Islet Function in Offspring of Mothers on Low-Protein Diet During Gestation

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A low-protein diet (8 vs. 20%) administered during pregnancy affects the structure and function of the endocrine pancreas of the offspring. At 21.5 days of gestation, we reported a reduction of cell proliferation, islet size, islet vascularization, and pancreatic insulin content. In this study, we demonstrated an impairment of insulin secretion of these fetal islets when stimulated in vitro with amino acids such as arginine and leucine. If the offspring is kept on the same low-protein diet during suckling, weaning, and adulthood, fasting insulin levels remain low in the presence of normal blood glucose levels. Glucose tolerance at 70 days is impaired, with lower insulin response. In addition, permanent functional damage seems to be induced in utero by a low-protein diet, because a normal diet given from birth to adulthood does not restore normal insulin response after a glucose challenge. Our experimental results stress the impact of a balanced diet with qualitative and quantitative amino acid composition for the fetal endocrine pancreas to develop normally, without lasting functional and structural consequences in adulthood. *Diabetes* 40 (Suppl. 2):115–20, 1991

The complex biological adaptation of the endocrine pancreas of the mother to pregnancy was masterfully described by Freinkel et al. (1). An alteration of the metabolic state may induce morbid events and lead to structural and functional changes in the endocrine pancreas of the fetus. The adverse effects of hyperglycemia in the mother is a striking example, i.e., hyperplasia and hypertrophy in the islets of the fetus and high insulin or C-peptide levels in the cord blood are induced (2,3). High birth weight and macrosomia are consequences of this chain of events. The birth weight may also be reduced. In low–birth weight infants born to diabetic or nondiabetic mothers, a reduced islet cell mass with a reduced β-cell number and a low insulin content in the endocrine pancreas and the cord blood are found (4). Small for gestational age babies are born to mothers whose diets during pregnancy are calorie or protein deficient (5), totaling 15% of the births in developing countries, which remains a world problem to be solved. However, information about the development of the fetal endocrine pancreas in these specific conditions is not well documented. Therefore, this problem was investigated in a rat model.

In a previous experimental approach (6), we demonstrated that a diet containing 8% protein instead of 20% administered to rats from the 1st day of gestation and throughout has deleterious consequences for the fetus. The weight of the 21.5-day-old fetus or newborn was decreased. The structure of the endocrine pancreas of these fetuses was altered: β-cell proliferation, islet size, vascularization, and pancreatic insulin content were significantly reduced. The impaired structural development of the endocrine pancreas due to the low-protein diet during gestation raises two fundamental questions. *1*) Are these structural changes associated with alterations in insulin secretion? *2*) Have these altered islet cells acquired a damage lasting to adulthood? Therefore, dietary modifications during pregnancy would have a permanent imprint on the function and structure of β-cells. The experimental approach that follows addressed both these issues. We analyzed the insulin secretion of the fetal islets in vitro in response to secretagogues and demonstrated that this function is altered. Consequently, we verified that the islet alterations acquired in utero persist to adulthood.

RESEARCH DESIGN AND METHODS

FETAL ISLET INSULIN SECRETION IN VITRO

Animals and diets. Female Wistar rats were fed with either a normal diet (20% protein) or an isocaloric low-protein diet

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(8% protein) from the 1st day of gestation until the end, as described previously (6).

Fetal islet culture and insulin secretion. At 21.5 days gestation, the fetal pancreas was removed, digested with collagenase, and cultured for 6 days in RPMI-1640 medium supplemented with 10% fetal calf serum according to the method of Hellerström et al. (7). Batches of 10 neoformed islets were picked up and incubated at 37°C in Krebs medium containing various secretagogues: different concentrations of glucose (0, 2.8, 5.6, and 16.7 mM), leucine (10 mM), arginine (10 mM), and theophylline (2 mM).

Insulin secretion into the incubation medium after 30 and 120 min was analyzed by radioimmunoassay, following the method of Hales and Randle (8) with rat insulin standard (Novo-Nordisk, Copenhagen). The islet insulin content was also determined after the incubation. For the latter, the same batches of 10 islets were sonicated in ethanol-acid solution. Insulin release during incubation was expressed as percentage of the total islet insulin content.

