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Original Research

Maternal protein restriction during gestation impairs female offspring pancreas development in the rat

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ABSTRACT

A maternal low-protein (LP) diet programs fetal pancreatic islet β-cell development and function and predisposes offspring to metabolic dysfunction later in life. We hypothesized that maternal protein restriction during pregnancy differentially alters β- and α-cell populations in offspring by modifying islet ontogeny and function throughout life. We aimed to investigate the effect of an LP maternal diet on pancreatic islet morphology and cellular composition in female offspring on postnatal days (PNDs) 7, 14, 21, 36, and 110. Mothers were divided into 2 groups: during pregnancy, the control group (C) was fed a diet containing 20% casein, and the LP group was fed an isocaloric diet with 10% casein. Offspring pancreases were obtained at each PND and then processed. β and α cells were detected by immunohistochemistry, and cellular area and islet size were quantified. Islet cytoarchitecture and total area were similar in C and LP offspring at all ages studied. At the early ages (PNDs 7-21), the proportion of β cells was lower in LP than C offspring. The proportion of α cells was lower in LP than C offspring on PND 14 and higher on PND 21. The β/α-cell ratio was lower in LP compared with C offspring on PNDs 7 and 21 and higher on PND 36 (being similar on PNDs 14 and 110). We concluded that maternal protein restriction during pregnancy modifies offspring islet cell ontogeny by altering the proportions of islet sizes and by reducing the number of β cells postnatally, which may impact pancreatic function in adult life.

1. Introduction

The islets of Langerhans are the endocrine cells of the pancreas. Although they comprise only 1% to 2% of pancreatic tissue, their endocrine secretions play a vital role in regulating metabolism throughout the course of life [1]. The main cell types in endocrine pancreas are insulin-secreting β cells, glucagon-secreting α cells, somatostatin-secreting delta cells,

Abbreviations: ANOVA, analysis of variance; C, control; LP, low protein; NOM, official Mexican guideline; PND, postnatal day.

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and pancreatic polypeptide-secreting cells. β Cells secrete insulin in response to stimulation by increased circulating glucose and amino acids, whereas α cells secrete glucagon in response to decreased circulating glucose. The coordinated function of both cell types is essential for maintaining normal glucose levels [2,3]. In addition, it is known that both cell types regulate paracrine signaling [4–6].

Changes in the mass and cellular composition of the islets of Langerhans result from apoptosis [7,8], proliferation [9], and neogenesis [1,10]. In response to environmental challenges that occur during the perinatal period, islets of Langerhans can undergo significant changes during the development of an organism. Pancreatic growth and function of offspring can be developmentally programmed by reduced maternal nutrition in several species, including rat [11–13], sheep [14,15], and nonhuman primates [16]. Developmental programming can be defined as adaptive responses to specific environmental challenges at critical time windows of development. These challenges can result in persistent alterations in phenotype and thus have lifelong effects on progeny health [17–19]. For this reason, the offspring of undernourished mothers are predisposed to metabolic diseases such as diabetes and obesity [20].

We hypothesized that a maternal low-protein (LP) diet during pregnancy would differentially alter β- and α-cell populations and modify ontogeny of islets in the pancreases of female rat offspring. The objective of this study was to investigate the effect of a maternal LP diet during pregnancy on the morphology and cellular composition of pancreatic islets in female rat offspring during weaning, puberty, and early adulthood. Understanding and establishing morphologic and physiologic relationships in the developing endocrine pancreas could explain, at least in part, how events in early life help determine adult islet physiology and metabolism.

2. Methods and materials

2.1. Animal care and management

All animal procedures were approved by the Animal Experimental Ethics Committee of Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán," in accordance with the Official Mexican Guideline for the care and use of laboratory animals (NOM-062-ZOO-1999). Virgin female albino Wistar rats (15-17 weeks old, weighing 220-260 g) were housed under controlled temperature (22°C-23°C) and humidity (30%-50%) conditions with 12-hour light/dark cycles and had ad libitum access to water and food (Purina Laboratory 5001 rodent chow, Purina Mexico). Females were mated overnight with proven male breeders on postnatal day (PND) 120. Pregnant rats were transferred to individual cages and allocated at random to 1 of 2 groups (n = 15 each): the control group (C) was fed a chow diet (with 20% casein), and the second group of rats was fed an LP, isocaloric diet (10% casein) [21] (composition of diets is shown in Table 1). After birth, both groups of offspring had ad libitum access to the control diet. Food was provided as large flat biscuits, which were retained behind a grill through which rats could nibble. Parturition occurred 21 days after conception, which was designated as PND 0. To ensure homogeneity of evaluated offspring, all litters were adjusted to 10 pups per dam at PND 2, with an equal number of males and females whenever possible. Female offspring were killed by decapitation [22] for tissue collection at PNDs 7, 14, 21, 36, and 110 (for each group, n = 6 offspring from different litters).

2.2. Tissue samples and immunohistochemistry

Pancreatic tissue from offspring was collected and fixed in 4% wt/vol formaldehyde in phosphate-buffered saline and paraffin-embedded [23]. Sections were cut 5 μm thick, which were then dewaxed and rehydrated in graded ethanol solutions. For antigen retrieval, slides were heated by microwave radiation for 10 minutes in 0.01 M citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked using 0.3% H_2O_2 in methanol for 30 minutes. To prevent nonspecific antibody binding, sections were blocked with bovine serum albumin (Sigma-Aldrich Co, St Louis, Mo, USA) for 60 minutes. Slides were incubated overnight at 4°C with primary antibodies: anti-insulin (1:100 dilution; Santa Cruz Biotechnology CA, USA; sc-9168) and antiglucagon (1:8000; Sigma-Aldrich; G2654). After washing with phosphate-buffered saline, the primary antibody was detected with an appropriate secondary antibody for 60 minutes at 37°C. After washes, slides were incubated with the 3,3′-diaminobenzidine tetrahydrochloride chromogen (Zymed/Invitrogen Inc, San Francisco, CA, USA) [24] and then rinsed in distilled water and counterstained with hematoxylin. Sections were mounted with a coverslip using a synthetic mounting medium (Entellan; Merck, Darmstadt, Germany; OB046327). Negative controls were processed without the addition of the primary antibody.

2.3. Image capture and morphometric analysis

Digital images were made of a longitudinal section of the entire pancreas through its maximal width [25]. The images were captured with a CoolSnap Pro CF (Roper Scientific, Trenton, NJ, USA) camera attached to a Nikon Eclipse E600 microscope using a ×20 objective. The total number of β (insulin positive) and α (glucagon positive) cells was counted. The number of cells/islet area (mm^2) was calculated using ImageJ software (NIH, Bethesda, MD, USA). For each time point, the ratio of β/α cells and the total islet area were

Table 1 – Ingredient composition of the diets fed to rats in g/kg diet

Ingredient	Control diet	LP diet
Casein	200	100
Cystine	3	1.5
Choline	1.65	1.65
Vitamin mix (AIN 93G)	10	10
Mineral mix (AIN 93VX)	50	50
Cellulose	50	50
Corn oil	50	50
Corn starch	317.6	373.4
Dextrose	317.6	363.4

Ingredients are expressed as grams per kilogram. The control and LP (50% protein) diets are isocaloric, 16.747 kjoules g^{-1}.