A low protein diet in early life delays the onset of diabetes in the non-obese diabetic mouse

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Abstract

Dietary insult in early life can affect the development and future function of the endocrine pancreas. We maintained pregnant non-obese diabetic (NOD) mice on a low protein (LP, 8% protein versus control, 20%) diet from conception until the weaning of pups at day 21. Serum insulin and pancreatic insulin content were reduced in LP-fed NOD offspring at 8 weeks, as were serum interferon γ and pancreatic tumor necrosis factor α, while the number of pancreatic islets demonstrating peri-insulitis, and the degree of invasiveness were reduced. To determine if LP caused early morphometric changes in the pancreas, we measured mean islet area at days 3 and 21. Mean islet size did not differ with diet, but by 8 weeks of age LP-fed NOD females exhibited a significantly reduced islet number and mean islet area, and a lower fractional area of pancreas occupied by both α- and β-cells than control-fed mice. The onset of diabetes was delayed in NOD mice of both genders fed LP diet. The mechanism is likely to involve both altered β-cell morphology and function and changes in cytotoxic cytokines. Journal of Endocrinology (2009) 201, 231–239

Introduction

Type 1 diabetes originates with the autoimmune-mediated destruction of pancreatic β-cells, and is characterized by a change in cytokine secretion from a helper T-cell (Th) 2 phenotype with relatively low levels of interferon γ (IFNγ) and tumor necrosis factor α (TNFα) but high levels of interleukin 10 (IL-10), to a Th1 phenotype where these ratios are reversed (Pearl-Yafe et al. 2007). Apoptosis of β-cells is mediated by a number of intracellular pathways, including the generation of nitric oxide, in response to the actions of IFNγ, TNFα and IL-1β. The non-obese diabetic (NOD) mouse is a well-studied model of spontaneous type 1 diabetes in which predominantly the female animals develop an insulinitis between 5 and 8 weeks of age, and most will become clinically diabetic by age 20–30 weeks (Delovitch & Singh 1997). Environmental factors such as viruses and dietary antigens have been shown to precipitate autoimmune diabetes both clinically and in animal models such as the NOD mouse and BioBreeding (BB) rat (Lefèvre et al. 2006). However, food antigens can also interact with the gut immune system, resulting in a Th1 cytokine pattern of expression within the Peyer’s patches of young NOD mice (Chakir et al. 2005, Barbeau et al. 2007).

Examples of food antigens that can advance the onset of diabetes in NOD mice include wheat flour–based diets and soya, while limiting exposure reduced the incidence of diabetes (Flohé et al. 2003, Schmid et al. 2004). Feeding of wheat proteins resulted in intestinal enteropathy and higher mucosal levels of proinflammatory cytokines (Maurano et al. 2005), while gluten-free diets were protective against diabetes (Funda et al. 1999). The amount of dietary carbohydrates was not important for the timing of diabetes in the BB rat and the NOD mouse (Scott et al. 1989).

The type of dietary protein has a major impact on the incidence of diabetes in the NOD mouse with meat meal or casein resulting in a high rate of onset while casein hydrolysate, a denatured form, or lactalbumin-based diets being relatively protective (Elliott et al. 1988, Beales et al. 2002). Elliott et al. (1988) postulated that the presence of cow’s milk casein in commercial mouse chow may be a dietary trigger of diabetes in the NOD mouse if it is introduced at weaning. Although casein hydrolysate was protective against spontaneous diabetes in NOD females the ability of T-cells from these animals to adoptively transfer diabetes was not changed (Hermitte et al. 1995), suggesting that the effects might be due to the alterations in islet biology rather than on T-cell selection and activation. Less attention has been given to the quantity of protein within the diet, although a relatively high-protein diet accelerated the onset of diabetes in the pregnant NOD mouse (Schneider et al. 1996).

