Low-Protein Diet during Early Life Causes a Reduction in the Frequency of Cells Immunopositive for Nestin and CD34 in Both Pancreatic Ducts and Islets in the Rat

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Feeding a low-protein (LP) diet to pregnant and lactating rats impairs pancreatic islet mass and insulin release in the offspring, leading to glucose intolerance as adults. We hypothesized that an LP diet changes the number of pancreatic endocrine precursor cells or cells supporting endocrine cell neogenesis. Pregnant rats were given LP (8% protein) or a control (20% protein) diet from conception until postnatal d 21. Cells containing nestin, CD34, or c-Kit were quantified in pancreata of the offspring. Stellate cells immunoreactive for nestin were seen to be adjacent to ductal epithelium and were resident within the islets. These were proliferative and immunonegative for cytokeratin 20, fibronectin, tyrosine hydroxylase, pancreatic duodenal homeobox 1, Nk homeodomain transcription factor 6.1, or insulin, but expressed vimentin. Approximately 20% of islet nestin-positive cells also expressed the endothelial cell marker platelet endothelial cell adhesion molecule-1. Both ducts and islets also contained CD34- and c-Kit-positive cells with similar morphology to those expressing nestin. Offspring from rats fed the LP diet had significantly less nestin/CD34-positive cells and reduced expression of nestin mRNA. Within islets, there was an associated decrease in cell proliferation and in cells immunopositive for pancreatic duodenal homeobox 1. Nestin-positive cell number within islets correlated positively with the percent area of β-cells. Supplementation of pregnant and lactating rats with taurine reversed the deficits in mean islet area and nestin-positive cells caused by the LP diet within the islets of the offspring. Nutritional programming of postnatal β-cell mass may involve an altered abundance of cells expressing nestin and/or CD34, which may limit endocrine cell development. (Endocrinology 145: 3004–3013, 2004)

LOW BIRTH WEIGHT for gestational age is a risk factor for type 2 diabetes in later life (1, 2), suggesting that the intrauterine environment can modulate programming of the metabolic axis. Susceptibility to type 2 diabetes may result from an altered development and insulin-secreting capacity of the endocrine pancreas or altered insulin sensitivity of target tissues. Experimental protein restriction in fetal and neonatal life is a documented model for the induction of adult glucose intolerance in rat, with resulting long-lasting changes in the morphology and function of pancreatic β-cells, in addition to impaired insulin sensitivity in insulin target tissues (3, 4). We showed that pregnant rats fed an isocaloric, low-protein (LP) diet (8% protein) compared with control (C) diet (20% protein) produced offspring with a significantly reduced birth weight and islet size (5, 6). The small islet size probably resulted from a reduction in cell proliferation, a greater apoptotic rate, and a reduced pancreatic expression of IGF-1 and IGF-II, which were shown to be mitogenic for β-cells while blocking apoptotic pathways (6, 7). Offspring of LP-fed rats also showed a reduced islet capillary density and a lower expression of vascular endothelial growth factor and its receptor, Flk-1, on islet cells (8, 9). Endothelial cells and vascular endothelial growth factor have been found to promote islet cell neogenesis during pancreatic development (10), suggesting that a defect may exist at the level of endocrine precursor cells in offspring of LP-fed rats. The objective of this study was to determine whether exposure of pregnant and lactating rats to an LP diet altered the abundance of pancreatic cells in their offspring expressing nestin, CD34, or c-Kit, possible markers of precursor cells or cells enabling endocrine cell differentiation. The development of cells within the ductal epithelium or within existing islets into an endocrine lineage is controlled by a specific expression sequence of transcription factors, one of the most important being pancreatic duodenal homeobox 1 (Pdx-1) (11–13). Subsequent expression of neurogenin 3, Nkx2.2, and β2/NeuroD is associated with the migration of small islets away from the ducts and the commitment and expansion of endocrine cell types. Final commitment to the separate endocrine cell phenotypes of β-cell, α-cell, δ-cell, and pancreatic polypeptide/adrenomedullin cells depends on the differential expression of additional transcription factors that for the β-cell include Nkx homeobox transcription factor 6.1 (Nkx6.1), Pdx-1, and Pax-4. Putative islet progenitor cells exist in association with the pancreatic ducts and within islets, whereas new islet cells may also derive by trans-differentiation from exocrine tissue (14, 15).
markers of such progenitors include the intermediate filament protein nestin, which is abundantly expressed in neuroepithelial stem cells during embryogenesis, but is absent from mature central nervous system cells (16), the hemopoietic cell lineage marker CD34, and the stem cell growth factor receptor, c-Kit (17).

