Genetic Selection for Diabetogenic Traits in Yucatan Miniature Swine

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SUMMARY

Yucatan miniature pigs have been selectively bred for reduced and increased glucose clearance during an intravenous glucose tolerance test. Pigs with low glucose clearance rates (low K) have been selected through the F-5 generation. They have a significantly reduced rate of glucose clearance and a blunted peripheral IRI increase in response to the challenge. Through the F-5 generation, heritability of glucose tolerance is estimated at 0.26. Sporadic fasting blood glucoses in excess of 100 mg/dl are seen in later generations of this group. High glucose clearance pigs (high K) have been selected through the F-4 generation. They have significantly increased rates of glucose clearance and a greater increase in peripheral IRI than the low K animals. The estimated heritability for high K through the F-4 generation is 0.31. No evidence of fasting hypo- or hyperglycemia has been seen in the high K animals. DIABETES *28*:1102–1107, December 1979.

Diabetes has been increasingly recognized as one of the serious chronic diseases of man.[1] It is a multifaceted condition with genetic, nutritional, environmental, and viral components. All the varieties of human diabetes are recognized as alterations in the metabolism of glucose (inappropriate hyperglycemia) linked to alterations in insulin synthesis, release, or utilization by the body.

Increased awareness of the extent of diabetes in the human population has intensified diabetes research. A byproduct of this awareness has been enhanced interest in animal models that may simulate or be closely analogous to one or more types of human diabetes. One recent symposium highlighted some of the models currently under study.[2] Most of the readily available natural diabetic models are rodents. Although such animals provide information regarding particular types of inappropriate hyperglycemia, they have short life spans; many do not seem to develop the chronic manifestations of diabetes seen routinely in the human diabetic. Their dietary habits and lipid metabolism also differ greatly from those of man. Their small size may be limiting for studies requiring chronic blood sampling or significant blood volume per sample. Individual *Macaca nigra*, a Celebes ape, have been found to be diabetic. They have longer life spans and develop cardiovascular lesions similar to those seen in man.[3] Unfortunately, these animals are quite limited in number and relatively unavailable for general research.

In 1972, we initiated a program of selective breeding in miniature swine to develop an additional model for studying genetic, metabolic, and cardiovascular lesions associated with diabetes mellitus in humans. This approach was chosen for several reasons:

1. Groups of swine, given glucose tolerance tests, showed a bimodal population distribution.[4,5]
2. Swine stressed with high glucose over a period of time developed reduced glucose tolerance.[6]
3. Swine have many similarities to man with respect to their cardiovascular system.[7]
4. Swine have a tendency toward obesity. Obesity is associated in humans with maturity-onset diabetes and pathology of the cardiovascular system.
5. Swine are omnivorous and will readily consume a "human-type" diet.
6. Swine have a sufficiently long life span that they might develop chronic diabetic pathology. Dogs and primates do eventually develop such pathology. In dogs with induced[8] or spontaneous[9] diabetes, retinopathies did not occur until the animal had been diabetic for over 4 yr. Spontaneously diabetic Celebes apes do not have obvious vascular lesions until late in the disease. Capillary basement membrane thickening has been demonstrated in these animals.[10] On the basis of the clinical onset of vascular lesions resulting from chronic diabetes in man, dog, and Celebes apes,

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it seems unlikely to anticipate "human-type" vascular lesions to appear rapidly in other diabetic models.

7. There are well-established familial, genetic, and genetic-dietary predispositions toward diabetes in man and in existing experimental animal models of this disease. These include the Chinese hamster, obese mouse, spiny mouse, sand rat, and dog. Logically, swine will also have this tendency.

8. Miniature swine have a mature body weight that is quite similar to that of man.

9. Compared with primates, swine have a faster genetic turnover with sexual maturity at 4–6 mo, a large number of offspring, and a lower per unit production and maintenance cost.