Reduced dietary protein content has developmental effects on the pancreatic β-cells of the rat in early life, involving reductions in both islet size and β-cell mass resulting in impaired insulin release and glucose tolerance (Snoeck et al. 1995). Examples of food antigens that can advance the onset of diabetes in the NOD mouse include wheat flour–based diets and soya, while limiting exposure reduced the incidence of diabetes (Flohé et al. 2003, Schmid et al. 2004). Feeding of wheat proteins resulted in intestinal enteropathy and higher mucosal levels of proinflammatory cytokines (Maurano et al. 2005), while gluten-free diets were protective against diabetes (Funda et al. 1999). The amount of dietary carbohydrates was not important for the timing of diabetes in the BB rat and the NOD mouse (Scott et al. 1989).

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Fronczak et al., 1990, Petrik et al., 1999, Boujendar et al., 2002, 2003, Hales & Ozanne, 2003), but the effects on the mouse pancreas have not been described. The deficits in rats were reversible following supplementation of a maternal low protein (LP) diet with the amino-acid, taurine (Arany et al., 2004). The effects of a LP diet in early life on susceptibility to autoimmune diabetes in rodent models have not been described. Given the greater apoptotic rate seen in β-cells of neonatal rats exposed to LP (Boujendar et al., 2002) it might be anticipated that the onset of diabetes would occur earlier in the NOD mouse. However, total calorie restriction of the pregnant NOD mouse delayed the onset of diabetes in the female offspring and reduced insulitis at 12 weeks of age (Oge et al., 2007). A human correlate would be the lower incidence of type 1 diabetes seen in individuals born of low birth weight or subjected to micro-nutritional deficiencies (Dahlquist et al., 1996, Fronczak et al., 2003).

The aim of this study was therefore to examine the effects of a LP diet on neonatal pancreatic development in NOD mice, the incidence of insulitis and the age of onset of diabetes.

Materials and Methods

Animals

Male and virgin female NOD mice were purchased from Robarts Research Institute (London, ON, Canada) at 4–6 weeks of age. BALB/c mice of the same age were purchased from Charles River (Montreal, PQ, Canada) and were bred at the Lawson Health Research Institute, London, ON, Canada. The animals were housed in temperature-controlled rooms with a 12 h light:12 h darkness cycle and were given food and water ad libitum. At 8 weeks of age, breeding pairs were caged individually and females were checked daily for the presence of sperm in the vaginal smear. When pregnancy was confirmed, the animals were randomly divided into two groups and fed immediately with a defined diet. The control animals (C) were fed with a 20% (w/w) protein diet. The second group received an 8% (w/w) isocaloric protein diet (LP). Diets were purchased from Bioserv (Frenchtown, NJ, USA). Equal amount of calories were given in both diets by increasing the carbohydrate content in the 8% protein diet. Protein restriction was implemented by reducing casein, which is the main protein source in the 20% diet. The proportion of the amino-acid methionine was also reduced in the LP diet. For each experimental group 7–10 litters of animals were used. The body weight, litter size, and gender of the offspring were recorded at birth. At 21 days of age, all pups from both C and LP groups were weaned and fed with the 20% protein diet until the end of the study. Weight gain of pregnant animals was monitored on a daily basis and for all offspring each week until 8 weeks of age. All procedures were performed with the approval of the animal ethics committee of the University of Western Ontario, and in accordance with the guidelines of the Canadian Council on Animal Care.

BALB/c or NOD mice fed with either LP versus C diet were killed at postnatal day 3 by decapitation and at day 21 by CO2 asphyxiation. At 8 weeks of age, males and females from litters of NOD mice were fasted overnight and blood drawn by lancing the tail vein for blood glucose measurement using a 2 μl volume on a hand held glucometer (Ascencia Dex2, Bayer), and some were killed by CO2 asphyxiation. Their litters were monitored every week for the onset of diabetes determined by polyuria on two consecutive days followed by a confirmation of glucosuria (6–14 mmol/l) using urine glucose strips (Diastix, Ames, Toronto, Canada). Diabetes was confirmed after an overnight fast when blood glucose was in excess of 11 mM, after which animals were killed by CO2 asphyxiation. Non-diabetic mice were killed at 45 weeks of age. At postnatal days 3 and 21 and at 8 weeks, the pancreas was dissected and cut longitudinally and fixed in 10% formalin for further analysis. Serum was collected for insulin and cytokine determinations.

