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RESEARCH ARTICLE

Overnutrition in spiny mice (*Acomys cahirinus*): β-cell expansion leading to rupture and overt diabetes on fat-rich diet and protective energy-wasting elevation in thyroid hormone on sucrose-rich diet

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Abstract

Previous studies The investigation of diabetes propensity in spiny mice, performed in Geneva and Jerusalem colonies, is reviewed. Spiny mice live in semi-desert regions of the eastern Mediterranean countries. Those transferred to Geneva in the 1950s were maintained on a rodent diet supplemented by fat-rich seeds. They became obese, exhibited pancreatic islet hyperplasia and hypertrophy. Low insulin secretion response was characteristic of this species, despite ample pancreatic content of insulin. After a few months, diabetes with ketosis occurred, often suddenly, in association with islet cell disintegration. In Jerusalem the spiny mice were collected from their native habitat and placed on diets containing 50% sucrose or fat-rich seed diets. On a sucrose-rich diet, spiny mice developed hepatomegaly, lipogenic enzyme hyperactivity, and elevation in very low density lipoproteins as a result of metabolism of the fructose component mainly in the liver. No overt diabetes or pancreatic islet disintegration were observed, although insulin content and β-cell hypertrophy and hyperplasia were apparent. On a fat-rich diet, spiny mice exhibited marked weight gain, adipose tissue growth and low hepatic lipogenesis. The obesity was accompanied by mild hyperglycemia and hyperinsulinemia with glucose intolerance leading to an occasional glucosuria after several months on the diet.

Novel experiments The sucrose diet induced an extrathyroidal elevation of triiodothyronine (T_3). Serum T_3 level and hepatic T_4–T_3 conversion were increased, while serum T_4 levels tended to decrease. The activity of the T_3-inducible hepatic mitochondrial FAD-glycerophosphate oxidase and K^+/Na^+-ATPase, as well as body temperature were increased, indicating that the sucrose diet was associated with enhanced thermogenesis and energy-wasting metabolic cycling. The sucrose-rich diet might exert an adaptive thermogenesis-mediated defense mechanism, protecting against excessive weight gain and disruptive pancreatic islet lesion. After 18 months maintenance on sucrose-rich versus fat-rich diets the number of animals surviving was significantly higher on the sucrose diet whereas on the fat diet a significant number of animals succumbed to expansive islet cell disruption and diabetes. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords nutritional diabetes; sucrose-rich diet; fat rich diet; spiny mice; beta cell proliferation; T_3 elevation; T_3 effects; hepatic lipogenesis; islet insulin content; islet cell disruption

Introduction

Spiny mice (*Acomys cahirinus*) live in the arid areas of eastern Mediterranean countries. They are relatively large light-brown mice, weighing between 30 and 50 g, with fur bristles on their backs, hence their common 'spiny' name. The interest in spiny mice as a model of diabesity (diabetes + obesity) arose when overweight, glucosuria and ketosis were discovered by chance by Gonet *et al*. [1] and Pictet *et al*. [2] from the Renold group in Geneva. The diabetic mice were kept as pets in bird cages and maintained on bird food, rich in pumpkin, sesame and sunflower seeds. The mice were transferred to laboratory rodent chow supplemented by seeds, believed to enhance their fertility. In a series of studies performed in Geneva, it was established that spiny mice manifest low insulin secretion capacity, despite pancreatic islet hypertrophy and hyperplasia, prior to the appearance of overt diabetes [1–4]. Since up to 15 years and about 40 generations elapsed between the transfer of the wild mice from Israel to Switzerland and the discovery of diabetes, it was suggested by Renold *et al*. [5] that spiny mice might have undergone a mutation on the affluent nutrition in captivity, characterized by defective insulin release and compensatory islet replication.

It is the purpose of this article to present an overview of the diabetes research performed in spiny mice and to present the islet insulin accumulation and hyperplasia as a model for the induction of diabetes. The deleterious effects of the fat- and sucrose-rich diets on the endocrine pancreas and possible islet disintegration are compared. The sucrose-rich diet did not lead to the outbreak of overt diabetes and ketosis. In novel investigations, it was found that T_3 elevation occurs in association with sucrose intake leading to several energy wasting mechanisms. Islet insulin content, replication and hypertrophy were greater on fat-rich than on sucrose-rich diets. It is probable that the thermogenesis and energy-wasting processes, induced by T_3 elevation, may have exerted a protective effect against the pancreatic breakdown occurring in animals on a fat-rich diet.

Spiny mice of the Geneva colony

In Geneva, nonketotic intermittent hyperglycemia was evident at the age of 9–12 months in 10 to 20% of the animals in the colony followed by a gradual or often sudden lapse into ketosis, with disintegration of the endocrine pancreas. Plasma insulin levels in the normoglycemic mice were not elevated and no overt insulin resistance was seen, apart from impaired glucose tolerance. The mice showed a low response to various secretagogues in the initial and late phases of insulin release, including glucose, arginine, glucagon and cyclic AMP both *in vivo* and *in vitro* [6–10]. This was attributed to several factors, such as low islet adenylate cyclase activity, low cAMP response to glucose, low amount of vincristine precipitable material in β-cell microtubuli through which insulin granules are extruded, and scarcity of autonomic islet innervation [11–13]. These findings were interpreted as resulting from a genetic mutation in the animals maintained in captivity, but such responses of the islets may also be a native characteristic of animals living in a desert environment, aimed at the protection of the pancreas against sporadic overstimulation. The liver of spiny mice was quite sensitive to the inhibition of glucose production by insulin and its maximal stimulation by glucagon was low. These observations were interpreted as a compensation for the impaired insulin secretion in spiny mice [14]. In later experiments, low insulin response could be potentiated by priming with high glucose concentration [15,16]. The possibility to amplify insulin secretion from isolated islets indicates that the secretion defect is functional rather than inherent.