A subpopulation of nestin-positive, putative precursor cells was identified within the rat pancreatic ductal epithelium, within mature rat islets, or from second trimester human fetal pancreas that consists mostly of primitive ductal structures (18–20). Once enriched within cultures, these cells proliferated rapidly and were reported to differentiate into pancreatic endocrine, exocrine, and hepatic phenotypes in vitro. Nestin-positive cells were also described in isolated adult human islets, and the multipotential cells were identified as a subtraction expressing the ATP-binding cassette transporter (ABCC2) (21). More detailed analyses of the nestin-positive fractions in human fetal and adult pancreas have concluded that they do not represent endocrine cell precursors (22, 23), but contribute to the microvasculature (24). Selander and Edlund (25) reported that nestin-positive cells in the pancreas of the mouse embryo were mesenchymal, not epithelial, and Lardon et al. (26) found similar results in normal and regenerating adult rat pancreas. Nestin is additionally expressed within newly formed capillaries during islet regeneration and was reported to colocalize to vascular endothelium and to cells coexpressing CD34 in the adult human pancreas (27). This may indicate that one population of resident endocrine precursors originates in mesenchymal tissue, such as bone marrow. Marrow-derived stem cells were shown to repopulate the irradiated mouse and contribute Pdx-1-positive, insulin-producing cells within the pancreatic islets (28). We recently found that a transplanted CD34/c-Kit-positive marrow cell fraction could reverse diabetes in the mouse, but that this was primarily due to a regeneration of endogenous islets (29). Such regeneration was clustered around primitive endothelial-like cells within the pancreas that were derived from hemopoietic stem cells. Shared phenotypic markers between nestin-positive cells and endothelial cells within the intact pancreas suggest that the nestin-positive cells may act as inducers of endocrine cell neogenesis, rather than representing the precursors themselves.

We therefore hypothesized that the changes in islet cell morphology induced by feeding the LP diet in early life may involve alterations in the numbers of endocrine precursor/inducer cells expressing nestin, CD34, or c-Kit within the pancreas, thus limiting islet cell plasticity.

**Materials and Methods**

**Animal model**

The model of pre- and postnatal exposure to an LP diet has been described by us previously (5, 6). Adult female Wistar rats bred at the Lawson Health Research Institute and Catholic University of Louvain were given food and water ad libitum and were housed at 24 °C with 60% humidity and a 14-h light, 10-h dark cycle. Nulliparous rats of 200–250 g maintained on standard laboratory diet were time-mated and randomly allocated to one of the two groups on d 1 of gestation until 21 d after birth of the offspring. A control group (C) was provided with a 20% protein diet and a second group (LP) was given an 8% protein diet (Hope Farms, Woerden, The Netherlands). The two diets had a similar fat content and were made isocaloric by the addition of carbohydrates to the LP diet. Food consumption did not appreciably differ between the C and LP groups. An additional group of C or LP-fed animals were supplemented with 2.5% (wt/vol) taurine (Sigma-Aldrich Corp., St. Louis, MO) in the drinking water throughout gestation and lactation. Taurine supplementation did not alter either food or water intake.

At birth, the litters from both diet groups were reduced to eight pups, and these remained with the lactating females until death at up to postnatal (PN) d 21. Pregnant rats were anesthetized with pentobarbital (55 mg/kg body weight) on d 21.5 of gestation, and the fetuses were removed and decapitated with scissors. After birth, pups were killed by decapitatio, and animals of both sexes were used. All procedures were performed with approval of the animal ethics committees of Catholic University of Louvain and University of Western Ontario and in accordance with the guidelines of the Canadian Council on Animal Care. At the time of death, the animals were weighed, and the pancreas was removed from each animal, weighed, and fixed for immunohistochemistry. Tissues were placed in ice-cold fixative (4% paraformaldehyde in 70 mm phosphate buffer, pH 7.4, containing 0.2% glutaraldehyde) for 16 h at 4 °C, followed by four washes at 4 °C in PBS over a 48-h period. Fixed tissues were dehydrated through a graded ethanol series, impregnated with butanol, and embedded in paraffin.

