Influence of Genetics and Diet on the Development of Diabetes in Yucatan Miniature Swine[1]

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ABSTRACT The intent of this study was to examine the effect of a diet nutritionally similar to that consumed by most Americans on Yucatan miniature swine that have been bred for enhanced and impaired glucose tolerance. Seventeen Yucatan miniature pigs from the glucose-intolerant line (low *K*) and 17 from the glucose-tolerant line (high *K*) were selected. The oral glucose tolerance (OGT) of both groups of pigs was determined. After the OGT test (OGTT), 13 low *K* pigs and 12 high *K* pigs were placed on the American Swine Diet (ASD). The remaining animals were left on the regular swine diet. After 5 months, the pigs were again tested with an OGTT. Mean plasma glucose levels observed during the OGTT showed significant differences between the low *K* and high *K* groups on ASD at all time intervals from 60 to 150 minutes postdosing. Serum insulin levels in the high *K* group were significantly elevated over those in the low *K* group during the OGTT at 10, 20, and 30 minutes prediet, and at 10 minutes after 5 months on diet. Both low and high *K* groups showed a dietary effect with an elevated plasma glucose response to an OGTT, although the mean increase was not significant in the high *K* group. In the low *K* group, plasma glucose levels were significantly elevated ($P < 0.05$) over prediet values at 20, 30, 60, 90 and 120 minutes. In addition, 7 out of the 10 low *K* pigs tested at 5 months developed insulin resistance, and noninsulin-dependent diabetes. J. Nutr. *112:* 2307–2313, 1982.

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The impact of dietary composition on the clinical expression of diabetes mellitus or impaired glucose tolerance has been assessed in humans as well as other mammalian species (1–4). More specifically, manipulation of the carbohydrate, fiber and fat components causes alterations in circulating levels of both glucose and insulin in normal and in diabetic subjects (5, 6). Burkitt et al. (7) and Trowell et al. (8) have suggested that fiber depleted diets play a causative role in the development of diabetes mellitus. A number of other investigators have demonstrated that fiber-enriched diets lower plasma glucose levels postprandially and during an oral glucose tolerance test (OGTT) (9–13).

Cohen (14) states that not only is the type of carbohydrate a critical factor in the expression of diabetes, but also the genetic make-up of the recipient. He found that chronic feeding of high sucrose, fructose, or glucose diets to a selected population of rats with a genetic predisposition for diabetes resulted in development of diabetes and diabetic angiopathy. Siblings of that population fed a high starch diet did not develop hyperglycemia or other symptoms of diabetes.

The diabetogenic effects of high fat diets have also been investigated. Blazquez and Lopez-Quijada (15) found that rats fed a high fat diet had decreased circulating levels of plasma insulin and increased plasma glucose.

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Zaragoza and Felber (16) also noted an impairment of glucose metabolism in rats fed a high fat diet.

The intent of this study was to examine the effect of a diet called the American Swine Diet (ASD), which is nutritionally similar to that consumed by most Americans on Yucatan miniature swine (17), which have been bred for enhanced and impaired glucose tolerance (18). The diet is a low fiber diet with a higher sucrose and fat content and a significantly lower fiber content than our standard swine diet. It is not, however, as high in either sucrose or fat as some stress diets used for rodent studies (14, 15).

MATERIALS AND METHODS

For all experimental procedures the pigs were restrained in canvas slings (17) and the anterior vena cava catheterized for sample withdrawal and glucose infusion. All catheterization procedures and tests were conducted on fully conscious, untranquilized animals previously accustomed to sling restraint, which is routine procedure in this laboratory.

Seventeen Yucatan miniature pigs from the glucose-intolerant line (low K)[2] and seventeen from the glucose-tolerant line (high K) were selected for the study. The mean K values (glucose clearance rates) during an intravenous glucose tolerance test (IVGTT), prior to initiating the diet study, were 1.09 ± 0.29 and 3.48 ± 0.46, respectively. Blood samples are taken at 10, 15, 20, 25, 30, 45 and 60 minutes following an intravenous pulse injection of 0.25 g/kg glucose.

