Renal Na-K-ATPase hyperactivity in diabetic *Psammomys obesus* is related to glomerular hyperfiltration but is insulin-independent

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Abstract

Psammomys obesus, a desert rodent, develops diabetes when displaced from its natural environment and fed a high energy diet in the laboratory. This study was designed to examine variations in renal function in relation to the diabetic state with emphasis on changes in Na-K-ATPase activity.

The following groups of *Psammomys* were studied: (1) Animals fed a saltbush diet; a low energy/high salt diet (natural). (2) Animals fed a low energy/low salt diet (laboratory). Both 1 and 2 were normoglycemic and normoinsulinemic and thus served as control. (3) Animals fed a high energy diet (group C) who were hyperglycemic and hyperinsulinemic; this group was divided into two subgroups: C1 presented with glomerular hyperfiltration rate and C2 with glomerular hypofiltration rate. (4) Animals fed a high energy diet presenting with hyperglycemia-hypoinsulinemia (group D). (5) Group D+I, similar to group D but treated with external insulin (2 U/24 h).

Groups D and C1, whose glomerular filtration rose above normal by 30% and 70% respectively, exhibited

metabolic similarity to Type I and Type II diabetes. In these groups, Na-K-ATPase activity in the cortex increased by 80–100% and in the medulla by 180% ($P < 0.001$ vs control). In group C2 with reduced glomerular filtration rate (GFR), Na-K-ATPase activity did not differ from control. In group D+I, with normalized glomerular filtration rate, Na-K-ATPase activity was similar to control. There was a linear and significant correlation between GFR and Na-K-ATPase activity both in the cortex and in the medulla.

These experiments present a well defined animal model of diabetes mellitus. Variations in glucose and in insulin did not correlate with Na-K-ATPase activity. These results clearly demonstrated that Na-K-ATPase activity in the diabetic *Psammomys* was determined by glomerular filtration but was independent of plasma glucose or insulin levels.

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Introduction

Psammomys obesus is prone to develop obesity, hyperglycemia and hyperinsulinemia when fed a high energy (HE) diet (2.93 kcal/g) (Kalman *et al.* 1993). In nature the *Psammomys* is a herbivorous rodent, subsisting on a low energy, high salt diet. In the Israeli desert the *Psammomys* feeds mainly or solely on saltbush (*Atriplex halimus*). We have isolated two defined genetic lines of the *Psammomys*: a diabetic prone (DP) line and a diabetes resistant (DR) line (Ziv & Shafir 1995). The selection process was done in laboratory by feeding the animals the HE diet. The diabetic state that develops can be prevented, and/or reversed by feeding the animals LE diet (2.3 kcal/g). The development of diabetes and obesity can be understood by the 'thrifty metabolism' theory. Our findings show that the *Psammomys* behaves according to this theory. The animals of the DP line use the food components more efficiently than the animals of the DR line (by 50%) (Kalman *et al.*

1993). It leads to the intensive and continuous storage of fat, and to obesity in the DP animals. Primary insulin resistance in *Psammomys* is an innate species characteristic that enables the animals to survive and breed in its natural low energy habitat (Ziv *et al.* 1996). Insulin resistance becomes a disadvantage when *Psammomys* is continuously fed a HE diet. A prolonged state of hyperglycemia leads to several secondary complications in the *Psammomys* model, such as nephropathy and kidney failure.

Na-K-ATPase, the enzyme that is responsible for the active transport of sodium and potassium (Skou 1965), is present in high concentrations in the kidney (Schmidt & Dubach 1969). It has been implicated in the process of active reabsorption of sodium from the glomerular filtrate by renal tubules (Katz & Epstein 1967). In a previous study from our laboratory (Wald *et al.* 1983), evidence was presented to support the concept that the kidney responds to chronic changes in the filtered load of sodium with an adaptive increase in the specific activity of the transport

Table 1 Different experimental *Psammomys* groups

Group	No. of animals	Digestible energy (kcal/g)	Type of diabetes	Blood glucose after fast	Blood glucose after food	Insulin levels
Saltbush diet (SB)	11	1·92	–	Normal	Normal	Normal
Laboratory artificial diet. Low energy (LE)	12	2·38	–	Normal	Normal	Normal
High energy diet (HE). Group C	24	2·93	Type II	Normal	High	High
High energy diet (HE). Group D	8	2·93	Type I	High	High	Low
High energy diet (HE) +insulin 2 U/24 h. Group D+I	8	2·93	Type II	Normal	High	High

enzyme Na-K-ATPase. A remarkable increase in the glomerular filtration rate (GFR) has been observed in the early stage of streptozotocin-induced diabetes mellitus in the rat (Canney *et al.* 1979, Wald & Popovtzer 1984). The increase in GFR was accompanied by a commensurate increase in the filtered load of sodium.

