Programming of defective rat pancreatic β-cell function in offspring from mothers fed a low-protein diet during gestation and the suckling periods

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ABSTRACT

Poor fetal and infant nutrition has been linked to impaired glucose tolerance in later life. We studied the effect of protein deficiency during gestation and the suckling period in a rat model and found that poor nutrition ‘programmes’ pancreatic β-cell GK (glucokinase; known as the glucose sensor) and glucose-stimulated insulin secretion response in newborn, suckling and adult rat offspring. Pregnant female rats were divided into three groups: a control group was kept on a normal protein (20%) diet, another group was fed a low-protein (LP) (6%) diet during gestation and suckling periods (LP-G + S group) and another was fed a LP diet during gestation then a normal protein diet during the suckling period (LP-G group). The pulsatile glucose-stimulated insulin secretion response was acutely disrupted and the peak insulin secretion was markedly decreased in newborn and 3-week-old offspring of the LP-G + S group compared with the control group. Also, there was an altered pulsatile secretory response in adults of the LP-G + S and 3-week-old and adult offspring of the LP-G groups compared with the control group. GK protein levels, detected by Western blotting, were decreased in newborn and 3-week-old offspring of both LP-G + S and LP-G groups compared with the control groups. The $K_m$ and $V_{max}$ of GK were altered. The prenatal and postnatal LP diet appeared to have a permanent effect in increasing the affinity of GK for glucose (indicated by decreased $K_m$ values) and decreasing the $V_{max}$. This showed that the critical period of programming of the function of GK was after birth and during the postnatal weaning period, since the adult offspring of the LP-G + S group when fed a normal protein diet showed no reversal in the $K_m$ values of the enzyme. Similar experiments in adult offspring of the LP-G group showed normalization of the $K_m$ values of GK at 3 weeks of age.

In conclusion, fetal and infantile nutrition ‘programmes’ pancreatic β-cell function; poor nutrition during this period caused irreversible effects on glucose homoeostatic mechanisms in the offspring, which may predispose the offspring to diabetes in later life.

INTRODUCTION

Epidemiological studies have shown previously that poor fetal and infant growth is associated with the development of impaired glucose tolerance, non-insulin-dependent diabetes mellitus and the insulin-resistance syndrome later in adult life [1–6]. These pathophysiological alterations have been attributed to a mechanism known as

Key words: glucokinase, irregular pulsatile insulin secretion, low-protein diet, pancreatic β-cell function, reaction kinetics.

Abbreviations: GK, glucokinase; GSIS, glucose-stimulated insulin secretion; HBSS, Hanks balanced salt solution; HK, hexokinase; HRP, horseradish peroxidase; LP, low protein; LP-G, LP diet during gestation and normal protein diet during suckling; LP-G + S, LP diet during gestation and suckling.

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metabolic entrainment or ‘programming’, whereby at critical periods of fetal and early development certain metabolic processes are programmed for the organism to accommodate a particular level of nutrition through the rest of life, thus providing a survival advantage [7,8]. However, subsequent improvement in nutrition during adult life apparently turns out to be a major cause of obesity, diabetes and cardiovascular disease [9].

Previous studies on rats, where pregnant dams were fed a low-protein (LP) diet during gestation and/or the suckling period have demonstrated impairment in islet cell development with a decrease in β-cell mass, altered structure of the fetal pancreas and decreased fasting plasma insulin concentrations in the offspring [10–15]. When the offspring were exposed to a high-carbohydrate or high-fat diet coupled with aging, impaired glucose tolerance was observed.

GSIS (glucose-stimulated insulin secretion) in the pancreatic β-cell is widely believed to be controlled by the low-affinity glucose phosphorylating enzyme GK [glucokinase; HK (hexokinase) type IV], which with its intrinsic catalytic properties acts as a ‘glucose sensor’ [16–18]. There are two isoforms of GK, which have liver and pancreas tissue-specific promoters and are regulated differentially. Previous studies have shown [19,20] that GK activity in the liver was permanently decreased in rat offspring of mothers fed a LP diet during gestation. A mutant form of GK, in which its catalytic properties are affected, results in a form of diabetes known as MODY (maturity onset diabetes of the young) [21,22]. In the present study, we describe, for the first time, permanent alterations in GSIS, as well as in the total and specific activities and reaction kinetics of GK, in pancreatic islets isolated from offspring born of rat dams fed a LP diet during the gestation and/or the suckling period.

