

β -Cell Secretory Dysfunction in the Pathogenesis of Low Birth Weight–Associated Diabetes

A Murine Model

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Low birth weight (LBW) is an important risk factor for type 2 diabetes. We have developed a mouse model of LBW resulting from undernutrition during pregnancy. Restriction of maternal food intake from day 12.5 to 18.5 of pregnancy results in a 23% decrease in birth weight ($P < 0.001$), with normalization after birth. However, offspring of undernutrition pregnancies develop progressive, severe glucose intolerance by 6 months. To identify early defects that are responsible for this phenotype, we analyzed mice of undernutrition pregnancies at age 2 months, before the onset of glucose intolerance. Fed insulin levels were 1.7-fold higher in mice of undernutrition pregnancies ($P = 0.01$ vs. controls). However, insulin sensitivity was normal in mice of undernutrition pregnancies, with normal insulin tolerance, insulin-stimulated glucose disposal, and isolated muscle and adipose glucose uptake. Although insulin clearance was mildly impaired in mice of undernutrition pregnancies, the major metabolic phenotype in young mice of undernutrition pregnancies was dysregulation of insulin secretion. Despite normal β -cell mass, islets from normoglycemic mice of undernutrition pregnancies showed basal hypersecretion of insulin, complete lack of responsiveness to glucose, and a 2.5-fold increase in hexokinase activity. Taken together, these data suggest that, at least in mice, primary β -cell dysfunction may play a significant role in the pathogenesis of LBW-associated type 2 diabetes. *Diabetes* 54: 702–711, 2005

Human studies from both developed and underdeveloped nations demonstrate a strong link between low birth weight (LBW) and increased risk for impaired glucose tolerance and diabetes during adult life (1–4). Barker et al. (5) proposed that

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CEACAM1, cell adhesion molecule 1; KRH, Krebs-Ringer-Hepes; LBW, low birth weight.

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disease risk begins during fetal life as a result of "programming," or long-term changes in gene expression resulting from a suboptimal metabolic milieu. Thus, either maternal undernutrition or abnormal uteroplacental function can reduce nutrient delivery to the fetus and may produce secondary adaptations in metabolism and gene expression that may be beneficial during intrauterine life but contribute to disease risk in later life.

Because LBW is a significant risk factor for type 2 diabetes, understanding the pathophysiology of LBW-associated glucose intolerance is important for both prevention and therapy. Metabolic studies in LBW humans have demonstrated both glucose intolerance and hyperinsulinemia (6–8). Whereas insulin sensitivity is reduced in some cohorts of both children and adults (2,7,9,10), other studies have highlighted β -cell dysfunction as a major contributor to LBW-associated type 2 diabetes (11–13). For example, β -cell function is reduced in 7-year-old African LBW children (14). Coexisting abnormalities in both insulin action and secretion have been found in young men (13) and in LBW children with catch-up growth, even as early as 1 year of age (15). Together, these data illustrate the apparent heterogeneity of LBW-associated diabetes and suggest that both insulin resistance and secretory dysfunction contribute to the final phenotype in humans.

In rats, either uterine artery ligation or maternal nutrient restriction during pregnancy and lactation (16–21) results in glucose intolerance or type 2 diabetes with aging, usually associated with reduced β -cell mass and function (20,21). By contrast, insulin action is variable, ranging from insulin resistance (22–24) to normal or improved insulin sensitivity (21,25).

Given the heterogeneous metabolic phenotype in LBW humans and the importance of LBW as a risk factor for type 2 diabetes, we generated a mouse model of maternal undernutrition during pregnancy with LBW. The long-term goal of these studies is to identify key contributors to the type 2 diabetes risk phenotype and to test the role of specific candidate genes using gene deletion techniques. The aim of the present study was to characterize, in a longitudinal manner, the metabolic defects that result from intrauterine undernutrition to determine the primacy of insulin resistance versus insulin secretory dysfunction in the pathogenesis of LBW-associated type 2 diabetes in this model.

RESEARCH DESIGN AND METHODS

Protocols were approved by the Joslin Animal Care and Use Committee. Mice were housed in an Office of Laboratory Animal Welfare–approved facility with controlled temperature, humidity, and light-dark cycle. Virgin female ICR mice (age 6–8 weeks) were caged with ICR male mice. Pregnancy was dated with vaginal plugs (day 0.5), and pregnant female mice were housed individually with ad libitum access to Purina 9F (9% fat) chow. On pregnancy day 12.5, female mice were randomly assigned to either control or undernutrition groups; weight did not differ between control and undernutrition mothers pre-pregnancy or at group assignment. Food intake of undernutrition mothers was restricted to 50% that of controls, calculated on a per-gestational day basis, from days 12.5 to 18.5. After delivery, litter size was equalized to eight in both control and undernutrition groups. Mothers received chow ad libitum after delivery. Pups nursed freely, were weaned at 3 weeks onto 9F chow ad libitum, and were followed longitudinally up to age 9 months. All experimental procedures were performed in male mice, except as indicated.

In vivo metabolic testing. Glucose tolerance was assessed after glucose injection (2 g/kg intraperitoneally) in unrestrained awake mice after a 16-h fast. Intraperitoneal insulin tolerance tests (1 unit/kg for young mice, 3 units/kg for 9-month-old mice; Humulin R; Eli Lilly, Indianapolis, IN) were performed in fed mice (2–3 P.M.).

Euglycemic-hyperinsulinemic clamp. Euglycemic-hyperinsulinemic clamps were performed in awake, unrestrained, catheterized mice ($n = 6$ control and 8 undernutrition mice) after an overnight fast (26). Insulin levels achieved did not differ between control and undernutrition mice (6.2 ± 0.7 vs. 7.2 ± 0.3 ng/ml; $P = 0.16$). Glucose turnover rate, hepatic glucose production, and tissue-specific glucose uptake ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were calculated (26).

Ex vivo glucose uptake. Soleus and epididymal fat were dissected from mice that were killed in the random-fed state. Muscles were mounted with resting tension 0.25 g; glucose uptake was measured at 0, 1.8, and 120 nmol/l insulin (27). Adipocytes were isolated by collagenase digestion, and ^3H -2-deoxyglucose transport was measured (28).