Islet and β-cell proliferation along with the elevation of the pancreatic insulin content were extensively investigated in the Geneva spiny mice colony. An electron-microscopic study revealed the presence of dense lysosome-rich bodies both in β and α-cells [17] raising the possibility that the excessive insulin storage in β-cell granules is contained in part by intracellular digestion. Also, the diminished α-cell activity could moderate the ketosis. However, spiny mice at the stage of diabesity were hyperinsulinemic and the prominent ultrastructural alterations were islet proliferation, overdeveloped Golgi apparatus, hypergranulation, and increased insulin content with remarkable cell polymorphism [1–5].

Spiny mice of the Jerusalem colony

A colony was established in Jerusalem of spiny mice collected in the Judean desert near the Dead Sea shore to investigate whether the progression to diabesity is a result of genetic changes during relatively affluent long-term *ad libitum* nutrition in captivity or may be evident also in newly collected animals. The spiny mice were fed a standard rodent chow, on which they grew and multiplied well during >10 years of observation. In contrast to the Geneva colony, no spontaneous hyperglycemia or glucosuria were seen in about 300 mice [18–20]. To increase the nutrient challenge, the mice were placed on a purified carbohydrate-rich diet containing 50% sucrose and on a mixture of seeds containing 29% glycerolipids. The sucrose-rich regimen resulted in a remarkable induction of hepatic enzymes of glycolysis and lipogenesis leading to hyperlipidemia, mainly the elevation of very low density lipoproteins [18–22]. The magnitude of enzyme induction and of hyperlipidemia far exceeded that in spiny mice maintained on an isocaloric starch diet and was also more prominent than in similarly sucrose-fed rats or albino mice [21]. The general metabolite and endocrine changes in spiny mice on different diets and the extensive induction of hepatic enzymes of glycolysis and lipogenesis are recapitulated and supplemented in Tables 1 and 2, and commented on, in conjunction with the novel experiments. Briefly, the sucrose-rich diet resulted in substantial elevation of pancreatic insulin content and in islet cell hyperplasia, but only in mild

Table 1. General changes in spiny mice maintained on regular, sucrose- and fat-rich diets for 6 weeks

	Regular chow	Sucrose-rich	Fat-rich
Food consumption, g/week	31.2 ± 2.5	36.3 ± 2.7[a]	28.1 ± 2.0
Initial body wt, g	34.1 ± 2.2	34.9 ± 2.1	33.9 ± 2.4
Weight gain at 6 weeks	6.0 ± 1.3	4.8 ± 1.2	9.6 ± 1.4[ab]
Liver wt, g/100 g	3.1 ± 0.2	4.6 ± 0.3[a]	3.3 ± 0.2
Epididymal fat wt, mg/100 g	785 ± 60	654 ± 50	1190 ± 95[ab]
Serum glucose, mg/dl	128 ± 5	131 ± 6	135 ± 7
Serum FFA, μmol/l	480 ± 35	765 ± 98[a]	530 ± 72
Serum triglycerides, mg/dl	146 ± 16	428 ± 48[a]	92 ± 6[a]
Serum cholesterol, mg/dl	136 ± 8	246 ± 11[a]	160 ± 12[a]
Liver triglycerides, mg/g	6.4 ± 0.3	28.5 ± 2.7[a]	10.6 ± 0.7[a]
Liver glycogen, mg/g	46 ± 3	55 ± 4	35 ± 4[a]
Serum insulin, mu/l	13 ± 5	39 ± 11[a]	49 ± 12[a]
Pancreas insulin, U/g	2.2 ± 0.3	3.9 ± 0.4[a]	4.4 ± 0.5[a]

Values are means ± SE for 12 male spiny mice in each group in nonfasting condition.
[a]Difference significant from chow-fed animals ($p < 0.05$ at least).
[b]Difference significant between sucrose- and fat-fed animals ($p < 0.05$ at least).
FFA = free fatty acids.

Table 2. Activity of liver glycolytic, glucogenic and lipogenic enzymes in spiny mice on different diets

	Regular	Sucrose-rich	Fat-rich
Pyruvate kinase	158 ± 13	1265 ± 67[a]	162 ± 28
NADP-malate dehydrogenase	14 ± 2	240 ± 23[a]	7 ± 1[a]
ATP-citrate lyase	1.3 ± 0.2	16.8 ± 1.9[a]	0.8 ± 0.1[a]
Acetyl-CoA carboxylase	1.1 ± 0.2	6.9 ± 0.7[a]	0.6 ± 0.2[a]
Fatty acid synthase	2.4 ± 0.2	14.5 ± 1.1[a]	1.8 ± 0.3
Glucose-6-phosphatase	26 ± 2	44 ± 3[a]	56 ± 7[a]
PEPCK	140 ± 9	92 ± 5[a]	185 ± 7[a]

Values are nmol/min per mg protein, in the appropriate homogenate as specified in the methods, given as means ± SE for groups of 12–16 spiny mice maintained on the diets for 6 weeks.
[a]denotes a significant difference from mice on regular diet. $p < 0.05$ at least.
PEPCK = phosphoenolpyruvate carboxykinase

serum insulin increase. There was virtually no adipose tissue gain on the sucrose-rich regimen. In contrast, the fat-rich seed diet resulted in marked weight gain, impaired glucose tolerance and moderate hyperinsulinemia associated with an increase in the pancreatic insulin content, larger than on sucrose-diet.

