Preconception weight loss improves fertility and maternal outcomes in obese mice

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Abstract

Women with obesity have higher incidences of infertility, with longer time to conception and increased risk of pregnancy complications compared to women with normal body weight. There is a lack of evidence demonstrating the benefit of preconception maternal weight loss on fertility and pregnancy outcomes. We aimed to determine if preconception weight loss, either with diet modification or glucose-like peptide 1 receptor agonist liraglutide, improves maternal weight, fertility, and pregnancy outcomes. C57BL/6 female mice were fed either a high-fat diet (HFD) or chow for 8 weeks. HFD-fed dams were administered liraglutide (0.3 mg/kg, s.c., for 4 weeks) or switched to chow to induce weight loss. Prior to mating, liraglutide was ceased and mice continued on HFD. Mice in the ‘diet switch’ group continued on chow. Pregnancy rates were recorded. Maternal anthropometry and glucose tolerance were measured before and after the intervention and at late gestation. Offspring outcomes were assessed. Liraglutide or diet switch led to weight reduction, improved insulin resistance (P < 0.001), and enhanced fertility, particularly in the liraglutide group (P < 0.005). Liraglutide-treated mice had significantly higher gestational weight gain (GWG) compared to the diet switch group (P < 0.05), with similar weight and glucose tolerance in late gestation to HFD mice. In contrast, diet switch maintained similar weight and glucose tolerance in late gestation to control mice. Pre-pregnancy weight intervention with liraglutide was effective at restoring fertility. Diet modification also improved fertility and avoided catch up weight gain in pregnancy. Liraglutide may be a therapeutic strategy for weight loss to prepare for pregnancy. However, our study provides caution about the potential for excessive GWG without diet intervention in pregnancy.

Introduction

The pandemic of obesity affects all stages of the lifespan, with unique considerations in women of reproductive age. In countries such as Australia and Canada, nearly one in five women of the reproductive age have obesity (Kuhle et al. 2019, Australian Institute of Health and Welfare 2021). Complications of maternal obesity are well established, impacting pregnancy-related and long-term outcomes for mothers and offspring (Poston et al. 2011). Maternal complications include miscarriage, gestational diabetes mellitus, hypertension, and preeclampsia;
Obesity is further associated with subfertility; women with obesity take longer to conceive than women with healthy body weights, with time to pregnancy commensurate with the severity of obesity (Broughton & Moley 2017). Women with obesity have reduced success with assisted reproductive therapy, significantly lower pregnancy and live birth rates, and higher miscarriage rates compared to women with normal body weight (Talmor & Dunphy 2015, Mushtaq et al. 2018).

Preconception weight loss has the potential to reduce many obesity-related complications associated with conception and pregnancy (Practice Committee of the American Society for Reproductive Medicine 2015). National guidelines on the management of obesity recommend attempting lifestyle modification prior to pregnancy (Practice Committee of the American Society for Reproductive Medicine 2015, RANZCOG 2017). However, this is not an ideal method for pre-pregnancy weight reduction, with 6–12 months of lifestyle intervention required to achieve 5–10% body weight reduction (Practice Committee of the American Society for Reproductive Medicine 2015). Research shows that women with overweight or obesity are not willing to delay conception plans for longer than 3 months, leaving a narrow therapeutic window in which to affect meaningful change (Sacha et al. 2018). Strategies for assisting obese women to lose weight prior to pregnancy are thus required. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are an attractive option in this context. They are widely used in the treatment of obesity and T2D, affecting a range of tissues to optimise metabolic function, including adipose tissue and the pancreas (Mancini & de Melo 2017, Drucker 2018).

Thus, we hypothesised that obese females treated with diet modification or liraglutide in the preconception period will have body weight reduction and improved glucose tolerance prior to pregnancy, improved fertility rates, improved maternal metabolic outcomes in pregnancy, compared to those without any intervention. The aim of this study was to determine if weight loss prior to pregnancy, with diet modification or pharmacotherapy with liraglutide can improve preconception, conception, and pregnancy-related outcomes in a rodent model of maternal obesity.

Methodology

Female C57Bl/6 mice were obtained at 4 weeks of age (Kearns Facility, Kolling Institute, Royal North Shore Hospital, St Leonards, NSW, Australia). They were housed in a stable environment maintained at 22 ± 1°C with a 12h light:12 h darkness cycle in groups of three to four mice per cage, with ad libitum access to food and water. All animal procedures were approved by the Animal Care and Ethics Committee of the Northern Sydney Local Health District (Approval No. RESP/18/148).