FOLLOW-UP OF OFFSPRING

Animals and diets. Three groups of rats were used in this study: *1*) a control group consisting of the offspring of mothers fed a normal diet, nursed by normal mothers, and fed with a normal diet after weaning; *2*) a low-protein (LP) group consisting of the offspring of mothers fed a low-protein diet, nursed by a mother fed a low-protein diet, and fed with a low-protein diet after weaning; and *3*) a recuperation (R) group consisting of the offspring of mothers fed a low-protein diet but nursed by normal mothers and fed with a normal diet after weaning.

Growth and glucose and insulin secretion. The weight gain of these animals was calculated from birth to 84 days in male and female offspring. After 15 h of fasting on days 28, 56, and 84, blood glucose concentration was determined by a glucose oxidase method (Boehringer Mannheim, Mannheim, Germany; 9), and blood insulin levels were analyzed by radioimmunoassay.

An oral glucose tolerance test was performed at the adult age of 70 days in the three groups. After 15 h of fasting, 350 mg glucose/100 g body wt was administered orally. Samples of blood were collected after 30, 60, and 120 min for glucose and insulin levels.

Volume density of endocrine pancreas. At 84 days, the pancreas of the offspring was removed, weighed, and fixed with Bouin's solution for light microscopy. Seven-micron-thick sections were stained according to Masson's trichrome stain method. With a Kontron electronic planimeter, the surface area of the total pancreas (TS) and the total area of the islet (IS) per section were measured. The volume density of the endocrine pancreas was expressed as a percentage and calculated with the formula IS/TS × 100.

Statistical analyses. The data were analyzed by two- or three-way analysis of variance (ANOVA 2 or 3) as indicated, followed by Neuman-Keuls or Scheffé's tests for individual differences between means.

RESULTS

FETAL ISLET INSULIN SECRETION IN VITRO

Control group. ANOVA 3 did not reveal any significant difference in the fractional insulin release at 30 or 120 min from islets incubated with various concentrations of glucose (0, 2.8, 5.6, and 16.7 mM; Fig. 1). By contrast, when 5.6 mM glucose was combined with 10 mM arginine or leucine, insulin secretion was significantly increased at 30 and 120 min ($P < 0.01$), with arginine slightly more potent than leucine. When 2 mM theophylline was added to 5.6 mM glucose and 10 mM amino acid solutions, it potentiated the effect of amino acids. The mean ± SE insulin content per 10 islets was 260 ± 13 ng ($n = 86$) and was similar in each group whether or not the islets were exposed to secretagogues.

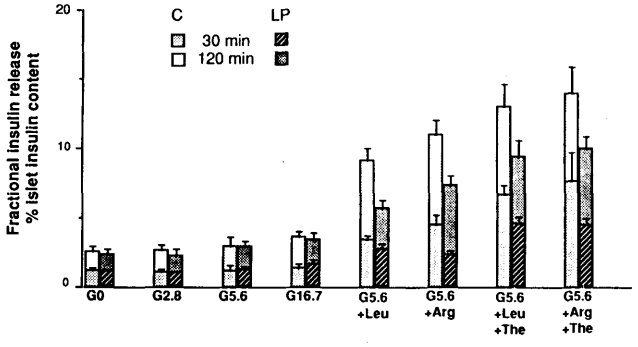

FIG. 1. Insulin release by cultured fetal islets (21.5 days) incubated in medium containing various concentrations of glucose (G, 0–16.7 mM), amino acids (leucine [Leu] and arginine [Arg], 10 mM), or theophylline (The, 2 mM). C, islets of fetuses from mothers fed normal diet (20% protein); LP, islets of fetuses from mothers fed low-protein (8%) diet. Values are means ± SE; $n = 4$ cultures with 3 batches of 10 islets/culture. Data were analyzed with 3-way analysis of variance.