10. Since the inception of our program, swine have been shown to develop a diabetes-like syndrome during pregnancy.[11] This may be analogous to human diabetic pregnancy, a condition often linked to poor carbohydrate-lipid control and often a precursor to diabetes.[12]

The data reported in this paper represent a summary of the progress made to date toward development of a genetically selected diabetic miniature swine. Preliminary information has been presented elsewhere.[13,14]

METHODS AND MATERIALS

Testing. Initial screening of potentially diabetic miniature swine was based on an intravenous glucose tolerance test (IVGTT). The tests were conducted after a fasting period of 24, 48, or 72 h. Selection was eventually based on results gained after a 48-h fast. It was determined, through necropsy, that the high grain ration fed to these pigs is slowly digested and that gastric emptying and the establishment of a true fasting condition requires 48 h. Before testing, the pigs were restrained in a canvas sling and a time-0 blood sample was removed by venipuncture or catheterization of the anterior vena cava. Fifty percent glucose at 0.5 g/kg body wt was infused through a pediatric catheter (Argyle Venocut infusion set, Sherwood Medical Industries, St. Louis, Missouri) into an ear vein; the infusion required 30–60 s. Serial blood samples were obtained at various intervals from the anterior vena cava. Blood samples were immediately centrifuged (Microfuge model 152A, Beckman Instruments, Fullerton, California) and the plasma was removed and frozen for subsequent analysis. Glucose disappearance from the blood after pulse injection follows first order kinetics; therefore, when the data are plotted on semilogarithmic graph paper, it is linear. The rate of disappearance may be mathematically described by $Y_t = Y_0 e^{-kt}$, where Y_t is the glucose concentration at any time t, Y_0 is the glucose concentration at time 0, and k is the rate constant. The dimensions of k are reciprocal time (1/t). By multiplying k by 100, K is obtained. K represents the percentage of glucose that disappears per unit of time, in this case, %/min. Glucose was measured using a glucose oxidase method and a glucose analyzer (Analyzer model ERA = 2001, Beckman Instruments, Fullerton, California). One modification was made that greatly decreased the cost of analysis.[15]

More recently, insulin has been assayed on an aliquot of the blood samples using radioimmunoassay, utilizing a preincubated double antibody system purchased from CIS Radiopharmaceutical, Inc. (Bedford, California). The procedure employed was adopted from Hales and Randle.[16] The method is as follows: 0.1 ml of standard or sample is combined with preincubated antibody complex and incubated for 6 h at 4°C. Following the initial incubation, 0.1 ml of ^{125}I-labeled insulin is added to the reaction mixture and the incubation is continued at 4°C for an additional 16 h. After buffer dilution and centrifuge sedimentation, the microprecipitate is counted on a Beckman Biogamma II and the results analyzed via the method set forth by Duddleson et al.[17]

The parent stock from which our colony of miniature pigs originated was imported to the United States from the Yucatan region of Mexico in 1960.[7] They appear to be indigenous to much of Southern Mexico and Central America. Their physical characteristics have been described;[18] they are tractable by nature and accommodate easily to testing procedures.

The colony is maintained at Colorado State University in indoor and outdoor facilities. The animals are fed a standard swine ration,[18] weaned at approximately 6 wk of age, and initially tested for glucose tolerance between 4 and 6 mo of age.

In the initial field selection of pigs to serve as parent stock, no information was available regarding genetic relationships between animals. Those with comparatively "poor" glucose tolerance were designated for the low K line. Pigs chosen to serve as parents were selected over the summers of 1972 and 1973. Subsequent breeding of selected animals has been conducted by tandem generation matings. For instance, both F-2 and F-3 animals are bred in a single mating season to produce F-3 and F-4 offspring. This practice tends to decrease variability among the pigs in several ways. First, environmental factors that vary from year to year, such as climate, are, in effect, minimized. Observed differences between two generations produced concurrently may be described as essentially genetic and most likely heritable. Second, under this tandem system, repeat breedings of effective matings may be easily accomplished.

As the second year of the project ended, a decision was made to change the selection of the control line from that of animals with an average K value to selection for animals with high K values. It was decided at this time that there was potential value in producing as great a diversity between the lines as possible. It is also conceivable that selection for rapid glucose utilization could result in a hypoglycemic strain; hypoglycemia is a well-established medical problem of the human population.[19] It has been determined, in subsequent matings, that an "average K" animal may be produced easily as a first-generation offspring of a high and low K cross.

RESULTS

Each animal was tested for glucose tolerance a minimum of 2 times before selection for retention in the colony and for the breeding program. Figure 1 presents data on animals that underwent repeat tests between 4 and 12 mo of age. A correlation was made between K values obtained from successive IVGTT in a number of animals, where the two tests were conducted within 1 mo. The repeatability is generally greater at the lower K value, as illustrated in Figure 1.

