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Decreased Intravenous Glucose Tolerance and Low Plasma Insulin Response in Spiny Mice* (*Acomys cahirinus*)

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Summary. A technique was developed to permit estimation of intravenous glucose tolerance and the concomitant immunoreactive insulin (IRI) response in the Geneva colony of the semi-desert rodent *Acomys cahirinus*. Following intravenous injection of glucose 1.5 g/kg, young *Acomys* (2—3 months, 25—35 g body weight) had a significantly lower glucose tolerance ($p < 0.01$) and a smaller 30 min integrated plasma IRI response ($p < 0.001$) than age and weight matched albino mice. With increasing age (8 and 21 months) and body weight (57 to 100 g) of the *Acomys*, glucose tolerance and plasma IRI response decreased further, and in the 21 month old group there were 2 overtly diabetic animals. There was a significant negative correlation between intravenous glucose tolerance and body weight in older *Acomys*. The results suggest that pancreatic B-cell responsiveness to glucose is impaired in *Acomys* of all ages, and that this is associated with poor glucose tolerance which may be aggravated by obesity.

Key words: Spiny mouse (*Acomys cahirinus*), intravenous glucose tolerance, insulin in plasma, obesity, endocrine pancreas, insulin secretion.

Introduction

The occurrence of a syndrome of carbohydrate intolerance and obesity, associated with considerable congenital hyperplasia of the islets of Langerhans, has previously been described in our colony of spiny mice, *Acomys cahirinus* [5, 8, 10]. Earlier experiments suggested that insulin release might be impaired in the *Acomys* [13], and more recently Cameron *et al.* [2, 3] demonstrated that plasma IRI responses to *intraperitoneally* administered glucose, theophylline, isoprenaline, or cyclic AMP were lower in *Acomys* than in Swiss albino mice. These experiments were limited by the absence of superficial veins in this semi-desert rodent, the inability to use intravenous injections hampering detailed analysis of glucose tolerance and associated insulin responses. The present experiments were made possible by the development of a technique permitting *intravenous* glucose administration and serial blood sampling in the *Acomys*.

Materials and Methods

Intravenous glucose tolerance was compared between normoglycemic *Acomys* and age- (2 to 3 months) and weight- (25 to 35 g) matched Swiss albino mice. In addition, older *Acomys* at the age of 8 months (57 to 79 g) and of 21 months (57 to 100 g) were tested. In the latter group there were two diabetic animals. All animals were bred in our own laboratory and fed *ad libitum* until the time of experimentation between 9—10 a.m. The animals were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Illinois, U.S.A.) 50 mg/kg injected intraperitoneally and 25 mg/kg subcutaneously. A femoral vein was exposed and, using a butterfly infusion set with a G 25 short needle (Abbott), glucose 1.5 g/kg was injected as a 20% solution during 50 to 60 sec. Injection was carried out by visualization through a binocular dissecting microscope. After the test the wound was closed with metal clips. The animals tolerated the procedure well.

Sequential blood sampling was performed by puncture of the retro-orbital venous plexus [12] using heparinized hematocrit capillary tubes before and 2, 5, 15 and 30 min after the injection of glucose. A total of 500 to 600 µl of blood was withdrawn from each animal in one test. The individual blood samples were immediately transferred to heparinized microcentrifuge tubes, kept at 4° C and, within 30 min, centrifuged for 3 min in a microcentrifuge (Greiner Electronic, Langenthal, Switzerland).

Plasma glucose was determined on 5 µl samples in duplicate using a glucose-oxidase method (GOD-POD kits, kindly supplied by Boehringer Mannheim, Germany).

Immunoreactive insulin (*IRI*) was measured on 10 µl samples in duplicate, using the charcoal separation method of Herbert *et al.* [6] adapted to small volumes. Assay incubations were carried out in small