METHODS

Animals and diet

All animal procedures were performed under license, in accordance with the Home Office Animal Act (1986). Female Wistar rats were caged with males overnight and coitus was determined by inspection of vaginal plugs, day 0 of gestation being the first day after coitus. A control group of female rats received a diet containing 20 % protein and a LP group received an isocaloric diet containing 6 % protein from day 0 of gestation and throughout gestation. The details and full dietary composition have been reported previously [23]. Diets were obtained from Dyets Inc. (Bethlehem, PA, U.S.A.) in pellet form and were administered ad libitum.

Experiments are based on both male and female offspring and the data relates to islets pooled from both sexes. In total, the control group consisted of approx. 160 animals (80 males, 80 females) from 20 litters and the LP group consisted of approx. 240 animals (120 male, 120 female) from 30 litters. Each set of experiments represents pancreatic islets pooled from a litter and not from individuals. Results represent data from a mean of 4–6 litters unless otherwise stated. For studies on newborn offspring, two litters were used for each set of experiments, as islet mass was small. After birth, litters of offspring were either subjected to laboratory investigation or were standardized to eight offspring/litter consisting of four males and four females for subsequent study. The control group continued to receive the 20 % protein diet and the LP group was split into two, where one group continued to receive the LP diet during the suckling period (LP-G group) and the other group was switched to the 20 % protein diet during the suckling period (LP-G + S group). At 3 weeks of age, offspring were either subjected to laboratory investigation or weaned on to normal laboratory chow. The adult offspring at 6–8 weeks of age were used for laboratory studies. All offspring and mothers were weighed twice weekly during gestation and suckling periods and once a week after weaning.

Total plasma protein concentration was measured using a bicinchoninic acid kit (Sigma), which is based on the colorimetric change due to reduction of Cu(II) to Cu(I) by protein.

Pancreatic islet preparation

Pancreatic islets were isolated from newborn, 3-week-old and adult offspring by a modification of the method described previously [24,25]. Briefly, the pancreas from 3-week-old and adult offspring was perfused via the pancreatic-biliary duct with 5 ml of HBSS (Hanks balanced salt solution) digestion solution [HBSS (pH 7.4), 1 mg/ml collagenase (type XI; Sigma) and 0.1 mg/ml DNase (Amersham Biosciences)]. The pancreas was excised and digested at 37 °C for approx. 17 min. To stop digestion, warm HBSS digestion solution was decanted off and ice-cold HBSS was added and the preparation kept on ice. The pancreas was then shaken vigorously and filtered to remove the islets. The islet preparation was washed with fresh HBSS and islets were removed manually using a stereomicroscope. To obtain islets from newborn offspring, the method described by Tu and Tuch [26] was used, whereby pancreases were isolated, pooled, minced and digested in a solution containing HBSS (pH 7.4), 0.8 mg/ml collagenase (type V; Sigma) and 0.1 mg/ml DNase, and was shaken during incubation at 37 °C for approx. 8 min. Digestion was stopped by placing the suspension on ice, and islets were removed from the suspension. Islet cell viability was determined by Trypan Blue exclusion.

Measurement of insulin secretion

An adaptation of the method described previously by Holmes et al. [27] was used. Samples containing 70 (from
newborns and 3-week-olds) or 50 (from adults) freshly isolated islets were transferred to a perfusion chamber. KRB (Krebs Ringer bicarbonate) solution pH 7.4 containing 0.1 % BSA and either 1.5 or 16 mM glucose was gassed with 95 % air/5 % CO₂ and perfused at 1 ml/min through the chambers during three consecutive periods as follows: 1.5 mM glucose for 60 min, 16 mM glucose for 60 min and, finally, 1.5 mM glucose for 60 min. The effluent from the chamber was sampled at 10 min intervals during the entire perfusion period with additional samples being taken at 1, 3, 7 and 9 min after the switch to high glucose. Insulin concentration in the samples was measured by ELISA using rat anti-insulin antibody and HRP (horseradish peroxidase)-conjugated antibody (Crystal Chem, Downers Grove, IL, U.S.A.).

