Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

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

Kim Ramil C. Montaniel[1,2], Matthew Bucher[3], Elysse A. Phillips[1], Cun Li[4,5], Elinor L. Sullivan[6,7,8], Paul Kievit[6], Sandra Rugonyi[9], Peter W. Nathanielsz[4,5] and Alina Maloyan[1,2]

[1]Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR, 97232, USA; [2]Physiology and Pharmacology Graduate Program, Oregon Health & Science University, Portland, OR, 97232, USA; [3]Department of Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR, 97232, USA; [4]Texas Biomedical Research Institute and Southwest National Primate Research Center, San Antonio, TX, 78227, USA; [5]Department of Animal Sciences, University of Wyoming, Laramie, WY, 82071, USA; [6]Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, 97006, USA; [7]Department of Psychiatry, Oregon Health & Science University, Beaverton, OR, 97006, USA; [8]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR, 97232, USA and [9]Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR, 97232, USA

Original Article

Cite this article: Montaniel KRC, Bucher M, Phillips EA, Li C, Sullivan EL, Kievit P, Rugonyi S, Nathanielsz PW, and Maloyan A. (2022) Dipeptidyl peptidase IV inhibition delays developmental programming of obesity and metabolic disease in male offspring of obese mothers. *Journal of Developmental Origins of Health and Disease* **13**: 727–740. doi: 10.1017/S2040174422000010

Received: 31 August 2021
Revised: 18 November 2021
Accepted: 24 December 2021
First published online: 24 January 2022

Keywords:
maternal obesity; developmental programming; metabolic disease; DPPIV; DDPIV inhibitors

Address for correspondence:
Alina Maloyan, PhD, FAHA, Associate Professor, Knight Cardiovascular Institute, Center for Developmental Health, Oregon Health & Science University, Portland, OR 97232, USA
Email: maloyan@ohsu.edu

Abstract

Maternal obesity programs the offspring to metabolic diseases later in life; however, the mechanisms of programming are yet unclear, and no strategies exist for addressing its detrimental transgenerational effects. Obesity has been linked to dipeptidyl peptidase IV (DPPIV), an adipokine, and treatment of obese individuals with DPPIV inhibitors has been reported to prevent weight gain and improve metabolism. We hypothesized that DPPIV plays a role in maternal obesity-mediated programming. We measured plasma DPPIV activity in human maternal and cord blood samples from normal-weight and obese mothers at term. We found that maternal obesity increases maternal and cord blood plasma DPPIV activity but only in male offspring. Using two non-human primate models of maternal obesity, we confirmed the activation of DPPIV in the offspring of obese mothers. We then created a mouse model of maternal high-fat diet (HFD)-induced obesity, and found an early-life increase in plasma DPPIV activity in male offspring. Activation of DPPIV preceded the progression of obesity, glucose intolerance and insulin resistance in male offspring of HFD-fed mothers. We then administered sitagliptin, DPPIV inhibitor, to regular diet (RD)- and HFD-fed mothers, starting a week prior to breeding and continuing throughout pregnancy and lactation. We found that sitagliptin treatment of HFD-fed mothers delayed the progression of obesity and metabolic diseases in male offspring and had no effects on females. Our findings reveal that maternal obesity dysregulates plasma DPPIV activity in males and provide evidence that maternal inhibition of DPPIV has potential for addressing the transgenerational effects of maternal obesity.

Introduction

Obesity is a central driver of cardiometabolic syndrome, a cluster of chronic disorders that includes type 2 diabetes mellitus (T2DM), dyslipidemia, and cardiovascular diseases (CVD)[1]; it is a silent pandemic that produces immense health and economic burdens[2]. In the US alone, the WHO estimates that by 2030, 50% of people will be obese and 25% morbidly obese[3], making >50% of the US population at risk of developing obesity-associated chronic diseases. Addressing the obesity pandemic is of paramount importance, albeit a tremendous challenge as the disease exists in diverse forms and its primary pathogenic factors may include genetic abnormalities, adverse lifestyle factors, and early-life exposure to adverse conditions[2].

In 2018, more than 65% of US women of childbearing age were either overweight or obese[4], and it is now believed that obesity during pregnancy is a major driving force behind the obesity pandemic[5]. Intrauterine exposure to maternal obesity is linked to development of obesity and associated chronic diseases later in life – a process called *developmental programming*[6]. This induces a vicious cycle of obesity: daughters of obese mothers have increased likelihood of developing obesity themselves, which, in turn, predisposes their future children to obesity[7]. However, while the phenomenon of developmental programming is well-described, the precise mechanisms, by which it is mediated, remain unclear. This knowledge gap poses a major obstacle to the development of effective strategies for addressing the generational perpetuation of obesity programming.

Table 1. Clinical information of human donors of maternal and cord blood samples. Data presented as mean (range). Sample size is shown. *, $p < 0.05$

Maternal Weight Class	Normal Weight		Obese	
Fetal Sex	*Males*	*Females*	*Males*	*Females*
Number of samples (n)	17	13	16	13
Maternal Pre-Pregnancy BMI (kg/m²)	22.38 (19.5-25)	22.57 (18.3-24.8)	35.12 * (31.07-42.58)	37.22* (30.03-43.4)
Maternal Age (years)	34.12 (23-46)	33.38 (27-41)	31.85 (26-38)	30.69 (23-38)
Gestational Age (weeks)	38.84 (36-39.57)	38.85 (37-39.71)	38.43 (37-39.14)	38.58 (36-39.57)
Birthweight (grams)	3393 (2470-3941)	3540 (2525-4975)	3382 (2520-4070)	3400 (2370-4550)
Gestational Weight Gain (kg)	12.66 (5.5-20.4)	13.62 (2.3-30.7)	12.08 (-0.5-38.9)	12.18 (1.4-45.4)

Dipeptidyl peptidase IV (DPPIV), also known as CD26, is a serine protease that has crucial roles in the regulation of metabolism, appetite, food intake, energy expenditure, and body composition[8]. DPPIV is produced by hepatocytes, adipocytes, intestinal K cells, placental cytotrophoblasts, and endothelial cells, among others, and exists in both soluble (s-DPPIV) and plasma membrane-bound (m-DPPIV) isoforms[9]. These two isoforms have distinct functions: m-DPPIV regulates intercellular communication by acting as a receptor, while s-DPPIV facilitates intercellular and inter-organ crosstalk by cleaving protein substrates in bodily fluids such as plasma and/or binding to DPPIV receptors on cell membranes[9]. In the context of obesity, DPPIV has been shown to exacerbate fat retention and metabolic abnormalities[10–13]. Evidence from epidemiological studies revealed that plasma DPPIV activity is increased in obese children[14], in obese adults[12], and in patients with T2DM[11–13,15], hypertension[16], and renal diseases[17,18]. Furthermore, emerging evidence suggests that elevated plasma DPPIV activity in childhood is a marker of increased risk for later-life obesity[12,13,19]. In mice, greater plasma DPPIV activity is observed in models of genetic obesity (*ob/ob*) and diet-induced obesity (DIO)[20]; meanwhile, mice that lack one or both *Dpp4* alleles in adipocytes and/or hepatocytes exhibit reduced plasma DPPIV activity and are protected from DIO[21]. DPPIV has also been extensively studied in the context of T2DM and insulin signaling, and DPPIV inhibitors such as sitagliptin are effective, United States Food and Drug Administration (FDA)-approved second-line treatments for T2DM[22–27]. In line with the hypothesis that DPPIV is an active player in obesity progression, results from clinical trials indicate that DPPIV inhibitors can improve weight control[28–34], systemic metabolism[22–27], and inflammatory responses[35–43] – all critical processes that become progressively dysregulated in the context of obesity. Nonetheless, despite extensive research efforts, the precise role of DPPIV in obesity progression remains to be determined.

In this respect, whether DPPIV has a role in developmental programming is an entirely novel direction of inquiry. Here, we determined the effect of maternal obesity on maternal and offspring plasma DPPIV activity in humans and non-human primates, and further tested the hypothesis that DPPIV plays a critical role in the progression of obesity and glucose intolerance in male offspring of obese mothers, and that inhibition of DPPIV will have potential as preventive therapeutics against obesity-related developmental programming. Our findings indicate that DPPIV activity is increased in obese mothers, and also in their offspring in an offspring sex- and age-dependent manner. Using a mouse model of high-fat diet (HFD)-induced maternal obesity, we show that administration of the DPPIV inhibitor sitagliptin to HFD-fed mothers during the immediate perinatal period delays progression of obesity and metabolic disorders in their male offspring, while female offspring remain unaffected. Importantly, our findings highlight a critical role for DPPIV in developmental programming of maternal obesity and provide supporting evidence that DPPIV inhibitors merit further examination as potential therapeutics to address developmental programming and the progression of obesity and metabolic disorders in offspring of obese mothers.

Results

Maternal obesity dysregulates plasma DPPIV activity in humans and non-human primates

To determine if maternal obesity has an effect on plasma DPPIV activity in humans, we collected maternal and cord blood plasma from normal-weight (Hum-Mat-NW, body mass index [BMI]<25, n = 25) and obese (Hum-Mat-Ob, BMI > 30, n = 13) mothers upon C-section *at term*. The clinical characteristics of the study participants are presented in Table 1. Indications for Caesarean section included only elective repeat or breech presentation, and no differences in indications were observed between normal-weight and obese groups. By experimental design, the groups differed significantly in terms of BMI. No differences were observed in maternal and gestational age, birth weight, or gestational weight gain.

Using an amino-methylcoumarin fluorogenic assay[44], we found that relative to normal-weight mothers, plasma DPPIV activity was significantly increased in obese mothers carrying male fetuses but decreased in mothers with female fetuses (Fig. 1A). Cord plasma DPPIV activity was likewise increased in male babies born to obese mothers but showed no difference in females (Fig. 1B). Next, we asked whether this activation of DPPIV in offspring persists beyond birth. Due to a lack of human samples, we addressed this question using two well-described non-human primate models of maternal diet-induced developmental programming: baboons (Bab, *Papio hamadryas*) and Japanese macaques (JM). Both of these model primate species have been extensively studied in the setting of maternal obesity due to high genetic and physiological similarities with humans[45,46]. Phenotype details for the baboon[47–51] and Japanese macaque[52–56] models of maternal HFD feeding vs. maternal regular diet (RD) are extensively