In a previous publication (Wald *et al.* 1993), we examined the effect of long-term diabetes on rat Na-K-ATPase activity in individual nephron segments and the effect of insulin treatment on that activity. In rat, after the induction of diabetes mellitus with streptozotocin (STPZ-DM), GFR rose and Na-K-ATPase activity increased in all nephron segments studied. Partial correction of blood glucose with external insulin replacement (2 U/24 h) led to a decrease in Na-K-ATPase in the proximal tubules and distal convoluted tubules, while partial correction of blood glucose in the presence of elevated GFR did not alter the Na-K-ATPase activity in the loop of Henle.

In general, the first stage of insulin-dependent diabetes mellitus (Type I, IDDM) is characterized by glycosuria and natriuresis, followed by increase in Na-K-ATPase activity in the proximal convoluted and proximal straight tubules. Subsequently, during the diabetic state, there is an increase in GFR and Na-K-ATPase activity of the thick ascending limb of Henle's loop.

In non-insulin-dependent diabetes mellitus (Type II, NIDDM), which is characterized by high blood glucose levels after food intake, the plasma insulin levels can vary from normal or high to low in long-term diabetes.

It was of interest in this context to characterize and to study the kidney function in the diabetic state of the *Psammomys* from the diabetic prone line. The transition of *Psammomys* to diabetes has been classified by Kalderon *et al.* (1986) into four consecutive states: A: normoinsulinemia and normoglycemia; B: hyperinsulinemia and normoglycemia, C: hyperinsulinemia and hyperglycemia, and D: hyperglycemia and hypoinsulinemia, due to loss of beta-cell insulin secretory capacity. The progression of the nutritionally induced diabetes in *Psammomys* in many respects resembles the development of the hyperglycemic-hyperinsulinemic stage in human populations. The

investigation of the primary insulin resistance and the secondary complications that develop in the diabetic *Psammomys* may provide better understanding of Type II diabetes mellitus in the human population.

The present study was undertaken to further characterize (a) the renal function and Na-K-ATPase activity in the kidney of the diabetic *Psammomys* from the diabetic prone line, and (b) the effect of varying severity of glycemic abnormality and insulin levels in the *Psammomys* on above measurements.

Materials and Methods

The animal experiments were approved by the Institutional Animal Welfare Committee.

Male *Psammomys obesus* (sand rat) of the Hebrew University strain were used in all the experiments. The animals were weaned at 3 weeks of age to one of the following groups (Table 1).

(1) Normoglycemic, normoinsulinemic *Psammomys* fed its native diet, the saltbush (SB; *Atriplex halimus*) leaves, picked from their natural habitat, the Dead Sea area. This group is equal to low energy, high salt diet and represents the basic natural state of *Psammomys* (SB group). The Na content in the saltbush leaves is 61·7 ± 2·1 mg/g dry matter and the K content 36 ± 7·7 mg/g dry matter (Degen 1988).

(2) Normoglycemic, normoinsulinemic *Psammomys* fed artificial commercial, low energy, low salt laboratory diet (LE group) (Koffolk, Tel Aviv, Israel). The Na content is 2·4 mg/g dry matter, and the K content 14·6 mg/g dry matter. The composition of the diets is given in Table 2.

(3) Hyperglycemic, hyperinsulinemic *Psammomys* fed for 5 weeks from weaning with commercial laboratory high energy (HE) diet (2·93 kcal/g) (Table 2) (Koffolk). The Na content is 2·4 mg/g dry matter and K content 14·6 mg/g dry matter (Kalman *et al.* 1993). In this group the glucose level was normal in fasted state and high (>200 mg/dl) following food intake (group C) (Kalderon *et al.* 1986). This group of animals was used as a model of Type II diabetes mellitus.

