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ORIGINAL ARTICLE

Cross-fostering and improved lactation ameliorates deficits in endocrine pancreatic morphology in growth-restricted adult male rat offspring

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Uteroplacental insufficiency and poor postnatal nutrition impair adult glucose tolerance and insulin secretion in male rat offspring, which can be partially ameliorated by improving postnatal nutrition. Uteroplacental insufficiency was induced in the WKY rat on day 18 of pregnancy (*Restricted*) compared to sham-operated *Controls*. Pups were then cross-fostered onto *Control* or *Restricted* mothers one day after birth resulting in: (*Pup*-on-*Mother*) *Control*-on-*Control*, *Control*-on-*Restricted*, *Restricted*-on-*Control* and *Restricted*-on-*Restricted.* Endocrine pancreatic morphology and markers of intrinsic β-cell function and glucose homeostasis were assessed in male offspring at 6 months. Pancreatic and hepatic gene expression was quantified at postnatal day 7 and 6 months. *Restricted* pups were born 10–15% lighter than *Controls* and remained lighter at 6 months. Relative islet and β-cell mass were 51–65% lower in *Restricted*-on-*Restricted* compared to *Controls* at 6 months. Non-fasting plasma C-reactive protein levels were also increased, suggestive of an inflammatory response. Overall, the average number of islets, small islets and proportion of β-cells per islet correlated positively with birth weight. Intrinsic β-cell function, estimated by insulin secretion relative to β-cell mass, was unaffected by *Restriction*, suggesting that the *in vivo* functional deficit was attributable to reduced mass, not function. Importantly, these deficits were ameliorated when lactational nutrition was normalized in *Restricted*-on-*Control* offspring, who also showed increased pancreatic *Igf1r, Pdx1* and *Vegf* mRNA expression at 7 days compared to *Control*-on-*Control* and *Restricted*-on-*Restricted*. This highlights lactation as a critical period for intervention following prenatal restraint, whereby deficits in endocrine pancreatic mass and associated impaired *in vivo* insulin secretion can be ameliorated.

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Introduction

Uteroplacental insufficiency causes much of the intrauterine growth restriction observed in Western society and increases the predisposition to adult metabolic diseases.[1–3] Furthermore, fetal growth restriction in other species can also impair whole body insulin sensitivity, insulin secretion and glucose tolerance in offspring.[4–6] Type 2 diabetes is characterized by compromised insulin inhibition of hepatic glucose production and stimulation of skeletal muscle glucose uptake, as well as impaired insulin secretion from the β-cell.[7] In fact, Type 2 diabetes is increasingly regarded as a disease of insulin insufficiency with failure to compensate for insulin resistance, due to inherent pancreatic β-cell dysfunction and reduced β-cell mass.[8,9] Local and systemic inflammation may be another important mechanism in the development of Type 2 diabetes in many individuals.[10] Increased circulating C-reactive protein (CRP) as a marker of systemic low-grade inflammation is associated with β-cell dysfunction and is an independent predictor of diabetes.[11,12]

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Bilateral uterine vessel ligation in late gestation of rats to induce uteroplacental insufficiency restricts fetal growth and induces insulin secretory defects leading to impaired glucose tolerance in adult male offspring who are cross-fostered onto Control dams.[4,5] When we cross-foster *Restricted* male offspring onto a different *Restricted* mother, this also adversely impacts on insulin secretion and glucose control compared to *Control*-on-*Control* male offspring.[13] In contrast, we have demonstrated that glucose tolerance, peripheral insulin sensitivity and relative adiposity are unchanged in growth-restricted offspring induced by bilateral uterine vessel ligation that remain with their biological mothers.[14] We have shown that following uteroplacental insufficiency in late gestation, dams produce less milk per pup with altered composition.[15] Improving postnatal nutritional quality and quantity, however, by cross-fostering growth-restricted offspring onto a *Control* mother partially corrected the impaired glucose tolerance and compromised insulin secretion in *Restricted*-on-*Restricted* male offspring. Hence, adult glucose control in these rats may be modifiable by the early postnatal environment.

In rodents, regulation of β-cell mass is particularly susceptible to nutritional and hormonal disturbances during late gestation when the majority of β-cell formation occurs.[16,17]

Growth and remodeling of pancreatic β-cells continue into the immediate postnatal period in the rat.[18] It is during this time that the perinatal β-cell population, which is somewhat unresponsive to glucose, is replaced with mature β-cells, which are highly glucose responsive.[8] Growth-restricted offspring are commonly born with a reduced β-cell mass that is less capable of proliferating at a normal rate, due to altered β-cell gene transcription and metabolism.[4,19,20] We have recently shown that the percentage of pancreatic islets per whole pancreas and β-cell mass was reduced by 45–65% in male rats exposed to uteroplacental insufficiency that remained with their biological mothers, when compared to *Controls* at 9 and 24 weeks of age.[21] As greater metabolic stress is exerted later in life, β-cell exhaustion of the limited population may occur, leading to the impairment of glucose tolerance.[8] The question that remains, however, is whether β-cell mass and key underlying molecular determinants can be restored or normalized by improving postnatal nutrition via cross-fostering. The latter include insulin-like growth factors (IGFs), vascular endothelial growth factor (Vegf) and the transcription factor, pancreatic duodenal homeobox-1 (Pdx1).[21–29] β-cell mass expansion is reliant on normal glucose uptake in the β-cell via the glucose transporter (Glut2) and the preservation of insulin signaling, including the downstream protein kinase, v-akt murine thymoma viral oncogene homolog 2 (Akt2). Upon insulin binding to its receptor in the liver, activation of Akt2 inhibits transcription of phosphoenolpyruvate carboxykinase (Pepck) and glucose-6-phosphatase, thus inhibiting hepatic glucose production.[5,30–33] These mechanisms are also required to maintain adaptive hyperinsulinemia during worsening insulin sensitivity.

