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Diabetic Syndrome in Sand Rats*

III. Observations on Adipose Tissue and Liver in the Non-Diabetic Stage**

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Summary. Normoglycemic sand rats of approximately 100, 140, and 220 grams body weight were found to have increased circulating insulin levels compared with albino rats of matched ages. Adipose tissue *in vitro* demonstrated decreased insulin sensitivity, which became further diminished with increasing age in both type of rats, but this effect was much more marked in the sand rats. Liver enzyme determinations and metabolic studies on liver slices showed identical results in both rats, except for decreased glucose incorporation into glycogen in the sand rat.

Syndrome diabétique chez le rat du sable (Psammomys obesus): III. Etude du tissu adipeux et du foie au stade non-diabétique.

Résumé. Chez des rats des sables pesant environ 100, 140, et 220 gr, avec une glycémie normale, nous avons observé des taux élevés d'insuline circulante par rapport aux taux que l'on observe chez des rats albinos du même âge. Chez ces animaux, le tissu adipeux incubé *in vitro* n'est que peu sensible à l'insuline, par comparaison avec la sensibilité du tissu adipeux du rat albinos. La sensibilité à l'insuline diminue dans les deux espèces avec l'âge, mais cet effet est beaucoup plus marqué chez le rat des sables. Les dosages d'enzymes hépatiques et les études métaboliques sur tranches de foie ont donné des résultats semblables pour les deux espèces à l'exception d'une incorporation diminuée de glucose en glycogène hépatique pour le rat des sables.

Das diabetische Syndrom bei der Sandratte (Psammomys obesus): III. Beobachtungen am Fettgewebe und an der Leber bei nicht-diabetischen Tieren.

Zusammenfassung. Sandratten mit normalem Blutzucker und einem Körpergewicht von ungefähr 100, 140 und 220 Gramm wurden mit Albinoratten desselben Alters verglichen. Dabei wurde beobachtet, daß die Sandratten auch unter diesen Bedingungen erhöhte Serum-insulinspiegel aufwiesen. Ihr Fettgewebe zeigte *in vitro* eine verminderte Insulinempfindlichkeit, die mit zunehmendem Alter weiter fiel. Diese altersbedingte Abnahme war bei Sandratten ausgeprägter als bei Albinoratten. Untersuchungen von Leberstoffwechsel an Leberschnitten oder an Leberenzymmessungen deuteten nicht auf ein unterschiedliches Verhalten der Leber der Sandratte, mit der einzigen Ausnahme eines verminderten Glucose-Einbaues in Glykogen.

Key-words: Spontaneous Diabetes, Sand rat, Psammomys obesus, Prediabetes, Preclinical diabetes, Insulin in serum, Adipose tissue in vitro, Insulin resistance, Liver enzymes, Liver metabolism in vitro, Obesity.

In previous publications from this laboratory (Miki et al., 1966, 1967) and from Duke University (Hackel et al., 1965; Haines et al., 1965), the appearance of "spontaneous" diabetes in the sand rat was attributed to feeding a diet high in calories. The diabetic state was associated with a marked elevation in immunoreactive insulin in serum and a low concentration of insulin stored in the pancreas (Miki et al., 1966; Like and Miki, 1967); although the latter, by electron microscopy, presented a picture compatible with excessive protein synthesis (Like and Miki, 1967). Assembling these facts into a possible sequence, it appears reasonable that the excess calories induce excessive insulin output by the pancreatic Beta cell. If the Beta cell can maintain increased production, mild or no diabetes occurs; if the Beta cell falls behind, severe diabetes ensues. Another factor contributing to an increased demand for circulating insulin would be the presence of tissues less sensitive to insulin, and the previous paper demonstrated this fact (Miki et al., 1967). Since, in addition, *age* is also associated with decreased insulin sensitivity, this paper deals with adipose tissue responses to insulin in sand rats and in albino rats obtained from a commercial source, of different ages, as well as with certain liver enzymes and glucose metabolism of liver slices obtained from these animals.

Animals and Methods

Male albino rats were obtained from the Charles River Breeding Farms (CD strain, Wilmington, Massachusetts). Sand rats were either bred in our laboratory or obtained as described previously (Miki et al., 1967). All animals were fed Purina Lab Chow *ad libitum*. The sand rats were also given fresh vegetables daily. The male sand rats were specifically selected in that they had not developed diabetes, as judged by blood glucose measured once or twice monthly. Immunoreactive serum insulin levels, blood glucose determinations, and techniques for metabolic studies of isolated adipose tissue *in vitro* were performed as described (Miki et al., 1967).