Comparison of Geneva and Jerusalem spiny mice colonies

The mice and the diets of the Geneva and Jerusalem colonies were exchanged and fed *ad libitum* the Jerusalem standard chow or the Geneva diet, which was a rodent chow supplemented with seeds comprising 15% of the total fat in the diet. The fat seeds in the Geneva diet were preferably consumed by spiny mice and caused a threefold weight gain at 8–10 months of age compared with albino mice on the rodent chow alone and a twofold gain compared with spiny mice fed the standard, unsupplemented Jerusalem rodent diet [23]. The hormonal, enzymatic and metabolic patterns in the two colonies were very similar, as determined by their nutritional condition, but one of the major differences was the higher body weight on the Geneva diet. Insulin resistance preceded the hyperglycemia and was associated with mild hyperinsulinemia, proceeding with time to more severe serum insulin elevation. Insulin resistance, associated with insulin receptor defect, as described in several obese animal species and obese humans [24–26], was considered to be the cause of overt diabetes in the Geneva spiny mice maintained on the fat-rich seed supplemented diet. Indeed, a correlation between insulin sensitivity and body fat content was demonstrated [23]. It is of interest that the propensity to obesity on the seed diet might be specific for spiny mice, either because of their desert derivation or particular taste, because albino mice fed a seed supplemented diet gained only moderately in weight [23]. These comparative dietary studies in the two spiny mice colonies lead to the conclusion that the low insulin response to glucose and other secretagogues as well as the lapse into diabetes are a characteristic trait of desert species subjected to nutritional stress rather than to a genetic aberration.

Novel experiments

Animal maintenance, diets and methods

Spiny mice were maintained on a regular rodent diet in the Hebrew University-Hadassah Medical School Animal Facility in Jerusalem. They were descendants of specimens collected in 1968 in the southern desert region and Dead Sea shores of Israel. All experiments reported here were performed during 1980–1990, according to the regulations of the Institutional Animal Care Committee.

The *regular stock diet* was Amrod 931 pellets (Anbar Mills, Hadera, Israel) containing by % dry weight, 4.5 fat, 20.7 protein, 64.0 carbohydrates as starch, 5.5 fiber, 4.1 minerals (ash) and 1.2 vitamin additions. The caloric value of this diet was 3.79 cal/g.

The *sucrose-rich diet* was American Institute of Nutrition AIN 76 pellets, (US Biochemical Corp., Cleveland, OH, USA), which contained by % dry weight: 5.0 fat, 20.0 protein, 15.0 starch, 50.0 sucrose, 5.0 fiber, 3.5 ash and 1.5 vitamin and salt additions. The caloric value of this diet was 3.85 cal/g, practically isocaloric with the Amrod 931 chow.

The *fat-rich diet* was a mixture of sunflower, sesame and pumpkin seeds containing by % dry weight 29.1 fat, 25.0 protein, 37.0 carbohydrate, 4.7 fiber, 4.1 ash amounting to 5.10 cal/g. Vitamins were supplemented in drinking water. Analysis was done on decorticated seeds.

The animals were placed on the diets when 1–2 months old and were fed *ad libitum* for up to 18 months.

Triiodothyronine (T_3) treatment

Spiny mice on a regular diet were s.c. injected with purified T_3 (Sigma, St Louis, MO, USA) dissolved in buffered 0.9% NaCl solution, at 2.5 μg/100 g body weight for 6 days.

Tissue preparation, metabolite and enzyme assays

The mice were decapitated in non-fasting condition between 9.00 and 10.00 a.m. and blood, liver, pancreas and epididymal adipose tissue were collected. Serum levels of glucose (hexokinase method), cholesterol (cholesterol oxidase method) and triglycerides (lipase-glycerokinase method) were determined with procedures and reagents from Boehringer (Mannheim, Germany). A portion of liver was extracted in chloroform : methanol (2 : 1, v/v) and the triglycerides determined in the evaporated extract with Boehringer reagents. Liver glycogen content in the extracted tissue was determined enzymatically [27]. Serum and pancreas insulin levels were determined by a double antibody radioimmunoassay (Amersham Radiochemical Centre, UK). Human insulin was used as standard since dilutions of spiny mice serum gave curves parallel to those of human insulin with a cross-reactivity of $>90\%$. The pancreas was extracted in acid ethanol [28] by sonication and insulin determinations carried out after the appropriate dilution. Serum-free fatty acid (FFA) levels were determined by the the radiochemical method of Ho *et al*. [29].

Liver enzyme assays

The liver was homogenized (1 : 3. w/v) in ice-cold 0.2 M sucrose solution containing 20 mM triethanolamine (pH 7.4), 1 mM disodium EDTA and 1 mM diethioerythreitol. The homogenates, after removing the debris, were centrifuged at 10 000 g for 10 min to collect the mitochondria. Part of the 10 000 g supernate was saved at -70°C for the determination of hepatic T_3 content and assays of Na^+/K^+ATPase and T_4 monodeiodinase. The other part was centrifuged at 100 000 g for 45 min and the supernatant fluid was used for cytosolic enzyme assays. The microsomal pellet was washed in the homogenate fluid, recentrifuged and resuspended for glucose-6-phosphatase assay. Enzyme activities were determined by spectrophotometric or radiochemical methods used previously [30] and were expressed as nmol/min of the substrate metabolized per mg of protein at 37°C. Glucose-6-phosphatase activity was determined radiochemically in the hepatic microsomal fraction [31] and expressed as μmol/min·g fresh liver weight.