LP group. As in the control group, no significant effect of glucose on insulin secretion appeared, but leucine and arginine stimulated insulin release ($P < 0.01$), which was potentiated by theophylline. However, insulin secretion in response to the individual amino acids and to the combination of these amino acids and theophylline was significantly lower in the LP group after 30 and 120 min of incubation compared with the control group ($P < 0.01$). The mean ± SE insulin content per 10 islets was 259 ± 14 ng ($n = 107$). It was similar in each experimental group and was not different from that of the control group.

FOLLOW-UP OF OFFSPRING

Food intake and weight gain. ANOVA 2 revealed that in each experimental group, female rats ingested significantly less food per day than male rats ($P < 0.01$) and that their body weight gain was lower ($P < 0.01$; Fig. 2). Moreover, female and male rats of the LP group ingested less food than those of the control group, and this difference increased with age ($P < 0.01$). The female and male rats of the R group ate the same quantity of food as those of the control group. Body weight gain was significantly lower in the LP group than in the control and R groups ($P < 0.01$; Fig. 2).

BLOOD GLUCOSE AND INSULIN LEVELS AND GLUCOSE TOLERANCE TEST

The glucose and insulin levels were identical in male and female rats; therefore, the results of both sexes were pooled for each parameter. The fasting blood glucose level, which increased significantly with age, did not differ in the three

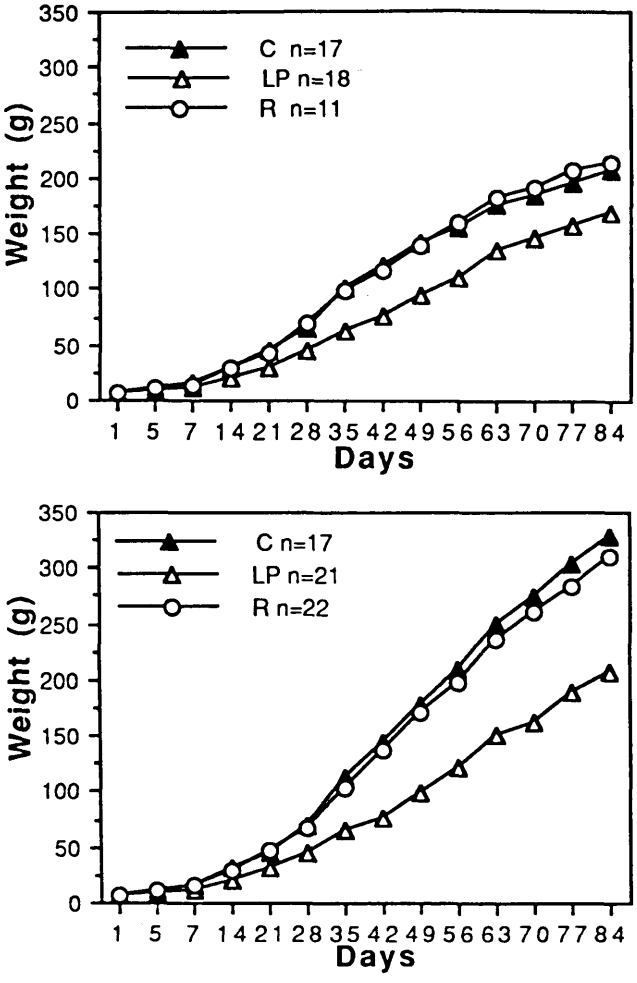

FIG. 2. Body weight gain in female (*top*) and male (*bottom*) rats in relation to age in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definition. Values are means ± SE. Data were analyzed with 2-way analysis of variance.

experimental groups (ANOVA 2, $P > 0.05$; Fig. 3). In the control group, fasting blood insulin level remained constant between 28 and 84 days (Fig. 4). In the LP group, fasting blood insulin concentration was significantly lower than in the control group at each day analyzed ($P < 0.01$). In the R group, although fasting insulin level was similar to that of the LP group at 28 days, it increased progressively and significantly ($P < 0.01$) and reached the level of the control group at 84 days.

In the control group, 30 min after oral glucose challenge, plasma glucose increased to a peak of 6.9 mM and subsequently declined to 5.3 mM at 120 min (Fig. 5). The insulin level reached a peak of 305 pM at 30 min and returned to 174 pM at 120 min (Fig. 6). In the LP group, ANOVA 2 revealed impaired glucose tolerance with a peak value of 9 mM glucose after 30 min that did not return to the basal level at 120 min. These animals did not exhibit a significant insulin secretory response to glucose ($P < 0.01$).

