Nutritional Origins of Insulin Resistance: A Rat Model for Diabetes-Prone Human Populations\textsuperscript{1,2}


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ABSTRACT While there has been little success identifying the genetic bases of noninsulin-dependent (type-2) diabetes, current epidemiological data and animal models implicate fetal undernutrition in the development of type-2 diabetes. We examined the effects of fetal undernutrition on insulin responses and glucose tolerance in adulthood in genetically normal rats. Control rats were adequately nourished in utero and consumed nutritionally adequate (N) diets throughout life. Experimental rats (F1 generation) were undernourished in utero and consumed either N or high-energy, high-fat (HF) diets postweaning. The offspring of the experimental rats (F2 generation) received the respective diets of their parent. Body weights of experimental F1 rats at d 4 were 40% less than that of control pups, and they remained significantly smaller than controls throughout adulthood. The experimental F1 rats consuming N diets postweaning had a reduced insulin response (-30%) at 30-min postglucose challenge in adulthood (P < 0.05). However, their offspring (F2 generation) displayed a markedly elevated insulin response (130% at 30 min (P < 0.001) and +250% at 120 min (P < 0.001) postglucose challenge). The insulin response of the F2 generation rats fed the high-energy, HF diet was even more pronounced (+130% at 30 min (P < 0.003) and +250% at 120 min (P < 0.001) postglucose challenge). Thus, undernourishment in utero produces striking insulin resistance in genetically normal, well-nourished second-generation rats. J. Nutr. 130: 741–744, 2000.

KEY WORDS: • type 2 diabetes • insulin resistance • rats

The clustering of noninsulin-dependent (type-2) diabetes mellitus in families and the high concordance rates noted for identical vs. fraternal twins implies a genetic etiology for type-2 diabetes (Barnett et al. 1981, Newman et al. 1987). These findings, in conjunction with numerous epidemiological studies which have consistently found high type-2 diabetes prevalence rates in populations moving from traditional, non-Western diets and active lifestyles to rich, Western diets and more sedentary lifestyles, provide the basis for the highly influential “thrifty genotype” hypothesis (Neel 1962). Originally proposed to explain the unusually high incidence and prevalence rates of type-2 diabetes among many Native American populations after WWII, the “thrifty genotype” was hypothesized as having been “selected for” when the earliest Native Americans encountered particularly harsh feast or famine conditions as they moved into North America from Asia thousands of years ago. This environment was presumed to have favored individuals with a “quick insulin trigger” which resulted in greater fat storage when plasma glucose levels were highest during times of abundance. The fat then served as an energy reserve to be called up in more difficult times.

However, while this special, genetically-based capacity promoted survival advantage under aboriginal feast or famine conditions, when coupled with stable, energy-adequate (or more than adequate) diets and sedentary lifestyles (common in reservation communities beginning after WWII), it promotes chronic hyperglycemia, hyperlipidemia, hypertension, obesity and type-2 diabetes. Others have refined and reformulated the original hypothesis (Brand Miller and Colagiuri 1994, O’Dea 1991, Reaven 1998, Rittenbaugh and Goodby 1989, Saath off 1990, Wendorf and Goldfine 1991); however, Neel’s emphasis on the role of genetics and natural selection in the etiology of type-2 diabetes remains the central concept.

After years of intensive work by many laboratories, however, there has been little success identifying type-2 diabetes susceptibility genes (Elbein 1997, Neel 1999). Moreover, important questions have been raised as to the methodological biases and conclusions drawn from nonpopulation-based twin studies which have often been cited as evidence of a strong genetic basis of the disease (Hopper 1999). As a result, support for the “thrifty genotype” hypothesis may be eroding. Hales and Barker (1992) proposed an alternative to Neel’s hypothesis, the “thrifty phenotype,” to accommodate the current epidemiological findings and experimental animal data. The “thrifty phenotype” implicates fetal undernutrition, not genetics, in the development of type-2 diabetes. Hales and Barker (1992) argue that metabolic adaptations by the undernourished fetus serve to increase fuel availability in utero, but this “programming” of pancreatic endocrine function persists throughout life, elevating risk for the later development of type-2 diabetes.

In human populations, low birth weight is considered a reflection of compromised nutrition in utero, and numerous reports have demonstrated a relationship between a low birth weight and impaired glucose tolerance in later life. In men aged 59 to 70 y (n = 370), the prevalence of type-2 diabetes or impaired glucose tolerance ranged from 40% if birth weight...
was less than or equal to 2.5 kg to < 14% if birth weight was > 4.3 kg (Hales et al. 1991). A follow-up report in men and women aged 50 to 58 y (n = 266) indicated that the percentage of subjects with type-2 diabetes or impaired glucose tolerance fell from 27 to 6% as birth weight increased from < 2.5 kg to over 3.4 kg (Phipps et al. 1993). Recently Rich-Edwards et al. (1999) reported an inverse association between birth weight and risk for type-2 diabetes in a cohort (n = 69,526) from the Nurses’ Health Study. Relative risk for type-2 diabetes, adjusted for age, adult body mass index and maternal history of diabetes, was significantly greater at low birth weights (<2.3 kg) vs. high birth weights (>3.2 kg) (relative risk: >1.8 and ≤ 1.0, respectively).