Table 2 Composition of the saltbush native (SB), low energy (LE) and high energy (HE) diets

	SB	LE	HE
Dietary			
Protein %	23.3	16.2	23.6
Fat %	2.9	3.1	2.4
Carbohydrate %	53.3	70.0	68
NaCl %	8.0	1.1	1.1
Ash %	12.5	9.6	5.0
Digestible energy (kcal/g)	1.92	2.38	2.93

(4) Hyperglycemic, hypoinsulinemic *Psammomys* fed HE diet for 8 weeks. Their blood glucose was high (>300 mg/dl) even after overnight fasting and after food intake. This group of animals was used as a model of Type I diabetes mellitus (group D). These animals, after developing a hypoinsulinemic state, survived for only several days.

(5) Hyperglycemic hyperinsulinemic *Psammomys* fed HE diet for 8 weeks, similar to group D but on the day following appearance of hyperglycemia (at the fasting state) an insulin release tablet (2 U/24 h) was implanted subcutaneously for one additional week (group D+I) (Wang 1989). Following this procedure the animals became normoglycemic at the fasted state (similar to group C).

Blood glucose and insulin levels were monitored weekly for 3–9 weeks. Blood was drawn from the tail vein after fasting and after food intake. At the end of the experimental period (each group in their suitable time), the animals were housed in individual polypropylene cages for kidney function determinations. Urine collection was performed for 24 h. The *Psammomys* were fed their respective diet (SB, LE or HE) and allowed to drink water *ad libitum*.

At the end of the experimental period blood was drawn from the aorta under ether anesthesia and the animals were killed. The kidneys were removed immediately, decapsulated, weighed and kept on ice. Slices of cortex and outer medulla were cut and stored separately for preparation of microsomal ATPase.

Preparation of microsomes

Preparation of microsomal ATPase was carried out according to Jørgensen & Skou (1969). The tissues from two kidneys of each individual animal in the experimental and control groups, at least six animals, were homogenized in 10 volumes of medium containing 0.25 mol/l sucrose, and 2 mmol/l EDTA buffered with 5 mmol/l Tris-HCl to a pH of 7.4–7.5. The homogenate was centrifuged at 7000 *g* for 15 min; the supernatant was decanted and the sediment centrifuged at 48 000 *g* for 40 min. The pellet was re-suspended in an equal volume of the above solution and again homogenized in 10 volumes of desoxycholate 0.1% containing 2 mmol/l EDTA and 25 mmol/l Tris-HCl

(pH 7.0). After incubation at 37 °C for 30 min, the suspension was centrifuged at 25 000 *g* for 30 min. The pellet was suspended in the above sucrose-EDTA-Tris. This final suspension was frozen at –20 °C until assayed.

Assay of ATPase

ATPase activity was determined by the amount of inorganic phosphate (P_i) released during incubation at 37 °C in a shaking, thermostatic bath, as previously described (Gutman *et al.* 1973). All assays were run in duplicates. The P_i release was studied in the presence or absence of K^+ in the medium. The standard incubation medium consisted of 100 mmol/l NaCl, 10 mmol/l KCl, 4 mmol/l $MgCl_2$ and 4 mmol/l ATP. Enzymatic activity was stopped by the addition of 10% trichloroacetic acid. P_i was determined according to the method of Fiske & Subbarow (1925). Enzymatic protein was assayed according to Lowry *et al.* (1951). Na^+K^+ -ATPase was estimated as the difference of P_i release with and without K^+ in the medium.

Metabolic clearance studies

Sodium and potassium concentrations in serum and urine were determined by flame photometry (Corning 48) and creatinine in blood and urine by automated picric acid method (Cobas-Mira, Hoffman La-Roche, Basel, Switzerland). Urine protein levels were detected automatically by Pyrogallol Red direct colorimetric method in urine and in the same autoanalyser as mentioned above (Cobas-Mira, Hoffman La-Roche).

Plasma glucose was measured using Glucometer Elite (Bayer Corporation, Elkhart, IN, USA) and plasma insulin was measured by radioimmunoassay utilizing antibodies against human insulin (Medgenix, Brussels, Belgium) and human insulin as standard. Dilutions of *Psammomys* serum gave a curve paralleling those of dilutions of human insulin. Cross-reactivity with purified human insulin used as standard was 90–95% (Kalderon *et al.* 1986).

