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SUPPLEMENTATION WITH A PREBIOTIC (POLYDEXTROSE) IN OBESE MOUSE PREGNANCY IMPROVES MATERNAL GLUCOSE HOMEOSTASIS AND PROTECTS AGAINST OFFSPRING OBESITY

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ABSTRACT

Objectives: We hypothesised that maternal diet-induced-obesity has adverse consequences for offspring energy expenditure and susceptibility to obesity in adulthood, and that the prebiotic polydextrose (PDX) will prevent the consequences of programming by maternal obesity.

Methods: Female mice were fed a control (Con), or obesogenic diet (Ob) for 6 weeks prior to mating and throughout pregnancy and lactation. Half the obese dams were supplemented with 5% PDX (ObPDX) in drinking water throughout pregnancy and lactation. Offspring were weaned onto standard chow. At 3 and 6 months, offspring energy intake (EI) and energy expenditure (EE by indirect calorimetry) were measured, and a glucose-tolerance-test performed. Offspring of control (OffCon), obese (OffOb) and PDX supplemented (OffOb*P*) dams were subsequently challenged for 3-weeks with Ob, and energy balanced reassessed. Potential modifiers of offspring energy balance including gut microbiota and biomarkers of mitochondrial activity were also evaluated.

Results: 6-month-old male OffOb demonstrated increased body weight (BW, $P<0.001$) and white adipose tissue mass ($P<0.05$), decreased brown adipose tissue mass (BAT, $P<0.01$), lower night-time EE ($P<0.001$) *versus* OffCon, which were prevented in OffOb*P*. Both male and female OffOb showed abnormal GTT (peak [Glucose] $P<0.001$; AUC, $P<0.05$) which was prevented by PDX. The Ob challenge resulted in greater BW gain in both male and female OffOb *versus* OffCon ($P<0.05$), also associated with increased EI ($P<0.05$) and reduced EE in females ($P<0.01$). OffOb*P* were protected from accelerated BW gain on the OB diet compared with controls, associated with increased night-time EE in both male ($P<0.05$) and female OffOb*P* ($P<0.001$). PDX also prevented an increase in skeletal muscle mtDNA copy number in OffOb vs. OffCon ($P<0.01$) and increased the percentage of Bacteroides cells in faecal samples from male OffOb*P* relative to controls.

Conclusions: Maternal obesity adversely influences adult offspring energy balance and propensity for obesity, which is ameliorated by maternal PDX-treatment with associated changes in gut microbiota composition and skeletal muscle mitochondrial function.

INTRODUCTION

Maternal obesity constitutes the most common obstetric risk factor in developed countries with direct implications not only for maternal and neonatal morbidity and mortality but also for increased risk of obesity in the next generation (1-3). Mother-child cohort studies suggest the acquisition of obesogenic traits from mother via an undefined association between maternal body mass index (BMI) in pregnancy and risk of obesity in childhood and beyond (2). Increasing experimental evidence suggests that exposure to maternal obesity *in utero* and during lactation, especially maternal hyperglycaemia and insulin resistance (4) associated inflammation and metabolic dysfunction, may contribute to this relationship (5), impacting Global Sustainable Development Goals, in terms of health and wellbeing of current and future generations (6). Interventions are therefore urgently sought.

In view of this unmet clinical need, we have investigated the potential of a dietary supplement to improve the maternal metabolic profile in obese pregnant mice and thereby prevent deleterious effects on offspring metabolism, inflammation and energy balance. Polydextrose (PDX) is a low calorie, neutral tasting, condensation polymer of D-glucose, sorbitol, and citric acid, which is water soluble, resistant to digestion in the small intestine, but partially fermented by endogenous microbiota in the large intestine, leading to its classification as a soluble dietary fibre (7). Randomised placebo-controlled trials and two recent meta-analysis of studies in adult humans have reported increased satiety,

and improved glucose homeostasis and lipid profiles with PDX supplementation (7-11). Therefore, PDX supplementation in obese women offers the potential to improve metabolic profile and inflammation during pregnancy to positively impact on the developing offspring (12, 13).

We have previously reported cardiometabolic dysfunction in the offspring of mice with diet-induced obesity (14-17). In this study we have addressed the effect of obesity and PDX supplementation on offspring metabolic function, with a focus on energy balance, both intake and expenditure. Energy expenditure has been relatively under-explored, in models of maternal/offspring obesity. A recent meta-analysis addressing the effect of maternal obesogenic diets in rodents on offspring food intake and body mass concluded that, overall, effects on appetite are modest, whereas the increase in offspring body weight are consistent with permanent alterations in metabolism (18).