In addition to epidemiological research, rat studies have demonstrated that the structure of the endocrine pancreas in undernourished fetuses is altered: beta cell numbers are reduced as are islet size and vascularization (Snoeck et al. 1990). Insulin secretion is impaired in these fetal islets when stimulated in vitro (Dahari et al. 1991). Furthermore, 70-d-old rats undernourished in utero display impaired glucose tolerance and a low-insulin response (Dahari et al. 1991). Thus, structural changes in the pancreata of undernourished fetuses lead to functional deficiencies in adulthood.

In the present study, we examined the effects of fetal undernutrition on birth weights, insulin responses and glucose tolerance in adult life. Rats were undernourished in utero but consumed nutritionally adequate diets postweaning and throughout adulthood. The same variables were also examined in well-nourished second-generation animals, i.e., the offspring of rats undernourished in utero, to investigate the persistence of the “thrifty phenotype.”

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (Arizona State University colony, Tempe, AZ) consumed either a nutritionally adequate (N) diet (20% protein; TD 91352, Harlan Teklad, Madison, WI) or an isocaloric, low-protein diet (8% protein; TD 93033, Harlan Teklad) from 1 d of pregnancy through lactation (Table 1). The day after delivery, all litters were reduced to 10 pups. Control pups consumed the N diet ad libitum postweaning (controls). Pups undernourished during gestation consumed either the N diet ad libitum postweaning (N: F1) or a high-fat (HF) diet (42% of energy from fat, TD 88137; Harlan Teklad) ad libitum postweaning (HF: F1) (Table 1).

At 70 d of age, control and N: F1 rats were food-deprived overnight and subjected to glucose tolerance tests. Rats were killed under CO2 anesthesia at time 0 and at 30 and 120 min post-glucose injection (30% w/v; 2 g/kg body weight, i.p.). Blood was collected by cardiac puncture. Several female F1 rats consuming N or HF diets were mated at 70 d of age and fed on their respective diets throughout gestation and lactation. Their offspring, the F2 generation, continued on their respective diets throughout adulthood. The same variables were also examined in well-nourished second-generation animals, i.e., the offspring of rats undernourished in utero, to investigate the persistence of the “thrifty phenotype.”

### TABLE 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>20% Protein1</th>
<th>8% Protein</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g/kg diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>230.0</td>
<td>92.0</td>
<td>195.0</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>3.0</td>
<td>1.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>431.7</td>
<td>551.8</td>
<td>341.5</td>
</tr>
<tr>
<td>Corn starch</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>52.3</td>
<td>53.7</td>
<td>-</td>
</tr>
<tr>
<td>Anhydrous milkfat</td>
<td>-</td>
<td>-</td>
<td>210.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>37.9</td>
<td>55.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin mix2</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix3</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix, Ca-P-deficient4</td>
<td>13.4</td>
<td>13.4</td>
<td>-</td>
</tr>
<tr>
<td>Calcium phosphate, dibasic</td>
<td>16.6</td>
<td>20.9</td>
<td>-</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.1</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Ethoxyquin (antioxidant)</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 The 20 and 8% protein diets are isocaloric with adjustments for the phosphorus contributed by the different amounts of casein. In comparison, the high-fat diet supplies 20% more energy by weight. (Harlan Teklad, Madison, WI).

2 Provided (g/kg diet): 1.017 ascorbic acid; 0.0004 biotin; 0.030 vitamin B-12; 0.066 calcium pantothenate; 3.497 choline dihydrogen citrate; 0.002 folic acid; 0.110 inositol; 0.050 menadione; 0.089 niacin; 0.110 para-aminobenzoic acid; 0.022 pyridoxine HC; 0.022 riboflavin; 0.022 thiamin HCl; 0.040 retinyl palmitate; 0.004 cholecalciferol; 0.242 tocopheryl acetate; 4.667 corn starch.

3 AIN-76; provided (g/kg diet): 17.50 calcium phosphate, dibasic; 2.59 sodium chloride; 7.70 potassium citrate, monohydrate; 1.82 potassium sulfate; 0.84 magnesium oxide; 0.123 manganous carbonate; 0.210 ferric citrate; 0.058 zinc carbonate; 0.011 cupric carbonate; 0.00035 potassium iodate; 0.00035 sodium selenite; 0.01925 chromium potassium sulfate; 4.13 finely powdered sucrose.

