The influence of sub-optimal protein nutrition on insulin hypersecretion evoked by high-energy/high-fat feeding in rats

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Received 12 September 1996

Abstract Low (8%) protein feeding during pregnancy impairs the functional development of the fetal endocrine pancreas. Continued low-protein feeding post-natally decreases pancreatic insulin content and secretion, whereas transfer to standard diet evoked β-cell recuperation. Glucose-stimulated insulin secretion (GSIS) and insulin action were examined in vivo at 28 days after transfer from 8% protein diet to a high-energy/high-fat/standard (20%)-protein diet (HEF diet). HEF feeding dramatically enhanced GSIS after intravenous glucose in control rats, but not in rats previously maintained on the low-protein diet. However, glucose disappearance after intravenous glucose, and glucose production and whole-body glucose disposal during euglycaemic-hyperinsulinaemic clamps were unaffected by prior protein malnutrition. In conclusion, impaired insulin secretion after protein malnutrition is exacerbated by high-energy/high-fat feeding, but this response is not linked to enhanced whole-body insulin resistance.

Key words: Insulin secretion; Euglycaemic-hyperinsulinaemic clamp; Glucose kinetics; High-fat feeding; Low-protein feeding

1. Introduction

The provision of an isocaloric diet containing 8% rather than 20% protein during pregnancy in the rat leads to a profound impairment in the structural and functional development of the fetal endocrine pancreas [1], in association with reduced fetal growth and a low birth weight [1–4]. The provision of a diet containing the normal protein content from weaning to adulthood leads to recovery of the insulin secretory capacity [1]. In contrast, the continued provision of the same diet post-natally leads to an impaired growth rate, decreased pancreatic insulin content and reduced insulin secretion by islets when challenged in vitro with a range of secretagogues, including glucose [1], and the progeny at maturity exhibit low insulin levels and impaired glucose tolerance after oral glucose tolerance [1].

Because of an association between low birth weight and the later development of the combination of poor glucose tolerance, hypertriglyceridaemia and hypertension [5,6], it has been suggested that nutritional inadequacy (which may include lack of adequate dietary protein) imposed on an individual during early life may increase the risk of becoming diabetic in adulthood, particularly in the presence of inappropriate nutrition and obesity [7]. The objective of the present study was to determine the impact of sub-optimal protein nutrition from conception to adulthood on the insulin secretory response and glucose tolerance after an intravenous glucose challenge and insulin responsiveness during euglycaemic-hyperinsulinaemic clamps after switching for 4 weeks to a diet containing the standard amount of protein but an increased ratio of fat to carbohydrate as a dietary challenge (high-energy/high-fat diet; HEF).

2. Materials and methods

Glucose assay kits were obtained from Boehringer Corp. (London), Lewes, Sussex, UK. Kits for determination of plasma insulin concentrations were from Phadeseph Pharmacia, Uppsala, Sweden. Other biochemicals and chemicals were from Boehringer Corp. or from Sigma Chemical Corporation, Poole, Dorset, UK. Radiolabeled [3-^{3}H]glucose was from Amersham International, Amersham, Bucks, UK. Female Wistar rats were purchased from Charles River Ltd., Margate, Kent, UK.

The control diet contained 20% protein (casein supplemented with DL-methionine (0.2 g/100 g diet)) and 4.3% lipid (soybean oil) by weight, and other dietary components as specified elsewhere [1,2]. The experimental low-protein/normal-energy diet contained 8% protein (casein supplemented with DL-methionine (0.08 g/100 g diet)) and 4.3% lipid (soybean oil) by weight. Isocaloricity was maintained by increasing the carbohydrate content (see [2]). Energy content (kcal/100 g diet) was 367 for the control diet and 365 for the low-protein diet. The HEF diet (419 kcal/100 g diet) contained the standard proportion of protein (20%) but 22% lipid by weight, namely lard, together with a corresponding decline in carbohydrate content to 33%. Corn oil (2% by weight) was included to prevent essential fatty acid deficiency (see [8] for further details). The control and low-protein diets were prepared in pellet form by Hope Farms BV, Woerden, The Netherlands, whereas the HEF diet was prepared at 3-day intervals from individual components supplied by Special Diet Services, except the lipid components which were purchased locally.