At the time of death, 50 μl blood were collected for glucose and precipitated in 500 μl HClO (0.33 n). Glucose concentrations were measured using a glucose oxidase test kit (Sigma-Aldrich Corp.). Insulin was measured by RIA in a modification of the method of Hales and Randle (30) as described by us previously (31). Rat insulin (Nordisk, Mississauga, Canada) was used for the standard curve.

**Immunohistochemistry**

Histological sections of pancreas (5 μm) were cut from paraffin blocks and mounted on glass microscope slides (SuperFrost Plus, Fischer Scientific, Nepean, Canada). Immunohistochemistry was performed to localize cells staining for insulin, nestin, CD34, c-Kit, proliferating cell nuclear antigen (PCNA), cytokertatin 20, Pdx-1, Nkx2.2, Nkx6.1, neuron-specific enolase, platelet endothelial cell adhesion molecule (PECAM-1), vimentin, fibronectin, or tyrosine hydroxylase by a modified avidin-biotin peroxidase method previously described for pancreas (6, 8). The primary antibodies used were guinea pig antihuman insulin (1:50 dilution; provided by Dr. Thomas McDonald, University of Western Ontario), monoclonal antibody against human cytokertatin 20 (1:50; DAKO, Santa Barbara, CA), monoclonal antibody against nestin (1:200 dilution; BD PharMingen, Mississauga, Canada), rabbit antihuman CD34 (1:30 dilution; Santa Cruz Biotechnology, Montréal, Canada), monoclonal antibody against PCNA (1:750 dilution; Sigma-Aldrich Corp.), rabbit anti-human Pdx-1 (1:50 dilution; Santa Cruz Biotechnology, Montréal, Canada), monoclonal antibody against Ki-67 (1:200 dilution; BD PharMingen, Mississauga, Canada), monoclonal antibody against Nkx2.2 (1:200 dilution; BD PharMingen, Mississauga, Canada), and rabbit antihuman nestin (1:50 dilution; Santa Cruz Biotechnology, Montréal, Canada).

**Fig. 1.** Mean area (±SEM) of islet in pancreata from fetal (F) or PN animals after feeding of control (●) or LP (▲) diets. Data are derived from eight to 10 animals for each age. ANOVA showed a significant difference between LP and C (P < 0.001).

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antirat Nkx6.1 (1:2000 dilution; provided by Dr. O. Madsen, Hagedorn Research Institute, Gentofte, Denmark), rabbit antirat Pdx-1 (1:2500 dilution; provided by Dr. C. Wright, Vanderbilt University, Nashville, TN), monoclonal antibodies against human neuron-specific enolase or tyrosine hydroxylase (both 1:50 dilution; Novocastra Laboratories, Newcastle upon Tyne, UK), monoclonal antibody against endothelial cell PECAM-1 (1:50; DAKO), rabbit antihuman c-Kit (1:50; DAKO), or monoclonal antibodies against vimentin (Hagedorn Research Institute) or fibronectin (1:50; DAKO). Controls included substitution of primary antisera with nonimmune serum and omission of the secondary antiserum.

Dual staining for nestin and insulin was performed by first performing immunohistochemistry for nestin as described above using diaminobenzidine as the chromagen. Before counterstaining and dehydration, the sections were subjected to immunohistochemistry for insulin using a fluorochrome, Vector Red alkaline phosphatase (Vector Laboratories, Inc., Burlingame, CA). Costaining for nestin and PCNA was achieved using blue alkaline phosphatase to visualize PCNA and Vector Red for nestin. In these studies no counterstain was used. For costaining of nestin with vimentin or PECAM-1, nestin was visualized using goat antimouse Alexa Fluor 488 (green; Molecular Probes, Inc., Eugene, OR) and the second antigen with goat antirabbit Alexa Fluor 555 (red). Sections were examined using fluorescence microscopy.

