

Maternal glucose intolerance reduces offspring nephron endowment and increases glomerular volume in adult offspring

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Received: 2 November 2015

Revised: 9 March 2016

Accepted: 25 March 2016

Abstract

Background Animal studies report a nephron deficit in offspring exposed to maternal diabetes, yet are limited to models of severe hyperglycaemia which do not reflect the typical clinical condition and which are associated with foetal growth restriction that may confound nephron endowment. We aimed to assess renal morphology and function in offspring of leptin receptor deficient mice (*Lepr^{db/+}*) and hypothesized that exposure to impaired maternal glucose tolerance (IGT) would be detrimental to the developing kidney.

Methods Nephron endowment was assessed in offspring of C57BKS/J *Lepr^{db/+}* and *+/+* mice at embryonic day (E)18 and postnatal day (PN)21 using design-based stereology. Transcutaneous measurement of renal function and total glomerular volume were assessed in 6-month-old offspring. Only *+/+* offspring of *Lepr^{db/+}* dams were analysed.

Results Compared with *+/+* dams, *Lepr^{db/+}* dams had a 20% and 35% decrease in glucose tolerance prior to pregnancy and at E17.5 respectively. Offspring of IGT *Lepr^{db/+}* dams had approximately 15% fewer nephrons at E18.5 and PN21 than offspring of *+/+* dams. There was no difference in offspring bodyweight. Despite normal renal function, total glomerular volume was 13% greater in 6-month-old offspring of IGT *Lepr^{db/+}* dams than in *+/+* offspring.

Conclusions IGT throughout gestation resulted in a nephron deficit that was established early in renal development. Maternal IGT was associated with glomerular hypertrophy in adult offspring, likely a compensatory response to maintain normal renal function. Given the increasing prevalence of IGT, monitoring glucose from early in gestation may be important to prevent altered kidney morphology. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords kidney development; renal function; maternal diabetes; impaired glucose tolerance; developmental programming; nephron number

Abbreviations AUC, area under the curve; E, embryonic day; IGT, impaired glucose tolerance; *Lepr*, leptin receptor; NGT, normal glucose tolerance; PN, postnatal day; PNA, peanut agglutinin; STZ, streptozotocin

Introduction

The worldwide epidemic of obesity and increases in maternal age mean that impaired glucose tolerance (IGT) during pregnancy is an increasingly common condition. Gestational diabetes is reported in 5–17% of pregnancies, and pre-gestational type 2 diabetes is diagnosed in 2% of pregnancies [1–5], with these figures set to rise in coming decades. Given a proportion of women of reproductive age exhibit pre-diabetes [6,7], and that gestational diabetes is typically not screened until mid-late gestation, a significant number of pregnancies may be complicated by undiagnosed pre-existing glucose intolerance.

Renal anomalies and urinary tract malformations are particularly prevalent in offspring of pre-gestational and gestational diabetic women [8–13]. Exposure to hyperglycaemia *in utero* may also impair offspring kidney development in a less overt manner by potentially altering certain cell populations and nephron endowment, which may, in turn, impact on adult renal health and disease [14,15].

We and others have reported a deficit in nephron endowment in offspring of rodent models of diabetic pregnancy [16–18]. These studies have, with the exception of one continuous glucose infusion model [17], utilized the streptozotocin (STZ)-induced type 1 diabetic model of severe fasting hyperglycaemia. These studies typically report a nephron deficit in conjunction with growth restriction [16–19], which is not a frequent neonatal outcome of human diabetic pregnancy. As there is a known positive correlation between birth weight and nephron endowment [20] it is likely that foetal growth restriction in the STZ model of diabetic pregnancy confounds the effect on nephron number.

To date, no animal study has investigated the effect of maternal glucose intolerance on foetal renal development. Analysis of kidney development in animal models that better mimic the increasingly common human condition of maternal glucose intolerance are required, where the maternal phenotype is characterized by mild-moderate hyperglycaemia, insulin resistance and post-prandial hyperglycaemic excursions, and in the absence of offspring growth restriction.

To explore the effect of IGT on the developing kidney, we utilized the leptin receptor deficient mouse (*Lepr^{db/+}*). These mice have a heterozygous mutation in the leptin receptor and spontaneously develop glucose intolerance during gestation. *Lepr^{db/+}* mice are reported to exhibit hyperphagia, increased weight gain and elevated glucose and insulin levels in late gestation compared with wild-type mice [21–24]. Moreover, there is reported to be an increased prevalence of macrosomic offspring [21,22,24–27], making this an attractive model of human gestational diabetes. To determine the effect of IGT on

offspring nephron endowment, kidney development was assessed in offspring of *Lepr^{db/+}* mice using design-based stereology. Renal function and metabolic parameters were also assessed in adult offspring to investigate the long-term consequences of exposure to maternal glucose intolerance.

Materials and methods

Animals and experimental protocol

All animal handling and experimental protocols were approved by the Animal Ethics Committee of Monash University (SOBS A/2011/122) and conformed to guidelines of the National Health and Medical Research Council of Australia. C57BKS/J-*Lepr^{db/+}* + / + *Dock7^m* mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA) at 6 weeks of age. In this strain *Lepr^{db}* is maintained in repulsion with the *misty Dock7^m* mutation. *Dock7^m*/*Dock7^m* mice are reported to have reduced body weight, body length and adipose mass compared with wild-type mice [28]. To therefore avoid the confounding effects of *Dock7^m* on growth traits and to clearly define control mice, in the present study *misty* was removed from the C57BKS/J-*Lepr^{db/+}* + / + *Dock7^m* population through cross-breeding with standard C57BKS/J mice (also purchased from The Jackson Laboratory). Consequently, colonies of C57BKS/J-*Lepr^{db/+}* + / + mice and C57BKS/J mice (hereafter abbreviated as *Lepr^{db/+}* + / + mice and + / + mice, respectively) were established. Mice had *ad libitum* access to standard chow and water with a 12-h light/dark cycle. Age-matched female *Lepr^{db/+}* + / + mice were mated overnight with male + / + mice. The presence of a vaginal plug the following morning was counted as embryonic day (E) 0.5.