Mitochondrial FAD-glycerol phosphate oxidase assay was based on the reduction of 2-*p*-iodo, 3-*p*-nitro, 5-phenyltetrazolium chloride (INT; Sigma Chemical Co., USA) to formazan, in the presence of sn-glycerolphosphate, by a modified procedure of Fried and Antopol [32]. The reaction mixture, in a final volume of 1 ml, contained: 0.06% INT 45 nmol/l phosphate buffer, pH 7.5, 0.01 mM phenazine methyl sulfate, 0.1 mM NaCN, 50 mM glycerolphosphate and 100 to 250 μl of mitochondrial suspension. After 10 and 20 min incubation at 37°C the reaction was stopped with 0.2 ml of 30% trichloroacetic acid, the formazan extracted into 2.5 ml ethylacetate and read at 490 nm against formazan standards. A reaction mixture without glycerolphosphate served as a blank. The activity was expressed as μg/min formazan formed per mg mitochondrial protein.

Na^+/K^+ ATPase activity was measured by a modified method of York *et al*. [33]. The postmitochondrial 10 000 g liver homogenate (0.1 to 0.4 ml) was incubated in a final volume of 1 ml containing 5 mM $MgCl_2$ 120 mM NaCl, 12.5 mM KCl, 5 mM sodium azide, 5 mM ATP 25 mM Tris, pH 7.4, and 2 mM EDTA. After 5 min preincubation, the reaction was started by the addition of ATP at a final concentration of 5 mM and was stopped after 30 min with 100 μl of 50% trichloroacetic acid, cooled on ice and centrifuged at 2000 g. The phosphate and protein in the supernatant were determined by standard methods [34,35]. ATPase activity (μmol/h phosphate released per mg protein) was the difference between the phosphate released with and without 1 mM ouabain.

T_4 monodeiodinase assay was performed on the 10 000 g supernatant of liver homogenate according to Hufner *et al*. [36] with minor modifications. The reaction, in 500 μl buffer of 0.1 M phosphate buffer, pH 7.5, 1 mM mercaptoethanol and 1 mM disodium EDTA) was started by the addition of 1 μg/100 μl T_4 solution. At 10 and 20 min, 200 μl of absolute ethanol were added. After centrifugation at 3000 g, the T_3 produced in the reaction mixture was determined by the Gammacoat immunoassay kit. A blank sample was incubated without T_4 to determine the basal level of T_3 in the homogenate, which was subtracted from that obtained in the presence of T_4.

Body temperature was determined in conscious spiny mice on fat-rich and sucrose-rich diets. Prior to the measurement, the mice were lightly attached to a plastic net by a girdle around the abdomen allowing free movement of the upper part of the body. A thermistor (Yellow Springs Instrument Co., Yellow Springs, OH, USA) was inserted about 3 cm into the rectum and the animals were allowed to relax for 10 min at room temperature (24°C) before the measurement was recorded for 3 min.

Pancreas staining. For morphological examination of the endocrine pancreas on the sucrose-rich and fat-rich diets, the pancreases were fixed in buffered formaldehyde and embedded in paraffin. Sections of 5 μm were stained with hematoxylin-eosin.

All results are presented as means ± SE. Comparisons were made by non-paired Student's *t*-test considering $p<0.05$ as a significant difference.

Results

Spiny mice on sucrose-rich diet

Previous data (Table 1) revealed that spiny mice maintained on the high sucrose diet for 6 weeks consumed 5–10% more of the sucrose than regular diet by weight (five weekly averages of 12 mice in three cages) but did not gain in the proportion of their fat tissue in contrast to

the fat-rich seed diet-fed animals (Table 1). The table shows that the average consumption of the sucrose-rich diet was higher than that of regular chow in caloric terms (14.0 versus 11.8 kcal/week) and that of fat-rich diet was also higher and similar to that of sucrose-rich diet amounting to 14.3 kcal/week.

The sucrose-induced 48% increase in the liver weight has to be emphasized. Although the fat content of the liver rose, the liver size increased mainly due to cellular hypertrophy and hyperplasia [37], as evident by the increase in liver protein content from 224 ± 6 mg/total liver on the regular diet to 344 ± 14 mg/total liver on the sucrose diet. There was also an increase in liver triglyceride and cholesterol content on the sucrose diet as reported previously [22], but this increase added only a small proportion to the liver weight.

Serum glucose levels did not change appreciably on the sucrose diet, but the levels of circulating insulin increased both on sucrose- and fat-rich diets, in agreement with the reported glucose intolerance and insulin resistance [21,22]. Also, the rise in serum FFA on the sucrose diet indicated an increased adipose tissue lipolysis and triglyceride turnover in accord with insulin resistance in adipose tissue, as noted previously. The increased fat mobilization, contributing to the hyperlipidemia, might have been involved in the failure of spiny mice to gain weight in spite of the increased nutrient consumption. Serum FFA levels also rose significantly in spiny mice on the high fat diet, in association with weight gain (Table 1). The increase in the pancreatic content of insulin after 6 weeks both on the sucrose and fat-rich diets is notable.