**Measurement of pancreatic GK activity**

GK activity was determined according to the fluorimetric method described previously [26,28]. Freshly isolated islets (200–300) chosen randomly were used. The islets were homogenized on ice by applying 100 manual stokes in a hand-held glass homogenizer in 160 µl of homogenization buffer (20 mM K₂HPO₄, 1 mM EDTA, 110 mM KCl and 5 mM dithiotheitol, pH 7.7.) Specific activity was measured as protein content and expressed as nmol·mg of protein⁻¹·h⁻¹. Protein content was measured by the bicinchoninic acid assay (Sigma) using BSA as a standard. The kinetic parameters Vₘₐₓ and Kₐₚ were determined as described previously [18] using the Hanes–Woolf plot of V versus V/[S]. GK activity was obtained by measuring HK activity at concentrations between 0.05–0.5 mM glucose and the HK Vₘₐₓ was subtracted from GK activity measured at concentrations between 5–60 mM glucose.

**Detection of GK protein**

GK protein was identified by Western blotting, by using the immunodetection procedure described previously [29]. Islets (300–500) were lysed in 100 µl of buffer containing 5 % (w/v) SDS, 80 mM Tris/HCl (pH 6.8), 1 mM PMSF, 0.2 mM N-ethylmaleimide and 5 mM EDTA. Protein aliquots (20 µg) were resolved by SDS/PAGE [10 % (w/v) polyacrylamide gel] and were electroblotted on to a nitrocellulose membrane (Amersham Biosciences) [29].

A polyclonal sheep antiserum (1:2500 dilution) against rat GK (a gift from Dr M. Magnuson, Vanderbilt University, Nashville, TN, U.S.A.) was biotinylated (Amersham Biosciences) to increase sensitivity and was used as a primary antibody, followed by a 1:1500 dilution of streptavidin-linked HRP (Amersham Biosciences). Bound antibody was detected by ECL (enhanced chemiluminescence), and band intensity was quantified by densitometry using Scion Image software. Rat liver extract (40 µg) was used as a GK reference, as liver tissue is more abundant and easier to procure than islet tissue. Results were calculated and expressed as a percentage of the band density of GK in liver.

**Statistical analysis**

Values are means ± S.E.M. Statistical significance was established using an unpaired Student t test.

**RESULTS**

**Weight gain profile**

The LP diet caused a 14.9 % decrease in maternal weight gain [91.7 ± 3.6 g for control-fed mothers (n = 16) compared with 78.2 ± 3.8 g for LP-fed mothers (n = 32); P < 0.03]. However, only a small decrease in weight was observed in newborn offspring [control-fed mothers, 5.3 ± 0.06 g for offspring (n = 88) compared with offspring from LP-fed mothers, 5.1 ± 0.05 g (n = 150); P < 0.008]. The progeny index, i.e. the number of offspring in a litter, showed no significant difference among mothers fed the normal and LP diet during pregnancy. Offspring from the recovery group (LP-G group) showed no significant decrease in weight at 3 weeks of age. Weight was significantly decreased (P < 0.0001) in offspring of the LP-G + S group (21.63 ± 4.54 g, n = 35; 17 male and 18 female) at 3 weeks by approx. 40 % compared with the control group (45.45 ± 1.64 g, n = 40; 20 male, 20 female), and the decrease in weight continued into adulthood [228.08 ± 4.16 g for control group (n = 40; 20 male, 20 female) and 137.9 ± 8.9 g for LP-G + S group (n = 32; 16 male, 16 female)], even after the offspring were placed on to a 20 % protein control diet. There was no decrease in plasma protein levels in newborn LP-fed offspring, but there was a small decrease observed at 3 weeks of age in the LP-G + S group (29.1 ± 5.1 mg/ml compared with 36.38 ± 4.6 mg/ml in the control group).

