A dietary intervention (high carbohydrate) during the neonatal period causes islet dysfunction in rats

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Aalinkeel, Ravikumar, Malathi Srinivasan, Satish C. Kalhan, Suzanne G. Laychock, and Mulchand S. Patel. A dietary intervention (high carbohydrate) during the neonatal period causes islet dysfunction in rats. Am. J. Physiol. 277 (Endocrinol. Metab.): E1061–E1069, 1999.—Artificial rearing of 4-day-old rat pups on a high-carbohydrate (HC) milk formula results in the immediate onset of hyperinsulinemia. To evaluate these early changes, studies on pancreatic function were carried out on 12-day-old HC rats and compared with age-matched mother-fed (MF) pups. The plasma insulin and glucagon contents were increased sixfold and twofold, respectively, in HC rats compared with MF rats. There was a distinct leftward shift in the glucose-stimulated insulin secretory pattern for HC islets. HC islets secreted insulin in the absence of any added glucose and in the presence of Ca2+ channel inhibitors. The activities of glucokinase, hexokinase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate dehydrogenase complex were significantly increased in HC islets compared with MF islets. The protein contents of GLUT-2 and hexokinase were significantly increased in HC islets. These findings indicate that a nutritional intervention in the form of a HC formula only during the suckling period has a profound influence on pancreatic function, causing the onset of hyperinsulinemia.

Newborn rats; nutritional modification; insulin secretion; β-cell metabolism

The endocrine pancreas undergoes significant structural modifications during the late intrauterine and suckling periods in the rat, involving differentiation, neogenesis, and apoptosis (13). These changes modulate the insulin secretory capacity of the pancreas. Nutrition during the early phases of life is of major importance for proper tissue development and functional maturation (9). Earlier studies from this laboratory have demonstrated the consequences of a dietary modification in the form of a high-carbohydrate (HC) milk formula given only during the suckling period (12, 29). The “pup in a cup” model, adapted in this laboratory, involves the artificial rearing of 4-day-old pups on a HC milk formula (56% of the total calories being derived from carbohydrates compared with 8% in rat milk) until postnatal day 24 when they are weaned onto lab chow. This nutritional intervention, only during the suckling period, results in the immediate onset of hyperinsulinemia, which persists into adulthood without any further nutritional stimulus (12, 29). The growth rate of HC rats increases around postnatal day 55, and these animals are distinctly obese by day 100 (12, 29). In 100-day-old HC rats, hypertrophy of the β-cells and an altered glucose-stimulated insulin secretory pattern have been reported (12, 16). HC females spontaneously transmit these characteristics to their progeny (30). In addition, alterations in the insulin-signaling pathway for the activation of glycogen synthase in liver, muscle, and adipose tissue have also been reported in 100-day-old HC rats (25, 26).

Because the timing of the dietary modification in this rat model coincides with the neonatal endocrine pancreatic development, it appears that this HC milk formula potentiates certain changes in the pancreas (both structural and functional), causing the onset of hyperinsulinemia in these pups. It also appears that these changes are “imprinted” into adulthood. It follows that hyperinsulinemia is an early response in this model, leading to the onset of obesity and insulin resistance in adult life. Hence, understanding the mechanism(s) that causes the initial onset of hyperinsulinemia in these rats is fundamentally pertinent.

Insulin secretion from β-cells in the endocrine pancreas is regulated by nutrient and nonnutrient secretagogues (19). The insulinotropic action of glucose and other nutrient secretagogues requires their metabolism in the β-cells (the fuel hypothesis), resulting in an increase in the ATP-to-ADP ratio in the β-cell, which closes the KATP channels; this leads to membrane depolarization, opening of voltage-dependent Ca2+ channels, influx of Ca2+, and a rise in the cytosolic free Ca2+ concentration; the elevation of intracellular Ca2+ concentration directly triggers insulin exocytosis (21). To understand the effect of the HC milk formula on the insulin secretory pattern, insulin secretion in response to various stimuli (a range of glucose concentrations and modulators of Ca2+ and K+ channels) was studied in islets isolated from 12-day-old HC rats and compared with the pattern obtained from the islets isolated from age-matched MF rats.