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

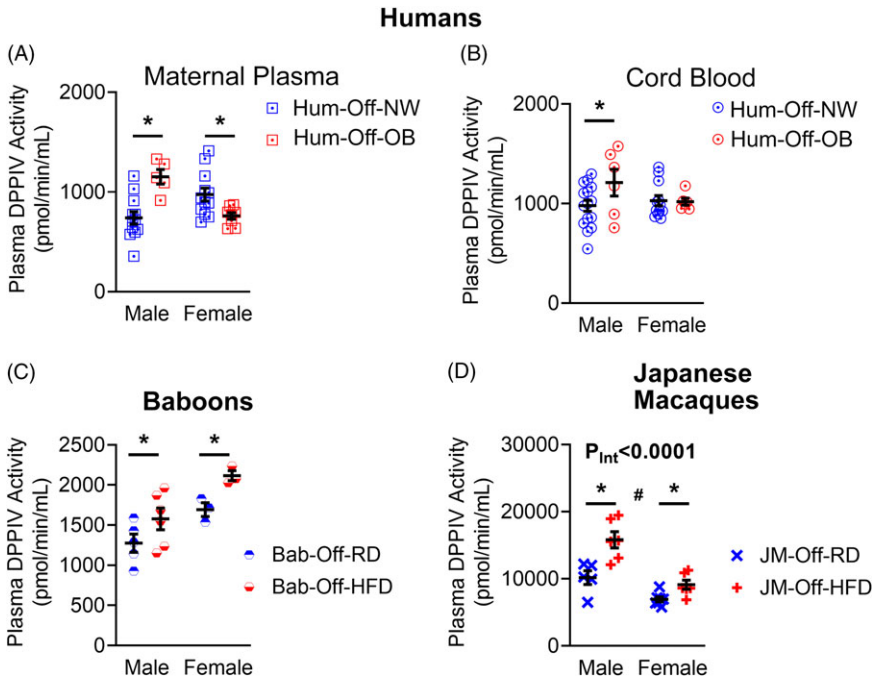

Fig. 1. Maternal obesity dysregulates plasma activity of dipeptidyl peptidase IV in humans and non-human primates. DPPIV activity was measured in maternal plasma samples (A) from normal-weight (Hum-Mat-NW) and obese (Hum-Mat-Ob) mothers *at term* and prior to delivery by C-section, and in cord plasma (B) from male and female fetuses born to normal-weight (Hum-Off-NW) and obese mothers (Hum-Off-Ob). Sample size is shown in Table 1. *, $p < 0.05$ NW vs. OB women. C-D, Plasma DPPIV activity was measured in six-month-old male and female baboon offspring (C) of regular diet (RD)-fed mothers (Bab-Off-RD, n = 6 males and 3 females) and high-fat diet (HFD)-fed mothers (Bab-Off-HFD, n = 6 males and 3 females), and in 36-month-old male and female Japanese macaque offspring (D) of RD-fed (JM-Off-RD, n = 5 males and 7 females) and HFD-fed (JM-Off-HFD, n = 6 males and 6 females) mothers. *, $p < 0.05$ offspring of RD-fed mothers vs. offspring of HFD-fed mothers.

described in the cited reports. In both models, the offspring of HFD-fed mothers (Off-HFD) in relation to offspring of a RD-fed mothers (Off-RD), exhibit programmed diseases whose development bears a striking resemblance to those occurring in human offspring of obese mothers. In six-month-old (pre-weaning) baboons both male and female offspring of HFD-fed mothers (Bab-Off-HFD) exhibited increased plasma DPPIV activity relative to offspring of RD-fed mothers (Bab-Off-RD) (Fig. 1C). Similarly, in three-year-old (pre-pubescent) JM, we found increased plasma DPPIV activity in both male and female offspring of HFD-fed mothers (JM-Off-HFD) relative to offspring of RD-fed mothers (JM-Off-RD) (Fig. 1D).

Mouse model of maternal high fat DIO

To determine if activation of DPPIV contributes to developmental programming, we developed a mouse model of maternal HFD-induced obesity. Specifically, we fed female wild-type Friend virus B (FVB/NJ) mice either a RD (13% kcal from fat) or a HFD (45% kcal from fat) starting from six weeks of age and continuing throughout the study (Fig. 2A). Composition of diets is shown in Table S1. After eight weeks of dietary intervention (i.e. at 14 weeks of age) and prior to breeding, HFD-fed females (Mat-HFD) exhibited no change in body weight (Fig. 2B) relative to RD-fed females (Mat-RD) but had increased body fat composition (Fig. 2C) as visualized using microcomputed (Fig. 2C) measured and quantified using echo magnetic resonance (EchoMRI, Fig. 2D). To confirm that feeding a HFD does not lead to pre-gestational diabetes, we assessed glycemic control using both an oral glucose tolerance test (OGTT) (Fig. 2E and F) and an insulin tolerance test (Fig. 2G and H). No significant differences were observed between Mat-RD and Mat-HFD in their responses to either test.

After eight weeks of dietary intervention, 14-week-old RD- and HFD-fed females were bred to RD-fed males. During pregnancy, the Mat-HFD group gained more weight than did Mat-RD females (Fig. 2I). At birth, pups born to HFD-fed mothers (Off-HFD) exhibited a 16% reduction in birthweight compared with pups born to RD-fed mothers (Off-RD) (Fig. 3A, $p < 0.001$). Pups were weaned at three weeks and thereafter were fed only the RD. As of weaning and into pre-pubescence (two months of age), no significant differences in body weight were evident in either male or female Off-HFD relative to Off-RD (Fig. 3B-C); however, at adulthood (i.e. from 4 through 11 months of age), both male and female Off-HFD were continuously heavier than their Off-RD counterparts (Fig. 3B-C).

At two months of age, we visualized offspring body composition using microcomputed tomography, which revealed increased fat mass in Off-HFD males and to a lesser degree in Off-HFD females (Fig. 3D). At four months of age, we measured body composition again using EchoMRI and found total fat mass to be significantly increased and lean mass decreased ($p < 0.05$ for both) in Off-HFD males with no changes in females (Supp. Fig. 1A-C). As adiposity in the Off-HFD could be attributed to changes in 'lifestyle choices' such as food intake, energy expenditure, or overall physical activity, we performed metabolic and behavioral phenotyping in four-month-old Off-RD and Off-HFD mice using indirect calorimetry (Promethion systems) (Supp. Fig. 1). We observed no significant differences between the groups in terms of food intake (Supp. Fig. 1D-E), energy expenditure (Supp. Fig. 1F-G), or overall activity (Supp. Fig. 1H-I), suggesting that the increased adiposity in Off-HFD was induced by exposure to maternal HFD, and is not a

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

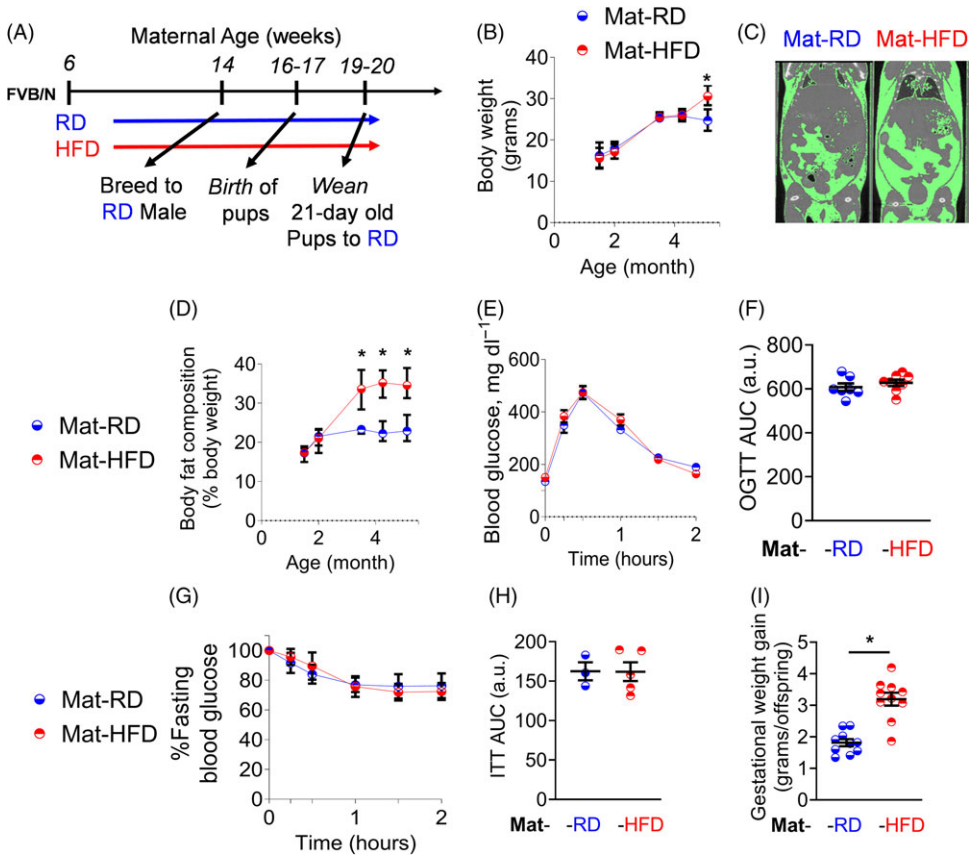

Fig. 2. HFD feeding of female mice leads to increased maternal adiposity without metabolic dysfunction. Study design (A). Panels illustrate body weights (B), representative microcomputed tomography radiographs (C), body fat composition (D), OGTT glycemic excursion curves (E), OGTT AUC values for E (F), ITT glycemic excursion curves (G), ITT AUC (H), and gestational weight gain (I) of Mat-RD (n = 7) and Mat-HFD (n = 8). *, $p < 0.05$ HFD-fed females vs. RD-fed females.

direct effect of lifestyle factors such as food consumption and/or physical activity.

Since children born to obese mothers are prone to developing metabolic dysregulations[57], we measured glucose tolerance and insulin sensitivity in male and female Off-RD and Off-HFD mice across a range of ages. At three weeks of age, glucose tolerance appeared comparable between groups for males and females alike (Fig. 3F-H). At two months of age, however, male Off-HFD exhibited a 44% decrease in glucose tolerance (Fig. 3I,K) and a 34% decrease in insulin sensitivity (Fig. 3L,N) relative to their Off-RD counterparts ($p < 0.05$), whereas no significant changes were evident in females (Fig. 3J,K,M,N). At 11 months of age, male Off-HFD likewise exhibited 40% decreased glucose tolerance (Fig. 3O,Q) along with OGTT glycemic excursion curves similar to mice having type 2 diabetes[58], while females continued to demonstrate no significant difference in either measure (Fig. 3P-Q) suggesting that Off-HFD male offspring appearing to be more metabolically compromised than female offspring through at least 11 months of age.

A maternal HFD in mice leads to activation of offspring plasma dipeptidyl peptidase DPPIV

Having established our mouse model of maternal obesity, we next examined the effect of maternal HFD on plasma activity of DPPIV. After eight weeks of dietary intervention and prior to breeding, HFD-fed females showed a tendency to increased plasma DPPIV activity but did not achieve statistical significance ($p = 0.1$, Fig. 4A). In three-week-old offspring, we found that plasma DPPIV activity was significantly increased in Off-HFD males relative to Off-RD males; these results are similar to observations in human babies, and suggest that DPPIV activation precedes metabolic dysregulations in these mice (Fig. 4B). Significantly reduced DPPIV activity was observed in three-week-old Off-HFD females ($p < 0.05$, Fig. 4B), but at 11 months of age, both male and female Off-HFD showed significant increases in DPPIV plasma activity ($p = 0.007$ and $p = 0.05$ respectively, Fig. 4C). These results indicate that, consistent with human and non-human primate data, maternal HFD-induced adiposity in mice dysregulates plasma DPPIV activity in a manner dependent on offspring sex and age. Hence, we reasoned that DPPIV might play a role in the developmental programming of obesity and metabolic diseases, at least in the maternal HFD context.