Animal weight was recorded prior to mating and throughout pregnancy. Gestational weight gain was calculated by subtracting the weight of dams at E0.5 from their weight at E17.5. Food intake was measured every second day from E0.5 to E17.5.

Glucose tolerance test

Glucose tolerance tests were performed before pregnancy and on E17.5. Mice were fasted for 6 h and injected intraperitoneally with glucose (2 mg/g body weight). Blood was sampled from the tail vein prior to injection and 30, 60, 90 and 120 min after injection, and blood glucose concentration was measured immediately using a glucometer (Accu-Chek Mobile Blood Glucose Monitor, Roche Diagnostics).

Incomplete penetrance of glucose intolerance in *Lepr^{db}/+* mice

Glucose tolerance tests at E17.5 revealed an incomplete penetrance of glucose intolerance in *Lepr^{db}/+* dams. Subsequently, a glucose area under the curve (AUC) value was calculated for each dam derived from the E17.5 glucose tolerance curve. Based on AUC levels greater than 1 standard deviation above the mean for *+/+* mice, *Lepr^{db}/+* mice were categorized with normal glucose tolerance (NGT; $AUC \leq 877$ mmol/l.min) or IGT ($AUC > 877$ mmol/l.min). Forty-five percent of *Lepr^{db}/+* mice displayed an elevated glucose profile. Fourteen percent of *+/+* mice presented with E17.5 glucose $AUC > 877$ mmol/l.min and were excluded from the study.

Tissue collection

Offspring were collected at E17.5 to assess foetal insulin and leptin levels and at E18.5 and postnatal day (PN) 21 to assess kidney development. Early glomerular number was measured at E18.5, the day prior to birth, to assess the maximal effect of the intrauterine environment on kidney development. Nephron formation is complete by PN5–6 in the mouse; hence, final glomerular endowment was measured at weaning (PN21). Embryos were removed, placenta and embryos were weighed and foetal blood was collected. Metanephroi were removed and fixed in 4% paraformaldehyde in phosphate buffered saline. PN21 offspring were anaesthetized, perfusion fixed with paraformaldehyde–glutaraldehyde solution (Karnovsky's Fixative) and the kidneys were collected. To avoid the confounding effect of genotype on offspring development, only *+/+* offspring of *Lepr^{db}/+* dams were analysed.

Plasma insulin and leptin analyses

Maternal and foetal plasma insulin and leptin levels were analysed at E17.5 following a glucose tolerance test. Samples were measured by commercial ELISA (Crystal Chem, Downers Grove, IL, USA). Foetal samples from the one litter were pooled and run in duplicate. The inter-assay coefficient of variation was 3%.

Estimation of glomerular number and volume: histochemistry and stereology

Total glomerular number was determined at E18.5 and PN21 using an unbiased stereological method as previously described [29,30]. Briefly, kidneys were processed to paraffin and exhaustively sectioned at 4 μ m (E18.5)

or 5 μ m (PN21). Ten evenly spaced section pairs were systematically sampled and histochemically stained with the lectin peanut agglutinin (Sigma Aldrich, Castle Hill, NSW, Australia) to localize the plasma membrane of glomerular podocytes. Sections were counterstained with haematoxylin. Section pairs were used to estimate the number of peanut agglutinin-positive glomeruli using the physical disector/fractionator combination. Mean glomerular volume and total glomerular volume were estimated as described previously [31,32].

Assessing metabolic and renal function in adult offspring

To investigate the long-term consequences of maternal glucose intolerance, a cohort of offspring were weaned at PN21 and renal function and metabolic parameters of body weight, glucose tolerance and body composition assessed at 6 months of age. Offspring were fed standard laboratory chow from weaning onwards. Adult mice underwent a glucose tolerance test as previously described. Body composition was measured by dual-energy X-ray absorptiometry (PIXImus2; Lunar, Madison, WI, USA). Renal function was assessed by the non-invasive transcutaneous measurement of FITC-sinistrin clearance (expressed as half life) in conscious mice [33,34]. Spot urine albumin/creatinine ratio was measured by ELISA (Exocell, Philadelphia, PA, USA). Offspring were subsequently perfusion fixed with Karnovsky's Fixative and the kidneys collected for analysis of total glomerular volume as described earlier.

Genotyping for the *Lepr^{db}* mutation

A modified version of Horvat and Bunger's PCR-restriction fragment length polymorphism assay was used to identify the *Lepr^{db}* genotype [35]. DNA was extracted from tail tissue and amplified using RED Extract-N-Amp Tissue PCR kit (Sigma Aldrich, Castle Hill, NSW, Australia). PCR product was digested with *AccI* restriction enzyme overnight. Digests were analysed in a 3.5% agarose gel containing Gel Red (Biotium, Hayward, CA, USA). Samples with 85- and 24-bp fragments were identified as *+/+* and samples with 85-, 58-, 27- and 24-bp fragments were identified as *Lepr^{db}/+*.

Statistical analysis

Statistical analyses were performed with SPSS 20, GraphPad Prism 6 and STATA 13 software. Glucose tolerance tests were analysed by repeated measures ANOVA.

Other maternal parameters were analysed by independent samples *t*-test or one-way ANOVA, as appropriate. Offspring parameters were analysed by two-way ANOVA with offspring sex and maternal genotype as main effects and incorporated a mixed linear model to account for any intra-litter bias [36]. Spearman's rank correlation coefficient was used to measure associations between maternal and offspring variables. Throughout *n* refers to the number of dams or litters. Data are presented as mean \pm standard error of the mean. $P < 0.05$ was considered statistically significant.

Results

Data are presented for $+/+$ mice, and $Lepr^{db}/+$ mice with normal glucose tolerance at E17.5 (NGT $Lepr^{db}/+$) or IGT at E17.5 (IGT $Lepr^{db}/+$).

