Diabetologia 3, 135–139 (1967)

Diabetic Syndrome in Sand Rats*

II. Variability and Association with Diet**

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Summary. Compared with our previous report, a milder form of diabetic syndrome induced by diet in Egyptian sand rats is described. In sand rats fed vegetables for a prolonged period after capture, pancreatic insulin content was significantly increased. When fed chow, one-third of young animals developed hyperglycemia to a variable degree. Hyperinsulinemia was even more marked than in the previously reported group (Miki et al., 1966) and persisted even when the blood glucose returned to normal following fasting. Pancreatic insulin content was decreased in hyperglycemic sand rats. Insulin refractoriness of adipose tissue was discussed in relation to the pathogenesis.

Syndrome diabétique chez le rat des sables (Psammomys obesus). II. Variabilité et relation avec le régime.

Résumé. Nous avons induit par modification du régime chez le rat des sables égyptien un syndrome diabétique plus modéré que celui décrit dans la précédente communication (Miki et al., 1966). Maintenus à un régime végétal pendant une longue période après leur capture, la teneur en insuline du pancréas de ces animaux augmente de façon significative. Par contre, lorsqu'ils sont soumis au régime standard de laboratoire (granulés-comprimés) une hyperglycémie de degré variable s'installe chez environ un tiers des jeunes rats. L'hyperinsulinémie s'est révélée être encore plus élevée chez ces animaux que chez ceux décrits antérieurement et elle persiste même après un retour à la normale de la glycémie au cours du jeûne. Chez les rats hyperglycémiques, le contenu du pancréas en insuline diminue. Le rôle possible de la résistance du tissu adipeux à l'action de l'insuline dans la pathogénèse de ce syndrome est discuté.

Das diabetische Syndrom bei der Sandratte (Psammomys obesus). II. Unterschiedliches Verhalten und Zusammenhänge mit der Diät.

Zusammenfassung. Eine mildere Form von diätinduziertem diabetischen Syndrom in der Ägyptischen Sandratte wird beschrieben. In Sandratten, die für längere Zeit nach der Gefangennahme mit Gemüse gefüttert worden waren, war der Pankreasinsulingehalt stark erhöht, der Blutzucker aber normal. Ein Drittel der jungen Tiere, hingegen, die mit Labor-Keks gefüttert wurden, entwickelte Hyperglykämie verschiedenen Grades. Hyperinsulinämie war sogar noch stärker ausgeprägt, als in der vorher beschriebenen Gruppe (Miki et al., 1966) und dauerte selbst dann an, wenn der Blutzucker durch Fasten auf normale Werte zurückgebracht wurde. Der Pankreasinsulingehalt hyperglykämischer Sandratten war gegenüber der Norm erniedrigt. Die Insulinempfindlichkeit des Fettgewebes wird im Zusammenhang mit der Pathogenese diskutiert.

Key-words: Spontaneous diabetes, Sand rat, Psammomys obesus, Insulin in serum, Insulin in pancreas, Insulin resistance, Adipose tissue in vitro, Obesity, Diet and diabetes, Fasting.

Previously it was reported from this laboratory (Miki et al., 1966) that sand rats imported from the United Arab Republic (U.A.R.) in August of 1964 developed a fulminating diabetic syndrome soon after arrival if fed regular laboratory chow instead of fresh vegetables. This was associated with a decrease in pancreatic insulin content within a few days of initiation of chow feeding and with a marked elevation in serum immunoreactive insulin, followed by a later decrease. The present report deals with results obtained from another group of the same species imported in 1965 and in animals born in this country of this latter 1965 group. The diabetic syndrome observed was much milder and, in general, the hyperglycemia returned to normal after brief fasting; however, the relative hyperinsulinemia persisted even after blood glucose decreased to the normal range during fasting.

* Supported in part by grants-in-aid from the United States Public Health Service T1-AM-5077-10, AM-09584-02, AM-09748-01 and by the John A. Hartford Foundation, New York, New York and the Adler Foundation, Inc., Rye, New York.

** Paper I of this Series is Miki et al. 1966.

Materials and Methods

On August 20, 1965, a total of 232 sand rats were imported from the U.A.R. All were maintained on a vegetable diet *ad libitum* consisting of fresh spinach, beets and carrots. Of the 190 animals surviving after one month, 24 apparently healthy males were placed into individual metabolic cages and given regular rat chow (Purina Chow Company). Ten animals were later added when the first group failed to show early hyperglycemia.