**RNA extraction and RT PCR**

Total RNA was extracted from whole pancreas (30–75 mg) using TRIzol reagent (Invitrogen, Carlsbad, CA). Purified RNA (100 µg) was extracted using the RNeasy Mini Kit (Qiagen, Mississauga, Canada) to remove any contamination of genomic DNA, and the integrity was verified by separation using gel electrophoresis and visualization by ethidium bromide staining. The amount of RNA was estimated by absorbance at 260 nm, and samples were stored at −80°C. Three micrograms of total RNA were reverse transcribed with 300 U SuperScript

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**Fig. 2.** Immunohistochemical localization of nestin (A and B), CD34 (C and D), or c-Kit (E and F) in sections of rat pancreas demonstrating islets (A, C, and E) or ducts (B, D, and F). Animals were taken on PN d 12 and were maintained on the C diet. Arrows indicate representative cells immunoreactive for the antigen shown. **Magnification bar:** A, 25 µm; C, 20 µm; B and D–F, 10 µm.
II ribonuclease H', reverse transcriptase (Invitrogen) using 0.5 μg oligo(deoxythymidine)12–18 primers (Invitrogen) according to the manufacturer's instructions. For multiplex PCR, the following reaction components were used: 32 μl water, 1.5 μl MgCl₂ (50 mM), 1 μl deoxy-NTP mix (10 mM), and 5 μl 10× PCR buffer (without Mg). Three microliters of nestin primers (50 μM) and then 5 μl of the RT samples were added to 39.5 μl of the above reaction mixture. The primers used were as follows: rat nestin: sense, 5'-TTC CCT TCC CCC TTG CCT AAT ACC-3'; antisense, 5'-TGG GCT GAG CTG TTT TCT ACT TTT-3' (expected product size, 464 bp); and β-actin: sense, 5'-GAC GGG GTC ACC ACT GTG CCC ATC TA-3'; antisense, 5'-CTA GAA GCA TTT GCG GTG GAC GAT GGA GG-3' (expected product size, 660 bp). After a denaturation step of 3 min at 95°C, 0.5 μl Taq DNA polymerase (Invitrogen) was added at 80°C to perform a hot start. After seven cycles of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 1 min at 72°C for extension, 2 μl β-actin primers (50 μM) were added to complete 35 more cycles and extension for the last 10 min at 72°C. To establish a linear range of amplification, several different cycle numbers of PCR (20, 25, 30, 35, 40, 42, and 45) were run. Negative controls without reverse transcriptase and without cDNA were run, and no genomic DNA contamination was found (data not shown). The β-actin primers covered exon/intron boundaries, and no signal at 970 bp of genomic DNA contamination was found (data not shown) compared with the expected product size of 660 bp. The amplified PCR products were visualized on 2% agarose gels stained with ethidium bromide and analyzed by a gel imaging system (AlphaEaseFC-FluorChem 8800 software, Alpha Innotech Corp., San Leandro, CA). The ratio between the signals for nestin and β-actin was taken from the same sample, allowing for a semiquantitative assessment.

Morphometric and statistical analysis

Morphometric analysis was performed using a transmitted light microscope (Zeiss, New York, NY) at a magnification of x250 or x400. Analyses were performed with Northern Eclipse version 6.0 morphometric analysis software (Empix Imaging Co., Mississauga, Canada). The percentage of islet cells or the islet cell area immunopositive for each antigen was calculated at each age for up to four sections of each pancreas, representing predominantly the head regions. We previously showed that the impact of LP diet on islet size is greater in the head than in the tail of the pancreas (5). The sections used contained at least five islets, and pancreata from up to 10 animals were examined for each age. Individual cell area and total areas of immunoreactive cells within islets...
were circled for image analysis and selected by gray level threshold. Differences between mean values for variables within individual experiments were compared statistically by two-way ANOVA. Mean values are given (± SEM) immunopositive for CD34 (D), tyrosine hydroxylase (E), or enolase (F) in pancreata from fetal (F) or PN animals after feeding of the C (■) or LP (□) diet. Data are derived from eight to 10 animals for each age. * ANOVA showed a significant difference between LP and C for CD34-positive cells (P < 0.001), but not for tyrosine hydroxylase- or enolase–positive cells.

