Changing Pattern of Prevalence of Insulin Resistance in *Psammomys obesus*, a Model of Nutritionally Induced Type 2 Diabetes

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Psammomys obesus **(a desert gerbil, nicknamed the "sand rat") with innate insulin resistance was transferred to a high-energy (HE) diet at a young (8 to 20 weeks) and older (38 to 45 weeks) age. The young *Psammomys* progressed to in vivo insulin resistance, followed by pronounced hyperglycemia and hyperinsulinemia, as described previously. Analysis of the time dependency of these changes in response to the HE diet showed that the increase in serum glucose preceded the increase in insulin and plateaued earlier, reverting to normal together with insulin in the older *Psammomys*. Implants releasing insulin 2 IU/24 h did not induce appreciable hypoglycemia, a decrease in free fatty acids (FFAs), or a suppression of hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity in young animals after 5 hours, despite a markedly increased circulating insulin. However, in the older *Psammomys*, the exogenous hyperinsulinemia produced a significant decline in serum glucose and FFA and a suppression of hepatic PEPCK activity. A euglycemic-hyperinsulinemic clamp confirmed that hepatic glucose production (HGP) was lower in older *Psammomys* versus the young and was almost completely abolished by insulin (from 5.6 ± 0.6 to 0.2 ± 0.1 mg · min^{-1} · kg^{-1} *v* 10.9 ± 0.8 to 3.9 ± 0.5 mg · min^{-1} · kg^{-1}). This indicates that HGP, rather than glucose underutilization, was the main contributor to the hyperglycemia and that the hepatic insulin resistance in *Psammomys* is attenuated with age. In relation to the human condition, these findings point out that while the type 2 diabetes prevalence in Western populations generally increases with age, the excessive nutritional intake in high-risk populations produces a pattern of diabetes prevalence that tapers off with age. As such, the nutritionally induced diabetes in *Psammomys* represents a similar model for a differing pattern of the age-related prevalence of diabetes.**

OFTEN NICKNAMED "sand rat" because of its sand dune habitat, *Psammomys obesus* is a herbivorous desert gerbil with innate insulin resistance.[1] *Psammomys* develops hyperinsulinemia and hyperglycemia when transferred from its native saltbush (*Atriplex halimus*) to a laboratory rodent diet, which is higher in energy (3.1 kcal/kg) relative to its native food (~1.9 kcal/g).[2-4] Four stages of progression to diabetes on the high-energy (HE) diet and the failure of insulin to activate the signaling pathway at the receptor level have been recently described.[5-7] In the final stage, the HE diet induces a loss of insulin secretory function and apoptosis of pancreatic β cells.[8-10]

Most of the hitherto published experiments have been performed with relatively young *Psammomys* by placing them on the HE diet 2 to 3 weeks after weaning. As reported previously,[1] administration of exogenous insulin by slow-release implants caused severe hypoglycemia in laboratory albino rats and an almost complete reduction in the activity of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting hepatic gluconeogenic enzyme. This indicated the effectiveness of insulin in restraining gluconeogenesis while promoting peripheral glucose uptake to the point of hypoglycemia. In contrast, the *Psammomys*, even on a low-energy (LE) diet, did not experience hypoglycemia under similar conditions and exhibited only partial (~60%) suppression of PEPCK activity. The *Psammomys* used in this study was relatively young, a few weeks after weaning.

We have observed in preliminary experiments that in older *Psammomys*, the transfer to a HE diet is not diabetogenic and insulin resistance is not expressed.[11] The aim of the present study is to show that insulin resistance and susceptibility to diabetes in this desert rodent disappears with age and pancreatic function is preserved even on a HE diet.

MATERIALS AND METHODS

Animals and Diets

Psammomys obesus, originating from the desert shores of the Dead Sea, were raised in a colony established at the Animal Farm of the Hebrew University Hadassah Medical School. All animals were from the genetically selected diabetes-prone (DP) line,[4] and were housed in individual polypropylene cages with water and food supplied ad libitum. All experimental procedures were authorized by the Institutional Animal Care Committee. The animals were fed ad libitum either on the LE diet supplying digestible energy of 2.4 kcal/g or on a regular rodent chow supplying digestible energy of 3.1 kcal/g, referred to as a HE diet. The composition of the LE diet was protein 16.7%, fat 3.1%, carbohydrate 70.0% (of which 64.0% was digestible), and ash 10.2%. The composition of the HE diet was protein 22.4%, fat 2.1%, carbohydrate 66.6% (of which 80.5% was digestible), and ash 6.9%.[4] Both diets were low in fat; the main difference in the diets was the higher fraction of digestible carbohydrate in the HE diet.