MATERIALS AND METHODS

Animal husbandry

All studies were approved locally by the Animal Welfare and Ethics Committee (AWERB) and were conducted under UK Home Office License (Taylor, PPL 70/7090). Power calculations were performed based on previous in vivo data to estimate sample size. Female C57BL/6J mice were fed either a standard chow diet (RM1, Special Dietary Services, UK) or a semisynthetic obesogenic diet (approx. 16% fat, 33% simple sugars, 15% protein, total energy 16.7 kJ/g (4.0 kcal/g), as previously described (14) (Supplementary methods and Supplementary Table 1). Following successful mating, a sub-group of obesogenic diet-fed dams were randomly assigned to supplementation with PDX (5% w/v) in the drinking water throughout gestation and lactation (n=34), generating three experimental groups; control (Con); obese (Ob) and obese + PDX, (ObP,

Figure 1). This concentration of PDX has previously proven efficacious in reducing insulin resistance in adult non-pregnant rats, without adverse effects or alteration in calorific intake (19).

Offspring of Control dams (OffCon), Obese dams (OffOb) and Obese PDX supplemented dams (OffObP) were weaned and maintained on standard chow, and one male and one female from each litter studied at time points 30 days, 3 and 6 months of age. Therefore, no evaluation included more than one subject of each sex from each litter

Indirect Calorimetry

Energy expenditure (EE), respiratory exchange ratio (RER) and food intake in the offspring, were measured using LabMaster® Automated Home Cage Phenotyping (TSE Systems, Bad Homburg, Germany).

Organ Collection

At each time point, animals were killed by rising concentration of CO_2 or cervical dislocation, in accordance with Schedule 1 of UK Home Office guidelines. All animals were sacrificed mid-morning, blood was taken by cardiac puncture, organs were removed and immediately snap frozen in liquid nitrogen for deoxyribonucleic acid (DNA) extraction and the fat pads (perineal, gonadal, inguinal and subcutaneous) and the skeletal muscle *tibialis anterior* were weighed.

Glucose tolerance test

PDX has been shown to improve glucose tolerance in mice (20). To determine whole body glucose tolerance, an intra-peritoneal glucose tolerance test (ipGTT) was performed in the dams at gestational day 16 (GD16) and in the offspring at 30, 90 and 180 days of age. Animals were injected (i.p.) with a glucose load (1 g/kg; 10% glucose solution). Blood

glucose was measured at 15, 30, 60 and 120 minutes after glucose injection using an AlphaTRAK® Glucose meter (Abbott Animal Health).

Cytokine Profile

To assess the impact of PDX on inflammatory cytokines in obese pregnancy, a subgroup of dams (n=5) were killed at gestational day 16, by a rising concentration of CO2 and maternal blood samples were taken by cardiac puncture and serum stored at -80 °C. Twenty-four adipocytokines were measured from pooled serum samples, using a Proteome Profiler Mouse Adipokine Array kit (R&D Systems) (see Supplementary methods for details).

Obesogenic dietary challenge

In a separate cohort of offspring (OffCon,OffOb and OffObP) at 3 months of age, 1 male and 1 female from each litter were provided *ad libitum* access to the maternal obesogenic diet (see above) for three weeks, to assess the impact of an obesogenic dietary challenge on the adult phenotype.

Quantitative real-time PCR

Greater brown fat distribution and activation may influence energy expenditure due to increased metabolic activity. We therefore evaluated expression of relevant brown fat genes (see Supplementary Table S2 for primers and sequences). Total RNA was extracted from BAT samples with the RNeasy mini kit (QIAGEN). RNA (1 µg) was reverse transcribed into cDNA with the Superscript II kit (Invitrogen). Semi-quantitative real-time PCR with SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich) was used to detect and amplify target cDNA. Relative gene expression was calculated using the ΔΔ Ct method. Genes of interest were normalised to the housekeeping gene Cyclophilin B.

177

178 **Mitochondrial DNA Copy Number in Offspring Skeletal Muscle**

179 MtDNA content varies between different cell types depending on the bioenergetic
180 needs, but can also change in response to physiological stimuli, leading to
181 alterations of mtDNA being employed as a biomarker of mitochondrial
182 dysfunction (21, 22). Total genomic DNA was isolated from skeletal muscle using the
183 DNeasy blood and tissue kit (Qiagen, UK) according to the manufacturer's
184 guidelines, and treated by sonication to minimise effects of dilution bias. Absolute
185 mtDNA copy number was determined by real time qPCR. The primers (see
186 Supplementary Table 2 for sequences) used were specific to mouse
187 mitochondrial and nuclear genome targets (mMitoF1/R1 and mB2MF1/R1
188 respectively), as detailed previously (22).

189

190 **Analysis of offspring faecal microbiota**

191 Since PDX is hypothesised to influence the maternal microbiome (13) with vertical
192 transfer to neonates, we investigated broad spectrum faecal microbiota profiles in
193 offspring at weaning, 3 months and 6 months of age. Offspring faecal samples were snap
194 frozen and stored at -80°C. Samples were quantified for broad-spectrum gut bacterial
195 species using probes targeting six phylogenetic groups (for detailed methods, targets and
196 specific probes see Supplementary Methods). Phylogenetic characterisation was
197 performed using 16S rRNA in *situ* hybridisation and whole cell fluorescence *in situ*
198 hybridisation (FISH) combined with flow cytometry as described by Rigottier-Gois and
199 colleagues (23) employing 16S rRNA-targeted oligonucleotide probes, and targets for
200 rRNA dot-blot hybridisation (Panel of group- and species-specific 16S rRNA-targeted
201 oligonucleotide probes, Supplementary Table S4).

Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was performed with GraphPad Prism 5, (GraphPad Software Inc. San Diego, California, USA). When comparing more than two groups, one-way ANOVA followed by Bonferroni post hoc test was employed. When comparing two groups, Student's t-tests was used. Normal distributions and equality of variance between groups were checked by visual inspection of scatter plots. Statistical significance was considered when P value <0.05. χ^2 test was used to test differences in reproductive outcomes between experimental groups.

RESULTS

Maternal Characteristics

Body weight, food intake in Pregnancy

There was no difference in gestational weight gain or calorific intake during gestation between the obese dams and the obese dams supplemented with PDX (Figure 2A, 2B).

Glucose Tolerance in Pregnancy

The obese dams receiving PDX demonstrated improved glucose tolerance (Figure 2C) and a reduced area under the glucose curve (AUC) 2 hours after the i.p. glucose load compared to the obese dams GD16 (Figure 2C).

Reproductive success

Maternal obesity affected both fertility and pup survival rates and was associated with increased rates of cannibalism in the obese dams. Control dams had 89% successful pregnancies and only 6% cannibalization compared to 44 % and 18 % respectively, for obese dams. Administration of PDX in obese pregnant and lactating dams improved

fertility rates by 14% and reduced cannibalization of the newborn pups ($P<0.05$, Chi-squared test, data not shown).

Maternal Cytokine profile at Gestational day 16

Inflammatory markers were decreased in obese dams following PDX dietary supplementation; notably, TNF-α and CSF-1 showed a 4 and 3 –fold decrease respectively (figure 2F).

Offspring Characteristics

Birth weight and litter size

There was no influence of maternal obesity or PDX on the birth weight of offspring. There was a reduction in the litter size due to maternal obesity, which was partially reversed by maternal dietary supplementation with PDX during pregnancy (figure 2D and 2E).

Body Composition, Energy Balance and Glucose Tolerance at 30 days and 3 months

At 30 days of age there was no difference in bodyweight, calorific intake, EE or glucose tolerance between offspring of obese and lean dams (data not shown).

At 3-months-of-age offspring did not differ between groups in bodyweight or body composition (percentage fat mass) as measured by bio-impedance (see Supplementary methods) or in calorific intake (data not shown).

Following ipGTT at 3 months, male OffOb showed an increase in the peak blood glucose concentration compared to OffCon although the area under the glucose curve was not different from OffCon (figure 3A, inset). In female OffOb, both peak blood glucose

concentration after 15 minutes and AUC were elevated compared to OffCon. Maternal dietary supplementation of PDX resulted in lower peak blood glucose concentration in female OffObP at 15 minutes compared with OffOb (figure 3A).

There was no effect of maternal obesity on male or female 3-month-old offspring EE compared to controls. However, maternal dietary PDX supplementation in obese dams was associated with an increase in EE in male OffObP compared to OffOb during both day and night-time (Figure 3B). There was no effect of maternal PDX supplementation on EE in female OffOb (figure 3C).

Both male and female OffOb showed a significant reduction in respiratory exchange ratio compared to OffCon, which was not observed in female OffObP (figure 3B and C).

Body Composition, Energy Balance and Glucose Tolerance at 6 months of age

Bodyweight of 6-month-old male OffOb was increased compared to OffCon. Maternal dietary supplementation with PDX was associated with a reduction in body weight in male OffObP only ($P<0.001$, figure 4A).

The increase in male OffOb bodyweight was reflected in greater white adipose tissue mass (WAT) (Figure 4B) compared to OffCon, with an increase in the visceral fat pad mass (mesenteric fat mass [g]: OffOb: 0.92 ± 0.08, n=6 *versus*, OffCon 0.65 ± 0.03, n=7, $P<0.05$). Maternal PDX supplementation prevented the rise in male offspring WAT mass and mesenteric fat mass secondary to maternal obesity (Figure 4B, mesenteric fat mass [g]: OffOb 0.65 ± 0.03, n=7, *versus* OffObP 0.51 ± 0.06, n=7, $P<0.05$).

Male OffOb had decreased brown adipose tissue (BAT) compared with OffCon (Figure 4C) when corrected for bodyweight. Maternal dietary PDX supplementation normalised BAT

weight relative to controls and resulted in male offspring with higher BAT weight compared to OffOb.

Both male and female OffOb demonstrated a greater peak glucose concentration in response to a glucose load (i.p.GTT, Figure 4D) and a greater AUC compared with OffCon. Maternal dietary PDX supplementation normalised offspring glucose profiles following the GTT (Figure 4D).

Maternal obesity resulted in lower EE in male OffOb during day and night compared with OffCon. Maternal PDX supplementation prevented the reduced EE associated with maternal obesity during both the active night-phase and the day-time rest-phase. There was no difference in EE between the female offspring at 6 months (Figure 4E and F).