Administration of the DPPIV inhibitor sitagliptin to male offspring of HFD-fed mothers ameliorates progression of obesity and improves glycemic control

To determine if DPPIV is involved in the metabolic abnormalities observed in Off-HFD mice, we treated the same cohorts of 2- and 11-month-old Off-RD and Off-HFD mice that were assayed for glucose control, with sitagliptin, a DPPIV inhibitor, (acute treatment, Ac-Sita, i.p. 0.3 mg/kg), then one hour later repeated the glucose tolerance test (Fig. 3). Sitagliptin administration noticeably improved glucose tolerance in both Off-RD and Off-HFD males, by 39 and 49% respectively at two months of age (Fig. 4D and

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

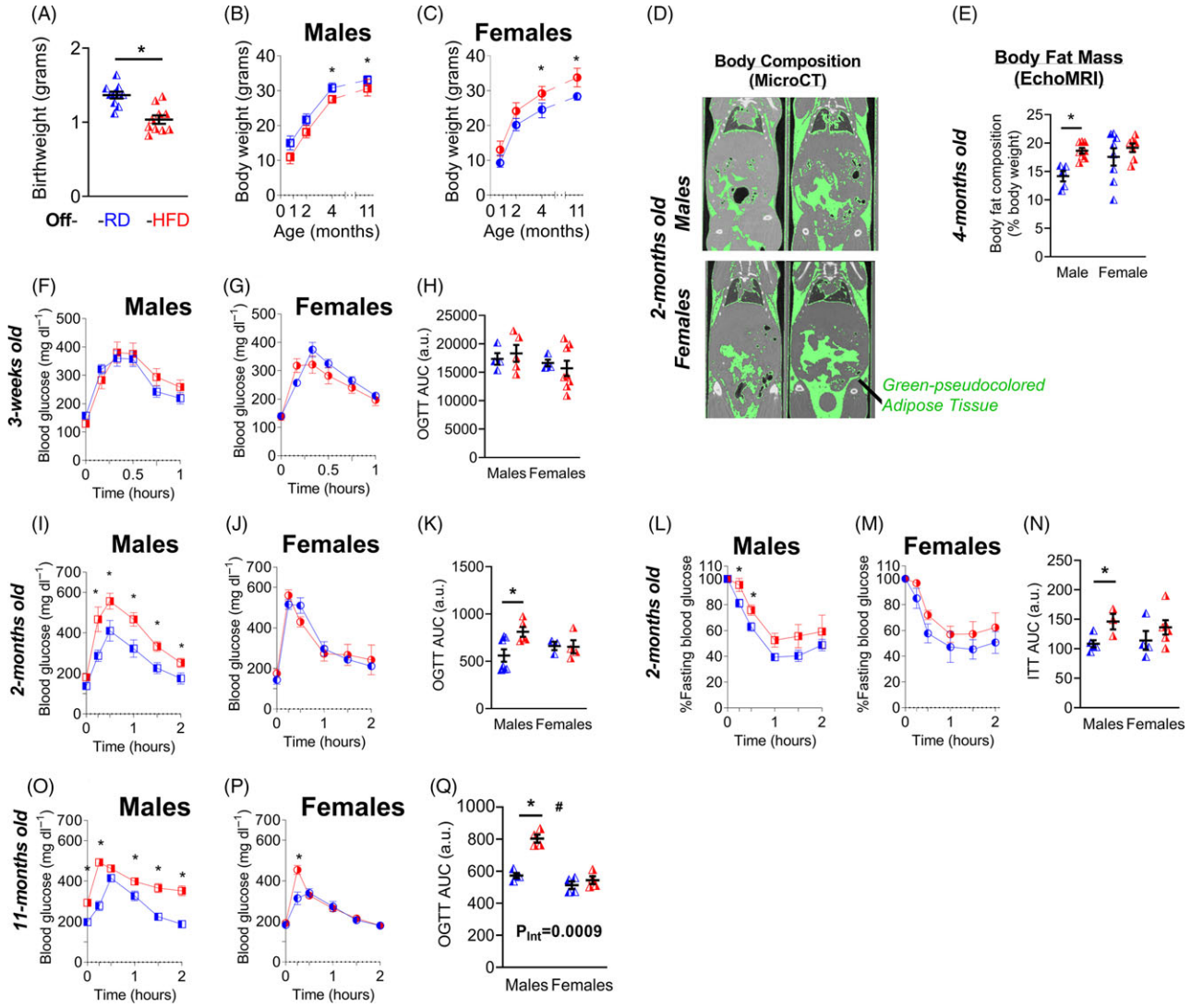

Fig. 3. In mice, maternal HFD and obesity leads to programming of obesity and metabolic dysfunction in male offspring. Data were collected from offspring of mothers that underwent the experimental protocol shown in Fig. 2A. Average birthweight of Off-RD and Off-HFD pups in each litter (A). N = 10/group of maternal diet. Body weights of male (B) and female (C) Off-RD/-HFD at three weeks old and at two, four and 11 months old. Representative microcomputed tomography radiographs at two months of age (D) and EchoMRI body fat mass quantification at four months of age (E). OGTT glycemic excursion curves of three-week-old male (F) and female (G) Off-RD/HFD, and OGTT AUC values (H). OGTT glycemic excursion curves of two-month-old male (I) and female (J) Off-RD/-HFD, and AUC values (K). ITT glycemic excursion curves of two-month-old male (L) and female (M) Off-RD/-HFD, and AUC values (N). OGTT glycemic excursion curves of 11-month-old male (O) and female (P) Off-RD/HFD, and AUC values (Q). N = 10/group/sex. *, $p < 0.05$ offspring of HFD-fed mothers vs. offspring of RD-fed mothers. #, $p < 0.05$ males vs. females within the same group of maternal diet.

E) and by 3.9% and 11.2% respectively at 11 months of age (Fig. 4F and G). No significant effects of Ac-Sita treatment were observed in females (Fig. 4H and I).

Next, we asked whether chronic administration of sitagliptin from a young age would similarly improve glycemic control and furthermore prevent the progression of obesity and metabolic abnormalities in Off-HFD mice. We treated male and female Off-RD and Off-HFD with sitagliptin (Sita, 0.3 mg/kg) or vehicle (Veh, DMSO) via drinking water, beginning at three weeks of age and extending into adulthood. No differences in the amount of water consumed each day were observed between sexes or between experimental groups.

At four months old, the Off-HFD vehicle-treated (Off-HFD + Veh) males exhibited a 7% increase in body weight (Fig. 5A) and a 56% increase in body fat percentage (measured as the percentage of body weight accounted for by inguinal adipose tissue) (Fig. 5C) compared with their Off-RD counterparts (Off-RD + Veh). However, sitagliptin-treated Off-HFD males showed a 50% reduction in body weight ($p < 0.05$, Fig. 5A) and 44% reduction in adiposity vs. Off-HFD + Veh ($p < 0.05$, Fig. 5C). Meanwhile, no differences in body weight or body composition were observed in four-month-old Off-HFD females, whether treated with sitagliptin or vehicle (Fig. 5B). Surprisingly, in Off-RD females, sitagliptin treatment reduced adiposity by 46% ($p = 0.04$, Fig. 5D) vs. vehicle-treated mice.

To determine the effects of chronic sitagliptin treatment on glycemic control, we measured glucose tolerance and insulin sensitivity at two months of age and compared the results with previously collected data (Fig. 3). Sitagliptin-treated Off-HFD males showed significant improvement, with the area under the curve for the glucose tolerance test being decreased by 10.4% ($p = 0.03$, Fig. 5E,G) and that for the insulin sensitivity test by 11% ($p = 0.05$, Fig. 5I,K)

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

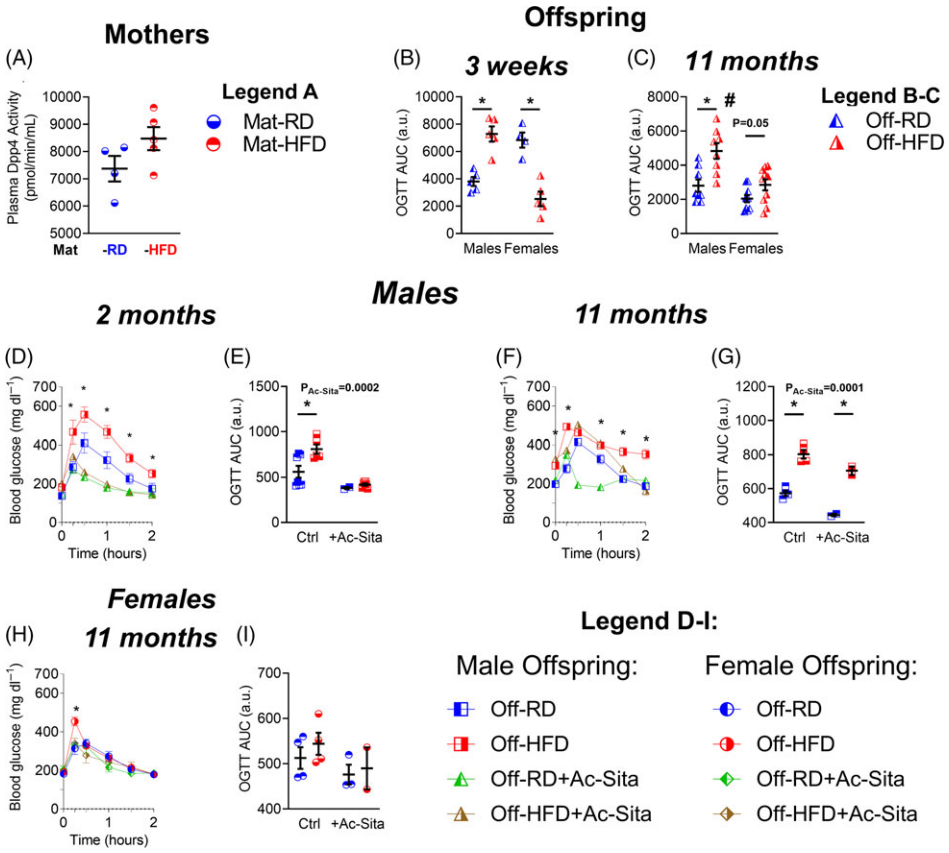

Fig. 4. In mice, maternal HFD dysregulates offspring plasma DPPIV activity in a sex- and age-dependent fashion, and acute DPPIV inhibition improves glucose tolerance in male Off-HFD. Plasma DPPIV activity of 14-week-old RD- and HFD-fed female FVB/n mice (A), and of three-week-old (B) and 11-month-old (C) Off-RD (n = 5-8/sex) and Off-HFD (n = 6-11/sex). Data in D-I were collected from two- and 11-month-old male and female Off-RD (n = 4-8/sex) and Off-HFD (n = 5/sex). Data for untreated Off-RD/-HFD groups were previously presented in Figure 2. OGTTs were first conducted on untreated mice (Off-RD/-HFD), then after a two-day rest were repeated on the same mice after one-hour pretreatment with sitagliptin (i.p. 30 mg/kg) (Off-RD/-HFD + Ac-Sita). OGTT glycemic excursion curves and respective AUC quantifications for two-month-old males (D, E), 11-month old males (F, G) and 11-month-old females (H, I). *, $p < 0.05$ offspring of HFD-fed mothers vs. offspring of RD-fed mothers. #, $p < 0.05$ males vs. females within the same group of maternal diet.

relative to vehicle-treated Off-HFD males. In females, neither glucose tolerance (Fig. 5F,H) nor insulin sensitivity (Fig. 5J,L) were affected by maternal obesity, and likewise no changes were observed with sitagliptin treatment.