In this study, we explored the effects of uteroplacental insufficiency on endocrine pancreas morphology, intrinsic β-cell and metabolic function and its molecular determinants in the pancreas of cross-fostered male offspring at 6 months of age. In addition, we examined molecular determinants of metabolic function in the pancreas one week after cross-fostering to determine the earlier effects of growth restriction. We also examined molecular determinants of hepatic contributions to metabolic control at these ages. We hypothesized that cross-fostering *Restricted* offspring onto *Control* mothers would rescue the pancreatic phenotype via differential regulation of its molecular determinants. Our previous studies have shown a clear metabolic phenotype in adult male cross-fostered offspring.[13] Of particular relevance to this study was the deficit in first-phase insulin secretion with concomitant impaired glucose tolerance in *Restricted*-on-*Restricted* males, which was absent in female offspring. This was then ameliorated by improving postnatal nutrition, by cross-fostering Restricted male pups onto a Control mother.[13] Therefore, this study analyzed samples from male offspring only. This is the first time that this characterization has been performed in a well-established rat model of growth restriction, incorporating cross-fostering protocols to delineate both prenatal and postnatal influences.

Method

Animals and growth measurements

All experiments were approved by The University of Melbourne Animal Ethics Committee prior to commencement. Wistar Kyoto rats (11 weeks of age) were obtained from the Australian Resource Centre (Murdoch, WA, Australia). They were provided with 12 h light/dark cycle and had access to food and water *ad libitum*. On day 18 of gestation, pregnant rats were randomly allocated to *Restricted* or *Control* (sham surgery) groups. The *Restricted* group underwent bilateral uterine artery and vein ligation to induce uteroplacental insufficiency as described previously.[34–36] Although the uteroplacental insufficiency surgery induced a reduction in litter size, our previous studies have shown no clear effect on metabolic outcomes of including a matched litter size control in addition to a sham-surgery control.[13] Pups were cross-fostered one day after birth onto a different sham-operated or *Restricted* mother as previously described.[13,34,37,38] This generated four experimental groups: (*Pup*-on-*Mother*) *Control*-on-*Control*, *Control*-on-*Restricted*, *Restricted*-on-*Control* and *Restricted*-on-*Restricted*, with a similar number of male and female pups in each litter. Offspring studied at 6 months of age were weaned at postnatal day 35, as in previous studies with only one randomly selected male offspring from each litter studied from eight independent litters ($n = 8$ total offspring studied).[13,34,39] A separate cohort was generated on postnatal day 7 in which tissues collected from male offspring were pooled within the same litter ($n = 8$ total litters studied), with weights and dimensions also recorded.

Post-mortem tissue and blood collection and analyses

Non-fasted male rats analyzed at 6 months of age were anesthetized with an intraperitoneal injection of Ketamine (30 mg/kg body weight) and Ilium Xylazil-20 (225 mg/kg body weight). Blood was collected via cardiac puncture at post-mortem (6 months) and plasma stored at −20°C. The whole pancreas was rapidly excised, weighed and a representative portion ($\sim$1 cm^3) from the hepatic end was fixed in 10% NBF for immunohistochemical analyses at 6 months. The liver and remaining pancreatic tissue at 6 months, and the liver and whole pancreata at 7 days pooled from males of the same litter, were snap-frozen in liquid nitrogen and stored at −80°C. Circulating CRP, total cholesterol, non-esterified fatty acids (NEFA), pancreatic-amylase and triglycerides were measured in duplicate by colorimetric enzymatic analysis on an automated centrifugal analyser (COBAS Mira, Roche Diagnostics, Castle Hill, NSW, Australia) in non-fasted plasma samples collected at post mortem at 6 months. Although all assays were designed for measuring specified metabolites in human plasma, validation confirmed 100% cross-reactivity with rat plasma in our analyses. The lower detection limits (sensitivity) and ranges for each of the assays were as follows: CRP 0.4–239 ng/ml; total cholesterol

3.43–5.45 mmol/l; NEFA 0.81–1.24 mmol/l; pancreatic-amylase 3–2386 mmol/l; triglycerides 1.23–2.52 mmol/l.

Immunohistochemistry and morphometric analysis

At 6 months, one pancreatic section (10 μm; $n = 5$–8 per group) was immunostained for insulin using polyclonal guinea-pig anti-rat insulin as previously described.[25] Samples were code-blinded to remove potential bias while performing analyses. Stained sections were visualized using a Light Zeiss microscope, camera and software (AxioCam MRc5, Carl Zeiss Pty. Ltd, North Ryde, NSW, Australia) at 20 × magnification. For each section, pancreatic islet number and area were expressed relative to total sectional area (per mm^2) with islet area arbitrarily divided into small ($<5000\ \mu m^2$), medium (5000–$10{,}000\ \mu m^2$) and large ($>10{,}000\ \mu m^2$), similar to Chamson-Reig *et al.*[29] Random-systematic sampling was then used to select 50 fields per section and relative islet and β-cell volume density (Vd) were quantified by point-counting morphometry using a 700-point grid (700 points/field, Vd equals the number of intercepts on an islet or insulin-positive cells as a proportion of intercepts on a pancreas).[25] Under the assumption that 1 cm^3 tissue weighs approximately 1 g, Vd and pancreatic weight can be multiplied to determine the absolute islet and β-cell mass, expressed in milligrams.[40] Percent of islet occupied by β-cells was also determined.