Obesogenic dietary challenge

Body weight

Male and female OffOb had greater body weight after three weeks' exposure to the obesogenic dietary challenge than similarly challenged OffCon. The exaggerated weight gain in both male and female OffOb on the obesogenic diet was prevented by maternal dietary PDX supplementation. (Figure 5A).

Energy Intake

Calorific intake increased across all offspring groups following the obesogenic dietary challenge. Female, but not male, OffOb (figure 5B) increased calorific intake by 25% compared with OffCon fed the same hyper-calorific diet. Maternal dietary supplementation with PDX in the obese dams prevented the increased food intake in females on the high calorie diet.

Energy expenditure

Male and female offspring, in all experimental groups, showed decreased EE during their active (night-time) phase following the high fat dietary challenge. An observed reduction in EE after dietary challenge in adult male and female OffOb offspring compared to control was prevented by maternal PDX (Figure 5C) such that OffObP was similar to control.

Respiratory Exchange Ratio

The dietary challenge normalised respiratory exchange ratio across all groups, such that male and female OffOb no longer showed the reduction in RER observed at baseline (figure 5D).

Skeletal Muscle Mitochondrial DNA Copy number

Mitochondrial DNA copy number was investigated as a potential determinant of the observed reduction in energy expenditure and glucose tolerance in OffOb. At 30 days-of-age, prior to any phenotypic change in the OffOb, MtDNA copy number ratio in male OffOb skeletal muscle was markedly increased compared to controls (Figure 6A). This was prevented by maternal PDX supplementation, such that OffObP males were similar to control. There was no significant effect of maternal diet on mitochondrial copy number ratio in female OffObP, although when sexes were combined there was a highly significant effect of maternal obesity on offspring skeletal muscle Mt/N ratio at 30 days, which was prevented by PDX.

Biomarkers of Brown Fat activation

An increase in Dio2 mRNA expression in 6-month-old male OffOb, a gene encoding Type 2 iodothyronine deiodinase involved in thermogenesis, was prevented by maternal dietary PDX supplementation. Pgc-1a mRNA expression (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) was increased in female OffOb and was

similarly prevented by maternal PDX (Figure 6B). Mitochondrial UCP-1, involved in non-shivering thermogenesis was up-regulated in male OffObP compared to OffCon (Figure 6B) but unaffected by maternal obesity alone.

Gut Microbiota- faecal analysis of broad-spectrum gut bacterial species

Maternal dietary PDX supplementation increased the percentage of *Bacteroides* in the male offspring bacterial population compared to OffCon at weaning (Figure 6C)

In 6-month-old offspring of obese dams the microbiota showed marked differences compated with controls (Figure 6C). Male and female OffOb demonstrated a higher percentage of *Eubacterium rectale-Clostridium coccoides* group compared with OffCon. There was no apparent influence of maternal PDX treatment on OffObP at 6 months.

DISCUSSION

Here we report, in a mouse model, the influence of maternal obesity on offspring energy expenditure and the potential therapeutic benefit of maternal dietary intervention with the prebiotic polydextrose. Our main findings were firstly, that PDX improves glycaemic control and reproductive function in obese pregnancy, without affecting calorific intake or gestational weight gain; secondly, that maternal PDX treatment improves glucose homeostasis in both male and female offspring; and thirdly, that maternal PDX treatment prevents offspring weight gain, via sex specific changes in energy intake and energy expenditure. Lastly, maternal PDX supplementation provided protection against the effects of an obesogenic diet in adulthood.

363 ***Maternal Phenotype***

364 Polydextrose has been shown to improve adult glucose metabolism (24) but not
365 previously in pregnant women or obese pregnant animals. In the present study obese
366 dams showed greatly improved glucose tolerance after supplementation with
367 PDX. This was associated with an improvement in inflammatory cytokine profile in late
368 gestation. Maternal glycaemia (and fetal hyperinsulinaemia) together with inflammatory
369 mediators have been implicated in life-long obesity risk through the altered fetal
370 hypothalamic neurodeveoplment leading to disturbance of anabolic, adipogenic and
371 neurotrophic pathways and permanent influences on metabolic and physiological
372 development (25-27).

373

374 PDX also improved reproductive success in obese pregnant mice, with beneficial effects
375 on fertility and litter size. Obesity perturbs the hypothalamic-pituitary-gonadal axis and
376 ovarian cycle, reducing FSH and LH in the follicular and ovulatory phase whilst also
377 shortening the luteal phase to reduce progesterone levels. It is possible, therefore, that
378 PDX, either directly or indirectly, may influence reproductive hormones in gestation to
379 improve reproductive capacity (28).

380

381 ***Effect of maternal Obesity on Offspring Body Composition & Glucose tolerance***

382 Human cohort studies demonstrate that maternal overweight and obesity is associated
383 with greater adiposity in offspring (5, 29). We found that male offspring were heavier
384 with increased white adipose tissue mass and reduced BAT mass at 6 months of age. The
385 impaired glucose tolerance observed in both males and females, at 3 months, antedates
386 any observed changes in body composition (BIA) suggesting an alternative cause,
387 potentially pancreatic beta cell dysfunction previously implicated in this model (30) or
388 the early changes in mitochondrial function observed.