Immunohistochemistry and β -cell mass. Pancreata were dissected from anesthetized mice (pentobarbital 40 mg/kg), weighed, spread in anatomical orientation, fixed (Bouin's solution 4 h, formalin 10% overnight), and embedded. Seven-micrometer sections were immunostained for glucagon and somatostatin (Linco Research, St. Charles, MO), with anti-rabbit secondary and diaminobenzidine for visualization. β -Cell mass was measured by point counting morphometry. A single "full footprint" section from each mouse was covered systematically in nonoverlapping fields at $\times 420$ magnification using a 90-point grid to obtain number of intercepts over β -cell, endocrine non- β -cell, exocrine tissue, and nonpancreatic tissue. Each full footprint section of these spread pancreata yielded ~ 200 fields over pancreas. The β -cell relative volume was calculated by dividing the intercepts over β -cells by intercepts over total pancreatic tissue; the β -cell mass was estimated by multiplying the β -cell relative volume by the corrected pancreatic weight. Pancreatic weight was corrected by subtracting a correction factor obtained by multiplying pancreatic weight by the ratio of intercepts over nonpancreatic tissue to intercepts over total tissue. All sections were quantified by a single blinded observer. A nomogram relating number of points counted to volume density and expected relative SE in percentage of mean ($<10\%$) had been used to determine the number of intercepts needed for a representative sampling (29,30).

Insulin clearance. One microgram of biotin-labeled insulin (Sigma, St. Louis, MO) in 200 μl of 0.9% NaCl was injected into the tail vein after a 6-h fast. Seventy-five-microliter blood samples were taken at 5, 15, and 60 min. Twenty-five microliters of serum were added to streptavidin-coated plate wells (Reacti-Bind NeutrAvidin Coated, no. 15508; Pierce, Rockford, IL) for 1 h. The microplate was washed three times with PBS-0.05% Tween-20, incubated overnight with primary guinea pig anti-insulin antibody (Crystal-Chem, Chicago, IL), and processed for insulin ELISA (CrystalChem).

Western blot. Tissue homogenates were prepared from frozen liver (31). Western blots were probed with anti-insulin receptor (α and β) and cell adhesion molecule 1 (CEACAM1) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and quantified using PhosphorImager/ImageQuant (Molecular Dynamics, Sunnyvale, CA).

Islet isolation, insulin secretion, and enzymatic assays. Islets were isolated from 2- and 6-month-old mice (32) after intraductal collagenase. Freshly isolated islets of similar size were hand-picked under a stereomicroscope. Islets (20 per tube) were preincubated in Krebs-Ringer-Hepes (KRH) buffer for 30 min (37°C), washed, and incubated in 1 ml of fresh KRH that contained indicated glucose. Fifty microliters of medium was removed for insulin enzyme-linked immunosorbent assay. Islets were extracted in acid ethanol (4°C) and stored (-20°C) for insulin content assay. Glucokinase and hexokinase activities were measured in sonicated homogenates (33).

Serum metabolites. C-peptide and glucagon levels were measured by radioimmunoassay (Linco). Insulin was measured in 5- μl serum samples by

ELISA (CrystalChem). Blood glucose was measured with Glucometer Elite (Bayer, Elkhart, IN).

Body composition. Body composition was analyzed by carcass digestion, using Folch and micro-Kjeldahl methods for lipid and nitrogen analysis (34). **Statistical analysis.** Results are expressed as means \pm SE. Group comparisons were performed using a two-tailed t test or ANOVA (Statview); $P < 0.05$ was considered significant.

RESULTS

We have developed a mouse model of LBW via maternal undernutrition during the final week of gestation (mean food intake: control 6.5 ± 0.3 vs. undernutrition 3.2 ± 0.1 g). Birth weight of undernutrition offspring was reduced by 23% (control 1.64 ± 0.01 vs. undernutrition 1.26 ± 0.02 g; $P < 0.001$; Fig. 1A). Weight in undernutrition offspring was reduced as early as 4 days after restriction (embryonic day 16.5; control 0.90 ± 0.02 vs. undernutrition 0.66 ± 0.01 g; $P < 0.001$; Fig. 1A). Whereas fetal number at embryonic day 16.5 did not differ (control 12.0 ± 0.4 vs. undernutrition 13.2 ± 0.3), pup number at birth was significantly decreased in undernutrition litters (control 12.7 ± 0.1 vs. undernutrition 10.2 ± 0.2 pups; $P < 0.001$). Duration of gestation was similar (control 18.0 ± 0.2 vs. undernutrition 18.7 ± 0.2 days). We observed no increase in neonatal mortality or maternal neglect in undernutrition litters. All litters were equalized to $n = 8$ to reduce litter size-related variability in postnatal nutrition.

Differences in weight between groups disappeared by weeks 3–4 of life, indicating "catch-up" growth in mice of undernutrition pregnancies; there were no differences in weight up to 5–6 months of age (Fig. 1B). Carcass analysis at 2 and 6 months revealed no difference in fat, protein, or water content between groups on chow diet (Fig. 1C).

Whole-body glucose homeostasis. At age 1 month, glucose and insulin levels were similar between control mice and mice of undernutrition pregnancies (data not shown). However, by age 2 months, fed glucose and insulin levels were significantly increased in mice of undernutrition pregnancies (Table 1). These differences became more pronounced at 6 months, when both fasting and fed glucose levels and fed insulin levels were significantly higher in mice of undernutrition pregnancies (Table 1). Fasting glucagon levels did not differ. Glucose tolerance in mice of undernutrition pregnancies progressively declined from normal to severe glucose intolerance, from 2 to 9 months (Fig. 2A). Glucose intolerance was present but less severe in female mice (data not shown); thus, further analysis was performed only in male mice.

Insulin resistance and/or defects in insulin secretion could contribute to severe glucose intolerance and hyperinsulinemia in mice of undernutrition pregnancies. We assessed insulin sensitivity by several methods, including insulin tolerance at 2, 4, 6, and 9 months (Fig. 2C) and the "gold standard" hyperinsulinemic-euglycemic clamp at 3 months (Fig. 3A). Insulin tolerance did not differ between control mice and mice of undernutrition pregnancies. During the clamp, despite equivalent glucose levels, both glucose infusion rates and achieved insulin levels were slightly higher in mice of undernutrition pregnancies. However, glucose disposal at steady state (final 30 min) was equivalent when normalized for insulin levels achieved (glucose infusion rate normalized for achieved insulin level: control 9.0 vs. undernutrition $10.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per $\text{pg} \cdot \text{ml}^{-1}$), indicating normal insulin-stimulated