Immunohistochemistry

Pancreata were fixed in 10% formalin and embedded in paraffin using a standardized protocol, and sections of 5 μm cut and mounted on Superfrost–plus slides (Fischer Scientific, Toronto, ON, Canada). Dual immunohistochemistry was performed to localize glucagon and insulin. Glucagon presence was identified by a modified avidin–biotin peroxidase method and insulin by an alkaline–phosphatase method. In brief, slides were incubated overnight at 4 °C in a humidified chamber with mouse anti-glucagon antibody in a 1:2000 dilution (Sigma) followed by biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA) for 2 h at room temperature. Slides were washed and incubated with extravidin peroxidase (Sigma) for 30 min at room temperature. Immunoreactivity was then visualized with diaminobenzidine solution (BioGenex, San Ramon, CA, USA). Slides were then incubated overnight at 4 °C in a humidified chamber with rabbit anti-insulin antibody at a 1:200 dilution (Santa Cruz, CA, USA) followed by biotinylated goat–anti rabbit IgG (Vector) for 2 h at room temperature. Slides were washed and incubated with Vectastain ABC–AP reagent (Vector) for 30 min at room temperature. Immunoreactivity was then visualized with Vector Red AP substrate Kit I (Vector). Tissue sections were counter-stained with Carrazi’s hematoxylin and mounted under glass coverslips with Euikit (Ruth Wagener Ent, Newmarket, ON, Canada). To establish specificity of the antisera, controls included substitution of the primary antibody with non-immune serum, omission of the secondary antibody, and pre-absorption of the antibody with an excess of homologous antigen.

Morphometrical analysis

The tissue sections were analyzed by light microscopy at a magnification of ×25 or ×400. Analyses were performed...
with Northern Eclipse (version 6.0) morphometric analysis software (Empix Imaging, Mississauga, ON, Canada). For each animal, five sections per pancreas were examined, taken at 50–60 section intervals to represent the entire tissue. For each section of pancreas, the number of islets with or without insulitis was calculated; as well as the total tissue area, the area of each islet present, the area within each islet occupied by $\alpha$- or $\beta$-cells, and the area of insulitis present within each pancreas section. Insulitis was quantified in NOD mice at 8 weeks of age. At this time, this consisted of predominantly peri-insulitis and occasional non-aggressive insulitis with lymphocytic infiltration in <50% of the islet.

**Hormone and cytokine measurement**

Blood samples from each animal were collected by killing after overnight fast, and serum was separated and stored at $-20^\circ C$. Circulating insulin measurement was performed with a sensitive rat insulin RIA kit (Linco Research Inc., St Charles, MO, USA). Following extraction of pancreas tissue with RNA Trizol the total pancreatic protein was recovered from the protein phase as described by the manufacturer (Life Technologies, BRL). Total insulin content was assayed by RIA and expressed per $\mu$g tissue protein. IFN$\gamma$, TNF$\alpha$, IL-4, and IL-10 were measured within serum or pancreatic tissue using ELISA assays for mouse cytokines (BD Biosciences, Mississauga, ON, Canada) according to the instructions of the manufacturers. The sensitivities of detection were as follows: IFN$\gamma$ (1 pg/ml), TNF$\alpha$ (5 pg/ml), IL-4 (4 pg/ml), IL-10 (0.05 pg/ml). Intra- and inter-assay coefficients of variation were between 4–10% and 6–13% respectively.