Insulin Implants

Insulin implants (bovine insulin; Linshin, Scarborough, Canada) were inserted subcutaneously into *Psammomys* in the scurf area around the neck of nonfasted animals maintained on the LE diet, with free access to food and water during the implantation. Each implant releases insulin continuously for up to 60 days at a rate of 2 IU/24 h.[12] The animals were killed by decapitation after 5 hours. Blood was collected for glucose and insulin assays. The liver was excised for measurement of glycogen content and enzyme activity.

Hyperinsulinemic-Euglycemic Clamp

Hyperinsulinemic-euglycemic clamp studies were performed on nonanesthetized, unstressed catheterized animals according to the method of Rossetti and Giaccari.[13] The catheters were surgically inserted 5 to 8 days before the clamp study. *Psammomys* were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). Indwelling catheters were inserted into the right jugular

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vein and the left carotid artery. On the day of the experiment in overnight-fasted animals, an infusion of 6 μCi 3-^{3}H-D-glucose (14 Ci/mmol; New England Nuclear, Boston, MA) was initiated and followed by a continuous infusion of 3-^{3}H-D-glucose at a rate of 0.2 μCi/min for 40 minutes to allow a steady state of the glucose tracer to be established. Blood samples were taken for measurement of glucose turnover and calculation of basal hepatic glucose production (HGP). Human insulin (Act-Rapid HM; Novo Nordisk, Bagsvaerd, Denmark) was then infused, first as a bolus of 18 mU and then at a constant rate of 4.4 mU · min^{-1} · kg^{-1}. A 10% glucose solution was also infused. The constant infusions of 3-^{3}H-D-glucose and insulin were performed together. Blood samples were taken every 10 minutes to measure glucose levels and maintain euglycemia by adjusting the glucose infusion rate. Basal glucose turnover, the insulin-stimulated total rate of glucose disappearance, and HGP were measured as described by Rossetti and Giaccari.[13]

Assays

Serum glucose and triglyceride (TG) levels were measured by enzymatic methods (Roche, Basel, Switzerland). Serum free fatty acids (FFAs) were determined using a Randox kit (Randox Laboratories, Ardmore, UK) based on the conversion to and oxidation of acyl coenzyme A. Serum insulin was determined by radioimmunoassay using anti–human insulin antibodies (Medgenix, Brussels, Belgium) and human insulin as a standard. Dilutions of *Psammomys* serum and pancreatic insulin yielded curves parallel to those for dilutions of human insulin. Cross-reactivity with purified human insulin used as a standard was 90% to 95%. Liver PEPCK activity was measured by the rate of enzymatically catalyzed exchange between potassium oxalate and H^{14}CO$_3$ in the presence of inosine triphosphate, as outlined previously.[14] Liver glycogen was determined enzymatically[15] after digestion of a portion of the liver in 33% potassium hydroxide and precipitation of the glycogen by ethanol.

RESULTS

Table 1 shows that the insulin implants in older *Psammomys,* already after 5 hours of severe hypoglycemia, caused a significant 26% suppression of PEPCK activity and a compensatory decrease in liver glycogen. No such response to insulin was observed in the young *Psammomys* at a level of hyperinsulinemia similar to that in aged *Psammomys.* Thus, compared with the young *Psammomys,* the responsiveness to insulin significantly improved with age.

Psammomys exhibits a pronounced insulin resistance, accompanied by hyperglycemia and hyperinsulinemia, when transferred from a LE to a HE diet.[5-7] Table 2 shows that these changes are prominent in the young *Psammomys,* but in contrast, no hyperglycemia or appreciable hyperinsulinemia was elicited by the HE diet in older *Psammomys.* Body weight in the young animals transferred to the HE diet increased somewhat due to fat accretion, as inferred from a 50% increase in the relative weight of epididymal fat tissue. With age, the slight weight gain in *Psammomys* on the HE diet was due to total body growth, since the increase in adipose tissue was proportional to the body weight gain. It should be emphasized that these changes occurred in young *Psammomys* along with the development of insulin resistance, but did not precede it. They were evident in the absence of appreciable changes in food consumption following the transfer from the LE to HE diet and expressed either per day or per animal weight (Table 2), indicating the absence of hyperphagia on the HE diet.