DPPIV inhibition during pregnancy and lactation in HFD-fed mothers delays progression of obesity and metabolic dysfunction in male offspring

In light of the results obtained from long-term sitagliptin treatment in offspring, we hypothesized that maternal inhibition of DPPIV would impede the progression of obesity and metabolic disorders in offspring. To address this hypothesis, we conducted a pre-clinical study, in which HFD- or RD-fed female mice were administered with either sitagliptin (Sita, 0.3 mg/kg) or vehicle (Veh, DMSO) starting one week prior to breeding and continuing throughout pregnancy and lactation (study design is shown in Fig. 6A). This treatment regimen is henceforth referred to as "Mat-Sita."

In RD and HFD-fed mothers, Sita did not affect body weight (Fig. 6B) or glucose tolerance prior to pregnancy (Fig. 6D and E) when compared with respective Veh-treated controls. Meanwhile, gestational weight gain was decreased by Sita in HFD-fed mothers (Mat-HFD + Sita) vs. Veh-treated HFD-fed mothers (Mat-HFD + Veh), with no effect observed in RD-fed mothers (Fig. 6C). Sita treatment likewise had no effect on birthweight in either Off-RD or Off-HFD mice (Fig. 7A), and reduced body weights at weaning in males ($p < 0.05$, Fig. 7B) with no changes in females (Fig. 7C) pups. However, where two-month-old male offspring of the Mat-HFD + Veh group showed a 25% increase in body weight vs. Mat-RD + Veh ($p < 0.05$), that change was ameliorated in the male offspring of Mat-HFD + Sita mice ($p < 0.05$, Fig. 7D). No changes were observed in females (Fig. 7C,E). Interestingly, at ten months of age, both male and female offspring of the Mat-HFD + Veh group demonstrated significantly higher body weights (Fig. 7F and G) and body fat composition (Fig. 7H and I), that were reversed in the offspring of Mat-HFD + Sita mothers. No changes in lean mass were detected under either treatment.

To determine if maternal administration of sitagliptin affects DPPIV activity in young offspring, we assessed plasma DPPIV activity at weaning. Consistent with the results illustrated in Fig. 5, we found maternal HFD to increase plasma DPPIV activity in male offspring and decrease it in female offspring relative to sex-matched offspring of RD-fed mothers ($p = 0.01$, Fig. 7J). Maternal Sita administration decreased plasma DPPIV activity in 3-week-old male pups born to HFD-fed mothers but not in those born to RD-fed mothers. Surprisingly, for female offspring, maternal Sita treatment decreased plasma DPPIV activity in pups born to RD-fed mothers and increased it in pups born to HFD-fed mothers ($p = 0.003$) (Fig. 7K).

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

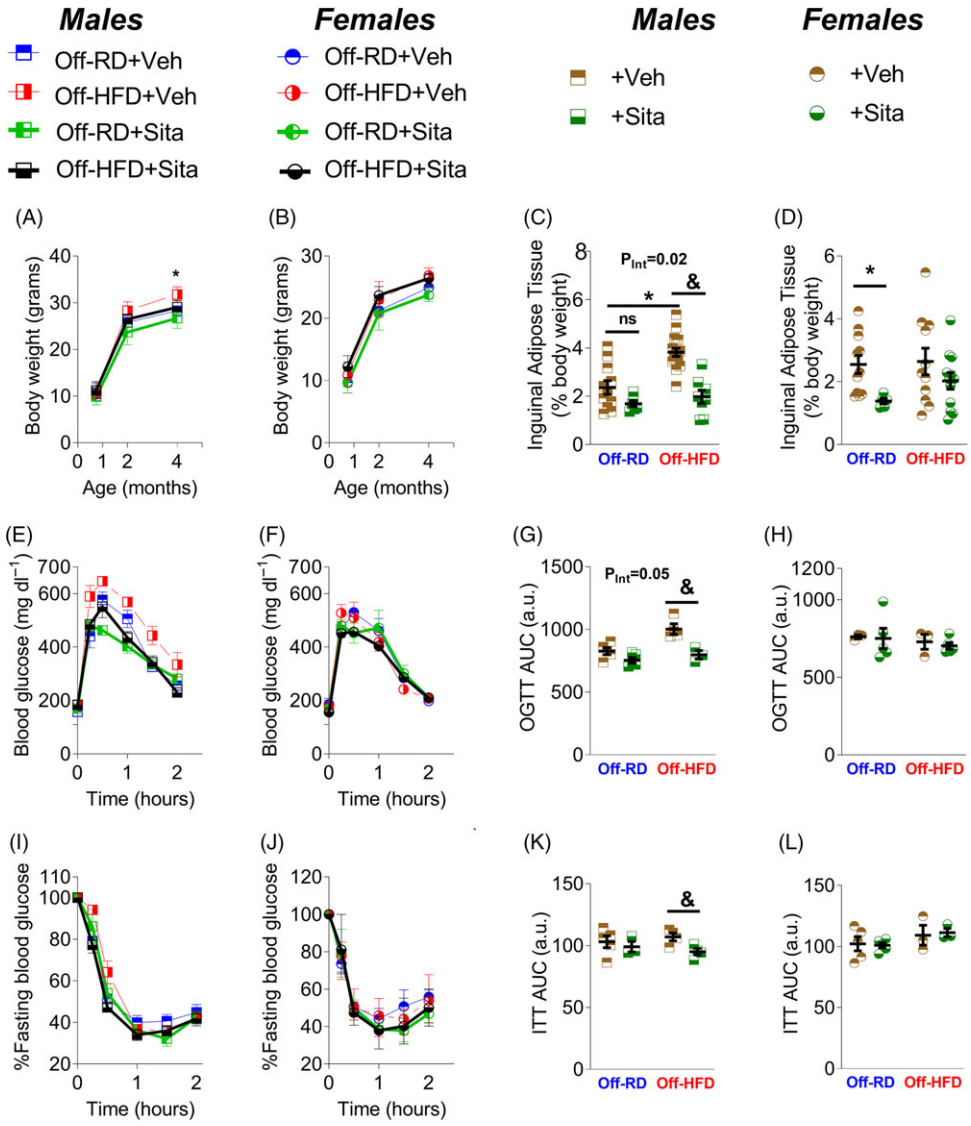

Fig. 5. DPPIV inhibitor treatment of male Off-HFD mice downregulates weight gain, glucose intolerance, and insulin resistance. Data were collected from Off-RD (n = 4-5/sex) and Off-HFD (n = 5/sex) that were treated with sitagliptin (+Sita) at a dosage of 30-45 mg/kg/day or its vehicle (+Veh, DMSO) in drinking water from weaning (three-weeks-old) until endpoint. Body weight trends of male (A) and female (B) offspring from all groups. OGTT glycemic excursion curves (E, F) and respective AUC quantifications (G, H) of two-month-old male (E, G) and female (F, H) offspring from all groups. ITT glycemic excursion curves (I-J) and respective AUC quantifications (K, L) of two-month-old male (I, K) and female (J, L) offspring from all groups. *, $p < 0.05$ offspring of HFD-fed mothers vs. offspring of RD-fed mothers. &, $p < 0.05$ sitagliptin-treated mice vs. vehicle-treated mice.

To investigate if maternal sitagliptin modifies offspring glycemic control, we measured offspring glucose tolerance and insulin sensitivity at weaning (three weeks of age), two months, and ten months of age. At weaning, maternal HFD did not affect glucose tolerance in either Veh- or Sita-treated groups, and Mat-Sita improved glucose tolerance by 24% in male Off-RD mice ($p = 0.01$) but not in any other group (Fig. 8A-D). In Veh-treated groups at two months of age, male Off-HFD mice exhibited significant impairment in both glucose tolerance (Fig. 8E,G) and insulin sensitivity (Fig. 8M,O) vs. their Off-RD counterparts, while female offspring exhibited no changes in either glucose tolerance (Fig. 8F,H) or insulin sensitivity (Fig. 8N,P). Importantly, Mat-Sita improved glucose tolerance in both male and female Off-HFD mice, by 21% (Fig. 8E,G) and 28% (Fig. 8F,H) respectively, while insulin sensitivity was unaffected (Fig. 8M-P). At ten months of age, both male (Fig. 8I,K) and female (Fig. 8J,L) offspring of Veh-treated HFD-fed mothers exhibited increased glucose intolerance (28 and 17% respectively), but Mat-Sita treatment produced no significant effect (Fig. 8I-L).

Discussion

Exposure to an adverse intrauterine environment such as maternal obesity is now recognized as a major driving force behind the rapidly rising incidence of obesity and associated chronic diseases. However, the underlying mechanisms by which maternal obesity programs obesity in offspring yet remain unclear, and there are consequently no effective strategies for addressing the passage of obesity from obese mothers to their children. A major goal in treating such detrimental developmental programming is to delay and/or reverse the progression of programmed diseases. Here, we found that plasma DPPIV activity is increased in male offspring of obese mothers. Using two well-established non-human primate models of maternal obesity, we further revealed that such dysregulation

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

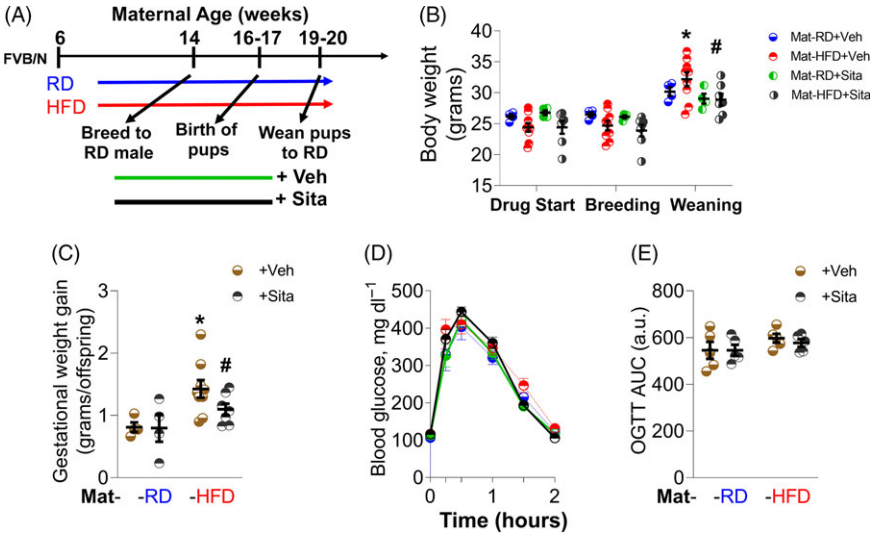

Fig. 6. DPPIV inhibitor treatment of HFD-fed female mice does not affect gross morphology and glycemic control, but decreases gestational weight gain. Study design (A), in which RD- and HFD-fed mice were treated with either sitagliptin (+Sita) at a dosage of 30-45 mg/kg/day or its vehicle (+Veh) (DMSO) in drinking water. Body weights (B), body fat composition (C), gestational weight gain (C), OGTT glycemic excursion curves (D), and OGTT AUC values (E) of mothers from all groups. N = 4-7/group. *, $p < 0.05$ offspring of HFD-fed mothers vs. offspring of RD-fed mothers. #, $p < 0.05$ offspring of sitagliptin-treated mothers vs. offspring of vehicle-treated mothers.