Real-time polymerase chain reaction (PCR) analysis

Total RNA was extracted from the pancreata and liver at postnatal day 7 and 6 months using the Micro-to-Midi Total RNA Purification System kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Reverse transcription and real-time PCR were performed as previously described.[36,41] Primers and TaqMan® probes (Biosearch Technologies, Novato, CA, USA) were designed from GenBank gene sequences for *18S*, *Akt2*, *Glut2*, *Hprt1*, *Igf1*, *Igf1r*, *Insr*, *Pdx1*, *Pepck* and *Vegf* (see Table 1). Optimal concentration for forward and reverse primers was 300 nM and for TaqMan® probes it was 100 nM. Results were analyzed using the sequence detector software (Rotor-Gene v6, Corbett Research, Mortlake, NSW, Australia). Relative quantification of gene expression was performed by the comparative threshold cycle (ΔΔCT) method with *18S* rRNA and hypoxanthine guanine phosphoribosyl transferase-1 (*Hprt1*) optimized and validated as suitable endogenous controls for the liver and pancreas, respectively.

Statistical analyses

All measures were analyzed using one-way ANOVA, with Duncan's *post hoc* analysis where appropriate. Relationships between size at birth, gene expression, pancreatic morphology and other outcomes were analyzed using Pearson's correlations ($n = 20$–32). All data were normally distributed and presented as mean ± S.E., with the level of significance set at $P < 0.05$.

Results

Body, organ weights and dimensions

The effects of uteroplacental insufficiency on litter size and birth weight have been previously reported.[13,34,36,39,41] Briefly, uteroplacental insufficiency reduced ($P < 0.05$) total litter size (mean ± S.E.; 5.6 ± 0.5 *v.* 8.2 ± 0.7) and male offspring birth weight (3.6 ± 0.1 g *v.* 4.3 ± 0.1 g) compared to sham-operated *Controls*. *Restricted*-on-*Control* and *Restricted*-on-*Restricted* remained lighter ($P < 0.05$) than *Control*-on-*Control* males at post mortem on postnatal day 7 and 6 months (Table 2). *Restricted*-on-*Restricted* males had shorter crown rump length (−13.5%, $P < 0.05$), head length (−12%, $P < 0.05$) and hind limb length (−14%, $P < 0.05$) compared to *Controls* on postnatal day 7, whereas *Restricted*-on-*Control* head length and hind limb length were intermediate between *Restricted*-on-*Restricted* and *Control*-on-*Control* demonstrating a positive effect of improved lactation on growth (Table 2). This cross-foster effect on growth was absent by 10 weeks as reported previously.[13] Absolute, but not relative, pancreas and liver weights were lower ($P < 0.05$) in *Restricted*-on-*Control* and *Restricted*-on-*Restricted* than *Control*-on-*Controls* on postnatal day 7 (Table 2). Absolute and relative pancreas and liver weights were not different across groups at 6 months of age (Table 2).

Plasma analyses

At 6 months, non-fasted plasma CRP in *Restricted*-on-*Restricted* and *Control*-on-*Restricted* offspring was 45–47% higher ($P < 0.05$) than in *Control*-on-*Control* (Fig. 1a). There were no differences in circulating concentrations of total cholesterol, NEFA, pancreatic-amylase or triglycerides (Fig. 1b–e).

Endocrine pancreatic morphology

Pancreatic β-cells comprised 73–77% of total islet mass at 6 months of age with no differences across groups, which is comparable to that previously reported.[42,43] Mean pancreatic islet number was 51% less ($P < 0.05$) in adult *Restricted*-on-*Restricted* male offspring than *Controls* (Fig. 2a). This substantial decrease in islet number in *Restricted*-on-*Restricted* males was in parallel with decreased ($P < 0.05$) absolute islet (−62%) and β-cell (−65%) mass compared to *Control*-on-*Control* males at 6 months (Fig. 2b and c). Exposing *Restricted* offspring to improved lactation attenuated these decreases, with increased (+60%; $P < 0.05$) islet and β-cell mass in *Restricted*-on-*Control* males when compared to *Restricted*-on-*Restricted*, equating to 95–98% of *Control*-on-*Control* pancreatic mass. Importantly, we observed comparable outcomes when islet and β-cell mass were expressed as absolute or relative to body weight (data not shown). There were no differences in the relative number of small, medium or large islets across groups at 6 months (Fig. 2d). Birth weight correlated positively with the average number of total islets ($r = 0.566$, $P = 0.005$, $n = 23$), average number of small

Table 1. *GenBank accession numbers, primer and probe sequences for genes quantified using real-time PCR relative to 18S or Hprt1*