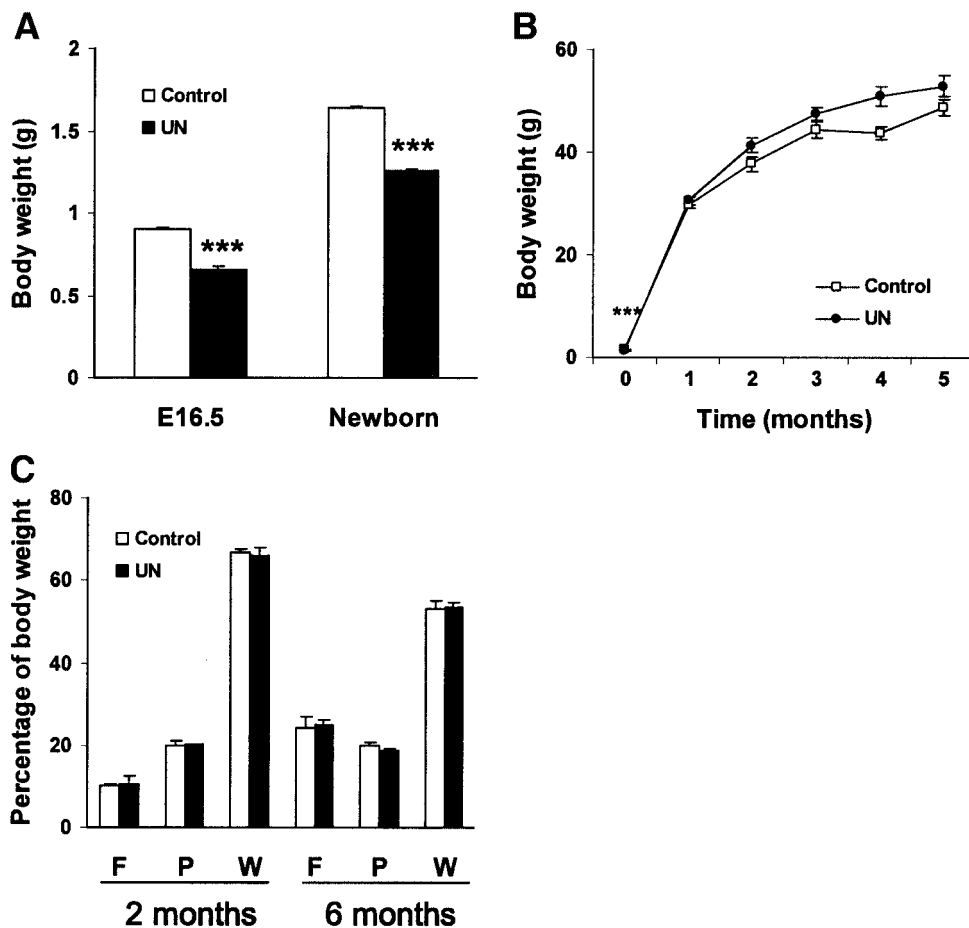


FIG. 1. Body weight and composition in offspring of control vs. undernutrition (UN) group mothers. *A:* Weight of fetuses (embryonic day 16.5 [E16.5]) and newborns from control or UN litters ($n > 18/\text{group}$). *** $P < 0.001$ vs. control. *B:* Growth of control and UN males to 5 months of age ($n > 24/\text{group}$). *C:* Body composition analysis at age 2 or 6 months ($n = 4/\text{group}$): fat (F), protein (P), and water (W) content is expressed as percentage of weight.

whole-body glucose disposal in mice of undernutrition pregnancies. Similarly, neither in vivo ^{14}C -2-deoxyglucose uptake (Fig. 3B) nor in vitro insulin-stimulated ^3H -2-deoxyglucose uptake into soleus or adipocytes differed between groups (Fig. 3C and D). Moreover, the relative contributions of glycolysis and nonoxidative metabolism, assessed during the clamp, did not differ, and insulin suppressed hepatic glucose production equally in both control and mice of undernutrition pregnancies (data not shown).

To evaluate the contribution of insulin secretory defects to glucose intolerance in mice of undernutrition pregnancies, we measured insulin levels during glucose tolerance testing. Insulin levels were normal in fasted mice of undernutrition pregnancies ($t = 0$) but were significantly increased 30 min postglucose in mice of undernutrition pregnancies at 2 months (Fig. 2C). With aging, both fasting

and postinsulin levels increased progressively in control mice. By contrast, insulin levels in mice of undernutrition pregnancies remained unchanged and thus were inappropriately low given the age-related increase in insulin resistance.

Insulin clearance. Hyperinsulinemia may also result from decreased clearance of insulin. C-peptide levels were 1.5-fold higher in mice of undernutrition pregnancies (control 364 ± 68 vs. undernutrition 530 ± 133 pmol/l; $P = 0.2$; Table 1, Fig. 4A). Although this elevation was similar in magnitude to that of insulin, the insulin-to-C-peptide molar ratio was slightly higher in mice of undernutrition pregnancies (control 1.38 ± 0.28 vs. undernutrition 1.86 ± 0.34 ; $P = 0.2$). Insulin levels achieved during the clamp were higher in mice of undernutrition pregnancies, also suggesting differences in clearance. We therefore assessed clearance of biotin-labeled insulin in vivo in 3-month-old

TABLE 1
Serum metabolites

	2 months		6 months	
	Control	UN	Control	UN
Fed glucose (mg/dl)	137 ± 3.4	147 ± 4.1*	170 ± 6.4	205 ± 16*
Fasting glucose (mg/dl)	84.2 ± 5.4	95.1 ± 9.4	71.5 ± 4.5	98.8 ± 8.6†
Fed insulin (ng/ml)	2.95 ± 0.5	4.43 ± 0.6*	3.69 ± 1.5	5.93 ± 1.5*
Fasting insulin (ng/ml)	0.72 ± 0.1	0.71 ± 0.2	1.99 ± 0.8	0.85 ± 0.1
Fed C-peptide (pmol/l)	364 ± 68	529 ± 133	ND	ND
Fasting glucagon (pg/ml)	47.1 ± 4.0	51.1 ± 4.8	94.6 ± 8.2	94.4 ± 4.2

Data are means ± SE. * $P < 0.05$, † $P < 0.01$ vs. controls. UN, undernutrition mice.