Rats were housed in a temperature-controlled room (21 ± 2°C) on a standard 12-h light/12-h dark cycle (light from 08.00 h). First-generation rats were time-mated by the appearance of sperm plugs (day 0 of pregnancy), immediately randomly assigned to isocaloric 8% protein or 20% protein diets, and maintained on these diets throughout pregnancy and lactation. The provision of the low-protein diet did not influence maternal food intake or body weight gain during pregnancy [2], but led to a reduction in the mean fetal weight at day 19 of gestation [2,4]. The mean litter sizes per dam during lactation were not affected (results not shown). At 26 days after birth female offspring were weaned onto the maintenance diet with which their mothers had been provided (i.e. either control or low-protein diet). The male offspring were culled at weaning. Exposure to the low-protein diet during fetal life and weaning resulted in a 33% reduction ($P < 0.001$) in body weight of the female offspring at weaning (control diet, 38.7 ± 1.3 g ($n = 16$); low-protein diet, 26.0 ± 0.3 ($n = 22$)). The female offspring continued to be maintained on either control or low-protein diet for approx. 150 days, and are termed the C or LP groups respectively. At 150 days of age both C and LP groups of offspring were transferred to the HEF diet and maintained on the HEF diet for 28 days.

Each rat was fitted with two chronic indwelling jugular cannulas (for infusion and sampling respectively) under Hypnorm (fentanyl citrate (0.315 mg ml^{-1})/fluanisone (10 mg ml^{-1}); 1 ml kg^{-1} i.p.) and diazepam (5 mg ml^{-1}; 1 ml kg^{-1} i.p.) anaesthesia after 21–23 days of HEF feeding, i.e. between 5 and 7 days before the studies were performed at 28 days of HEF feeding. Intravenous glucose tolerance

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PII S0014-5793(96)01067-8

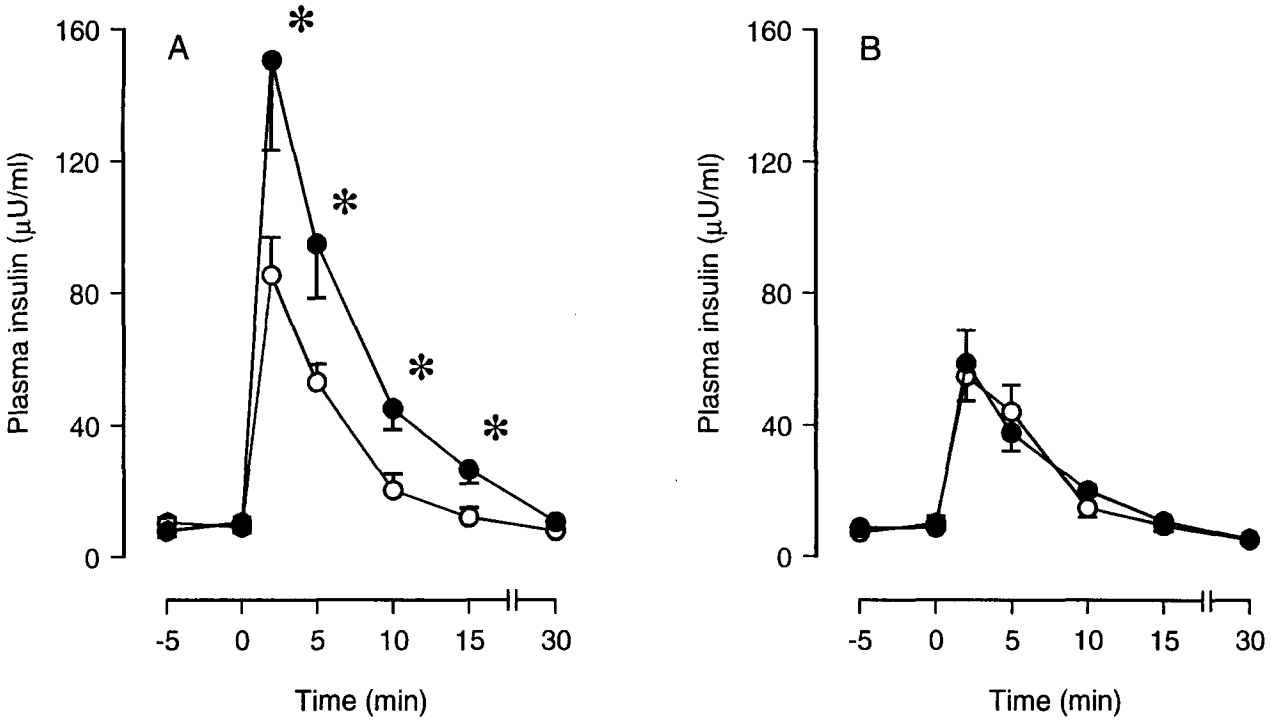

Fig. 1. Plasma insulin concentrations before and during an intravenous glucose tolerance test performed in the post-absorptive state in C (A) and LP (B) female rats with (closed symbols) or without (open symbols) transfer to HEF diet for 28 days. Values are means ± S.E.M. Statistically significant effects of HEF feeding are indicated: $^{*}P < 0.05$.