Maternal glucose intolerance is present before pregnancy

Glucose tolerance tests were performed in a cohort of mice before mating. IGT $Lepr^{db}/+$ mice were found to have mild glucose intolerance prior to pregnancy compared with $+/+$ mice (Figure 1a and b). Peak glucose was 26% higher and

glucose AUC was 17% greater in IGT $Lepr^{db}/+$ mice compared with $+/+$ mice before mating. NGT $Lepr^{db}/+$ mice showed similar pre-pregnancy glucose tolerance to $+/+$ mice and IGT $Lepr^{db}/+$ mice (Figure 1a and b).

Following selection criteria, IGT $Lepr^{db}/+$ dams exhibited glucose intolerance at E17.5 with mild fasting hyperglycaemia (Figure 1c and d). Peak glucose was 62% higher in IGT $Lepr^{db}/+$ dams than in $+/+$ dams. Glucose AUC was 35% greater in IGT $Lepr^{db}/+$ dams. When compared with NGT $Lepr^{db}/+$ dams, IGT $Lepr^{db}/+$ dams had elevated glucose tolerance curves and glucose AUC at E17.5 (Figure 1c and d).

Increased weight gain and food intake in pregnancy

Maternal parameters were assessed in $+/+$ and IGT $Lepr^{db}/+$ dams (Table 1). IGT $Lepr^{db}/+$ mice were heavier prior to mating and at E17.5. IGT $Lepr^{db}/+$ dams had a greater pregnancy weight gain, yet also had significantly greater litter sizes. Weight gain minus uterus weight was elevated in IGT $Lepr^{db}/+$ dams. $Lepr^{db}/+$ dams had a greater food intake compared with $+/+$ dams, regardless of glucose tolerance in late gestation.

Maternal parameters were compared between NGT $Lepr^{db}/+$ and IGT $Lepr^{db}/+$ dams to determine if they predict glucose intolerance in late gestation (Table 1).

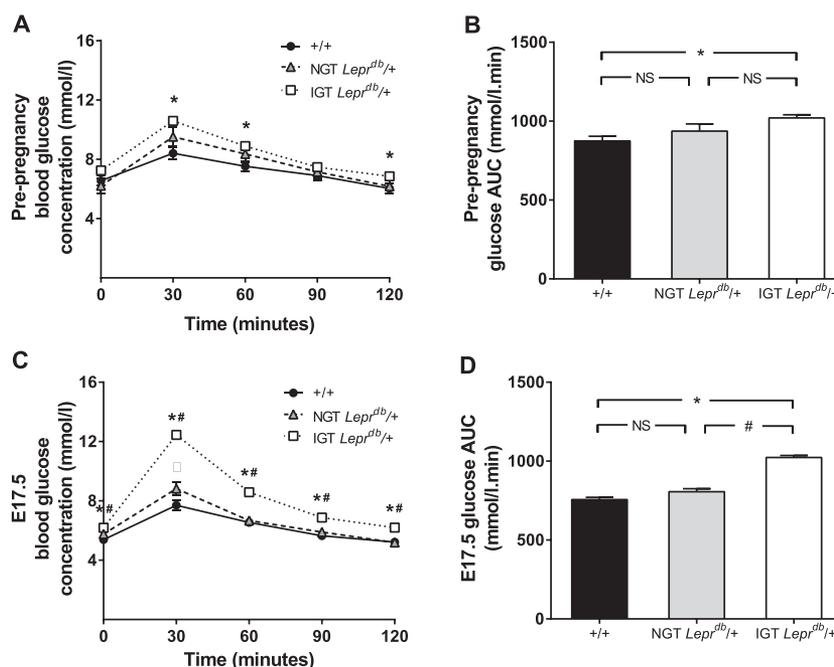


Figure 1. IGT $Lepr^{db}/+$ mice are glucose intolerant prior to pregnancy and in late gestation. (a) Pre-pregnancy glucose tolerance tests and (b) pre-pregnancy glucose AUC in $+/+$ ($n = 13$), NGT $Lepr^{db}/+$ ($n = 6$) and IGT $Lepr^{db}/+$ ($n = 28$) mice. (c) E17.5 glucose tolerance tests and (D) E17.5 glucose AUC in $+/+$ ($n = 36$), NGT $Lepr^{db}/+$ ($n = 27$) and IGT $Lepr^{db}/+$ ($n = 50$) mice. Values are mean \pm SEM. * $P < 0.05$ $+/+$ versus IGT $Lepr^{db}/+$. [†] $P < 0.05$ NGT $Lepr^{db}/+$ versus IGT $Lepr^{db}/+$. # $P < 0.05$ $+/+$ versus NGT $Lepr^{db}/+$

Maternal Diabetes Reduces Nephron Endowment

Table 1. Maternal parameters in +/+, NGT *Lepr^{db}/+* and IGT *Lepr^{db}/+* mice

| | +/+ (n) | NGT <i>Lepr^{db}/+</i> (n) | IGT <i>Lepr^{db}/+</i> (n) | <i>P</i> _{ANOVA} |
|---|-----------------|------------------------------------|------------------------------------|---------------------------|
| Weight prior to pregnancy (g) | 20.3 ± 0.3 (34) | 22.2 ± 0.4 (26) | 23.5 ± 0.3 (49) * ** | <0.0001 |
| Weight E17.5 (g) | 32.5 ± 0.5 (34) | 35.3 ± 0.6 (23) * | 37.4 ± 0.4 (48) * ** | <0.0001 |
| Pregnancy weight gain (g) | 12.3 ± 0.3 (33) | 13.2 ± 0.4 (22) | 14.0 ± 0.3 (47) * | 0.001 |
| Uterus weight (including conceptus) (g) | 9.1 ± 0.7 (8) | 9.3 ± 0.8 (6) | 9.8 ± 0.6 (11) | 0.73 |
| Weight gain – uterus weight (g) | 3.6 ± 0.4 (8) | 3.8 ± 0.5 (6) | 4.9 ± 0.4 (11) * | 0.05 |
| Litter size | 5.7 ± 0.2 (34) | 6.2 ± 0.5 (10) | 6.5 ± 0.2 (43) * | 0.05 |
| Total food intake (g) | 38.7 ± 1.6 (8) | 46.2 ± 2.3 (4) * | 45.3 ± 1.4 (11) * | 0.01 |

Values are mean ± SEM. Bold highlights statistically significant *P* values.