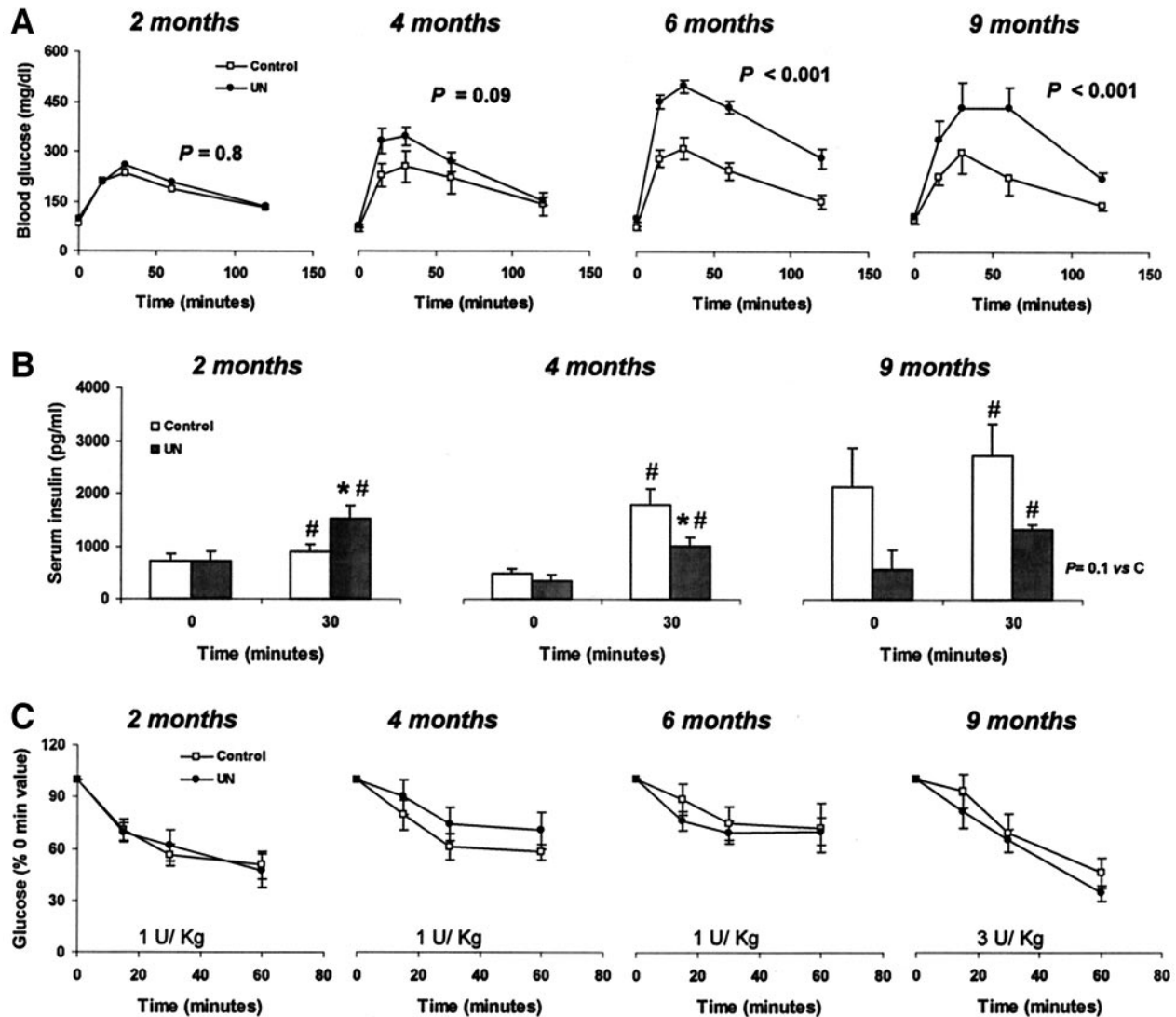


FIG. 2. Glucose and insulin tolerance tests in control mice and mice of undernutrition pregnancies. *A*: Intraperitoneal glucose tolerance tests in fasted mice at 2, 4, 6, and 9 months of age. *B*: Insulin levels at baseline and at 30 min postglucose. * $P < 0.05$ vs. control; # $P < 0.01$ vs. insulin value at $t = 0$. *C*: Intraperitoneal insulin tolerance tests were performed in mice that were fasted for 6 h. All data are means \pm SE from more than eight mice. Statistical significance was evaluated with ANOVA in *A* and *C*.

mice. In all mice, glucose levels decreased after injection of biotin-labeled insulin (control $31 \pm 6\%$ decrease vs. undernutrition $28 \pm 5\%$ at 15 min), indicating bioactivity. Controls cleared the insulin almost completely in 1 h (Fig. 4B); clearance was mildly impaired in mice of undernutrition pregnancies ($P = 0.03$). Because liver is the primary site for insulin clearance, we assessed the hepatic uptake of biotin-labeled insulin. Insulin staining progressively increased over time in sections from both control mice and mice of undernutrition pregnancies; this pattern was mildly delayed in mice of undernutrition pregnancies (Fig. 4C). Expression of α and β subunits of the insulin receptor, which constitute the first step in insulin clearance, was similar in control mice and mice of undernutrition pregnancies (data not shown). However, expression of CEACAM1, a major regulator of hepatic insulin clearance (35), was reduced by 30% in mice of undernutrition pregnancies at both 2 and 6 months of age (Fig. 4D).

β -Cell function. To define further the potential secretory defects that could contribute to progressive glucose intolerance and hyperinsulinemia in mice of undernutrition

pregnancies, we assessed pancreatic weight, β -cell mass, and insulin content and secretion. Pancreas weight was normal at 2 months (control 705 ± 69 vs. undernutrition 613 ± 71 mg) but significantly reduced in mice of undernutrition pregnancies by 6 months (control 957 ± 45 vs. undernutrition 622 ± 45 mg; $P < 0.001$). In contrast, β -cell mass, assessed by point morphometry, did not differ at either 2 or 6 months of age (Fig. 5A and B), and non- β -cell mass was similar in both groups (data not shown). Pancreatic insulin content was reduced in mice of undernutrition pregnancies by 30% at 2 months ($P = 0.02$), with a similar trend at 6 months ($P = 0.05$; Fig. 5C). This reduction cannot be explained solely by reduced pancreatic weight, because insulin concentration was also lower in mice of undernutrition pregnancies ($P = 0.03$ at 2 months; Fig. 5D).

We next evaluated glucose-stimulated insulin secretion *ex vivo*, in freshly isolated islets from 2- or 6-month-old mice. In controls, insulin secretion increased with glucose exposure in a dose-dependent manner, as expected (Fig.