Pre-pregnancy mouse model of obesity and preconception weight loss

Mice were randomly allocated into two groups to receive either a high-fat diet (HFD, 19 kJ/g, 43% fat, SF04-001; Specialty Feeds, WA, Australia) or a standard chow diet (Fig. 1). Following an 8-week period, an intraperitoneal glucose tolerance test (IPGTT) was performed to ensure the efficacy of the HFD model of obesity as previously described (Rasmussen & Lindenberg 2014, Glastras et al. 2016). Briefly, mice were weighed on the test day, fasted for a period of 6 h, 50% glucose (2 g/kg) was administered at time 0 min via intraperitoneal injection, and tail tip blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min using a Roche AccuCheck Performa Meter. Thereafter, HFD-fed mice underwent 4 weeks of weight loss intervention with either liraglutide or with a change of diet from HFD to chow. HFD-fed mice were divided into three groups (1:1:1 ratio, n = 36 each): (i) HFD-L: HFD in combination with liraglutide; (ii) HFD-V: HFD-vehicle; (iii) HFD-C: HFD switch to chow. Control (C) mice continued on chow (n = 24). The HFD-L group underwent weight-based daily s.c. liraglutide injections, while continuing on HFD. To reduce adverse effects of liraglutide such as nausea and gastrointestinal discomfort, dose escalation comprised of 3 days of 0.1 mg/kg/day, followed by 3 days of 0.2 mg/kg/day, to a full treatment dose of 0.3 mg/kg/day, as previously described (Fransson et al. 2014). The three other groups received saline injections. Following treatment, just prior to pregnancy, a repeat IPGTT was performed, to assess the efficacy of liraglutide therapy and preconception weight loss. Following a 6-h fasting period, eight mice per group were killed humanly at this time. Blood, kidneys, liver, and fat (retroperitoneal, gonadal, and brown) were harvested and weighed. The remaining mice went on to the pregnancy stage of the model.
Figure 1
Study design schematic outlining the progression of the four mouse groups through the preconception and pregnancy period of the mouse model.
Pregnancy

Following a 1-week washout period (to minimise the risk of teratogenic effects of liraglutide), dams were co-housed with an 8–12-week-old male for 3 days. The presence of a vaginal plug indicated pregnancy; if the pregnancy was not achieved, two further cycles of 3 days of mating were attempted. Once pregnancy was confirmed, pregnant mice were housed individually. HFD-L and HFD-V mice were continued on HFD. HFD-C and control mice were continued on chow diet. On day 18 of gestation, a proportion of pregnant mice underwent an IPGTT after 6-h fasting.

Following a 6-h fast, eight pregnant dams in each group were sacrificed at gestational days 19–20. Blood, kidneys, liver, fat (retroperitoneal, gonadal, and brown), placentae, and feti were harvested and weighed. The remaining dams delivered their litters.

Fertility was assessed in all mice by pregnancy rates after the first attempt of mating, and fecundity score was calculated as the number of mice pregnant after the maximal number of attempts at mating. Pregnancy outcomes were assessed by litter size and the number of live offspring. Infanticide rates were determined by the number of remaining pups alive in the litter at day 3 of life.

Bioassays

Serum insulin was measured using ELISA (Ultrasensitive) (Crystal Chem, IL, USA) (interassay and intraassay CV <10%). Homeostatic model assessment for insulin resistance (HOMA-IR) score was calculated using the following formula:

\[
\text{HOMA-IR} = \frac{\text{Fasting blood glucose (mmol/L)}}{\text{Fasting plasma insulin (U/mL)}} \times 22.5
\]

Plasma non-esterified fatty acids (NEFA) were measured using a NEFA kit (WAKO, Osaka, Japan) (interassay and intraassay CV 1.5%). Serum cholesterol, triacylglyceride (TAG), and HDL were measured using the Architect C16000 Clinical Chemical Analyzer (Abbott Laboratories) (interassay and intraassay CV <5%). LDL was calculated using the Friedewald calculation:

\[
\text{LDL} = \text{Total Cholesterol} - (\text{TAG}/2.2) - \text{HDL}
\]

Statistical methods

All results are expressed as mean ± S.E.M. Differences in mean for each group were compared using one-way analysis of variance (ANOVA) with post hoc Tukey’s tests. The trapezoidal rule was used to determine the area under the curve (AUC) for IPGTT results for each mouse (Andrikopoulos et al. 2008). For differences in plasma glucose levels during the IPGTT, an ANOVA with repeated measures was performed and the significance was determined using Tukey’s post hoc test. Chi-square tests were used for fertility analyses. All analyses were carried out using GraphPad Prism 9.0 (GraphPad Software) and P value <0.05 was considered statistically significant.