Nutrient transport and metabolism are important components of the insulin secretory process in the islets. Because the onset of hyperinsulinemia is an early response to the HC milk formula in the HC rat, we focused our attention in this study on the insulin secretory pattern, as well as glucose transport and metabolism, in islets from 12-day-old rats. The hypothesis is that changes in the insulin secretory pattern and
in glucose transport and/or metabolism are responsible for the onset and persistence of hyperinsulinemia in this rat model.

Our results show significant changes in the insulin secretory pattern with concomitant changes in glucose transport and metabolism in HC islets. These observations indicate that a dietary modification during the suckling period has a profound influence on pancreatic function in these pups and that these changes have implications for the onset of pathological conditions in adulthood.

MATERIALS AND METHODS

Materials. Collagenase type IV was from Worthington Biochemicals (Freehold, NJ). GLUT-2 antibody was from Biogenesis (Sandown, NH). The antibody to the low Michaelis-Menten constant (Kₘ) hexokinase was a kind gift from Dr. J. E. Wilson (Michigan State University). The insulin radioimmunoassay kit was from Linco Research (St. Louis, MO). 2-Deoxy-o-glucose, mannoheptulose, glibenamide and iodoacetate, and kits for assay of glucose and triglycerides and all other reagent grade chemicals were from Sigma (St. Louis, MO). The kit for the assay of free fatty acids (FFA) was from Boehringer-Mannheim (Indianapolis, IN). Nimodipine was from Calbiochem (San Diego, CA), and 1,2-bis(2-amino-phenoxo)ethane-N,N',N' ,N'-tetraacetic acid BAPTA was from Molecular Probes (Eugene, OR). The protein assay kit was from Bio-Rad (Hercules, CA). [1-14C]Pyruvate and the reagents for chemiluminiscence were from NEN-Du Pont (Boston, MA).

Animal protocols. All animal protocols were approved by the Institutional Animal Care and Use Committee. Timed pregnant Sprague-Dawley rats were obtained from Zivic Miller Laboratories (Zelienople, PA). The newborn pups were pooled and assigned randomly to control and experimental groups. In the mother-fed (MF) control group, pups were reared by their nursing mothers, whereas pups in the experimental groups. In the mother-fed (MF) control group, pups were reared by their nursing mothers, whereas pups in the experimental group were reared artificially on a high-fat milk formula derived from carbohydrate, 24% from protein, and 20% from fat. Pancreatic islets were isolated from the same batch of mixed MF rats and from the mother-fed (MF) control group, and from 4-day-old pups by a modification of the method described previously (29) and 12-day-old pups with 3 mg of collagenase (type IV, Worthington) in Hank's buffer, pH 7.4, at 37°C in a shaking water bath. The digestion was stopped after 10–12 min by the addition of ice-cold Krebs-Ringer bicarbonate (KRB) buffer containing 0.2% bovine serum albumin (BSA), pH 7.4. After being washed two times with the same buffer, islets were picked manually under a stereomicroscope.

Insulin secretion. Four batches of islets were isolated, and islets from the same batch were used to compare insulin secretion at the different glucose concentrations. Equal numbers (30) of freshly isolated islets from 12-day-old MF and HC pups were preincubated at 37°C in KRB buffer containing 16 mM HEPES, 1 mM glucose, 0.01% BSA, pH 7.4, for 30 min under an atmosphere of 95% O₂-5% CO₂ in a shaking water bath. The islets were then resuspended in fresh KRB buffer (0.5 ml), and an aliquot of the buffer was removed for determination of zero-time insulin levels. Islets were further incubated with glucose at final concentrations of 1, 2.8, 5.5, or 16.7 mM, and aliquots of buffer were withdrawn at 10 and 60 min for determination of insulin. Zero-time insulin levels were subtracted from insulin levels determined at later times for determination of insulin release.