An oral glucose tolerance test (OGTT) was also administered to all animals in the fasted state. After catheterization and withdrawal of a control blood sample, 1.5 g glucose per kilogram was mixed in warm tap water to make a 10% solution and administered via stomach tube. Serial blood samples were withdrawn at 10, 20, 30, 60, 90, 120 and 150 minutes postdosing.

The same procedure was used on all blood samples collected during both the IVGTT and OGTT. A portion was placed in a heparinized tube, immediately centrifuged, and the plasma removed and frozen. The remaining whole blood was allowed to clot, and the serum was separated and frozen for insulin analysis. Plasma glucose concentration was measured by glucose oxidase, and serum insulin concentration by radioimmunoassay as previously reported (18).

Following collection of base-line data, the pigs were fed the ASD (table 1) or continued on regular diet (table 2) for 5 months and tested again. The ASD nutritional composition is based on data reported by the 1965 USDA survey of food consumption by Americans (19).

Crude fiber (CF) of the regular swine diet has been determined at 8.2% compared to 1.9% CF in the ASD. The primary source of carbohydrate in the regular diet is cracked corn (starch) as 64.8% of the total, whereas the ASD contains only 8.7% corn with 29% sucrose as the primary carbohydrate source.

Prior to receiving the low fiber diet the pigs were fed the standard swine diet shown in table 2 at approximately 2% body weight daily. When placed on the low fiber diet, the pigs were fed a quantity (approx. 454 g/pig) adequate for normal growth and maintenance twice daily at approximately 0900 and 1500 hours.

All animals were housed in a heated, ventilated, indoor facility designed for swine with thermostatically heated flooring, standard steel slats and a pit-flush system for waste removal. They were grouped according to sex, but randomly mixed by genetic line and age so as to minimize environmental influences. All animals were sexually mature at the onset of the study ranging from 8 to 24 months of age.

Statistical analysis was conducted utilizing the multivariate analysis of variance (MANOVA) procedures of the statistical packages for the social sciences (SPSS) program. Analysis was directed to detect significant interactions by genetically selected group (selection K values, low, high), time on diet (control, 5 months), and the combination of these two at each sample time. Significant interaction ($P < 0.05$) was further analyzed by calculation of Tukey's honestly significant difference[3] (HSD) at the ($P < 0.05$) confidence level.

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[2] K value is the slope of the natural log of the plasma glucose values versus time multiplied by 100.

[3] $\text{HSD}_{0.05} = Q_{k,v}$ EMS/n where Q, critical values of the studentized range; k, number of means being compared; v, degrees of freedom for EMS (MANOVA); EMS, error mean squared; n, number of cases/mean.

RESULTS

All pigs readily consumed the ASD. Plasma glucose and serum insulin response to the OGTT conducted prediet and after 5 months on diet are presented in figures 1A, 1B (low *K* pigs); figures 1C, 1D (high *K* pigs). Eleven of the 12 low *K* pigs on ASD are reported in the control data and 9 at 5 months on the diet. Four of the original 17 low *K* pigs were maintained on regular diet. Data from OGTTs conducted on that group are shown in figures 2A and 2B. In the high *K* group 8 of the pigs on ASD are reported in the control data and 7 at 5 months on diet. Four from the high *K* group were maintained on regular diet (figures 2C and 2D). The animals not included in the control data were excluded due to an inadequate number of good blood samples or sample assay problems. Those excluded from the 5-month test were in late gestation.

Significant differences in plasma glucose response were observed in the pigs being fed the ASD for 5 months between the low *K* and high *K* groups at all time intervals during an OGTT from 60 to 150 minutes. At these time intervals the low *K* animals mean plasma glucose was 40, 53, 64 and 64 mg/dl higher than the mean glucose concentration seen in the high *K* group.

Serum insulin levels in the high *K* group were significantly elevated over those seen in the low *K* group at the following time intervals: 10, 20, and 30 minutes prediet, and at 10 minutes after 5 months on ASD. No significant differences were observed between the groups on regular diet at either period of time.