In the R group, plasma glucose and insulin levels were 7.39 mM and 262 pM, respectively, at 30 min. These values were intermediate between those of the control and LP groups. Statistical analysis showed impaired glucose tolerance ($P < 0.01$) and a lower insulin response to glucose challenge compared with the control group ($P < 0.01$).

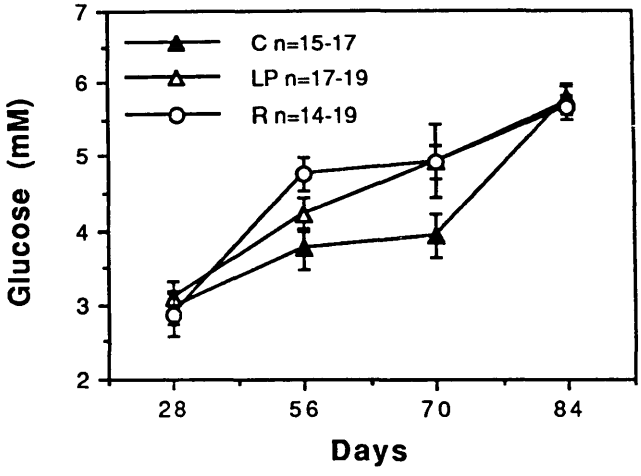

FIG. 3. Fasting blood glucose levels in relation to age in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definition. Values are means ± SE. Data were analyzed with 2-way analysis of variance.

During the oral glucose tolerance test, the insulin-glucose ratio in the blood showed a normal dynamic response in the control rats at 70 days (Fig. 7). In the pups of the LP group the ratio indicated the deficiency of the response.

MORPHOMETRICAL ANALYSIS

When rats were submitted to protein deficiency during fetal and postnatal life (LP group), the mean ± SE volume density of the endocrine pancreas (0.62 ± 0.40%) in adulthood was significantly reduced by 40% compared with the control group (1.01 ± 0.12%). When the protein deficiency was applied only during fetal life (R group), the volume density of the endocrine pancreas (0.72 ± 0.10%) was reduced by only 24% compared with the control group, which was not significant. The mean ± SE weight of the total pancreas was reduced by 15% in the LP group (0.68 ± 0.02 g) and

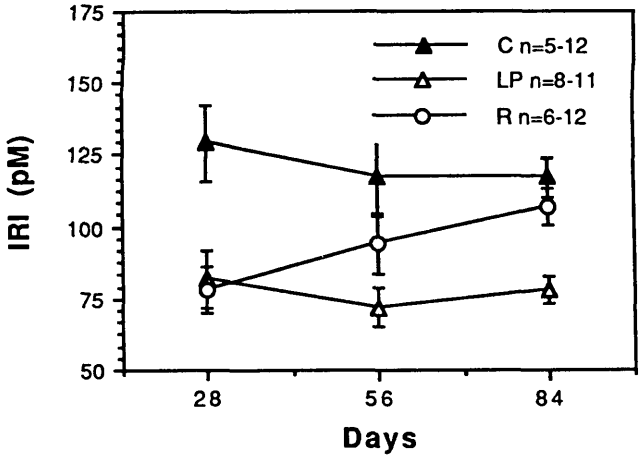

FIG. 4. Fasting blood immunoreactive insulin (IRI) levels in relation to age in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definition. Values are means ± SE. Data were analyzed with 2-way analysis of variance.