**Auto-antibody detection**

A micro-assay for insulin auto-antibody was performed as described by Yu et al. (2000). In brief, $[^{125}]$I insulin was incubated with serum (5 $\mu$l diluted 1:5) with or without unlabeled insulin for 3 days at 4 $^\circ C$. Protein A (50%) and Protein G Sepharose (50 $\mu$l; Pharmacia Biotech Inc.) were added to the incubation and antibody complexes separated on 96-well filtration plates (Millipore, Nepean, ON, Canada). After washing, scintillation liquid was added to each well and radio-labeled insulin quantified by liquid scintillation counting. The presence of auto-antibodies against glutamic acid decarboxylase 65 (GAD65) in serum from NOD mice was similarly determined (Koczwara et al. 2003).

**Statistical analysis**

Data are presented as mean±s.e.m. for all offspring when considering weight gain or the time of onset of diabetes. For analyses of circulating insulin or cytokines, or pancreatic histology, two or three animals per litter were examined representing both genders and a total number of animals between 15 and 30. Animals selected were those closest to the mean litter body weight at that age. For histological assessments, five pancreatic tissue sections were used per organ. This number of sections was shown to yield equivalent mean value estimation to more extensive sectioning accounting for the entire pancreas. Growth curves of pregnant mice or offspring were compared by two way repeated measures of ANOVA followed by a Holm–Sidek post hoc analysis. Serum cytokine levels were compared by Kruskal-Wallis non-parametric test followed by Dunn’s post test. The analysis of the cumulative incidence of diabetes was performed by the comparison of survival curves.
with a Log rank test. For other results, group means were compared by \( t \)-test or two-way ANOVA according to the number of data sets. Upon significant interactions on the two-way ANOVA, differences between individual group means were analyzed by Duncan’s test. In all cases, differences were considered statistically significant at the level of \( P<0.05 \).

**Results**

No differences in food intake were found between LP or control diets for NOD mice within pregnancy or lactation. However, the body weight gain of NOD mice during pregnancy was significantly reduced by the day of birth in animals fed LP diet compared with C-fed animals (Fig. 1A). Despite this, the mean birth weight of the offspring did not change with maternal diet (Fig. 1B and C). Mean litter size and gender ratio were also not altered by diet and the number of surviving offspring after 6 days did not differ (Table 1).

When islet histomorphometry was analyzed at 8 weeks of age, LP-fed NOD female mice had a significantly lower number of islets per unit area of pancreas and a lower mean islet area (Fig. 2A and B). The percentage areas of pancreas occupied by insulin-immunopositive \( \beta \)-cells was lower in NOD females (53\% ± 2\%) than in BALB/c female mice (67\% ± 1\%). No such differences were seen in males (NOD 65\% ± 5\%, BALB/c 75\% ± 3\%). Exposure to LP diet did not significantly alter mean islet area in NOD or BALB/c mice at either age (Table 2). This suggests that \( \beta \)-cell mass might already be lower in NOD female mice compared with a non-diabetic strain prior to the development of insulin, and that this was not significantly reduced further by LP exposure.

At 8 weeks of age, serum insulin levels in the NOD offspring were significantly lower in animals that received LP diet compared with C diet regardless of gender (Fig. 3A), as was the pancreatic insulin content (Fig. 3B). Animals not yet diabetic at 45 weeks of age had lower circulating insulin levels than at 8 weeks and this did not differ with gender or diet. In non-diabetic NOD mice, mean blood glucose values were similar between males and females at 8 and 45 weeks of age in animals fed either LP or C diet (not shown). At the time of

**Table 1** Mean litter size (\( \pm \) S.E.M.) at birth, number of pups surviving after 6 days, and gender ratio for offspring of non-obese diabetic (NOD) mice fed either control of low protein (LP) diet from conception until weaning

<table>
<thead>
<tr>
<th></th>
<th>Pups born</th>
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<th>Pups survived</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LP</td>
<td>Control</td>
<td>LP</td>
</tr>
<tr>
<td>Number of pups</td>
<td>8.71 ± 0.78</td>
<td>7.50 ± 0.82</td>
<td>5.71 ± 0.52</td>
<td>5.50 ± 0.89</td>
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<tr>
<td>Gender ratio (F/M)</td>
<td>1.09 ± 0.11</td>
<td>1.31 ± 0.36</td>
<td>1.04 ± 0.19</td>
<td>1.10 ± 0.40</td>
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</tbody>
</table>

Values represent 10–15 litters of animals.

