Failure of Genetically Selected Miniature Swine to Model NIDDM

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SUMMARY

Ten young adult miniature swine from a line reported to be genetically selected for glucose intolerance and eight normal controls were obtained from Colorado State University. They were consecutively exposed to 4 mo of a high-fiber, low-fat standard swine diet; 4 mo of a high-sucrose, high-fat, low-fiber diabetogenic diet; and 4 mo of excess diabetogenic diet for obesification. Results of oral glucose tolerance and intravenous insulin tolerance tests conducted at the end of each regimen were compared. Hyperglycemia was not observed in any animals after any manipulation. Insulin sensitivity was also not influenced by diet. We conclude that F_7 low-K miniature swine from this colony fail to model human non-insulin-dependent diabetes. *Diabetes* 36:284–87, 1987

There is a paucity of animal models of non-insulin-dependent diabetes (NIDDM) (1,2). Whereas several models of NIDDM exist in rodents, large animal models for this disease have been identified only recently (2). The Yucatan miniature swine is of particular interest. Miniature swine are an excellent model for the study of human disease. Pigs have remarkable anatomic and physiologic similarities to humans with respect to the cardiovascular system, digestive tract, skin, nutritional requirements, feeding habits, bone development, and mineral metabolism. Recently, two lines of Yucatan miniature swine were developed by genetic selection for increased and decreased glucose clearance (3,4). It has been reported that glucose intolerance in this animal is directly related to diet (5). When fed a low-fiber diet that contained 40% cal from saturated fat, the low-K Yucatan miniature swine was reported to become glucose intolerant. Despite dietary and adrenergic intervention, we were unable to induce glucose intolerance or hyperinsulinemia in these animals.

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MATERIALS AND METHODS

Eighteen young adult Yucatan miniature swine were obtained from Colorado State University. Ten (6 male, 4 female) of these animals were from a line reported to be genetically predisposed to glucose intolerance (low K), and eight (2 male, 6 female) were from a normal line (normal K) (3,4). All low-K pigs were F_7 generation.

Method. The pigs were housed in a controlled environment (21 ± 2°C, 12-h light-dark period). The animals were fed daily at 0800 h. Fresh drinking water was always available. The pigs were sequentially exposed to three different diet regimens. Regimen 1 consisted of a standard swine diet that was relatively high in crude fiber (8.2%), high in starch (64.8% cracked corn), and low in fat (none added). The diet was supplied in amounts necessary to maintain body weight [~ 1 kg/day per pig or 2% of their body weight daily on a dry-matter basis (5)]. After the completion of diet regimen 1, regimen 2 was instituted. Regimen 2 consisted of the American Swine Diet, which is a low–crude-fiber (1.9%), low-starch (8.7% cracked corn), high-sucrose (28.7%), high-fat (20.1%) diet (5). This diet was also fed to maintain body weight [~ 0.5 kg/day (5)] for 4 mo. Diet regimen 3 was identical to diet regimen 2 except that the American Swine Diet was fed in amounts intended to obesify the animals (50–75% more than fed in regimen 2). Body weight was monitored every 2 wk throughout the study. At the end of each feeding period, precaval blood sampling catheters (polyethylene tubing, 0.86 mm ID, 1.27 mm OD, Intramedic Clay-Adams) were aseptically implanted with 10–15 mg/kg i.m. ketamine hydrochloride (Vetalar, 100 mg/ml, Parke-Davis, Detroit, MI) for analgesia. Atropine sulfate (0.05 mg/kg i.m.) was administered simultaneously. Additionally, local anesthesia was provided with 2% lidocaine hydrochloride. The catheters were flushed with heparinized saline solution (20

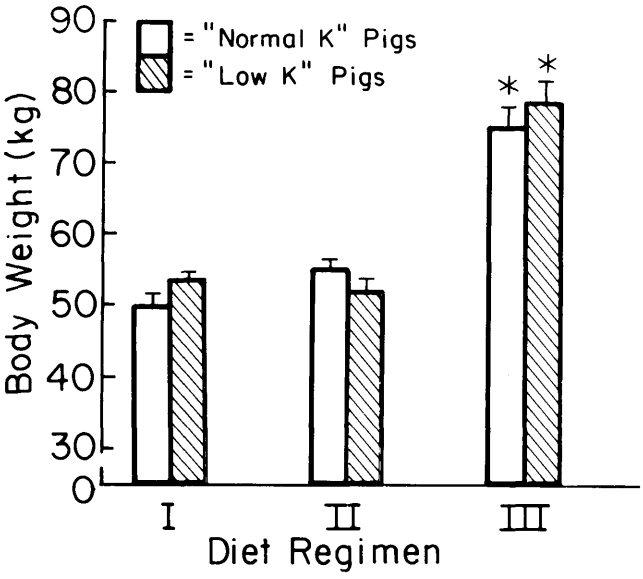

FIG. 1. Body weights (group means ± SE) after exposure to 3 different diet regimens. *Significantly different ($P \leq .05$).