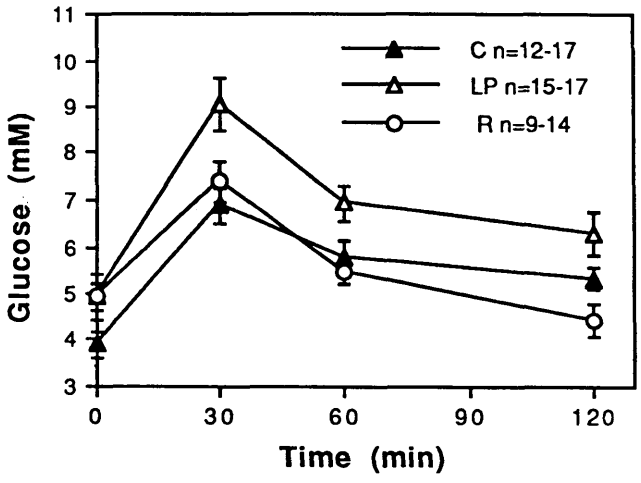

FIG. 5. Blood glucose levels during oral glucose tolerance test performed at 70 days of age in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definition. Values are means ± SE. Data were analyzed with 2-way analysis of variance.

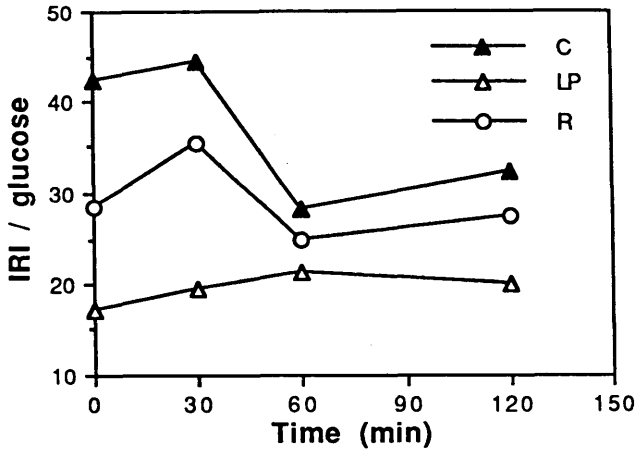

FIG. 7. Immunoreactive insulin (IRI)-glucose ratio in blood during oral glucose tolerance test in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definitions.

increased by 15% in the R group (0.93 ± 0.03 g) compared with the control group (0.80 ± 0.03 g).

DISCUSSION

Our previous studies showed major alterations in structure and function of the fetal endocrine pancreas as a consequence of a low-protein isocaloric diet administered during gestation (6). In this study, our objective was to evaluate whether these structural changes are associated with functional deficiencies at the perinatal stage and in adulthood.

It is known from previous studies that islets of pups from normally fed mothers exhibit no (10) or poor (11,12) insulin release in response to high concentrations of glucose compared with normal adult islets. In our experiments, after 7 days of culture, the islets of pups from normally fed pregnant rats did not release insulin in the presence of glucose. However, an enhanced insulin secretion was observed when leucine or arginine was added to the incubation medium. Insulin secretion was further increased when theophylline was added to arginine or leucine. These results agree with those reported by others (11,13).

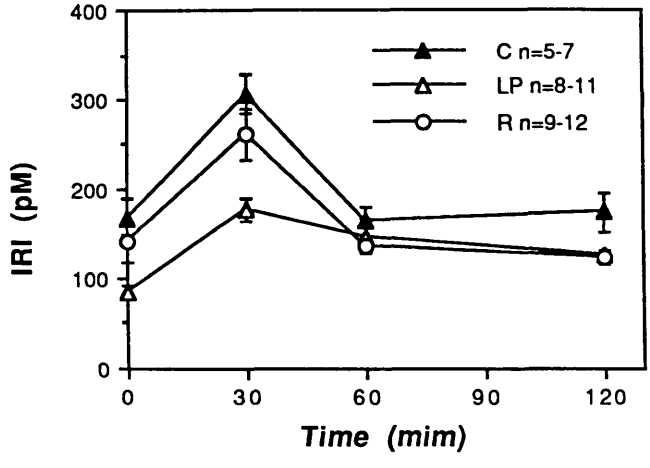

FIG. 6. Blood immunoreactive insulin (IRI) levels during oral glucose tolerance test performed at 70 days of age in control (C), low-protein (LP), and recuperation (R) groups. See METHODS for group definition. Values are means ± SE. Data were analyzed with 2-way analysis of variance.