Spiny mice manifested a considerable increase in the glycolytic and lipogenic enzymes on the sucrose diet as presented in Table 2. The induction of these enzymes in spiny mice was several-fold greater than in albino mice placed on similar diets [21]. The activity of glycolytic and lipogenic enzymes on the fat-rich diet decreased or tended to be lower than on the regular laboratory chow. Of the gluconeogenic enzymes, glucose-6-phosphatase activity was increased reflecting the channeling of fructose derived precursors into glucose [37] but the activity of the PEPCK enzyme declined on the sucrose diet. The activity of both glucogenic enzymes rose on the fat-rich diet, consistent with enhanced gluconeogenesis from 3-carbon units.

Serum and liver elevation of T_3

Table 3 demonstrates the surprising finding of a rise in the circulating T_3 on the sucrose-rich diet in contrast to the fat-rich diet. This was evident after 6 weeks on the diets and persisted during the 12 months of observation (see below). The rise in T_3 levels was associated with a decrease in T_4 levels, suggesting that the T_3 elevation is not a result of increased release from the thyroid gland. The metabolic effects of the rise in T_3 were further investigated by measuring the activity of the hepatic mitochondrial FAD-glycerolphosphate oxidase, one of the regulatory enzymes of the electron transfer chain is highly dependent on T_3 [38–40]. The activity of Na^+/K^+-ATPase reflecting the T_3 induced thermogenesis [41–43] was also measured (Table 3). The activity of both these enzymes rose markedly on the sucrose diet but did not significantly change on the fat-rich diet. An increase in adipose tissue FAD-glycerolphosphate oxidase was also evident.

A similar experiment was performed with albino mice of the Hebrew University strain. As seen in Table 4, the sucrose-rich diet produced a similar elevation in the circulating T_3 level. The induction of FAD-glycerolphosphate oxidase was somewhat lower and that of ATPase somewhat higher than that observed in spiny mice. This experiment demonstrates that the T_3 elevation was

Table 3. Effect of sucrose- and fat-rich diets on serum T_3 level and T_3-dependent enzymes in spiny mice

Diets	Serum T_3 (ng/ml)	Serum T_4 (μg/dl)	Liver mitochondrial FAD-glycerol-phosphate-oxidase (μg formazan/min·mg)	Adipose tissue FAD glycerol-phosphate-oxidase (μg formazan/min·mg)	Liver membrane Na^+/K^+ ATPase (μmol phosphate/h·mg)
Regular chow	1.05 ± 0.09	3.5 ± 0.2	14.0 ± 1.3	9.2 ± 0.7	9.4 ± 0.7
Sucrose-rich	1.48 ± 0.09^b	2.6 ± 0.2^a	48.3 ± 5.7^b	17.8 ± 2.1^b	14.9 ± 1.0^a
Fat-rich (seeds)	1.16 ± 0.04	4.4 ± 0.3	17.9 ± 2.2	6.5 ± 0.8	10.5 ± 0.9

Values are means ± SE for groups of six to eight animals maintained on the diets for 6 weeks.
$^a p < 0.05$; $^b p < 0.01$ – compared with regular chow by Student nonpaired *t*-test.

Table 4. Effect of sucrose- and fat-rich diets on serum T_3 level and T_3-dependent enzymes in albino mice

Diets	Serum T_3 (ng/ml)	Serum T_4 (μg/dl)	Liver mitochondrial FAD-glycerol-phosphate-oxidase (μg formazan/min·mg)	Adipose tissue FAD glycerol-phosphate-oxidase (μg formazan/min·mg)	Liver membrane Na^+/K^+ ATPase (μmol phosphate/h·mg)
Regular chow	1.06 ± 0.08	4.5 ± 0.3	11.3 ± 1.1	5.8 ± 0.7	12.5 ± 1.1
Sucrose-rich	1.35 ± 0.07^a	3.6 ± 0.3^a	33.3 ± 2.9^b	9.2 ± 0.8^b	19.1 ± 1.5^a
Fat-rich	1.12 ± 0.04	4.3 ± 0.3	15.9 ± 1.2	4.9 ± 0.6	8.8 ± 0.9

Values are means ± SE for groups of eight to ten animals maintained on the diets for 6 weeks.
$^a p < 0.05$; $^b p < 0.01$ – compared with regular chow.

dependent on sucrose feeding and was not particular to the spiny mice species.

To ascertain the physiological thermogenic effect of T_3 elevation, the temperature of spiny mice, maintained for 6 weeks on sucrose- and fat-rich diets, was compared with that of animals maintained on regular rodent chow. Measurements were performed on groups of 12 nonfasting mice at room temperature of 24°C, showing that the mean temperature on regular chow was 37.1 ± 0.2°C, and on the fat-rich diet was 37.0 ± 0.2°C, whereas in spiny mice on the sucrose-rich diet it was 37.9 ± 0.3°C. The difference was significant at $p < 0.05$.

The basis for the elevation of T_3 in the circulation of spiny mice on the sucrose diet was investigated by measuring the activity of hepatic iodothyronine deiodinase, the enzyme responsible for the extrathyroidal T_3 delivery into the circulation (Table 5). The T_3 content in the liver was increased, particularly when expressed per total liver weight, since the mean liver size in spiny mice on sucrose diet was about twice that of mice kept on the laboratory chow or fat-rich diets. The activity of the deiodinase enzyme, converting T_4 to T_3 was also increased on the sucrose diet, significantly so when expressed per total liver weight rather than per protein unit.

