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Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Malinowski, R. M., Ghiasi, S. M., Mandrup-Poulsen, T., Jensen, P. R., & Ardenkjær-Larsen, J. H. (2017). Probing of biochemical pathways in clonal pancreatic β -cells by quantitative dDNP of metabolite extracts. Abstract from EUROMAR 2017, Warsaw, Poland.

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Probing of biochemical pathways in clonal pancreatic β -cells by quantitative d DNP of metabolite extracts

Ronja M. Malinowski¹, Seyed M. Ghiasi², Thomas Mandrup-Poulsen², Pernille R. Jensen¹, Jan H. Ardenkjær-Larsen^{1,3}

¹Technical University of Denmark, Kgs. Lyngby, Denmark, ²University of Copenhagen, Copenhagen, Denmark, ³GE Healthcare, Denmark

Anaplerotic flux into the tricarboxylic (TCA) cycle is crucial for glucose-stimulated insulin secretion (GSIS) in pancreatic β -cells [1]. Although it is well-established that GSIS requires oxidation of carbohydrates in the mitochondria to drive ATP production, studies have suggested intermediates generated directly or indirectly from anaplerosis may act as second messengers linking glucose oxidation to insulin release [2].

In this study, dissolution Dynamic Nuclear Polarization (d DNP) is used to visualize the metabolic fate of [¹³C₆, D₆]glucose (¹³C-Glc) in cell extracts from the clonal pancreatic cell-line INS-1 from rat at two different ¹³C-Glc concentrations (11.7 mM and 17 mM). The extracts were obtained by incubation of 10 mio. cells in Eppendorf tube for 2, 4 and 8 h, at which the reaction was stopped with perchloric acid and the metabolites were extracted, and the sample extracts were hyperpolarized. The metabolites were quantified according to an internal standard (Fig. 1). Glc was linearly consumed at an average rate of 0.44 mM/h (initial concentration 11.7 mM) and 0.48 mM/h (initial concentration 17 mM) at the two incubation concentrations, while the output in terms of a sum of observable metabolites has increased by 66% from 11.7 mM to 17 mM ¹³C-Glc incubation. ($p = 0.02$).

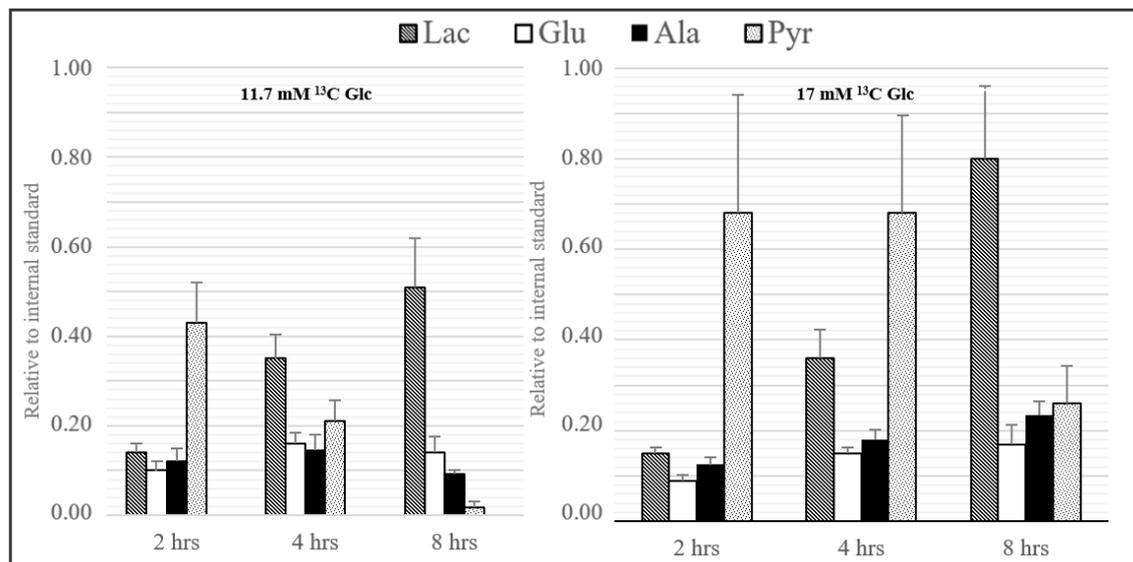


Fig. 1 - Metabolites generated from ¹³C Glucose 11.7 mM (left) and 17 mM (right) incubations of INS-1 cells at various time points. (Lac – lactate, Glu – glutamate, Ala – alanine, Pyr – pyruvate)

In both cases, lactate increases linearly, with no significant difference ($p > 0.05$). However, pyruvate decreases over time, but is significantly ($p = 0.02$) increased at 17 mM vs 11.7 mM by 155% (sum of pyruvate for the two experiments). Therefore, the intersection when lac = pyr occurs later in 17 mM Glc, than 11.7 mM Glc. Glc derived pyruvate has been suggested to have a prominent role in GSIS as several pathways involving pyruvate has been described [3], and the upregulation is expected since GSIS correlates directly with Glc concentration and pyruvate cycling [4].

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