tests (IVGTT; 0.5 g glucose/kg body weight) were performed at 5–7 days after cannulation in awake, unstressed rats in the post-absorptive state at 6 h after food withdrawal [3,4]. The insulin and glucose responses during IVGTT were calculated as the incremental areas under the blood glucose and plasma insulin curves respectively (using the trapezoidal rule) from data obtained during the 30-min period following glucose injection. Rates of glucose disappearance (K values) were calculated from the slopes of the regression lines obtained with the log-transformed blood glucose values between 2 and 15 min after glucose administration. Endogenous glucose production (R_a) and whole-body glucose disposal rates (R_d) were estimated in the post-absorptive state (at 6 h after food withdrawal) and during a euglycaemic-hyperinsulinaemic clamp. The rats were awake and allowed to move freely throughout the study. Full details of the procedures are given in [2,4]. The blood glucose metabolic clearance rate (MCR) was calculated by dividing R_d by the blood glucose concentration.

Blood glucose concentrations during the clamp were measured using a glucose analyser (YSI, Yellow Springs, OH, USA). Blood samples for determination of tracer concentrations were deproteinised immediately with $ZnSO_4/Ba(OH)_2$ and immediately centrifuged. An aliquot of the supernatant was added to scintillant (Optiphase HiSafe 3) for counting in a liquid scintillation spectrometer. To measure plasma [^{3}H]glucose, supernatants (0.25 ml) were dried down at 60°C before counting to remove any ^3H_2O. The dry residue was dissolved in 0.25 ml distilled water and counted with 10 ml of Optiphase HiSafe 3.

Results are expressed as means ± S.E.M. Statistical significance of differences was assessed with Student's t-test.

3. Results

3.1. Effects of low-protein/normal-energy diet on post-natal growth before and after transfer to high-energy/high-fat diet

Body weights at 150 days after weaning were 13% lower if rats were exposed to low-protein diet from conception (C, 306 ± 4 g (n = 16); LP, 267 ± 4 g (n = 18); $P < 0.001$). During the 28 days period of HEF feeding the daily energy intakes of the rats previously maintained on low-protein diet were 15% lower than control (C, 96.1 ± 3.0 kcal/day (n = 16), LP 81.6 ± 2.5 kcal/day (n = 18); $P < 0.001$), but when expressed as a proportion of body weight were not significantly different

Table 1
Plasma insulin and blood glucose concentrations before and during an intravenous glucose tolerance test in control and low-protein fed rats after 28 days of high-energy/high-fat diet

Group	C	LP
n	7	6
Peak plasma insulin concn. (μU/ml)	150 ± 27	59 ± 10**
IAUC plasma insulin (μU/min per l)	281 ± 55	90 ± 17**
Peak blood glucose concn. (mM)	10.3 ± 0.5	9.4 ± 0.7
K (%/min)	3.2 ± 0.3	3.3 ± 0.3
IAUC blood glucose (mmol/min per l)	14.4 ± 0.5	9.9 ± 1.5*

Female rats were maintained on either 20% protein diet (C) or 8% protein diet (LP) during fetal and post-natal life as indicated. At 150 days of age both groups of rats were transferred to a high-energy/high-fat (HEF) diet and maintained on this diet for a further 28 days. Blood glucose and plasma insulin concentrations were measured in the post-absorptive state. Further details are given in Section 2. Values are means ± S.E.M. Statistically significant effects of exposure to LP diet prior to HEF feeding are indicated: $^{*}P < 0.05$; $^{**}P < 0.01$.

from control rats. Body weight gain after transfer to the HEF diet (expressed as a percentage of initial body weight) was unaffected (C, 9.3 ± 2.0% (n = 16); LP, 8.2 ± 2.8% (n = 18)). After 28 days of HEF feeding body weights of LP rats were 15% lower than control (C, 336 ± 7 g (n = 16); LP, 267 ± 4 g (n = 18); $P < 0.001$).