Gene	GenBank accession	Primer sequence (300 nm)	Probe sequence (100 nm)
18S	V01270.1	Fwd: 5′-GCATGGCCGTTCTTAGTTGG-3′ Rev: 5′-TGCCAGAGTCTCGTTCGTTA-3′	5′FAM-TGGAGCGATTTGTCTGGTTAATTCCGA-BHQ1-3′
Akt2	NM_017093	Fwd: 5′-ACCCAACACCTTTGTCAT-3′ Rev: 5′-GCCCGTATCCACTCTT-3′	5′FAM-TGCCTGCAGTGGACCACAGT-BHQ1-3′
Glut2	NM_012879	Fwd: 5′-GCCCTGGGCACTCTTCAC-3′ Rev: 5′-TGAGGCCAGCAATCTGACTA-3′	5′FAM-CAACTGGCTCTTGTCACAGGCA-BHQ1-3′
Hprt1	NM_012583	Fwd: 5′-CAGCCCCAAAATGGTTAAGGTTGCA-3′ Rev: 5′-GCTTTCCTTGGTCAAGCAGTA-3′	5′FAM-GAAGTGTTGGATACAGGCCAGA-BHQ1-3′
Igf1	X_06043	Fwd: 5′-CCAGCGCCACACTGACATG-3′ Rev: 5′-GGGAGGCTCCTCCTACATTC-3′	5′FAM-CCCAAGACTCAGAAGGAAGTACACTTGA-BHQ1-3′
Igf1r	NM_052807	Fwd: 5′-CCGCAGGATGGCTATCTGTTC-3′ Rev: 5′-CGGCGTACTTTCTGATGGGTAT-3′	5′FAM-CGGCACAACTACTGCTCCAAAGACA-BHQ1-3′
Insr	NM_017071	Fwd: 5′-GCTGCTCATGTCCTAAG-3′ Rev: 5′-CACGTTGTGCAGGTAA-3′	5′FAM-ACTGACTCTCAGATCCTGAAGGAGC-BHQ1-3′
Pdx1	NM_008814	Fwd: 5′-AACCGCGTCCAGCTCCCTTT-3′ Rev: 5′-CTCGGGTTCCGCTGTGTAAG-3′	5′FAM-ATGAAATCCACCAAAGCTCACGCGT-BHQ1-3′
Pepck	NM_198780	Fwd: 5′-GCAAGCTGAAGAAATATGAC-3′ Rev: 5′-GCTCTTGGGTAATGATGAC-3′	5′FAM-ACTGTTGGCTGGCTCTCACTGACC-BHQ1-3′
Vegf	NM_031836	Fwd: 5′-GGAGCAGAAAGCCCATGAAGT-3′ Rev: 5′-GATGTCCACCAGGGTCTCAA-3′	5′FAM-TCATGGACGTCTACCAGCGCA-BHQ1-3′

Table 2. *Effect of prenatal and postnatal growth restriction and cross-fostering on body weight, dimensions and organ weights on postnatal day 7 and at 6 months*

Pup-on-*Mother*	*Control*-on-*Control*	*Control*-on-*Restricted*	*Restricted*-on-*Control*	*Restricted*-on-*Restricted*
Postnatal day 7				
Body weight (g)	10.53 ± 0.66	9.34 ± 0.19	7.47 ± 0.34*	6.61 ± 0.39*
Crown rump length (mm)	46.62 ± 0.99	45.21 ± 0.59	41.99 ± 0.62*	40.33 ± 0.97*
Hind limb length (mm)	14.60 ± 0.24	14.19 ± 0.11	13.22 ± 0.21**	12.50 ± 0.26**
Head length (mm)	16.55 ± 0.23	16.71 ± 0.33	15.73 ± 0.48*	15.18 ± 0.31*
Pancreas weight (g)	0.016 ± 0.002	0.015 ± 0.001	0.011 ± 0.001*	0.009 ± 0.001*
Relative pancreas weight (%)	0.17 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Liver weight (g)	0.31 ± 0.01	0.28 ± 0.01	0.24 ± 0.01*	0.21 ± 0.01*
Relative liver weight (%)	3.00 ± 0.17	3.03 ± 0.12	3.14 ± 0.05	3.28 ± 0.19
6 months				
Body weight (g)	386.1 ± 7.0	368.9 ± 2.3	355.4 ± 7.1*	353.9 ± 6.9*
Pancreas weight (g)	1.19 ± 0.07	1.26 ± 0.06	1.15 ± 0.06	1.10 ± 0.06
Relative pancreas weight (%)	0.31 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.31 ± 0.01
Liver weight (g)	8.67 ± 0.15	8.49 ± 0.16	8.03 ± 0.13	8.34 ± 0.17
Relative liver weight (%)	2.32 ± 0.03	2.35 ± 0.04	2.29 ± 0.03	2.42 ± 0.05

Body weight, crown rump length, hind limb length, head length, absolute pancreas and liver weight, relative pancreas and liver weight measured for male offspring. All data are expressed as mean ± S.E. ($n = 8$).

*Indicates significantly different ($P < 0.05$) to *Control*-on-*Control*.

**Indicates significantly different ($P < 0.05$) to all other groups.

islets ($r = 0.522$, $P = 0.011$, $n = 23$) and proportion of β-cells per islet ($r = 0.538$, $P = 0.010$, $n = 22$, data not shown). Both absolute ($r = 0.581$, $P = 0.005$, $n = 21$) and relative ($r = 0.543$, $P = 0.009$, $n = 21$) β-cell mass correlated positively with post-mortem body weight, despite having no direct relationship to birth weight (data not shown).

Pancreatic and hepatic gene expression

Pancreatic gene expression of *Glut2*, *Igf1r*, *Insr*, *Pdx1* and *Vegf* was elevated ($P < 0.05$) in *Restricted*-on-*Control* offspring on postnatal day 7 compared to *Control*-on-*Controls* (Fig. 3). Pancreatic *Insr* and *Igf1r* mRNA expression were also upregulated ($P < 0.05$) in *Restricted*-on-*Restricted* offspring at this age compared to *Control*-on-*Controls* (Fig. 3). In contrast, there were no differences in pancreatic gene expression observed at 6 months, whether expressed in absolute terms (Fig. 4) or relative to β-cell mass (data not shown). Furthermore, absolute and relative β-cell mass did not correlate with pancreatic gene expression at 6 months (data not shown). *Restricted*-on-*Restricted* offspring had decreased ($P < 0.05$) hepatic *Glut2* mRNA compared to *Control*-on-*Controls* on postnatal day 7 with no other differences identified on postnatal day 7 or at 6 months (Table 3).