For the liver slice experiments, 1 gram of slices were cut with a Stadie-Riggs microtome and were incubated in a Krebs-Ringer bicarbonate buffer containing K^+, 110 mEq/L and Na^+, 25 mEq/L, and randomly-labelled glucose-^{14}C, 10 mM (86000 DPM/μM). The slices were shaken for 90 minutes at 75 oscillation/minute in a metabolic incubator at 38° C, and tissue

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glycogen, fatty acids, as well as medium CO_2 analyzed for radioactivity by methods previously described (Snyder and Cahill, 1965). Hepatic glucose phosphotransferase activities were also determined (Lauris and Cahill, 1966).

Table 1

	Albino rat			Sand rat		
n =	3	3	3	3	3	3
Age, days	30	50	75	35 – 52	71 – 75	124 – 160
Weight, g	100	200	300	94 ± 10	134 ± 10	220 ± 7
Blood glucose, mg/100 ml	154 ± 4	110 ± 20	172 ± 5	84 ± 10	63 ± 18	84 ± 12
Immunoreactive insulin, μU/ml	20 ± 2	23 ± 4	60 ± 6	32 ± 8	50 ± 9	108 ± 29

Results

In Table 1 are listed the mean weights and ages for the albino and sand rats with respective levels of blood glucose and serum insulin. The albino rats were selected for their weights, and their ages were estimated from standard growth curves of this strain. Both the albino and sand rats demonstrated progressive hyperinsulinemia with age, but it should be emphasized that the sand rats compared with the albino rats exhibited lower and more stable blood glucose values. Thus, as they age, there appears to be a relative hyperinsulinemia in both types of rats, although the sand rats have significantly higher levels.

Results from the insulin response *in vitro* of epididymal adipose tissue from the albino rats are shown in Table 2. It can be seen that 500 μU/ml of insulin stimulated marked increases in glucose oxidation to CO_2 and incorporation into glycogen or lipid in the youngest animals. In the older animals, the baseline decreased, as well as the percentage of the insulin stimulation. In all animals, a maximal stimulation was achieved by 500 μU/ml of insulin.

Table 2. *Adipose tissue response to insulin in vitro, albino rats, varying ages (3 rats each group)*

Albino rat	CO_2[1]	Glycogen[1]	Lipid[1]
100 g			
Control	37 ± 16	2 ± 0.4	48 ± 17
500 μU/ml	309 ± 78	85 ± 22	256 ± 76
5000 μU/ml	433 ± 8	122 ± 18	325 ± 8
50000 μU/ml	352 ± 70	100 ± 21	319 ± 122
200 g			
Control	31 ± 5	1 ± 0.2	37 ± 4
500 μU/ml	203 ± 34	43 ± 5	125 ± 26
5000 μU/ml	210 ± 26	46 ± 5	106 ± 24
50000 μU/ml	206 ± 20	45 ± 4	107 ± 19
300 g			
Control	24 ± 7	0.3 ± 0.1	11 ± 4
500 μU/ml	38 ± 10	4 ± 2	20 ± 8
5000 μU/ml	48 ± 19	6 ± 3	26 ± 11
50000 μU/ml	51 ± 13	6 ± 2	28 ± 9

[1] Values in CPM/mg wet weight adipose tissue, medium defined in text.

Table 3 lists similar data obtained in epididymal adipose tissue from sand rats. The youngest animals already demonstrated a decreased response to insulin, and it is of interest to point out that with insulin concentrations as high as 50 000 μU/ml, a progressive but blunted response could still be elicited. Thus, not only was there a decreased sensitivity to insulin, but the apparent Km (treating the entire sequence as a single enzymatic event) was increased for glucose oxidation to CO_2 and incorporation into glycogen and lipid.

Table 3. *Adipose tissue response to insulin in vitro, sand rats, varying ages (3 rats each group)*

Sand rats	CO_2[1]	Glycogen[1]	Lipid[1]
94 ± 10 grams			
Control	39 ± 4	2 ± 0.3	26 ± 3
500 μU/ml	75 ± 9	11 ± 2	55 ± 9
5000 μU/ml	105 ± 18	19 ± 4	78 ± 13
50000 μU/ml	140 ± 16	29 ± 5	110 ± 14
134 ± 10 grams			
Control	21 ± 1	0.6 ± 0.03	13 ± 1
500 μU/ml	34 ± 3	3 ± 1	24 ± 3
5000 μU/ml	39 ± 3	6 ± 2	34 ± 3
50000 μU/ml	48 ± 3	8 ± 3	39 ± 3
220 ± 7 grams			
Control	12 ± 2	0.4 ± 0.06	11 ± 1
500 μU/ml	16 ± 2	0.6 ± 0.06	13 ± 1
5000 μU/ml	19 ± 2	1.7 ± 0.2	18 ± 1
50000 μU/ml	21 ± 3	2.4 ± 0.3	22 ± 2

[1] Values CPM/mg adipose tissue, wet weight, medium defined in text.