Microsomal renal Na-K-ATPase activity was determined for each animal separately. In the final analysis the results for all animals were combined and expressed as means $\pm$ s.e. for each experimental or control group. Thus the results represent six different determinations added to obtain means $\pm$ s.e. for a given group. Analysis of variance was performed for statistical evaluation between the different groups. Results between individual groups were compared by a non-paired Student's *t*-test with modified levels of significance according to the Bonferroni method (Godfrey 1985).

Results

Glucose levels

Blood glucose and insulin levels in fed and fasted state for all groups studied are given in Table 3. There was no

Table 3 Blood glucose and plasma insulin levels in fed and fasted state of the *Psammomys* in all experimental groups

Group	No. of animals	Glucose (mg/dl)	Insulin (μ U/ml)
Saltbush diet (SB)	11	Fed 86 ± 3 Fast 69 ± 5	23 ± 8 8 ± 2
Laboratory artificial diet.	12	Fed 89 ± 4	28 ± 8
Low energy (LE)	25	Fast 71 ± 5	9 ± 2
High energy diet (HE).	24	Fed $351 \pm 13^{*}\ddagger$	$418 \pm 62^{*}$
Group C	10	Fast 89 ± 10	60 ± 8
High energy diet (HE).	8	Fed $437 \pm 10^{*}$	$92 \pm 15^{*}$
Group D	10	Fast 395 ± 55	28 ± 9
High energy diet (HE)	8	Fed $425 \pm 8^{*}$	$318 \pm 61^{*}$
+insulin 2 U/24 h. Group D+I	12	Fast 110 ± 20	320 ± 40

* $P < 0.001$ vs groups SB and LE; $\ddagger P < 0.001$ vs groups D and D+I.

Table 4 Biochemical data in normal and diabetic *Psammomys*

Group	No. of animals	Blood Na (mEq/l)	Creatinine clearance (ml/min)	$U_{Na}V$ (μ Eq/min)	U_KV (μ Eq/min)	$U_{prot.}$ (10^{-3} g/24 h)	Kidney weight (g)
Saltbush diet (SB)	11	151.2 ± 3.1	0.44 ± 0.04	$6.0 \pm 0.5^{*}$	$2.08 \pm 0.20^{*}$	0.49 ± 0.06	$1.06 \pm 0.03^{*}$
Laboratory artificial diet.	12	150 ± 2.2	0.52 ± 0.07	0.32 ± 0.07	0.99 ± 0.12	0.40 ± 0.40	0.93 ± 0.03
Low energy (LE)							
High energy diet (HE).	10	152.2 ± 1.1	$0.96 \pm 0.07^{*}$	$1.19 \pm 0.10^{*}$	$1.94 \pm 0.19^{*}$	$3.81 \pm 0.96^{*}$	1.03 ± 0.06
Group C1 (GFR $\uparrow$)							
High energy diet (HE).	14	150.8 ± 0.8	$0.27 \pm 0.03^{*}\ddagger$	0.59 ± 0.07	0.89 ± 0.08	$1.48 \pm 0.50^{*}$	0.98 ± 0.03
Group C2 (GFR $\downarrow$)							
High energy diet (HE).	6	150.1 ± 3.0	0.66 ± 0.11	0.52 ± 0.16	1.01 ± 0.05	$3.75 \pm 0.75^{*}$	$1.05 \pm 0.05^{**}$
Group D							
High energy diet (HE)	8	149 ± 2.1	0.54 ± 0.20	0.38 ± 0.13	0.64 ± 0.14	$4.35 \pm 1.50^{*}$	$1.25 \pm 0.06^{*}$
+insulin 2 U/24 h. Group D+I							

* $P < 0.001$ vs group LE.

$\ddagger P < 0.001$ vs groups LE and C1.

** $P < 0.05$ vs group LE.

$U_{Na}V$, urinary Na excretion; U_KV , urinary K excretion; $U_{prot.}$, urinary protein excretion.

difference in the glucose levels of the *Psammomys* fed the native, saltbush diet, low energy/high salt diet (SB group) and in those fed low energy/low salt artificial laboratory diet (LE group). The blood glucose levels of the animals in the above two groups at the fed state were less than 140 mg/dl. The glucose level following food intake in the Type II diabetic *Psammomys* (group C) was high (351 ± 13 mg/dl).