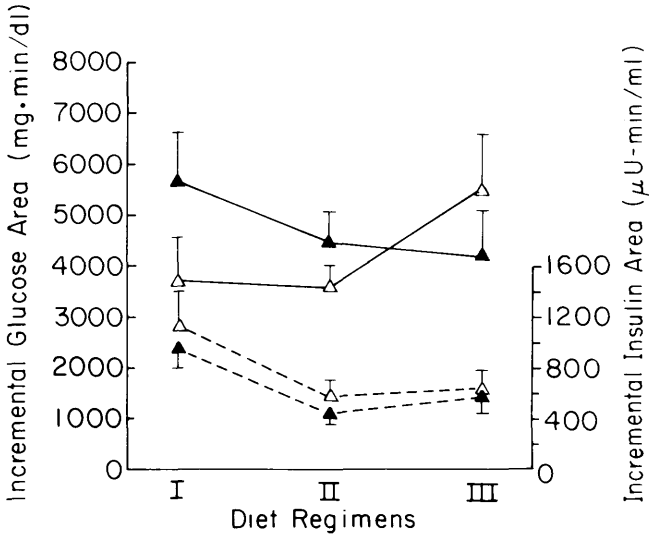

FIG. 2. Incremental glucose (*solid line*) and insulin (*dashed line*) areas (group means ± SE) in normal-K (△) and low-K (▲) pigs subsequent to 3 different diet regimens. Values were not significantly different ($P \geq .05$) between diet regimens.

U/ml), capped, and secured over the shoulders with tape. Three to 4 days later, after a 24-h fast, the pigs were placed in slings to which they had been previously accustomed. A standard oral glucose tolerance test (OGTT) was administered after a 1-h acclimatization/control period, during which three blood samples were obtained from the sampling catheter. A solution of 10% glucose was given via stomach tube (1.5 g glucose/kg body wt), and serial blood samples were drawn at 10, 20, 30, 60, 90, 120, and 180 min. Plasma was frozen for subsequent glucose assay (Beckman autoanalyzer), and serum was frozen for insulin assay (6).

To further highlight the glucose intolerance of the low-K strain, the OGTT was repeated on another day, during which epinephrine was infused [E/OGTT (6)]. The E/OGTT was conducted after the pigs had been returned to their normal diets for 1 day and then fasted for 24 h after the first OGTT. During this procedure, the OGTT was conducted as described above, and epinephrine was infused at the rate of 0.25 $\mu g \cdot m^{-2}$ body surface area $\cdot$ min^{-1} for 5 h. Ascorbic acid (1 mg/ml) was added to the infusate to protect against oxidation. The OGTT was conducted 2 h after the initiation of the epinephrine infusion.

The next day the fast was continued for a total of 48 h, before the initiation of the next study to ensure complete gastric emptying (5). An intravenous insulin tolerance test (IVITT) was then performed with 0.1 U regular insulin/kg body wt (8). This test is a simple measure of insulin sensitivity. Blood samples were withdrawn at 5-min intervals for 30 min and analyzed for plasma glucose. After the IVITT, catheters were removed, and the pigs were returned to their pens.

Glucose tolerance was estimated by calculation of incremental glucose area (the area under the glucose tolerance curve above the fasting plasma glucose) obtained during the OGTT. Glucose-stimulated insulin secretion was estimated by calculation of the incremental insulin area from serum insulin values obtained during the same OGTT. The glucose-disposal constant in response to insulin (K_i) was derived by multiplying the slope of the initial linear portion of the regression line of log n plasma glucose vs. time by 100. This represents the percent per minute fall in plasma glucose. Statistical analyses were performed with analysis of variance (ANOVA).

RESULTS

Despite the fact that the pigs were fed a diabetogenic diet (regimen 2) and allowed to increase their body weight by 50% (regimen 3), we did not observe fasting or glucose-stimulated hyperglycemia in normal-K or low-K animals (see Figs. 1 and 2 and Tables 1 and 2). Results of insulin sensitivity testing as measured by K_i showed no significant difference between normal-K and low-K animals in the baseline state (9.03 ± 0.87%/min for normal-K animals; 8.00 ± 1.15%/min

TABLE 1
Plasma glucose concentrations from oral glucose tolerance tests after three different diet regimens

	Regimen 1 (min)		Regimen 2 (min)		Regimen 3 (min)	
Pigs	0	120	0	120	0	120
Normal K	80.2 ± 3.5*	105.9 ± 3.0‡	92.3 ± 4.9‖	102.9 ± 2.7¶	67.0 ± 1.9*‖**	89.7 ± 3.9¶‡
Low K	89.8 ± 2.1*†	119.2 ± 4.2§	98.2 ± 2.5†**	99.9 ± 5.7§	83.9 ± 1.4**	113.8 ± 8.9‡

Values are in mg/dl and are group means ± SE. Values sharing the same superscript in the same row or the same column are significantly different ($P \leq .05$).