When islets of pups from mothers fed a low-protein diet during gestation were challenged in vitro by leucine or arginine with or without theophylline after 7 days of culture, insulin release was significantly lower than in the control group. The observed lower insulin response of fetal β-cells to the secretagogues should not be attributed to a lower insulin content. This experiment does not allow us to detect a difference between a defect in the insulin secretory capacity and a lower sensitivity of the β-cells to secretagogues. In any case, this study shows that the consequences of the low-protein diet during gestation persist even when the fetal islets are withdrawn from the disturbed metabolic maternal environment.

Other metabolic alterations in the mother have been shown to modify structure or function of the fetal islets. Mild diabetes in pregnant rats induces disturbances in the neonatal pancreas, such as a high percentage of endocrine tissue, high β-cell volume density (14–16), and high insulin content (13,17). An increased proliferative capacity of the fetal endocrine pancreas in vivo is observed. This excessive proliferative rate still persists in vitro when verified after 7 days of culture (18). In contrast, experimental severe diabetes during pregnancy may be associated with low cell proliferation in islets in vitro (19), reduced islet cell mass (15,16), diminished pancreatic insulin content (13), and low birth weight of the neonates (15). In both conditions (mild or severe diabetes during gestation), the imprint given to the fetal β-cells during pregnancy remains even when the influence of the maternal environment disappears (15,18,19).

The long-lived effect of the low-protein diet during gestation is more clear when the offspring of such mothers are analyzed in adulthood. The low-protein diet administered only during gestation did not affect the body weight of the offspring in adulthood, but it had a specific impact, because glucose intolerance with low insulin levels was observed.

The abnormal response to an oral glucose tolerance test, which could be considered as a prediabetic state, should be related to the lower insulin secretion demonstrated in vitro for fetal islet cells.

In developing countries, malnutrition occurs not only during gestation but also afterward. Therefore, we also analyzed the effect of a low-protein diet in the rat model. In our follow-up study of rats from mothers on a low-protein diet during gestation and receiving a similar diet during and after weaning, the fasting insulin levels were reduced in adulthood compared with control rats. These offspring also exhibited glucose intolerance with a low insulin level after a glucose challenge. Because we observed a lower total pancreatic weight, proportional to the lower body weight (results not included), and the volume density of the endocrine pancreas (ratio of endocrine tissue to exocrine tissue) was reduced by 40% in the experimental group, we suspect that the total endocrine cell mass should be specifically decreased. These results show that the low-protein diet associated with a lower calorie intake during weaning and later still enhances the effect that the low-protein isocaloric diet of the mother had on the fetal endocrine pancreas.

The role of the low-protein diet is also apparent when it is given only after weaning during 3 wk in the growing phase. In this instance, fasting insulin levels are lower (20), and glucose tolerance is impaired. A normal diet after this experimental period does not normalize the glucose and insulin levels (21,22), and the reduced β-cell mass and β-cell size did not revert to normal (23). When a low-protein diet was administered from weaning for 14 wk, the rats failed to release insulin after intravenous glucose. During perfusion studies, their pancreases also secreted less insulin than normal (24). In addition, when a low-protein diet was given to diabetes-prone rats, glucose tolerance became impaired. It was further disturbed when sucrose was given instead of starch (25). Furthermore, in adult animals, islet size was increased with a protein-rich diet compared with a fat-rich diet. This was more apparent in females than in males. A carbohydrate-rich diet increased islet size even more (26). A similar effect of the diet was seen for the insulin content of the pancreas (27).

The effect of amino acids on the development of the fetal endocrine pancreas is also highlighted in in vitro studies. Arginine and leucine enrichment of the culture medium favor the differentiation of fetal rat pancreas rudiments. High concentrations promote growth and secretion of β-cells in vitro (28,29).

In conclusion, this study demonstrates clearly that a mother fed a low-protein diet during gestation gives birth to an offspring with an abnormal endocrine pancreas that features aftereffects such as functional deficiencies in adulthood.

Our observations may be relevant to humans living in the tropical and subtropical zones, where protein-deficient diets are endemic. Further confounding factors may enhance the deleterious effect of a low-protein diet, which could be partially responsible for the high incidence of diabetes in these countries. This public health aspect of environmental factors already operative during pregnancy should be addressed soon.

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