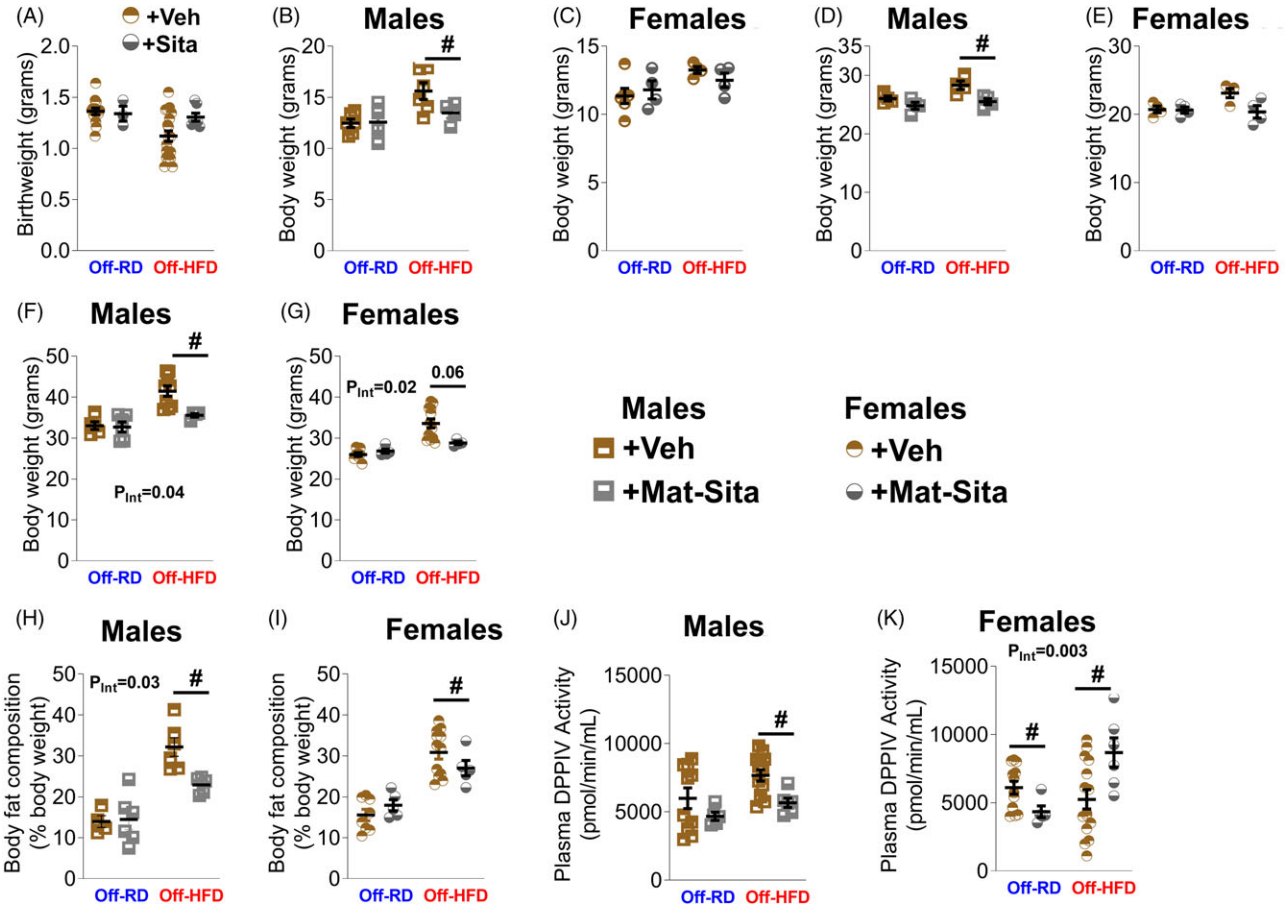

Fig. 7. In mice, administration of DPPIV inhibitor to HFD-fed mothers delays progression of obesity in male offspring. Data were collected from male and female offspring of mothers that underwent the experimental protocol illustrated in Fig. 6A. Average birthweights of all pups in litters from all groups (A). Body weights of three-week-old males (B) and females (C), two-month-old males (D) and females (E), and ten-month-old males (F) and females (G) from all offspring groups. Body fat composition of ten-month-old males (H) and females (I) from all offspring groups. Plasma DPPIV activity of three-week-old males (J) and females (K) from all offspring groups. N = 5-15/group/sex. #, $p < 0.05$ offspring of sitagliptin-treated mothers vs. offspring of vehicle-treated mothers.

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

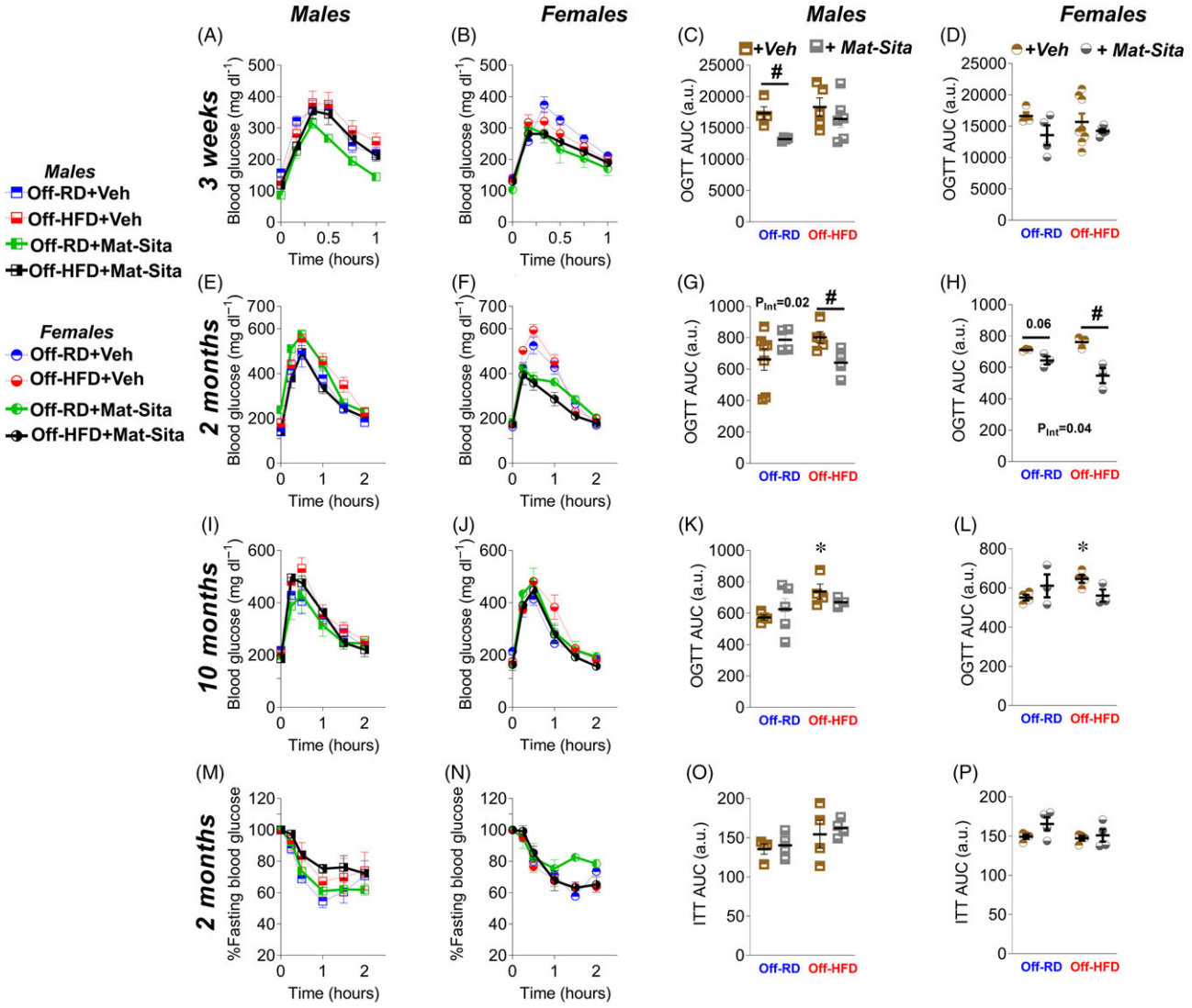

Fig. 8. In mice, DPPIV inhibitor treatment of HFD-fed mothers delays progression of metabolic abnormalities in male offspring. Data were collected from male and female offspring of mothers that underwent the experimental protocol illustrated in Fig. 6A. OGTT glycemic excursion curves and respective AUC quantifications of three-week-old males (A, C) and females (B, D), two-month-old males (E, G) and females (F, H), and ten-month-old males (I, K) and females (J, L) from all offspring groups. ITT glycemic excursion curves and respective AUC quantifications of two-month-old males (M, O) and females (N, P) from all offspring groups. N = 5-15/group/sex. #, $p < 0.05$ offspring of sitagliptin-treated mothers vs. offspring of vehicle-treated mothers.

can persist into adult life for offspring born to obese mothers. Hence, we reasoned that DPPIV likely plays an active role in developmental programming mediated by maternal obesity. To investigate the effects of pharmacologic DPPIV inhibition on the progression of programmed offspring obesity, we performed a pre-clinical study in a mouse model of maternal high fat DIO for which we used the FDA-approved anti-diabetic drug sitagliptin to inhibit DPPIV activity *in vivo*. We demonstrated that both long-term DPPIV inhibition in offspring starting from a young age and short-term maternal inhibition of DPPIV during gestation and lactation blunt the progression of obesity and metabolic disorders in male offspring of HFD-fed mothers. Thus, our findings highlight dysregulation of DPPIV as being at least partly responsible for the sex-dependent programming of obesity and metabolic disorders in offspring of obese mothers. Furthermore, we suggest that DPPIV inhibitor therapy could be a putative strategy for halting and/or inhibiting this process.

Our finding that maternal obesity leads to dysregulation of plasma DPPIV activity in both mother and offspring is not surprising. DPPIV is strongly linked to obesity[12]; prior studies have reported increased s-DPPIV in obese adults[11], in lean children that subsequently develop obesity[14], and in mice with DIO[59,60]. Both obesity and/or hypercaloric diets have been shown to increase levels of plasma DPPIV by upregulating production of s-DPPIV and/or by accelerating shedding of m-DPPIV[15,61] from DPPIV-expressing cells. Moreover, children born to obese mothers often exhibit abnormalities in regulation of blood glucose, blood lipids and body fat mass[57] – all of which are regulated by DPPIV. In the present study, one of our novel findings is that early-life dysregulation in plasma DPPIV activity precedes metabolic abnormalities in male Off-HFD mice, suggesting that DPPIV could be a culprit in the progression of programmed diseases stemming from maternal obesity. Furthermore, our observation that DPPIV is dysregulated in both obese mothers carrying male fetuses and their

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

newborn male offspring suggests that DPPIV could be involved in developmental programming in males even prior to birth.