Progeny through the F-5 generation have been tested in the low K line. Table 1 presents, by generation, the mean K value, the range of K in selected animals, the percentage of

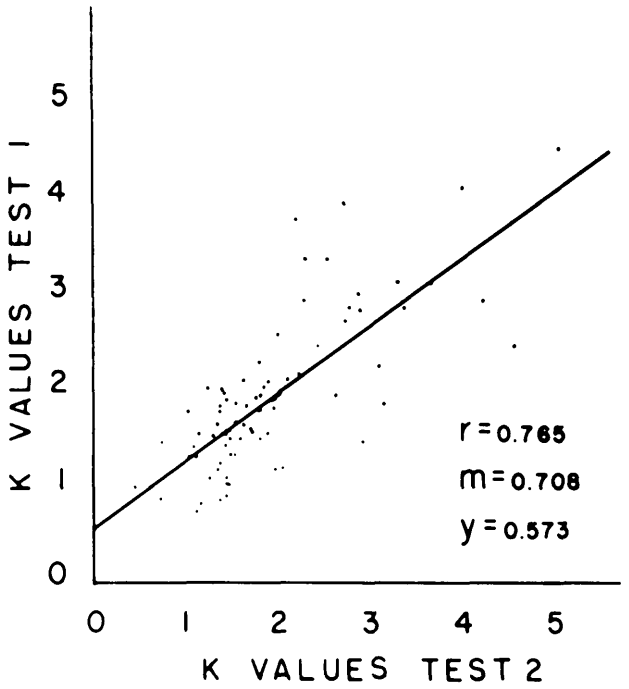


FIGURE 1. A comparison of the repeatability of two intravenous glucose tolerance tests conducted on a number of animals within a 4-wk period is shown. K is the percent glucose that disappears from the blood per minute. K is more repeatable at lower values.

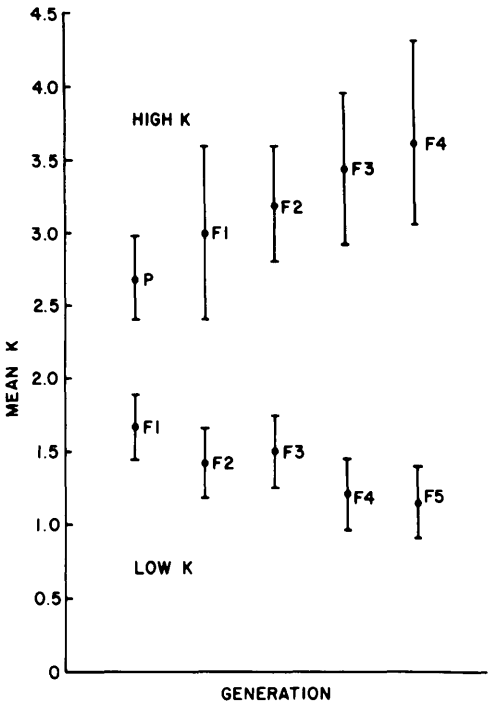


FIGURE 2. Changes in mean K of selected pigs by generation and line. The low and high K animals were highly significantly different at the time of animal selection as to serve as high K parents. The two lines have continued to diverge by generation.

tested animals that were selected, and the 48-h fasting blood glucose values for the low K animals. There has been a general increase in the percentage of tested animals selected as breeding stock, and the upper limit of K that is accepted has decreased. The increase in fasting blood glucose seen in the F-5 generation is due to several individual animals with values greater than 100 mg/dl. The range of fasting blood glucose in the selected F-1 was 57–99 mg/dl, while the range of those selected in the F-5 was 61–123 mg/dl.

Progeny through the F-4 generation have been tested in the high K line. It must be appreciated that the parent stock for the high K line are those F-1 animals from the low K line that had the most rapid rate of glucose utilization, as there was no initial selection for high K, that is, the extremes of the F-1 low K line were used. The eight lowest K animals produced F-2 low K, and the eight highest K animals were designated as parents for the high K line. This can be seen in Tables 1 and 2. Data are presented on the generational changes seen in mean K values, the range of K in tested animals that were selected, and the 48-h fasting blood glucose values for the selected animals (Table 2). Again, as in the low K line, there has been an increase in percent selected and an upward shift of the values used for selection criteria. At this time, there is no evidence of swine developing hypoglycemia. A comparison of the data on changing K values between the low and high K lines by generation is presented in Figure 2. The two lines had significantly different K values, $P < 0.001$, when compared at the initiation of the high K line, and have shown increasing divergence since that time.