Discussion

Despite previously reporting hypoinsulinemia and impaired glucose tolerance in *Restricted*-on-*Restricted* rats,[13] there is no adverse effect on intrinsic β-cell function, estimated by insulin secretion relative to β-cell mass in this study. This suggests that the lower β-cell mass in overall fewer islets in these offspring impairs the ability of the pancreas to meet normal metabolic demands following uteroplacental insufficiency. Importantly, we have shown that improving lactation for *Restricted*-on-*Control* male rat offspring can attenuate deficits in adult pancreatic islet number, islet mass and β-cell mass induced by uteroplacental insufficiency. Together, these data support previous studies in the rat, in which growth-restricted offspring had reduced pancreatic islet number and β-cell mass in adulthood,[4,19,20] as well as our previous findings of improved glucose tolerance in *Restricted*-on-*Control* rats.[13] Furthermore, the average number of total islets, small islets and proportion of β-cells per islet correlated positively with birth weight in our adult male offspring. However, these deficits in endocrine pancreatic morphology were in the absence of any changes in regulatory gene expression in the whole pancreas or relative adiposity.

Male offspring born small and provided with improved milk quality and quantity (*Restricted*-on-*Control*)[15] showed a marked increase in relative pancreatic gene expression of the β-cell specific transcription factor (*Pdx1*) and growth factors (*Igf1r* and *Vegf*) on postnatal day 7 compared to all other groups. This upregulation above that of the *Control*-on-*Control* male pups may be a compensatory response following exposure to a poor intrauterine environment, in the face of improved postnatal nutrition. Following this lactational improvement and upregulation of pancreatic genes early in life, pancreatic islet and β-cell mass in *Restricted*-on-*Control* offspring were increased when compared to *Restricted*-on-*Restricted*

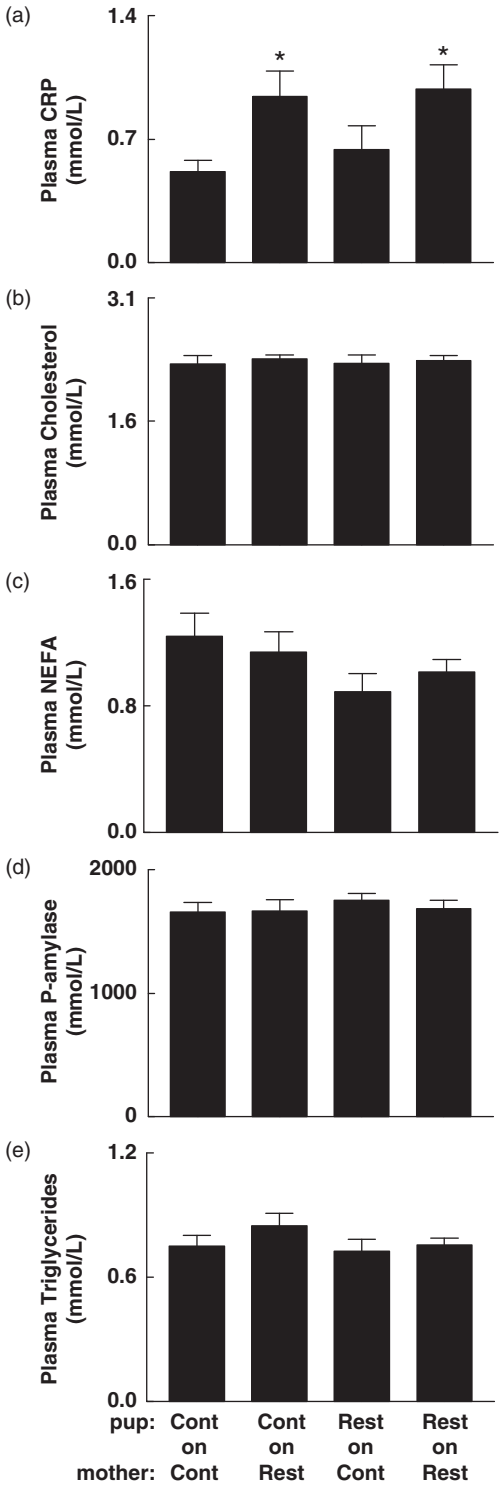

Fig. 1. The effect of prenatal and postnatal growth restriction and cross-fostering on plasma metabolite concentrations in non-fasted male offspring at 6 months. (*a*) C-reactive protein (CRP), (*b*) total cholesterol, (*c*) non-esterified fatty acids (NEFA), (*d*) pancreatic-amylase and (*e*) triglycerides measured at 6 months of age for male offspring. Cont = *Control*; Rest = *Restricted*, *Pup*-on-*Mother*. All data are expressed as mean ± S.E. ($n = 8$/group). *Indicates significantly different ($P < 0.05$) to *Control*-on-*Control*.