Figures 1 and 2 show, respectively, the time course of the age dependence of changes in serum glucose and insulin and in relative epididymal fat tissue weight on the transfer to the HE diet. The effect of this diet on the elevation of serum glucose was prominent at 19 weeks of age, but did not persist at 38 or 45 weeks of age. The elevation of circulating insulin was evident at 23 weeks, with a peak at 30 weeks, but receded at 38 and 45 weeks. A similar time course was also evident with respect to fat accumulation, with a decrease in fat content relative to total body weight at 23 to 30 weeks of age. At 38 and 45 weeks, there was no increase in epididymal fat in relation to total body weight. No changes relative to body weight were found for the liver and kidneys throughout the total period of 19 to 45 weeks (data not shown).

Values in Figs 1 and 2 are the mean values for the whole group of animals transferred from the LE to the HE diet, including some that did not become hyperglycemic and/or hyperinsulinemic during the 35 days of exposure to the HE diet. From our previous experience, it is known that in the DP line of *Psammomys,* almost all of the animals become diabetic on the HE diet; however, in some animals, it may take longer than 35 days to fully elicit this effect. Thus, at 19 weeks, only eight of 14 animals became hyperglycemic, whereas 12 of 14 became markedly hyperglycemic and hyperinsulinemic at 31 weeks. However, consistent with the reduced susceptibility of the older *Psammomys* to the HE diet, the percentage of those becoming

Table 1. Effect of Slow-Release Insulin Implant on Serum Glucose and Hepatic Gluconeogenesis Enzyme Activity in Young and Older *Psammomys*

Parameter	Young *Psammomys*			Older *Psammomys*		
	Without Implant	With Implant	*P*	Without Implant	With Implant	*P*
Blood glucose at start (mmol/L)	4.2 ± 0.4	4.4 ± 0.3	NS	4.0 ± 0.3	4.3 ± 0.5	NS
Serum glucose at 5 h (mmol/L)	4.2 ± 0.2	3.8 ± 0.3	NS	3.6 ± 0.4	1.9 ± 0.4	<.001
Serum FFA at 5 h (μmol/L)	288 ± 16	240 ± 12	NS	216 ± 10	130 ± 11	<.05
Serum TG at 5 h (mmol/L)	169 ± 32	173 ± 22	NS	102 ± 7	51 ± 6	<.01
Serum insulin at 5 h (pmol/L)	789 ± 60	2,256 ± 118	>.001	546 ± 120	2,160 ± 282	<.01
Liver glycogen at 5 h (mg/g)	51.3 ± 3.1	42.3 ± 2.5	NS	56.5 ± 4.5	42.1 ± 2.8	<.05
Liver PEPCK (nmol · min^{-1} · mg^{-1})	326 ± 35	314 ± 28	NS	274 ± 24	202 ± 20	<.05

NOTE. Values are the mean ± SE for groups of 6 animals. The experiment was from 8:30 AM to 1:30 PM. The nonfasted animals had free access to the LE diet during the experiment. The age of young *Psammomys* was 10-12 weeks and that of older *Psammomys* 42-46 weeks. The respective body weights were 180 ± 6 and 233 ± 13 g.

Abbreviation: NS, nonsignificant.

Table 2. Effect of HE Diet on the Progression to Diabetes in Young and Older *Psammomys*

Parameter	Young *Psammomys*			Older *Psammomys*		
	LE Diet	HE Diet	*P*	LE Diet	HE Diet	*P*
Age (wk)	19	19		45	45	
Blood glucose (mmol/L)	4.7 ± 0.3	14.7 ± 1.5*	<.01	4.5 ± 0.3	5.8 ± 0.8	NS
Serum insulin (pmol/L)	696 ± 44	3,174 ± 348*	<.001	952 ± 290	862 ± 152	NS
Body weight (g)	246 ± 5	268 ± 7*	<.05	252 ± 7	273 ± 8	NS
Epididymal fat (% body weight)	1.6 ± 0.1	2.4 ± 0.2*	<.02	1.1 ± 0.1	1.2 ± 0.1	NS
Food consumption						
g/d	14.1 ± 0.5	13.8 ± 0.6	NS	15.4 ± 0.8	15.9 ± 0.9	NS
g/100 g	6.6 ± 0.4	5.6 ± 0.6	NS	6.1 ± 0.3	5.8 ± 0.3	NS

NOTE. Values are the mean ± SE for groups of 12-14 animals, which were transferred from the LE to HE diet 35 days before the indicated age. Statistical significance (*) was calculated by Student's *t* test for nonpaired groups.

hyperglycemic on the HE diet within the span of 35 days decreased with age, two of 10 at 38 weeks and zero of 12 at 45 weeks.