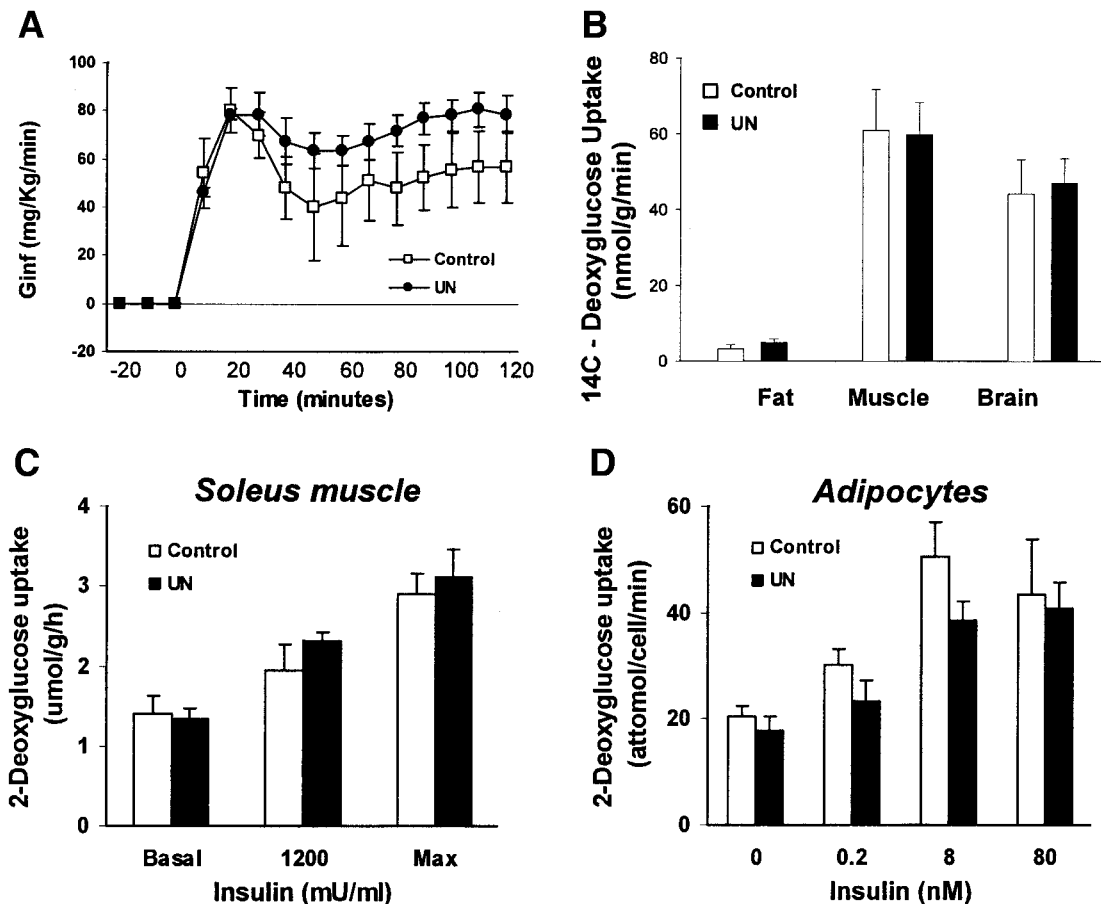


FIG. 3. Assessment of insulin sensitivity. *A*: Whole-body insulin sensitivity is represented by the glucose infusion rate (Ginf) required to maintain euglycemia during hyperinsulinemic clamp. *B*: In vivo 2-deoxyglucose uptake was measured in epididymal fat, gastrocnemius, and brain during the clamp. *C*: Insulin-stimulated 2-deoxyglucose uptake was measured in soleus muscle. *D*: Epididymal adipocytes ex vivo in 2-month-old mice. Data are means \pm SE from five (control) and eight undernutrition (UN) mice.

6A). By contrast, undernutrition islets showed 1) a completely flat response to glucose and 2) higher insulin secretion in response to very low glucose concentrations when compared with controls. Because the glucokinase/hexokinase complex is rate limiting for glucose phosphorylation and metabolism in β -cells, we measured their enzymatic activity (V_{max}). Glucokinase activity was not statistically different between groups (control 7.6 ± 1.9 vs. undernutrition 4.8 ± 0.6 mU/mg protein; $P = 0.15$; Fig. 6B). However, hexokinase activity was increased 2.5-fold in undernutrition islets (control 1.2 ± 0.36 vs. undernutrition 4.2 mU/mg protein; $P = 0.03$).

DISCUSSION

We describe a murine model of intrauterine growth restriction and LBW, generated by transient undernutrition during the final week of gestation. With this simple manipulation, birth weight was reduced by 23% in undernutrition offspring. This reduction was present as early as embryonic day 16.5 (4 days after the beginning of the diet restriction), demonstrating that fetal growth is highly dependent on maternal nutrient supply. Litter size was slightly decreased in mice of undernutrition pregnancies, suggesting that maternal undernutrition results in some fetal mortality over the final 4 days of gestation.

Eighty to 90% of LBW children exhibit “catch-up growth,” reaching height and weight equivalent to that of normal

birth weight children (36). Similarly, our mice of undernutrition pregnancies exhibited catch-up growth, reaching body weight equivalent to controls between 3 and 4 weeks of age. Whereas some human studies demonstrate increased risk for obesity in LBW children (37), our mice of undernutrition pregnancies that were fed standard chow during postnatal life maintained equivalent weight and did not develop increased adiposity, as assessed by carcass analysis at 2 and 6 months of age.

Despite achieving similar adult weight and adiposity, mice of undernutrition pregnancies developed progressive, severe glucose intolerance by 6 months. To identify early defects that are responsible for this phenotype, we characterized carbohydrate metabolism at age 2 months, before the onset of glucose intolerance. Fed insulin levels were 50% higher in mice of undernutrition pregnancies, despite similar glucose levels. These data initially suggested that peripheral insulin resistance might also underlie glucose intolerance in our model, as reported in some studies of LBW humans (1,6,9). Surprisingly, however, our mice of undernutrition pregnancies had normal peripheral insulin sensitivity, as assessed by four independent methods: 1) insulin tolerance test, up to age 9 months; 2) hyperinsulinemic-euglycemic clamp at age 3 months; 3) insulin-stimulated 2-deoxyglucose uptake into muscle and gonadal fat in vivo; and 4) insulin-stimulated glucose uptake in soleus muscle and isolated adipocytes ex vivo.