**Table 2** Mean islet area (\( \mu m^2 \pm S.E.M. \)) at postnatal days 3 and 21 for BALB/c or non-obese diabetic (NOD) mice. Animals were the offspring of mice fed either control of low protein (LP) diet from conception until weaning

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 21</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LP</td>
</tr>
<tr>
<td>BALB/c male</td>
<td>6244 ± 933</td>
<td>5621 ± 363</td>
</tr>
<tr>
<td>BALB/c female</td>
<td>8965 ± 348</td>
<td>7583 ± 277</td>
</tr>
<tr>
<td>NOD male</td>
<td>4864 ± 1073</td>
<td>5119 ± 622</td>
</tr>
<tr>
<td>NOD female</td>
<td>6012 ± 1811</td>
<td>5395 ± 448</td>
</tr>
</tbody>
</table>

Values represent 10–15 animals derived from five separate litters of animals. *\( P<0.05 \) versus BALB/c mice of the same gender.
onset of diabetes, there was also no significant difference in serum insulin with diet or gender (Fig. 3A).

Since cytotoxic cytokine presence is associated with autoimmune destruction of β-cells in the NOD mouse, we measured the circulating levels of IFNγ, TNFα and IL-4 in pre-diabetic animals and at the diagnosis of diabetes. Circulating levels of IL-4, thought to be protective against the onset of diabetes in the NOD mouse, were significantly greater in NOD females, but not males, fed LP diet at 8 weeks of age (Fig. 4). IL-4 levels were generally lower in non-diabetic animals at 45 weeks, and following the onset of diabetes, and did not differ with diet. At 8 weeks of age, circulating levels of IFNγ were significantly lower in LP-fed diabetes-prone female offspring (Fig. 4), but not so in males. At diabetes onset serum IFNγ was lower in both male and female mice in animals that had received LP diet. This did not occur in mice of either sex that had not become diabetic by 45 weeks. Serum levels of TNFα were substantially increased at the onset of diabetes compared with non-diabetic female mice, regardless of diet, and less so in males (Fig. 4). However, the pancreatic content of TNFα in 8-week female offspring was significantly lower in LP-fed mice (control 94 ± 6 μg/mg protein, LP 42 ± 2 μg/mg, *P < 0.05 versus control; mean ± S.E.M., n = 5). Circulating IL-10 levels did not significantly differ with gender or diet at 8 weeks of age or at the onset of diabetes (not shown). These findings suggest a selective change occurred in the levels of certain cytotoxic cytokines occurred in both serum and pancreas of pre-diabetic animals previously exposed to LP diet.

Antibodies against insulin were not detected in serum of any diabetic or pre-diabetic NOD mouse. Prior to diabetes, GAD65 antibody was present in the majority of female and in 50% of male NOD mice both following C diet at 8 weeks of age, and this did not differ with LP diet. GAD65 antibody was detectable in all animals once diabetic, but not in those that had not become diabetic by 45 weeks of age, regardless of diet.

The number of pancreatic islets showing evidence of peri-insulitis at 8 weeks of age in NOD female mice was substantially reduced in animals that had received LP diet (Fig. 5A, C and D). Similarly, when the area of pancreas occupied by insulin was quantitated it was significantly reduced following LP diet (Fig. 5B). Male animals showed a similar percentage of islets with peri-insulitis at 8 weeks of age to females and this did not differ with diet (C, 13 ± 5%; LP 8 ± 3%).

The cumulative incidence of diabetes was compared for NOD males and females previously given LP or control diet (Fig. 6). By 45 weeks of age, 86% of female NOD mice that
had received C diet had become diabetic, but only 63% of LP-fed animals. Of the diabetic animals, the age at which 50% of these had become diabetic was 23.3 weeks following C diet but 30.8 weeks following LP diet. Only 60% of male animals receiving C diet were diabetic by 45 weeks and 15% of animals receiving LP diet.