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(350 µl capacity) plastic microcentrifuge tubes (Beckman Corp., Palo Alto, California, U.S.A.) in a total volume of 110 µl: 10 µl plasma or insulin standard (kindly donated by Dr. J. Schlichtkrull, Novo Industri, Copenhagen, Denmark), 50 µl anti-pork insulin guinea pig serum (kindly donated by Dr. P. Wright, Indianapolis, Indiana, U.S.A.), and 50 µl (about 5 pg) ^{125}I-insulin, iodinated by the method of Hunter and Greenwood [7] and purified on G-25 fine Sephadex (Pharmacia, Uppsala, Sweden). Human insulin was used as standard for assay of *Acomys* plasma samples, since *Acomys* plasma dilutions were parallel to human (or pork) insulin standards (but not to rat or mouse standards), assayed with anti-pork insulin serum. Mouse insulin was used as standard for assay of albino mouse plasma samples. Insulin standards, antiserum and ^{125}I-insulin were diluted in 0.2 M glycine buffer pH 8.8, containing 0.25 g/100 ml human serum albumin (Swiss Red Cross, Bern, Switzerland). After incubation for 2 to 3 days at 4°C, 20 µl of beef serum (obtained from the local abattoir) was added as a protein carrier to tubes with insulin standards, and 20 µl of beef serum diluted with an equal volume of glycine buffer was added to tubes with plasma samples. Antibody-bound insulin was then separated from the unbound fraction by addition of 50 µl of a suspension of 3.0 g charcoal (C 5260 Sigma Chem., St. Louis, Missouri, U.S.A.) and 0.25 g dextran-70 (Pharmacia, Stockholm, Sweden) in 100 ml glycine buffer, pH 8.8, without human serum albumin. After a 3 min centrifugation in the microcentrifuge the supernatants were aspirated and the charcoal pellets counted for 5 min in a Gamma Scintillation Spectrometer (Packard, Downers Grove, Illinois, U.S.A.). The minimal sensitivity of this assay was 0.5 ng/ml. Between 0.5 and 2.0 ng/ml, the interassay coefficient of variation was 13% and the intraassay coefficient was 8%.

Results

Fig. 1 compares plasma glucose (mean ± SEM) after intravenous injection of 1.5 g/kg of glucose into the femoral vein of albino mice (shaded area) and *Acomys* of different ages. Preinfusion (0 min) plasma glucose values were similar in the different groups. 2 min after the glucose infusion, plasma glucose levels were significantly higher for the 8 and 21 month old *Acomys* than for the 2 to 3 month old *Acomys* or albino mice ($p < 0.001$), possibly reflecting the decrease in extracellular space relative to body weight in the older, more obese *Acomys*. The subsequent decreases of plasma glucose are calculated as per cent of glucose cleared per min (K_g) as shown in Table 1. Glucose disposal (K_g) was significantly lower ($p < 0.01$) in 2 to 3 month old *Acomys* (1.98 ± 0.22% per min) than in age- and weight- matched albino mice (3.08 ± 0.25% per min). Glucose disposal was decreased further in the older *Acomys*, although the difference between *Acomys* of different ages was not significant. There was, however, a significant negative correlation between glucose disposal and body weight ($r = -0.51$, $p < 0.01$) in the older *Acomys* (8 and 21 months) (Fig. 3, upper panel).

Table 1 indicates that preinfusion plasma glucose values were similar in all four normoglycemic animal groups, but were significantly elevated ($p < 0.001$) in the two 21 month old diabetic *Acomys*. Furthermore, Table 1 indicates that preinfusion plasma IRI levels were similar in the young *Acomys* (1.4 ± 0.2 µg/ml) and the age and weight matched albino mice (1.7 ± 0.4 ng/ml). 8 months old *Acomys* had significantly higher preinfusion plasma IRI levels (3.7 ± 0.4 ng/ml, $p <$ 0.01), than did *Acomys* aged 2 to 3 months, whereas the elevation was less in the 21 month old *Acomys* group (2.0 ± 0.4 ng/ml, NS). In the two diabetic animals there was no elevation of preinfusion plasma IRI (1.67 and 0.75 ng/ml.

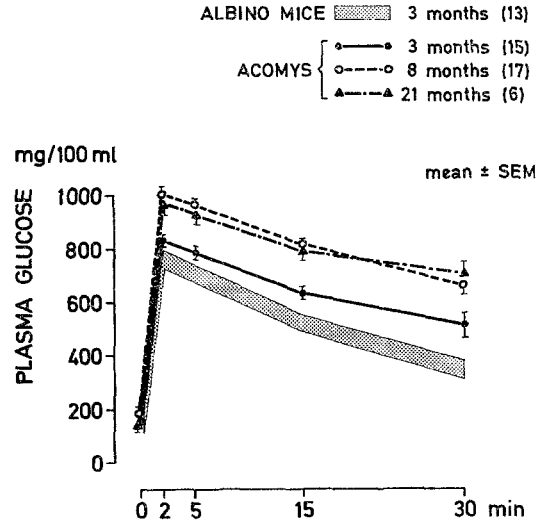

Fig. 1. Plasma glucose before, and at 2, 5, 15 and 30 min after intravenous injection of glucose 1.5 g/kg in albino mice, age 2–3 months (shaded area, representing mean ± SEM, n = 13); *acomys*, age 2–3 months (●——● n = 15); *acomys*, age 8 months (o------o n = 17); and *acomys* age 21 months (▲—·—▲ n = 6). Mean ± SEM are given