Results
The effects of LP diet on body and pancreatic size were similar to those reported previously (6). The mean body weight of offspring from rats receiving the LP diet was not significantly lower at 21.5 d gestation compared with that of control-fed animals, but by postnatal d 14, LP animals were smaller (C, 23 ± 3 g; LP, 17 ± 2 g; P < 0.05; n = 10). Mean pancreatic weight did not differ between groups at birth, but by PN d 14, this was also significantly lower in offspring of LP-fed rats (C, 82 ± 4 mg; LP, 65 ± 7 mg; P < 0.05; n = 10). Blood glucose and plasma insulin levels in offspring of LP and C rats did not differ significantly at any age (not shown). The mean islet area in pancreata from offspring of C animals increased from approximately 8,000 μm² at 21.5 d gestation to approximately 11,000 μm² on PN d 14, and this was reduced in LP animals throughout this period (Fig. 1). The lower mean islet size in LP rats was due to a relative absence of β-cells, as determined by the mean percentage of cells
within islets that were insulin immunoreactive between fetal d 21.5 and PN d 14 (PN d 14: C, 82 ± 2%; LP, 75 ± 1%; \( P < 0.01; n = 10 \)). The mean size of individual \( \beta \)-cells was unchanged, with an area of approximately 120 \( \mu m^2 \).

Immunohistochemistry showed that subpopulations of cells adjacent to the ductal epithelium contained nestin, CD34, and c-Kit, which have previously been used as indicators of stem and precursor cell presence (Fig. 2). These cell populations did not coexpress the ductal epithelial marker, cytokeratin 20 (not shown). Approximately 10% of ductal-associated cells were nestin- or CD34-positive, and 15% were c-Kit-positive. Within the islets, a population of nestin-immunoreactive cells (6–8% of cells) was found that had a stellate appearance (Fig. 2). CD34-positive cells had a similar morphology, distribution, and frequency, suggesting that they were an overlapping cell population, whereas c-Kit-positive cells were more rounded and less abundant, representing less than 4% of islet cells. Islet cells immunoreactive for nestin, CD34, and c-Kit did not colocalize cytokeratin 20, which, as we reported previously (32), was localized to cells.

**Fig. 6.** Immunohistochemical localization of nestin (A and C) or Pdx-1 (B and D) in consecutive sections of rat pancreas demonstrating islets. Animals were taken on PN d 12 and were maintained on a C diet (A and B) or a LP diet (C and D). Magnification bar, 10 \( \mu m \).
at the periphery of the islet (not shown). Tissue sections were costained for the presence of PCNA, an indicator of DNA synthesis, and nestin. Many ductally associated and islet nestin-positive cells showed a copresence of PCNA (Fig. 3) regardless of whether animals had received the C or LP diet (C: duct, 62 ± 3% of nestin-positive cells immunopositive for PCNA; islets, 54 ± 4%; LP: duct, 56 ± 4%; islets, 48 ± 4%; n = 5–6 animals). Similar results were found for CD34-positive cells (C: duct, 66 ± 4% immunopositive for PCNA; islets, 51 ± 3%; LP: duct, 64 ± 4%; islets, 46 ± 4%) and those immunopositive for c-Kit, confirming that these could be cell populations linked to endocrine cell neogenesis.

Within islets, nestin immunoreactivity colocalized in cells also expressing vimentin (Fig. 4, A–D). About 20% of nestin-positive cells also colocalized the endothelial cell marker PECAM-1 (Fig. 4, E–H), suggesting that nestin-expressing populations included some cells with an endothelial phenotype. No nestin-positive cells expressed the mesenchymal cell marker fibronectin (not shown). As nestin expression is also a characteristic of immature neuronal cells, its location in islets relative to cells expressing the neuronal markers tyrosine hydroxylase or neuron-specific enolase was also determined (Fig. 5). Although a dispersed subfraction of cells contained both ligands, these differed in distribution and morphology from those containing nestin. Nestin-positive cells in islets did not colocalize the transcription factor Pdx-1 (Fig. 6), which is expressed in both β-cell precursors and mature, functional β-cells. Similarly, nestin-positive cells did not colocalize with cells expressing Nkx6.1, a transcription factor required for the later stages of β-cell lineage commitment and maturation (Fig. 7). Costaining of nestin and insulin within the same tissue sections showed that each resided in distinct cell types (Fig. 7). These findings suggest that nestin-, CD34-, and c-Kit-positive cells within the pancreas of fetal and neonatal rats could not be identified as endocrine, neuronal, or stromal cell types, but were proliferative and possibly mesenchymal in origin. Some nestin-positive cells appeared to be endothelial in nature.