**GSIS**

**Newborn offspring**

Figure 1 clearly demonstrates that the pulsatile GSIS was remarkably dampened in the newborns of the LP-fed group compared with those of the control group. The initial basal secretion levels were similar in both groups. The overall GSIS was decreased significantly (P < 0.05) from 1404 ± 225.4 pg in the control group (n = 4 litters; 16 male, 16 female) to 399.7 ± 151.4 pg in the LP group (n = 4 litters; 16 male, 16 female), and was accompanied by a significant decrease (P < 0.05) in peak secretion levels from 41.58 ± 7.35 pg·ml⁻¹·min⁻¹ in the control group to 10.86 ± 4.34 pg·ml⁻¹·min⁻¹ in the LP group. Similarly, on switching to a low-glucose concentration, although the islets from the control group exhibited a classical fall in the insulin secretion, the pattern in the LP group demonstrated no significant difference (Figure 1). In brief, these observations highlight two
important issues: first, a significant decrease in the overall insulin synthesis and secretion in the islets from the LP group and, secondly, a complete loss of pulsatile pattern of GSIS and adaptation to a dampened diffuse pattern in the offspring from the LP groups.

3-week-old offspring
The LP-G + S group showed an approx. 40% decrease ($P < 0.05$) in the overall insulin secretion ($602 \pm 118.7$ pg, $n = 5$ litters; 20 male, 20 female) compared with the control group ($1019 \pm 132.8$ pg, $n = 5$ litters; 20 male, 20 female), as well as a significant decrease ($P < 0.05$) in the peak secretion level ($13.56 \pm 2.89$ pg·ml$^{-1}$·islet$^{-1}$, $n = 5$ litters; 20 male, 20 female) compared with the control group ($33.11 \pm 6.59$ pg·ml$^{-1}$·islet$^{-1}$, $n = 5$ litters; 20 male, 20 female; Figure 2a). The LP-G group, where the mothers were fed the normal diet during the suckling period, showed no significant decrease in the overall insulin secretion ($1084 \pm 340$ pg), but there was a wide spread of secretion levels at different time points (Figure 2b). A closer examination of the data, however, revealed two different populations of animals with respect to GSIS within this group. Litters from three mothers were found to have a normal pattern of GSIS, whereas litters from two other mothers showed a loss of pulsatile GSIS, similar to that seen in the offspring from the LP-G + S group.

Adult group
The three groups showed some variation in the overall levels of insulin secretion [control, $1716 \pm 353$ pg; LP-G + S, $1996 \pm 286$ pg; and LP-G, $1362 \pm 395$ pg; all groups with $n = 5$ litters, with 20 male and 20 female]. Although the animals from the LP-G group appeared to secrete less insulin than those in the control and LP-G + S groups, the decrease in insulin secretion did not reach statistical significance. To appropriately illustrate the effect of the LP diet, Figure 3 shows the individual results for the pooled litters. A comparison of the data shows that, although there was some difference in the overall secretion levels of insulin within the control group, the pulsatile pattern of GSIS was normal (Figure 3a). On the other hand, in both LP-G + S and LP-G groups, the pulsatile pattern of GSIS with respect to ‘switching on’ and ‘switching off’ time was completely disrupted (Figures 3b and 3c). In addition, the internal variations within each group with respect to the overall insulin secretion levels, ensuring the response to ‘switching on’ and ‘switching off’ was either completely discordant or diffused (Figures 3b and 3c). These data clearly demonstrate that, although the maternal LP diet during the gestation period alone and gestation and suckling periods together had no statistically significant effects on the overall synthesis and secretion of insulin, it had a profound effect on GSIS and on the ‘glucose-sensing’
Pancreatic β-cell function and low-protein diet

Figure 3  Insulin secretion in response to 16 mM glucose in adult rats
(a) Control group litters, (b) LPG + S group litters, and (c) LP-G (recovery) group litters. 1.5 mM and 16 mM indicate the glucose concentration. Data sets represent mean duplicate readings of individual litters. Vertical lines represent time of glucose concentration switch.

response of islets in the adult group when transferred to a normal protein diet.

Reaction kinetic parameters of pancreatic islet HK and GK
A summary of the \( K_m \) and \( V_{\text{max}} \) values of high-affinity HK and low-affinity GK from pancreatic islets is shown in Table 1 and is accompanied by the Hanes–Woolf plots for GK (Figure 4) to illustrate the change in enzyme behaviour.