The reduced insulin resistance in aging *Psammomys* is further demonstrated by the euglycemic-hyperinsulinemic clamp studies (Table 3). Basal HGP was lower in the aged versus young *Psammomys* and was almost completely abolished by the hyperinsulinemia produced by 1 hour of insulin infusion during the clamp. Total glucose transport (TGT), representing the overall tissue glucose utilization, was generally increased by insulin infusion during the clamp studies. The exogenous glucose supply was similar to the TGT in both young and older *Psammomys*. Since the exogenous glucose supply corresponds to TGT minus HGP, in both young and older *Psammomys*, there was a good correlation between the measured TGT and the infused amount of glucose. The relative percentage of hyperinsulinemic HGP to total-body glucose homeostasis was markedly reduced, from 22.6% in the young *Psammomys* to 1.4% in the older *Psammomys* (Table 3). In each group, the relative percentage of basal HGP to hyperinsulinemic HGP was 36% versus 3% (Fig 3).

DISCUSSION

Our study shows that 38- to 45-week-old *Psammomys* exhibit markedly reduced insulin resistance, in contrast to the severe insulin resistance in young animals investigated at 8 to 31 weeks of age. This was demonstrated by the inability of exogenous insulin, administered by implants, to produce hypoglycemia, to suppress the activity of the hepatic gluconeogenesis enzyme PEPCK, and to induce the decrease of circulating FFA and TG, most probably by inhibiting FFA release from adipose tissue. On the other hand, the HE diet failed to elicit hyperglycemia and hyperinsulinemia in the older *Psammomys*. A remarkable observation was the almost complete shutdown of hepatic gluconeogenesis during the euglycemic-hyperinsulinemic clamp in the older *Psammomys*, while at the same prevalent circulating insulin concentration, the young animals responded with a reduction to 36% of basal HGP (Fig 3). Thus, the hepatic insulin resistance of *Psammomys*, attributed to the thrifty genotype of a desert species[1] and accentuated by the HE diet, declined with age.

The restored ability of insulin to suppress PEPCK activity

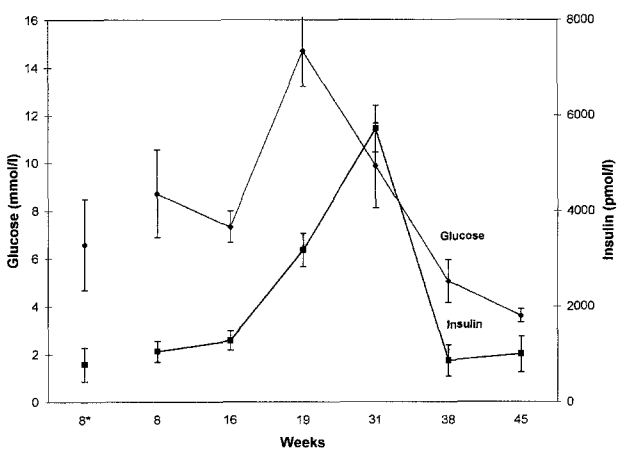

Fig 1. Serum glucose and insulin levels in *Psammomys obesus* upon transfer from the LE to HE diet. *The animals were maintained on LE diet and the basal values are shown at 8 weeks postweaning. The animals were transferred to the HE diet for 5 weeks and killed by decapitation in the nonfasting state at the age indicated in Table 2. The increase in serum glucose on exposure to the HE diet was significant at 8, 16, 19, and 31 weeks at $P < .005$ *v* the initial value. Increases in serum insulin were significant at 19 and 31 weeks at $P < .005$.

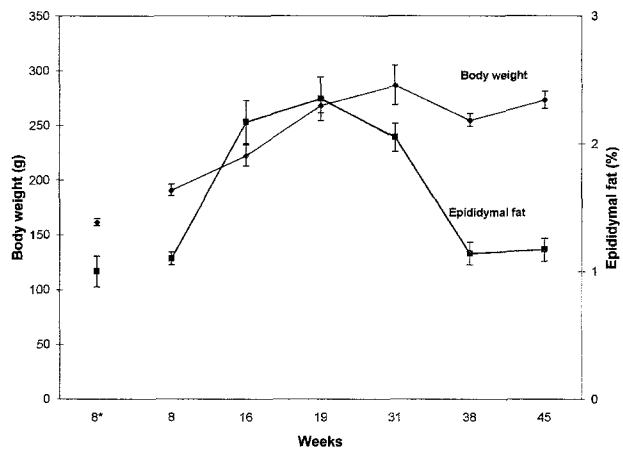

Fig 2. Changes in body weight and epididymal fat weight relative to total body weight in the same *Psammomys* age groups in Fig 1 upon transfer to the HE diet. Total body weight increased during the period of observation, reflecting the growth of the animals. Epididymal fat weight increased particularly in young animals upon transfer to the HE diet, reflecting the association of insulin resistance with fat accumulation, but decreased relatively to total body weight in older animals. The increase in epididymal fat weight *v* the initial value was significant at 16, 19, and 31 weeks of age at $P < .001$.