Both low and high *K* groups on ASD showed a dietary effect with an elevated plasma glucose response to an OGTT, although the mean increases were (multiple time points) not significant in the high *K* group. In the low *K* group, plasma glucose levels were significantly elevated over prediet values at 20, 30, 60, 90, and 120 minutes.

The serum insulin response in the low *K* group after diet showed a significant elevation when compared to prediet values at 30 and 60 minutes of the OGTT.

Six of the 9 low *K* animals on the ASD, had diagnostic signs of diabetes based on human standards of classification (20). They had plasma glucose levels in excess of 200 mg/100 ml during a minimum of two time periods during the first 2 hours of the OGTT. The summed areas of the glucose and insulin curve during an OGTT are shown in figure 3. None of these animals would have been classed as diabetic prior to diet modification. No differences between males and females of either line were observed.

TABLE 1

American Swine Diet[1]

Ingredients	Amount (% of diet)
Sucrose	28.7
Corn (fine chop)	8.7
44% soybean oil meal	40.0
Lard	19.0
Soy oil	1.0
Cholesterol	0.1
Vitamin-mineral supplement[2]	2.5

[1] Crude fiber 1.89%. [2] Nutrena, super swine premix (Cargill, Nutrena Feed Division, Minneapolis, MN) guaranteed analysis: (by percent): Ca, 21.0–25.0; P, 8.0; NaCl, 13.5–16.0; I, 0.0088; Fe, 0.6; Zn, 0.4; Cu, 0.1; Mn, 0.3; (per pound): vitamin A, 125,000 IU; cholecalciferol, 25,000 IU; vitamin E, 225 IU; riboflavin, 66 mg; *d*-pantothenic acid, 220 mg; niacin, 660 mg; vitamin B-12, 0.5 mg; vitamin K, 111 mg; and *l*-lysine, 0.784%.

TABLE 2

Regular swine diet[1]

Ingredients	Amount (% of diet)
Corn (fine chop)	64.9
Dehydrated alfalfa meal	14.9
Soybean oil meal	12.5
Wheat bran	4.9
Vitamin-mineral supplement[2]	2.8

[1] Crude fiber 8.2%. [2] Nutrena, super swine premix guaranteed analysis: see table 1, footnote 2.

DISCUSSION

After 5 months of ASD, the animals from the glucose-intolerant line had a prolonged elevation in plasma glucose concentration in spite of an increased peripheral insulin response. These changes shown in figure 1 are similar to changes seen in human maturity onset diabetes with the apparent develop-