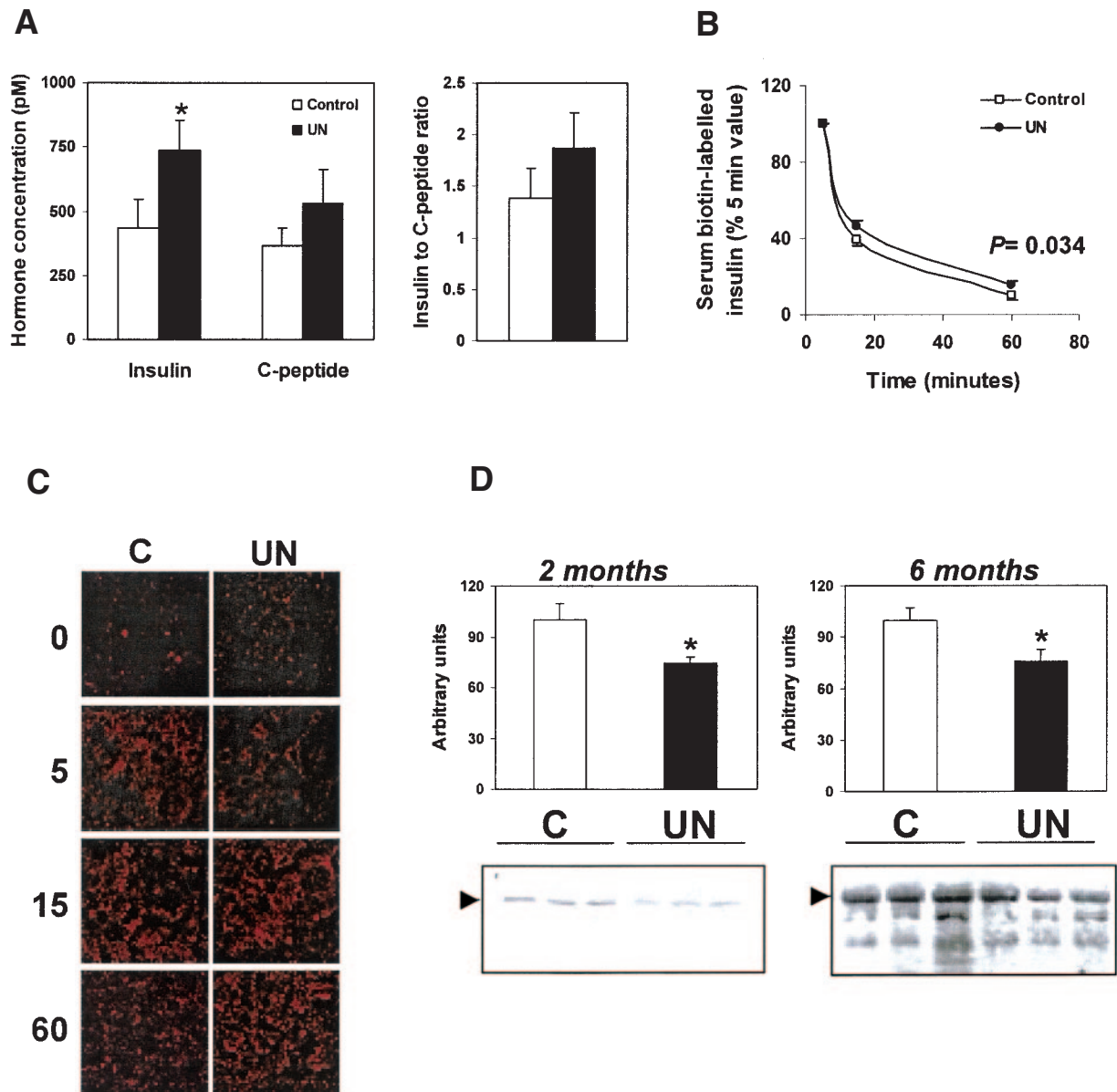


FIG. 4. Assessment of insulin clearance. **A:** Serum insulin and C-peptide were measured in random-fed 2-month-old mice. **B:** In vivo insulin clearance was assessed in fasted mice after the injection of biotinylated insulin (1 μ g). *P* value indicates differences between curves (ANOVA). **C:** Liver was removed at the indicated times after insulin injection and processed for immunodetection of biotinylated insulin (streptavidin conjugated to Texas Red). **D:** CEACAM1 expression was assessed with anti-CEACAM1 Western blotting, with enhanced chemiluminescence detection and quantification by densitometry. Results are means \pm SE of more than six mice. **P* < 0.05 vs. control. UN, undernutrition mice.

Moreover, there was no evidence for significant hepatic insulin resistance. Taken together, these data demonstrate that insulin sensitivity is normal in chow-fed mice of undernutrition pregnancies and suggest that in our mouse model, hyperinsulinemia is not preceded by or secondary to either peripheral or hepatic insulin resistance.

It is interesting that despite normal insulin sensitivity and fed hyperinsulinemia, we did not detect any hypoglycemia in mice of undernutrition pregnancies. Because glucagon levels did not differ between control mice and mice of undernutrition pregnancies, it is possible that other components of the counterregulatory response are upregulated to compensate for hyperinsulinemia in mice of undernutrition pregnancies. Alternatively, we cannot completely exclude the possibility that hyperinsulinemia in mice of undernutrition pregnancies occurs in the con-

text of subtle and transient insulin resistance occurring within the 1st month of life, before the dysregulation of insulin secretion. Further investigation in younger animals will be required to further explore this possibility. In addition to the normal insulin sensitivity as measured by hyperinsulinemic clamp, we find no evidence for impaired insulin signal transduction in muscle from mice of undernutrition pregnancies, with normal insulin-stimulated insulin receptor and Akt phosphorylation in muscle (data not shown). These data further suggest that muscle insulin resistance is not the major initiating defect in our murine undernutrition model.

Our data contrast with some human studies linking LBW to insulin resistance. Many early studies assumed that hyperinsulinemia in LBW subjects reflected insulin resistance or demonstrated insulin resistance using the less precise