Table 3. Hyperinsulinemic-Euglycemic Clamp Results in Young and Older *Psammomys*

Parameter	Young *Psammomys*	Older *Psammomys*	*P*
Clamp serum glucose (mmol/L)	5.4 ± 0.3	4.4 ± 0.3	NS
Clamp serum insulin (pmol/L)	1,848 ± 110	1,584 ± 132	NS
Basal HGP (mg · min^{-1} · kg^{-1})	10.9 ± 0.8	5.6 ± 0.6	<.01
Insulin HGP (mg · min^{-1} · kg^{-1})	3.9 ± 0.5	0.2 ± 0.1	<.001
TGT (mg · min^{-1} · kg^{-1})	17.2 ± 2.4	14.8 ± 1.7	NS
Exogenous glucose supply (mg · min^{-1} · kg^{-1})	13.3 ± 2.2	14.6 ± 1.9	NS

NOTE. Values are the mean ± SE for groups of 9 animals. Serum glucose in the nonfasted young and older *Psammomys* before the clamp procedure was 4.5 ± 0.3 and 4.1 ± 0.3 mmol/L, respectively. Values in the table are for 18-hour fasted animals before starting the glucose infusion. All animals were maintained on the LE diet. The older *Psammomys* were 38-45 weeks old and weighed 222 ± 13 g, *v* young *Psammomys,* 19-22 weeks old and weighing 165 ± 5 g.

and HGP in the older *Psammomys* strongly indicates that the hepatic insulin resistance was improved and excessive HGP was prevented. Thus, of the dual control mechanisms of glucose homeostasis, HGP rather than peripheral glucose uptake, represented by the TGT, appears to be the major factor determining the circulating glucose level in the aged *Psammomys.* The importance of HGP in determining the level of diabetic hyperglycemia also has been demonstrated in other species[16,17] and in the human.[18-20]

Changes in HGP in *Psammomys* maintained on the HE diet could be related to alterations in circulating FFAs, which are known to enhance gluconeogenesis.[21] However, as reviewed by Boden,[22] this occurs when plasma FFA levels are elevated, inhibiting peripheral glucose utilization and enhancing HGP, particularly when pancreatic insulin output is reduced. This is unlikely to occur in *Psammomys,* since plasma FFA levels are

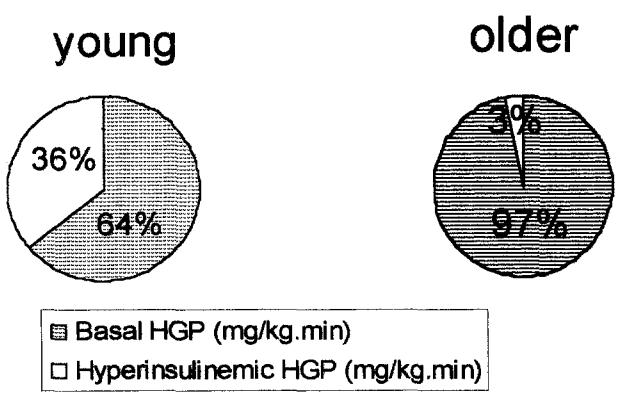

Fig 3. Relative percentage of basal HGP and hyperinsulinemic HGP during the hyperinsulinemic-euglycemic clamp in young (14-19 weeks) and older (38-45 weeks) *Psammomys.* Note the almost complete suppression of HGP by the clamp hyperinsulinemia with age, associated with normoglycemia on the HE diet in older *Psammomys.*

generally low (Table 1) and do not appreciably increase on HE diets (E. Shafir, E. Ziv, unpublished observations, December 1998). In aging human subjects, FFAs increase and their competition with the tissue utilization of other substrates seems related to the enlarged adipose tissue mass,[23] which was not the case in *Psammomys.*

Barzilai et al[24] reported that long-term caloric restriction, to 55% of the diet of control rats fed ad libitum, reverses hepatic insulin resistance in Sprague-Dawley rats by decreasing the visceral fat content. The possibility that the reduced insulin resistance in older *Psammomys* was a corollary of protracted caloric restriction during growth, by maintenance on the LE diet, is highly unlikely. The LE diet is richer than the diet in their native habitat (digestible energy, 2.4 *v* 1.9 cal/g, respectively) and does not constitute caloric restriction. The older *Psammomys* reached a weight similar to that of young *Psammomys* (Table 2).