**P* < 0.05, +/+ versus IGT *Lepr^{db}/+*.

***P* < 0.05 NGT *Lepr^{db}/+* versus IGT *Lepr^{db}/+*.

Lepr^{db}/+ dams that were deemed glucose intolerant at E17.5 were found to be slightly heavier prior to pregnancy and at E17.5. However, there was no difference in pregnancy weight gain, litter size or food intake between *Lepr^{db}/+* dams that did or did not exhibit abnormal glucose handling.

Maternal and foetal hyperinsulinaemia and hyperleptinaemia

IGT *Lepr^{db}/+* dams were hyperinsulinaemic and hyperleptinaemic at E17.5 compared with +/+ dams (Figure 2). Foetal plasma was reflective of the maternal environment (Figure 2).

No effect of glucose intolerance in pregnancy on early offspring growth

Foetal body weight (Figure 3a), placental weight and foetal : placental weight ratio were similar in offspring of +/+, NGT *Lepr^{db}/+* and IGT *Lepr^{db}/+* dams at E18.5. Male offspring and placentae were heavier than female offspring. Only offspring of +/+ dams and IGT *Lepr^{db}/+* dams were assessed in the early postnatal period. At PN2, pups of IGT *Lepr^{db}/+* dams were significantly heavier than pups of +/+ dams (Figure 3b) although it should be noted that offspring genotypes were not known at this time. At PN21, there was no effect of maternal glucose intolerance on offspring body weight (Figure 3c). Male offspring were significantly heavier than female offspring at both postnatal time-points.

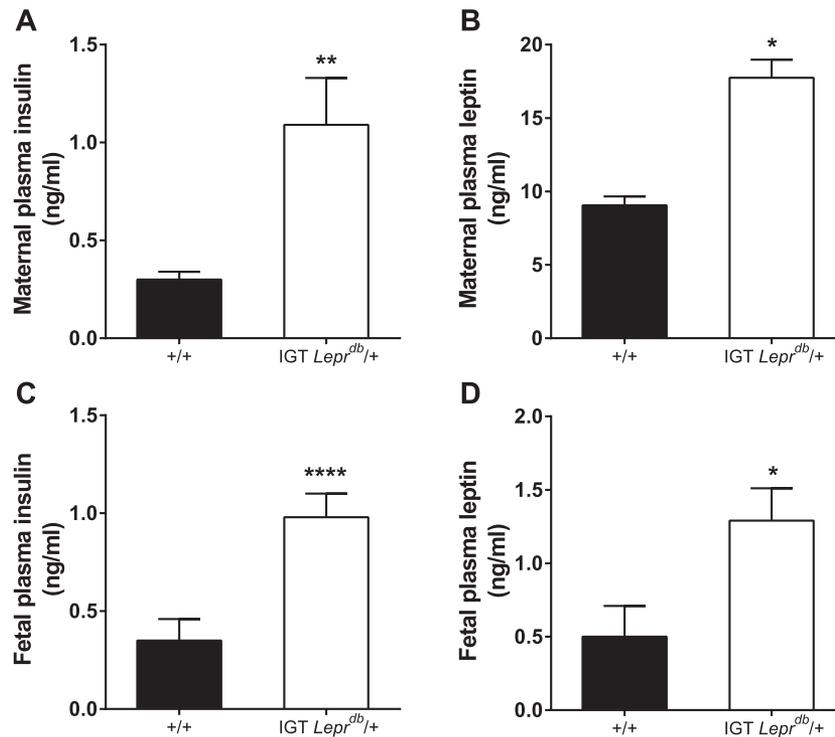


Figure 2. Maternal and foetal hyperinsulinaemia and hyperleptinaemia. (a) Maternal plasma insulin and (b) leptin in +/+ (n = 12) and IGT *Lepr^{db}/+* dams (n = 12) at E17.5. (c) Foetal plasma insulin and (d) leptin in +/+ (n = 6 litters) and IGT *Lepr^{db}/+* dams (n = 6 litters) at E17.5. Values are mean ± SEM. **P* < 0.05

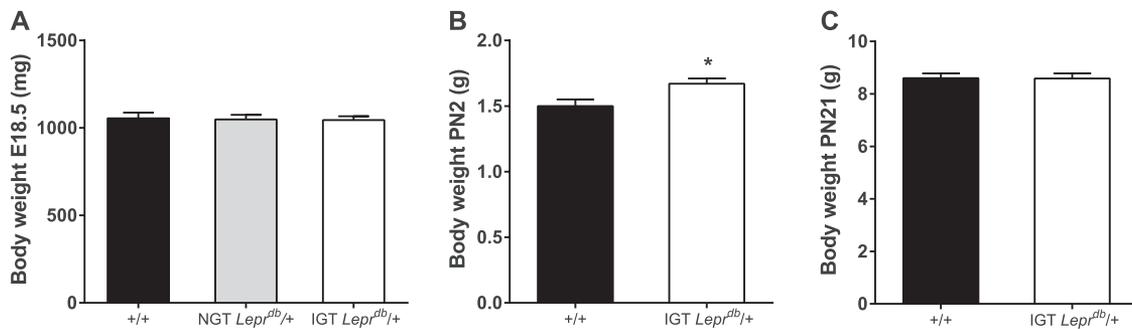


Figure 3. No effect of glucose intolerance in pregnancy on early offspring growth. (a) Body weight of offspring of +/+ ($n = 7$ litters), NGT *Lepr^{db}/+* ($n = 5$ litters) and IGT *Lepr^{db}/+* ($n = 10$ litters) dams at E18.5. (b) Body weight at PN2 and (c) at PN21 of offspring of +/+ ($n = 23$ litters) and IGT *Lepr^{db}/+* ($n = 25$ litters) dams. Values are mean \pm SEM. * $P = 0.05$