The *Psammomys* which developed Type I diabetic state (group D) showed very high blood glucose levels in the fasting state compared with all other groups studied, 395 ± 55 mg/dl, and external insulin supplement reduced it dramatically to 110 ± 20 mg/dl.

Insulin levels

The plasma insulin levels were similar in both control groups (low energy/high salt and low energy/low salt

diets: SB and LE). The plasma insulin levels were higher in the groups consistent with the diabetic state except for group D in which the hyperglycemia was accompanied with relative hypoinsulinemia. Still, in this group the values of insulin levels were higher compared with the control groups at fed state (92 ± 15 μ U/ml vs 23 ± 8 or 28 ± 8 , $P < 0.001$).

Kidney function

Data regarding kidney function are listed in Table 4. The natural saltbush diet (SB) is actually a low energy/high salt diet with a similar caloric content as the low energy/low salt diet (LE) (Table 2). According to the data in Table 4, the animals that were fed on saltbush diet (low energy/high salt diet) represent the basic natural state of the *Psammomys*. The results show also that there were no differences between the GFR of saltbush animals (low

Table 5 Blood Na levels, GFR, Na excretion, filtered load of Na (FL of Na) and absolute reabsorption of Na (AR of Na) in normal and diabetic *Psammomys*

Group	No. of animals	Blood Na (mEq/l)	GFR (ml/min)	FL of Na (mEq/24 h)	Na excretion (mEq/24 h)	AR of Na (mEq/24 h)
Saltbush diet (SB)	11	151.2 ± 3.1	0.44 ± 0.04	95.67 ± 10	8.64 ± 0.9	87.03 ± 10.2
Laboratory artificial diet.	12	150 ± 2.2	0.52 ± 0.07	112.32 ± 5.2	0.46 ± 0.08	111.86 ± 4.9
Low energy (LE)						
High energy diet (HE).	10	152.2 ± 1.1	0.96 ± 0.07	210.40 ± 20*	1.71 ± 0.12	208.69 ± 19*
Group C1 (GFR↑)						
High energy diet (HE).	14	150.8 ± 0.8	0.27 ± 0.03	58.63 ± 5.2	0.85 ± 0.06	57.78 ± 6.1
Group C2 (GFR↓)						
High energy diet (HE).	6	150.1 ± 3.0	0.66 ± 0.11	142.60 ± 7.8*	0.75 ± 0.15	141.85 ± 8*
Group D						
High energy diet (HE) + insulin 2 U/24 h. Group D+I	8	149 ± 2.1	0.54 ± 0.20	115.86 ± 11	0.55 ± 0.12	115.31 ± 11.2

* $P < 0.05$ vs SB and LE.

energy/high salt diet) and the low energy diet *Psammomys* (i.e. low energy/low salt diet) which shows that the saltbush diet fed animals handled themselves exactly as the animals on low energy/low salt diet. In the *Psammomys* fed saltbush (SB) diet, sodium excretion in the urine was 20 times higher compared with that in animals fed the low energy (LE) diet. In the SB fed *Psammomys* there was also an increase in kidney weight as compared with animals fed laboratory diet. In contrast, the sodium blood levels did not differ between SB diet animals and LE diet *Psammomys*.

In the early phase of diabetes, usually after the appearance of hyperglycemia, GFR is found to be elevated above that of age-matched normal controls. In established diabetic nephropathy GFR declines progressively and relentlessly towards end-stage renal failure (Viberti *et al.* 1994).

Accordingly, the *Psammomys* that were fed HE diet and developed Type II diabetes (group C) were divided into two subgroups consistent with their GFR: (a) group C1 with GFR higher than the control (0.96 ± 0.07 ml/min, $P < 0.001$ vs control LE group), and (b) group C2 with GFR values below that in the control group (0.27 ± 0.03 ml/min, $P < 0.001$ vs control group). In both groups there was a tendency to an increase in kidney weight as compared with animals fed LE diet. The difference, however, did not reach a statistical significance. The diabetic state of these *Psammomys* was also characterized by an increase in urinary protein excretion as compared with non-diabetic animals (3.81 ± 0.96 and 1.48 ± 0.15 respectively, $P < 0.001$).