HK
There was no statistically significant difference in the \( K_m \) values for HK among the three groups (control, LP-G and LP-G + S) or between the newborn, 3-week-old and adult offspring. Similarly, the \( V_{\text{max}} \) values for HK showed no statistically significant differences among all age groups in the control and LP-G + S groups. However, in the case of the LP-G group, although the \( V_{\text{max}} \) values were not statistically different between the newborn offspring in the control and LP groups, the \( V_{\text{max}} \) values for 3-week-old and adult offspring of the LP-G groups were significantly higher and lower respectively, compared with the corresponding age of control and LP-G + S groups (Table 1). The biological significance of this finding is not clear, except that it may reflect a generalized and/or transient change in the glucose metabolic homoeostasis in the LP-G group.

GK
The analysis of data on GK or the ‘glucose sensor’ enzyme showed some interesting variations in the three experimental groups (Table 1). The \( K_m \) values for glucose of GK from newborn offspring of the LP-G group and 3-week-old and adult offspring of the LP-G + S group were significantly decreased (\( P < 0.05 \)) and showed an approx. 5-fold decrease compared with the values for the corresponding control groups. In contrast, 3-week-old and adult offspring of the LP-G groups showed no such changes in \( K_m \) values of GK (Table 1). A similar analysis of \( V_{\text{max}} \) values of GK revealed a significant decrease (\( P < 0.05 \)) in the 3-week-old and adult offspring of both LP-G and LP-G + S groups compared with the control group. A change in the \( K_m \) of GK for glucose, i.e. the binding affinity of the enzyme for its substrate, is important in determining the glucose-sensing ability of the enzyme. The 1.7–5-fold decrease in the \( K_m \) value would result in a profound difference in the behaviour of GK as a glucose sensor.

GK protein expression
The levels of expression of GK protein were determined by Western blots, followed by immunodetection using an anti-GK antibody. The analysis of the data (Figure 5) showed that GK protein expression in islets of newborn offspring from the LP-G group was decreased by approx. 71% (8.6 ± 5.83 %, \( n = 5 \) litters; 20 male, 20 female) compared with the control group (30.53 ± 5.83 %, \( n = 5 \) litters; 20 male, 20 female). A similar decrease in GK protein expression was also observed in islets of 3-week-old offspring from the LP-G (42.49 ± 18.4 %, \( n = 5 \) litters; 20 male, 20 female) and LP-G + S (81.72 ± 51.79 %, \( n = 5 \) litters; 20 male, 20 female) groups compared with the corresponding control animals.
Table 1  Kinetic parameters of pancreatic HK and GK
Values are means ± S.E.M. of 3–6 experiments on litters consisting of an equal ratio of males to females. *P < 0.05 compared with control group of relevant age group.

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>$K_m$ (mM glucose)</th>
<th>$V_{max}$ (nmol · mg of protein$^{-1} · h^{-1}$)</th>
<th>$K_m$ (mM glucose)</th>
<th>$V_{max}$ (nmol · mg of protein$^{-1} · h^{-1}$)</th>
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<tbody>
<tr>
<td></td>
<td>HK</td>
<td></td>
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<td>GK</td>
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</tr>
<tr>
<td>Newborn</td>
<td>Control</td>
<td>0.07 ± 0.02</td>
<td>3.84 ± 0.69</td>
<td>14.94 ± 3.31</td>
<td>1.37 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>0.07 ± 0.02</td>
<td>5.35 ± 0.95</td>
<td>2.94 ± 1.56*</td>
<td>2.17 ± 1.08</td>
</tr>
<tr>
<td>3 Weeks</td>
<td>Control</td>
<td>0.13 ± 0.02</td>
<td>4.82 ± 1.39</td>
<td>16.49 ± 3.85</td>
<td>4.36 ± 1.43</td>
</tr>
<tr>
<td></td>
<td>LP-G + S</td>
<td>0.10 ± 0.03</td>
<td>6.88 ± 0.91</td>
<td>2.67 ± 0.79*</td>
<td>0.84 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>LP-G</td>
<td>0.12 ± 0.02</td>
<td>12.39 ± 3.51*</td>
<td>10.23 ± 3.83</td>
<td>0.99 ± 0.38*</td>
</tr>
<tr>
<td>Adult</td>
<td>Control</td>
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<td>11.25 ± 1.54</td>
<td>14.47 ± 2.29</td>
<td>2.6 ± 0.56</td>
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<td>LP-G + S</td>
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<td></td>
<td>LP-G</td>
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<td>2.42 ± 0.37*</td>
<td>8.18 ± 2.83</td>
<td>0.88 ± 0.45*</td>
</tr>
</tbody>
</table>

