Short Communication

Emergence of ageing-related changes in insulin secretion by pancreatic islets of male rat offspring of mothers fed a low-protein diet

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(Submitted 7 April 2011 – Final revision received 27 July 2011 – Accepted 31 July 2011 – First published online 9 September 2011)

Abstract
Maternal low-protein (LP) diets programme β-cell secretion, potentially altering the emergence of ageing of offspring pancreatic function. We hypothesised that isolated pancreatic islet β-cell secretory responses are blunted in offspring exposed to LP during development and age-related reduction is influenced by the developmental stage of exposure to decreased nutrition. We studied male offspring of rats fed control (C) or LP protein (R) diets in pregnancy, first letter and/or lactation second letter of CC, RR, CR or RC groups. Serum glucose, insulin and homeostatic model assessment (HOMA) were measured. Pancreatic islets were isolated and 
in vitro
 insulin secretion quantified in low (LG – 5 mM) or high glucose (HG – 11 mM). Body weight and serum values between groups were similar at all ages. Insulin and HOMA rose with age and were highest at postnatal day (PND) 450 in all groups. At PND 36, insulin secretion was greatest in RR and RC. Only CC increased insulin secretion to HG. By PND 110, restricted groups responded less to LG but increased secretion to HG. By PND 450, CC offspring alone increased secretion to HG. Despite minimal differences in circulating insulin and glucose, reduced maternal protein intake affected insulin secretion at all ages. In addition, ageing reduced function in all R groups compared with CC by PND 110 and further by PND 450 most markedly in RC. We conclude that maternal LP diet during pregnancy and/or lactation impairs offspring insulin secretory response to a glucose challenge and alters the trajectory of ageing of pancreatic insulin secretion.

Key words: Rats; Insulin; Developmental programming; Islets; Undernutrition; Glucose; Ageing

The fetal and neonatal pancreas shows developmental plasticity and responsiveness to its metabolic environment, including poor maternal nutrition. Low-protein (LP) maternal diets decrease fetal β-cell mass¹² and isolated islet insulin secretion at term³. Life-time consequences of poor fetal pancreatic development can impair offspring carbohydrate metabolism predisposing to diabetes even when the diet is normalised at weaning¹⁵. To our knowledge, ageing-related changes in 
in vitro
 insulin secretion following LP diets in pregnancy and lactation have not been studied. Studies on pancreatic programming by LP maternal diet have studied a single postnatal time point or limited range of early life up to postnatal day (PND) 56⁶.²⁰

We hypothesised that temporal patterns of β-cell secretory responses are blunted in offspring from protein-restricted mothers and that reduced function with ageing would be influenced by both the nature of the dietary challenge and the developmental stage in which nutrition was altered. We examined 
in vitro
 insulin and glucose levels and secretory response to culture in low (LG) and high glucose (HG) by isolated islets from offspring exposed to LP during pregnancy and/or lactation. Since male offspring are more susceptible to islet damage⁵, we studied male offspring at puberty at postnatal day (PND) 36, full maturity at PND 110 and relative old age at PND 450.

Materials and methods
Pregnant Wistar rats (PND 120) were assigned to control (C) (20 % casein; CC) or a restricted (R) (10 % casein; RR)
isoenergetic diet\(^7\) in pregnancy and lactation, respectively. C and R in pregnancy and lactation, (CR), R in pregnancy and C in lactation (RC). Litters were adjusted to ten pups at PND 2. After weaning, pups ate the C diet. At PND 36 (450) male pups were euthanised by guillotine (Thomas Scientific, Swedesboro, NJ, USA) and trunk blood obtained. A bile duct catheter was introduced and pancreatic islets isolated and collected individually microscopically following collagenase digestion and cultured overnight\(^8\). \textit{In vitro} insulin release was measured in groups of ten isolated islets/well, in LG (5 mM) or HG (11 mM) for 1 h. Medium and serum insulin were measured by RIA (Millipore, Billerica, MA, USA). Inter- and intra-assay CV were <4 and <6\%, respectively. Fasting serum glucose was measured using the hexokinase method (Beckman Coulter, Company, Brea, CA, USA). Intra- and inter-assay CV were <2 and <3\%, respectively. Homeostasis model assessment (HOMA) was calculated as HOMA = glucose (mmol/l) \(\times\) insulin (\(\mu\)U/ml)/22.5. All procedures were approved by the Animal Experimentation Ethics Committee, Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City.