4 Modification of mineral mix AIN-76; when used at the rate of 13.36 g/kg of diet, mineral elements other than Ca and P will be supplied to the diet at rates similar to those provided by 35.0 g/kg of AIN-76.

RESULTS

Body weight was significantly lower than controls in N: F1 and HF: F1 pups at age 4 d (Table 2). This difference in body weight persisted throughout the 65 d of the study (Table 2).

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 4</th>
<th>Day 65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls2</td>
<td>20</td>
<td>9.64 ± 0.23b</td>
<td>320.50 ± 18.40b</td>
</tr>
<tr>
<td>N/F13</td>
<td>20</td>
<td>5.64 ± 0.22c</td>
<td>226.43 ± 12.22c</td>
</tr>
<tr>
<td>HF/F1</td>
<td>20</td>
<td>5.64 ± 0.22c</td>
<td>250.52 ± 13.42c</td>
</tr>
<tr>
<td>N/F2</td>
<td>19</td>
<td>9.00 ± 0.16b</td>
<td>322.82 ± 16.79b</td>
</tr>
<tr>
<td>HF/F2</td>
<td>20</td>
<td>12.36 ± 0.25a</td>
<td>381.05 ± 27.38a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Means in a column with different superscript letters are significantly different, P < 0.05.

2 A single generation of control rats was used in the statistical analyses.

3 F1 pups were separated into groups at weaning; hence, mean d 4 body weights are the same for the N/F1 and HF/F1 groups.
weight persisted into adulthood. Body weights at d 4 did not differ between N: F2 and control rats, but body weights at d 4 for HF: F2 pups were significantly higher than that for all other groups (Table 2). Body weights at 65 d did not differ in second-generation rats consuming N or HF diets, while body weights at 65 d were 20% higher in HF: F2 rats compared to control rats (P < 0.05).

The baseline insulin concentration did not differ between control and N: F1 rats; however, the baseline plasma insulin concentration tended to be 100% greater in N: F2 rats compared to controls and N: F1 rats (0.05 < P < 0.10) (Fig. 1). Furthermore, the baseline plasma insulin concentration in second-generation experimental rats consuming the HF diet was twice that of the second-generation experimental rats consuming the N diet (P < 0.05). At 30-min postglucose challenge, the plasma insulin concentration was insignificantly lower (−30%) in N: F1 rats vs. controls (P = 0.395), whereas insulin concentrations were significantly elevated in both sets of second-generation rats compared to first-generation experimental rats and controls. This pattern persisted at 120-min postglucose challenge.

Baseline plasma glucose concentrations did not differ among controls, N: F1 or N: F2 rats, but they were 40–60% greater in second-generation rats fed HF diets (Fig. 1). At 30-min postglucose challenge, glucose concentrations were significantly reduced in all experimental groups compared to controls. There were no differences among groups at 120-min postglucose challenge.

The baseline insulin/glucose (I/G) ratio was significantly raised in second-generation rats fed HF diets compared to controls and N: F1 animals (Fig. 1). At 30-min postglucose challenge, second-generation rats fed HF diets had a significantly higher I/G ratio than all other groups. By 120-min postglucose challenge, both groups of second-generation rats had I/G ratios significantly higher than those of control and N: F1 rats.

**DISCUSSION**

Previous epidemiological studies and experimental animal research have demonstrated that undernutrition in utero is related to a reduced insulin secretion in adulthood and, in humans, an elevated risk for type-2 diabetes. We found that undernutrition in utero in rats was related, but not significantly, to a reduced insulin response (−30%), at 30-min postglucose challenge in adulthood. However, the offspring of these rats (the second-generation rats) displayed a markedly elevated insulin response following a glucose challenge in adulthood. Thus, we have demonstrated that the subtle endocrine pancreatic dysfunction noted in first-generation rats undernourished in utero produces striking insulin resistance in well-nourished second-generation rats.

Snoeck et al. (1990) observed that beta cells from neonates born to dams fed low-protein diets had reduced proliferation and islet size compared to control neonates. Additionally, islet vascularization was reduced dramatically (−50%) in the low-protein-exposed neonates. Thus, a low-protein diet during gestation impaired the normal maturation of the fetal beta cell. Further investigation revealed that in vitro stimulation of insulin secretion was reduced 20–30% in fetal islets extracted from offspring of dams fed low-protein diets (Dahri et al. 1991). Islets from low-protein-exposed offspring fed control diet from birth until d 84 also exhibited reduced insulin secretion when challenged in vitro, indicating that the functional deficit noted at birth persisted into adulthood (Dahri et al. 1995). An oral glucose challenge in these rats generated a normal blood glucose and blood insulin response in the male rats, but a hyperglycemic and hypoinsulinemic response in the female rats (Dahri et al. 1995).