To investigate whether changes similar to those seen on the sucrose-rich diet can be elicited by T_3 treatment, a group of spiny mice received T_3 injections . As shown in Table 6 the administration of T_3, 2.5 μg/100 g for 6 days, resulted in an elevation of circulating T_3 of similar magnitude to that attained by a 6 week sucrose diet, whereas, the T_4 level was reduced. The elevation in the activities of mitochondrial FAD-glycerolphosphate dehydrogenase and Na^+/K^+ ATPase produced by T_3 was similar in magnitude to that of the sucrose-rich diet. However, the activities of hepatic enzymes concerned with lipogenesis, though raised by T_3 treatment, were much below those attained by the sucrose diet.

Table 5. Hepatic content of T_3 and activity of T_4-monodeiodinase in spiny mice on sucrose-rich and fat-rich diets

	T_3 content		T_4 to T_3 conversion	
Diet	pg/mg protein	ng per total liver	pg T_3/min·mg protein	ng T_3/min per total liver
Regular chow	167 ± 6	35 ± 3	4.5 ± 0.6	0.95 ± 0.18
Sucrose-rich	191 ± 11	69 ± 5[a]	5.8 ± 0.7	2.09 ± 0.28[a]
Fat-rich	178 ± 8	39 ± 4	4.9 ± 0.7	1.09 ± 0.18

Values are means ± SE for 12 animals maintained on diets for 6 weeks.
[a]denotes significant difference from the regular chow or fat-rich diet fed animals at $p < 0.005$. Liver weight per 100 g body wt in chow-fed mice was 3.1 ± 0.2, sucrose-fed mice 4.6 ± 0.3, and fat-rich seed-fed mice was 3.3 ± 0.2 g.

Long term sucrose- and fat-rich diets

Groups of spiny mice were maintained on regular chow, sucrose-rich and fat-rich diets for 18 months, with intermittent assessment of body weight, serum glucose and insulin, as well as pancreatic insulin levels (Table 7). Initially, 15 animals were sacrificed to obtain basal values at 1 month of age. The diet experiment was started with 40 animals in each group of which five animals were sacrificed at 3, 6, 12 and 18 months. On the regular chow the gain in body weight at 18 months was 49.3%, on sucrose diet 26.6% and on the fat-rich diet 140.2% (Table 7). Mean serum glucose levels on the regular chow remained without significant change, whereas they increased on the sucrose diet from 136 to 181 mg/dl and on the fat-rich diet from 139 to 230 mg/dl at 18 months. Serum triglycerides rose moderately on the regular chow-fed animals, whereas in the sucrose-fed mice there was a considerable hypertriglyceridemia at 3 months which subsided with age. In mice on the fat-rich diet the triglyceride elevation was evident at 6 to 18 months, in apparent association with obesity. Serum insulin levels increased somewhat with age in the regular chow-fed mice, moderately in the sucrose-fed mice but markedly in fat-fed mice surviving for 18 months. With respect to pancreatic insulin, there was about a twofold increase between 3 and 18 months in chow-fed mice, but greater than a fourfold increase in the sucrose-fed mice to a level of 10.31 U/g at 18 months. The increase in pancreatic insulin on a high fat diet was even larger, amounting to 14.68 U/g at 18 months, indicating a striking pancreatic cell expansion. It should be emphasized, however, that these values were obtained only in surviving animals. The mortality of the sucrose-fed mice, between 12 and 18 months was four of 23, which did not appear to be related to hyperglycemia or hypertriglyceridemia. On the other hand, the mortality of spiny mice on the fat-rich diet was higher, amounting to two of 35 between 3 and 6 months, nine of 28 between 6 and 12

Table 6. Induction of liver enzymes in spiny mice by treatment with T_3 compared with sucrose-rich diet effect

	Control regular diet 6 weeks	Sucrose-rich diet 6 weeks	Control + T_3 2.5 μg/100 g 6 days
Serum T_3 (ng/ml)	0.86 ± 0.03	1.55 ± 0.10[a]	1.85 ± 0.14[b]
Serum T_4 (μg/dl)	3.5 ± 0.2	2.7 ± 0.2[a]	2.5 ± 0.3[b]
FAD-glycerolphosphate oxidase (μg formazan/min·mg)	4.8 ± 0.5	16.2 ± 1.4[a]	12.5 ± 0.8[a]
Na^+/K^+ ATPase (μmol phosphate/h·mg)	9.4 ± 0.7	15.6 ± 1.2[a]	13.5 ± 0.8[b]
NADP-malate dehydrogenase (nmol/min·mg)	11.3 ± 0.6	212 ± 14[a]	25.3 ± 3.1[b]
ATP-citrate lyase (nmol/min·mg)	1.54 ± 0.12	18.6 ± 1.5[a]	3.85 ± 0.50[b]
Acetyl-CoA carboxylase (nmol/min·mg)	1.64 ± 0.20	8.35 ± 1.1[a]	2.56 ± 0.35
Fatty acid synthase (nmol/min·mg)	3.65 ± 0.28	26.5 ± 2.4[a]	4.83 ± 0.42

Values are means ± SE for ten to 12 mice. [a]denotes significant difference ($p < 0.05$ at least) between sucrose-fed and regular chow-fed mice; [b]denotes significant difference ($p < 0.05$ at least) between T_3-treated and regular chow-fed mice. Note the large increment in enzyme activity values of the sucrose-fed compared with T_3-treated mice.