The percent presence of CD34- or nestin-positive cells, either associated with the pancreatic ducts or present within the islets, did not significantly differ between fetal d 21 and PN d 14 (Figs. 5 and 8). Within both tissue compartments, significantly lower numbers of nestin-immunopositive cells were found in offspring from LP rats compared with C animals (Fig. 8). This was accompanied by a reduced presence of mRNA encoding nestin within pancreas obtained from LP vs. C animals between PN d 1 and 21 (Fig. 9) and a reduced expression on PN d 21 compared with the day of birth. Cells immunopositive for CD34 were similarly reduced in pancreata from LP-fed animals (Fig. 5). The reduction in the presence of these putative precursor-associated cell populations was accompanied by many fewer islet cells expressing Pdx-1 (Figs. 6 and 10). The number of nestin-positive cells within islets correlated with the number of insulin-positive cells within the same islet when LP and control animals were considered together (r = 0.65; P < 0.05). Although age-related changes were seen in the number of neuronal cells containing either tyrosine hydroxylase or enolase (Fig. 5), both being most abundant on PN d 12, there were no differences between C and LP animals.

Supplementation of pregnant and lactating rats given the LP diet with taurine rescued the deficit in mean islet area seen in neonatal offspring and caused a recovery in the frequency of islet cells immunopositive for nestin (Table 1). Taurine supplementation to a C diet did not significantly alter either the mean islet area or the number of nestin-positive islet cells.
Discussion

There is presently no consensus on the identity of precursor cells with the potential to generate new islet endocrine cells, especially β-cells, within the pancreas, or whether more than one such population exists. In the embryonic mouse and normal and regenerating adult rat pancreas, the nestin-positive cells with a stellate morphology possess mesenchymal, not epithelial, characteristics (25, 26), whereas in the adult human pancreas these were shown to include vascular endothelium (26). However, when nestin-positive cell-rich populations of putative islet precursor cells were isolated from adult rat islets or the human fetal pancreas, they were reported to give rise to insulin-expressing neoislets with epithelial characteristics (19, 20), although it has recently been shown that these cells are unlikely to be direct endocrine precursors (22, 23).

To demonstrate the effect of fetal and neonatal dietary restriction on putative precursor cell types we considered several possible cell phenotypes, including cells expressing nestin, c-Kit, or CD34. We found that nestin-positive and
TABLE 1. Mean area of islets and frequency of islet cells immunopositive for nestin in neonatal rats after exposure of the mothers to a C or LP diet, with or without supplementation with taurine (T)

<table>
<thead>
<tr>
<th>Age (d)</th>
<th>Islet area (μm²)</th>
<th>Nestin (% of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7916 ± 308</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>LP</td>
<td>6182 ± 277a</td>
<td>3.2 ± 0.3a</td>
</tr>
<tr>
<td>C + T</td>
<td>7531 ± 212</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>LP + T</td>
<td>8146 ± 339b</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10886 ± 438</td>
<td>8.1 ± 0.3</td>
</tr>
<tr>
<td>LP</td>
<td>6365 ± 311a</td>
<td>4.9 ± 0.2a</td>
</tr>
<tr>
<td>C + T</td>
<td>9871 ± 262</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>LP + T</td>
<td>11159 ± 289f</td>
<td>7.8 ± 0.3b</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM (n = 5).

a P < 0.05 vs. C diet.
b P < 0.05 vs. LP diet.
c P < 0.01 vs. LP diet.