Pancreatic insulin content. A piece of the pancreas was accurately weighed and homogenized in 200 µl of acid alcohol solution (75 ml ethanol, 1.5 ml 12 N HCl, and 23.5 ml of distilled water). The pancreatic extracts were centrifuged at 10,000 rpm for 5 min at 4°C, and the supernatants were stored at −20°C until assayed for insulin as described earlier.

Glyceroldehyde-3-phosphate dehydrogenase assay. Glyceroldehyde-3-phosphate dehydrogenase (GAPDH) activity was carried out as described previously (5). Briefly, islets (~100) were washed with homogenization buffer containing 50 mM glycolytic, pH 7.0, 10 mM EDTA, 100 mM sodium fluoride, and 0.5 mM dithiothreitol and were homogenized by sonication for 10 s in 100 µl of the same buffer. The homogenate was centrifuged at 10,000 rpm for 30 s, and the supernatant was used for enzyme assay. GAPDH activity was measured in buffer containing 50 mM triethanolamine pH 7.6, 50 mM arsenate, 100 mM glyceroldehyde 3-phosphate, 2.4 mM reduced glutathione, and homogenate (1–3 µg protein). The tubes were preincubated in a water bath for 5 min at 37°C after which a baseline fluorometric reading was recorded at
347 nm excitation and 448 nm emission. NAD$^+$ (250 µM final concentration) was added to start the reaction, and the linear change in fluorescence was recorded for 5 min.

Assay for glucose-phosphorylating activities. Glucose-phosphorylating activity measurements were carried out by a modification of the method described previously (27). Briefly, islets (~300) were sonicated in ice-cold buffer containing 20 mM K$_2$HPO$_4$, 1 mM EDTA, 110 mM KCl, and 5 mM diethytritol (pH 7.4). The sonicated material was then centrifuged at 12,000 g at 4°C for 20 min. The pellet was resuspended in homogenization buffer. Ten microliters of supernatant or the resuspended pellet was added to 200 µl of assay buffer containing 50 mM HEPES, pH 7.4, 100 mM KCl, 7.4 mM NaHCO$_3$ buffer (pH 9.4), and fluorescence was measured at 347 nm excitation and 448 nm emission. NAD$^+$ (250 µM final concentration) was added to start the reaction, and the linear change in fluorescence was recorded for 5 min.

Table 2. Biochemical characteristics of 12-day-old MF and HC rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MF</th>
<th>HC</th>
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<tbody>
<tr>
<td>Plasma insulin, pM</td>
<td>61 ± 4.0</td>
<td>382 ± 87†</td>
</tr>
<tr>
<td>Plasma glucagon, pM</td>
<td>157 ± 12</td>
<td>341 ± 61†</td>
</tr>
<tr>
<td>Plasma glucose, mmol</td>
<td>65.4 ± 0.1</td>
<td>67.4 ± 0.2</td>
</tr>
<tr>
<td>Plasma FFA, mM</td>
<td>0.67 ± 0.11</td>
<td>0.24 ± 0.00†</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/l</td>
<td>53.7 ± 5.0</td>
<td>10.0 ± 2.8*</td>
</tr>
<tr>
<td>Pancreatic insulin, µg/g tissue</td>
<td>22.0 ± 4.5</td>
<td>38.4 ± 3.0*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>23.0 ± 0.6</td>
<td>21.8 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SE of 6 rats. Animals were weighed before they were killed, trunk blood was collected in heparinized tubes, and plasma was separated for analysis of the various parameters as indicated under MATERIALS AND METHODS.* P < 0.001, † P < 0.01, ‡ P < 0.05 compared with mother-fed (MF).

