Primary mouse islets

Mouse islets were isolated from cohorts of equal numbers of male and female C57BL/6N mice (Charles River). Mice were housed 5 animals per cage on woodchip bedding. Mice were maintained on a standard chow diet (PicoLab Rodent Diet 20 pellets, #5053). The young mouse cohort was 4-weeks-old and the old cohort was 10-15 months of age. For islet isolations, Liberase TL (Roche) was perfused into pancreata at a working concentration of 0.655 units/mL through the common hepatic bile duct. Pancreata were then removed and dissociated at 37^{o}C for roughly 15 minutes (dissociation time depends on age and size of pancreas). Islets were separated onto a gradient composed of HBSS (Cellgro) and Histopaque (Sigma) layers. Purified islets were then handpicked under a dissection microscope to minimize acinar cell contamination. For islet experiments, islets were pooled from different mice where n represents the number of biological replicates using islet pools (i.e. 2 mice per 1 biological replicate, n=1). All animal experiments were approved by the Institutional Animal Care and Use Committees of the University of California, San Diego.

U-^{13}C Glucose Tracing and Metabolite Measurements

Islets were isolated, pooled from 10 mice per group, and incubated overnight in RPMI 1640 media supplemented with 8 mM glucose, 10% FBS, 2 mM L-glutamine, 100 U/mL Pen/Strep, 1 mM sodium pyruvate, 10 mM HEPES, and 0.25 mg/mL amphoterecin B. Islets were starved for 1 hour in KRBH supplemented with 2.8 mM glucose as for GSIS assays. Islets were then transferred to petri dishes containing U-^{13}C-labeled glucose as indicated and immediately seeded into wells of non-treated 12-well plates at a density of 100 size-matched islets per replicate in a final volume of 500 µl KRBH containing basal (2.8 mM) or stimulatory (16.8 mM) concentrations of labeled glucose. Tracing was performed for the indicated durations at 37^{o}C with 5% CO_2. Following the trace, islets were rapidly chilled by swirling plate to resuspend islets then transferring entire contents of each well to an Eppendorf tube on ice. Islets were then pelleted by centrifugation at 500 x *g* for 2 min at 4^{o}C, washed in 1 mL of ice cold 0.9% NaCl, centrifuged as before, then pellets were frozen in a dry ice/ethanol bath and stored at -80^{o}C.

Metabolites were extracted from pellets using a bligh and dyer-based methanol/chloroform/water extraction with inclusion of norvaline as a polar internal standard. Briefly, 250 µl MeOH, 250 µl $CHCL_3$, 100 µl water containing norvaline were added to pellets. This was vortexed for 10 minutes followed by centrifugation at 10,000 g for 5 minutes at 4°C. The upper phase was separated and dried under vacuum at 4°C until dry. Polar metabolites were derivatized in 2% (w/v) methoxyamine hydrochloride in pyridine and incubated at 45°C for 60 minutes. Samples were then silylated with N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide (MtBSTFA) with 1% tert-butyldimethylchlorosilane (tBDMCS) at 45°C for 30 minutes. Polar derivatives were analyzed by GC-MS using a DB-35MS column (30m x 0.25 mm i.d. x 0.25 μm) installed in an Agilent 7890B gas chromatograph (GC) interfaced with an Agilent 5977A mass spectrometer (MS) with an XTR EI source using the following temperature program: 100 °C initial, increase by 3.5 °C/min to 255 °C, increase by 15 °C/min to 320 °C and hold for 3 min. The % isotopologue distributions of each polar metabolite was determined and corrected for natural abundance using in-house algorithms adapted from (Fernandez et al., 1996). Negative isotopologue distributions resulting from natural abundance corrections were rounded to zero. The metabolite ion fragments used are summarized below:

Metabolite fragment ions used for GC-MS.

Metabolite	Carbons	Formula	m/z
Pyruvate	1,2,3	C6H12O3NSi	174
Alanine	1,2,3	C11H26O2NSi2	260

Glycine	1,2	C10H24O2NSi2	246
Aspartate	1,2,3,4	C18H40O4NSi3	418
Glutamate	1,2,3,4,5	C19H42O4N1Si3	432
Citrate	1,2,3,4,5,6	C20H39O6Si3	459
Malate	1,2,3,4	C18H39O5Si3	419
Fumarate	1,2,3,4	C12H23O4Si2	287

REFERENCES

Fernandez, C.A., Des Rosiers, C., Previs, S.F., David, F., and Brunengraber, H. (1996). Correction of 13C mass isotopomer distributions for natural stable isotope abundance. J Mass Spectrom *31*, 255-262.