The effect of maternal protein deficiency during pregnancy and lactation on glucose tolerance and pancreatic islet function in adult rat offspring

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Abstract
To test the hypothesis that poor foetal-neonatal nutrition predisposes adult animals to impaired glucose tolerance or diabetes, pregnant and lactating rats were fed a low (5%) protein diet and glucose tolerance and pancreatic islet function then assessed in the adult offspring. To expose any underlying defects the offspring were allowed access to a sucrose supplement (35%) or fed a high fat diet. Offspring born to low protein-fed females had significantly lower body weights than controls. In islets from previously malnourished rats, insulin release in batch incubations or perfusion was not significantly different to controls. In islets from previously malnourished animals fed sucrose, glucose-stimulated insulin release was reduced in perfusion by 66% (P<0.01) and batch incubations by 26–52% (6–16 mmol/l glucose, P<0.01). Similarly, impaired secretory responses were found in islets from previously malnourished animals fed a high fat diet. These did not result from a reduced pool of releasable insulin, as arginine-stimulated secretion was not impaired. Rats previously malnourished showed a normal glucose tolerance. Glucose tolerance was impaired, however, in previously malnourished rats fed sucrose (area under the glucose tolerance test curve was increased by 42%, P<0.05) but despite the reduced islet secretory responses was not significantly different to sucrose-fed controls (area increased by 54%, P<0.05). Glucose tolerance was impaired in previously malnourished animals fed high fat diet (area increased by 48%, P<0.05) more so than in high fat-fed-controls (28% increase, NS). These data support the hypothesis that poor foetal-neonatal nutrition leads to impaired pancreatic β-cell function which persists into adult life. Alone this is not sufficient to produce diabetes, but an inability to respond to a highly palatable fat diet may tip the balance towards impaired glucose tolerance. Journal of Endocrinology (1997) 154, 177–185

Introduction
Non-insulin-dependent diabetes (NIDDM) is characterised by both impaired insulin secretion from pancreatic β-cells and insulin resistance in peripheral tissues (Leahy 1990). Although it is generally believed to be a genetic disorder, the impact of dietary status on the development of diabetes may be pronounced (Leahy 1990, Porte 1991). The relationship between overnutrition, obesity and diabetes is well recognised whereas the role of malnutrition is less well understood. Children with syndromes of protein malnutrition such as Kwashiorkor exhibit impaired glucose tolerance and poor insulin secretory responses (Baig & Edozien 1965, Milner 1972). Alternatively, it has been documented, as in the case of Ethiopian immigrants to Israel (Cohen et al. 1988), that the incidence of diabetes increases on changing from a diet where poor nutrition is prevalent to one high in refined carbohydrate. Recently, Hales, Barker and colleagues have addressed the role of adequate foetal nutrition for programming the subsequent glucose homeostatic mechanisms of the individual and the involvement of this process in NIDDM (the thrifty phenotype hypothesis) (Hales & Barker 1992). These authors have proposed that NIDDM may not have a genetic basis but rather that poor foetal (and neonatal) nutrition impairs normal pancreatic β-cell development and predisposes the individual to the subsequent development of diabetes in later life. Poor maternal diet can impair the developing foetal endocrine pancreas (Snoeck et al. 1990) and can impair secretory responses in foetal pancreatic islets (Dahri et al. 1991). It has also been shown, however, that maternal malnutrition does not lead to impaired secretory responses in islets isolated from the offspring (Rasschaert et al. 1995) or to impaired glucose tolerance in the adult offspring (Langley et al. 1994, Holness 1996). We have tested the thrifty phenotype hypothesis by first producing a model of foetal and neonatal malnutrition (by feeding pregnant then lactating rats a diet low in protein) and then examining pancreatic islet function and glucose tolerance in the adult offspring.
Our approach has been to try to expose any underlying abnormalities in pancreatic β-cell function and thus glucose tolerance by feeding diets to the adult animals which place additional demand on the mechanisms of glucose homeostasis, diets which are high in carbohydrate or high in fat content.