Results

Pre-pregnancy model of obesity

Body weight measurements after 8 weeks showed a 49.3% body weight gain in all HFD-fed mice (all three groups averaged), compared to 29.9% in chow-fed mice (P < 0.005; Fig. 2A). To confirm the weight loss effect of liraglutide treatment, body weight measurement after 4-week treatment indicated negligible body weight change in the HFD-L group compared to 16% body weight gain in the HFD-V group (P < 0.01). There was no significant difference in body weight after 4 weeks of treatment between the C, HFD-L, and the HFD-C groups.

Anthropometry in the preconception period after treatment

Kidney and liver weights were similar between groups (Table 1). Retroperitoneal adipose tissue pads were larger in the HFD-V group compared to the control group (P < 0.0001). Liraglutide lowered retroperitoneal fat mass compared to HFD-V (P < 0.0005), an effect greater than diet change (HFD-C vs HFD-V, P < 0.005). There was no significant difference in retroperitoneal fat mass among the C, HFD-L, and HFD-C groups. Similarly, gonadal fat mass was significantly higher in the HFD-V group compared to the chow (P < 0.0001). Liraglutide-treated mice and mice with diet switch had smaller gonadal fat masses compared to HFD-V (both P < 0.0001). There was no significant difference in gonadal fat mass between C, HFD-L, and HFD-C groups. There was no difference in brown adipose tissue weight between groups.
Figure 2
Preconception weight over time with glucose tolerance testing pre- and post-treatment (either liraglutide or diet switch). (A) Body weight trajectory over time, (Bi) intraperitoneal glucose tolerance test (IPGTT) prior to treatment. (Bii) Area under the curve (AUC) IPGTT prior to treatment. (Ci) IPGTT following preconception treatments with liraglutide or diet change, (Cii) AUC IPGTT post-treatment. n = 8, *P < 0.05, **P < 0.005, ***P < 0.0005, ****P < 0.0001, *between C and HFD-V, #between HFD-L and HFD-V, +between HFD-C and HFD-V.

Table 1  Metabolic measures and anthropometry in mice at the preconception period 4-weeks post-treatment. Results are expressed as mean ± s.e.m. n = 8 per group for metabolic measurement. P value for ANOVA.

<table>
<thead>
<tr>
<th>Metabolic measurements</th>
<th>C (mean ± S.E.M.)</th>
<th>HFD-L (mean ± S.E.M.)</th>
<th>HFD-V (mean ± S.E.M.)</th>
<th>HFD-C (mean ± S.E.M.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum fasting glucose (mmol/L)</td>
<td>8.0 ± 0.1</td>
<td>5.7 ± 0.4</td>
<td>9.1 ± 0.2</td>
<td>7.9 ± 0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum insulin (mU/L)</td>
<td>2.7 ± 0.8</td>
<td>1.2 ± 0.3</td>
<td>7.3 ± 2.1</td>
<td>4.4 ± 2.0</td>
<td>0.089</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.0 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>3.0 ± 0.7</td>
<td>1.7 ± 0.8</td>
<td>0.0045</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1.14 ± 0.08</td>
<td>1.09 ± 0.08</td>
<td>1.08 ± 0.06</td>
<td>1.32 ± 0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Left kidney (g)</td>
<td>0.16 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.45</td>
</tr>
<tr>
<td>Right kidney (g)</td>
<td>0.16 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.39</td>
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<tr>
<td>Retroperitoneal fat (g)</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.32 ± 0.03</td>
<td>0.12 ± 0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gonadal fat (g)</td>
<td>0.47 ± 0.08</td>
<td>0.49 ± 0.06</td>
<td>1.67 ± 0.15</td>
<td>0.53 ± 0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Brown fat (g)</td>
<td>0.08 ± 0.01</td>
<td>0.1 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Metabolic markers in the preconception period after treatment