3.2. Intravenous glucose tolerance tests after 28 days of high-energy/high-fat feeding

The exposure to low-protein diet from conception to adulthood led to a 31% decline in the incremental area under the curve (IAUC) for insulin and a 36% decline ($P < 0.05$) in the peak plasma insulin concentration observed at 2 min after intravenous glucose administration (Fig. 1). However, despite an impaired insulin secretory response, the LP rats were associated with a 54% increase in glucose disappearance rates (the K value) (control rats, 2.57 ± 0.18%/min (n = 9); low-protein rats, 3.96 ± 0.36%/min (n = 5); $P < 0.01$). The transfer of control rats to HEF diet for 28 days led to a marked increase in glucose-stimulated insulin secretion during IVGTT: peak insulin concentrations, observed at 2 min after glucose injection, were increased by 76% ($P < 0.05$; Fig. 1A) and IAUC for insulin was increased 2.2-fold ($P < 0.05$; Table 1). In contrast, transfer of LP rats to HEF diet for 28 days was without effect on glucose-stimulated insulin secretion during IVGTT (Fig. 1B). As a consequence, after transfer to HEF diet, the peak plasma insulin concentration after intravenous glucose challenge was 61% lower ($P < 0.01$) and the IAUC for insulin was 48% lower ($P < 0.01$) in LP rats compared with C rats (Table 1). Despite the marked differences in the plasma insulin profiles between C and LP rats, peak blood glucose concentrations (observed at 2 min after glucose administration in both groups) and glucose disappearance rates (the K value) after IVGTT did not differ significantly between the C and LP groups (Table 1).

3.3. Glucose turnover in the post-absorptive state after 28 days of high-energy/high-fat feeding

Table 2 shows results of kinetic studies in which rats were infused with [3-^{3}H]glucose in the post-absorptive state. Trends towards lower blood glucose and plasma insulin concentrations in the LP group after HEF feeding in the post-absorptive state did not achieve statistical significance. Despite being provided with the same (HEF) diet for the 28 days preceding the kinetic measurements, glucose turnover rates ($R_a = R_d$) in the post-absorptive state were significantly (1.9-fold; $P < 0.01$) higher in the LP group. Similarly, MCR values in the post-absorptive state were 2.1-fold higher ($P < 0.001$) in the LP group compared with the C group after HEF feeding.

3.4. Whole-body insulin action after 28 days of high-energy/high-fat feeding

At steady-state hyperinsulinaemia, the glucose infusion rate required to maintain euglycaemia between 90 and 120 min (GIR) did not differ significantly between the C and LP groups after HEF feeding (Table 2). Hyperinsulinaemia led to a 3.0-fold increase in R_d in the C group ($P < 0.001$) compared with a 1.8-fold increase in R_d in the LP group ($P < 0.001$). There was no statistically significant difference in R_d between C and LP groups in the hyperinsulinaemic state. Hyperinsulinaemia led to a 2.9-fold increase in MCR values in the C group ($P < 0.001$) compared with a 1.4-fold increase in MCR values in the LP group after HEF feeding ($P < 0.01$). Essentially complete suppression of R_a was observed during hyperinsulinaemia in both groups (Table 2).

Table 2
Glucose kinetics in the post-absorptive state and after euglycaemia-hyperinsulinaemia in control and low-protein fed rats after 28 days of high-energy/high-fat feeding

Group	C	LP
n	6	6
Basal state		
Blood glucose (mM)	3.8 ± 0.1	3.5 ± 0.2
Plasma insulin (μU/ml)	14 ± 2	10 ± 1
$R_a = R_d$ (mg/min per kg)	6.5 ± 0.9	12.1 ± 1.2**
MCR (ml/min per kg)	9.5 ± 1.2	19.8 ± 0.9***
Hyperinsulinaemic state		
Blood glucose (mM)	3.9 ± 0.2	4.2 ± 0.2
Plasma insulin (μU/ml)	64 ± 4	70 ± 5
GIR (mg/min per kg)	19.0 ± 1.2	19.9 ± 2.0
R_d (mg/min per kg)	19.4 ± 0.8	21.4 ± 1.7
R_a (mg/min per kg)	0.4 ± 1.0	1.5 ± 1.4
MCR (ml/min per kg)	27.8 ± 1.6	28.2 ± 2.3

Glucose kinetics were measured in post-absorptive state and after euglycaemia-hyperinsulinaemia by infusion of [3-^{3}H]glucose. The mean coefficient of variation (± S.E.M.) of glucose specific activity in the post-absorptive state was 10.1 ± 2.0% for the LP group and 7.0 ± 1.4% for the C group. The mean coefficient of variation (± S.E.M.) of glucose specific activity during the euglycaemic-hyperinsulinemic clamp was 9.6 ± 0.9% for the C group and 3.6 ± 0.8% for the LP group. Further details are given in Section 2. Values are means ± S.E.M. Statistically significant effects of exposure to LP diet prior to HEF feeding are indicated: **$P < 0.01$; ***$P < 0.001$.