Materials and Methods

Materials

BSA, hexokinase/glucose 6-phosphate dehydrogenase and NADP were supplied by Boehringer Mannheim Ltd (Lewes, East Sussex, UK). Heps, Hanks' balanced salts solution, ATP and collagenase type I A were all supplied by Sigma Chemical Co. (Poole, Dorset, UK). Sagatal was supplied by Southern Veterinary Supplies (Lewes, East Sussex, UK). Millicell culture inserts were from Millipore (Milton Keynes, Bucks, UK). 125I-Labelled insulin (225 µCi/µg) was supplied by Lifescreen (Aylesbury, Bucks, UK). Anti rat insulin antibody was generously provided by Dr R. Turner (University of Oxford, Oxford, UK). Rodent diets were supplied by Special Diet Services (Witham, Essex, UK).

Animals

Pregnant and lactating rats (Sprague–Dawley) were allowed free access to either a control diet (w/w protein, 18%; corn starch, 45%; oil, 2.5%; cereal product, 28.5%; vitamins/minerals, 5% and DL-methionine, 0.3%) or a low protein diet (w/w protein 5%; casein, 2.5%; corn starch, 60%; vegetable protein, 2.5%; soya oil, 5%; cereal product, 24.5%; vitamins/minerals, 5% and DL-methionine, 0.3%). Twenty breeding pairs fed the low protein diet produced on average 8.5 ± 0.6 pups per litter (83 male and 77 female pups in total) which was similar to that observed in control breeding pairs (10.5 ± 0.8 pups per litter). Male and female offspring were weaned onto control diet at age 21–28 days. This diet was fed ad libitum until age 10 weeks, when some animals (primarily males although the experiments were confirmed in females) were allowed free access to a carbohydrate supplement, in the form of a 35% sucrose solution (in addition to normal solid food and drinking water). Alternatively, a number of rats were fed control diet until age 13 weeks, when their diet was changed to one with a high fat content to which they had free access. The composition of the diet was (w/w) lard, 30%; casein, 20%; corn starch, 18%; sucrose, 14.7%; solkaffloc, 5%; soya oil, 5%; vitamins/minerals, 5% and DL-methionine, 0.3%. All animals were allowed free access to drinking water.

This protocol produced six groups of animals: controls (fed control diet throughout the study); controls+ sucrose (fed control diet throughout, sucrose additionally provided at age 10 weeks); controls+high fat (fed control diet, until high fat diet was provided at age 13 weeks); previously malnourished (mothers fed low protein diet during pregnancy and lactation, control diet from weaning to the end of experiment); previously malnourished+ sucrose (low protein diet until weaning, sucrose provided at age 10 weeks); previously malnourished+ high fat (low protein diet until weaning, high fat diet provided at age 13 weeks). Rats were maintained on normal diet with sucrose supplementation for 3 weeks, or on high fat diet for 4 weeks, before the experiments described below were carried out.

Glucose tolerance test

An intraperitoneal glucose tolerance test was carried out on rats that had been fasted overnight using a dose of 2 g glucose per kg body weight (Hughes et al. 1994). Rats were anaesthetised by Sagatal injection and blood samples (50 µl) collected into heparinised tubes from cut tail tips. Sampling was carried out every 30 min over a 2 h period. Blood samples were mixed with ice cold perchloric acid at a final concentration of 3.84% (v/v), centrifuged at 2500 g for 5 min, and the supernatant collected and stored at −20 °C prior to assay. Samples were assayed for glucose using the hexokinase method on a Cobas Bio auto-analyser (Roche, Welwyn Garden City, UK). Areas under glucose tolerance test curves were calculated by the trapezoidal rule (Thorburn et al. 1990).

Islet isolation

Islets of Langerhans were isolated from males and females; either non-fasting rats, or from fasting rats that had previously undergone a glucose tolerance test. Rats were anaesthetised with Sagatal, and islets prepared by collagenase digestion following injection of collagenase into the pancreas through the bile duct (Sutton et al. 1986). Free islets were collected under a dissecting microscope with a 20 µl pipette into Heps-buffered Hanks' balanced salts solution containing 0.5% BSA.