TABLE 2
Mean plasma glucose and serum insulin concentrations of normal and diabetic Yucatan minipigs exposed to oral glucose tolerance testing after three different diet regimens

Group type	Diet regimen	Plasma glucose (mg/dl)					Serum insulin (μU/ml)				
		0 min	30 min	60 min	120 min	180 min	0 min	30 min	60 min	120 min	180 min
Diet regimen	1										
D		89.8 ± 2.1	108.2 ± 2.5	122.1 ± 5.3	119.2 ± 4.2	120.6 ± 8.8	9.4 ± 0.8	17.3 ± 1.8	14.2 ± 2.5	18.4 ± 1.7	15.6 ± 1.7
N		80.2 ± 3.5	107.2 ± 3.2	97.3 ± 5.4	105.9 ± 13.0	96.4 ± 10.1	7.1 ± 1.1	18.9 ± 2.2	14.0 ± 2.3	14.6 ± 3.0	15.1 ± 3.0
Diet regimen	2										
D		98.2 ± 2.5	123.4 ± 6.8	124.8 ± 11	99.9 ± 5.7	116.8 ± 7.0	6.6 ± 0.4	11.7 ± 1.4	11.9 ± 1.1	8.9 ± 1.4	8.3 ± 0.9
N		92.3 ± 4.9	105.6 ± 4.9	99.1 ± 4.5	102.9 ± 2.7	99.6 ± 4.3	6.8 ± 0.6	10.6 ± 1.2	9.2 ± 1.9	12.4 ± 1.7	9.9 ± 0.9
Diet regimen	3										
D		83.9 ± 1.4	116.1 ± 7.9	107.1 ± 7.2	113.8 ± 8.9	108.5 ± 7.5	9.6 ± 0.5	17.7 ± 1.3	14.2 ± 1.9	12.6 ± 1.4	13.2 ± 2.2
N		67.0 ± 1.9	101.9 ± 6.7	92.1 ± 9.7	89.7 ± 3.9	88.6 ± 2.9	10.9 ± 0.7	17.4 ± 1.4	12.9 ± 2.9	13.3 ± 1.8	11.6 ± 1.1

D, pigs genetically selected for diabetes; N, normal pigs. Regimen 1: 4-mo standard swine diet fed for body weight maintenance; 2: 4-mo American Swine Diet fed for body weight maintenance; 3: American Swine Diet fed for obesification.
Data tabulated are group means ± SE.

for low-K animals). No significant change occurred after dietary manipulation. However, as shown in Table 1, low-K pigs tended to show higher fasting and glucose-stimulated glucose values than normal-K pigs. Epinephrine challenge failed to alter responses to OGTT in either group of animals.

DISCUSSION

The pig is usually considered an excellent animal model of human disease. Recently it was reported that a line of Yucatan miniature swine with decreased glucose tolerance had been developed by genetic selection (3,4). As in human non-insulin-dependent diabetes, glucose intolerance in this animal is reported to be directly related to diet (5). In a previous report, a significant increase in plasma glucose concentration (195 mg/dl) at 120 min after an oral glucose challenge was found in low-K swine after 5 mo on the American Swine Diet. A 120-min value of 81 mg/dl was noted when the pigs consumed their standard diet. In contrast, in our study, 2-h plasma glucose values in low-K pigs were significantly lower (100 mg/dl) after 4 mo on the same American Swine Diet compared with values on the standard diet (119 mg/dl).

Our studies included various manipulations known to aggravate glucose intolerance. As in previous studies with this animal, we used a low-fiber, high-sucrose, high-fat diet (American Swine Diet) as a chronic hyperglycemic stimulus. In addition, we allowed animals to obesify by feeding the challenge diet in excessive amounts. We also included a direct glucose challenge alone and in combination with an adrenergic challenge that had previously been shown to produce glucose intolerance in other species (7,9). Surprisingly, we were unable to induce glucose intolerance in these pigs. One difference between this study and the one reported previously is the duration of exposure to the American Swine Diet. Our protocol called for 4 mo versus the 5-mo period used by Panepinto et al. (5). However, an additional 4 mo of the American Swine Diet was fed in amounts that resulted in significant obesification. Therefore, this study design should have allowed a glucose-intolerant genotype to manifest itself phenotypically.

The discrepancy between the results of this study and those previously reported may be accounted for by the breeding program employed subsequent to generation F_5. The heritability estimate reported for generation F_5 was 0.26 ± 0.49 (3), which is low and contains considerable variance. To obtain even this level of heritability, each animal was tested at least twice for glucose intolerance before inclusion into the breeding program. Subsequent generations were simply line bred but still classified as low K, even though untested (R. W. Phillips and L. M. Panepinto, personal communication). Based on the results of our study, any prior genotypic glucose intolerance was lost to breeding, and the F_7 Yucatan swine from this colony appear to be unsuitable as a model for NIDDM.

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