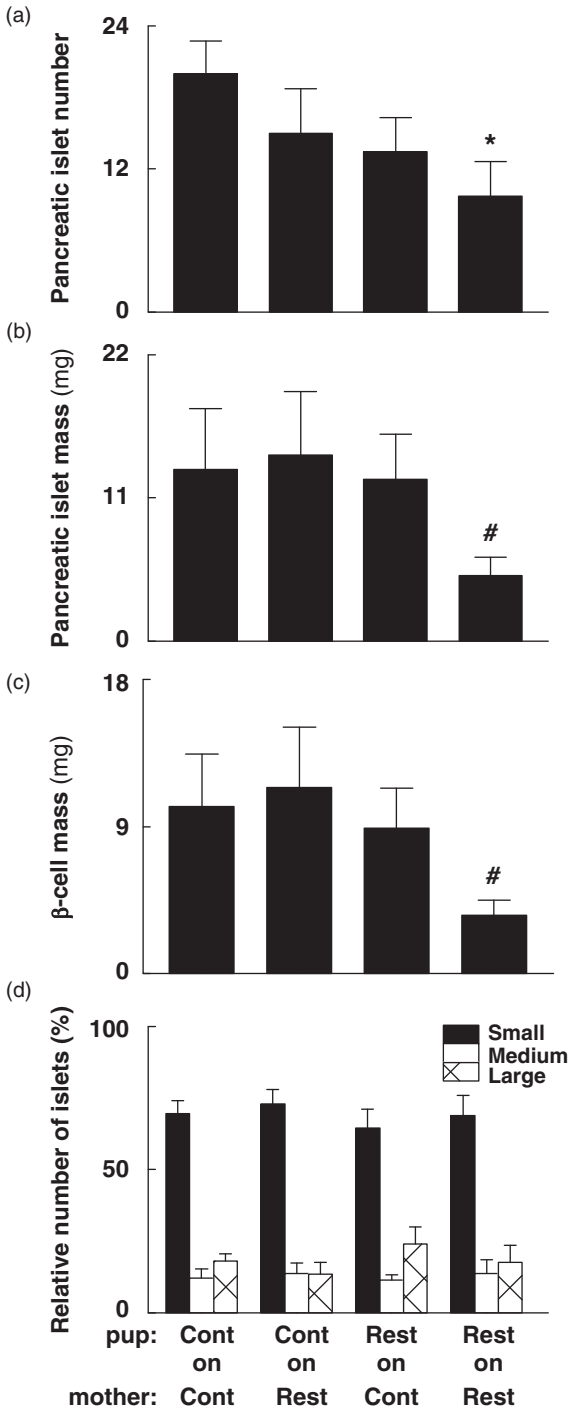

Fig. 2. The effect of prenatal and postnatal growth restriction and cross-fostering on (*a*) islet number per section area, (*b*) absolute islet mass, (*c*) absolute β-cell mass and (*d*) percentage of small, medium and large islets in male offspring at 6 months. Small ($<5000\ \mu m^2$), medium ($5000–10{,}000\ \mu m^2$) and large ($>10{,}000\ \mu m^2$) pancreatic islets relative to the total number of islets. Cont = *Control*; Rest = *Restricted*, *Pup*-on-*Mother*. All data are expressed as mean ± S.E. ($n = 6–8$ for all groups). *Indicates significantly different ($P < 0.05$) to *Control*-on-*Control*. #Indicates significantly different ($P < 0.05$) to all other groups.

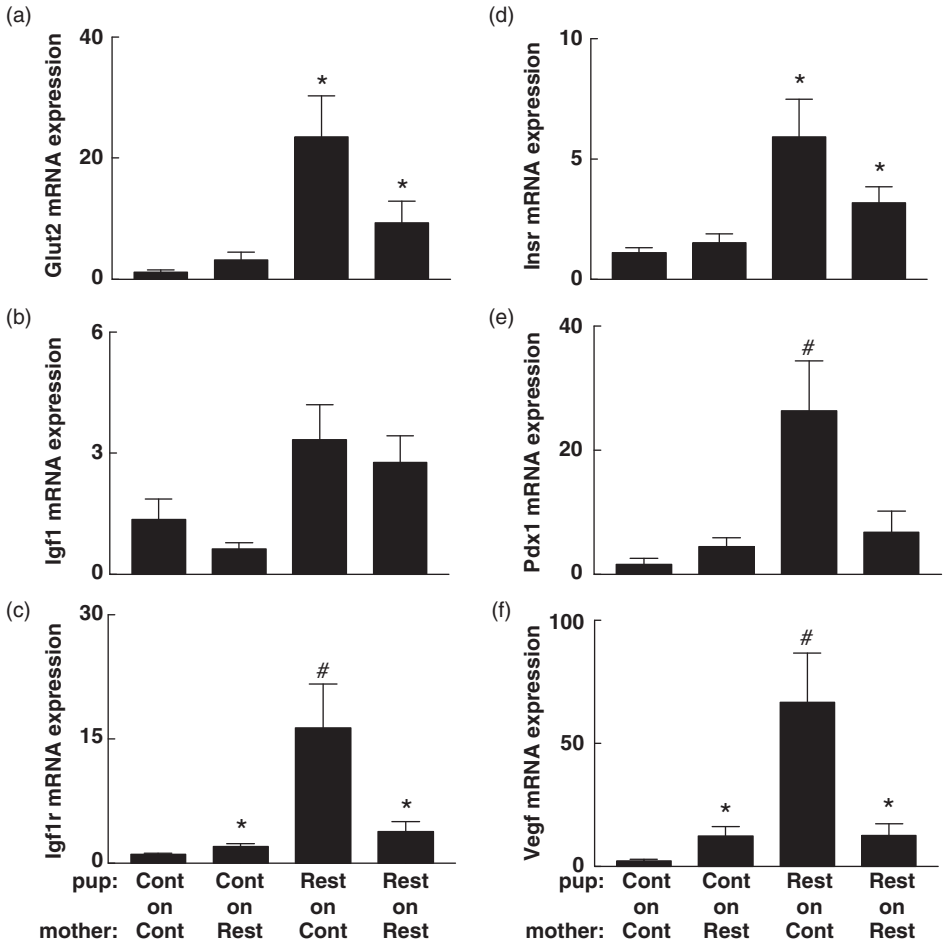

Fig. 3. The effect of prenatal and postnatal growth restriction and cross-fostering on pancreatic (*a*) *Glut2*; (*b*) *Igf1*; (*c*) *Igf1r*, insulin-like growth factor-1 receptor; (*d*) *Insr*, insulin receptor; (*e*) *Pdx1*, pancreatic duodenal homeobox-1 and (*f*) *Vegf*, vascular endothelial growth factor mRNA expression relative to the endogenous reference gene (*Hprt1*) in postnatal day 7 male offspring. Cont = *Control*; Rest = *Restricted*, *Pup*-on-*Mother*. All data are expressed as mean ± S.E. ($n = 6$–8 for all groups). *Indicates significantly different ($P < 0.05$) to *Control*-on-*Control*. #Indicates significantly different ($P < 0.05$) to all other groups.

offspring at 6 months of age. This correlates well with the improved metabolic function in these *Restricted*-on-*Control* male adults from the same rats.[13] Therefore, this demonstrates that both the intrauterine and lactational environments are important determinants of adult endocrine pancreatic morphology and metabolic function.