IPGTT demonstrated that the HFD-V group had impaired glucose tolerance compared to control, with significant differences seen at 15, 30, 60, and 90 min ($P < 0.05$, $P < 0.001$, $P < 0.01$, and $P < 0.05$, Fig. 2Ci), as well as AUC (HFD-V/L/C vs C, $P < 0.0001$, Fig. 2Bi). Following either 4 weeks of liraglutide therapy or diet change, the IPGTT results were similar between C, HFD-L, and HFD-C (Fig. 2Ci). At time point 30, 60, and 90 min of the IPGTT, plasma glucose levels in the HFD-V group were significantly higher than all other groups (at least $P < 0.0001$, $P < 0.0001$, $P < 0.001$, respectively). There was a significant difference in plasma glucose levels at 15 min between C and HFD-V ($P < 0.01$), and at 90 min between HFD-L and HFD-V ($P < 0.05$). In keeping with the known glycaemic benefits of liraglutide therapy, 4 weeks of treatment improved overall glucose tolerance significantly (AUC HFD-V, $P < 0.0001$, Fig. 2Cii). Preconception diet switch also resulted in a highly significant improvement in glucose tolerance ($P < 0.0001$). There was no significant difference in glucose tolerance between HFD-L and HFD-C groups.

As expected, the HFD-V group had higher fasting glucose levels ($P < 0.01$ vs control, Table 1). The HFD-L group had statistically lower fasting glucose levels compared to all other groups (vs control $P < 0.005$, vs HFD-V $P < 0.0001$, vs HFD-C $P < 0.001$). Serum insulin concentration was higher in the HFD-V group vs control ($P < 0.05$, Table 1). Liraglutide treatment had significantly lower fasting insulin levels (HFD-L vs HFD-V, $P < 0.05$). Insulin levels in the HFD-C group were not significantly different from the control, HFD-L, or HFD-V groups. The HOMA-IR score showed greater insulin resistance in the HFD-V group compared to both HFD-L and control ($P < 0.001$, $P < 0.01$, respectively). Liraglutide was more effective at reducing HOMA-IR than HFD-C ($P < 0.05$). There was no difference in the HOMA-IR score between HFD-C and HFD-V or HFD-C and control groups (Table 1).

As anticipated, fasting serum NEFA was higher in the HFD-V compared to control ($P < 0.01$, Fig. 3A). NEFA levels were normalised in the HFD-L and HFD-C groups ($P < 0.01$ vs HFD-V). Serum cholesterol was higher in the HFD-V group compared to the control ($P < 0.01$, Fig. 3B). Liraglutide and diet switch significantly reduced cholesterol levels compared to HFD-V ($P < 0.05$ and $P < 0.01$, respectively). There was no significant difference between HFD-L and HFD-C and control groups. Serum TAG levels were higher in the HFD-V group compared to the control ($P < 0.05$, Fig. 3C). Liraglutide therapy reduced serum TAG below HFD-V ($P < 0.001$). Serum TAG was lower in the HFD-C compared to the HFD-V group ($P < 0.05$), and there was no significant difference between HFD-C and control groups. Moreover, TAG levels in the liraglutide-treated mice were significantly lower than TAG levels in mice treated with diet change ($P < 0.01$). Serum LDL was higher in HFD-V vs control ($P < 0.002$, Fig. 3D). Preconception diet change and liraglutide therapy showed lower LDL compared to HFD-V. However, diet change was more effective at lowering LDL levels compared to liraglutide (HFD-V vs HFD-C, $P < 0.001$, HFD-L vs HFD-C, $P < 0.05$). HDL levels were not significantly different between groups.