Of 112 animals born in this laboratory by January 10, 1966, 45 animals were separated for metabolic studies and placed on one of two chow diets containing 3% (regular) or 11% (high) lipid (Purina Laboratory Chow or Old Guilford Chow, Guilford, Connecticut, respectively).

All animals were weighed three times a week. The amount of chow consumed was estimated by weighing the feed bins at the same time. Blood glucose was measured once a week using 0.05 ml of venous blood collected from the tail vein. The Somogyi-Nelson method and later the Autoanalyzer (Technicon Company, Chauncey, New York) were used for this analy-

sis. Serum immunoreactive insulin (IRI) was measured as in the previous report (SOELDNER and SLONE, 1965)[1]. Ketone bodies in the urine were detected with Ketostix® (Ames Company, Elkhart, Indiana).

After the development of hyperglycemia in some of the animals, a total of 15 sand rats, including vegetable-fed controls (14 males and 1 female, ranging between age 3 months, 15 days and 6 months, 13 days) were sacrificed together with 5 male albino rats of a similar weight. These sand rats consisted of three groups: 5 normoglycemic animals on vegetables alone, 5 on chow but not hyperglycemic (3 on regular and 2 on high fat chow), and 5 on chow but hyperglycemic not less than 5 weeks (2 on regular and 3 on high fat chow). The animals were sacrificed by decapitation. At the time of autopsy, serum and a portion of the pancreas were collected and frozen for subsequent insulin assay. Pancreatic insulin was extracted with acid ethanol (STEINKE and DRISCOLL, 1965).

Epididymal fat pads or equivalent tissues were excised, cut into small pieces weighing approximately 100 mg., and incubated in Krebs-Ringer bicarbonate buffer with gelatin containing varying amounts of insulin. Production of $^{14}CO_2$ was measured as described elsewhere (MARTIN et al., 1958). Total lipid was extracted according to FOLCH's method (FOLCH et al., 1957) and the ^{14}C counted in Liquifluor (Pilot Chemical Co., Watertown, Mass.) by a liquid scintillation counter. A Cab-o-Sil dioxane system (Cabot Chemicals, Boston, Mass.) was employed to suspend glycogen isolated as previously described (CAHILL et al., 1959) for liquid scintillation counting. Complete postmortem examinations were performed with conventional histologic studies of all major organs. Electron microscopic studies of retinal preparations and renal tissues are in progress. Ultrastructural studies of the pancreatic islets will be published elsewhere (LIKE and MIKI, 1967).

Results

Development of hyperglycemia in this study. Of the 24 in the first group on chow, only 2 animals developed hyperglycemia of a mild degree. The two animals that became hyperglycemic in the next group of 10 reverted spontaneously to normoglycemia after one or two weeks in spite of continuation of the chow diet.

Since this 1965 group of newly-imported adult animals consumed regular rat chow much less eagerly than the previous group in 1964, every effort was made to overcome this apparent decrease in appetite. Techniques included repeated trials of feeding with and without intermittent total or half starvation, timed feedings, tube feedings, substitution of another type of rat chow with a higher fat content (11%), which was found to be more diabetogenic in mice (GLEASON et al., 1967) (the Old Guilford Chow), and use of various cages that were presumed to be more suitable. In spite of these efforts, the acute diabetic syndrome of the preceeding year could not be reproduced.

Meanwhile, intensive efforts at breeding led to success in obtaining healthy young animals (MIKI et al., in press). One hundred and eighty animals were born and weaned by May 1966. Some were started on either chow diet before they were weaned. After 5 to 6 weeks of chow feeding *ad libitum*, 16 out of 45 young animals developed a hyperglycemia of more than 200 mg/100 ml. This mild hyperglycemia never progressed to severe ketosis and death in contrast with the 1964 group. On the contrary, 6 animals out of this group of 16 showed a return to euglycemia. As outlined in Table 1, even in those animals that remained hyperglycemic until sacrifice, caloric intake during and after development of hyperglycemia was found to decrease

Table 1. *Caloric intake of sand rats on chow*
(Cal/100 gram weight per day, mean of 7 days)

	Before the onset of hyperglycemia	During establishment of hyperglycemia	After establishment of hyperglycemia
Hyperglycemic animals	28.88 ± 1.78	22.57 ± 1.32	15.85 ± 1.97
Normoglycemic animals during same period	20.57 ± 0.53	18.83 ± 1.31	15.25 ± 1.20