Offspring of glucose intolerant dams have a lower glomerular endowment

Glomerular number was assessed in offspring of +/+, NGT *Lepr^{db}/+* and IGT *Lepr^{db}/+* dams at E18.5. Offspring of +/+ dams had a similar number of glomeruli as offspring of NGT *Lepr^{db}/+* dams. However, offspring of IGT *Lepr^{db}/+* dams had approximately 16% fewer glomeruli than offspring of +/+ and NGT *Lepr^{db}/+* dams (Figure 4a) and significantly fewer glomeruli per milligram of body weight. The negative association between maternal glucose AUC and offspring glomerular number at E18.5 is shown in Figure 4b. Representative images of sectioned E18.5 kidneys from offspring of +/+ and IGT *Lepr^{db}/+* dams are shown in Figure 5.

Final glomerular endowment was assessed at PN21 in offspring of +/+ and IGT *Lepr^{db}/+* dams. Offspring of IGT *Lepr^{db}/+* dams had 14% fewer glomeruli than offspring of +/+ dams (Figure 4c). Female offspring had more glomeruli than male offspring. As observed at E18.5, the glomerular number to body weight ratio was

significantly lower in offspring of glucose intolerant dams. There was no difference in kidney weight, kidney volume, mean glomerular volume or total glomerular volume at PN21 between the groups (data not shown).

The association between E17.5 maternal parameters (in +/+, NGT *Lepr^{db}/+* and IGT *Lepr^{db}/+* dams) and offspring parameters at E18.5 are presented in Table 2. Offspring glomerular endowment was found to have a significant negative association with maternal glucose AUC.

Adult offspring of glucose intolerant dams have normal metabolic function

A cohort of littermates from +/+ dams and IGT *Lepr^{db}/+* dams was weaned at PN21 and assessed at 6 months of age. Adult offspring had similar body weight trajectories (Figure 6a), with males heavier than females. Dual-energy X-ray absorptiometry revealed no difference in percentage body fat (Figure 6b) between offspring of

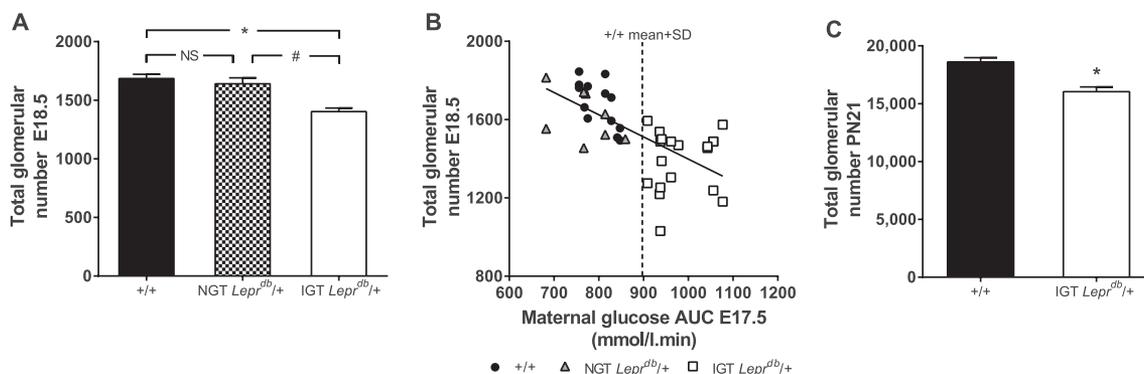


Figure 4. Offspring of glucose intolerant dams have a lower glomerular endowment. (a) Total glomerular number in kidneys of offspring of +/+ ($n = 7$ litters), NGT *Lepr^{db}/+* ($n = 5$ litters) and IGT *Lepr^{db}/+* ($n = 8$ litters) dams at E18.5. (b) Plot of maternal glucose AUC at E17.5 against E18.5 offspring glomerular number of +/+, NGT *Lepr^{db}/+* and IGT *Lepr^{db}/+* dams. $P < 0.0001$, $r_s = 0.74$. Dashed line indicates cut-off criteria used to identify glucose tolerant and glucose intolerant *Lepr^{db}/+* dams. Solid line denotes line of best fit. (c) Total glomerular number in kidneys of offspring of +/+ ($n = 7$ litters) and IGT *Lepr^{db}/+* ($n = 9$ litters) dams at PN21. Values are mean \pm SEM. * $P < 0.05$, +/+ versus IGT *Lepr^{db}/+*. # $P < 0.05$ NGT *Lepr^{db}/+* versus IGT *Lepr^{db}/+*