CD34-positive stellate cells located within the islets had an identical morphology and distribution, showed a high incidence of DNA synthetic activity, and may represent the same cell population. Under conditions of normal development we found that only a minority of nestin-positive cells within the islets represented PECAM-1-positive endothelial cells. The majority of nestin-positive cells found within islets do not colocalize PECAM-1, but do express CD34. Whether these cells originate from the pancreatic ducts or represent cells that have migrated via the circulation from hemopoietic tissue, such as fetal liver or bone marrow, is not known. However, as we recently showed that CD34/c-Kit-positive hemopoietic stem cells derived from mouse bone marrow have a limited capability to translocate to the pancreas of diabetic mice and form insulin-expressing cells within the islets (29), it is possible that some endocrine precursor cells may be derived from marrow. Similar studies demonstrated that marrow-derived stem cells will populate the diabetic mouse pancreas and contribute to new capillaries within an angiogenic response (33). Small cells immunoreactive for c-Kit were also found within islets, but were less numerous than those expressing nestin/CD34. Elongated cells immunopositive for nestin, CD34, and c-Kit and with high proliferative activity also occurred in juxtaposition with the pancreatic ductal epithelium. These may represent reactive pericytes that support, but do not represent, endocrine cell precursors.

LP feeding of rats in early life results in profound reductions in pancreatic weight, islet cell mass, and the relative contribution of β-cells to the islets (6). The resulting β-cells have impaired insulin release in vitro, and the animals have abnormal glucose tolerance once they become adults (3, 5). Our previous findings of a reduced capillary density in the pancreata of such animals together with altered cell cycle kinetics of β-cells (6, 9) suggested that the mechanism of action of the LP diet might be directed at the number or activation of endocrine precursor cells. A reduced population of nestin/CD34-positive cells in the pancreas of fetal and neonatal LP-fed rats and a lower expression of nestin mRNA are supportive of this. The reduction in nestin-positive cells within the islets cannot be accounted for solely by a reduction in vascularity, and a lower abundance of nestin-positive endothelial cells. Also, changes to nestin/CD34-positive cells were not representative of other pancreas cell types, as the numbers of cells expressing the neuronal markers tyrosine hydroxylase and neuron-specific enolase did not change between LP and C diets. Within LP islets, the association of fewer nestin/CD34-positive cells with a reduction in the number of cells expressing Pdx-1 and fewer β-cells support the likelihood of a functional linkage between these cells and endocrine cell formation.

If nestin-positive cells are not themselves precursors, they may function as inducers of endocrine cell neogenesis. During the induction of islet regeneration in the diabetic mouse using marrow-derived CD34/c-Kit-positive stem cells, focal points of islet cell generation were found to surround marrow-derived cells with characteristics of endothelial cell lineage (29), possibly providing morphogenic stimulation. Nestin/CD34-positive cells within developing islets may provide a similar stimulus to endocrine cell plasticity. We previously showed that supplementation of pregnant and lactating rats with the amino acid taurine will reverse the detrimental effects of the LP diet on islet capillary density, islet area, and islet cell DNA synthesis (8, 9, 34). As taurine supplementation was also able to reverse the deficit in nestin-positive islet cells caused by the LP diet, this further supports a causative link with endocrine cell mass. It is also possible that the LP environment results in the selection of a differing subpopulation of nestin- or CD34-positive precursor cells, giving rise to β-cells with a differing insulin release. An altered ratio of α-cells to β-cells in the islets of LP-fed rats in favor of the former (6) might also indicate that the ability of precursor cells to contribute equally to each endocrine lineage is altered. The true identity of endocrine cell precursors within ducts and islets remains unclear. In the human adult pancreas, Petropavlovskiaia and Rosenberg (35) have described small islet cells that coexpress all pancreatic endocrine hormones and Pdx-1, but not nestin, and have few neuroendocrine granules, whereas Du Viville et al. (36) found Pdx-1-positive, endocrine hormone-negative cells in neonatal rat islets that demonstrated a prolonged labeling with bromodeoxyuridine. The relationship between these and the c-Kit-positive cells identified in neonatal rat islets is unknown.

In summary, these studies suggest that dietary programming of postnatal β-cell mass may be initiated at the level of endocrine precursor cells during fetal and neonatal life and is related to the presence of nestin- and CD34-positive cells within the pancreas.

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