One novel therapeutic approach for type 1 diabetes is to replace destroyed β-cells. Native human islets or β-cells are the optimal choice; unfortunately, a severe shortage of such tissue precludes widespread clinical use of this approach, and places a premium on identifying appropriate surrogate β-cell sources. Because defining specific enzymatic pathways vital to insulin secretion is essential to understanding secretion mechanisms, there are investigations using surrogate insulinoma cell lines. NMR spectroscopy and isotopomer analysis [1] of cell extracts are well suited to quantitatively assess enzymatic pathways associated with the TCA cycle following metabolism of a ^{13}C-labeled substrate (e.g., glucose). Recently we published the first spectra of porcine islets (obtained with 100,000 islet equivalents on a 5 mm coil in a 500 MHz magnet), and have preliminary evidence that aspartate metabolism may play a role in insulin secretion [2]. However, islets are difficult to procure in large numbers to effectively characterize metabolic pathways with these techniques. The present work focuses to exploit the enhanced sensitivity of a low-temperature microcoil apparatus on a 600 MHz magnet, thereby significantly reduce the number of islets needed for such studies and permit us 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 of the extract was examined by ^{13}C NMR spectroscopy on a 600 MHz vertical bore Bruker magnet using a low-temperature microcoil apparatus. Data were visually inspected for sufficient signal to allow ^{13}C isotopomeric analysis.

Results and Discussion

It is difficult to procure human islets for research. As only one shipment (20,000 islets) was received this year (November) towards performing these NMR studies, only one trial of the experimental setup was attempted this calendar year. An advancement from last year is that previous sample preparation issues have been addressed and corrected. Although only 20,000 islets were present in the extract sample, there were a number of peaks visible in the spectrum. Importantly, the isotopomeric splitting of the glutamate peaks (through which the metabolic isotopomer analysis is accomplished) were visible, although not sufficiently above the noise to allow proper analysis. Improvements to the design would be to reduce the resuspension volume (from 20 microliters to ~8 microliters) and use a wider bore capillary tube to effectively double the volume near the sensitive region of the probe, thereby gaining at least a four-fold increase in signal. With these changes, we should be able to discern true isotopomer patterns from this small islet sample. To this end, the sample has been re-lyophilized and will be run again on the NMR system in January. Theoretically, we should be able to achieve sufficient signal with this small number of islets. This would be a great step toward obtaining fundamental information integral to understanding the links between reactions associated with the TCA cycle and insulin secretion in native islets.

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References

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