Maternal Diabetes Reduces Nephron Endowment

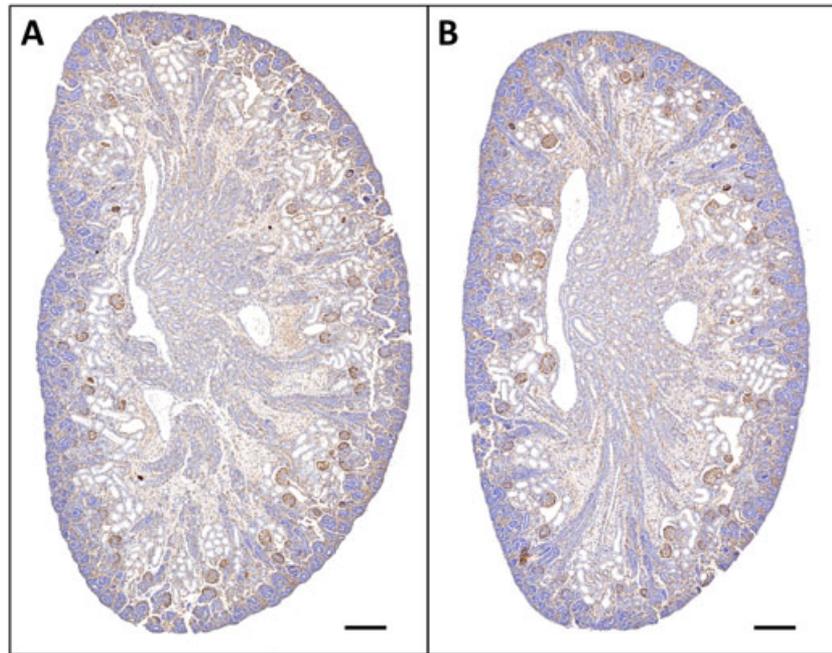


Figure 5. Representative images of sectioned E18.5 kidneys. Kidney sections from (a) offspring of +/+ dam and (b) offspring of IGT *Lepr^{db}/+* dam. Sections histochemically stained for peanut agglutinin to identify glomeruli from the S-shaped stage to mature stage. Scale bar = 200 μ m

Table 2. Associations between kidney development at E18.5 and offspring body weight and maternal factors

| | Offspring body weight E18.5 | Offspring nephron number E18.5 | Offspring kidney volume E18.5 | Maternal glucose AUC E17.5 |
|--------------------------------|-----------------------------|--------------------------------|-------------------------------|----------------------------|
| Offspring nephron number E18.5 | 0.153 | — | — | — |
| | 0.32 | — | — | — |
| Offspring kidney volume E18.5 | 0.138 | 0.526 | — | — |
| | 0.38 | 0.0004 | — | — |
| Maternal glucose AUC E17.5 | -0.183 | -0.704 | -0.301 | — |
| | 0.24 | < 0.0001 | 0.06 | — |
| Maternal pregnancy weight gain | -0.281 | -0.257 | -0.257 | 0.600 |
| | 0.08 | 0.0003 | 0.13 | < 0.0001 |

r_s (top value), P (bottom value). Negative values indicate a negative association. Values in bold are statistically significant.

+/+ and IGT *Lepr^{db}/+* dams. There was no difference in fasting glucose, glucose tolerance or glucose AUC (Figure 6 c and d). Male offspring had elevated glucose profiles and percentage body fat compared with female offspring.

Adult offspring of glucose intolerant dams have normal renal function but exhibit glomerular hypertrophy

Maternal glucose intolerance had no effect on kidney weight, renal function or urine albumin/creatinine ratio in offspring at 6 months (Table 3). Despite a nephron deficit, total glomerular volume was 13% greater in offspring of IGT *Lepr^{db}/+* dams compared with offspring of +/+ dams. Histological analysis of kidneys in offspring at 6 months of age showed no evidence of renal pathology (data not shown).

The association between maternal variables in pregnancy and parameters of offspring health at 6 months of age are presented in Table 4. Maternal pregnancy glucose AUC and maternal weight gain were weakly associated with total glomerular volume. Offspring body weight had a strong positive association with offspring glucose AUC. Offspring renal function had a negative association with offspring body weight and glucose AUC.

Discussion

The present study has three major findings: (i) *Lepr^{db}/+* dams exhibit pre-gestational glucose intolerance and incomplete penetrance of glucose intolerance in late gestation – phenotypes not previously described; (ii)

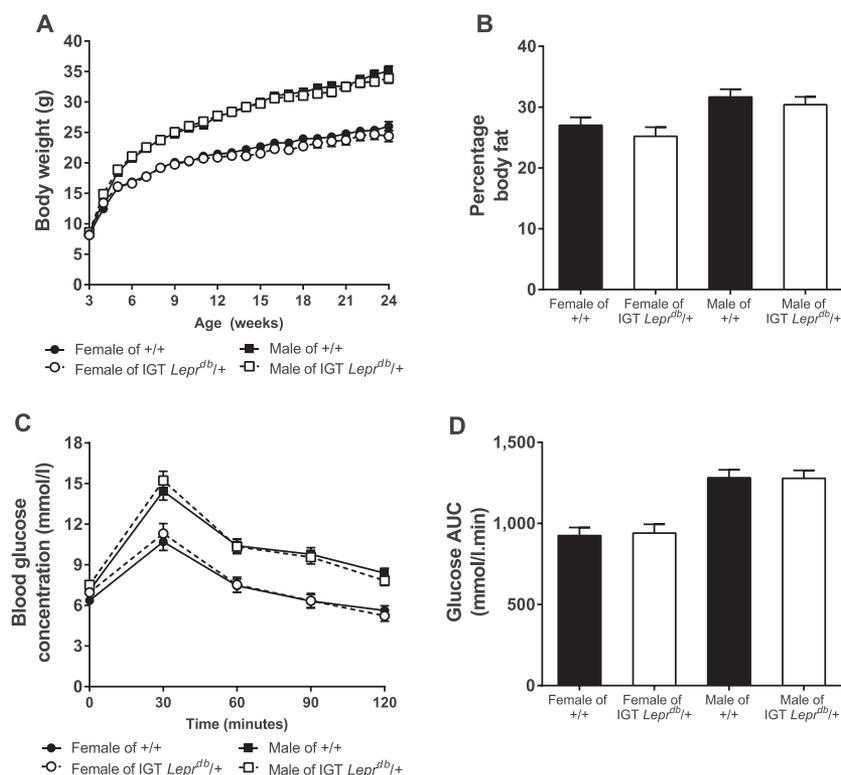


Figure 6. Normal body weight, body composition and glucose tolerance in adult offspring of glucose intolerant dams. (a) Growth trajectories, (b) percentage body fat, (c) glucose tolerance and (d) glucose AUC of offspring of +/+ dams ($n = 11$ litters) and IGT $Lepr^{db/+}$ dams ($n = 12$ litters) at 6 months. Values are mean \pm SEM