389

Effect of maternal Obesity and PDX on Offspring Energy expenditure

In this study we present novel evidence for the developmental programming of altered EE secondary to maternal diet-induced obesity, and prevention by maternal PDX supplementation. PDX influenced EE from as early as 3 months of age, preceding the subsequent changes in body composition, without affecting energy intake. Previously the scant literature in this area includes demonstration of reduced EE in 6-month-old infants born to overweight and obese mothers (31); in genetically altered mice following intrauterine exposure to gestational diabetes (32); and in 30 day old offspring of severely obesity rats (33). Changes in both RER and EE were associated with hepatic mitochondrial dysfunction, with reduced PGC-1α mRNA expression, and impaired fatty acid oxidation (33). Taken together, these findings suggest impaired nutrient sensing and fuel switching in offspring of obese dams. Compromised fatty acid oxidation would be consistent with the development of a fatty liver phenotype which we have previously described in this rodent model (15, 17).

Response to an obesogenic environment in adulthood: Energy intake on the obesogenic diet

Female offspring of obese dams demonstrated hyperphagia secondary to maternal obesity only when exposed to obesogenic dietary challenge, suggesting programming of sex specific effects on food preference, and implicating mesolimbic reward pathways (34). Perinatal 'junk food' exposure similarly increases the preference for palatable diets in juvenile and adult rat offspring, and we previously reported reduced Mυ-opioid receptor expression in the ventral tegmental area (VTA) of female 'junk-food' offspring only (35) (36). Moreover, we have previously reported in the offspring of obese rats, structural and functional deficits in neuronal development in the hypothalamic acruate and paraventricular nucleus associated with leptin resistance and hyperphagia (49).

Prevention of female hyperphagia by maternal PDX supplementation, therefore, could imply protection of central neurotrophic development in the neonatal brain.

Male offspring of obese mice had lower energy expenditure than controls. In man, a blunted glucose-induced thermogenesis has been observed in obese individuals, increasing susceptibility to obesity when consuming diets rich in sugars (37-39). Since OffOb males were not obese at three months, a programmed deficit in diet-induced thermogenesis or central insulin resistance at the level of the hypothalamus could underlie the reduction in night time EE during the obesogenic dietary challenge. Reduced physical activity can also play a role in reduced EE, however, this is unlikely in the murine model employed here, since we have previously reported that male offspring of obese mice have a hyperactive ADHD-like phenotype (40)

Mitochondrial biogenesis and activation

The observed increase in mitochondrial DNA copy number in skeletal muscle at 30 days of age in OffOb males is consistent with early developmental exposure to maternal high glucose-induced ROS, secondary to maternal obesity, and could reflect compensatory mitochondrial biogenesis in response to a decline in mitochondrial function (21, 41, 42). Alternatively, the increase in MtDNA may be non-functional and a maladaptive response to oxidative stress (43) or hyperglycaemia (44), which can lead to an increase in tissue MtDNA and inflammation through activating of mTOR pathways and induction of TNFα (45). Either way, the data suggest an independent influence of maternal obesity on skeletal muscle mtDNA levels and hence mitochondrial function prior to the development of other metabolic defects, which might suggest a primary mechanism in the developmental programming due to maternal obesity.

Biomarkers of Brown Fat activation

The increased expression Type 2 iodothyronine deiodinase (D2) which mediates adaptive thermogenesis in brown adipose tissue may reflect the increased sympathetic drive (46) previously described in this model (14, 47, 48). PDX may theoretically prevent this increased Dio2 gene expression by normalising hypothalamic development and sympathetic drive in OffOb (49). Indeed, others have shown that probiotics rescue neurogenesis and behavioural deficits in dysbiotic mice treated with antibiotics (50).

Mitochondrial UCP-1 expression was up-regulated in BAT of male offspring of obese dams treated with PDX compared to control offspring and may contribute to the increased energy expenditure observed. Prebiotics may increase thermogenic capacity in BAT by increasing UCP-1 expression (51) through altering microbiota and their by-products, short chain fatty acids, which can act as both energy source and receptor-mediated metabolic regulators of host energy metabolism involving processes such as hepatic gluconeogenesis and lipid metabolism via AMPK and PGC-1a activation (52) .

