**SUPPLEMENTAL MATERIAL for**

Dipeptidyl peptidase IV inhibition delays developmental programming of obesity and metabolic disease in the offspring of obese mothers

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*Supplementary Figures*

**Supplementary Figure 1: Food intake and energy expenditure of male and female Off-RD and Off-HFD.** Data were collected from four-month old male and female Off-RD/-HFD (n=5-8/group) using Promethion (Sable Systems – Las Vegas, NV). Body mass (A), Lean mass (B), and Fat mass (C) of males and females Off-RD/-HFD prior to data collection. Data in D-G represent the trends of hourly averages of each respective parameter from the beginning of data collection (Hour 0) until study endpoint (Hour 9360). Cumulative food intake of males (D) and females (E), energy expenditure of males (F) and female (G), and activity of males (H) and females (I) Off-RD/-HFD.

*Methods*

*Brief description*

DPPIV activity was measured using an AMC-fluorogenic assay, as previously described (1). Japanese macaque samples were generously provided by the group of Dr. Ellinor Sullivan, and were collected as previously described (2, 3). Baboon samples were provided by Dr. Peter Nathanielz, and were collected as previously described (4, 5). All mouse studies were conducted using wild type FVB/NJ mice that were housed in sterile conditions and have *ad libitum* access to food and water. Mice were kept on a nocturnal 12 hour light cycle. To model maternal obesity in mice, we fed female FVB/NJ mice a high-fat diet (HFD, 45% kcal from fat) or a regular diet (RD, 17% kcal from fat) starting from six weeks old and throughout the study. After eight weeks of dietary intervention, females were bred to a RD-fed male. Offspring were weaned at three-weeks old and were fed only a RD thereafter. Acute Sitagliptin (3 mg/kg) was administered using an intraperitoneal injection. Oral Sitagliptin (30-45mg/kg/day) was administered in the drinking water as previously described (6) Body weights were measured weekly. Body fat composition was measured using EchoMRI(7) and visualized using microcomputed tomography(8). Food intake and energy expenditure were measured by Vanderbilt University’s Mouse Metabolic Phenotyping Core using indirect calorimetry (Promethean) (9). All euthanasia and metabolic function studies were conducted on mice that were fasted for 4-6 hours. OGTT and ITT were conducted as previously described and following recommendations by the MMPC(10). Euthanasia was performed using isofluorane inhalation and cardiac exsanguination. Sample size in mouse studies reflect the number of mothers/litters studied. One representative male and one representative female offspring was chosen randomly and were designated for each experiment.

Studies using human samples

**Plasma collection**. Maternal blood, cord blood, and placentas were collected from labor and delivery units at the University of Texas Health San Antonio and Oregon Health & Science University under protocols approved by the respective Institutional Review Boards and with informed consent from the patients. Samples for this study were collected from subjects who: (1) Had either normal or high pre-pregnancy body mass index (BMI), respectively, grouped as normal weight (NW; BMI = 18.5–24.9) or obese (OB; BMI = 30–45); (2) Had an uncomplicated singleton pregnancy, and (3) Delivered by C-section. Exclusion criteria included multifetal gestation, gestational diabetes, preeclampsia, chronic inflammatory diseases, tobacco use, illicit drugs, or both; and recent bariatric surgery. The placentas were randomly sampled as described previously. Maternal blood was collected from fasting patients before C-section. Cord blood was collected and placed in EDTA-containing collection tubes. Plasma was immediately separated from whole blood by centrifugation at 2000 *g* for 10 min at 4°C, then flash-frozen in liquid nitrogen and stored at −80°C for further analyses. Clinical information of donors are provided in the section entitled “Human Donors Clinical Information.

Studies using primate samples

**Baboon model of maternal obesity**. Baboon plasma and liver samples were generously provided by Dr. Peter Nathanielsz. Details of this model are extensively described in these reports (4, 5).

**Japanese Macaque model of maternal obesity**. Japanese Macaque plasma samples were generously provided by Drs. Ellinor Sullivan and Paul Kievit. Details of this model are extensively described in these reports (2, 3).

Studies in mice

**Mouse model of maternal obesity**. All mice studies were done using wild type FVB/N mice. Mice were kept under a 12-hour light/dark cycle, between 18-23 degrees Celsius with 40-60% humidity, in stress-free/bacteria free conditions. Mice were caged in groups of three to five whenever possible. Food and water were given *ad libitum*. Body weights were collected weekly. To induce maternal obesity, high fat diet (Teklad Cat#TD.06415) or its control, a regular diet (RD, PicoLab® Laboratory Rodent Diet Cat # 5L0D) was given to virgin female FVB/NJ mice from the age of six-weeks old and throughout the entire study. Composition of diets is presented in Table S1. After eight weeks of dietary intervention, RD- and HFD-fed female mice were bred to an age-matched RD-fed male. Pregnant mothers were left undisturbed and were then housed individually in a birthing cage five to seven days prior to expected delivery. The number of females suffering from perinatal loss and the total number of pups born dead or alive were recorded. Perinatal death was defined as stillbirth or death during the first 24 hours. Litters with high percentage of stillbirth were excluded from the study. Newborn and adult mice with signs of distress, lethargy, unusual behavior, and weight loss >20% were also excluded from the study. Live pups were weighed at birth. Pups were weaned at three-weeks of age, and were given only a RD, thereafter. At weaning, one male and one female offspring from each mother were randomly selected for the study.