In the *Psammomys* that developed Type I diabetes (group D), there was an increase in kidney weight, in GFR, in sodium excretion and in proteinuria, as compared with the control group.

The *Psammomys* of group D+I treated with external insulin (2 U/24 h) still showed an increase in kidney weight and proteinuria, but their GFR and sodium excretion decreased to control values, 0.54 ± 0.2 ml/min and 0.38 ± 0.13 μ Eq/min respectively.

The diabetic kidney is characterized by hyperabsorption of sodium. Table 5 depicts the calculated absolute reabsorption of sodium in the kidney of the different groups studied. In the diabetic animals (group C1) the calculated absolute reabsorption is 208 ± 19 mEq/24 h and in group D it is 141.8 ± 8 mEq/24 h and it is high compared with low energy/low sodium (group LE) *Psammomys* in which absolute Na reabsorption is 111.86 ± 4.9 mEq/24 h ($P < 0.005$, group LE vs group C1 and group D), see also Table 5.

Na-K-ATPase activity

The Na-K-ATPase activity in the cortex and outer medulla in the different experimental groups is depicted in Table 6 and Figs 1 and 2.

The Na-K-ATPase activity was similar in the cortex and medulla of both low energy diet groups: *Psammomys* fed native saltbush diet and those fed the low energy artificial laboratory diet (groups SB and LE) (Fig. 1).

Table 6 Na-K-ATPase activity of *Psammomys* in the cortex and medulla in the different diabetic states

Group	No. of animals	Cortex (μ mol P_i /mg prot. per h)	Medulla (μ mol P_i /mg prot. per h)
Salt bush (SB)	6	38.7 ± 8	59.7 ± 5
LE	8	35.9 ± 3	52.5 ± 5
C1	8	72.5 ± 15 ^a	150.8 ± 16 ^c
C2	6	39.8 ± 5 ^e	77.2 ± 14 ^d
D	8	61.9 ± 8 ^a	158.2 ± 21 ^c
D+I	8	39.2 ± 8	94.2 ± 13 ^b

^a $P < 0.05$ vs SB and LE groups.^b $P < 0.02$ vs SB and LE groups.^c $P < 0.001$ vs SB and LE groups.^d $P < 0.005$ vs C1.^e $P < 0.05$ vs C1.

Figure 1 Microsomal Na-K-ATPase activity in renal cortex and medulla of *Psammomys* on saltbush (low energy/high salt) and on an artificial laboratory (LE) (low energy/low salt) diet. Bars are means $\pm$ S.E. For saltbush, $n=6$; and for LE, $n=8$. There were no differences in enzyme activity between the two groups, both in the cortex and in the medulla.

Figure 2 Regression analysis of GFR (ml/min) with Na-K-ATPase activity ($\mu\text{mol Pi/mg prot. per h}$) in the cortex and the medulla in all groups studied. ■, values for the cortex, $R=0.862$, $P<0.025$; ●, values for the medulla, $R=0.798$, $P<0.05$.

Figure 2 represents the regression analysis of GFR with Na-K-ATPase activity in the cortex and medulla in all groups studied. There was an increase in Na-K-ATPase activity in the cortex and the medulla of the Type I (group D) and Type II (group C1) diabetic *Psammomys* with the high GFR levels, while the enzyme activity in cortex and medulla was decreased towards control values in *Psammomys* with diabetes but lower or normal GFR (groups C2 and D+I) (Fig. 2, Table 6). The elevation in GFR was associated with a marked increase in Na-K-ATPase activity of the medulla and, to a lesser extent, of the cortex. The relationship between GFR and Na-K-ATPase activity was statistically significant ($P<0.025$ for the cortex, $P<0.05$ for the medulla). This verifies that this relationship is indeed real.