The mean K of the selected animals has decreased from 1.67 ± 0.25 in the F-1 generation to 1.14 ± 0.25 in the F-5 generation ($P < 0.001$). The percentage of animals falling within the selected range was significantly increased, so that over 34.0% of the tested animals were retained in the

TABLE 1
Low K line, percent selected by generation, and range (mean ± SD) of selected breeding stock

Generation	Number tested (N)	Percent selected (%)	FBG of selected animals*	$\bar{K}$ of selected animals	Range (K) of selected animals
F-1	67	11.9	79 ± 14	1.67 ± 0.25	1.22–2.05
F-2	110	20.0	83 ± 15	1.43 ± 0.26†	1.00–2.08
F-3	69	21.7	82 ± 12	1.51 ± 0.28	1.12–2.29
F-4	38	44.7	81 ± 9	1.20 ± 0.25‡	0.73–1.53
F-5	53	26.4	94 ± 15†	1.14 ± 0.25‡	0.61–1.53

* Fasting blood glucose.
† Different from F-1 ($P < 0.05$) and F-4 ($P < 0.02$).
‡ Different from F-2 and F-3 ($P < 0.01$) and F-1 ($P < 0.001$).

TABLE 2
High K line, percent selected by generation, and range (mean ± SD) of selected breeding stock

Generation	Number tested (N)	Percent selected (%)	FBG of selected animals*	$\bar{K}$ of selected animals	Range (K) of selected animals
Parent	67	11.9	79 ± 9	2.69 ± 0.31	2.10–3.00
F-1	49	16.0	79 ± 8	3.04 ± 0.74	2.50–4.74
F-2	46	35.0	82 ± 10	3.20 ± 0.51†	2.59–4.14
F-3	52	34.6	83 ± 10	3.43 ± 0.52†	2.80–4.58
F-4	20	70.0	81 ± 10	3.67 ± 0.63‡	2.64–4.98

* Fasting blood glucose.
† Significantly different from parent ($P < 0.05$).
‡ Significantly different from parent ($P < 0.001$).

F-4 and F-5 generations compared with 11.9% in the F-1 generation. The percentage selected would have been even greater if the initial selection criteria were still in use. We have imposed more intensive selection pressure in the later generations. For instance, in the low K F-5 generation, no animals were retained with a K greater than 1.53, while the highest low K pig selected in the F-1 generation had a K of 2.05 (Table 1). In the high K line, the change in the mean K value of the selected animals was highly significant from 2.69 ± 0.31 in the F-1 to 3.67 ± 0.63 in the F-4 generation, and the percentage of animals that were selected increased from 16% to 70.0%, although only 20 F-4 animals have been tested at this time.

Heritability estimates for reduced and increased glucose tolerance have been calculated. Heritability is that fraction of the phenotypic variation that is caused by genetic effects. It is a prediction of anticipated phenotypic expression of certain traits, based on past selection pressures. In reference to this study, heritability represents an estimate of the proportion of offspring that can be expected to exhibit impaired (low K) or enhanced (high K) glucose tolerance, as illustrated in Tables 3 and 4. Heritability for increased tolerance, or high K, is 0.31 ± 0.18, while that for low K is 0.26 ± 0.49. Both values indicate that the rate of glucose disappearance from the blood is a genetically transmittable trait. Based on evaluation by line, selection for low K and high K are not significant regressions, principally due to genetic progression through only five generations in each line, as shown in Tables 3 and 4. If the data from Figure 2 and Tables 3 and 4 are combined and considered not as heritability per se, but as the influence of genetic selection on glucose utilization, then a combined mathematical analysis can be made. This is presented in Figure 3 as a regression line showing the generation effect of selection for glucose tolerance in a reverse chronology for high K and a normal progression for low K. The "generation effect" using the heritability formula (Table 3) is 0.26 ± 0.06.[27] The correlation coefficient of the derived line through the nine generations is 0.97. No data are included for the low K parent stock, as the conditions under which they were tested and selected were not comparable with the tests used for subsequent selection.

Insulin data were not collected during the early years of the experiment; however, it is available on all selected progeny born in 1977, including both F-4 and F-5 low K and F-2 and F-3 high K animals. All insulin values have been obtained from blood samples taken before and after a glucose challenge and simultaneously with blood glucose sampling. Figure 4 presents the insulin response following an IVGTT on 11 animals with a mean K of 1.65 ± 0.61 (SD) compared with 10 animals with a mean K of 3.75 ± 0.61. The low K animals presented in this graph had a severely inhibited insulin secretory response to the glucose challenge. They also had a significantly decreased fasting serum insulin concentration before infusion of glucose (time-0 sample). All the subsequent samples through the 30-min period were also significantly lower in serum insulin in the low K group. Figure 5 is a plot of K value versus the net peak insulin value per pig. The correlation of the derived regression line was 0.72, indicating that the rate of glucose disappearance is associated with an increased peripheral serum insulin concentration.