Emerging evidence suggests that DPPIV activation in the setting of obesity is driven by excessive cellular workload or cellular stress and manifests within tissues as increased expression of endoplasmic reticulum (ER)-stress markers, accumulation of reactive oxygen species (ROS), and production of pro-inflammatory cytokines[63]. DPPIV is constitutively expressed in hepatocytes, endothelial cells, adipocytes immune cells, intestinal K cells, and placental cytotrophoblasts, among others; more than 90% of plasma s-DPPIV is derived from these cell types. Importantly, a growing body of evidence suggests that the cellular origin of s-DPPIV determines its activity and therefore its physiological effect. For instance, Mulvihill et al.[49] demonstrated that while enterocyte DPPIV plays no role in glucose homeostasis, hematopoietic DPPIV can cleave the incretin hormone gastric inhibitory peptide (GIP) but not glucagon-like peptide 1 (GLP-1), and endothelial s-DPPIV can degrade both hormones in insulin-resistant states. In a 2018 study using a mouse model of DIO, Ghorpade et al. demonstrated that hepatocyte-secreted s-DPPIV and factor-Xa cooperatively activate inflammation in adipose tissue macrophages, leading to obesity and insulin resistance[63]. It is yet unclear what exactly triggers the observed dysregulation of plasma DPPIV activity in obese mothers, from which cell/tissue the dysregulated enzyme is derived, and how the sex of the fetus determines the direction in which the dysregulation of maternal DPPIV will occur. Work to answer these important questions is presently underway in our laboratory.

In the meantime, we posit that maternal DPPIV may contribute to developmental programming in three ways. First, dysregulation of maternal m- and s-DPPIV can disrupt critical pregnancy-induced physiological states – the most important of which is maternal insulin resistance. Inter-organ crosstalk mediated by s-DPPIV regulates insulin production by degrading incretins[62] and insulin sensitivity[61,63]; as such, abnormalities in maternal DPPIV may directly alter control of maternal physiological states, which in turn indirectly affects fetal development. While we observed obese and normal-weight human mothers to have no differences in insulin sensitivity (measured by HOMA-IR)[64], our approach is limited in that maternal plasma was only collected *at term* and therefore our data are not informative regarding maternal insulin sensitivity throughout gestation. Second, dysregulation of maternal s-DPPIV could impair fetal-maternal crosstalk through the placenta. Although the role of DPPIV in such crosstalk has not been studied, it is likely that maternal plasma s-DPPIV affects cellular processes in the placenta. It has been shown that maternal s-DPPIV can cross the placenta (Januvia [package insert]. Kenilworth, NJ: Merck Pharmaceuticals, Inc. 2010), and that DPPIV receptors such as caveolin-1 are expressed on fetal stem cells[65]. Accordingly, we posit that as maternal blood perfuses placental capillaries, maternal plasma s-DPPIV will bind to caveolin-1 on the membrane of placental cytotrophoblasts[66], thereby inducing intracellular signaling cascades; in effect, this represents a communication axis between s-DPPIV-producing maternal cells and placental cytotrophoblasts. Ongoing studies in our laboratory focus on addressing the role of DPPIV in placental function, and our preliminary results show reduced mitochondrial respiration in cytotrophoblasts treated with DPPIV (Montaniel, Bucher & Maloyan, *manuscript in preparation*). As such, abnormalities in placental function represent a second possible consequence of dysregulated maternal s-DPPIV; such abnormalities have been previously linked to developmental programming[67,68]. Third, dysregulation of maternal s-DPPIV could permanently alter the epigenomic landscape of offspring stem cells, including mesenchymal, adipocyte, and hematopoietic stem cells, thereby altering the fate and phenotype of their future daughter cells, ultimately affecting essentially all cells in critical metabolic organs such as the liver and adipose tissues[69,70]. Hence, maternal s-DPPIV can potentially have life-long effects on the function of offspring cells and organs, which is consistent with our observations of progressive metabolic abnormalities in Off-HFD.

It is important to understand how *in utero* exposure to an obesogenic environment can induce such long-lasting dysregulation in offspring plasma DPPIV activity. Is there a unique factor or condition that mediates this process? In the setting of DIO, increased DPPIV production and shedding by metabolic cells such as hepatocytes and adipocytes is driven by ER stress and NFkB pathways[63]. Maternal obesity is reportedly associated with signs of increased ER stress and activation NFkB-driven pathways in offspring cells[71], which provides a partial explanation for our findings. It remains to be discovered how exposure to maternal obesity affects DPPIV production in different offspring cell populations, and how such a process could be diametrically opposite between male and female offspring.

Converging evidence strongly suggests that when exposed to adverse gestational conditions, male fetuses are more compromised than female fetuses[72,73]. Sex-specific differences exist at all levels of physiology, from the genomic to organ systems[74], all of which could affect DPPIV production in the setting of maternal obesity. At the organ and organ systems level, possible influencing factors include variations in the neuroendocrine milieu, placental function, fetal maturation, and maternal immune reactivity. At the tissue level, possible DPPIV-inducing stimuli induce dimorphic responses in males and females, with effects such as cellular dysplasia and excessive accumulation of ROS and pro-inflammatory cytokines[5]. Sex differences are also apparent at the cellular and intracellular levels[74]; for example, monocytes isolated from adult offspring of obese mothers respond differently to endotoxin challenge and express different epigenetic landscapes. As discussed earlier, we posit that exposure to maternal obesity may permanently alter epigenetic landscapes in offspring stem cells, which in turn permanently dysregulate offspring DPPIV. Maternal obesity is associated with fetal sex-specific changes in the activation of epigenome-regulatory factors such as histone deacetylases (HDACs)[75], which can potentially increase accessibility of the *DPPIV* locus to transcriptional regulators such as NFkB (p50/p65) the male offspring, which is also activated in obesity, thereby increasing transcription of *DPPIV* mRNA and eventually production of the bioactive s- and/or m-DPPIV protein. Understanding how exposure to maternal obesity induces persistent fetal sex-dependent dysregulation of offspring plasma DPPIV activity will be critical to addressing obesity progression in the offspring of obese mothers.

The mouse model of maternal obesity studied here bears a striking phenotypic resemblance to diverse pathologies observed both in humans[76,77] and in animal models of maternal obesity[78]. One of our novel findings is that Off-RD and Off-HFD mice are similar in terms of food consumption and energy expenditure, which suggests that the Off-HFD do not develop obesity because of 'obesogenic' lifestyle choices such as overeating and/or having insufficient physical activity. In addition, our observation that exposure to maternal obesity leads to metabolic dysfunction (increased adiposity, glucose intolerance, and insulin resistance) more so in male offspring than in their female siblings, along with

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

the sexual dimorphism in maternal and offspring activation of DPPIV, provides further evidence of sex differences in developmental programming[72,73]. Several prior studies have demonstrated sexual dimorphism in human offspring of obese mothers. Mingrone et al.[79] reported sex-dependent metabolic changes, with males showing significantly larger fat mass, higher levels of blood insulin, and higher total insulin secretion. A longitudinal analysis of children of obese mothers also reported increased body fat at 2-6 years of age in males but not in females[80]. The Helsinki Birth Cohort Study[81], which followed participants born in 1934–1944 and conducted follow-ups from 1971 to 2010, found sex-related differences in terms of the occurrence of coronary heart disease, type 2 diabetes, and stroke. Notably, the association of maternal BMI with offspring coronary heart disease was statistically significant only in males, whereas the association of maternal BMI with stroke was significant only in females. It appears possible that female offspring are more protected from the metabolic consequences of maternal obesity. For one, estrogen and its receptors have been found to be protective against obesity, type 2 diabetes, and CVD[82]. Estrogens moreover have significant effects on insulin sensitivity and on the body's response to changes in glucose levels, two key factors mediating the programmed effects of maternal obesity[83,84]. Actions of estrogens therefore could at least partially explain the sexual dimorphism observed in susceptibility to early-life programming and increase in DPPIV activity in 11-month-old female offspring.

Expanding on the role of glucose, DPPIV inhibitors such as sitagliptin are effective against T2DM[11–13,15]. Sitagliptin improved glycemic control in randomized controlled clinical trials involving adults with T2DM, indicated by a 20% reduction in hemoglobin A1C[22–27]; this protective effect has been attributed to decreased degradation of the incretin hormones GLP-1 and GIP, both of which potentiate glycemic control after a meal and promote satiety, acting over time to improve metabolic function[62]. However, the effects of sitagliptin on weight control remain unclear. Initial clinical reports indicate that sitagliptin is "weight neutral" in adults with T2DM[62]; however, in most such studies, the subjects have an advanced form of obesity involving co-morbidities such as insulin resistance that are known to respond poorly to weight-loss strategies. In contrast, some clinical reports along with studies in DIO mice support that sitagliptin therapy can promote weight loss[29,33]. Ultimately, it remains unclear whether sitagliptin can effectively reverse the progression of obesity and associated metabolic disorders.

In mice, increased DPPIV production in the absence of an obesogenic stimulus such as a HFD has been shown to lead to spontaneous development of obesity[60], indicating that increased DPPIV signaling can independently cause obesity. Inhibiting the catalytic activity of DPPIV can reverse this effect, which is consistent with our findings that chronic sitagliptin therapy can delay obesity progression in Off-HFD males. Furthermore, our data revealed that maternal inhibition of DPPIV improves offspring metabolic profiles, proving our hypothesis that maternal DPPIV plays a key role in developmental programming. It has been previously reported that maternal sitagliptin administration improves glucose metabolism in male offspring of HFD-fed mother[85], and our data supports this finding. However, in contrast to this report, we found no effect of maternal sitagliptin on insulin sensitivity in offspring. Different findings might be explained by selection of the animal model (rats vs. mice), duration of maternal HFD feeding prior to conception (4 weeks vs. 8 weeks), routes of drug administration (oral gavage vs. drinking water), or sitagliptin dosage (10 mg/kg vs. 0.3 mg/kg). Lack of effect of maternal sitagliptin on insulin sensitivity in male offspring suggests potentially complex role for DPPIV in the establishment of glycemic control mechanisms during gestation – a process that likely involves the development of the fetal liver, pancreas, skeletal muscles, and adipose tissue. Understanding how DPPIV regulates developmental programming is an area of future investigation that will be both important and fascinating to pursue and will be critical in our efforts to combat the pandemic of obesity and chronic disease.

In summary, it is clear that the environment during fetal and early neonatal life is a major determinant of health throughout the lifespan. Therefore, adverse early-life conditions such as exposure to maternal obesity must be considered in biomedical research and in clinical settings. As of 2018, more than half of pregnant mothers in the US are either overweight or obese[86]; it is thus likely that their offspring will be programmed to develop obesity. To halt the progression of this looming healthcare crisis, it is important to understand the etiologies of the various forms of obesity, including that induced by exposure to maternal obesity. Our findings highlight a role for DPPIV in the transgenerational propagation of obesity from obese mothers to their children, suggest that dysregulation of plasma DPPIV activity in pregnant mothers or in their newborn children can serve as a marker of developmental programming, and justify clinical trials using DPPIV inhibitors to address the adverse consequences of maternal obesity.

