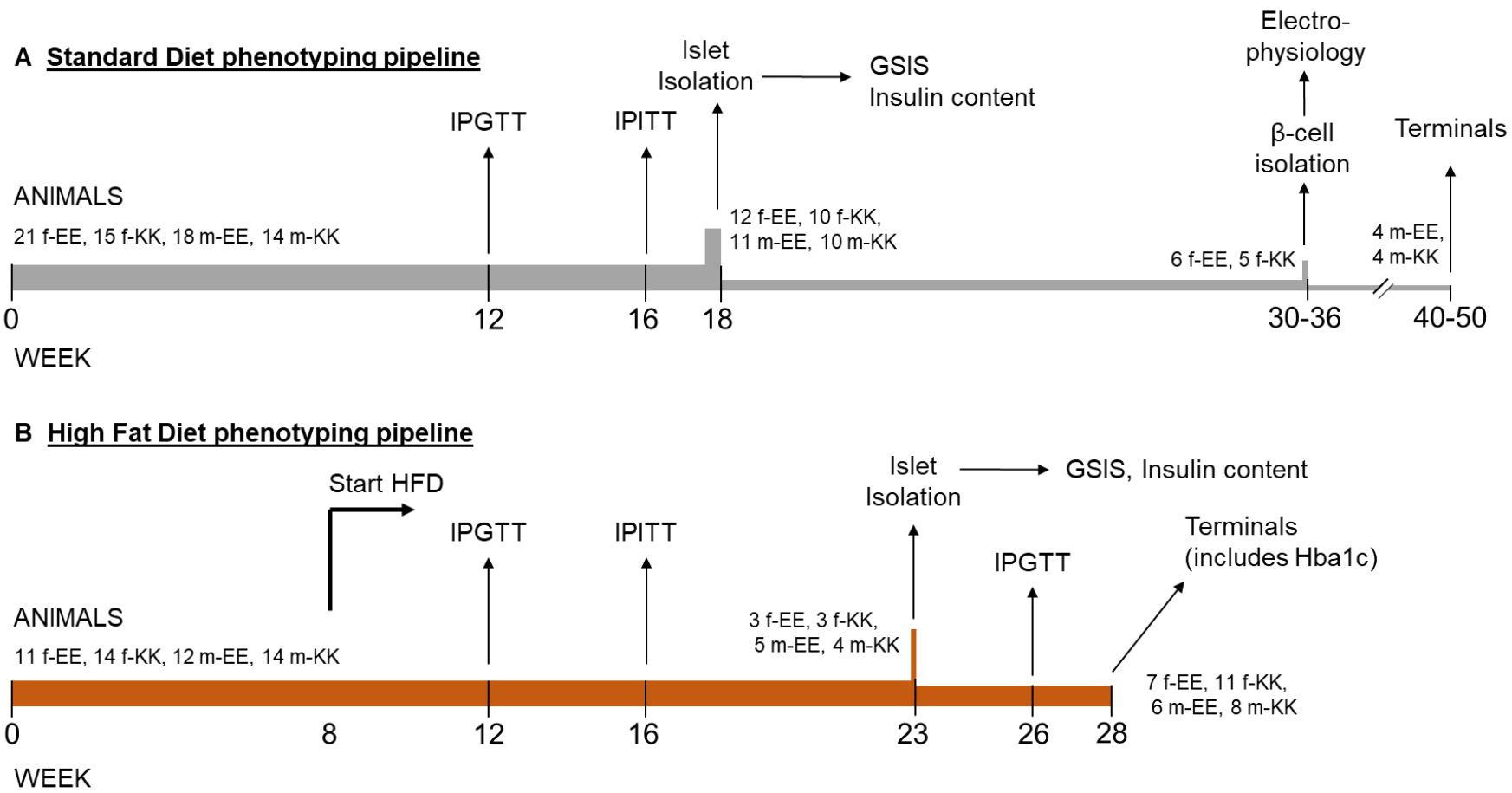
Online supplemental material, part 1 – Phenotyping pipelines, animal maintenance and experimental detail



Supplemental Figure – Phenotyping pipelines

Animals were assigned to experimental groups based on their sex (male or female) and genotype (EE or KK). These are abbreviated as f-EE (female EE genotype), f-KK (female KK genotype), m-EE (male EE genotype) and m-KK (male KK genotype). Minimum group sizes (10 mice) were derived from power calculations (statistical power 1-β > 90%, false type I error rate α < 5%) based on the relative effect sizes of E23K genotypes found in a human population study (Villareal et al., 2009) and typical in-house standard deviations for genetically uniform littermate mice (Girard et al., 2009).

A Timeline and mouse use for standard diet phenotyping. Not all SD animals were used for IPGTTs and IPITTs.

B Timeline and mouse use for high fat diet phenotyping.

IPGTT: Intra-Peritoneal Glucose Tolerance Test. IPITT: Intra-Peritoneal Insulin Tolerance Test. GSIS: Glucose Stimulated Insulin Secretion. 3 m-EE on SD and 1 m-EE, 2 m-KK and 1 f-EE on HFD had to be killed during the phenotyping pipeline for reasons unrelated to procedures (infection, weight loss).

Animal maintenance

Mice were housed in individually ventilated cages under specific opportunistic pathogen-free (SOPF) and controlled light (light 7am–7pm, dark 7pm–7am), temperature (21±2 °C) and humidity (55±10%) conditions. They had free access to water (9–13 ppm chlorine), and were fed ad libitum either on a standard diet (SD) containing 11.5 kcal% fat, 23.93 kcal% protein and 61.57 kcal% carbohydrate ("Rat and Mouse No. 3 Breeding diet", RM3; Diatex Int. Ltd., Witham, UK) or a high fat diet (HFD) containing 60 kcal% fat, 20.0 kcal% protein and 20 kcal% carbohydrate ("Rodent Diet with 60% kcal% fat", D12492; Research Diets, Inc., New Brunswick, NJ, USA).