Fig. 2 compares the increase in plasma IRI from preinfusion levels after infusion of glucose in the different animals. The differences in the plasma IRI responses between the group of albino mice and any of the group of *Acomys* were most marked at 2 min ($p <$ 0.001) and at 5 min ($p < 0.01$) after glucose injection. At 15 and 30 min the differences were smaller ($p < 0.05$) or not statistically significant. Plasma IRI responses are also calculated as insulinogenic indices, obtained by dividing the incremental plasma IRI values integrated over 30 min following the injection of glucose by the corresponding incremental integrated plasma glucose values. Table 1 shows that the insulinogenic index for 2 to 3 month old *Acomys* (47.6 ± 11.6) was significantly lower ($p < 0.001$) than that for albino mice ($137.2 \pm$ 17.6). The insulinogenic indices were further decreased

in the 8 months (11.4 ± 5.8) and 21 months (26.0 ± 14.5) *Acomys*, and the lowest indices were observed in the two diabetic *Acomys* (6.5 and 8.3).

Discussion

The intravenous injection procedure and a micro-assay for the measurement of IRI as described in this report enabled us to study the kinetics of insulin release during glucose tolerance tests in *Acomys cahirinus*. This rodent has previously been shown to be defective in insulin secretion [2] and to have an increased incidence of spontaneous diabetes [5]. Although none of the young *Acomys* tested were hyperglycemic or glucosuric, the intravenous glucose tolerance in these animals was clearly reduced when compared to age and weight matched albino mice. Obviously, since no criteria are available, we cannot state whether or not these animals should be considered as chemical diabetics, but the finding of two diabetic animals among the 21 month old *Acomys* group would favour this possibility. The insulin response to the sudden elevation of blood glucose appeared to be as rapid in the young *Acomys* as in the albino mice, but the

Table 1. *Glucose disposal (K_g-rate), preinfusion plasma glucose and IRI levels, and insulinogenic indices (Δ IRI/Δ Glucose) during intravenous glucose tolerance test in albino mice and Acomys*

Rodent	Age months	(n)	K_g rate % per min	p *vs.* mice	Preinfusion glucose mg/100 ml	p *vs.* mice	Preinfusion IRI ng/ml	p *vs.* mice	$\frac{\Delta \text{ IRI}}{\Delta \text{ Glucose}}$	p *vs.* mice
Albino mice	2–3	(13)	3.08 ±0.25	—	176.0 ± 13.3	—	1.7 ±0.4	—	137.2 ± 17.6	—
Acomys	2–3	(15)	1.98 ±0.22	< 0.01	153.7 ± 10.0	NS	1.4 ±0.2	NS	47.6 ± 11.6	< 0.001
Acomys	8	(17)	1.62 ±0.19	< 0.001	182.6 ± 13.9	NS	3.7 ±0.4	< 0.005	11.4 ± 5.8	< 0.001
Acomys	21	(6)	1.49 ±0.32	< 0.005	135.2 ± 9.8	NS	2.0 ±0.4	NS	26.0 ± 14.5	< 0.001
Acomys diabetic	21	(a)	0.6		533		1.67		6.5	
		(b)	1.22		534		0.75		8.3	

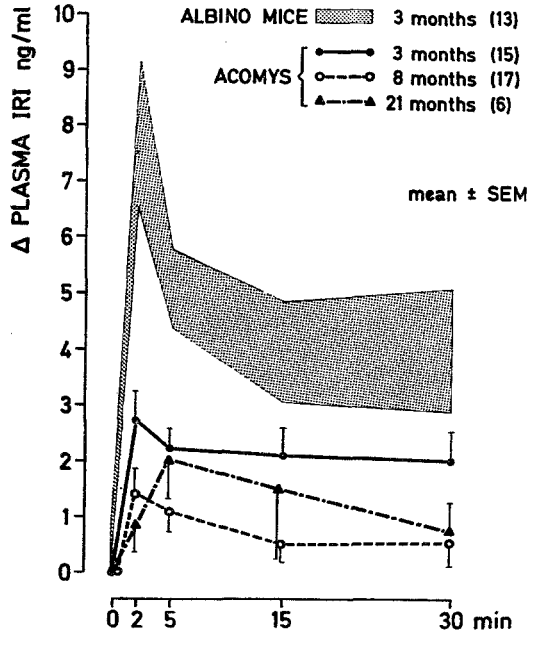

Fig. 2. Increase (Δ) in plasma IRI above preinfusion levels 2, 5, 15 and 30 min after intravenous injection of glucose. Symbols as for Fig. 1