Discussion

Nutritional sufficiency in early life can influence the incidence and time of onset of autoimmune diabetes in animal models (Pedersen et al. 1999, Oge et al. 2007). Altered dietary protein content has been shown to exacerbate diabetes, a high-protein diet accelerated the onset of disease (Schneider et al. 1996) while a protein-rich high-fat diet increased β-cell loss by apoptosis in pre-diabetic NOD mice (Linn et al. 1999). We report here that a LP but isocalorific diet can delay the onset of diabetes in the NOD mouse. However, it is not clear if the altered ontogeny of disease is primarily mediated through changes in the development and function of the pancreatic β-cell, by changes in T-cell trafficking or cytokine release, or both. It must also be considered that the deficiency of calories due to protein in the diet was balanced by additional carbohydrate, which may also have contributed to the delay in autoimmune diabetes, although a previous report using NOD mice suggested that carbohydrate content of diet did not change the onset of diabetes (Scott et al. 1989).

The provision of LP diet during gestation and lactation in the normal rat resulted in only a mild reduction in birth weight, but severe changes in pancreatic phenotype of the offspring (Snoeck et al. 1990). We found here that in the NOD mouse, feeding of the same LP diet limited maternal weight gain during pregnancy but did not significantly alter birth weight of the offspring, mean litter size, infant survival or gender ratio. The subsequent weight gain of the offspring from LP-fed animals was retarded, but only in males, who have a lower incidence of diabetes. Thus, the general body growth rate of the diabetes-prone females was unaltered.
Feeding of LP diet to both NOD and BALB/c mice in early life did not significantly reduce birth weight or neonatal islet morphology showing that the phenotype in the offspring in this species is noticeably different from that reported in rat. Regardless of diet, we observed that the mean islet area in both female and male NOD animals was significantly less than that found in BALB/c mice at day 21, with a reduced percent contribution of β-cells to islets in NOD compared with BALB/c females. NOD offspring have a higher islet endocrine mass in the first week of life compared with C57BL/6 or BALB/c strains, due in one report to greater numbers of small islets (Palegri et al. 2001) and in another to a greater mean area of larger islets (Geutskens et al. 2004). These differences were less apparent by weaning, but islets from NOD animals had a greater percentage composition of glucagon-positive α-cells suggesting that they were relatively immature (Palegri et al. 2001). We did not detect a greater mean area of islets in NOD versus BALB/c mice on day 3, but it is possible that this was missed since no further tissues were examined until day 21. The extracellular matrix composition of pancreas in neonatal NOD mice showed increased deposition of fibronectin around the islets (Geutskens et al. 2004). Such fibronectin-rich sites are associated with the localization of macrophages during insulitis, which might contribute to the precipitation of diabetes.

Substantial remodeling of rodent islets occurs between birth and weaning with β-cell turnover by apoptosis and islet replacement through neogenesis (Petrik et al. 1998). The rate of β-cell apoptosis at this time was elevated in the female NOD mouse, leading to a relatively reduced β-cell mass (Trudeau et al. 2000). When neonatal β-cell remodeling was prevented in the NOD mouse by trans-placental exposure to bafilomycin, a vacuolar-ATPase inhibitor, this was associated with an accelerated onset of diabetes, suggesting that disruption of β-cell ontogeny renders the adult cells more susceptible to cytokine-induced death (Hettiarachchi et al. 2008).

The mean pancreatic islet size was not significantly different in offspring of control versus LP-fed NOD mice on postnatal days 3 or 21, prior to the commencement of insulitis. This is in agreement with the intrauterine exposure of NOD mice to a reduced calorie diet where β-cell mass in the offspring was unaltered at postnatal day 14 relative to control-fed animals (Oge et al. 2007). However, by 8 weeks of age, female offspring exposed to LP diet had significantly reduced mean islet number and size compared with control-fed mice. This was not likely to be due to autoimmune loss of β-cells since the extent of insulitis was much reduced in LP-fed mice, and the smaller islet size was associated with a reduction in both β- and α-cell components. No such changes were seen in male offspring suggesting that there might be a differential developmental deficiency in the islets of the female NOD mouse.