Accordingly, adult female rats gestated by dams fed low-protein diets but themselves fed the control diet throughout life developed glucose intolerance during pregnancy (Dahri et al. 1995). The endocrine pancreas of the offspring (the F2 generation) exhibited a higher proportion of large islets and a significantly higher relative pancreatic insulin content vs. control tissue (Dahri et al. 1995, Eriksson and Swenne 1993).

Thus, protein-energy malnutrition during fetal development reduced the ability of the rats to increase insulin production to meet the needs of pregnancy; hence, increased amounts of glucose and other nutrients were transferred to the fetus, stimulating beta cell growth. Dahri et al. (1995) postulated that these fetal changes in the F2 generation would promote the development of a type-2 diabetes-like syndrome in adulthood. The data reported here expand on these indications and demonstrate that adult F2 rats do indeed develop an insulin resistance typical of type-2 diabetes in human.

Our findings are particularly interesting, given the fact that Native American populations with high prevalence rates for type-2 diabetes, such as the Pima, endured periods of starvation during pregnancy. The results are presented as means (n = 18 to 20) ± SEM Means at 0, 30 or 120 min without a common superscript letter are different, P < 0.05.

**FIGURE 1.** Plasma insulin and glucose concentrations and the insulin/glucose (I/G) ratio following a glucose challenge (300 g/L; 2 g/kg body weight, i.p.) at 70 d of age in control rats, experimental rats undernourished in utero but adequately nourished postweaning (N: F1), the adequately nourished offspring of N: F1 rats (N: F2) and the high-fat diet-fed offspring of rats undernourished in utero but overnourished postweaning (HF: F2). The results are presented as means (n = 18 to 20) ± SEM Means at 0, 30 or 120 min without a common superscript letter are different, P < 0.05.
tion at the turn of the century but did not experience high type-2 diabetes prevalence rates until over 50 y later. The Pima suffered severe, general malnutrition and even death due to starvation in the period 1870–1910 (Hackenberg 1983). Subsequently, most Pimas escaped such extreme privation, but income based on migratory labor remained low, as did energy intake, through WWII. Just 21 cases of diabetes were reported in 1940, and it has only been since the 1960s, when energy intake approached and then surpassed the national level, that diabetes became a major problem (Knowler et al. 1990). By that time, most of the 1870–1910 birth cohort had died, and the cases were drawn from their children and grandchildren. Furthermore, Lillioja et al. (1991) have demonstrated that nondiabetic Pimas are more insulin-resistant and have exaggerated early insulin release and elevated I/G ratios, compared to nondiabetic Caucasians.

This history of starvation and the subsequent development of diabetes are observed also among the Havasupai of Arizona and the Nauruans of Micronesia (Diamond 1992, Martin 1986, Smith 1970, Zimet et al. 1991), as well as other indigenous and migrant populations around the world (Griffiths 1995, Kidd 1997, Mayer 1961). In light of our findings in rats, insulin resistance and enhanced insulin secretory responses would be expected among offspring born to mothers who were gestated under starvation conditions at the turn of the century.

Obesity is a major risk factor for type-2 diabetes. Age-specific incidence rates of type-2 diabetes are 100–300% higher in individuals with body mass index >35 kg/m² vs. <25 kg/m² (Knowler et al. 1990). Total energy intake, particularly from fat, is a strong predictor of obesity development. In the present report, the consumption of HF, energy dense diets by rats gestated on low-protein diets and by their offspring (the F2 generation) was associated with a significant elevation in fasting blood glucose vs. controls and a significant elevation in fasting blood insulin vs. controls as well as F2 rats fed control diets. Hence, the diet-induced insulin resistance of the present study appeared to be exaggerated by feeding HF, high-energy diets, an association also observed among diabetic Pimas (Bennett et al. 1984).

Together, these data strongly indicate that fetal undernutrition in the rat is a strong risk factor for the development of insulin resistance in the subsequent F2 generation. Hattersley and Tooke (1999) argue that any association of low birth weight, an indication of poor maternal nutrition, to diabetes is genetically determined and that the “thrifty genotype” continues to be a valid hypothesis. Our data counter this argument in that insulin resistance developed in genetically normal rats via maternal undernutrition. The lack of a gene-insulin resistance interaction suggests that careful maternal monitoring may optimize human fetal development and break the familial chain of diabetes (Godfrey 1998). Buchanan et al. (1990) and others (Alpert et al. 1985, Maraes et al. 1985) have demonstrated the efficacy of brief energy deprivation in mothers with gestational diabetes in terms of reducing plasma glucose without creating a greater propensity for maternal ketosis. Careful exploration of maternal diet intervention strategies to reduce maternal hyperglycemia and optimize fetal environment is an important consideration for future research.

LITERATURE CITED