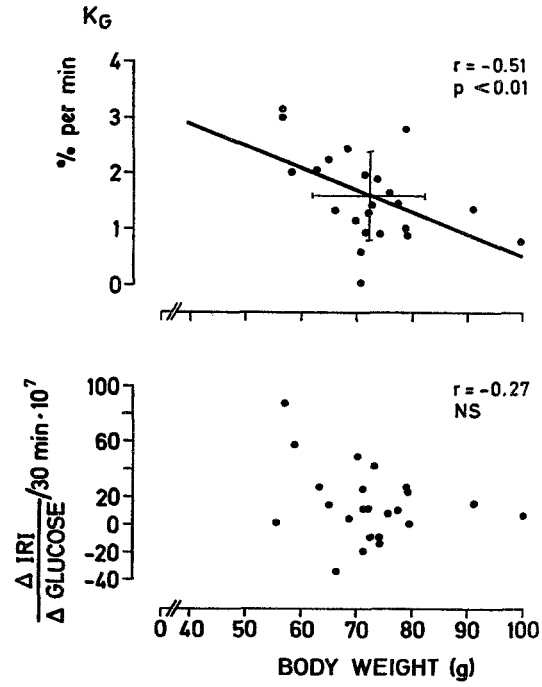

Fig. 3. Correlation between glucose disposal (K_g, panel above) or insulinogenic index (Δ IRI/Δ glucose, panel below) and body weight of individual *Acomys* age 8 and 21 months (n = 23) after intravenous injection of glucose 1.5 g/kg. Mean ± 1 S.D. are shown for K_g rate and body weight. Insulinogenic index is calculated as incremental plasma IRI values integrated over 30 min following injection of glucose, divided by the corresponding area under the plasma glucose curve

magnitude of the response was markedly diminished. Although in the *Acomys* the lessening of the insulin response was more pronounced soon after glucose administration, both its initial and late phases were affected. This is also shown by the markedly lower insulinogenic index values for the integrated (30 min) insulin response, which mainly reflects the late secretion phase. In these respects the *Acomys* is reminiscent of human subjects with chemical diabetes or low insulin response in whom glucose tolerance is slightly to moderately reduced, while insulin response to glucose is markedly diminished (with emphasis on the initial response) [4]. It must be borne in mind, however, that the glucose infusions presented in this report were performed while the animals were under Nembutal anesthesia, a condition which has been shown to affect both glucose tolerance and insulin secretion [1]. Therefore, whether the glucose and insulin data given here do represent the actual *in vivo* situation in the animals must remain an open question.

Acomys develop moderate obesity with increasing age [5], and obesity is known to be accompanied by enhanced insulin secretion in many animal species [3] and in man [9]. In the *Acomys* of older age groups (8 and 21 months) the level of circulating insulin before glucose administration was indeed higher than in the younger and leaner animals. However, no enhancement of the insulin response to glucose injection could be observed in these obese *Acomys*. There was no correlation between the insulinogenic index and body weight in the older animals (Fig. 3, lower panel) in spite of the wide range in weights (57 to 100 g). Nevertheless, the lower insulin response to glucose demonstrated in the older *Acomys* suggests that B-cell responsiveness decreases with age. This possibly masks the expected increase in insulin response with obesity. Furthermore, the B-cell defect presumably inherent in the *Acomys* may limit the free expression of obesity as a stimulating factor for insulin secretion. A similar situation appears to exist in mice of the obese hyperglycemic strain DBM in which the more hyperglycemic animals demonstrate higher basal (fasting) plasma insulin levels but smaller insulin responses to glucose [3].

Since obesity is associated with insulin resistance [11] one would expect deterioration of glucose tolerance in the *Acomys* whose insulin secretion was not enhanced by obesity. Indeed, as shown in Fig. 3, a significant negative correlation was found between glucose tolerance (K_g) and body weight in the higher age group of *Acomys*, where obesity is more apparent. Since glucose tolerance of 21 month old animals (the diabetic animals excluded) was not significantly different from that of 8 months old *Acomys*, it is probable that this lesser glucose tolerance may be more closely related to obesity than to ageing. The eventual development of marked or severe glucose intolerance in *Acomys* may depend on the balance between the gradual reduction in insulin response with age, the degree of enhancement of the islet function induced by obesity, and the severity of resistance to insulin that may accompany this state.

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