Figure 4  Hanes–Woolf plots illustrating $K_m$ and $V_{max}$ obtained from GK assays
$K_m$ is given by the x-axis intercept, and $V_{max}$ by 1/gradient. GK activity plots for (a) newborn, (b) 3-week-old and (c) adult offspring are shown. The plots are lines of best fit for six data points. Data for GK assays are given in Table 1.

Figure 5  Densitometric analysis of GK Western blots
Results are measured as a percentage of a GK liver control. *P < 0.03 compared with the control group. n = 3–5.

$n$ = 5 litters; 20 male, 20 female) groups showed a significant 82 % ($P < 0.0004$) and 66.5 % ($P < 0.02$) decrease respectively, in GK protein expression compared with the control group ($292.7 ± 83.42 %$, $n = 4$ litters; 16 male, 16 female).

DISCUSSION

Poor nutrition in early life has been implicated in poor fetal and infant growth with consequences, such as Type II diabetes and the metabolic syndrome in adult life [1]. Animal studies on the effect of restricted carbohydrate maternal diet have demonstrated [19,20] permanent changes in liver metabolism with respect to the glycolytic pathway. The present study reports, for the first time, the effect of a maternal LP diet during the gestation period alone and during the gestation and suckling periods together, on the metabolic functions of pancreatic islets in the offspring of rat dams.

The present study clearly demonstrates that a continuous maternal LP diet regimen during gestation and suckling periods (LP-G + S group) has a more serious effect on the growth and weight gain profile of the offspring which, even in adult life (6–8 weeks), was
significantly leaner than those that those weaned by mothers fed a normal protein diet during the suckling period (LP-G group). The offspring born to mothers fed on normal diets during the suckling period (LP-G group) appeared to catch up with offspring of the control group with respect to the weight gain profile. Similar variations were also observed in the overall insulin secretion and GSIS levels.

The design of the present study involved switching the diets of LP-fed mothers after birth. It is important to point out that an effect on the mothers own protein profile and milk production would not be instant and would take a few days to normalize. Therefore the observed effect in the LP-G group might not be entirely attributable to the LP diet during gestation, but also for the first few days of suckling. However, the results in the present study imply that it was the weaning period that might be critical for metabolic programming.

The maternal diet and the maternal physiological status during the postnatal 3-week weaning period seemed to be of crucial importance in imprinting some permanent effect on the metabolic outcome of the offspring. This implies the presence of additional switch-on/switch-off mechanisms during the postnatal period preceding the intra-uterine mechanism, which may determine the metabolic fate of the offspring in their adult life. The neonates during 3 weeks of postnatal suckling also appeared to respond in different ways when the mothers were put back on a normal protein diet during the suckling period (LP-G group). This demonstrates a difference in the molecular and biological response of the individual litters with respect to their ability or lack of ability to repair the initial intra-uterine defects. This phenomenon could give rise to the subgrouping and general variations observed in the case of LP-G group offspring at the post-weaning and adult age.

GSIS is not only dependent on the net synthesis of insulin or the glucose-sensing ability of GK, but also on a large number of other factors involving biosynthesis of insulin-secreting granules, their trafficking along the secretory pathway and their exocytosis. In addition, the non-glucose-stimulated mechanisms may also be responsible in eliciting dysregulated insulin secretion as a function of their malformation, because of a general deficit in protein synthesis in pancreatic islets. In conclusion, a maternal LP diet not only decreases overall levels of insulin secretion, but also acutely disrupts the pulsatile pattern of GSIS. The present findings correlate with other studies on the effect of poor nutrition in pregnant rat dams and their offspring [10–13], where altered development of islets resulted in decreased islet size and number and vascularization of islets and, notably, decreased insulin secretion in the offspring. The present study was designed to investigate the effect of maternal LP diet on in vitro β-cell function of the offspring, although it would have been interesting to look at the effects of physiological parameters, such as plasma insulin and glucose levels, these tests were not performed. However, similar studies [19,20] have already investigated and documented the effect on fasting plasma and glucose levels in offspring of rat dams fed pre- and post-natal LP diets.