**Sitagliptin treatments**. Sitagliptin (ApexBio Tech LLC, Cat No. A4036) or Vehicle (DMSO) was administered either acutely using an intraperitoneal injection (3 mg/kg) or chronically in the drinking water (0.3 mg/mL) – which results in a dose of 30-45 mg/kg/day.

**Mouse sample collection.** Mice were fasted for 4-6 hours. Body weight was measured immediately prior to euthanasia. Mice were euthanized by isoflurane anesthetization and cardiac exsanguination. Blood was collected from the left ventricle immediately after isoflurane anesthetization, treated with EDTA (final concentration 8 mM), centrifuged for 15 minutes at 2000 x g, and supernatant was collected and stored in -80oC until use. 1X Phosphate buffered saline (PBS, pH 7.4) was perfused into the left ventricle and out through the right atrium. Organs were weighed and preserved by either flash-freezing or formaldehyde fixation. Frozen tissues were stored in -80oC until use.

**Indirect calorimetry**. Experiments were conducted on 16-week old mice using Promethion from Sable Systems (Las Vegas, NV) at the Vanderbilt University’s Mouse Metabolic Phenotyping Core

**Body mass visualization using microcomputed tomography**. Micro-CT images of the entire body of mice were acquired to visualize body composition. Mice were euthanized using isoflurane inhalation and underwent imaging within 10 minutes. We acquired high-resolution (~10 μm) 3D scans using a Caliper Quantum FX Micro-CT system (Perkin-Elmer, Part#CLS140083) with 10 mm field of view, 140 μA current, 90 kV voltage, and a scan time of 3 min. We used the Amira 6.0 software platform (FEI Company) or Dragonfly 4.1 software (Object Research Systems, Quebec, Canada) to visualize these scans.

**Body fat mass quantitation using EchoMRI**. Body composition were measured using EchoMRI 4-in-1/1000 and as previously described (64).

**Oral glucose tolerance test.** OGTTs were performed based on standard protocols established by the NIH Mouse Metabolic Phenotyping Core. Mice were fasted for 4-6 hours. Blood glucose was measured from a tail clip and using a glucometer (AlphaTRAK). After measurement of fasting blood glucose, 20% glucose was orally administered through a gavage at a dose of 2g/kg. After which, blood glucose were measured after 15, 30, 60, 90 and 120 minutes. Physiological effort exerted were quantified as area under the curve (AUC).

**Insulin tolerance test.** ITTs were performed based on standard protocols established by the NIH Mouse Metabolic Phenotyping Core. Mice were fasted for 4-6 hours. Blood glucose was measured from a tail clip and using a glucometer (AlphaTRAK). After measurement of fasting blood glucose, Insulin (Humalin R – Eli Lilly R-100) was administered through an intraperitoneal injection at a dose of 1.5 U/kg. After which, blood glucose were measured after 15, 30, 60, 90 and 120 minutes. Physiological effort exerted were quantified as area under the curve (AUC).

Ex-vivo assays

**DPPIV activity assay**. H-Glycyl-Prolyl-7-amino-4-methylcoumarin (H-Gly-Pro-AMC) (Cat#4002520) was purchased from Bachem AG (Bubendorf, Switzerland). 7-amino-4-methylcoumarin (AMC) (Cat#164545) was purchased from Sigma Chemical Co., (St. Louis, MO., USA). Assay was carried out using either 10 μl plasma or protein lysate.[[9](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2941613/#CIT9)] Briefly, the samples were incubated for 5 minutes at 37°C in 90 μl of assay buffer (50 mM HEPES, 80 mM MgCl2, 140 mM NaCl, 1% BSA, 40 μM, H-Gly-Pro-AMC. Each sample was incubated in buffer containing Sitagliptin (0.3 mg/ml) or its vehicle (DMSO). AMC release was measured kinetically (1 read per minute for 30 minutes) using BioTek Synergy H1 (Part #8041000) at 380 nm excitation/460 nm emission, respectively. Absorbance values were transformed to AMC concentration using an AMC standard curve. Each sample’s specific absorbance (one to 30 minute) was calculated as the difference of each respective sample’s aliquot that was incubated with Sitagliptin and its vehicle (DMSO). DPPIV activity was calculated as the slope of AMC release over unit time, and are presented as pmol AMC/min/mL sample.

**References**

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