Insulin release

Insulin release from freshly isolated islets was measured in a perifusion system as described elsewhere (Hughes et al. 1992). In this system, 50 islets were housed in small chambers on Millicell culture inserts and perifused in basal medium (Krebs–Ringer containing 20 mmol/l Heps, pH 7.4, 5 mg/ml BSA and 2 mmol/l glucose) for 60 min at a flow rate of 1 ml/min at 37 °C prior to collection of fractions. Test agents were added as indicated. Fractions (2 ml) were collected at 2-min intervals and stored at −20 °C prior to assay for insulin by RIA (Ashcroft & Crossley 1974). At the end of the perifusion, islet insulin
Figure 1  Body weights of male (○, △) and female (●, ▽) offspring born to either control (○, ●) or low protein diet mothers (△, ▽). The inset enlarges the period before weaning. Data represent the mean ± s.e.m. of data from 50–100 animals. The body weights of offspring from low protein-fed dams were significantly lower than controls at all time points (P<0.001, Student's t-test).

Insulin release
The results of perifusion experiments on islets isolated from animals in the various groups are shown in Fig. 2. Neither sucrose nor high fat diet consumption by control animals had any significant effect on insulin release from isolated islets, whether stimulated by glucose or arginine (Fig. 2A). Control islets (50) released 4·53 ± 0·64 mU insulin (n=8) during the 40-min perifusion period in the presence of 10 mmol/l glucose. Islets from previously malnourished animals released only 2·25 ± 1·0 mU insulin/40 min (n=8) in response to perifusion with 10 mmol/l glucose, although this was not significantly different to controls (P=0·07; Fig. 2B) nor was the response of these islets to arginine significantly different to that of controls. Islets from previously malnourished animals fed sucrose released 1·54 ± 0·58 mU insulin in response to during the first 24 h being 164 ± 14 kJ (n=8)), leading to an increase in the total daily energy intake of these rats of 16%, and an increase in energy intake in the form of carbohydrate alone, of 50%. This did not, however, lead to any increase in the weight gained over the 3-week sucrose feeding period (Table 1). Similar observations were made in previously malnourished animals. In animals (both control and previously malnourished) fed a diet high in fat content, weight gain increased over the 4-week feeding period, compared with animals left on the normal diet (Table 1).

Statistical analysis
Results are given as means ± s.e.m. for the number of experiments indicated. Statistical analysis was carried out using Student’s t-test.

Results
Figure 1 shows the body weights of offspring born to mothers fed either a control diet during pregnancy and lactation, or a diet low in protein. Previously malnourished offspring were significantly smaller than control offspring at all points measured. The weight gain of both sets of animals accelerated when they were weaned onto a normal diet, but the previously malnourished animals remained smaller throughout. At 10 weeks of age a number of animals were allowed access to a carbohydrate supplement in the form of a 35% sucrose solution. This was readily consumed by the animals (intake in the controls was released by sonication and samples collected and stored for determination of total islet insulin content (by RIA).

Insulin release was also measured from batches of five islets incubated for 1 h at 37 °C in Krebs–Ringer medium containing 2 mg/ml BSA and various glucose concentrations. Incubations were stopped by cooling on ice. After centrifugation (1000 r.p.m., 1 min) aliquots of supernatant were collected and stored for insulin assay.

Results
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Table 1  Rat body weights before and after feeding regimes

<table>
<thead>
<tr>
<th></th>
<th>Sucrose regimen</th>
<th>High fat regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control+ sucrose</td>
</tr>
<tr>
<td>Male weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>282 ± 13 (11)</td>
<td>295 ± 7 (31)</td>
</tr>
<tr>
<td>End</td>
<td>364 ± 9</td>
<td>359 ± 8</td>
</tr>
<tr>
<td>Female weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>229 ± 4 (7)</td>
<td>220 ± 1 (3)</td>
</tr>
<tr>
<td>End</td>
<td>254 ± 4</td>
<td>256 ± 5</td>
</tr>
</tbody>
</table>

The number of animals in each group is shown in parentheses. *P<0·01 vs control for high fat study.
stimulation by glucose which was significantly lower than in islets isolated from controls ($P<0.01, n=6$), whereas the response to arginine was not significantly different (Fig. 2C). The insulin release from islets isolated from previously malnourished animals fed high fat diet is shown in Fig. 2C. The islets showed a significantly impaired response to glucose (1.50 $\pm$ 0.34 mU insulin released, $P<0.01, n=7$), but not to arginine when compared with islets isolated from controls.