Impact of weight loss on fertility

HFD-fed females were nearly 50% less likely to become pregnant on the first attempt compared to the control mice. However, preconception liraglutide fully restored fertility (HFD-V vs control, odds ratio (OR) 0.6, CI 0.3–0.9, $P < 0.05$; HFD-V vs HFD-L 1.8, CI 1.2–3.0, $P < 0.005$) (Fig. 4A and B). Liraglutide was more efficient in improving fertility than switching diet (OR 1.6, CI 1.1–2.5, $P < 0.05$). The HFD-L group required less time to become pregnant compared with the HFD-V group ($P < 0.05$). There was no difference between HFD-C and HFD-V in the time to fall pregnant. Fecundity in the control group was 100%, significantly higher than the HFD-V group, which achieved pregnancy in 84% of cases (OR 1.2, CI 1.1–1.2, $P < 0.05$, Fig. 4C and D). The fecundity rates were higher in the HFD-L group compared to the HFD-V (97% vs 84%, OR 1.2, CI 1.1–1.4, $P < 0.05$). Overall, 93% of HFD-C achieved pregnancy, which was not significantly different from C, HFD-L, or HFD-V groups.

Impact of weight loss on metabolic effects in late gestation

Gestational weight gain and late pregnancy body weight

Gestational weight gain (GWG) was greatest in the HFD-L group (33.6%), compared to the HFD-V group (29.7%, $P < 0.05$, Fig. 5A). There was no significant difference in GWG between HFD-V and the control group. The HFD-C group had GWG comparable to the control group.

HFD-L and HFD-V groups had analogous body weights by the end of pregnancy, and this was significantly greater than the control or HFD-C groups (both $P < 0.001$, Fig. 5B).

Maternal metabolic function in late pregnancy

In late pregnancy, IPGTT results were similar between HFD-V and HFD-L groups and significantly impaired
Preconception weight loss in obese mice

There was no significant difference in glucose levels between groups at 15 min of the IPGTT. By 30 min, there was a significant difference in glucose levels between HFD-V and C (31.3 mmol/L vs 24.2 mmol/L, \( P < 0.005 \)), and 90 min (20.8 mmol/L vs 9.8 mmol/L, \( P < 0.0001 \)). By 120 min, there was no difference in glucose levels between HFD-V and C. Following liraglutide therapy, there was a significant difference between HFD-L and C at 30 (29.13 mmol/L vs 25.2 mmol/L, \( P < 0.005 \)), 60 (29.3 mmol/L vs 13.9 mmol/L, \( P < 0.0001 \)), and 90 min (20.8 mmol/L vs 9.8 mmol/L, \( P < 0.0001 \)). There was no difference between HFD-L and HFD-V at any time point. Following diet change, HFD-C mice showed glucose levels at each time point akin to those of control mice.

Fasting serum glucose was higher in HFD-V vs control (\( P < 0.01 \), Table 2), with no significant difference between preconception and late gestation fasting glucose. The HFD-L group had lost the protective effect of liraglutide on glycaemic control by late pregnancy, with fasting levels significantly higher than preconception levels (\( P < 0.001 \), Tables 1 and 2). There was no significant difference in fasting glucose between HFD-L and HFD-V groups at late gestation. Fasting glucose was lower in the HFD-C group compared to HFD-L and HFD-V groups (both \( P < 0.001 \)), similar to control. There was no significant difference in fasting blood glucose levels between preconception and late gestation in the HFD-C group. Serum insulin was similar among the four groups. However, insulin resistance, as measured by HOMA-IR, was the most severe in the HFD-V group, compared to the C group (\( P < 0.01 \), Table 2). Despite similar body weights, the HOMA-IR score was lower in HFD-L compared to HFD-V (\( P < 0.0005 \)).
There was only a modest difference between HFD-L and HFD-C groups ($P < 0.05$). HOMA-IR scores were similar between the C and HFD-L, and between the C and HFD-C groups.

In late gestation, serum NEFA was similar between HFD-V and HFD-L groups (Fig. 6A). Serum NEFA was higher in the HFD-V and HFD-L groups compared to the control (both $P < 0.05$). In contrast, the HFD-C group had serum NEFA similar to the control group. Serum total cholesterol, HDL, and LDL were similar between groups in late gestation (Fig. 6B and D). Serum cholesterol levels were not significantly different in late gestation compared to the preconception period for C and HFD-C groups. Meanwhile, both HFD-L and HFD-V groups showed a reduction in levels at late gestation (both $P < 0.05$). Serum TAG was lower in the HFD-V and HFD-L groups compared to control mice (both $P < 0.01$, Fig. 6C). Both control and HFD-L groups had higher TAG at late gestation compared to preconception (both $P < 0.05$). HFD-C TAG levels were not statistically different in preconception and late gestation. HFD-V had reduced TAG levels by late gestation ($P < 0.05$).