Table 4. *Liver glucose phosphotransferases of albino and sand rats (3 rats in each group)*

	Hexokinase[1]	Glucokinase[1]	Glucose-6-Phosphatase[2]
Albino rats			
100 g	0.7 ± 0.2	2.2 ± 0.5	6.5 ± 0.3
200 g	0.3 ± 0.1	1.5 ± 0.6	6.4 ± 0.5
300 g	0.6 ± 0.2	1.7 ± 0.3	5.7 ± 0.5
Sand rats			
94 ± 10 g	0.2 ± 0.03	1.7 ± 0.2	7.0 ± 0.5
134 ± 10 g	0.5 ± 0.1	1.6 ± 0.7	6.5 ± 0.8
220 ± 7 g	0.1 ± 0.03	1.8 ± 0.4	7.2 ± 0.5

[1] Units/min/gram liver (Lauris and Cahill, 1966).
[2] μmoles P/minute/gram liver.

In view of these findings in adipose tissue, the metabolic state of liver was examined by determination of glucose phosphotransferase activities; and these are

listed in Table 4. No significant changes were found between albino and sand rats, suggesting that the "insulin effect" as reflected by these liver enzymes was similar in both species.

Table 5 summarizes the results of incubation *in vitro* of liver slices obtained from the animals in the youngest and oldest of both types of rats. In both, glucose oxidation to CO_2 and its incorporation into fatty acids and glycogen decreases with age. Of note is the significant difference between the albino and sand rats in the amount of glucose incorporated into glycogen, the latter showing 1/10 the rate of the former. In addition, there was a more rapid rate of glycogenolysis in the sand rat liver slices and, as expected, an increased rate of glucose production. Also of interest is the equal incorporation of glucose carbon into fatty acids, in marked contrast to the lesser activity of the adipose tissue.

Table 5. *Metabolism of glucose-U-^{14}C by liver slices from albino and sand rats incubated in vitro*

	Albino rats		Sand rats	
	100 g	300 g	94 ± 10 g	220 ± 7 g
Oxidation to $^{14}CO_2$ μM glucose/g/90 minutes	2.4 ± 0.1	1.6 ± 0.2	1.9 ± 0.1	1.1 ± 0.1
^{14}C in fatty acids μM glucose/g/90 minutes	0.80 ± 0.21	0.24 ± 0.06	0.91 ± 0.07	0.22 ± 0.04
^{14}C in glycogen μM glucose/g/90 minutes	31 ± 22	13 ± 1	2 ± 1	1 ± 0.1
Initial glycogen μM glucose/g	355 ± 31	388 ± 21	288 ± 22	268 ± 26
Decrease in glycogen μM glucose/g	20 ± 33	51 ± 22	115 ± 28	116 ± 17
Glucose production μM glucose/g/90 minutes	40 ± 8	49 ± 8	57 ± 3	55 ± 9

Discusssion

These data demonstrate that the sand rat, while normoglycemic, possesses a relative increase in immunoreactive serum insulin when compared with the commercial laboratory albino rat of similar age. Since sand rat insulin is not available, as a standard for the insulin assay, this higher serum insulin level might be due to an altered affinity of sand rat insulin to the pork insulin antibody. Refuting this possibility is the observation that this increased insulin level further progressed with age in the sand rat. This strongly suggests a peripheral resistance to circulating insulin, and is supported by the experiments *in vitro* with adipose tissue, demonstrating decreased basal activity as the animals became older, as well as a marked decrease in their response to exogenous insulin. Thus, there coexists an *absolute hyper*insulinism with an *effective hypo*-insulinism. That this relative "hypo"-insulinism is limited to peripheral tissues is supported by the data obtained from liver slices, as there appears to be no insulin deficiency as evidenced by normal enzymes and *l*ipogenesis from glucose-^{14}C. Only the decrease of glucose incorporation into glycogen is not in agreement with the above hypothesis; however, the more rapid rate of glycogenolysis in the sand rat liver may account for the decreased recovery of label in glycogen.

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