RESULTS

Effects of HC diet on physiological parameters and insulin secretion. Table 2 shows the physiological characteristics of the 12-day-old MF and HC rats. The characteristic feature is the marked increase (>6-fold) in the plasma insulin levels of HC rats compared with age-matched MF rats. An increase (>2-fold) in plasma glucagon level is also observed. The plasma insulin-to-glucagon molar ratio is increased from 0.4 in MF rats to 1.1 in HC rats. Pancreatic insulin levels are also higher in HC rats. Despite the hyperinsulinemia in the HC rats, plasma glucose levels are comparable between the two groups of rats. Plasma FFA and triglycerides are significantly decreased in the HC rats compared with age-matched MF rats. There is no significant change in the body weight of the HC rats compared with MF rats.

Fig. 1 depicts the glucose-stimulated insulin secretion by islets isolated from 12-day-old MF and HC rats at 10 and 60 min. MF islets did not secrete any measurable amount of insulin at 1 mM glucose (A: 10 min; B: 60 min) or at 2.8 mM glucose at 10 min and secreted a very small amount of insulin (0.23 fmol/30}
from each group were preincubated for 30 min in Krebs-Ringer buffer containing 5.5 mM glucose at 37°C in a shaking water bath under an atmosphere of 95% O2:5% CO2. After the islets were washed, they were further incubated in same medium containing different modulators at indicated concentrations for 60 min when an aliquot was withdrawn and frozen at −20°C for analysis of insulin. Mannoheptulose and 1,2-bis(2-aminophenylethyl)anthranilic acid (BAPTA) were included in the preincubation step. For medium containing no calcium, preincubation was carried out in KR buffer containing no calcium. For no glucose and 2-deoxy-D-glucose studies, preincubation was done in KR buffer containing no glucose and 2-deoxy-D-glucose respectively. ND, not detectable.

Table 3. Insulin secretory response by isolated islets from 12-day-old MF or HC rats to modulators of insulin secretion

<table>
<thead>
<tr>
<th>Glucose, mM</th>
<th>Addition/Deletion</th>
<th>Insulin Release, fmol·30 islets⁻¹·60 min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>None</td>
<td>0.45 ± 0.03, 3.87 ± 0.04</td>
</tr>
<tr>
<td>5.5</td>
<td>Mannoheptulose, 11 mM</td>
<td>4.20 ± 0.02, 4.19 ± 0.1</td>
</tr>
<tr>
<td>16.7</td>
<td>None</td>
<td>4.72 ± 0.20, 13.38 ± 0.9</td>
</tr>
<tr>
<td>16.7</td>
<td>Mannoheptulose, 11 mM</td>
<td>1.43 ± 0.14, 4.0 ± 0.1</td>
</tr>
<tr>
<td>5.5</td>
<td>Nimodipine, 1 µM</td>
<td>ND, 1.58 ± 0.33</td>
</tr>
<tr>
<td>5.5</td>
<td>EGTA, 500 µM</td>
<td>ND, 1.65 ± 0.11</td>
</tr>
<tr>
<td>5.5</td>
<td>Without calcium</td>
<td>ND, 1.53 ± 0.03</td>
</tr>
<tr>
<td>5.5</td>
<td>BAPTA, 1 µM</td>
<td>ND, 1.65 ± 0.03</td>
</tr>
<tr>
<td>5.5</td>
<td>Gilbendamide, 0.1 mM</td>
<td>3.61 ± 0.11, 3.03 ± 0.11</td>
</tr>
<tr>
<td>5.5</td>
<td>KCl, 25 mM</td>
<td>2.92 ± 0.07, 3.45 ± 0.11</td>
</tr>
<tr>
<td>0</td>
<td>None</td>
<td>ND, 1.63 ± 0.03</td>
</tr>
<tr>
<td>0</td>
<td>2-Deoxyglucose, 11 mM</td>
<td>ND, 1.76 ± 0.03</td>
</tr>
<tr>
<td>5.5</td>
<td>Iodoacetate, 1 mM</td>
<td>ND, 1.80 ± 0.07</td>
</tr>
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</table>

