Additional file 3: Palmitate-induced alterations in enzyme gene expression and activity follow dissimilar trends in INS-1E β-cells

A commonly used model for lipid-induced β-cell dysfunction, lipotoxicity, entails treatment of primary or clonal β-cells, such as INS-1E cells, with palmitate coupled to BSA. To investigate the action of lipotoxicity on INS-1E cells on the glucose metabolic enzyme fingerprint, we treated INS-1E cells according to a published lipotoxicity protocol for 24hrs before cell harvest. In parallel, we extracted RNA samples for comparison to mRNA levels.

Methods

Palmitate treatment

For the model of lipotoxicity in INS-1E cells palmitate was dissolved as a 200mM stock solution in ethanol (100%). For BSA-coupling, palmitate stock was diluted to 6mM concentration with BSA (10%, weight/volume) and shaken over night at 37°C. This corresponds to a molecular palmitate:BSA ratio of 4:1.The coupled palmitate was used for cell culture stimulation. For fatty acid treatment 48h after seeding, cells were stimulated for 24h with medium containing 0.25mM palmitate or BSA as control, as described. For the model of glucose toxicity in INS-1E cells; 24h after seeding medium was removed, cells washed with PBS and stimulated for 48h with medium containing either 5mM or 20mM glucose.

RNA extraction and RT-Q-PCR

For RNA isolation, medium was removed, and cells were harvested in 1mL TRI Reagent (SigmaAldrich). RNA extraction was performed as instructed by the manufacturer. RNA-concentration was determined using a Nanodrop ND-1000 spectrophotometer. Complementary DNA was synthesized from 1000 ng RNA using the SuperScript III First-Strand Synthesis kit (ThermoFisher) according to the manufacturer's instructions using random hexamers. Q-PCR reactions were carried out with the Agilent MxPro3005 instrument using Quantitect SYBR green (Qiagen) according to manufacturer's instructions. TFIIB was used as reference gene as it is unchanged by glucose and fatty acid treatment [1, 2]. Sequences (5' to 3') of used primers are

AldoA (forward: GGCTTCTTTCACTGCACCAC, reverse: CCAGCTCCTTCTTCTGCTCC),

AldoB (forward: CCGCTTGCAGGAACAAACAA, reverse: GTCCGAGATCCTCAGCACAG),

AldoC (forward: GCTTGGACTGAGCTACTGTCTG, reverse: GGTATGAGTGGGGCATGGT),

TFIIB (forward: GTTCTGCTCCAACCTTTGCCT, reverse: TGTGTAGCTGCCATCTGCACTT).

Array data

An mRNA array data from INS-1 cells treated with palmitate was obtained from Malmgren et al

(2013)[3] (E-MTAB-3232).

References

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