Discussion

The results of the present study demonstrated similar kidney function (Table 4) and Na-K-ATPase activity

(Table 6, Fig. 1) in *Psammomys* fed native saltbush (low energy/high salt) and those fed a low energy (LE) laboratory diet (low energy/low salt). In both groups the kidney responded in a similar fashion. Blood glucose, plasma insulin levels, creatinine clearance and urinary protein excretion did not differ between the two groups of *Psammomys* (Tables 3 and 4). However, as expected, there was a difference between the groups in urine sodium excretion reflecting differences in dietary sodium intake. Thus, the animals fed the laboratory (LE) diet (low energy/low salt) could serve as a control group for all other experimental groups.

In nature *Psammomys* obtain all their water from their solid food, the saltbush leaves. When transferred to laboratory conditions the transition to the high energy diet leads to the emergence of diabetes mellitus. This abnormality stems, at least partly, from primary insulin resistance and altered physical activity (Ziv *et al.* 1996).

The effect of diabetes on kidney function

Psammomys are prone to develop obesity, hyperglycemia and hyperinsulinemia when fed on a high energy diet (Kalman *et al.* 1993, Ziv & Shafir 1995). In our study the *Psammomys* that developed diabetes on a high energy (HE) diet were subdivided according to their kidney function: (1) group C1 with high creatinine clearance (0.96 ± 0.07 ml/min) typical of the early diabetic state in general, and (2) group C2 with low creatinine clearance (0.27 ± 0.03 ml/min) similar to diabetic chronic renal disease. The GFR distribution of group C was clearly bimodal. The cut-off point that was selected to divide the groups was a GFR of 0.5 ml/min. Animals with a GFR of less than 0.5 ml/min were in the low GFR group (C2) and those with a GFR exceeding 0.5 ml/min were in the high GFR group (C1). Proteinuria was evident in both subgroups (Table 4).

The evolution of diabetic nephropathy in men follows three stages (Viberti *et al.* 1994). The early stage is characterized by the presence of microalbuminuria and increased GFR. Insulin treatment with adequate metabolic control lowers the GFR almost to normal values (Schmitz *et al.* 1989). The subsequent stages are characterized by proteinuria above 0.5 g/day, followed by a decrease in GFR.

The *Psammomys* with diabetes and high GFR (group C1) represent the early stage of the diabetic kidney (high GFR and proteinuria), while the reduced kidney function of the *Psammomys* of group C2 (GFR lower than control and the proteinuria higher than control) represents the progression of diabetic nephropathy leading to chronic renal failure.

The present results in group C1 are compatible with similar findings both in men and experimental animals (Ditzel & Junken 1972, Morgensen 1972, Gartner 1978).

The average increment in GFR of group C1 was about 80% of control values.

Glomerular hyperfiltration is a common finding in clinical and experimental diabetes mellitus as reported by us and others (Morgensen 1972, Canney *et al.* 1979, Ku & Meezan 1984, Wald & Popovtzer 1984, Ku *et al.* 1986, Khaduri *et al.* 1987). However, the increase in GFR is poorly understood. In our present experiments, in the *Psammomys* the differences in GFR were not related to hyperglycemia or to hyperinsulinemia.

Another distinct diabetic subgroup that emerged in this work was characterized by hyperglycemia after fast with hypoinsulinemia (group D). These features are characteristic of Type I diabetes. Treatment with insulin, which lowered blood glucose levels to 110 ± 20 mg/dl after fast, corrected most of the metabolic parameters towards normal values including GFR. However, proteinuria and kidney weight remained high.

The relation between Na-K-ATPase and GFR

No difference was observed in the enzyme activity in the cortex and medulla or in the GFR between the animal groups fed native saltbush diet (SB; low energy/high sodium) and those on low energy laboratory diet (LE; low energy/low sodium) (Fig. 1, Table 4).

In the *Psammomys* with hyperglycemia and hyperinsulinemia (groups C1 and C2) the enzyme activity corresponded with variations in GFR. Thus, in group C1 with high GFR, the Na-K-ATPase activity was significantly higher both in the cortex and in the medulla as compared with control animals (SB and LE groups), while in group C2, with low GFR, it did not differ from the control animals (Table 6, Fig. 2).

The increase in Na-K-ATPase activity (in cortex and medulla) in diabetic *Psammomys* with high GFR (group C1) was similar to that which was previously observed in rats with streptozotocin-induced diabetes mellitus (Ku & Meezan 1984, Wald & Popovtzer 1984, Ku *et al.* 1986).