Data are means ± SE of 4 independent experiments. Thirty islets from each group were preincubated for 30 min in Krebs-Ringer bicarbonate (KR) buffer containing 5.5 mM glucose at 37°C in a shaking water bath under an atmosphere of 95% O2:5% CO2. After the islets were washed, they were further incubated in same medium containing different modulators at indicated concentrations for 60 min when an aliquot was withdrawn and frozen at −20°C for analysis of insulin. Mannoheptulose and 1,2-bis(2-aminophenylethyl)anthranilic acid (BAPTA) were included in the preincubation step. For medium containing no calcium, preincubation was carried out in KR buffer containing no calcium. For no glucose and 2-deoxy-D-glucose studies, preincubation was done in KR buffer containing no glucose and 2-deoxy-D-glucose respectively. ND, not detectable.

In contrast, islets isolated from 12-day-old HC rats secreted insulin at 1 and 2.8 mM glucose at both 10 and 60 min (Fig. 1). At 5.5 mM glucose, HC islets secreted ~15- and 9-fold more insulin compared with MF islets at 10 and 60 min, respectively. At 16.7 mM glucose, the response by HC islets was markedly higher compared with MF islets at both 10 and 60 min. Intragroup comparison showed that MF islets have a higher (16.7 to 5.5 mM glucose) ratio for insulin secretion at both 10 and 60 min (6- and 10.5-fold) compared with HC islets (1.4- and 3.46-fold). But for each glucose concentration studied, HC islets secreted significantly greater amounts of insulin compared with MF islets. These results clearly indicate an altered insulin secretory pattern for islets isolated from 12-day-old HC rats, with a marked leftward shift (1 mM glucose) for glucose-stimulated insulin secretion.

Effects of inhibitors and nonnutrient stimuli on insulin secretion. In the presence of Ca²⁺ channel inhibitors (nimodipine and BAPTA) HC islets secreted ~40% of the amount of insulin they secrete at 5.5 mM glucose (Table 3). Similar results were obtained in the presence of EGTA (500 µM) or Ca²⁺ free buffer. MF islets did not secrete any measurable amount of insulin under these conditions. The amount of insulin secreted by HC islets under these conditions is almost 3.5-fold higher than the amount of insulin secreted by MF islets in the presence of Ca²⁺ at 5.5 mM glucose. Under conditions where K⁺ channels are closed, as in the presence of 100 µM glibenclamide or 25 mM potassium chloride, and the membrane is in a depolarized state, MF islets secreted significantly more insulin compared with their basal levels, whereas this treatment had no effect on HC islets. In the presence of the glucokinase inhibitor, mannoheptulose, there was no effect on insulin secretion at 5.5 mM glucose, whereas at 16.7 mM glucose the insulin secreted was almost reduced to basal levels (5.5 mM glucose) in both MF and HC islets. It was also observed that HC islets could secrete insulin in the absence of added glucose, in the presence of the nonmetabolizable sugar 2-deoxyglucose, and also in the presence of iodoacetate (an inhibitor of glycolysis) in amounts similar to what they secrete in the presence of Ca²⁺ channel inhibitors (~1.60 fmol·30 islets⁻¹·60 min⁻¹; Table 3). On the basis of these findings, it is clear that basal insulin release is fundamentally altered in the HC islets.

Glucose metabolism in pancreatic islets. Glucose is transported into the pancreatic β-cell where it is metabolized via both the glycolytic pathway and the tricarboxylic acid cycle, resulting in ATP production and initiation of insulin secretion. Because glucose transport into the islet cells is the first step in the pathway leading to insulin secretion, GLUT-2 protein content was measured in islets from MF and HC pups. Western blot analysis of islet homogenates from 12-day-old MF and HC pups indicated a significant increase (~70%) in GLUT-2 protein content in HC islets compared with MF islets (Fig. 2).