Increased body weight and particularly the increment in adipose tissue have been suggested by Barzilai and Rossetti[25] to contribute to the development of insulin resistance in aging albino rats. A doubling of the insulin infusion rate was required during the euglycemic-hyperinsulinemic clamp to maintain HGP at a similar rate in 4- to 14-month-old rats. However, in older *Psammomys,* the body growth was not related to excessive fat tissue (Table 2 and Fig 2). This finding also does not conform to observations in aged human subjects, in whom glucose intolerance, insulin resistance,[26-28] and plasma lipoproteins[29] increase with aging and are mostly attributed to fat accumulation, particularly in the abdominal region. In *Psammomys,* fat accretion in excess of body growth was transient between 4 and 7 months of age, and plateaued with aging. This occurred along with but not as an outcome of the increase in insulin resistance, which repeatedly emphasizes that the disappearance of insulin resistance in older *Psammomys* was evident at normal body composition.

One of the major conclusions from our observations is that the liver insulin resistance leads to the elevation of HGP despite hyperinsulinemia and is responsible for the diabetic hyperglycemia in young *Psammomys.* With age, the diet-induced hyperinsulinemia suppresses HGP, whereas glucose uptake is not increased, as indicated from the unchanged TGT in our clamp experiments. The impaired muscle glucose uptake, assumed to characterize the thrifty metabolic genotype,[30] appears not to be reduced as evident from the normally functioning muscle GLUT-1 and GLUT-4 transporters in aged rats[31] and in humans.[32]

The relevance of our findings to the human condition must be discussed in the light of etiological factors inducing type 2 diabetes. Nutritionally induced diabetes may result in severe hyperglycemia and hyperinsulinemia, which lead to β-cell breakdown and apoptosis in *Psammomys* and other susceptible species but are reversible by restricting the food intake.[9] This etiology may be different from the irreversible genetically predetermined diabetes as exemplified by *db/db* mice, in which Leiter[33,34] demonstrated that genomic factors are responsible for the insulin resistance and β-cell loss.

Human populations in which nutritional affluence as a result of an improved lifestyle leads to a diabetes epidemic may be

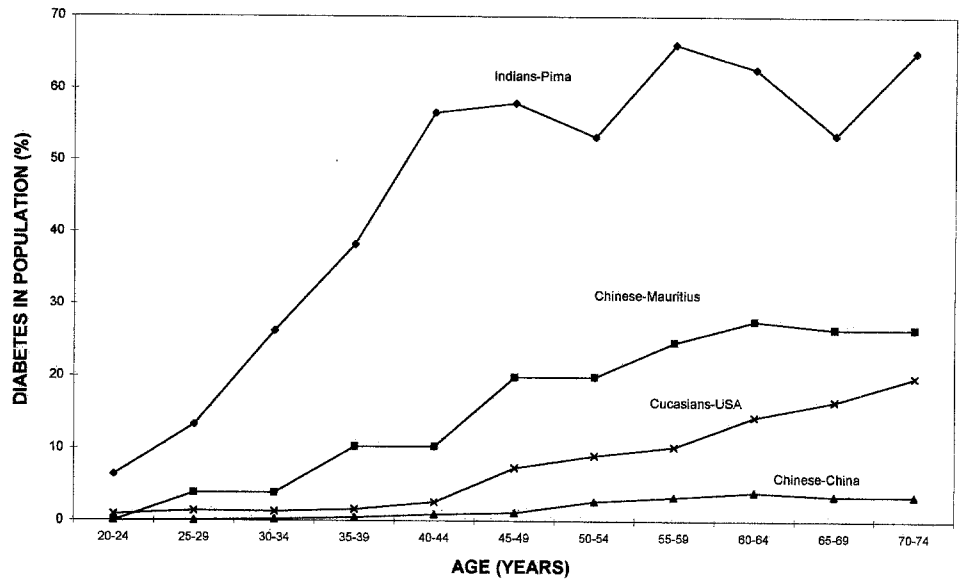

Fig 4. Effect of age on the plateau for the prevalence of diabetes in populations achieving improved nutritional conditions, US Pima Indians and Chinese of Mauritius and China, *v* rising prevalence in Caucasian Americans. Data from Zimmet et al.[38,39]

