Isotopically Tracing Ingested Fructose

After 3 days or 4 weeks of either HFD or CD feeding, each mouse was administrated a 1g/kg U-^{13}C fructose oral gavage. Mice were anesthetized with 2% isoflurane, and their abdominal cavities were opened at the specified time. Abdominal tissues were displaced to identify the portal vein, and a 27G needle was used to immediately collect portal vein blood samples (~100 µL). Immediately after, cardiac blood was collected from the right ventricle using a 25G needle (~1 mL). Jejunum and liver tissues were quickly resected and snap-frozen in liquid nitrogen. Blood samples were placed on ice in the absence of anticoagulant for 20 minutes and centrifuged at 16,000 × g for 10 minutes at 4 °C to separate the serum and plasma fractions of each sample. Serum and tissue samples were kept at −80 °C until further analysis.

Intestine-specific Khk-c knockout mice and control littermates were gavaged with 1 g/kg U-^{13}C fructose, and tail blood was collected at 0, 10, 30, 60, and 120 minutes after the gavage.

Metabolite Extraction

Metabolites were extracted using a protocol optimized for water-soluble polar metabolite analysis using liquid chromatography coupled with mass spectrometry. All extraction buffers were stored at -20°C prior to usage and immediately preceding the metabolite extraction.

For serum samples, a 10-µL serum aliquot was used for fructose tracing. Metabolites were extracted with 40 µL of ice-cold methanol and incubated at -20 °C for 20 minutes. The clean supernatant was collected after centrifugation for 10 minutes at the highest speed, and the leftover pellet was further treated with 200 µL cold extraction buffer (40:40:20 v/v/v methanol:acetonitrile:water solution) and left to incubate on crushed ice for an additional 10

minutes. Following an additional 10 minutes of centrifugation at the highest speed, the clean supernatant was collected and pooled with the supernatant from the first collection.

For tissue samples, the extraction buffer used was a (v/v/v) solution of 40:40:20 (methanol:acetonitrile:water) + 0.1 M formic acid. An aliquot volume equivalent to 20x the sample weight was added to the Eppendorf tube with the homogenized sample, vortexed for 10 seconds, and left to chill on crushed ice for 10 minutes. The samples were then centrifuged for 10 minutes at the highest speed at 4°C. The supernatant was transferred to a correspondingly labeled and chilled Eppendorf tube, and the process was repeated once more. The total volume of supernatant was then centrifuged for an additional 10 minutes. After centrifugation, a final 500µL aliquot of the homogenate was then pipetted to a second clean Eppendorf tube, to which 44µL of 15% (m/v) NH_4HCO_3 was added to neutralize the acid in the buffer and precipitate the protein. This is the final sample extract to be vialed and loaded to the instrument for analysis. Metabolite extracts were stored at -80 °C until analysis.

Metabolomics Analysis

UHPLC chromatography conditions. The HILIC separation was performed on a Vanquish Horizon UHPLC system (Thermo Fisher Scientific, Waltham, MA) with XBridge BEH Amide column (150 mm × 2.1 mm, 2.5 µm particle size, Waters, Milford, MA) using a gradient of solvent A (95%:5% H2O:acetonitrile with 20 mM acetic acid, 40 mM ammonium hydroxide, pH 9.4), and solvent B (20%:80% H2O:acetonitrile with 20 mM acetic acid, 40 mM ammonium hydroxide, pH 9.4). The gradient was 0 min, 100% B; 3 min, 100% B; 3.2 min, 90% B; 6.2 min, 90% B; 6.5 min, 80% B; 10.5 min, 80% B; 10.7 min, 70% B; 13.5 min, 70% B; 13.7 min, 45% B; 16 min, 45% B; 16.5 min, 100% B and 22 min, 100% B (Su et al., 2020). The flow rate was 300 µl/min. The

injection volume was 5 μL and the column temperature was 25 °C. The autosampler temperature was set to 4°C and the injection volume was 5 μL.

Fullscan mass spectrometry. The full scan mass spectrometry analysis was performed on a Thermo Q Exactive PLUS with a HESI source which was set to a spray voltage of -2.7kV under negative mode and 3.5kV under positive mode. The sheath, auxiliary, and sweep gas flow rates of 40, 10, and 2 (arbitrary unit), respectively. The capillary temperature was set to 300°C, and the aux gas heater was 360°C. The S-lens RF level was 45. The *m/z* range was set to 72 to 1000 *m/z* under both positive and negative ionization mode. The AGC target was set to 3e6, and the maximum IT was 200 ms. The mass resolution (full-width half maximum) was set to 70,000 @ *m/z* = 200.

Mass Spectrometry Imaging by IR-MALDESI-MSI

Pancreas tissues were first equilibrated to -15°C then sectioned to 20 μm thickness using a Leica CM1950 cryostat (Buffalo Grove, IL, USA). Cut sections were then thaw-mounted on clean microscope slides (1 mm height, plain, Fisher Scientific, Pittsburgh, PA) and stored at -80 °C until IR-MALDESI-MSI analysis. Tissues were first blocked into four groups, each containing one replicate of each condition. Tissues were both cut and imaged in randomized order within these blocks to minimize sampling bias.

Mass spectrometry imaging (MSI) was performed using the infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) source (Caleb Bagley et al., 2021; Robichaud et al., 2013) coupled to an Exploris 240 Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) to achieve high resolution and accurate mass measurements. To facilitate desorption of targeted analytes, a thin layer of ice was applied to the surface each tissue section by first purging the source enclosure with nitrogen gas to 10% humidity, stabilizing the sample stage at -8 °C, and

exposing the stage to ambient humidity until the ice matrix formed(Robichaud et al., 2014). The enclosure was then resealed and purged again until humidity returned to 10%, where it was maintained throughout analysis.

To ablate targeted tissue regions a 2970-nm wavelength laser was used with a single burst of ten pulses to produce 1 mJ of energy at a rate of 10 kHz. X and Y stage movements of 100 µm were used to achieve oversampling(Nazari and Muddiman, 2015). Ablated analytes were post-ionized in the orthogonal electrospray plume, established by applying a voltage of approximately 3 kV to the electrospray solvent (1 mM acetic acid in 50:50 water/acetonitrile.) Mass spectrometry analysis of ionized molecules was performed in negative mode with internal calibrant used to achieve high mass accuracy (<2.5 ppm) within the 85-225 *m/z* range. Automatic gain control (AGC) was disabled. A mass resolution power of $240{,}000_{FWHM}$ at 200 *m/z* was used with a fixed injection time (15 ms) to synchronize timing of the ablation plume with ion collection in the C-trap of the mass spectrometer.

Collected raw data files were converted from the XCalibur.RAW format to mzML files via the ProteoWizard tool, MSConvert(Chambers et al., 2012), and then to imzML format using imzMLConverter(Race et al., 2012). Images were generated from the imzML files in MSiReader v1.03c(Bokhart et al., 2018). To remain conservative, abundances of stable isotope-labeled peaks (M+3 glycerate, 108.0293 *m/z*) were corrected for potential signal overlap from the naturally abundant species of each molecule using the percent isotope enrichment (PIE) tool in MSiReader(Mellinger et al., 2021). Isotopic distributions were computed using the R-based IsoSpec package(Lacki et al., 2020). Tissue regions of interest were selected to include pancreas tissue only and exclude peripheral fat tissue found in adjacent tissue staining.