Table 7. Spiny mice maintained on regular, sucrose-rich and fat-rich diets for 18 months

Diet	No. of mice[a]	Body weight (g)	Serum glucose (mg/dl)	Serum T_3 (mg/ml)	Serum triglycerides (mg/dl)	Serum insulin (mU/l)	Pancreas insulin (U/g)
Regular chow Initial values (1 month, $n=15$)		35.3 ± 0.9	110 ± 4	0.98 ± 0.08	124 ± 9	26 ± 2	2.27 ± 0.31
3 months	35	45.5 ± 1.1	116 ± 7	1.10 ± 0.12	145 ± 23	28 ± 3	2.86 ± 0.42
6 months	30	47.3 ± 1.6	113 ± 8		180 ± 21	34 ± 4	3.61 ± 0.45
12 months	24	50.5 ± 1.4	118 ± 2	1.06 ± 0.11	185 ± 24	36 ± 5	4.65 ± 0.52
18 months	17	52.7 ± 1.7	120 ± 5		210 ± 22	40 ± 6	5.04 ± 0.54
Sucrose-rich							
3 months	35	44.0 ± 1.9	136 ± 7	1.38 ± 0.13	410 ± 22	29 ± 4	3.71 ± 0.80
6 months	30	46.2 ± 1.8	154 ± 16		435 ± 28	43 ± 6	5.89 ± 0.41
12 months	23	45.0 ± 1.7	162 ± 11	1.45 ± 0.15	354 ± 21	61 ± 7	8.55 ± 0.62
18 months	14	44.7 ± 1.5	181 ± 9		296 ± 16	78 ± 9	10.31 ± 1.15
Fat-rich							
3 months	35	47.1 ± 1.4	139 ± 6	1.07 ± 0.14	182 ± 12	24 ± 4	3.91 ± 0.45
6 months	28	62.5 ± 1.6	151 ± 15		240 ± 15	73 ± 11	7.45 ± 0.90
12 months	20	76.3 ± 1.8	185 ± 16	0.99 ± 0.10	320 ± 38	90 ± 15	11.61 ± 1.31
18 months	7	84.8 ± 5.5	230 ± 40		345 ± 41	138 ± 28	14.68 ± 1.87

Male spiny mice were kept on the indicated diets in individual cages. Five animals were sacrificed at random at each time period.
Values are means ± SE for five animals.
[a]Numbers listed in the column are those of mice surviving at each time period.
Body weight gain at 18 months, compared with the initial value on each diet was: regular chow, 49.3%; sucrose-rich diet, 26.6%; fat-rich diet, 140.2%.

months and as much as 18 of 20 between 12 and 18 months. The exact reason for mortality in all mice on the high fat diet could not be established with certainty but a large percentage of them were hyperglycemic above the mean of the animals surviving at 18 months. A few were ketonuric.

Pancreas examination in several instances of mice maintained for 12 months on the diets revealed differences in islet population. Figure 1 presents the islet distribution in mice on the regular rodent chow. Figure 2 shows the increased population of islets in a representative pancreas section from a sucrose-fed mouse. A high density of the intra-islet particles should be noted. Figures 3 and 4 show a disturbed morphologic pattern of hypertrophic islets of spiny mice on the fat-rich diet. The irregularity of the islet shape and intra-islet structure, probably preceding islet rupture and disintegration, is clearly discernible.

Discussion

Divergent patterns of overnutrition stress

The recorded literature on animal models progressing to diabetes as a result of relative overnutrition, indicates that

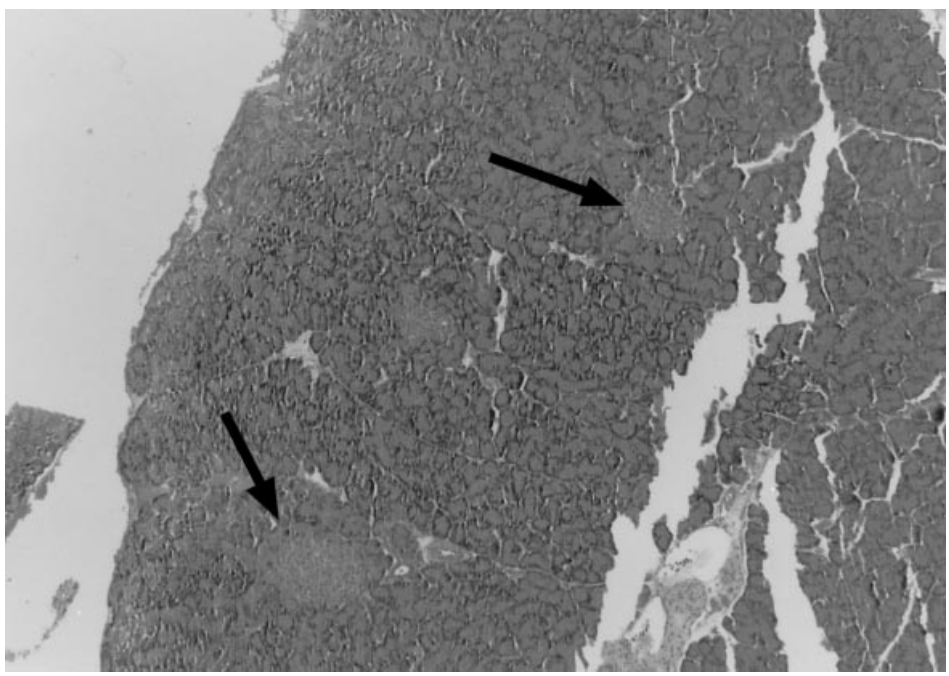

Figure 1. Islets of Langerhans in a pancreas section of a nondiabetic spiny mouse maintained on regular rodent chow for 12 months (Table 7). Hematoxylin-eosin stain. Magnification 50 ×