Insulin release was similar in males and females; secretion at 16 mmol/l glucose was 451 $\pm$ 38 or 412 $\pm$ 49 µU/islet per hour in control male (5) or female (3) islets respectively and 388 $\pm$ 43 or 468 $\pm$ 42 µU/islet per hour in previously malnourished male (5) or female (4) islets respectively. Data were therefore pooled. Increasing the medium glucose concentration led to a dose-dependent increase in the rate of insulin release from the islets in batch incubations, such that in control animals an increase from 2 to 16 mmol/l glucose caused a 35-fold increase in insulin secretion (Table 2). Consumption of sucrose by control animals had no significant effect on insulin release at the glucose concentrations tested. On the other hand, high fat consumption by control animals significantly increased the rate of insulin release from isolated islets, at 6 and 10 mmol/l, but not at 16 mmol/l glucose (Table 2).

Islets isolated from the offspring of low protein-fed mothers did not show a significant difference in insulin release at stimulatory glucose concentrations compared with controls. However, the basal insulin release at 2 mmol/l glucose was significantly 3-fold higher than in controls ($P<0.001$). For this reason 16 mmol/l glucose only induced a 12-fold increase in insulin release, as opposed to the 35-fold increase seen in controls. In previously malnourished animals fed sucrose there was a significant impairment ($P<0.01$) in insulin release from isolated islets at all stimulatory glucose concentrations (26–52% reduced compared with islets isolated from controls; Table 2). In islets from previously malnourished animals fed a high fat diet, the rate of insulin release in response to 16 mmol/l glucose was 71% of that observed in islets from controls ($P<0.05$, Table 2). In islets from these animals the rate of insulin release at 2 mmol/l glucose was significantly 2.7-fold higher than in controls ($P<0.001$).

The insulin contents of the islets in the different groups of animals (measured after the perfusion experiments) were not significantly different. These were: control, 2.4 $\pm$ 0.2 mU/islet; control+sucrose, 1.8 $\pm$ 0.2; control+ high fat 2.6 $\pm$ 0.3; previously malnourished, 2.4 $\pm$ 0.2;

**Figure 2** Insulin release in perfused islets from control and previously malnourished rats. Panel A: 50 islets from either control (○), sucrose fed (●) or high fat fed (△) rats were perfused with medium containing 2 mmol/l glucose for 65 min prior to stimulation with 10 mmol/l glucose followed by 19 mmol/l arginine. Panel B shows data from islets isolated from control (○), previously malnourished (△) or previously malnourished rats provided with a sucrose supplement (●). Panel C shows data from islets isolated from control (○), previously malnourished (△) or previously malnourished rats fed a high fat diet (●). Data are expressed as µU insulin released/min and represent the mean $\pm$ S.E.M. for 6–8 separate experiments.
Table 2. Insulin release in batches of islets incubated at various glucose concentrations from control and previously malnourished rats.

<table>
<thead>
<tr>
<th>Glucose Concentration (mmol/l)</th>
<th>Control</th>
<th>Control-sucrose</th>
<th>Control-high fat</th>
<th>Previously malnourished</th>
<th>Previously malnourished+sucrose</th>
<th>Previously malnourished+high fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12.6±2.5</td>
<td>70.3±6.7</td>
<td>341±22</td>
<td>36.3±5.3**</td>
<td>91.7±11.9</td>
<td>377±36</td>
</tr>
<tr>
<td>6</td>
<td>14.7±2.6</td>
<td>67.6±12.3</td>
<td>364±19</td>
<td>11.3±2.7</td>
<td>33.5±4.6**</td>
<td>253±35</td>
</tr>
<tr>
<td>10</td>
<td>126±21.2**</td>
<td>383±23</td>
<td>454±57*</td>
<td>34.2±5.0**</td>
<td>94.2±14.3</td>
<td>287±42</td>
</tr>
<tr>
<td>16</td>
<td>444±22</td>
<td>448±46</td>
<td>431±32</td>
<td>318±28**</td>
<td>431±32</td>
<td></td>
</tr>
</tbody>
</table>

Results are the mean ± S.E.M. of 20–36 observations from 5–8 separate experiments. *P<0.05, **P<0.01 compared with controls.