Maternal, feti, and placenta anthropometry in late gestation
There were no differences in kidney or liver weights between groups (Table 2). HFD-V mice had a larger gonadal fat mass compared to the control mice ($P < 0.005$). Gonadal fat mass was greater in the HFD-L mice compared to the control groups ($P < 0.005$). HFD-L mice reaccumulated gonadal fat deposits not seen in the HFD-C group ($P < 0.0005$). Similarly, retroperitoneal fat deposits were greater in the HLD-L group compared to the control ($P < 0.05$). There was significant reaccumulation of retroperitoneal fat in HFD-L compared to the HFD-C group ($P < 0.005$). There was no difference between BAT deposits between groups (Table 2).

HFD-V and HFD-L groups had lower number of developed feti compared to C mice ($P < 0.01$ and $P < 0.005$, respectively, Table 2). Diet switch resulted in intermediate numbers of developing feti, with no significant difference between HFD-C, HFD-L, or HFD-V. There were no significant differences between groups in the fetal body weight between the groups. There was a significant difference...
in the placental weights between HFD-C and HFD-L groups ($P < 0.005$).

**Live births**

There was no significant difference in maternal age at the time of delivery between groups (Table 2). Litter size in the HFD-L group was significantly smaller than in the control ($P < 0.005$, Table 2). There was no significant difference between litter sizes at birth between HFD-L and HFD-V, or HFD-C groups.

The control group demonstrated the best overall survival compared to HFD-V mice ($P < 0.05$). The surviving litter sizes, denoted litter size at weaning (Table 2), in HFD-L at wean was smaller than the control ($P < 0.005$), with a greater incidence of infanticide. There was no significant difference in survival between C and HFD-C groups. There was a greater survival of pups in the HFD-C group compared to the HFD-L group ($P < 0.05$).

**Discussion**

Our study employed a mouse model of maternal obesity to demonstrate that weight loss interventions prior to pregnancy can improve preconception body weight, metabolic profiles and restore fertility. As far as we are aware, this is the first study to use mice modelling to demonstrate the benefit of weight modulation prior to pregnancy with diet or liraglutide therapy. We highlighted liraglutide as a potent weight loss medication that improves glucose tolerance, lipid profile, and fertility when administered to obese female mice before pregnancy. However, preconception liraglutide therapy, in the absence of dietary modulation intrapartum, resulted in excessive GWG with less optimal outcomes, including impaired glucose tolerance and fewer live births compared to diet modulation. Pre-pregnancy diet modulation continuing throughout gestation is shown to be more effective in sustaining a lean phenotype throughout pregnancy and to improve glucose tolerance.
in late pregnancy and ultimately more successful birth outcomes.

Our study elucidates the benefit of pre-pregnancy liraglutide for fertility in obesity. In our study, liraglutide led to greater conception rates than diet change alone, suggesting liraglutide’s benefits on fertility beyond weight reduction. Preconception administration of liraglutide was most effective at restoring normal glucose tolerance, reducing insulin resistance, and lowering serum triglycerides with a sustained benefit on insulin resistance and triglyceride levels in late gestation compared with dietary modulation, which are well-known metabolic benefits of liraglutide therapy (Fadini et al. 2013, Li et al. 2017). Importantly, liraglutide was more effective at reducing HOMA-IR than HFD-C. Both liraglutide therapy and diet switch resulted in similar overall benefits in glucose tolerance test performance, as demonstrated by the differences in glucose levels during the 2-h glucose tolerance test and area under the curve analysis, mimicking the control group after 4 weeks. Similar metabolic benefits have been seen in women with obesity and PCOS, in which liraglutide has a greater effect on insulin resistance and lowering of androgen levels (Elkind-Hirsch et al. 2008, Rasmussen & Lindenberg 2014, Cena et al. 2020). A study favourably compared the addition of exenatide, another GLP-1RA, to metformin, on menstrual cycle regularity and hormone profile in PCOS, with greater conception rates (Liu et al. 2017, Cena et al. 2020). Our study thus suggests that liraglutide therapy may have an important role in fertility management in women with obesity.