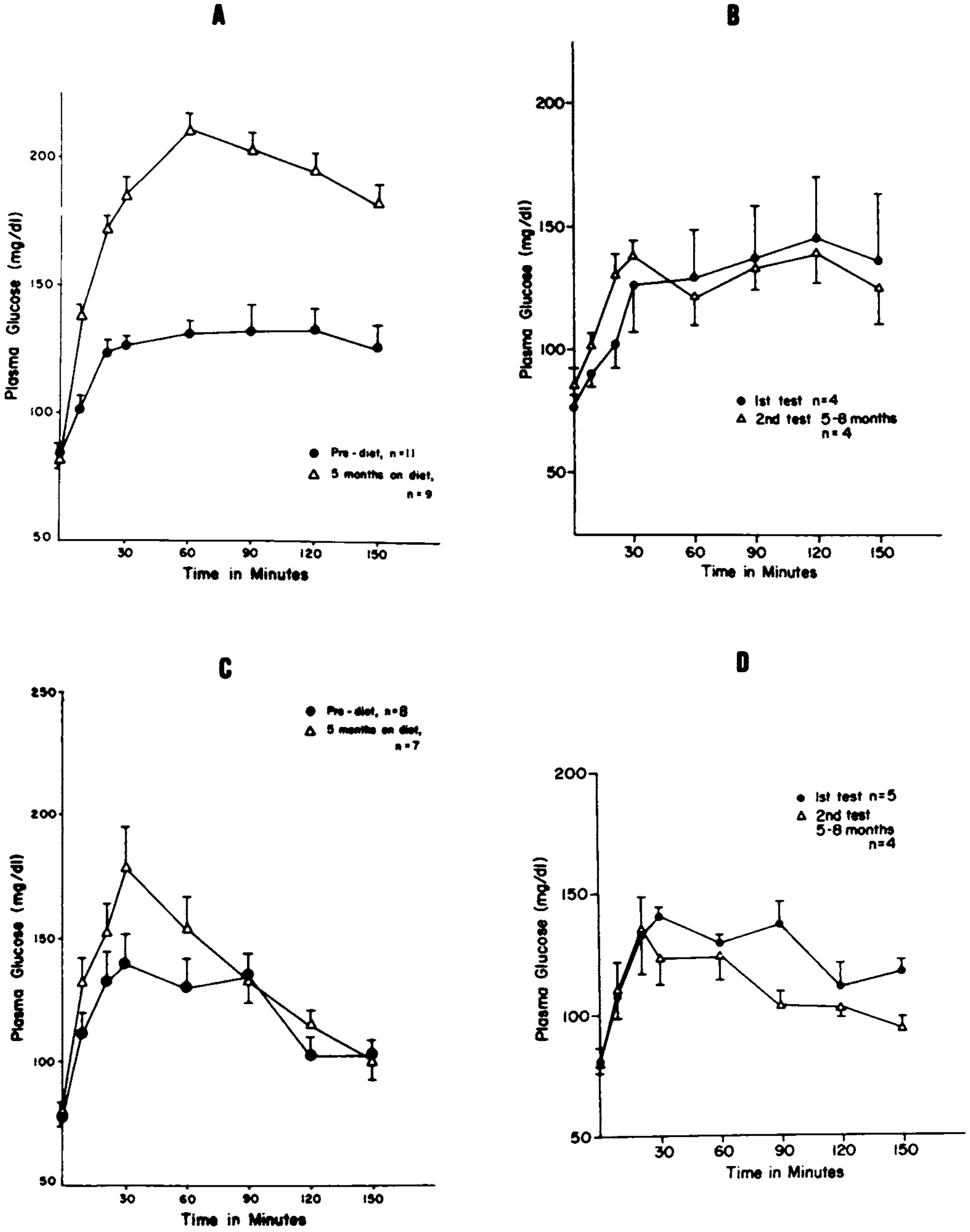

Fig. 1 Plasma glucose responses to oral glucose tolerance tests (OGTT) conducted at the beginning of the study and after 5 to 8 months of feeding the American Swine Diet (ASD) or a regular swine diet. 1*A* Glucose-intolerant (low *K*) pigs fed ASD. 1*B* Low *K* pigs maintained on regular swine diet. 1*C* Glucose-tolerant (high *K*) pigs fed ASD. 1*D* High *K* pigs maintained on regular swine diet.

ment of peripheral insulin resistance. The low fiber diet produced some elevation in plasma glucose levels after administration of glucose during an OGTT in high *K* pigs as well. Although the changes were not statistically significant in the insulin response to an OGTT in that group after 5 months on ASD, the high *K* pigs did have a more rapid