Glucose tolerance

There were no gender differences in glucose tolerance in control or previously malnourished rats. In control animals, sucrose feeding significantly increased blood glucose at several time points during the course of the test (Fig. 3A) and significantly increased the area under the glucose tolerance test curve by 54% (Fig. 3A, inset). High fat feeding of control animals (Fig. 3B) also increased blood glucose at several time points, but this effect was not as great as with sucrose feeding and the area under the glucose tolerance test curve was not significantly increased (Fig. 3B, inset).

In previously malnourished animals glucose tolerance was not significantly different to controls. The consumption of sucrose by previously malnourished animals led to a deterioration in glucose tolerance. The area under the glucose tolerance test curve was similar to that in controls fed sucrose and was significantly increased by 42% when compared with controls (Fig. 3A, inset). Changing the diet of previously malnourished animals to a high fat diet (Fig. 3B, inset) led to a deterioration in glucose tolerance; the area under the glucose tolerance test curve was significantly greater by 48% than that of control animals. The area was not significantly different to controls fed high fat diet.

Discussion

Poor nutrition during the foetal–neonatal period may predispose to the development of impaired glucose tolerance or diabetes in adult life (Hales & Barker 1992). In recent studies to test this hypothesis, Holness and Sugden (1996) reported that glucose utilisation was normal using the euglycaemic–hyperinsulinaemic clamp technique and that there was no deterioration in glucose tolerance in pregnant rats that were the offspring of animals fed a low protein diet during pregnancy. Conversely, Ozanne et al. (1996) reported that poor maternal nutrition led to a reduction in the sensitivity of glucagon stimulated-glucose production to inhibition by insulin in the livers of the male rat offspring. We have fed pregnant and lactating rats a 5% (w/w) low protein diet in order to investigate the effects of foetal–neonatal malnutrition on pancreatic islet function and glucose tolerance in the adult offspring. This regime is more severe than that used in the studies mentioned above (8% protein diet) and possibly models more severe human malnutrition. We show here that this approach leads to the birth of small pups, which do not recover normal body weight throughout adult life, even after weaning onto a control diet. This finding is different to that of a previous study (Dahri et al. 1991), where animals nursed by mothers fed a normal diet recovered normal body weight. This suggests that nutrition during the period immediately after birth may be an important determinant of body weight in the adult offspring. Our finding that glucose-stimulated insulin release in pancreatic islets isolated from previously malnourished rats is not different to controls confirms the study by Rasschaert et al. (1995). We show for the first time, however, that glucose-stimulated insulin release is impaired in islets from previously malnourished animals fed highly palatable fat or carbohydrate diets. Feeding such diets presumably places additional demand on the pancreatic β-cell and hence unmasks a secretory defect induced by poor nutrition during the foetal and neonatal period. The mechanism underlying the impaired insulin release in islets from previously malnourished rats is not addressed in the present study. It is, however, apparent that this is not related to a reduced capacity to secrete insulin since islet insulin content was not reduced, and the
response to arginine was not impaired indicating that the readily releasable pool of insulin was not reduced in size. Results from morphological studies do not agree on whether β-cell mass is reduced in animals exposed to protein-calorie malnutrition (Dixit & Kaung 1985, Swenne et al. 1992). It is unclear therefore whether the impairment of insulin release is due to functional or structural abnormalities.