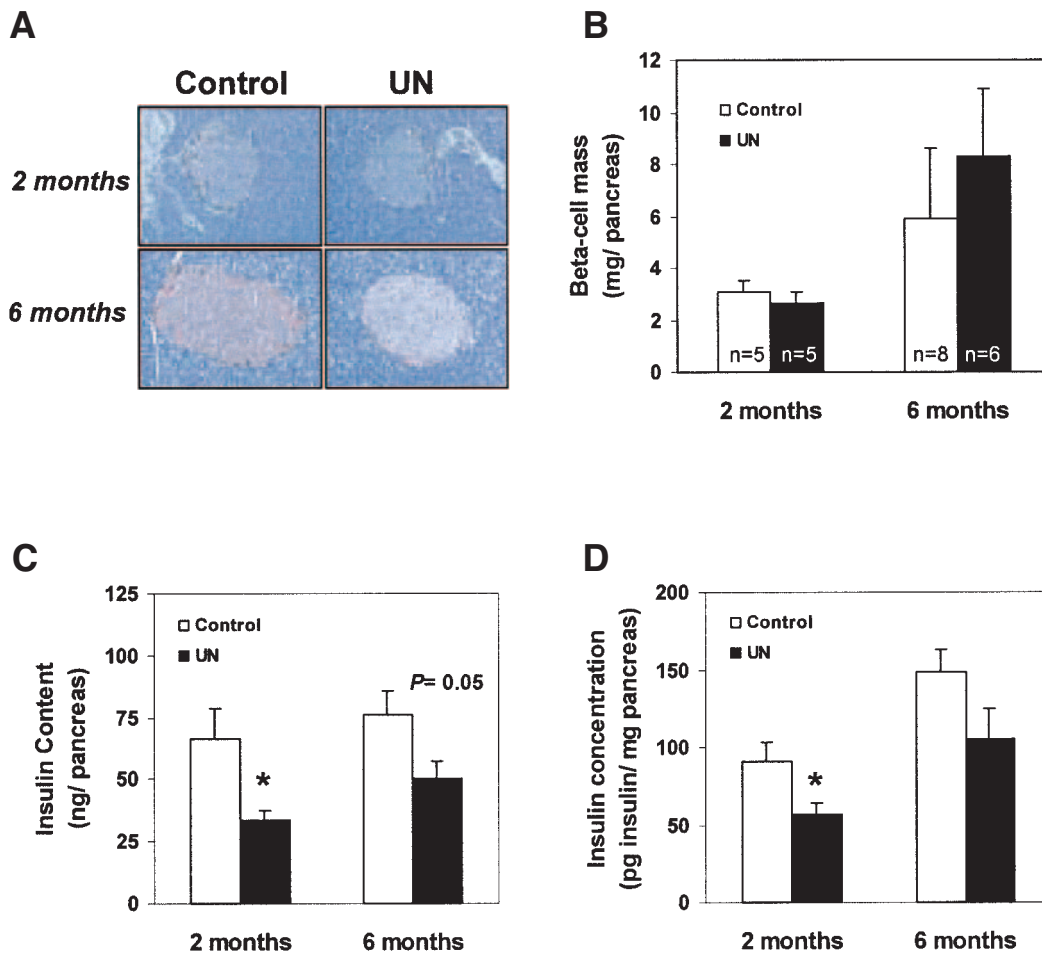


FIG. 5. Islet histology and insulin content. *A*: Representative photomicrograph of islets from 2- and 6-month-old mice, demonstrating normal architecture in both groups. Sections were stained with antibodies to glucagon and somatostatin and counterstained with hematoxylin. *B*: β-Cell mass was quantified by point morphometry from at least five mice per group as described. Pancreatic insulin content (*C*) and insulin concentration (*D*) were measured at the indicated ages (six per group). Results are means ± SE. **P* < 0.05 vs. control. UN, undernutrition mice.

homeostasis model assessment or intravenous glucose tolerance modeling approaches (2,6,10,15). However, even hyperinsulinemic-euglycemic clamp analysis in LBW subjects has yielded surprisingly discordant conclusions. Insulin-stimulated glucose disposal is reduced in some cohorts of prepubertal children (9,38) and in young adults with a history of LBW (7), whereas insulin sensitivity is normal in both 7-year-old LBW African children (12) and LBW white male individuals well matched for fat mass and aerobic capacity with control subjects (13). Still other studies demonstrate coexisting abnormalities in both insulin action and secretion (13), even in LBW children as early as 1 year of age (15). Similarly, intrauterine growth restriction in rats yields varied outcomes, with whole-body insulin action ranging from insulin resistance (22–24) to normal or improved insulin sensitivity (21,25). Potential contributors to the variability in insulin sensitivity in both LBW humans and rodents are likely to include the population and species under study, methods used for metabolic assessment, the underlying cause of aberrant fetal growth, postnatal catch-up growth (39–42), and other postnatal risk factors, including aging, obesity, and inactivity.

If not insulin resistance, then what is the origin of hyperinsulinemia and subsequent glucose intolerance and type 2 diabetes in mice of undernutrition pregnancies? We

have considered two principal possibilities: 1) abnormal insulin clearance and 2) abnormalities in β-cell function. Mice of undernutrition pregnancies displayed a very mild impairment of insulin clearance that could contribute to the development and/or maintenance of hyperinsulinemia, particularly in the fed state. It is interesting that mice of undernutrition pregnancies have a 30% reduction in CEACAM1 expression, because transgenic mice overexpressing a dominant negative, phosphorylation-defective form of CEACAM1 in liver are hyperinsulinemic, despite normal insulin sensitivity (35), and develop progressive glucose intolerance after 2 months of age. Thus, reduced CEACAM1 signaling may contribute to both hyperinsulinemia and secondary impairment in glucose tolerance.

We next evaluated the possibility that β-cell dysfunction could also contribute to the early hyperinsulinemia and glucose intolerance in mice of undernutrition pregnancies. Abnormal β-cell function or mass has been linked to LBW-related metabolic disorders in both humans (12–14) and rats (20,21). We explored the possibility of either 1) altered β-cell mass or 2) a functional β-cell defect resulting in abnormal glucose-stimulated insulin release. Quantitative β-cell mass in 2- and 6-month-old mice did not differ between control mice and mice of undernutrition pregnancies. These data are consistent with the majority