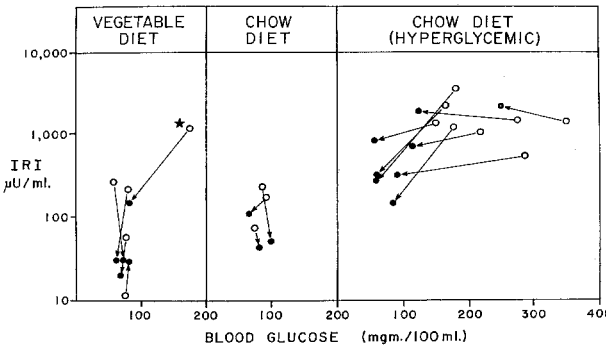

Fig. 1a. Correlation of blood glucose concentration in vegetable-fed sand rats with concentration of immunoreactive insulin (IRI). Fed *ad libitum* = open circles; after a 3-hour fast = closed circles. The one sand rat marked with an asterisk (*) is the only animal to develop hyperglycemia on vegetables alone

Fig. 1b. Blood glucose and insulin concentrations in chow-fed sand rats. These animals did *not* develop hyperglycemia. Fed *ad libitum* = open circles; after a 3-hour fast = closed circles

Fig. 1c. Blood glucose and insulin concentrations in chow-fed sand rats with persistent hyperglycemia. Fed *ad libitum* = open circles; after a 3-hour fast = closed circles

[1] Rat insulin used as standards was a gift from Dr. J. SCHLICHTKRULL, Novo Research Institute, Copenhagen, Denmark.

significantly when compared with the period before onset of hyperglycemia.

Blood glucose and serum insulin following brief fasting. As shown in Fig. 1 a, serum immunoreactive insulin (IRI) of vegetable-fed animals was not elevated, except for one animal, which exhibited transient hyperglycemia accompanying a very rapid weight gain. Fig. 1 b and 1 c show the relationship between blood glucose and IRI when fed *ad libitum*. As long as blood glucose remained normal, IRI was not elevated even on either of the chow diets. Serum IRI was significantly elevated in hyperglycemic sand rats. Following three to six hours fasting, in almost all hyperglycemic sand rats, blood glucose concentrations decreased to the normal range. It is to be emphasized that in spite of this normoglycemia, serum IRI remained elevated at levels of 150 μU/ml or above.

Pancreatic insulin concentrations. As shown in Fig. 2, pancreatic insulin concentration of albino rats ranged between 0.5 and 1.1 U/g pancreas in this series. Vegetable-fed sand rats had significantly elevated levels ranging between 4.4 and 21 U/g pancreas. In the chow-fed group, normoglycemic sand rats showed variable concentration between 2 and 22 U/g pancreas. Hyperglycemic animals, however, showed significantly lower values, ranging between 0.31 and 2.3 U/g pancreas.

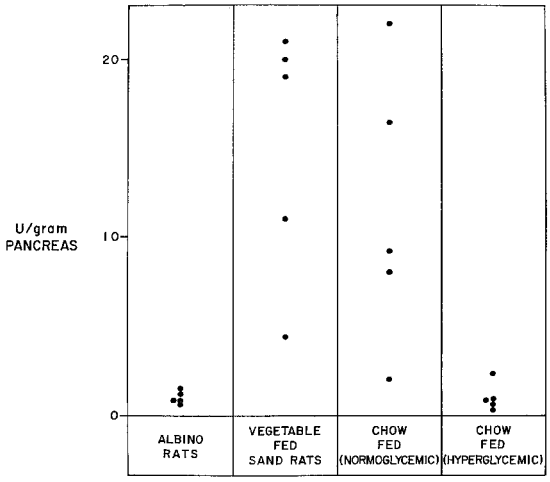

Fig. 2. Pancreatic insulin concentration in sand rats fed vegetables or chow (with and without hyperglycemia). In addition, 5 albino rats obtained from a commercial source were also fed chow *ad libitum* and their pancreas assayed

Responsiveness of adipose tissue to insulin. Metabolism by the adipose tissue of glucose-1-^{14}C in the incubation medium and in a medium also containing 500 μU/ml of insulin was compared (Fig. 3). Good