Table 3. Renal parameters of offspring of +/+ dams and IGT $Lepr^{db/+}$ dams at 6 months

| | Female of +/+ | Female of IGT $Lepr^{db/+}$ | Male of +/+ | Male of IGT $Lepr^{db/+}$ | P_{group} | P_{sex} | $P_{group*sex}$ |
|---|-------------------|-----------------------------|-------------------|---------------------------|-------------|-------------------|-----------------|
| Kidney weight (mg) | 340.1 \pm 22.2 | 312.8 \pm 25.6 | 494.0 \pm 22.3 | 527.4 \pm 23.2 | 0.92 | <0.0001 | 0.19 |
| Kidney weight: body weight (mg/g) | 14.0 \pm 0.6 | 13.8 \pm 0.7 | 16.4 \pm 0.6 | 16.4 \pm 0.6 | 0.87 | <0.0001 | 0.85 |
| Renal function, t half-life (min) | 8.7 \pm 0.6 | 9.1 \pm 0.6 | 12.8 \pm 0.5 | 11.8 \pm 0.5 | 0.61 | <0.0001 | 0.21 |
| Urinary albumin: creatinine (μ g/mg) | 11.0 \pm 1.9 | 12.2 \pm 2.0 | 16.4 \pm 1.7 | 17.1 \pm 1.9 | 0.61 | 0.01 | 0.87 |
| Total glomerular volume (mm^3) | 2.037 \pm 0.084 | 2.377 \pm 0.097 | 2.108 \pm 0.087 | 2.319 \pm 0.093 | 0.01 | 0.93 | 0.47 |
| Total glomerular volume: body weight (mm^3/g) | 0.084 \pm 0.004 | 0.099 \pm 0.005 | 0.067 \pm 0.004 | 0.072 \pm 0.005 | 0.04 | <0.0001 | 0.29 |

+/+ ($n = 11$ litters), IGT $Lepr^{db/+}$ ($n = 12$ litters). Values are mean \pm SEM. Bold highlights statistically significant P values.

offspring of glucose intolerant pregnancies have a deficit in nephron endowment established early in nephrogenesis; and (iii) adult offspring exposed to glucose intolerance during gestational kidney development have an elevated total glomerular volume suggestive of adaptive changes in renal morphology.

The leptin receptor deficient $Lepr^{db/+}$ mouse is repeatedly reported to be a model of gestational diabetes, with mice displaying normal plasma glucose levels and glucose handling prior to pregnancy and glucose intolerance in late gestation [21–24,26]. Here we present a model of pre-gestational IGT with selected $Lepr^{db/+}$ dams

exhibiting mild post-prandial hyperglycaemia prior to pregnancy, contrary to previous reports [22,24]. Glucose intolerance in $Lepr^{db/+}$ mice was exacerbated in late gestation owing to mild fasting hyperglycaemia and a marked elevation in 30-min peak glucose levels as previously reported [21–24,26]. Surprisingly, lower glucose AUC values were observed in +/+ dams in late pregnancy compared with pre-pregnancy, an observation not previously described in $Lepr^{db/+}$ mice. This decrease was not seen in IGT $Lepr^{db/+}$ mice and contributes to the marked glucose intolerance observed in $Lepr^{db/+}$ dams compared with controls in late gestation.

Maternal Diabetes Reduces Nephron Endowment

Table 4. Associations between offspring metabolic and renal function at 6 months of age and maternal variables

| | Offspring body weight | Offspring glucose AUC | Offspring renal function | Offspring total glomerular volume | Maternal glucose AUC E17.5 |
|-----------------------------------|---------------------------------|-------------------------------|--------------------------|-----------------------------------|-------------------------------|
| Offspring glucose AUC | 0.806 < 0.0001 | — | — | — | — |
| Offspring renal function | -0.550 0.0002 | -0.425 0.006 | — | — | — |
| Offspring total glomerular volume | 0.125 0.40 | 0.237 0.11 | -0.057 0.72 | — | — |
| Maternal glucose AUC E17.5 | -0.035 0.81 | 0.111 0.46 | -0.069 0.66 | 0.351 0.02 | — |
| Maternal pregnancy weight gain | -0.121 0.43 | -0.046 0.76 | 0.024 0.88 | 0.321 0.03 | 0.502 0.0004 |

r_s (top value), P (bottom value). Negative values indicate a negative association. Values in bold are statistically significant.

Similar to previous reports, the glucose intolerance in *Lepr^{db}/+* dams was associated with excess maternal weight gain, hyperinsulinaemia and hyperphagia. It is important to note in our study, however, that food intake was elevated in *Lepr^{db}/+* mice regardless of glucose tolerance status. Pregnancy weight gain was confounded by larger litter sizes but is supported by the observed increase in weight gain minus uterus weight indicating greater fat mass in *Lepr^{db}/+* dams. *Lepr^{db}/+* mice had an elevated body weight prior to pregnancy, and it may be this novel phenotype that explains our model of pre-gestational glucose intolerance. The finding that IGT *Lepr^{db}/+* mice were heavier than NGT *Lepr^{db}/+* mice before pregnancy further supports this hypothesis. It should be noted that blood pressure was not measured during pregnancy. Chronic leptin infusion is previously shown to induce hypertension in pregnant rats, with an approximate 20-fold increase in serum leptin levels associated with a 20 mmHg increase in mean arterial pressure [37]. In the present study, *Lepr^{db}/+* dams exhibited a twofold increase in leptin levels because of leptin resistance; however, it is unknown what effect hyperleptinaemia may have on blood pressure in this mouse model. This warrants further investigation as maternal hypertension may contribute to the alterations reported in offspring development.