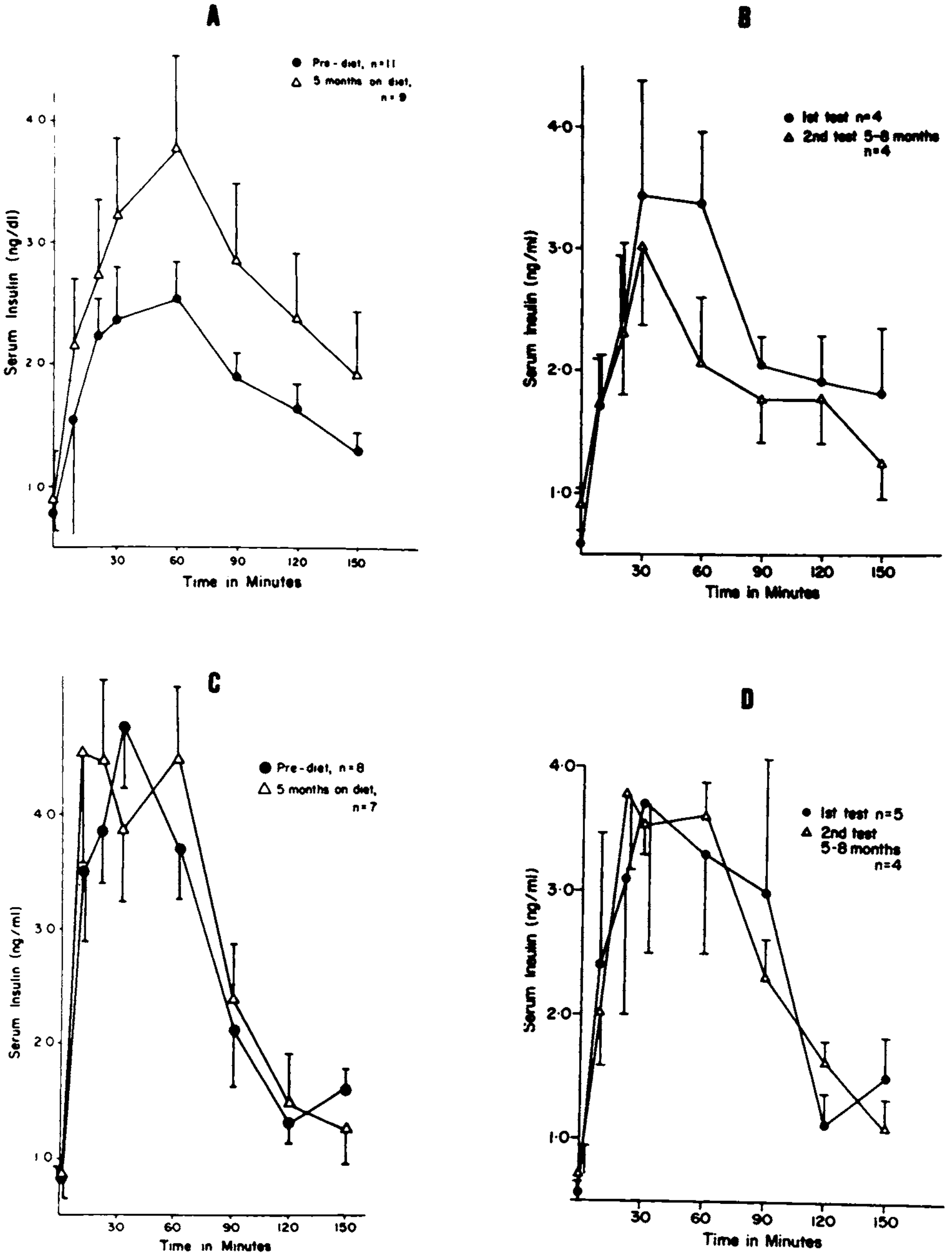

Fig. 2 Serum insulin responses to OGTTs conducted at the beginning of the study and after 5 to 8 months of feeding the ASD or a regular swine diet. *2A* Glucose-intolerant (low *K*) pigs fed ASD. *2B* Low *K* pigs maintained on regular swine diet. *2C* Glucose-tolerant (high *K*) pigs fed ASD. *2D* High *K* pigs maintained on regular swine diet.

increase in insulin after oral glucose administration. There were no significant changes seen in any of the pigs maintained on the regular swine diet. This observation further supports the dietary effect of the ASD, as all the animals were of a comparable age. No age-related changes were observed in this study.