Materials and methods

Study approval

Human maternal and cord blood samples were collected from labor and delivery units at the Oregon Health & Science University under protocol approved by the Institutional Review Boards and with informed consent from the patients. All animal experiments were approved by the Oregon Health & Sciences University's Institutional Animal Use Committee.

Methods

Extended descriptions of methods, sources of materials, and data analysis, are provided in Supporting Information.

Statistics

Means were compared using 2-way ANOVA and student's t-test (corrected for multiple comparisons). Error bars reflect standard errors of the mean (SEM). Means were compared using two-way analysis of variance (2-way ANOVA) and student's T-test (corrected for multiple comparisons), and results of both tests are indicated in each respective graph. Significance was set at an alpha of 0.05, which when relevant, was indicated by a symbol in each respective graph. P_{Int}=2-way ANOVA Interaction *p* value.

Supplementary material. For supplementary material accompanying this paper visit https://doi.org/10.1017/S2040174422000010

Acknowledgments. We thank Yem Alharithi for technical assistance, and laboratory of Daniel Marks, MD, PhD, for giving us an access to EchoMRI. We thank OHSU Division of Comparative Medicine, Physiology and Pharmacology Graduate Program, and Vanderbilt University Mouse Metabolic Phenotyping Core (MMPC).

Author Contributions. KRCM and AM conceived and planned experiments. KRCM, MB, and EP conducted the experiments, MB and EP collected human samples, CL, JB, ES, PK, and PWN provided samples from non-human

primates. SR contributed to the interpretation of the micro-CT results. KRCM and AM wrote the manuscript. All authors provided feedback and assisted with preparing final manuscript.

Financial support. This work was provided by the NIH HD099367 (AM) and NIDDK Mouse Metabolic Phenotyping Centers (National MMPC, RRID: SCR_008997, www.mmpc.org) under the MICROMouse Program, grants DK076169 (AM).

Conflicts of interest. The authors declared no conflict of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards described in the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals and have been approved by the Oregon Health & Sciences University's Institutional Animal Use Committee (IACUC Protocol IP0432). Human samples were collected from labor and delivery units at the Oregon Health & Science University under protocol approved by the Institutional Review Board (IRB ID STUDY00017798), and with informed consent from the patients.

References

1. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med*. 2015; 373(14), 1307–1317.
2. Bluher M. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol*. 2019; 15(5), 288–298.
3. Ataey A, Jafarvand E, Adham D, Moradi-Asl E. The relationship between obesity, overweight, and the human development index in world health organization eastern mediterranean region countries. *J Prev Med Public Health*. 2020; 53(2), 98–105.
4. Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. *BMJ*. 2017; 356, j1.
5. Gambineri A, Conforti A, Di Nisio A, *et al.* Maternal obesity: focus on offspring cardiometabolic outcomes. *Int J Obes Suppl*. 2020; 10(1), 27–34.
6. Friedman JE. Developmental programming of obesity and diabetes in mouse, monkey, and man in 2018: where are we headed? *Diabetes*. 2018; 67(11), 2137–2151.
7. King V, Dakin RS, Liu L, *et al.* Maternal obesity has little effect on the immediate offspring but impacts on the next generation. *Endocrinology*. 2013; 154(7), 2514–2524.
8. Varin EM, Mulvihill EE, Beaudry JL, *et al.* Circulating levels of soluble dipeptidyl Peptidase-4 are dissociated from inflammation and induced by enzymatic DPP4 inhibition. *Cell Metab*. 2019; 29(2), 320–34 e5.
9. Klemann C, Wagner L, Stephan M, von Hörsten S. Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. *Clin Exp Immunol*. 2016; 185(1), 1–21.
10. Lamers D, Famulla S, Wronkowitz N, *et al.* Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes*. 2011; 60(7), 1917–1925.
11. Lee JY, Jang BK, Song MK, Kim HS, Kim MK. Association between serum dipeptidyl Peptidase-4 concentration and Obesity-Related factors in health screen examinees (J Obes Metab Syndr 2017;26, 2018: 188–96). *J Obes Metab Syndr*. 2018; 27(1), 73–4.
12. Sanz B, Larrinaga G, Fernandez-Atucha A, *et al.* Obesity parameters, physical activity, and physical fitness are correlated with serum dipeptidyl peptidase IV activity in a healthy population. *Heliyon*. 2018; 4(5), e00627.
13. Sarkar J, Nargis T, Tantia O, Ghosh S, Chakrabarti P. Increased plasma dipeptidyl Peptidase-4 (DPP4) activity is an obesity-Independent parameter for glycemic deregulation in type 2 diabetes patients. *Front Endocrinol (Lausanne)*. 2019; 10, 505.
14. Iwabuchi A, Kamoda T, Saito M, *et al.* Serum dipeptidyl peptidase 4 activity in children with type 1 diabetes mellitus. *J Pediatr Endocrinol Metab*. 2013; 26(11-12), 1093–1097.
15. Williams KH, Vieira De Ribeiro AJ, Prakoso E, *et al.* Circulating dipeptidyl peptidase-4 activity correlates with measures of hepatocyte apoptosis and fibrosis in non-alcoholic fatty liver disease in type 2 diabetes mellitus and obesity: a dual cohort cross-sectional study. *J Diabetes*. 2015; 7(6), 809–819.
16. Balfour PC Jr., Rodriguez CJ, Ferdinand KC. Blood pressure and cardiovascular effects of new and emerging antidiabetic agents. *Curr Hypertens Rep*. 2014; 16(8), 455.
17. Lovshin JA, Zinman B. Blood pressure-lowering effects of incretin-based diabetes therapies. *Can J Diabetes*. 2014; 38(5), 364–371.
18. Kanasaki K. The role of renal dipeptidyl peptidase-4 in kidney disease: renal effects of dipeptidyl peptidase-4 inhibitors with a focus on linagliptin. *Clin Sci (Lond)*. 2018; 132(4), 489–507.
19. Koh G. Association between Serum Dipeptidyl Peptidase-4 Concentration and Obesity-Related Factors in Health Screen Examinees (J Obes Metab Syndr, 2017;26:188-96). *J Obes Metab Syndr*. 2018; 27(1), 71–72.
20. Schurmann C, Linke A, Engelmann-Pilger K, *et al.* The dipeptidyl peptidase-4 inhibitor linagliptin attenuates inflammation and accelerates epithelialization in wounds of diabetic ob/ob mice. *J Pharmacol Exp Ther*. 2012; 342(1), 71–80.
21. Takeda K, Sawazaki H, Takahashi H, *et al.* The dipeptidyl peptidase-4 (DPP-4) inhibitor teneligliptin enhances brown adipose tissue function, thereby preventing obesity in mice. *FEBS Open Bio*. 2018; 8(11), 1782–1793.
22. Yoshikawa K, Tsuchiya A, Kido T, *et al.* Long-Term safety and efficacy of sitagliptin for type 2 diabetes mellitus in Japan: results of a multicentre, Open-Label, observational Post-Marketing surveillance study. *Adv Ther*. 2020; 37(5), 2442–2459.
23. Derosa G, Tritto I, Romano D, D'Angelo A, Catena G, Maffioli P. Effects of sitagliptin on lipid profile in patients with type 2 diabetes mellitus after 7 years of therapy. *J Clin Pharmacol*. 2019; 59(10), 1391–1399.
24. Shi C, Zhang R, Bai R, *et al.* Efficacy and safety of sitagliptin added to metformin and insulin compared with voglibose in patients with newly diagnosed type 2 diabetes. *Clinics (Sao Paulo)*. 2019; 74(9), e736.
25. Takai M, Ishikawa M, Maeda H, *et al.* Efficacy and safety of adding sitagliptin in type 2 diabetes patients on insulin: Age-Stratified comparison at one year in the ASSIST-K study. *J Clin Med Res*. 2019; 11(5), 311–320.
26. Raji A, Long J, Lam RLH, O'Neill EA, Engel SS. Efficacy and safety of sitagliptin in Hispanic/Latino patients with type 2 diabetes: a pooled analysis from ten randomized, Placebo-Controlled phase 3 clinical trials. *Diabetes Ther*. 2018; 9(4), 1581–1589.
27. Tang Y, Huang X, Liu J, Shankar RR, Ganz ML, Rajpathak S. The effects of a sitagliptin formulary restriction program on diabetes medication use. *Am Health Drug Benefits*. 2017; 10(9), 456–462.
28. Lipscombe LL. In uncontrolled type 2 diabetes, adjunctive semaglutide reduced HbA1c and body weight vs sitagliptin. *Ann Intern Med*. 2019; 171(4), JC16.
29. Ferjan S, Janez A, Jensterle M. Dipeptidyl Peptidase-4 inhibitor sitagliptin prevented weight regain in obese women with polycystic ovary syndrome previously treated with liraglutide: a pilot randomized study. *Metab Syndr Relat Disord*. 2017; 15(10), 515–520.
30. Lind M, Matsson PO, Linder R, *et al.* Clinical effectiveness of liraglutide vs sitagliptin on glycemic control and body weight in patients with type 2 diabetes: a retrospective assessment in sweden. *Diabetes Ther*. 2016; 7(2), 321–333.
31. Hussain M, Atif MA, Tunio AG, Ali B, Akhtar L, and Serwar G. Effect Of Sitagliptin On Glycemic Control, Body Weight. Blood pressure and serum lipid profile in type 2 diabetic hyperlipidemic patients. *J Ayub Med Coll Abbottabad*. 2016; 28(2), 369–372.
32. Yanai H, Adachi H, Hamasaki H, *et al.* Effects of 6-month sitagliptin treatment on glucose and lipid metabolism, blood pressure, body weight and renal function in type 2 diabetic patients: a chart-based analysis. *J Clin Med Res*. 2012; 4(4), 251–258.
33. Seck TL, Engel SS, Williams-Herman DE, *et al.* Sitagliptin more effectively achieves a composite endpoint for A1C reduction, lack of hypoglycemia and no body weight gain compared with glipizide. *Diabetes Res Clin Pract*. 2011; 93(1), e15–7.