TABLE 3
Estimated heritability (h^2) of reduced tolerance to glucose (low K)

Generation	Number tested (N)	K tested (Y)	Number selected	$\bar{K}$ selected	Selection differential*	Cumulative selection differential (X)
F-1	67	2.18	8	1.67	—	—
F-2	110	2.08	14	1.41	0.51	0.51
F-3	69	1.98	14	1.51	0.67	1.18
F-4	38	1.58	17	1.20	0.47	1.64
F-5	53	1.77	14	1.14	0.38	2.03

Heritability (h^2) is estimated by the formula: $h^2 = [\Sigma XY - (\Sigma X \Sigma Y/N)]/\{\Sigma X^2 \cdot [(\Sigma X)^2/N]\}$; $h^2 = 0.26 \pm 0.49$.[27]
* Selection differential represents the difference between the mean K of all animals tested and the mean of those selected within a particular generation. It is also known as genetic "reach."

TABLE 4
Estimated heritability (h^2) of increased tolerance to glucose (high K)

Generation	Number tested (N)	$\bar{K}$ tested (Y)	Number selected	$\bar{K}$ selected	Selection differential*	Cumulative selection differential (X)
Parent	67	2.18	8	2.69	—	—
F-1	49	2.23	8	3.04	0.51	0.51
F-2	46	2.57	16	3.20	0.81	1.32
F-3	52	2.63	18	3.43	0.63	1.95
F-4	20	2.96	14	3.67	0.80	2.75

Heritability is estimated by the formula: $h^2 = [\Sigma XY - (\Sigma X \Sigma Y/N)]/\{\Sigma X^2 \cdot [(\Sigma X)^2/N]\}$; $h^2 = 0.31 \pm 0.18$.[27]
* Selection differential represents the difference between the mean K of all animals tested and the mean of those selected within a particular generation. It is also known as genetic "reach."

DISCUSSION

The research described herein has been designed to develop a reproducible, genetically induced miniature swine model for studying chronic complications of diabetes mellitus, a model that would be analogous to one or more types of human diabetes. The genetic selection program has progressed through five generations thus far in selecting toward glucose intolerance in Yucatan miniature pigs.

The increase in the percentage of animals falling within our selectable range, in both the low and high K lines, indicates that glucose clearance rate has a strong genetic component—a finding that has been previously demonstrated in rats.[20] Evidence of a genetic basis is further strengthened by the progressive changes in glucose tolerance in successive generations with increased selection pressure. This is true even when different generations are bred, farrowed, and tested under the same environmental circumstances during the same period of time. The individual line data, separately examined, are substantive. However, when combined and presented as the generation effect on glucose tolerance in Figure 3, it is convincing. Glucose tolerance is a heritable trait that can be selected for or selected against. Selection, to date, has resulted in a change of 0.265 K units/generation. It is unlikely that the low K line will continue to decrease at this rate. It would seem much more likely that the value will become asymptotic, and, in fact, it appears that this has already begun to occur in spite of the linearity of the regression line in Figure 3. The high K line, conversely, could theoretically continue to increase for several generations before reaching a maximum rate of glucose utilization. The mean K of 1.14 in the F-5 generation is considerably less than the mean K of 1.6 reported for human chemical diabetics by Jackson and his co-workers. All overt diabetics, and chemical diabetics that became overt, had K values less than 1.1. Insulin response to intravenous glucose was decreased in these chemical diabetics.[21]

The impaired insulin response seen in our line of glucose-intolerant pigs may be analogous to impaired insulin release reported in human diabetics.[22–24] In contrast, there was a more normal insulin response following a glucose challenge in our selected high K or control pigs. According to Kosaka and co-workers, "decreased insulin response to

FIGURE 3. A plot of the mean K value of the 10 groups of selected animals. It represents the "generation effect" of selection for low and high glucose utilization. The original parent stock is not included, as the testing techniques are not comparable.