Pgc1α is the master regulator of mitochondrial biogenesis and linked to adaptive thermogenesis, following 'BAT activation'. Increased Pgc1α expression in skeletal muscle of offspring of obese dams which was prevented by maternal PDX treatment appears counter-intuitive, as BAT activation would be expected to contribute to greater energy expenditure, if the observed increase in mtDNA were indeed functional. However, in addition to stimulating mitochondrial proliferation in skeletal muscle, PGC-1α activation favours enhanced lipid- over carbohydrate-mediated mitochondrial respiration in skeletal muscle in mice, and leads to intrinsic mitochondrial adaptations in fatty acid-induced uncoupling and a reduction in mitochondrial superoxide production (53). This 'fuel switching' is consistent with the observed reduction in RER, and thus increased lipid

oxidation, in offspring of obese mice and may represent a compensatory response to reduce ROS production, or a direct influence of the gut microbiota (52)

Offspring microbiota profile

Inheritable microbiota, passed from an obese mother to offspring during labour, may contribute to the modern patterns of human health and disease affecting gut barrier integrity and energy provision (54) but also maturation of the immune system (55), insulin sensitivity, energy expenditure and visceral adiposity (56). Indeed, we have previously implicated impaired innate immunity in offspring liver together with an increase in pro-inflammatory markers associated with NAFLD in offspring of obese mice (17). A recent landmark study demonstrated that transplanted gut microbiota from stool microbes of 2-week-old infants born to obese mothers increases inflammation and susceptibility to NAFLD in recipient germ-free mice (57).

Prebiotic effects of polydextrose on offspring microbiota

Maternal supplementation with PDX in obese pregnant mice resulted in increased abundance of *Bacteroides* compared to controls. Administration of prebiotics has previously been shown to improve pregnancy outcomes (58) and influence maternal transfer of microbiota and initial establishment of bifidobacteria in the infant (59). In obese humans an increase in bacteroides relative abundance is associated with weight-loss (60). We report a similar effect here, with maternal PDX intervention, in which obesity traits in the offspring were reduced associated with an increase in bacteroides relative abundance.

Conclusions

In this study, evidence has been presented that diet-induced maternal obesity in the mouse results in reduced EE, glucose intolerance and increased bodyweight in 6 month

male offspring compared to controls. Moreover, following a 3-week obesogenic dietary challenge, offspring of obese dams had reduced energy expenditure, increased calorific intake an increased weight gain compared to controls. The offspring obesogenic phenotype is preceded by evidence of early mitochondrial damage and changes in the gut microbiota, which are prevented by maternal polydextrose . Polydextrose is a synthetic indigestible glucose polymer, classified as a dietary fibre and therefore, safe for use in pregnancy. However, there is currently a lack of high-quality scientific data on the use of polydextrose, or indeed other prebiotics, in pregnant or breastfeeding women. The present study supports the safety and efficacy of polydextrose supplementation in obese pregnancy.

Supplementary information is available at International Journal of Obesity's website

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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703

704 **FIGURE LEGENDS**

705

706 ***Figure 1. Schematic representation of the experimental design***. Female dams were fed
707 either an obesogenic (n=34) or a control diet (n=18). Following successful mating a
708 subgroup of obese dams were supplemented with 5 % PDX (n=12) in the drinking water.
709 All offspring were weaned on to control diet and were followed up to 6 months. I male
710 and 1 female was studied at each timepoint: 30 days, 3 months and 6 months. At three
711 months after recording baseline characteristics, 1 male and 1 female from each litter
712 (n=10) were exposed to the obesogenic diet for three weeks and reassessed.

713

714 ***Figure 2. Maternal characteristics (A)*** Gestational bodyweights and **(B)** calorific intake
715 during gestation in obese (Ob) and obese supplemented with PDX (ObP) dams (n=6-7).
716 **(C)** Response to a glucose tolerance test (GTT) and the respective area under the curve
717 (AUC) on GD16 **(D)** Litter size and **(E)** Birthweight in control (Con) obese (Ob) and obese
718 supplemented with PDX (ObP) dams (n=8-16) **(F)** Cytokine profile from late gestation
719 obtained by 2 samples of pooled serum samples (n=5 per pool) from dams at day 16. Data
720 are expressed as mean ± SEM. *represents $P<0.05$, ** represents $P<0.01$, comparison with
721 the obese group.

722

723 ***Figure 3. Offspring Phenotype at 3 months (A)*** Response to a glucose tolerance test
724 (GTT) and the respective area under the curve (AUC, inset). Average energy expenditure
725 (EE) and RER during daytime or night-time (as indicated) in male **(B)** and female **(C)**
726 offspring of control (OffCon), obese (OffOb) and obese dams supplemented with PDX
727 (OffObP) at 3 months of age, n=6-15; Data are expressed as mean ± SEM. * represents
728 $P<0.05$; ** represents $P<0.01$ versus OffCon. Hash symbol ♯ represents $P<0.05$ male
729 OffObP vs OffOb.