subject to factors similar to those operating in the *Psammomys* on the HE diet. In Pima Indians, a large percentage of whom become diabetic when young, the prevalence of diabetes reaches a plateau with age, as noted initially by Bennet et al[35] and more recently by King and Rewers[36] (Fig 4). Also, the diabetes prevalence in Chinese and Hindu inhabitants of Mauritius diminishes with age, in contrast to other populations on this island in which the diabetes prevalence was not attenuated by age.[37] Zimmet et al[38,39] among the urbanized populations of Nauru and Western Samoa consuming diets markedly higher than optimal by Western standards, found that the prevalence of diabetes tapers off above 50 years of age. Thus, diabetes induced by improved food availability in high-risk population groups appears to show a different prevalence pattern, for which *Psammomys* may be a pertinent model. This is in contrast to the prevalence of diabetes in US Caucasians[40] (Fig 4) and other ethnic groups, eg, the population of Jerusalem,[41] which generally increases with age up to the seventh decade. It is also of interest that diabetes acquired by Australian Aborigines on a Western diet may be reversed by a return to the traditional diet,[42] which is characteristic of the diabetes induced by relative overnutrition and has been demonstrated likewise in *Psammomys*.[2,6]

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REFERENCES

1. Ziv E, Kalman R, Hershkop K, et al: Insulin resistance in the NIDDM model *Psammomys obesus* in the normoglycaemic, normoinsulinaemic state. Diabetologia 39:1269-1275, 1996

2. Shafrir E, Gutman A: *Psammomys obesus* of the Jerusalem colony: A model for nutritionally induced, non–insulin-dependent diabetes. J Basic Clin Physiol Pharmacol 4:83-99, 1993

3. Ziv E, Shafrir E: *Psammomys obesus:* Nutritionally induced NIDDM-like syndrome on a "thrifty gene" background, in Shafrir E (ed): Lessons From Animal Diabetes. London, UK, Smith-Gordon, pp 285-300, 1995

4. Kalman R, Adler H, Lazarovici G, et al: The efficiency of sand rat metabolism is responsible for development of obesity and diabetes. J Basic Clin Physiol Pharmacol 4:83-99, 1993

5. Kalderon B, Gutman A, Shafrir E, et al: Characterization of stages in the development of obesity-diabetes syndrome in sand rat (*Psammomys obesus*). Diabetes 35:717-724, 1986

6. Kanety H, Moshe S, Shafrir E, et al: Hyperinsulinemia induces a reversible impairment in receptor function leading to diabetes in the sand rat model of non–insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 91:1853-1857, 1994

7. Shafrir E: Development and consequences of insulin resistance: Lessons from animals with hyperinsulinemia. Diabetes Metab 22:122-131, 1996

8. Like AA, Miki E: Diabetic syndrome in sand rats. IV. Morphologic changes in islet tissue. Diabetologia 63:143-166, 1967

9. Bar-On H, Ben-Sasson R, Ziv E, et al: Irreversibility of nutritionally induced NIDDM in *Psammomys obesus* is related to β-cell apoptosis. Pancreas 18:259-265, 1999

10. Donath MY, Gross DJ, Cerasi E, et al: Hyperglycemia-induced β-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. Diabetes 48:738-744, 1999

11. Kalman R, Galer S, Ziv E, et al: Age related changes in susceptibility to diabetes are associated with severity of hepatic insulin resistance in *Psammomys obesus*. Acta Diabetol 35:S6, 1998 (suppl 1, abstr)

12. Wang PY: Implantable reservoir for supplemental insulin delivery on demand by external compression. Biomaterials 10:197-201, 1989

13. Rossetti L, Giaccari A: Relative contribution of glycogen synthesis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. J Clin Invest 85:1785-1792, 1990

14. Krausz Y, Bar-On H, Shafrir E: Origin and pattern of glucocorticoid-induced hyperlipidemia in rats. Dose-dependent bimodal changes in serum lipids and lipoproteins in relation to hepatic lipogenesis and tissue lipoprotein lipase activity. Biochim Biophys Acta 63:69-82, 1981

15. Huijing F: A rapid enzymatic method for glycogen determination in small tissue samples. Clin Chim Acta 30:567-572, 1970