Glucokinase (high K_m for glucose) and hexokinase (low K_m for glucose) activities were measured in the islet homogenates (supernatant and pellet fractions). Preliminary experiments indicated that glucokinase was present predominantly in the supernatant fraction and hexokinase was present in both the pellet and supernatant fractions. Glucokinase activities in the
supernatant fractions of MF and HC islet homogenates showed an increase of ~40% in activity for the HC rats (Fig. 3). Hexokinase activities in the supernatant and pellet fractions of the islet homogenates were observed in nearly equal amounts for both the groups but were increased significantly in both the supernatant and pellet fractions from HC islets compared with MF islets (an increase of ~100% in the supernatant fraction and 60% for the pellet fraction).

Because a significant difference was observed in the low K_m hexokinase activity in MF and HC islets, hexokinase protein was also quantified. Hexokinase protein was significantly increased (60%) in islets from HC rats (Fig. 4). No change was observed in the glucokinase protein content in islets from MF and HC rats (data not shown).

The glucose 6-phosphate formed as a result of glucokinase-hexokinase activities is further metabolized in the glycolytic pathway, and GAPDH is a key enzyme in this pathway. Because of the importance of GAPDH in the coupling of glucose metabolism to insulin secretion, its activity was measured in islet supernatants from 12-day-old MF and HC pups. A significant increase in GAPDH (~30%) was observed in islet extracts from HC pups compared with MF pups (Fig. 5).

Acetyl-CoA is formed from pyruvate by oxidative decarboxylation of pyruvate catalyzed by the PDC, and the activity of the active form of PDC is crucial for glucose oxidation. Hence, the active and total forms of PDC were measured in islet homogenates from 12-day-old MF and HC rats. The active form of PDC is increased by ~43% in islet homogenates from HC islets compared with MF islets (Fig. 6). The total form of the enzyme, obtained after conversion of the inactive form to the active form by treatment with a partially purified phosphatase, was also significantly increased by almost twofold in islet extracts from HC pups (Fig. 6). The above observations indicate significant changes in glucose transport and metabolism in HC islets.

**DISCUSSION**

Hyperinsulinemia is a very early event in the HC rats. Hence, this study was designed primarily to characterize the effects of the nutritional intervention, in the form of a HC milk formula during the suckling period, on pancreatic islet function. The influence of this HC formula on islet function becomes all the more significant because the suckling period is a criti-
The primary short-term regulation of insulin secretion is achieved by elevated glucose levels. However, this stimulus is absent in the HC rats, because their glucose levels were not significantly different compared with age-matched MF rats (Table 2). Hence, some other functional and/or structural changes must contribute to maintain this hyperinsulinemia. Earlier we had shown that the number of insulin-positive cells and islet size were increased by ~1.6-fold and 1.3-fold, respectively, in islets from 12-day HC rats (12). But these reported increases do not appear to account for an approximately sixfold increase seen in circulating plasma insulin levels.

A leftward shift in the glucose dose response for insulin secretion was observed in islets from 12-day-old HC rats (Fig. 1). The lowering of the threshold for glucose-stimulated insulin secretion (1 mM glucose for HC islets) may be responsible to a large extent for maintaining the >6-fold increase in circulating plasma insulin levels. This leftward shift in insulin secretion makes it possible for a large increase in insulin secretion at basal and subbasal glucose levels. Similar lowering in the threshold for glucose-stimulated insulin release has been reported in obese Zucker rats and in pregnancy (conditions in which hyperinsulinemia is present) (20, 31).