Body weight, fed blood glucose and haemoglobin glycation

Body weight was measured weekly (Denver Instruments Balance #SG-601, Fisher Scientific, UK, or equivalent). Free-fed blood glucose was measured between 1- 4pm on weeks 7, 9, 11, 13, 15, 17 (SD and HFD) and 28 (HFD only) from small tail incisions after administration of a local anaesthetic (EMLA cream, Eutectic Mixture of Local Anesthetics, Lidocaine/Prilocaine, AstraZeneca), using Lithium-Heparin microvette tubes (Sarstedt, #16.443). Plasma glucose was measured using AlphaTrek 2 meters (Zoetis UK). Glycated haemoglobin (HbA1c), expressed as a percentage of total haemoglobin, was measured in samples from terminal bleeds at the age of 28 weeks using a degradation resistant colorimetric assay (Mouse Haemoglobin a1c (HbA1c) Kit, Crystal Chem, #80310).

Electrophysiology

Freshly isolated islets were handpicked into 200 µL RPMI 1640 medium (Thermo Fisher Scientific, #11875093), washed with 10 mL with phosphate buffered saline (DPBS, Thermo Fisher Scientific, #14190-144), and incubated in 500 µl HBSS-based enzyme-free dissociation buffer (Thermo Fisher Scientific, #13150016) for 4 minutes. After manual trituration, cells were washed once in 10 mL complete RPMI medium. A cell number equivalent of 5 islets per dish was resuspended in 50 µL complete RPMI medium, then plated into individual 35 mm cell-culture treated dishes (Starlab, #CC7682-3340). After 2-4 hours, 1.5-2 mL RPMI culture medium was added per dish. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2/95% air and used 1–2 days after isolation.

K_{ATP} currents were recorded from excised inside-out patches at -60 mV with an Axopatch 200B patch clamp amplifier (Axon Instruments) and Axon Digidata 1550B acquisition system (Axon Instruments). The pipette solution contained (in mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$ and 10 HEPES (pH 7.4 with KOH). The internal (bath) solution contained (in mM): 107 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 EGTA and 10 HEPES (pH 7.2 with KOH) plus MgATP as indicated. Experiments were carried out at room temperature (20–22°C). K_{ATP} current rundown was corrected for by expressing the current amplitude (I) in the test solution as a fraction of the mean of the current amplitude in the control (ATP-free) solution (I_{max}) directly before and after ATP application. Individual concentration-response curves were fit with the Hill equation and the mean IC_{50} calculated as the mean of individual fits.

Intraperitoneal Glucose and Insulin Tolerance Tests (IPGTT and IPITT)

IPGTT were conducted after an overnight fast. Blood glucose was measured by tail incision, immediately before (T0), and then 30, 60 and 120 minutes after injection with 2 g of glucose per kg body weight (kg_{BW}) in dissolved form (20% w/v glucose in 0.9% w/v NaCl). Larger blood volumes (60 µL) for insulin quantification were also taken, both before and after glucose injection. To test for statistically significant differences in glycaemic control, the Area under Curve (AUC) above baseline (minimum overnight fasted glucose concentration for the experiment) was calculated.

Intraperitoneal insulin tolerance tests (IPITT) were conducted after a four hour fast at 16 weeks of age. Blood glucose was measured immediately before (T0) and 15, 30, 45, 60 and 90 minutes after intraperitoneal injection of insulin. To test for statistically significant differences, the Area under Curve (AUC) below baseline (4-hr fasted glucose concentration, normalized to 1) was calculated. The insulin dose was optimized to avoid hypoglycaemia and was 0.75 IU/kg_{BW} for females and 1.0 IU/kg_{BW} for males.

Islet isolation

Islets were isolated essentially as described (Solomou et al., 2016; Ravier & Rutter, 2010). In brief, pancreata were inflated with collagenase solution at 1 mg/ml (Collagenase NB 8 Broad Range, Nordmark, S1745602), dissected out and placed in a water bath at 37 °C for 10 min. After washing, islets were purified on a Histopaque-1119 (Merck, #11191) / Histopaque-1083 (Merck, #10831) gradient. The islet layer was recovered and islets incubated in culture medium (RPMI 1640 medium (Thermo Fisher Scientific, #11875093), 11mM glucose, 10%(v/v) FBS (Thermo Fisher Scientific, #10500064), 1%(v/v) penicillin-streptomycin solution (Thermo Fisher Scientific, #15140122) at 37 °C, in a humidified atmosphere of 5% CO_2/95% air. After 2–4 h, islets were hand-picked into fresh culture medium and cultured overnight.

Glucose Stimulated Insulin Secretion (GSIS)

After overnight culture, islets were pre-incubated for 1 hour in Krebs-Ringer-HEPES (KRH) buffer containing (in mM): 120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 KH_2PO4, 1.2 $MgSO_4$, 10 HEPES, and 25 $NaHCO_3$, pH 7.4 (with NaOH) plus 0.1% w/v BSA (Merck, #A3803) plus 2mM glucose. Subsequently, insulin secretion was measured in 1 hour batch incubations (SD: 10 islets each; HFD: 5 islets each) at 37°C in KRH buffer with glucose and glibenclamide as indicated. Total insulin content per batch was measured by sonication of islets followed by overnight extraction of insulin using acid ethanol (75% v/v EtOH; 15 mM HCL; 0.1% v/v Triton-X100) at -80°C. Insulin was quantified using a Mercodia Mouse Insulin ELISA (Mercodia, 10-1247-10).