**Project:**

Metabolomics of Human islets treated with DHT and high-glucose challenge

**Subject species:**

Homo Sapiens

**Sample type:**

Human pancreatic islets isolated from cadaveric human pancreata.

**Study Design:**

Human islets were treated overnight with 10nM DHT or EtOH in a humidified incubator containing 4% CO2 at 37°C and then batched in groups of 100 islets in microcentrifuge tubes and treated for either 30 or 60 minutes with media containing either 2.8mM or 16.7 mM 13C6 glucose; the same DHT or EtOH concentrations were maintained.

**Collection:**

Following the incubation period, tubes were briefly centrifuged at low speed and media was withdrawn. 150mM ammonium acetate in water were added to rinse residual media (150mM ammonium acetate is an osmolarity-matching, mass spectrometry compatible rinse buffer with near-neutral pH). Tubes were centrifuged, supernatant was withdrawn, and they were frozen by immersion in liquid nitrogen and stored at -80C prior to extraction.

**Sample preparation:**

*Sample prep summary:* Islet samples were extracted in 200μL of ice-cold 8:1:1 methanol:water:chloroform, with tissue disruption aided by immediate probe sonication for 20 seconds with a Branson 450 Sonifier set to output level 2, duty cycle 20%. The disrupted samples were allowed to incubate on ice for 10 minutes and were then centrifuged for 5 min at 14,000 xg to pellet cell debris. 180μL of supernatant were dried under a gentle stream of nitrogen gas at room temperature, and were then reconstituted in 45μL of 4:1 water:methanol and transferred to autosampler vials for analysis.

*Extraction method: Single-phase extraction using 8:1:1 methanol:water:chloroform*

*Extract storage: -80°C until extraction; 4°C after reconstitution*

**Chromatography:**

Analysis type: MS

Chromatography type: Ion pairing reversed-phase

Chromatography system: Agilent 1290 Infinity

Column: Agilent Zorbax Extend C18 1.8 µ RRHD, 2.1 x 150mm ID with matched guard column

MS Type: ESI

MS instrument type: Quadrupole-Time of Flight

MS instrument name: Agilent 6530 qTOF

Ion mode: Negative

Units: relative

Column temperature: 35*°C*

Flow gradient: 0-2min hold 100/0/0 (%A/%B/%C); 2-12 min linear to 1/99/0, 12-20 min hold 1/99/0, 20-20.5 min linear to 5/0/95, 20.5-26.1 5/0/95, 26.1-26.3 min linear to 100/0/0, 26.3-33 min hold 100/0/0

Flow rate: 0-20.5 min hold 0.25mL/min, [18 min switch flow direction to backflush], 20.5-21.5 linear to 0.4mL/min, 21.5-22.3 linear to 0.8 mL/min, [26 min switch flow direction to forward] 26-26.1 linear to 0.6mL/min, 26.1-26.3 linear to 0.4 mL/min, 26.3-31.8 min hold 0.4mL/min, 31.8-32 min linear to 0.25mL/min

Sample injection: 5 uL

Solvent A: 97:3 water:methanol with 10 mM tributylamine and 5mM acetic acid

Solvent B: methanol with 10 mM tributylamine and 5mM acetic acid

Solvent C: acetonitrile

Chromatography type: Ion pairing reversed-phase

**MS:**

*Instrument name:* Agilent 6530 qTOF

*Instrument type:* Quadrupole-Time of flight

*MS type:* ESI

*MS comments:* MS acquisition was performed in negative ion mode, scan rate 2 spectra/sec, mass range 50-1200 m/z. Source parameters were: drying gas temperature 250°C, drying gas flow rate 13 L/min, nebulizer pressure 35 psig, sheath gas temp 325°C and flow 12 l/min, capillary voltage 3500V, internal reference mass correction enabled. Data analysis was performed using Agilent Profinder 10.0 software in batch isotopologue extraction mode with automated natural isotope abundance correction enabled. Compound identification was performed by matching accurate mass and retention time to those of authentic standards analyzed using the same method.

*Ion mode:* Negative

*Capillary voltage:* 3500V

*Dry gas flow:* 13L/min

*Dry gas temp:* 250 C

*Fragment voltage:* 150V

*Nebulizer*: 35 psig

*Sheath gas temp:* 325C

*Sheath gas flow:* 12L/min