The higher rate of developmental β-cell turnover and cell death reported in the neonatal female NOD mouse (Trudeau et al. 2000) could act as an initiator for T-cell activation and

Figure 5 Percentage of islets (mean ± S.E.M.) that showed associated peri-insulitis (A) and the percentage of pancreas area accounted for by insulitis (B) in female offspring of control (open bars) or LP-fed (LPD, closed bars) NOD mice at age 8 weeks. *P<0.05 versus control fed mice (n=25 animals, 2–3 per litter). The immunohistochemical localization of insulin (red) and glucagon (brown) is shown within islets without (C) or with (D) peri-insulitis (arrow) in a control-fed animal. Magnification bar 100 μm.
later insulitis. This may result in a higher metabolic stress on individual β-cells resulting in additional cell damage and auto-antigen release. It has been shown that at 4–8 weeks of age, NOD and NOD/SCID mice present a paradoxical but transient hyperinsulinemia. This is manifested as a lower glycemia that is preceded by transient, perinatal β-cell hyperactivity, particularly in the females (Homo-Delarche 1997, Rosmalen et al. 2002). The beneficial effects of prior LP exposure on preventing insulitis may be related to a reduction in β-cell metabolic stress. We found that at 8 weeks of age serum insulin levels in the offspring were significantly lower in animals that received LP diet regardless of gender, as was the pancreatic insulin content. This might be expected to correlate with a reduced presence of islet auto-antibodies. However, antibodies to insulin were not detectable in these mice while anti-GAD65 antibodies were present in all females at 8 weeks of age, and some males, regardless of diet, suggesting that a primary effect of LP diet on the delay in diabetes is likely not due to altered GAD65 antibody presence.

It seems unlikely that β-cells in NOD females that received LP diet were more resistant to cytokine-induced damage than those of control-fed mice, leading to less auto-antigen release, since islets from rats fed LP diet in early life were more susceptible to cytokine-induced apoptosis (Merezak et al. 2004). However, the balance of cytotoxic cytokines might be altered in the pancreas of the LP-fed NOD mouse resulting in less insulitis and a delay in β-cell loss. A direct effect of LP diet on the development of the immune system is likely to contribute to the delayed onset of diabetes seen in these studies. The maternal immune system is known to be influenced by gestational nutritional deficiency, with a reduced immune function in the offspring (Chandra 1975). Lifelong food restriction reduces antigen-triggered memory T-cell responses and increases levels of the anti-inflammatory IL-2, while lowering levels of IL-6 and TNFα. Restriction of energy intake increases circulating corticosterone levels, which in turn enhances negative selection of potentially autoreactive CD4–CD8 cells in thymus (Wilder 1995). In our studies, female animals fed LP diet in early life had lower circulating levels of IFNγ at 8 weeks of age, and also at the onset of diabetes. Serum levels of TNFα were not altered, but the pancreatic content of TNFα was significantly lower in LP-fed female NOD mice, consistent with a reduction in the number of islets demonstrating insulitis, and the degree of insulitis, where this was present. While the onset of autoimmune diabetes is more rapid in the female NOD mouse, the ability of LP diet in early life to delay the onset was seen in both sexes, implying that effects of diet on subsequent cytotoxic cytokine levels are likely to be gender independent.

In summary, we found that feeding a LP diet to NOD mice throughout gestation and until weaning selectively decreased levels of cytotoxic cytokines, reduced insulitis, and delayed the onset of diabetes. In females, LP diet was also associated with a reduction in the number and size of pancreatic islets, a lower pancreatic insulin content, and lower serum insulin by 8 weeks of age. The mechanism by which LP diet delays the onset of diabetes is likely to involve both immune alterations and changes in β-cell development.

Declaration of interest

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

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Delay in onset of diabetes in NOD mice with LP diet · A CHAMSON-REIG and others

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