Ca$^{2+}$ is an important mediator of glucose-stimulated insulin secretion (32). From our data, it is evident that HC islets secrete a modest amount of insulin in the absence of extracellular Ca$^{2+}$ or when the intracellular Ca$^{2+}$ stores are depleted or the voltage-gated Ca$^{2+}$ channels are blocked (Table 3). This indicates two possibilities: 1) a Ca$^{2+}$-independent insulin secretion pathway is also operative in HC islets and/or 2) structural alterations in β-cells of HC rats facilitate insulin secretion in the absence of Ca$^{2+}$. Komatsu et al. (15) have shown that a Ca$^{2+}$-dependent and a Ca$^{2+}$-independent GTP-dependent insulin secretion pathways, both of which require glucose metabolism, exist in rat pancreatic islets. The fact that the HC islets secrete as much insulin in the presence of 2-deoxyglucose or in the absence of glucose or in the presence of iodoacetate, an inhibitor of glycolysis, as they do in the absence of Ca$^{2+}$ suggests some structural alterations in

![Pyruvate dehydrogenase complex activity in islets from 12-day-old MF and HC rats.](Image)

Data are means ± SE of 4 independent experiments. **P < 0.01 compared with MF.
HC islets. These results also suggest that a common mechanism may be responsible for insulin secretion under such conditions by HC islets. Depolarization of the membrane by glibenclamide or high K+ does not appear to alter insulin secretion by HC islets, indicating that Ca2+ stores are already elevated in the HC islets and may contribute partly to the elevated basal insulin release. Under similar conditions, insulin secretion by MF islets is significantly increased above basal levels. It has been suggested that neurotransmitters and incretins simultaneously activate protein kinase C and protein kinase A activities, causing an increase in GTP levels and insulin secretion (15). It is possible that a similar mechanism may be operative in the HC islets and account for the increased basal insulin secretion in the HC rats.

Due to the central importance of glucose metabolism in insulin secretion by the β-cells, it was of interest to evaluate the adaptive changes occurring in glucose metabolism in the islets of the HC rats (Fig. 7). It is plausible that initial changes in glucose metabolism are imprinted into adulthood and form the basis for the onset of pathological conditions. The first step in the pathway for glucose-stimulated insulin secretion by the β-cells is the facilitated diffusion of glucose by GLUT-2. Our studies indicate an approximate increase of 70% in GLUT-2 protein content in islets from 12-day HC rats (Fig 2). Weinhaus et al. (31) have reported a similar increase in GLUT-2 protein in islets of normoglycemic and hyperinsulinemic pregnant rats. In contrast, in the hyperinsulinemic fa/fa Zucker rats GLUT-2 expression and function were normal (20). In rodent models of overt hyperglycemia, the loss of glucose-induced insulin secretion has been attributed to a reduction in GLUT-2 protein content in the β-cells (18). However, islets cultured in a medium containing a high glucose concentration demonstrate an increase in GLUT-2 protein levels (34). It appears that the expression and function of GLUT-2 may be specifically modified according to the physiological and metabolic environment of the model being studied.

Glucose is metabolized in islets to serve as stimulus for insulin secretion. Both the low and high K_m glucose-phosphorylating activities were significantly increased in HC islets compared with MF islets (Fig. 3). Several reports suggest that under some conditions, increased activity of the low K_m glucose-phosphorylating enzyme could contribute to basal hyperinsulinemia (1, 2, 4, 8, 20). Overexpression of the hexokinase I gene in transfected rat islets caused a significant increase in insulin secretion at low glucose levels (1). Similar results were reported when yeast hexokinase B gene was overexpressed in islets of transgenic mice (8). Male JCR:LA/N-cp rats exhibited a fourfold increase in hexokinase activity and a leftward shift in glucose-induced insulin secretion (2). In the hyperinsulinemic Zucker diabetic and Zucker fatty rats, hexokinase activity was significantly increased in islets (20). In the preobese and prediabetic stage of the Zucker diabetic and Zucker fatty rats, increased hexokinase activity was associated with a leftward shift in insulin secretion (4). It appears that the marked increase in the low K_m hexokinase activity in both the supernatant and pellet fractions of islet homogenates from HC rats coupled with the significant increase in hexokinase protein content plays a significant role in sustaining basal hyperinsulinemia in the HC rats (Figs. 3 and 4). The modest increase in glucokinase activity may also have a role to play in the altered insulin secretory pattern in the 12-day-old HC rats (Fig. 3). Glucokinase activity is increased, without changes in hexokinase activity, in rat islets during pregnancy (31) and in the islets of spontaneously hypertensive rats (3), both of which are normoglycemic but hyperinsulnemic. Because both hexokinase and glucokinase activities are significantly increased in HC islets, it appears that the overall increase in the glucose-phosphorylating activities contributes significantly to the hyperinsulinemic state in the 12-day-old HC rats (Fig. 7).