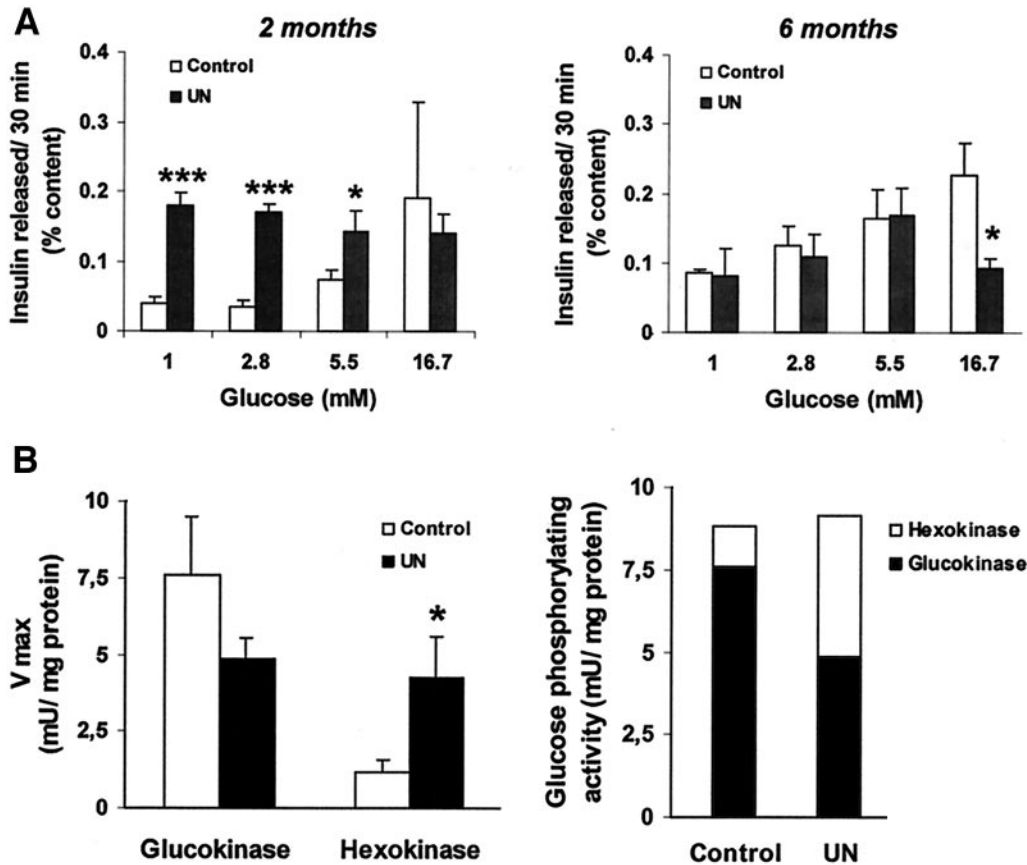


FIG. 6. Islet cell physiology at 2 and 6 months. **A:** In vitro glucose-stimulated insulin secretion. Isolated islets were incubated with the indicated glucose concentration for 30 min. Results are means \pm SE of eight replicates from at least two independent isolations. **B:** Glucokinase and hexokinase I enzymatic activities were assayed in isolated islets; results are means \pm SE of 10 replicates from two independent isolations. * $P < 0.05$, *** $P < 0.001$ vs. control. UN, undernutrition mice.

of rodent studies, which indicate that global reductions in maternal caloric intake result in normal islet growth but functional impairment in β -cells (21), whereas maternal protein malnutrition or global caloric restriction continued postnatally reduces β -cell mass (21) or impairs the normal age-related expansion of β -cell mass (43). Despite similar β -cell mass, insulin content was reduced by 25% in mice of undernutrition pregnancies. Reduced pancreatic insulin content has been previously reported in LBW rats, usually in association with reductions in β -cell mass (20,21). Whether this reduction is due to lower insulin gene transcription, biosynthesis, and/or accumulation is not yet known, but differences in insulin content clearly cannot account for hyperinsulinemia and instead point to intrinsic dysregulation of glucose-stimulated insulin secretion in mice of undernutrition pregnancies (44).

Ex vivo analysis of insulin secretion demonstrated that, as expected, control islets secreted insulin in a glucose-dependent manner. By contrast, islets from 2-month-old mice of undernutrition pregnancies showed 1) completely flat response to glucose and 2) higher insulin secretion at low glucose concentrations (1–5.5 mmol/l) compared with controls. By age 6 months, insulin secretion in response to 16.7 mmol/l glucose was significantly reduced in undernutrition islets. These data suggest that the secretory defect in undernutrition islets is initially characterized by inability to modulate insulin secretion relative to ambient glucose, with a secondary decline in glucose-stimulated insulin secretion with aging. Older mice of undernutrition pregnancies are unable to increase insulin secretion to compensate for age-related insulin resistance and thus develop progressive glucose intolerance.

Glucose-stimulated insulin secretion is regulated at the level of glucose phosphorylation by the glucokinase/hexokinase complex (45). Because glucokinase has a high K_m for glucose, the impaired secretion in undernutrition islets at 16.7 mmol/l glucose can be explained in part by the 36% reduction in glucokinase activity. More striking is the finding that islet hexokinase activity was increased 2.5-fold in young mice of undernutrition pregnancies. Because hexokinase has a very low K_m for glucose, undernutrition islets may secrete more insulin than controls at glucose levels between 1 and 5 mmol/l as a result of higher glucose phosphorylation. At these low glucose concentrations, glucokinase is poorly active, contributing little to insulin secretion. A similar pattern of glucokinase/hexokinase activity and secretion has been previously reported (46,47); for example, overexpression of hexokinase I in rat islets increases basal insulin secretion, with similar insulin secretion at 30 mmol/l glucose. It is interesting that humans with glucokinase mutations (48,49) and mice heterozygous for β -cell-specific glucokinase deletion also have reduced birth weight (50). These data have led to the hypothesis that reduced glucokinase expression during fetal life reduces fetal insulin secretion and, as a consequence, reduces fetal growth. Our undernutrition model suggests an alternative view of the fetal insulin hypothesis: maternal undernutrition results in a low fetal glucose and nutrient milieu, which “programs” (low) fetal glucokinase activity and (high) hexokinase activity in an attempt to ensure appropriate insulin secretion. Of course, alterations in expression/function of other key genes that regulate insulin synthesis/secretion and the in vivo environment of undernutrition islets may also modulate insulin secretion.

Taken together, our data highlight an important role for early insulin secretory defects, characterized by inappropriate secretion relative to ambient glucose, in the development of glucose intolerance and type 2 diabetes in our LBW mouse model. Although no single model recapitulates all features of LBW-associated type 2 diabetes in humans, our data are consistent with secretory defects described in young LBW rats (before reduction in β-cell mass) (16) and in LBW humans as early as 1 year of age (15). As with other features of the LBW metabolic phenotype, it is likely that the precise mechanisms used to induce experimental LBW or those that contribute to spontaneous LBW are critical in determining the final β-cell phenotype. Although we cannot entirely exclude the contribution of transient and subtle insulin resistance in early life to the metabolic phenotype, our data suggest that insulin secretory defects in adult LBW mice can be associated with maternal undernutrition even in the absence of coexisting insulin resistance, increased adiposity, or alterations in β-cell mass.

Thus, insulin secretory abnormalities in LBW mice may result from appropriate fetal adaptation (“programming”) to a suboptimal nutritional state during intrauterine life but ultimately are maladaptive when presented with a high-carbohydrate diet after weaning. With the superimposition of age-related or dietary insulin resistance, insulin secretory responses are inadequate, resulting in progressive glucose intolerance. Further analysis of cellular mechanisms underlying β-cell dysfunction in our model is likely to increase our understanding of the insulin secretory defects that contribute to human LBW-associated diabetes.

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