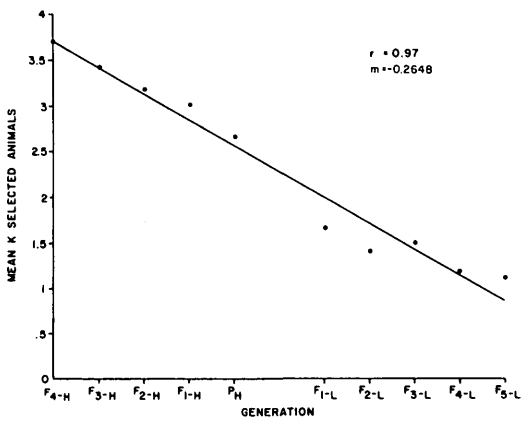
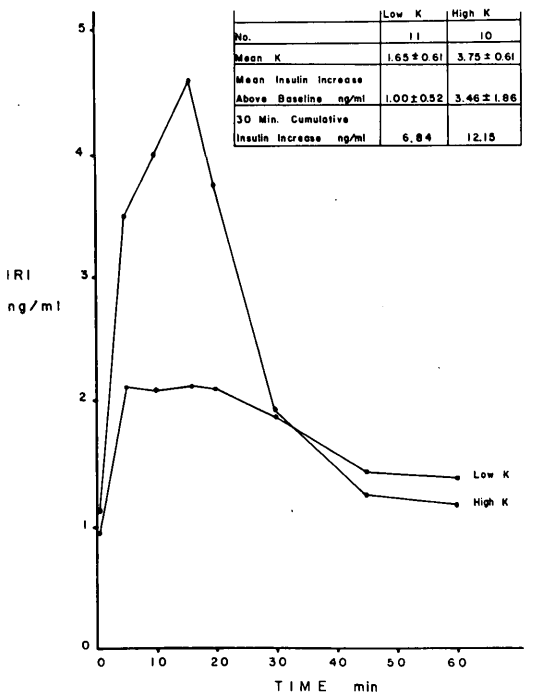


FIGURE 4. Changes in plasma immunoreactive insulin (IRI) during an intravenous glucose tolerance test on 11 low K and 10 high K animals. The low K animals have a blunted response to the glucose challenge with a significantly lower insulin increase and a lesser cumulative insulin increase over the first 30 min.



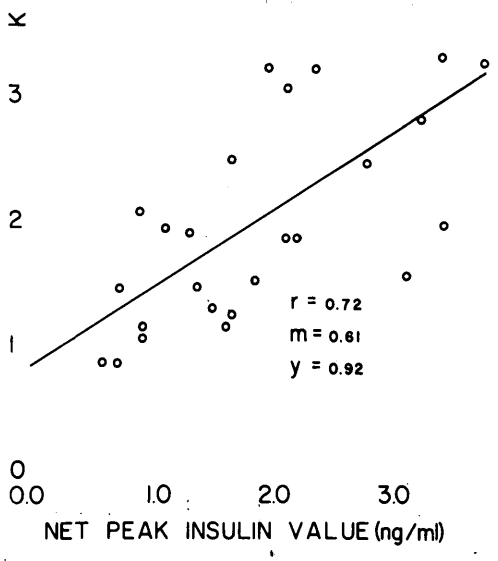


FIGURE 5. A comparison of the peak net IRI increase following an intravenous glucose tolerance test plotted against the K value obtained during that same test. This experiment used F-4 and F-5 low K and F-2 and F-3 high K pigs born in 1977.

glucose is a more inherent, stable, and specific characteristic of true diabetes than the impairment of glucose tolerance."[24]

Almost all genetic diabetes models currently in use are the result of the spontaneous occurrence of hyperglycemia in laboratory animals. Considerable research has been reported on the genetic basis of spontaneous hyperglycemia seen in animal models.[25] One exception to this spontaneity is the work of Goto et al., who produced spontaneously diabetic rats through a selective breeding program.[20] By the F-10 generation, the rats had a fasting blood glucose of 114 mg/dl and seriously impaired insulin response to an oral glucose load (Kakizaki, personal communication, 1977).

In our selected line of glucose-intolerant Yucatan swine, hyperglycemia has not been a common occurrence, although several animals in the latest generation, in the low K line, have had hyperglycemia following our standard 48-h fast. This would appear analogous to the occasional hyperglycemia reported in latent or chemical diabetics.[26]

The results of genetic selection for glucose intolerance in Yucatan miniature swine indicate the heritability of diabetogenic traits in these animals. The actual nature of the metabolic defect or pathology in these pigs, and its relevance to human diabetic conditions, is yet to be determined. The decrease in insulin concentration in the peripheral circulation, in response to an intravenous glucose challenge, indicates that the decreased tolerance is analogous to common findings in some humans with either overt or latent diabetes. It remains to be seen whether continued selection will provide overt diabetes in these animals.

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