4. Discussion

In a rat model of early undernutrition, the maintenance of pregnant dams on a diet containing just under half of the control amount of protein (8% vs. 20%) in an isocaloric diet leads to a low birth weight in conjunction with impaired development of the fetal endocrine pancreas, such that pancreatic β-cell number is decreased and the ability of pancreatic β-cells to divide is impaired [1]. The continued maintenance of the low birth weight offspring on the same 8% protein diet during suckling and after weaning results in lower fasting insulin concentrations and pancreatic insulin contents [1,9], and loss of tolerance to oral glucose at 84 days [1], but the transfer of offspring to standard diet at birth allows significant pancreatic islet recuperation and almost complete restoration of tolerance to oral glucose [1]. In the present study, a standard (20%) protein diet or an isocaloric low (8%) protein diet was administered during pregnancy and lactation and offspring were weaned onto the maintenance diet with which their mothers had been provided. At maturity (150 days), offspring were transferred to a diet containing the standard amount of protein (to facilitate pancreatic islet recuperation) but which was energy-dense (419 kcal/100 g diet vs. approx. 365 kcal/100 g diet), with a higher fat and lower carbohydrate content than normal. The rat groups were studied after a fixed period of 28 days on the high-energy/high-fat diet. Intravenous glucose challenge revealed a marked distinction in responses between the groups of rats that had experienced normal or sub-optimal protein nutrition prior to the transfer to high-energy/high-fat diet. Rats that had been maintained on a diet containing the standard amount of protein responded to the challenge of high-energy/high-fat feeding with dramati-

cally enhanced insulin secretion. This profile may bear parallels in man, where some insulin-resistant individuals exhibit an enhanced insulin secretory response to maintain normal glucose tolerance [10]. In contrast, rats maintained on low-protein diet prior to transfer to the high-energy/high-fat diet failed to respond with an enhanced insulin secretory response to intravenous glucose. Interestingly, if the period of exposure to protein malnutrition was limited to fetal and early life only followed by a period of recuperation on 20% protein diet from weaning to adulthood, subsequent transfer to high-energy/high-fat diet evoked an intermediate (1.5-fold) increase in the IAUC for insulin after intravenous glucose challenge (results not shown). The present results indicate that an impairment in the insulin secretory response to glucose introduced as a result of protein malnutrition is unmasked by the increased insulin secretory demand associated with the challenge of a high-energy/high-fat diet. In several animal models of type 2 diabetes, an impaired activity of β-cell mitochondrial glycerophosphate dehydrogenase, a key enzyme of the glycerol phosphate shuttle, has been implicated as a factor in the impairment of the islet insulin secretory response to glucose (see [9]) and within this context rats that have experienced prior sub-optimal protein nutrition may also show this anomaly, which is not rectified by transfer to diet of normal protein content [9].

Although high-energy/high-fat feeding led to a markedly lower insulin secretory response to glucose in the previously protein-malnourished group compared with control, rates of glucose disappearance after intravenous glucose challenge were similar in the two groups. The failure to observe impaired glucose tolerance may be related to a compensatory increase in whole-body insulin sensitivity induced by low-protein feeding: in the present study the rate of glucose disappearance after intravenous glucose challenge was significantly higher in rats fed the low (8%) protein diet compared to control rats. The subsequent transfer of rats that previously experienced sub-optimal protein nutrition to high-energy/high-fat diet was associated with a modest (18%) decline in the rate of glucose disappearance after IVGTT.

Uncertainty exists as to whether insulin resistance or defective insulin secretion is the primary defect in non-insulin-dependent diabetes mellitus (NIDDM). It is also not known whether impairments in insulin secretion and action have a causal link [10–12]. Measurements of the rate of glucose infusion required to maintain glycaemia and endogenous glucose production and whole-body glucose disposal rates during the euglycaemic-hyperinsulinaemic clamp in the LP group gave no indication that a history of protein malnutrition adversely affects whole-body, hepatic or peripheral insulin action in rats maintained on a high-energy/high-fat diet. The results clearly indicate that an impaired insulin secretory response is not of necessity linked to the development of hepatic or peripheral insulin resistance.

Acknowledgements: This work was generously supported by the British Heart Foundation.

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