A recent study [30] has demonstrated gender-specific insulin sensitivity in 20-week-old rat offspring exposed to protein restriction in early life. The relative insulin resistance and hyperinsulinaemia in adulthood was observed only in males. A mixed gender number in newborn rat offspring were used in the present study, and the main questions addressed in the present investigation included the effect of a maternal LP diet at the newborn and 3 and 6–8 weeks of age stages in offspring. Whether gender-specific differences can be observed in offspring in these earlier stages of life remains to be seen but, in order to maintain the comparative nature of the experimental protocol and evaluation of the data from the three groups, equal numbers of male and females per litter were used.

The amount of GK protein was also decreased in all groups fed a prenatal LP diet at all stages (newborns and 3-week old offspring). This appeared to be a permanent effect and it is likely that the down-regulation of GK protein synthesis or mRNA synthesis occurs at, or just before, birth. This correlates with previous studies of the activity and levels of liver GK [19,20]. These studies found liver GK to be programmed by prenatal nutrition; however, a change in GK enzyme kinetics were not reported in these investigations. The decreased level of GK protein observed in both experimental groups at 3 weeks and adulthood may also be responsible for the low $V_{\text{max}}$, which subsequently may have a bearing on the observed loss of GSIS pulsatility at these stages. GK from rats on a LP diet (newborn and 3-week-old offspring of LP-G+S group) also showed decreased $K_m$ for glucose. These data also correlate with the insulin secretory responses, indicating that, although the animal was in a state of malnutrition, it did not require GK to operate optimally at higher glucose concentrations, but required optimal function at lower glucose concentrations, hence the decreased $K_m$ value. This effect was not permanent in newborn rats, as the $K_m$ value normalized when the animal was placed on to a normal diet after birth as observed in the case of rats of the LP-G group. However, a prenatal and postnatal LP diet had a permanent effect on the substrate-binding ability of GK (as evident from the permanently decreased $K_m$ value in adults of the LP-G+S group), indicating that the critical period for the programming the affinity of GK for glucose was after birth and during the suckling period.

The effect of the LP diet on GK activity at a genetic level could occur by transcriptional regulation. Tissue-specific promoters in the liver and pancreas control GK gene transcription. Liver GK gene expression is
dependent on nutritional status [31], and insulin appears to be a key regulator in pancreatic GK gene transcription [32]. Genetic factors involved in development of GK maturity during gestation and early life could be involved, considering that this was the critical period for programming. As yet, these genetic factors are unknown; however, the transcription factor Foxa2 [33] and PPAR-γ (peroxisomal proliferator receptor-γ) [34] have been implicated.

Considering that the LP diet affected the $K_m$ of pancreatic GK implies plausible changes in protein interactions between GK and other putative regulatory proteins. It is, therefore, possible that the programming effect occurred at the post-translational level. The regulation of liver GK has been elucidated by the finding of the GKR (GK-regulatory protein) [35]. However, the same mechanism does not seem to function in the pancreas [36], and further work on the regulation of pancreatic GK is required.

Programming may involve a change in the protein conformation of the GK enzyme molecule with respect to its active site, a change in the interaction with another protein involved with GK regulation or with a cofactor required for GK activity. Possible candidate proteins that interact with GK have been identified in the pancreas [37–39], as well as the recent observation of GK association with insulin granules and NO (nitric oxide) production. [40,41].

A permanently decreased pancreatic GK activity may not necessarily bring on a diabetic state, but may lead to a decreased glucose tolerance. This is evident in MODY2 [42], where the affected people do not always develop symptoms until later in life and, in fact, the condition can sometimes go unnoticed. However, it is the additional factors (i.e. aging and obesity) in later life and the background of decreased GK activity that may result in diabetes.

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