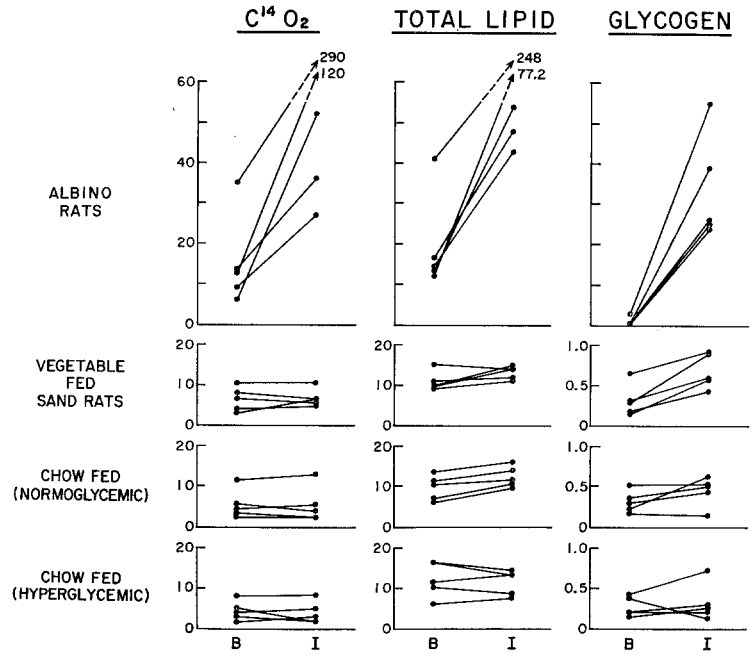

Fig. 3. Response of isolated epididymal adipose tissue from albino rats and sand rats fed vegetables or chow (normoglycemic or hyperglycemic) to 500 μU/ml insulin. All values expressed as disintegrations per minute recovered in the measured parameter. *B* = buffer; *I* = insulin. Note differences in scale between figures

responses were noted with fat pads from albino rats in all the parameters measured, i.e., oxidation to $^{14}CO_2$ and incorporation of ^{14}C into total lipid and into glycogen. In contrast, 500 μU/ml of insulin caused no increase in these same parameters in regular chow-fed sand rats. In five vegetable-fed sand rats, paired analysis showed a significant increase in incorporation of ^{14}C into glycogen. However, this response is far less pronounced than that of the five albino rats of similar weight.

A ten or hundred-fold increase in insulin concentration to 5000 or 50000 μU/ml in another experiment did not overcome this refractoriness of the adipose tissue to insulin, measuring both $^{14}CO_2$ production and ^{14}C incorporation into total lipid (DeFronzo et al., 1967).

Discussion

Differences in the diabetic syndrome. Sand rats imported in 1965 appear to develop a milder form of diabetes than those imported in 1964. However, the 1965 group ate less chow than did the earlier group. Although both groups were captured under almost identical conditions, the possibility of a genetic difference cannot be completely excluded at present in view of the possible existence of subspecies (Hoogstraal, 1963).

A more probable cause of this difference, however, might be the difference in conditions before they were fed chow. Animals imported in the summer of 1964 had been shipped shortly after capture and used for experiments soon after arrival. Following this success,

two successive shipments of 60—90 animals from the U.A.R. failed to survive. Animals imported in the summer of 1965 were kept in captivity for one to three weeks before shipment until they became familiar with the cage and to the presence of man. After arrival, they were maintained exclusively on vegetables for more than one month before experimental use. Since no spontaneous diabetes has been observed in sand rats in their natural habitat (SCHMIDT-NIELSEN et al., 1964), the development of diabetes in this species is almost certainly due to the intake of a high caloric diet (MIKI et al., 1966; HAINES et al., 1965; HACKEL et al., 1966), every effort was made as described in the methods to increase their food intake, but these were unsuccessful. Possibly, the adaptation to fresh vegetables played an important role in decreasing the eagerness of these animals to consume unfamiliar food. The importance of stress in producing the fulminating diabetic syndrome seen in 1964 was also considered in view of the role of epinephrine in producing pancreatic lesions (LOUBATIÈRES, 1965).