In the *Psammomys* with fasting hyperglycemia and hypoinsulinemia, that represent Type I diabetes mellitus (group D) with increased GFR (0.66 ± 0.11 ml/min), Na-K-ATPase activity was elevated both in the cortex and in the medulla. Insulin treatment in this group (group D+I) reduced GFR towards control values (0.54 ± 0.2 ml/min) and accordingly led to a fall in Na-K-ATPase toward control values in the cortex and in the medulla (Table 6, Fig. 2).

High blood glucose and insulin levels in *Psammomys* featuring Type II diabetes mellitus (group C2) with a fall in GFR (0.27 ± 0.03 ml/min) below the levels observed in control group did not affect Na-K-ATPase, which was similar to that of the control groups.

In groups C1 and C2 that were similar with regard to blood glucose and insulin levels, there was a significant difference in the enzyme activity that correlated directly

with the variations in GFR. Taken together, these results demonstrate that neither blood glucose control nor plasma insulin levels influence Na-K-ATPase activity in the present experiments.

Glomerular hyperfiltration that was observed in animals with Type II diabetes mellitus (group C1) and Type I diabetes mellitus (group D) was associated with increased Na-K-ATPase activity. By contrast, in Type II diabetes mellitus with diabetic nephropathy (group C2) manifested by reduced GFR, and in animals with diabetes mellitus Type I treated with insulin (group D+I) with normalized blood glucose levels (after fasting) and normalized GFR, Na-K-ATPase activity declined towards control levels (Table 6, Fig. 2).

Figure 2 depicts the regression analysis of GFR with Na-K-ATPase activity in the cortex and medulla of the different groups studied. An increase in GFR correlated with Na-K-ATPase activity in the medulla and in the cortex. The relationship between GFR and Na-K-ATPase activity was statistically significant ($P < 0.025$ for the cortex, $P < 0.05$ for the medulla). This verifies that this relationship is indeed real. These findings are similar to previous observations in rats with streptozotocin-induced diabetes mellitus (Wald & Popovtzer 1984, Wald *et al.* 1986, Khaduri *et al.* 1987). In the rat, Na-K-ATPase activity increased in the cortex and medulla parallel with an increase in GFR.

Our results show that in the different diabetic states in the *Psammomys*, Na-K-ATPase activity in the cortex and in the medulla are only influenced by GFR levels and not by blood glucose levels and/or plasma insulin levels. The major difference between rats with streptozotocin-induced diabetes mellitus and *Psammomys* with diabetes mellitus (group D) relates to the effect of hyperglycemia *per se* (before an increase in GFR occurs) which in rats enhances Na-K-ATPase activity in the proximal convoluted tubule (PC) and proximal straight tubule (PS), presumably due to stimulated proximal Na-glucose co-transport. This GFR-independent effect was not observed in *Psammomys*. Thus, the *Psammomys* resembles the rat with regard to its response to changes in GFR but differs with respect to its response to changes in serum glucose and/or insulin levels. These findings emphasize the importance of species differences in the study of diabetes mellitus.

The increased Na-K-ATPase activity reflects enhanced tubular reabsorption of Na along the nephron in the early phases of both Type I and Type II diabetes mellitus. Thus, it appears that glomerular hyperfiltration is a common denominator for the rearranged glomerulotubular balance in the diabetic kidney. It has been emphasized that in the diabetic kidney not only hyperfiltration but also hyperabsorption may play an important role in renal insufficiency by causing interstitial inflammations followed by fibrosis. There is a direct relationship between interstitial fibrosis and the degree of renal diseases in general (Schainuck *et al.* 1970). The Na-K-ATPase activity as

alluded to before reflects tubular Na reabsorption and therefore it may be a marker of an increased risk of renal damage. It has been suggested that hyperfiltration is the mechanism for progressive glomerular damage in diabetic nephropathy (Zatz *et al.* 1985). Therefore, the use of angiotensin converting enzyme (ACE) inhibitors that reduce intraglomerular pressure (Lewis *et al.* 1993) has been proposed as a protective measure to prevent progressive renal failure. Our findings suggest that the diabetic kidney is not only a hyperfiltering but also a hyperabsorbing organ. Both hyperfiltration and the resulting hyperabsorption, as suggested above, may play an important role in the progression of diabetic nephropathy.

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