High carbohydrate or high fat feeding did not impair glucose-stimulated insulin release in isolated islets from
control animals. Feeding sucrose as part of the solid diet has been shown to increase glucose-stimulated insulin secretion in vitro in the perfused pancreas preparation (Kergoat et al. 1987). Studies using sucrose given in solution (as in the present study) show no changes in fasting plasma glucose or insulin (Kanarek & Orthen-Gambill 1982), suggesting that with this approach, insulin secretion in response to a given glucose level is not increased. High fat feeding has been shown to decrease glucose-stimulated insulin secretion in mice (Capito et al., 1992), as has long-term lipid infusion (Sako & Grill 1990) and exposure of rat islets in culture to fatty acids (Zhou & Grill 1994). However, short-term infusion has been shown to increase glucose-stimulated insulin release (Sako & Grill 1990), as has long-term lipid infusion in normal humans under hyperglycaemic conditions (Boden et al. 1995). Indeed in our study, in islets isolated from the high fat-fed controls, insulin secretion was increased at certain glucose concentrations.

Previous studies looking at the effects of protein-calorie malnutrition on glucose tolerance have used short-term (Swenne et al. 1987, Escriva et al. 1991) or long-term (Younoszai & Dixit 1980, Levine et al. 1983, Okitolonda et al. 1987) exposure of weanling rats to a low protein diet. These studies show that animals consuming such a diet have either a normal (Younoszai & Dixit 1980, Levine et al. 1983), slightly impaired (Okitolonda et al. 1987, Swenne et al. 1987) or even improved (Escriva et al. 1991) tolerance to a glucose load and that after a period of recovery on a control diet, partial (Okitolonda et al. 1987) or complete (Swenne et al. 1987) recovery of glucose tolerance was observed. In studies using offspring of rats fed a low protein diet during gestation, animals that were fed a control diet from birth onwards or from weaning had normal (Dahri et al. 1991, Holness 1996) or lower (Langley et al. 1994) blood glucose levels at the end of a glucose tolerance test. In the present study, rats that were previously malnourished did not exhibit any significant reduction in glucose tolerance. It is possible that protein-calorie malnutrition early in life may lead to a reduced ability to respond to nutritional challenges (Swenne et al. 1987) such as in the case of Ethiopian immigrants to Israel (Cohen et al. 1988) where changing from conditions of poor nutrition to a diet high in refined carbohydrate, increased the prevalence of diabetes. In our study, the consumption of excess carbohydrate, or high fat diet, by previously malnourished rats after a period of recovery on normal diet led to a deterioration in glucose tolerance, such that it became significantly impaired compared with control animals.

A striking finding in the present study is that on feeding high carbohydrate diet, a similar reduction in glucose tolerance was observed in controls and in previously malnourished animals. Allowing normal animals access to a sucrose solution has been shown previously to impair glucose tolerance (Kanarek & Orthen-Gambill 1982), and when sucrose is incorporated into a solid diet, to decrease insulin sensitivity in adipose tissue (Reiser & Hallfrisch 1977) and liver (Kergoat et al. 1987). Given the poor secretory responses to glucose in islets isolated from the previously malnourished rats fed sucrose or high fat diet, one might expect a much larger deterioration in glucose tolerance than that observed in controls. A similar phenomenon has been shown previously in weanling animals fed a low protein diet (Younoszai & Dixit 1980, Levine et al. 1983, Swenne et al. 1987), and is seemingly due to increased sensitivity of both hepatic and peripheral tissues to stimulation by insulin (Levine et al. 1983, Okitolonda et al. 1987, Escriva et al. 1991). Thus it is possible that while the sucrose and high fat feeding promote insulin resistance, foetal–neonatal malnutrition may improve insulin sensitivity in peripheral tissues. Only in the case of high fat feeding to induce insulin resistance is this effect overcome and glucose tolerance deteriorates. Thus longer-term feeding on these regimes may overcome the increased sensitivity induced by malnutrition during the foetal–neonatal period and in concert with the reduced insulin secretion which we found may lead to a severe deterioration in glucose tolerance or even overt diabetes.

Acknowledgements

This study was supported by grants from the British Diabetic Association and Medical Research Council.

References


Leahy JL 1990 Natural history of beta cell dysfunction in NIDDM. *Diabetes Care* 13 992–1010.


Sako Y & Grill VE 1990 A 48-h lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and β cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology* 127 1580–1589.


Received 30 July 1996
Revised manuscript received 10 March 1997
Accepted 11 March 1997