\section*{Statistical analysis}

Data are presented as means with their standard errors. To avoid skewed effects from a single litter, one male from each litter was studied. Analysis of group changes according to age was by two-way ANOVA with the Holm-Sidak post hoc test with significance \(P<0.05\).

\section*{Results}

\subsection*{In vivo outcomes}

RC and CC offspring body weight was similar at all ages. Except for CR at PND 36, offspring of both groups restricted in lactation weighed less (Table 1). Serum glucose was generally similar between all age groups, but lower in CR at PND 36 and 450. Serum insulin was similar in all four groups at PND 36. By PND 110, serum insulin was higher in RC and lower in RR offspring. Serum insulin rose with age and was highest in all groups at PND 450. HOMA was lower in CR and RC than other groups at PND 36, as was RR at PND 110. HOMA rose in all four groups (\(P<0.05\)) by PND 450 and was highest in RC.

\subsection*{In vitro responses to glucose between groups at each postnatal age}

At PND 36, RR and RC responses to both glucose concentrations were increased compared to CC, while in CR insulin secretion was higher than CC only in HG (Fig. 1(A)) and the increment in insulin release in HG response compared with LG was only significant in CC (Fig. 1(D)). At PND 110, CC responses to both LG and HG were increased. In contrast, the LG response decreased in all restricted groups while the HG response was only lower in RR (Fig. 1(D)). A significant incremental insulin release to HG compared with LG occurred in all groups with no group differences (Fig. 1(D)). At PND 450, RR and RC islets responded less to LG than CC and all restricted groups showed blunted responses to HG (Fig. 1(C)). At this age, CC was the only group that increased insulin release to HG compared with LG (Fig. 1(D)).

\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
 & CC & & RR & & CR & & RC \\
 & Mean & SEM & Mean & SEM & Mean & SEM & Mean & SEM \\
\hline
\textbf{PND 36} & & & & & & & & \\
\text{Body weight (g)} & 97 & 6 & 72 & 2 & 87 & 6 & 93 & 2 \textsuperscript{*a} \\
Glucose (mmol/l) & 7.8 & 0.3 & 7.5 & 0.2 & 5.1 & 0.7 & 7.1 & 0.3 \textsuperscript{a} \\
Insulin (pmol/l) & 60 & 8 & 53 & 5 & 57 & 8 & 37 & 5 \\
HOMA & 3.3 & 0.3 & 3.0 & 0.2 & 2.0 & 0.3 & 1.9 & 0.3 \textsuperscript{a} \\
\textbf{PND 110} & & & & & & & & \\
\text{Body weight (g)} & 383 & 6 & 332 & 12 & 339 & 8 & 383 & 11 \textsuperscript{*b} \\
Glucose (mmol/l) & 6.5 & 0.4 & 6.9 & 0.2 & 6.9 & 0.2 & 7.2 & 0.4 \\
Insulin (pmol/l) & 220 & 20 & 85 & 28 & 130 & 33 & 454 & 14 \textsuperscript{a} \\
HOMA & 10.5 & 1.1 & 4.4 & 1.5 & 6.7 & 1.7 & 24.0 & 1.3 \textsuperscript{a} \\
\textbf{PND 450} & & & & & & & & \\
\text{Body weight (g)} & 561 & 5 & 452 & 18 & 482 & 12 & 585 & 28 \textsuperscript{b} \\
Glucose (mmol/l) & 7.0 & 0.4 & 6.8 & 0.1 & 5.8 & 0.2 & 6.0 & 0.2 \textsuperscript{a} \\
Insulin (pmol/l) & 363 & 333 & 333 & 47 & 352 & 81 & 739 & 20 \textsuperscript{a} \\
HOMA & 19 & 3.2 & 16 & 2.4 & 14.7 & 3.3 & 32.9 & 5.4 \textsuperscript{a} \\
\hline
\end{tabular}
\caption{Male offspring body weight, serum glucose and insulin concentration and homeostasis model assessment (HOMA) on postnatal days (PND) 36, 110 and 450 in offspring of mothers fed a control (C) or low-protein diet (R) during pregnancy and/or lactation, first and second letter, respectively (Mean values with their standard errors)}
\end{table}

\textsuperscript{a,b} Mean values with unlike superscript letters were significantly different at the same age group (\(P<0.05\)).