It has been well-documented that increased weight gain between the ages of 2 and 7 years in low birth weight children independently predicts an increased risk of cardiovascular disease, obesity and diabetes in adulthood.[44–47] Furthermore, placental restriction in the sheep is associated with rapid catch-up growth during the 1st month of life resulting in visceral obesity and insulin resistance.[6,48] In previous studies using the Sprague-Dawley rat, uteroplacental insufficiency induced by bilateral uterine artery ligation resulted in growth-restricted offspring catching up in weight to *Controls* by 7 weeks of age with concomitant mild glucose intolerance, when fostered onto an unoperated mother after birth.[4,49] By 10 weeks of age, these rats weighed more than *Controls*, developed insulin resistance and were obese with virtually absent acute first-phase insulin secretion by 6 months.[4] In contrast, we observed no accelerated growth or obesity in our cross-fostered *Restricted* offspring, and no evidence of insulin resistance following an insulin tolerance test,[13] supported by no alterations in key hepatic genes involved in the insulin-signaling cascade at 6 months of age. A reduction in hepatic *Pepck* mRNA expression in the *Control*-on-*Restricted* offspring on postnatal day 7 may be suggestive of impaired hepatic glucose production. However, no further glucose or insulin tolerance testing has been performed in this cohort at this age. These differences in growth profiles and subsequent metabolic control are potentially due to a number of factors including strain differences (Wistar-Kyoto *v.* Sprague-Dawley rats) in response to the bilateral uterine vessel ligation surgery, cross-fostering, varying litter sizes, altering lactation and increased adiposity.

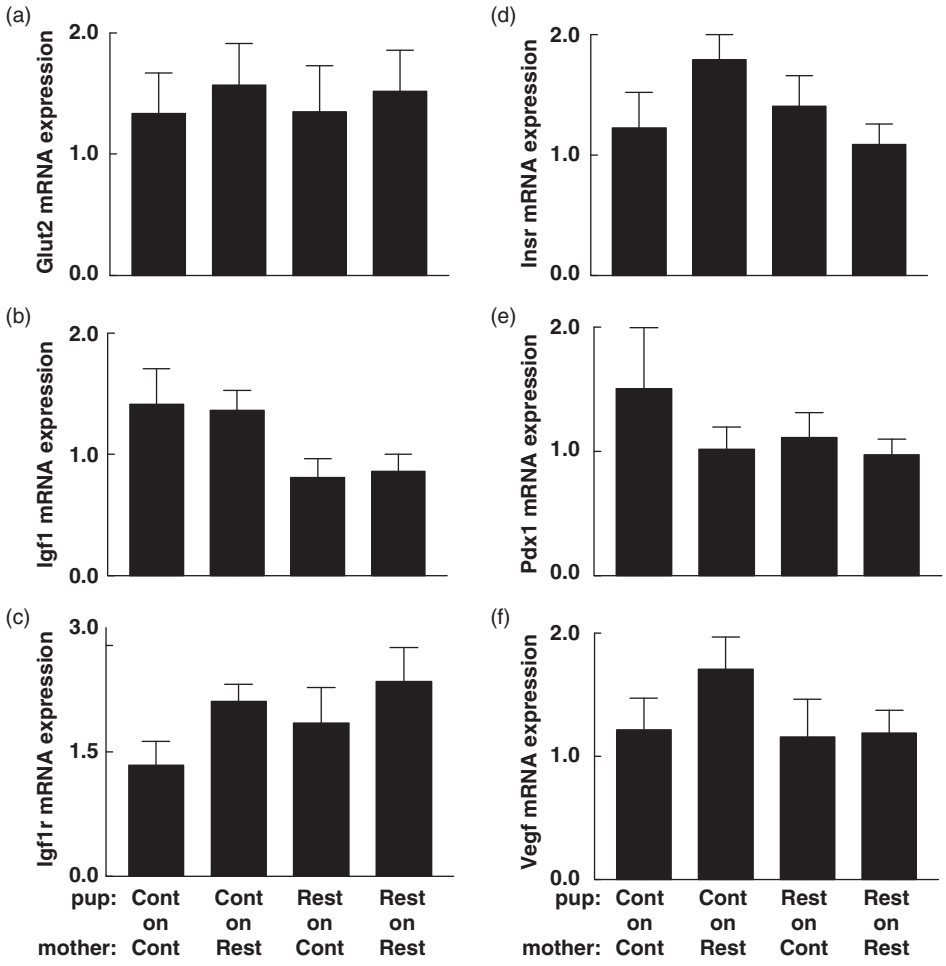

Fig. 4. The effect of prenatal and postnatal growth restriction and cross-fostering on pancreatic (*a*) *Glut2*; (*b*) *Igf1*; (*c*) *Igf1r*, insulin-like growth factor-1 receptor; (*d*) *Insr*, insulin receptor; (*e*) *Pdx1*, pancreatic duodenal homeobox-1 and (*f*) *Vegf*, vascular endothelial growth factor mRNA expression relative to the endogenous reference gene (*Hprt1*) in male offspring at 6 months. Cont = *Control*; Rest = *Restricted*, *Pup*-on-*Mother*. All data are expressed as mean ± S.E. ($n = 6$–8 for all groups). There were no differences in pancreatic gene expression across groups at 6 months.