730

731 ***Figure 4. Offspring Phenotype at 6 months*** Average **(A)** bodyweight **(B)** weight of
732 white adipose tissue (WAT) and **(C)** brown adipose tissue weight corrected for
733 bodyweight **(D)** GTT with AUC inset **(E)** Day-time Energy expenditure and (F) night-time
734 energy expenditure in male and female offspring of control (OffCon), obese (OffOb) and
735 obese dams supplemented with PDX (OffObP) at 6 months of age, (n=6-7), Data are
736 expressed as mean ± SEM. * represents P<0.05, * *represents P<0.01, *** represents
737 P<0.001.
738
739 ***Figure 5. Obesogenic Dietary Challenge***. Offspring Phenotype at 3 months after 3 weeks
740 on the obesogenic diet **(A)** Bodyweights and **(B)** average daily calorific intake **(C)** energy
741 expenditure and **(D)** RER in male and female offspring of control (OffCon), obese (OffOb)
742 and obese dams supplemented with PDX (OffObP) at 3 months of age and after three-
743 weeks exposure to obesogenic diet, n=7-10. Exposure to obesogenic diet (OD) and
744 maternal diet significantly accounted for variation (two-way ANOVA); Data are expressed
745 as mean ± SEM. * represents P<0.05, **represents P<0.01, *** represents P<0.001.
746
747 ***Figure 6. Potential mechanisms (A)*** MtDNA copy number in skeletal muscle from male
748 and female offspring of control (OffCon), obese (OffOb) and obese dams supplemented
749 with PDX (OffObP) at 30 days of age, (n= 5-6) ***(B)*** mRNA expression of brown adipose
750 tissue biomarkers of BAT activity at at 30 days of age. ***(C)*** Percentage of *bacteroides* (Bac+)
751 and ***(D)*** *Eubacterium rectale-Clostridium coccoides* (Erec+) in bacteria cells (EUB+)
752 identified in faecal samples from male and female offspring of control (OffCon), obese
753 (OffOb) and obese dams supplemented with PDX (OffObP) at ***(C)*** weaning and ***(D)*** 6
754 months of age (n=6). ***(E)*** Representative FACS plot. EUB+ and gated cells. FL1 histogram,
755 green fluorescence is the total number of bacteria hybridising with the EUB 338-FITC
756 probe. FL4 histogram, red fluorescence, shows the proportion of cells targeted by the

group Cy5-probe in the sample. Data are expressed as mean ± SEM. * represents P<0.05; ** represents P<0.01, *** represents P<0.001.

Supplementary Methods

Animal husbandry and diets

Female C57BL/6J mice were maintained under controlled conditions (22°C, 12-hour light/dark cycle) and fed either a standard chow diet (RM1, Special Dietary Services, UK, 7% simple sugars, 3% fat, 50% polysaccharide, 15% protein [w/w] energy 3.5 kcal/g; n=18) or a semisynthetic obesogenic diet (#824053, Special Dietary Service, 10% simple sugars, 20% animal lard 28% polysaccharide, 23% protein [w/w], Special Dietary Services, energy 4.5 kcal/g, n=46). The obesogenic diet was supplemented by *ad libitum* access to sweetened condensed milk, fortified to control levels with micronutrient mineral mix (AIN93G, Special Dietary Services, UK). The combined macronutrient and calorific composition of the highly palatable obesogenic diet (based on intake) was approximately 16% fat, 33% simple sugars, 15% protein, total energy 4.0 kcal/g. Diets were introduced 6 weeks prior to mating and throughout gestation and lactation, as previously described by Samuelsson *et al (2008) (14).* All females included in the study were proven breeders with one previous successful pregnancy. Following successful mating, with proven breeders, indicated by the presence of a copulation plug, a sub-group of obesogenic diet-fed dams were randomly assigned to the same obesogenic diet supplemented with PDX (5% w/v) in the drinking water throughout gestation and lactation (n= 34) leading to three groups; control (Con); obese (Ob) and obese +5% (*w/v*) PDX, ObP, *Figure 1*). This concentration of PDX has previously proven efficacious in reducing insulin resistance in adult rats without toxicity and without altering calorific intake (19).

All pups were weighed 48 hours after delivery (to avoid pup rejection), and each litter was then standardised to 3 males and 3 females where possible. Litters with less than four pups were not included in the study.

Indirect Calorimetry

At each time point, 6 age-matched animals were weighed, and then placed in the open circuit indirect calorimetry cages (CaloCages) for 24 hours of acclimatisation, followed by 24 hours of experimental recording. Data were recorded automatically every 20 minutes and hourly means calculated.

Mitochondrial DNA Copy Number in Offspring Skeletal Muscle

2ul sample DNA diluted to 10ng/ul was loaded in triplicate alongside a 5-point standard curve consisting of primer-specific amplicons of known copy number.

Detailed Glucose tolerance test

In order to determine whole body glucose tolerance, an intra-peritoneal glucose tolerance test (ipGTT) was performed using AlphaTRAK® Glucose meter (Abbott Animal health), which has been specially designed for and validated in mice and rats. Following an overnight fast, a cream containing lidocaine (2.5%) and prilocaine (2.5%) (EMLA cream 5%, AstraZeneca, UK) was applied as a topical anaesthetic to the tail. Once the analgesia had taken effect, fasting tail venous blood glucose was measured. Animals were then injected via the intraperitoneal (i.p.) route of administration with a glucose load (1 gram/kg) of glucose solution (10% glucose). Measurements of the blood glucose were taken at 15, 30, 60 and 120 minutes after the glucose injection using the glucose meter. Measurements were taken at each point while the animals were conscious and semi-restrained. Glucose tolerance tests were performed on the dams at gestational day 16 (GD16) and on the offspring at 30, 90 and 180 days of age.