Approximately one-third of the young animals born in this laboratory became hyperglycemic when fed regular chow. The appearance of hyperglycemia was always associated with a rather sharp increase in weight. Although the caloric intake of hyperglycemic animals before the onset of hyperglycemia was higher than that of normoglycemic animals during the same period, this decreased during and after establishment of the hyperglycemia. At slight variance with the report of SCHMIDT-NIELSEN et al., (1964), severe ketosiss or eventual death has never been encountered in our group of young sand rats although some animals were sacrificed rather early in the hyperglycemic stage and might have progressed to a more severe stage. Hyperglycemic levels were usually reached after two to three weeks of a transitional period, and then were fixed at a specific level for each animal. Instead of becoming more severely diabetic, several animals returned to normal blood glucose values. Since caloric intake per 100 g body weight in 1964 group was 38 $\pm$ 4 compared with 28.9 $\pm$ 1.8 of the hyperglycemic group in this series, mildness of diabetic syndrome in the latter group can be understood.

Concerning pathogenesis of this experimental diabetes. Hyperinsulinemia accompanying hyperglycemia was very marked in this group. This hyperinsulinemia persisted, although to a lesser degree, even after blood glucose values returned to normal after fasting. To this indirect evidence of peripheral tissue resistance to insulin, some direct evidence was obtained from incubation of adipose tissue *in vitro*, which showed a marked refractoriness to insulin even if obtained from an animal fed vegetables. Whether this refractoriness of the adipose tissue to insulin is inherent to this species was studied further, using animals of different ages (DEFRONZO et al., 1967). It is possible that adipose tissue became refractory when animals were three to six months of age, similar to albino rats (DOBRANSKI, 1966); however the epididymal fat pads of two adults animals sacrified shortly after arrival in 1965 and not included in this report were much smaller in size and more responsive to insulin than that of animals born in this laboratory and of apparently similar age.

This observation raises the possibility that the refractoriness may be related not only to the age of the animal but also to the nutritional state (DIGIROLAMO and RUDMAN, 1966). Even in the vegetable-fed group, almost all the animals exceeded the weight range observed in their natural state and the fat pads appeared enormously enlarged (SCHMIDT-NIELSEN et al., 1964).

In the hyperglycemic, animals, serum IRI values exceeding 2000 μU/ml were frequently observed. However, this hyperinsulinemia probably has no role in the development of insulin resistance as the hyperinsulinemia was not observed in normoglycemic sand rats even on chow, and increasing insulin concentrations did not overcome the refractoriness of the adipose tissue. Glucose uptake and conversion of glucose into glycogen in the diaphragm muscle were also not stimulated by insulin.

Since pancreatic insulin content of animals fed vegetables in 1964 ranged between 2 and 3 U/g pancreas, an increase in the pancreatic insulin content of the vegetable-fed group is striking. This is probably brought about by ingestion of an increasingly larger amount of vegetables over a long period. This might have afforded at least partial protection to this group of animals to acute diabetes. The amount of insulin in the pancreas was variable in the group fed regular chow but that were not hyperglycemic. This may represent different stages of insulin depletion as hyperglycemic animals had significantly reduced amounts of pancreatic insulin. This finding is essentially in agreement with the previous report, although the absolute values were higher in the present group.

In spite of the decrease in pancreatic insulin content, the pancreas appeared to be capable of producing immunologically measurable insulin at even enhanced rates in hyperglycemic animals as judged from serum IRI values. This suggests an increased potential for insulin synthesis by the pancreatic islets and a rapid turnover.

The hypothesis that the endocrine pancreas of this species is incapable of increasing its ability to produce sufficient insulin to avoid death in ketoacidosis was certainly true in the rapidly occurring, fulminating diabetic syndrome. However, over longer periods, the pancreatic islets in this species apparently can hypertrophy similar to many other rodents. It therefore appears logical to look for the pathogenesis of this diabetic syndrome in peripheral tissues rather than as a primary defect in the pancreas itself.

So far, it has not been possible to reproduce the fulminating diabetic syndrome as observed in 1964. This implies that, in spite of a heavy genetic predis-

position, environmental factors may play a role in determining the severity of diabetic syndrome in this animal, based on the assumption that the 1964 and 1965 groups were genetically not different. In this respect, in spite of the many types of spontaneous diabetes known to date, it will be of interest to continue to study this syndrome in the sand rat as a unique type of diabetes provoked possibly by a mild stress and a high coloric intake.

Acknowledgement. The authors acknowledge the skillful technical assistance of Mrs. MAIJA GRINBUSH, Mrs. MARTA GRINBERGS, Mr. ROBERT J. BRUNELLE and Miss JENNIFER CHAFFEE.

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