Both GAPDH and PDC (active and total) activities were significantly increased in islets from 12-day HC pups (Figs. 5 and 6). GAPDH activity was reported to be increased in islets from 100-day-old HC male rats (16). Moreover, β-cells cultured in a high glucose medium showed increased GAPDH expression (24). An increase in GAPDH is associated with increased ATP production, which mediates insulin secretion (6). PDC catalyzes the oxidation of pyruvate to acetyl-CoA for ATP.
production in the tricarboxylic acid cycle and has been shown to be decisive for glucose oxidation in several tissues (23). In the diabetic db/db mouse model, dimished glucose-induced insulin secretion has been attributed to a decrease in PDC activity in islets (35). Our results indicate that increased PDC and GAPDH activities support increased glucose metabolism and increased insulin secretion by islets from 12-day HC rats (Fig. 7).

Recently, Leahy et al. (17) reported that in the normoglycemic, hyperinsulinemic, and spontaneously hypertensive rats, pancreatic β-cell insulin content was increased, whereas no changes occurred in proinsulin mRNA levels, biosynthesis, or degradation. These observations suggest that some other regulatory mechanism(s) modulating insulin secretion may be operative in these rats. In the HC rat model, it appears that the onset of hyperinsulinemia is the primary event and is not a compensatory response to either insulin resistance or overt hyperglycemia in these 12-day-old HC rats. A recent study (21) showed that qualitative and quantitative differences in fatty acid content of the diet influence glucose-induced insulin secretion by islets. In the HC formula, fatty acids are qualitatively similar to rat milk (11), but the fat-derived calories are reduced compared with rat milk and are compensated for by an increase in carbohydrate-derived calories. Circulating FFA levels are reduced in the HC rat, and considering that the fat-derived calories are reduced in the HC formula, it appears that the increase in carbohydrate-derived calories primarily contributes to the basal hyperinsulinemia in this model. The lowering of the glucose threshold for the insulin secretory response sustained by a marked increase in hexokinase protein content and activity observed in these rats may, in part, contribute to the development of the hyperinsulinemic state. It is not clear what mechanism is responsible for the modest insulin release observed in the absence of any stimulus or in the presence of Ca2+ channel inhibitors by islets from HC rats (Table 3). However, insulin release observed under such conditions could contribute substantially to the basal hyperinsulinemia of the HC rats. Other regulatory factors, such as storage and trafficking of insulin, the influence of a variety of peptide factors produced by the β-cells, and the incretins produced by the gut, may have a role to play in the regulation of secretion of insulin by the HC islets. The diet-induced rat model for obesity reported from this laboratory differs from other reported animal models for obesity in that the increased consumption of carbohydrate-derived calories only during the suckling period causes the immediate onset of hyperinsulinemia, which is the primary event, and subsequently leads to adult onset obesity. Our results clearly emphasize the importance of the role played by early nutrition on the development of pancreatic function and the implications of changes in its function for the onset of obesity and insulin resistance in adulthood. It appears that altering the nature of the fuel source during the suckling period has profound implications for functional alterations of the pancreas. The HC milk given during the suckling period induces modifications in the β-cells resulting in the onset of hyperinsulinemia. This model therefore provides a unique opportunity to evaluate the importance of the early onset of hyperinsulinemia to the development of conditions like obesity, insulin resistance, and possibly non-insulin-dependent diabetes mellitus in adulthood.

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REFERENCES