16. Burcelin R, Eddouks M, Maury J, et al: Excessive glucose production, rather than insulin resistance, accounts for hyperglycemia in recent-onset streptozotocin-diabetes rats. Diabetologia 38:283-290, 1996

17. Cusin I, Rohner-Jeanrenaud F, Terrettaz J, et al: Hyperinsulinemia and its impact on obesity and insulin resistance. Int J Obes 16:S1-S11, 1992 (suppl 4)

18. Fery F: Role of hepatic glucose production and glucose uptake in the pathogenesis of fasting hyperglycemia in type 2 diabetes: Normalization of glucose kinetics by short term fasting. J Clin Endocrinol Metab 78:536-542, 1994

19. Consoli A, Nurjhan N, Capani F, et al: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes 38:550-557, 1989

20. Perriello G, Pampanelli S, Del Sindaco P, et al: Evidence of increased systemic glucose production and gluconeogenesis in an early stage of NIDDM. Diabetes 46:1010-1016, 1997

21. Prager R, Wallace P, Olefsky MJ: Direct and indirect effects of insulin to inhibit hepatic glucose output in obese subjects. Diabetes 36:607-611, 1987

22. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 45:3-10, 1996

23. Bonadonna RC, Groop LC, Simonson DC, et al: Free fatty acid and glucose metabolism in human aging—Evidence of operation of Randle cycle. Am J Physiol 266:E501-E509, 1994

24. Barzilai N, Banerjee S, Hawkins M, et al: Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. J Clin Invest 101:1353-1361, 1988

25. Barzilai N, Rossetti L: Age-related changes in body composition are associated with hepatic insulin resistance in conscious rats. Am J Physiol 270:E930-E936, 1996

26. Davidson MB: The effect of aging on carbohydrate metabolism: A review of the English literature and a practice approach to the diagnosis of diabetes mellitus in the elderly. Metabolism 28:688-705, 1979

27. Kohrt WM, Kirwan JP, Staten MA, et al: Insulin resistance in aging is related to abdominal obesity. Diabetes 42:273-281, 1993

28. Cefalu WT, Wang ZQ, Werbel S, et al: Contribution of visceral fat mass to the insulin resistance of aging. Metabolism 44:954-959, 1995

29. Lemieux S, Prud'homme DP, Moorjani S, et al: Do elevated levels of abdominal visceral adipose tissue contribute to age-related differences in plasma lipoprotein concentrations in men? Atherosclerosis 118:155-164, 1995

30. Neel JV: Diabetes mellitus: A "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 14:353-362, 1963

31. Wang ZQ, BellFarrow AD, Sonntag W, et al: Effect of age and caloric restriction on insulin receptor binding and glucose transporter levels in aging rats. Exp Gerontol 32:671-684, 1997

32. Eriksson J, Koranyi L, Bourey R, et al: Insulin resistance in type 2 (non–insulin-dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT 4) gene in human muscle. Diabetologia 35:143-147, 1992

33. Leiter EH: Analysis of differential survival of syngeneic islets transplanted into hyperglycemic C57BL/6J versus C57BL/KsJ mice. Transplantation 3:401-406, 1987

34. Leiter EH: The genetics of diabetes susceptibility in mice. FASEB J 3:2231-2241, 1989

35. Bennet PH, Burch TA, Miller M: Diabetes mellitus in American (Pima) Indians. Lancet 2:125-128, 1971

36. King H, Rewers M: Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. Diabetes Care 16:157-177, 1993

37. Dowse GK, Gareeboo H, Zimmet PZ, et al: High prevalence of NIDDM and impaired glucose tolerance in Indian, Creole, and Chinese Mauritians. Diabetes 39:390-396, 1990

38. Zimmet P, Taft A, Guinea W, et al: The high prevalence of diabetes mellitus on a Central Pacific island. Diabetologia 13:11-115, 1977

39. Zimmet P, Faaiuso S, Ainuu J, et al: The prevalence of diabetes in the rural and urban Polynesian population of Western Samoa. Diabetes 30:45-51, 1981

40. Harris MI, Goldstein DE, Flegal KM, et al: Prevalence of diabetes, impaired fasting glucose and impaired glucose tolerance in U.S. adults. Diabetes Care 21:518-524, 1998

41. Bar-On H, Friedlander Y, Kidron M, et al: Serum glucose and insulin characteristics and prevalence of diabetes mellitus and impaired glucose tolerance in the adult Jewish population of Jerusalem. Nutr Metab Cardiovasc Dis 2:75-81, 1992

42. O'Dea K: Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to traditional lifestyle. Diabetes 33:596-603, 1984