Foetal offspring were hyperinsulinaemic (suggestive of foetal pancreatic involvement) and hyperleptinaemic (reflective of the maternal environment or increased placental leptin production). Contrary to what has been reported in the majority of previous *Lepr^{db}/+* studies and despite foetal hyperinsulinaemia, offspring macrosomia was not observed in the present study. The discrepancy in foetal weight may be because of the inclusion of *Lepr^{db}/+* pups in previous studies [21–24,26], as a recent report found foetal genotype to be more important than the maternal environment in determining foetal overgrowth in offspring of *Lepr^{db}/+* dams [38].

In this study, the ability to predict which *Lepr^{db}/+* mouse would exhibit IGT in pregnancy was difficult.

Compared with NGT *Lepr^{db}/+* mice, IGT *Lepr^{db}/+* mice did not differ in weight gain or food intake and were only slightly heavier prior to pregnancy and in late gestation. Selecting mice based on their glucose profiles is somewhat cumbersome and requires a larger number of experimental animals. However, an advantage of *Lepr^{db}/+* dams exhibiting incomplete penetrance of glucose intolerance in late gestation was the opportunity to assess offspring of *Lepr^{db}/+* dams with normal glucose tolerance. This enabled the effects of maternal genotype and leptin receptor deficiency to be isolated from the effects of maternal glucose.

Offspring of glucose intolerant mice had a permanent deficit in nephron endowment. The reduction in glomerular number was observed early in nephrogenesis at E18.5, when only a small proportion of glomeruli had formed [39,40]. Importantly, nephron number was normal in offspring of NGT *Lepr^{db}/+* dams, indicating that the nephron deficit observed in offspring of IGT *Lepr^{db}/+* dams was a consequence of maternal glucose intolerance rather than maternal leptin receptor deficiency.

Previous studies investigating nephron number in rodent models of maternal diabetes have mostly utilized the Type 1 diabetes model of STZ-induced selective toxicity of pancreatic islet cells, which results in severe fasting hyperglycaemia. These studies reported a 15–40% deficit in nephron endowment that was proportionate to the magnitude of maternal fasting glucose (fasting values ranging from 11 to 28 mmol/l in STZ-treated dams), with reductions in offspring birth weight exacerbating (yet confounding) the nephron deficit [16–18,41]. An advantage of the present study is the lack of growth restriction in offspring of IGT dams. E17.5 dams had mild fasting hyperglycaemia with moderate postprandial hyperglycaemic excursions comparable to previous studies utilising *Lepr^{db}/+* mice. The mild 15% reduction in glomerular number reported in the present study supports the results observed in the STZ paradigm. Despite quite different glucose profiles (periodic, moderate increases in glucose after feeding *versus* constant and profound elevations in fasting glucose), our findings support

the hypothesis that an increase in glucose during kidney development may alter nephrogenesis and thereby nephron endowment.

Adult offspring of pre-gestational diabetic dams were assessed at 6 months of age and failed to demonstrate any long-term consequences on body weight, body composition or glucose handling. Few studies have assessed offspring born to *Lepr^{db}/+* dams in adulthood. Lambin *et al.* [25] and Nadif *et al.* [38] reported IGT and overweight in male but not female offspring of *Lepr^{db}/+* dams at 2–3 months of age, whereas Yamashita *et al.* [22] reported similar body weight but increased insulin levels and percentage fat in female but not male offspring at 6 months of age. Equivocal effects on glucose handling and body weight are also described in adult offspring born to STZ-treated dams [41–45].

The effect on offspring renal function and morphology is less ambiguous, with proteinuria, glomerular hypertrophy, reduced renal function, glomerulosclerosis and hypertension commonly reported in adult offspring exposed to severe fasting hyperglycaemia during kidney development [41–45]. Interestingly, these effects have been described in offspring with and without a reduction in nephron endowment and thus suggest that mechanisms other than a nephron deficit may contribute to the progression of kidney disease in offspring exposed to maternal diabetes. In the present study, renal function and albuminuria were comparable in offspring of glucose tolerant and glucose intolerant pregnancies. Total glomerular volume was elevated at 6 months of age despite a nephron deficit, suggesting a compensatory change in glomerular size and thereby glomerular filtration surface area in order to maintain normal renal function in adulthood. Although blood pressure was not measured in the present study, kidney sections from 6-month-old offspring were assessed for evidence of renal pathology. We found no indication of interstitial fibrosis, vascular thickening, tubular atrophy or glomerular lesions that would be expected in hypertensive animals. Future studies to assess further compensatory changes in renal function, glomerular morphology and blood pressure in ageing offspring of *Lepr^{db}/+* dams are required. Given the relatively mild maternal glucose profile of this study compared with that of STZ studies, it would also be interesting to challenge offspring with a high-salt or high-fat diet to assess

the physiological limits of offspring metabolic and renal function.

In conclusion, the *Lepr^{db}/+* phenotype reported in this study highlights the difficulty in obtaining a reliable and consistent mouse model of human gestational diabetes. We do, however, present a valuable model of pre-gestational diabetes, as the IGT *Lepr^{db}/+* mouse shares features of the increasingly common human condition; maternal overweight, insulin resistance and glucose intolerance before and during pregnancy. We present the first report of kidney development and renal function in offspring exposed to maternal glucose intolerance. We show that a moderate postprandial increase in glucose can have an adverse and permanent impact on nephron endowment and glomerular volume in adulthood and that these deficits are comparable to what is reported in offspring exposed to constant and severe fasting hyperglycaemia.

Acknowledgements

The authors acknowledge the facilities and members of the Histology Platform at Monash University. SH received an Australian Postgraduate Award to support her PhD candidature.

Funding

This study was funded by Diabetes Australia Research Trust (Y13G-CULL).

Author contributions

SH, JAA, JFB and LAC-M contributed to the study's conception and design. SH, NA and KF performed all experiments. SH analysed results and drafted the manuscript. All authors revised the article's intellectual content and approved the final version. LAC-M is the guarantor of this work.

Conflicts of interest

The authors declare that there is no duality of interest associated with this manuscript.

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Maternal Diabetes Reduces Nephron Endowment

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