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

34. Waters SB, Topp BG, Siler SQ, Alexander CM. Treatment with sitagliptin or metformin does not increase body weight despite predicted reductions in urinary glucose excretion. *J Diabetes Sci Technol*. 2009; 3(1), 68–82.
35. Ferreira L, Teixeira-de-Lemos E, Pinto F, *et al.* Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). *Mediators Inflamm*. 2010; 2010(2), 592760–11.
36. Dobrian AD, Ma Q, Lindsay JW, *et al.* Dipeptidyl peptidase IV inhibitor sitagliptin reduces local inflammation in adipose tissue and in pancreatic islets of obese mice. *Am J Physiol Endocrinol Metab*. 2011; 300(2), E410–21.
37. Marques C, Mega C, Goncalves A, *et al.* Sitagliptin prevents inflammation and apoptotic cell death in the kidney of type 2 diabetic animals. *Mediators Inflamm*. 2014; 2014(106), 538737–15.
38. Tremblay AJ, Lamarche B, Deacon CF, Weisnagel SJ, Couture P. Effects of sitagliptin therapy on markers of low-grade inflammation and cell adhesion molecules in patients with type 2 diabetes. *Metabolism*. 2014; 63(9), 1141–1148.
39. Best C, Struthers H, Laciny E, Royal M, Reeds DN, Yarasheski KE. Sitagliptin reduces inflammation and chronic immune cell activation in HIV+ adults with impaired glucose tolerance. *J Clin Endocrinol Metab*. 2015; 100(7), 2621–2629.
40. Kelany ME, Hakami TM, Omar AH, Abdallah MA. Combination of sitagliptin and insulin against type 2 diabetes mellitus with neuropathy in rats: neuroprotection and role of oxidative and inflammation stress. *Pharmacology*. 2016; 98(5-6), 242–250.
41. Esposito G, Cappetta D, Russo R, *et al.* Sitagliptin reduces inflammation, fibrosis and preserves diastolic function in a rat model of heart failure with preserved ejection fraction. *Br J Pharmacol*. 2017; 174(22), 4070–4086.
42. Goncalves A, Almeida L, Silva AP, *et al.* The dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin ameliorates retinal endothelial cell dysfunction triggered by inflammation. *Biomed Pharmacother*. 2018; 102(2), 833–838.
43. Prakash S, Rai U, Kosuru R, Tiwari V, Singh S. Amelioration of diet-induced metabolic syndrome and fatty liver with sitagliptin via regulation of adipose tissue inflammation and hepatic Adiponectin/AMPK levels in mice. *Biochimie*. 2020; 168(1), 198–209.
44. Lorey S, Faust J, Buhling F, Ansorge S, Neubert K. A new type of fluorogenic substrates for determination of cellular dipeptidyl peptidase IV (DP IV/CD26) activity. *Adv Exp Med Biol*. 2000; 477, 111–115.
45. Booz GW, Massoud GP, Altara R, Zouein FA. Unravelling the impact of intrauterine growth restriction on heart development: insights into mitochondria and sexual dimorphism from a non-hominoid primate. *Clin Sci (Lond)*. 2021; 135(14), 1767–1772.
46. Disotell TR, Tosi AJ. The monkey's perspective. *Genome Biol*. 2007; 8(9), 226.
47. Schlabritz-Loutsevitch NE, Lopez-Alvarenga JC, Comuzzie AG, *et al.* The prolonged effect of repeated maternal glucocorticoid exposure on the maternal and fetal leptin/insulin-like growth factor axis in papio species. *Reprod Sci*. 2009; 16(3), 308–319.
48. Puppala S, Li C, Glenn JP, *et al.* Primate fetal hepatic responses to maternal obesity: epigenetic signalling pathways and lipid accumulation. *J Physiol*. 2018; 596(23), 5823–5837.
49. Maloyan A, Muralimanoharan S, Huffman S, *et al.* Identification and comparative analyses of myocardial miRNAs involved in the fetal response to maternal obesity. *Physiol Genomics*. 2013; 45(19), 889–900.
50. Li C, Jenkins S, Considine MM, *et al.* Effect of maternal obesity on fetal and postnatal baboon (Papio species) early life phenotype. *J Med Primatol*. 2019; 48(2), 90–98.
51. Huber HF, Jenkins SL, Li C, Nathanielsz PW. Strength of nonhuman primate studies of developmental programming: review of sample sizes, challenges, and steps for future work. *J Dev Orig Health Dis*. 2020; 11(3), 297–306.
52. True C, Arik A, Lindsley S, Kirigiti M, Sullivan E, Kievit P. Early High-Fat diet exposure causes dysregulation of the orexin and dopamine neuronal populations in nonhuman primates. *Front Endocrinol*. 2018; 9, 508–.
53. True C, Dean T, Takahashi D, Sullivan E, Kievit P. Maternal High-Fat diet effects on adaptations to metabolic challenges in male and female juvenile nonhuman primates. *Obesity (Silver Spring, Md)*. 2018; 26(9), 1430–1438.
54. Rivera HM, Kievit P, Kirigiti MA, *et al.* Maternal high-fat diet and obesity impact palatable food intake and dopamine signaling in nonhuman primate offspring. *Obesity (Silver Spring, Md)*. 2015; 23(11), 2157–2164.
55. Sullivan EL, Rivera HM, True CA, *et al.* Maternal and postnatal high-fat diet consumption programs energy balance and hypothalamic melanocortin signaling in nonhuman primate offspring. *Am J Physiol Regul Integr Comp Physiol*. 2017; 313(2), R169–R79.
56. Campodonico-Burnett W, Hetrick B, Wesolowski SR, *et al.* Maternal obesity and western-Style diet impair fetal and juvenile offspring skeletal muscle insulin-Stimulated glucose transport in nonhuman primates. *Diabetes*. 2020; 69(7), 1389–1400.
57. Herring SJ, Oken E. Obesity and diabetes in mothers and their children: can we stop the intergenerational cycle? *Curr Diab Rep*. 2011; 11(1), 20–27.
58. Winzell MS, Ahrén B. The High-Fat diet-Fed mouse. *Diabetes*. 2004; 53(suppl 3), S215–S219.
59. Pospisilik JA, Ehses JA, Doty T, McIntosh CH, Demuth HU, Pederson RA. Dipeptidyl peptidase IV inhibition in animal models of diabetes. *Adv Exp Med Biol*. 2003; 524, 281–291.
60. Conarello SL, Li Z, Ronan J, *et al.* Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci U S A*. 2003; 100(11), 6825–6830.
61. Mulvihill EE, Varin EM, Gladanac B, *et al.* Cellular sites and mechanisms linking reduction of dipeptidyl Peptidase-4 activity to control of incretin hormone action and glucose homeostasis. *Cell Metab*. 2017; 25(1), 152–165.
62. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. *Endocr Rev*. 2014; 35(6), 992–1019.
63. Ghorpade DS, Ozcan L, Zheng Z, *et al.* Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance. *Nature*. 2018; 555(7698), 673–677.
64. Bucher M, Montaniel K, Myatt L, Weintraub S, Tavori H, Maloyan A. Dyslipidemia, insulin resistance, and impairment of placental metabolism in the offspring of obese mothers. *J Dev Orig Health Dis*. 2021; 12(5), 738–747. https://doi.org/10.1017/S2040174420001026
65. Baker N, Tuan RS. The less-often-traveled surface of stem cells: caveolin-1 and caveolae in stem cells, tissue repair and regeneration. *Stem Cell Res Ther*. 2013; 4(4), 90.
66. Naing Z, Hamilton ST, van Zuylen WJ, Scott GM, Rawlinson WD. Differential expression of PDGF Receptor-alpha in human placental trophoblasts leads to different entry pathways by human cytomegalovirus strains. *Sci Rep*. 2020; 10(1), 1082.
67. Muralimanoharan S, Guo C, Myatt L, Maloyan A. Sexual dimorphism in miR-210 expression and mitochondrial dysfunction in the placenta with maternal obesity. *Int J Obes (Lond)*. 2015; 39(8), 1274–1281.
68. Muralimanoharan S, Gao X, Weintraub S, Myatt L, Maloyan A. Sexual dimorphism in activation of placental autophagy in obese women with evidence for fetal programming from a placenta-specific mouse model. *Autophagy*. 2016; 12(5), 752–769.
69. Boyle KE, Patinkin ZW, Shapiro ALB, *et al.* Maternal obesity alters fatty acid oxidation, AMPK activity, and associated DNA methylation in mesenchymal stem cells from human infants. *Mol Metab*. 2017; 6(11), 1503–1516.
70. Baker PR 2nd, Patinkin Z, Shapiro AL, De La Houssaye BA, Woontner M, Boyle KE. Maternal obesity and increased neonatal adiposity correspond with altered infant mesenchymal stem cell metabolism. *JCI Insight*. 2017; 2(21), 2570.
71. McCurdy CE, Schenk S, Hetrick B, *et al.* Maternal obesity reduces oxidative capacity in fetal skeletal muscle of japanese macaques. *JCI Insight*. 2016; 1(16), e86612.
72. Dunn GA, Morgan CP, Bale TL. Sex-specificity in transgenerational epigenetic programming. *Horm Behav*. 2011; 59(3), 290–295.
73. Rodriguez-Gonzalez GL, Reyes-Castro LA, Bautista CJ, *et al.* Maternal obesity accelerates rat offspring metabolic ageing in a sex-dependent manner. *J Physiol*. 2019; 597(23), 5549–5563.
74. Penaloza C, Estevez B, Orlanski S, *et al.* Sex of the cell dictates its response: differential gene expression and sensitivity to cell death inducing stress in male and female cells. *FASEB J*. 2009; 23(6), 1869–1879.

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press

75. Dearden L, Bouret SG, Ozanne SE. Sex and gender differences in developmental programming of metabolism. *Mol Metab*. 2018; 15, 8–19.
76. Wankhade UD, Thakali KM, Shankar K. Persistent influence of maternal obesity on offspring health: mechanisms from animal models and clinical studies. *Mol Cell Endocrinol*. 2016; 435(Suppl. 2), 7–19.
77. Santangeli L, Sattar N, Huda SS. Impact of maternal obesity on perinatal and childhood outcomes. *Best Pract Res Clin Obstet Gynaecol*. 2015; 29(3), 438–448.
78. Taylor PD, Matthews PA, Khan IY, Rees D, Itani N, Poston L. Generation of maternal obesity models in studies of developmental programming in rodents. *Methods Mol Biol*. 2018; 1735, 167–199.
79. Mingrone G, Manco M, Mora ME, *et al.* Influence of maternal obesity on insulin sensitivity and secretion in offspring. *Diabetes Care*. 2008; 31(9), 1872–1876.
80. Andres A, Hull HR, Shankar K, Casey PH, Cleves MA, Badger TM. Longitudinal body composition of children born to mothers with normal weight, overweight, and obesity. *Obesity*. 2015; 23(6), 1252–1258.
81. Eriksson JG, Sandboge S, Salonen MK, Kajantie E, Osmond C. Long-term consequences of maternal overweight in pregnancy on offspring later health: findings from the helsinki birth cohort study. *Ann Med*. 2014; 46(6), 434–438.
82. Morselli E, Santos RS, Criollo A, Nelson MD, Palmer BF, Clegg DJ. The effects of oestrogens and their receptors on cardiometabolic health. *Nat Rev Endocrinol*. 2017; 13(6), 352–364.
83. Clegg DJ, Riedy CA, Smith KA, Benoit SC, Woods SC. Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes*. 2003; 52(3), 682–687.
84. Sandoval DA, Ertl AC, Richardson MA, Tate DB, Davis SN. Estrogen blunts neuroendocrine and metabolic responses to hypoglycemia. *Diabetes*. 2003; 52(7), 1749–1755.
85. Zhang Q, Xiao X, Zheng J, *et al.* Maternal sitagliptin treatment attenuates offspring glucose metabolism and intestinal proinflammatory cytokines IL-6 and TNF-α expression in male rats. *PeerJ*. 2020; 8, e10310–e.
86. Yoshida S, Kimura T, Noda M, Takeuchi M, Kawakami K. Association of maternal prepregnancy weight and early childhood weight with obesity in adolescence: a population-based longitudinal cohort study in Japan. *Pediatr Obes*. 2020; 15(4), e12597.

https://doi.org/10.1017/S2040174422000010 Published online by Cambridge University Press