Biochemical analysis (Cytokine Profile)

Briefly, the Proteome Profiler Mouse Adipokine Array allows the simultaneous measurement of relative expression levels of 38 mouse adipokines. Capture and control antibodies were spotted in duplicate on nitrocellulose membranes. Serum samples were diluted, mixed with a biotinylated detection antibody, and incubated overnight with the Proteome Profiler Mouse Adipokine Array. The next morning the membrane was washed in order to remove unbound material. Streptavidin-HRP and chemiluminescent detection reagents were applied allowing the production of a signal at each capture spot corresponding to the amount of protein bound. The density of each protein was measured with Image J.

Analysis with flow cytometry

The analysis was performed with flow cytometry as described by Rigottier-Gois et al (23). Cells were pelleted and resuspended in PBS for data acquisition by flow cytometry (HTS Fortessa, Becton Dickinson, USA).

A total of 20 000 events EUB 338-FITC positives were stored in list mode files. Subsequent analyses were conducted using FlowJo software (Tree Star, USA). Cell enumeration was performed by combining, in one hybridisation tube, one group Cy5-probe with the EUB 338-FITC probe. An FL1 histogram (green fluorescence) was used to evaluate the total number of bacteria hybridising with the EUB 338-FITC probe. A gate was designed in this histogram representing the total number of bacterial cells in the sample and was used to build an FL4 histogram (red fluorescence) to directly estimate the proportion of cells targeted by the group Cy5-probe in the sample. The proportion of cells was corrected by eliminating background fluorescence, which was measured using the negative control NON 338-Cy5 probe. Results were expressed as cells hybridising with the group-Cy5

838 probe as a proportion of the total bacteria hybridising with the EUB 338-FITC bacteria
839 domain probe or normalised to the basal conditions as indicated.
840

841 **Supplementary tables**

842

Diet	Control (RM1)	Obesogenic Diet (Pellets)	Obesogenic Diet (Sweet Condensed milk)
Protein (%)	15	23	7.8
Total Carbohydrate (%)	61.73	38.83	55.3
Polysaccharides (%)	57.68	28.34	-
Simple Sugars (%)	7	10.49	55.3
Fat (%)	2.78	22.21	8.1
Soya oil (%)	-	4.32	-
Lard (%)	-	17.89	5.6
Corn oil (%)	2.78		
Crude Fibre (%)	4.65	6.17	traces
Energy (kcal/g)	3.5	4.5	3.3

843 ***Table S 1.*** *Composition of the experimental diets presented as percentages by weight. The*
844 *obesogenic diet consisted of both pellets and sweetened condensed milk. Due to varying*
845 *moisture and nitrogen free extract content, rows will not sum to 100%.*

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847
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Primers	Accession number	Sequences
mouse mitochondrion complete genome	NC_005089.1	**Forward primer:** 5‘-CTAGAAACCCCGAAACCAAA -3‘ **Reverse primer:** 5‘-CCAGCTATCACCAAGCTCGT-3‘
mouse β2M (beta-2 microglobulin)	NC_000068.8	**Forward primer:** 5‘-CTAGAAACCCCGAAACCAAA -3‘ **Reverse primer:** 5‘-CCAGCTATCACCAAGCTCGT-3‘

852 ***Table S2*** *Oligonucleotide primer sequences used to determine mitochondrial copy number*
853 *in skeletal muscle (tibialis anterior) samples.*
854

855

Gene	Forward Primer	Reverse Primer
Cyclophilin B	TGGAGAGCACCAAGACAGACA	TGCCGGAGTCGACAATGAT
Dio2	CCTACAAACAGGTTAAACTGGG	CTCTGCACTGGCAAAGTC
Pgc1a	TGAAAGGGCCAAACAGAGAGA	TAAATCACACGGCGCTCTT
Ucp1	AATACTGGCAGATGACGTCC	TTACCACATCCACTGGAGAG
Zic1	CACATGAAGGTCCATGAGTCC	GGGTTGTCTGTTGTGGGAG

Table S3 *Semi-quantitative real-time PCR primers and sequences for BAT tissue samples.*

Probe	Sequence	Target	Label 5'
EUB 338 pB-00159	GCTGCCTCCCGTAGGAGT	Domain Bacteria	FITC
NON 338 pB-00243	ACATCCTACGGGAGGC	Negative probe	Cy5
Bac 303 pB-00031	CCAATGTGGGGGACCTT	*Bacteroides*	Cy5
Erec 482 pB-00963	GCTTCTTAGTCARGTACCG	*Clostridium coccoides–Eubacterium rectale*	Cy5
Lab 158 pB-03928	GGTATTAGCAYCTGTTTCCA	*Lactobacillus-Streptococcus group*	Cy5
Bif 164 pB-00037	CATCCGGCATTACCACCC	*Bifidobacterium*	Cy5

Table S4. *16S rRNA-targeted oligonucleotide probes, and targets for rRNA dot-blot hybridisation (Panel of group- and species-specific 16S rRNA-targeted oligonucleotide probes)*