Weight loss by diet modulation has long been advocated as preconception care for women with obesity (Kominariak & Chauhan 2016, RANZCOG 2017, Denison 2018), albeit hard to achieve (Sand et al. 2017). Studies demonstrate maternal benefits including a lower risk of hyperlipidaemia (Hillemeier et al. 2008, van Oets et al. 2018). Our data are consistent with human observations, that preconception diet change was an effective means of facilitating weight loss, as well as optimising preconception insulin resistance and serum lipids. Dietary modulation improved time to conception, though not to the same extent as liraglutide, and is consistent with published findings in human studies (Moran et al. 2011, Kort et al. 2014). Importantly, diet modification before and during pregnancy restored normal glucose tolerance, with improvement in both first and second phase insulin secretion, insulin resistance, and serum lipids to a greater extent than liraglutide in late gestation with improved live birth outcomes. Therefore, it is an effective, if hard to achieve, strategy with benefits in preconception and late pregnancy.

In this study, liraglutide was not administered during pregnancy as it is currently contraindicated in pregnancy due to potential teratogenic effects (Peterson & Pollom 2010). After conception, the group with preconception liraglutide treatment exhibited ‘catch up’ GWG, which was significantly greater than in all other groups, resulting in similar body weight and fat mass in late gestation to the HFD-fed group without weight loss treatment prior to pregnancy. However, short-term liraglutide treatment showed benefits with less insulin resistance in late pregnancy. In contrast, the ‘diet switch’ group did not display ‘catch up’ GWG in pregnancy. These results suggest that ongoing diet modulation is required to facilitate optimal pregnancy outcomes. In practice, women often find diet modification during pregnancy challenging in the context of an environment with plentiful access to high caloric density foods. Hence, the results seen in the pre-pregnancy liraglutide do demonstrate a role for liraglutide when lifestyle modification is not achievable.

Infanticide in mice is a maladaptive maternal behaviour that can occur shortly after birth (Weber et al. 2013). We noted a higher rate of infanticide in obese mice with or without pre-pregnancy liraglutide treatment, consistent with previous studies (Bellisario et al. 2015, Saben et al. 2016, Powell & Choudhury 2019). A weakness of this study is that investigation into the cause of mortality was not captured, which would have required filming of mice in the peripartum period. The observed cannibalism of dead offspring also precluded post-mortem analyses. Another limitation of the study is that offspring body weight was not recorded directly after birth because in our experience pup handling soon after birth increases infanticide rates.

A strength of our study is the simplicity of our model of maternal obesity and pre-pregnancy weight modulation recapitulating the human experience; women frequently seek medical attention for weight management at times of great motivation, such as preconception. C57Bl/6 mice are well suited to study diet-induced obesity modelling as they are prone to develop obesity, elevated adiposity, glucose intolerance, and moderate insulin resistance (Surwit et al. 1988, Winzell & Ahrén 2004). This polygenic obesity-prone mouse strain demonstrates subfertility that mimics the human context (Weber et al. 2013, Lai et al. 2014), contrasting to well-known monogenic models such as the db/db and Ob/Ob mouse models that exhibit severe obesity but infertility, making them unsuitable for use in studies of maternal obesity and reproductive health (Swerdlaff et al. 1976, Dubuc 1977, Gat-Yablonski & Phillip 2008). Our study did not include a group receiving liraglutide therapy.
and diet switch in the preconception/pregnancy period as we considered the degree of weight loss pre-pregnancy by combined treatments to be unsafe for normal pregnancy and outcomes.

In summary, liraglutide improves pre-pregnancy body weight, glucose tolerance, and fertility. Furthermore, the striking finding of improved fertility in the absence of any diet change elucidates the utility of liraglutide in a sub-fertile obese population. Pre-pregnancy liraglutide treatment alone is not protective of GWG and euglycaemic control associated with HFD consumption in pregnancy. However, it does confer metabolic benefits with respect to insulin sensitivity. Our study suggests that preconception liraglutide therapy is a potentially useful tool in the armamentarium to confer metabolic benefits and facilitate improved fertility in women with obesity. Continuous diet change throughout the prenatal and antenatal period does offer the optimal beneficial effect in late gestation. Further human studies are required to demonstrate if these findings can be translated to benefit human reproduction in women with obesity.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
N R and S G were responsible for the design of the animal protocol, the animal work and laboratory analysis, analysis of results and the writing of the paper. H C aided with the animal work and writing of the paper. C P aided with review of results and writing of the paper.

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