Altered growth profiles in late gestation, early postnatal life and the environments that induce these can severely impact on adult metabolic health, as previously reviewed.[3,50] The critical period around birth is important for the development and later function of pancreatic β-cells, the primary determinants of glucose tolerance, in a number of different species and paradigms.[8] Nutritional perturbations, such as uteroplacental insufficiency and maternal undernutrition during gestation and lactation in rodents, can adversely affect endocrine pancreas development, resulting in reduced β-cell mass,[4,51–53] impaired glucose tolerance and first-phase insulin secretion.[4,53] It appears that the insulin secretion deficiency and resulting impaired glucose tolerance in our adult male rats with prenatal and postnatal restraint may be due to a pancreatic β-cell mass deficit programmed around birth, since there was no impairment in intrinsic β-cell function. The importance of the immediate postnatal environment for later metabolic function is highlighted in a study which exposed growth-restricted rat offspring to the long-acting glucagon-like peptide-1 analog, Exendin-4, in early postnatal life.[17] They showed that the rate of β-cell proliferation was normalized, reversing the adverse consequences of perinatal insults, thereby preventing the development of diabetes in adulthood.[17] This strongly supports our results, which clearly show amelioration of pancreatic deficits following cross-fostering of *Restricted* offspring onto a *Control* mother.

It seems likely that the intrauterine nutritional environment can not only program β-cell mass in offspring via altered organogenesis of the endocrine pancreas but also change to key lineage determinants, such as Pdx1.[29] There is evidence that intrauterine growth restriction induced by uteroplacental insufficiency can both increase and decrease *Pdx1* mRNA expression within the pancreas depending upon the stage of development and life examined.[54] Stoffers *et al.* observed that *Pdx1* mRNA in the pancreatic islet is reduced by more than 50% before birth, remains downregulated

Table 3. *Effect of prenatal and postnatal growth restriction and cross-fostering on hepatic mRNA expression on postnatal day 7 and at 6 months*

Pup-on-*Mother*	*Control*-on-*Control*	*Control*-on-*Restricted*	*Restricted*-on-*Control*	*Restricted*-on-*Restricted*
Postnatal day 7				
Akt2	1.014 ± 0.083	1.020 ± 0.065	1.113 ± 0.309	0.812 ± 0.084
Glut2	1.070 ± 0.186	0.826 ± 0.090	0.576 ± 0.160	0.509 ± 0.131*
Pepck	1.026 ± 0.110	0.658 ± 0.053*	1.139 ± 0.309	0.927 ± 0.229
6 months				
Akt2	1.859 ± 0.581	1.907 ± 0.658	1.634 ± 0.460	1.112 ± 0.454
Glut2	1.951 ± 0.633	1.738 ± 0.596	1.440 ± 0.388	0.839 ± 0.375
Pepck	1.633 ± 0.446	2.085 ± 0.721	1.224 ± 0.348	1.034 ± 0.414

Akt2, v-akt murine thymoma viral oncogene homolog-2; *Glut2*, glucose transporter-2; *Pepck*, phosphoenolpyruvate carboxykinase. Values are expressed as mean ± S.E. ($n = 8$ per group), relative to a calibrator (the control group).

*Indicates significantly different ($P < 0.05$) to *Control*-on-*Control*.

postnatally and is almost absent by adulthood in growth-restricted rats.[17] This initial downregulation and sustained repression of *Pdx1* gene transcription in the pancreatic islet appears to be acting via epigenetic modifications, particularly histone deacetylation and methylation that generally suppress gene transcription.[55] Furthermore, this process is reversible at the neonatal stage, defining a critical developmental window for potential intervention. Presently, however, we found no evidence of uteroplacental insufficiency suppressing *Pdx1* gene transcription or of any other growth factor or functionally relevant gene studied in the whole early postnatal or adult pancreas. This may be complicated by the fact that pancreatic islets were not isolated and that the pancreas predominantly comprises exocrine tissue. Although pancreatic and hepatic gene expression does not appear to be adversely affected by uteroplacental insufficiency or cross-fostering at 6 months of age, whole pancreatic *Pdx1*, *Igf1r* and *Vegf* mRNA expression was increased in *Restricted*-on-*Control* pups when compared to *Restricted*-on-*Restricted* at postnatal day 7. This upregulation of key genes did not persist into adulthood, suggesting that it was a transitory response to improved milk quality and quantity, but one that nevertheless appears to prevent later structural and functional deficits observed in adult *Restricted* offspring exposed to a poor lactational environment.

Adult growth-restricted offspring born to mothers with 50% global food reduction throughout pregnancy have increased plasma CRP levels, despite being nursed by Control dams.[56] Although it is not known how CRP specifically affects insulin signaling and β-cell function, the increased circulating CRP levels in both the *Control*-on-*Restricted* and *Restricted*-on-*Restricted* male offspring at 6 months of age may be predictive of low-grade systemic inflammation and later impaired glucose tolerance.[10,11] When lactation was improved in *Restricted* male offspring cross-fostered onto a *Control* mother, circulating CRP concentrations returned to *Control*-on-*Control* levels, concomitant with restored insulin secretion and glucose tolerance as reported earlier.[13] Considering that we previously found that *Restricted*-on-*Restricted* male offspring were not insulin resistant or obese,[13] it was not surprising that there were no other differences in circulating total cholesterol, NEFA or triglycerides.

In summary, we have shown that adult male offspring exposed to uteroplacental insufficiency followed by a poor lactational environment (*Restricted*-on-*Restricted*) have endocrine pancreatic mass deficits that are ameliorated by improved lactation (*Restricted*-on-*Control*). We have characterized an underlying deficit in the mass of the endocrine pancreas that may be responsible for the insulin secretion deficiency and impaired glucose tolerance observed in these growth-restricted adult male offspring. Further determination of the mechanisms involved in this amelioration by improved early postnatal nutrition may provide additional insight into other early life interventions that can modulate these long-term consequences for low birth weight offspring.

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Statement of Interest

None.

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