* Mean values were significantly different from those of PND 36 in the same experimental groups (\(P<0.05\)).

† Mean values were significantly different from those of PND 110 in the same experimental groups (\(P<0.05\)).

\textsuperscript{‡} Mean values were significantly different from those of PND 36 and 110 in the same experimental groups (\(P<0.05\)).
Changes in in vitro response to glucose with ageing

Major changes occurred in response to both LG and HG between PND 36 and 110. LG and HG responses rose in CC but fell in all restricted groups except HG in CR (Fig. 1). The increase in response to HG compared with LG remained significant in all groups at PND 110. The only changes between PND 110 and 450 were decreased responses to LG in CC and increased response in CR. By PND 450, only CC retained the ability to increase insulin release to HG.

Discussion

The developing and the developed worlds are experiencing an explosion of type 2 diabetes and obesity(9,10). The developmental programming hypothesis proposes that poor maternal nutrition impairs the function of key organs including the pancreatic islets, leading to decreased function in later life. Emergence of problems in any organ system may depend on an environmental change that constitutes a second hit, e.g. poor diet or lack of exercise in childhood and later life. Animal studies of programming during development can avoid second-hit effects by controlling experimental conditions at all stages. We controlled for a nutritional second hit by feeding all groups the same post-weaning diet.

The majority of studies on developmental programming of insulin metabolism as a result of poor maternal diet have been in vivo. All groups we studied showed the previously reported increase in insulin levels required to maintain normal blood glucose with age(11). Despite a similar weight to CC, RC offspring developed the greatest insulin resistance(4,5,7). This lack of differences in the peripheral blood disguises changes in both pancreatic insulin secretion and peripheral resistance, providing the justification for in vitro islet secretion studies such as the one we have conducted.

Neonatal rat plasma insulin and β-cell mass peak around PND 28 (11). Increased responsiveness at PND 36 of groups restricted in pregnancy may reflect a delay of this peak activity resulting from LP in fetal life or an increased insulin responsiveness to glucose as a result of attempts to compensate for insulin resistance that is already present in these groups.

Compared with extensive in vivo studies, in vitro experiments of isolated pancreatic islets to establish age-related changes in direct secretory responses to glucose are few and conducted over a much shorter and earlier period of life than the studies we report here(6). It is of interest that CR offspring show normal responses to LG at PND 36 and is the only restricted group to show responses that do not differ from CC to HG at PND 110 and LG at PND 450, suggesting that...
neonatal LP is better tolerated than LP in fetal life. At PND 36, all restricted groups presented insulin hypersecretion to HG and RR and RC to LG compared with CC; however, insulin response to HG is blunted in all restricted groups, which probably reflects a lack of reserve due to the increased responsiveness to LG.

By adulthood, all restricted groups show decreased secretory responses and reserve capacity indicated by the differences in response to LG and HG. Comparison of LG and HG responses shows that all restricted groups lose secretory reserve which, in contrast, is well maintained in CC. The profile of changes in CC offspring with age differed from all other groups showing the influence of developmental programming on ageing of islet function. By the relatively early age of PND 110, restricted groups showed maximal decrease in response to LG. The comparisons of islet function within a group across the three ages studied clearly show that the major ageing changes occur between PND 36 and PND 110. At PND 110 and 450, all three restricted groups show similar reductions in insulin secretion to both glucose concentrations, the only difference being that RC had a lower secretion to LG than CR at PND 450. A similar conclusion can be drawn from the loss of the ability to increase insulin secretion to HG in all three restricted groups. Thus, in contrast to the marked effect that restriction in pregnancy has at PND 36, further ageing changes were similar in all three dietary restriction groups.

In conclusion, while further studies on altered gene and signalling function related to the dietary challenges imposed here are needed, the present study demonstrates age-related decreases in in vitro pancreatic islet function over a large proportion of normal rat life and emphasizes that developmental programming must be considered as a major factor in predisposition to dysfunctional insulin metabolism across the life-course.

Acknowledgements

The present study was supported by CONACyT-48839 México and NIH-HD21350. L. G., T. C. S., L. A. R., G. L. R.-G. and A. M. conducted research on the data; S. M., P. W. N. and E. Z. wrote, reviewed and edited the manuscript. All authors declare that there are no conflicts of interest.

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