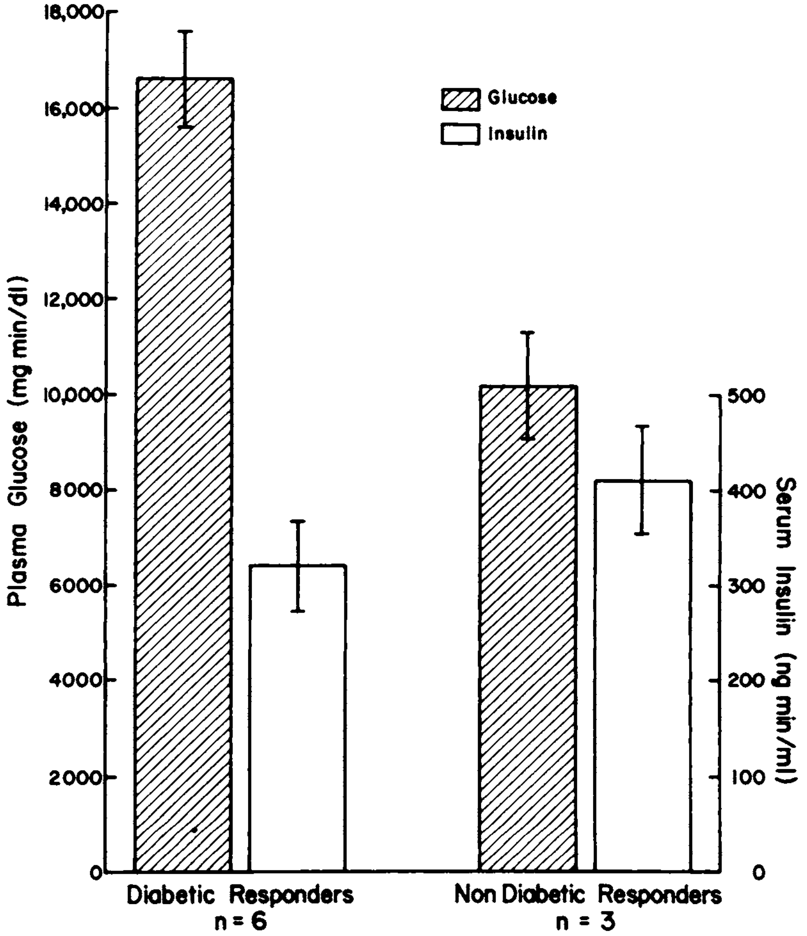

Fig. 3 Six low *K* pigs exhibiting diagnostic signs of diabetes mellitus. Summed areas of the glucose and insulin curves during an OGTT.

Many non-insulin dependent human diabetics maintained on a high fiber diet do not exhibit clinical signs of diabetes and often remain at normoglycemia without other therapy (5, 21–23). In a 1978 clinical trial Anderson and Ward (12) subjected 10 diabetic patients to a high carbohydrate, high fiber diet. After an average of 15 months on the diet, 7 of the 10 patients were managed without insulin or sulfonylureas.

The heritability of glucose intolerance as well as the heritable nature of increased tolerance to glucose in Yucatan swine has been reported by Phillips et al. (18).

Cohen as well as Laube et al. (6, 14) have suggested that high levels of sucrose in the diet (at least 50%) are required before diabetogenic effects occur in rats. Earlier work from this laboratory (24) has shown that replacing starch with up to 33.7% sucrose in the diet does not significantly alter glucose tolerance in Yucatan pigs. The alteration of fiber and fat content are thereby implicated as more significant factors influencing the changes that occurred.

The diabetogenic effects of the ASD seen in the low *K* pigs are similar to changes seen in some humans (13). That is the ingestion of low fiber, higher fat foods is diabetogenic in susceptible individuals. It may result in a modification of insulin sensitivity in all, but only cause clinical signs in the genetically predisposed.

The reasons for decreased insulin response to an oral glucose load when patients are maintained on fiber-enriched diets are not clear. One theory proposed by Hall et al. (26) is that fiber increases portal blood flow, thereby decreasing peripheral serum insulin concentration because of increased hepatic extraction. This could be interrelated with other factors such as fiber-induced modification of the secretion rates of gut hormones (11), which also might affect subsequent handling of insulin in the body. Munoz et al. (11) have suggested that dietary fiber may reduce the postprandial blood sugar increase due to slowed or decreased intestinal absorption, as well as an enhanced insulin release and/or peripheral insulin activity.

Anderson et al. (5) have proposed that decreased postprandial plasma glucose values in patients on high fiber foods may be due to increased tissue sensitivity to insulin. After 5 months on the low fiber diet, the enhanced insulin secretion with the decrease in glucose clearance is indicative of increased insulin resistance in the low *K* pigs. The mechanism of increased resistance and its relationship to a lack of dietary fiber is not yet understood (25) but seems to be a significant factor in the development of diabetes in many humans (26) and in swine bred for impaired glucose tolerance.

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