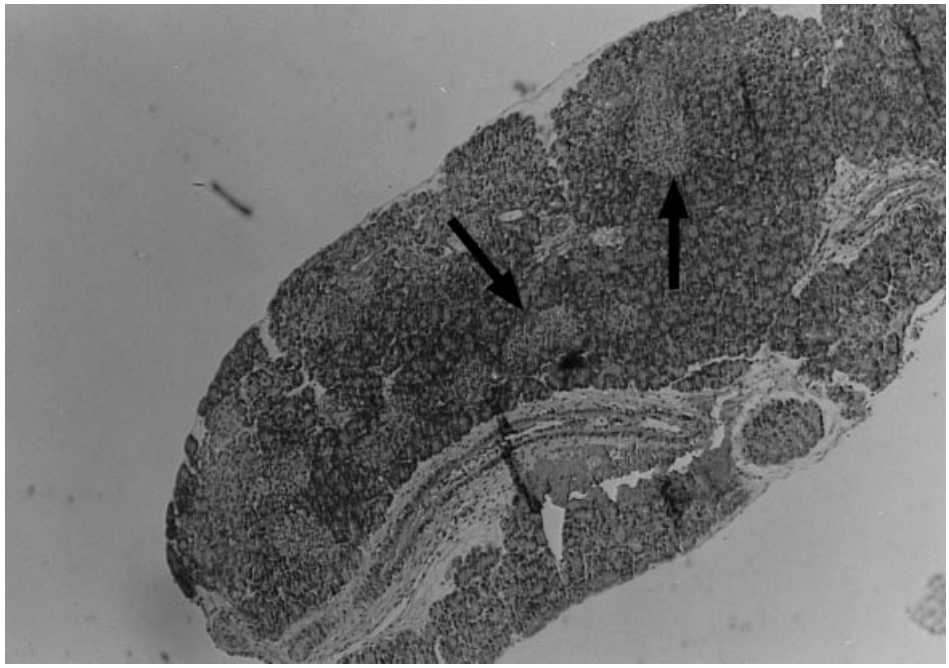

Figure 2. Islets of Langerhans in a pancreas section of a spiny mouse with moderately elevated serum glucose and insulin levels, maintained on sucrose-rich diet for 12 months (Table 7). Note the multiple islets, some increased in size, in the representative pancreas section. Dense granulation but normal shape of the islets is apparent. Hematoxylin-eosin stain. Magnification 50 ×

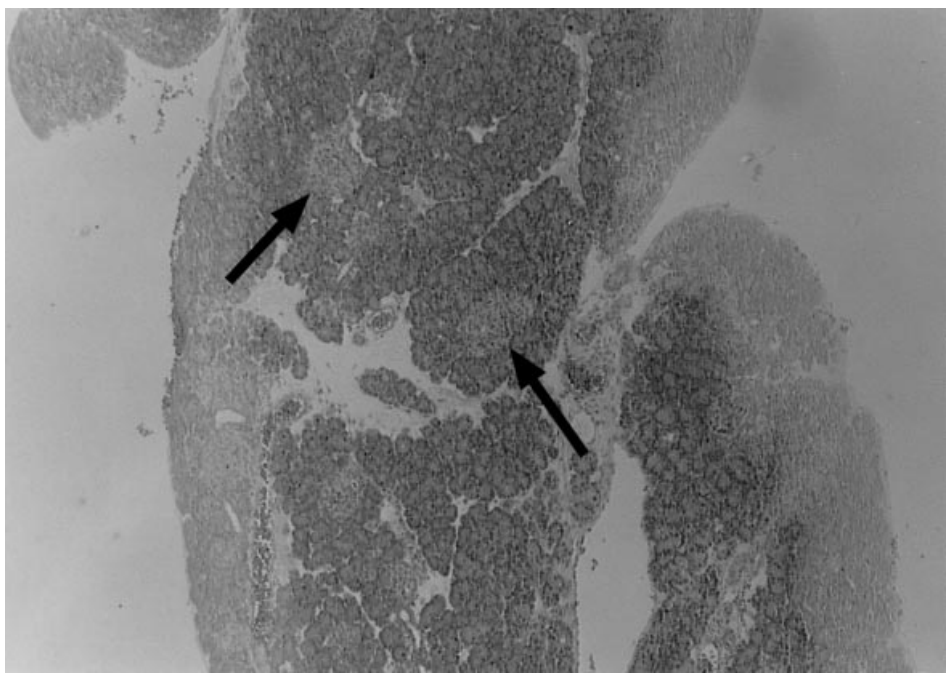

Figure 3. Islets of Langerhans in a representative pancreas section of a spiny mouse with elevated seum glucose and insulin levels maintained on fat-rich diet for 12 months (Table 7). Note the enlarged and irregular shape of the islets and irregularity of the intra-islet granulation. Hematoxylin-eosin stain. Magnification 50 ×

the impact of caloric surplus may lead to several modes of defect in endocrine pancreatic function.

1. *Psammomys obesus* (sand rat) exhibits marked insulin resistance, insulin receptor malfunction, hyperinsulinemia and hyperglycemia leading to β-cell overstimulation and apoptosis with a lapse into ketotic diabetes [44–46].
2. *Zucker diabetic rats* (ZDF) were also recently shown to exhibit marked pancreatic lesion possibly involving apoptosis [47] probably effected through lipotoxicity [48].

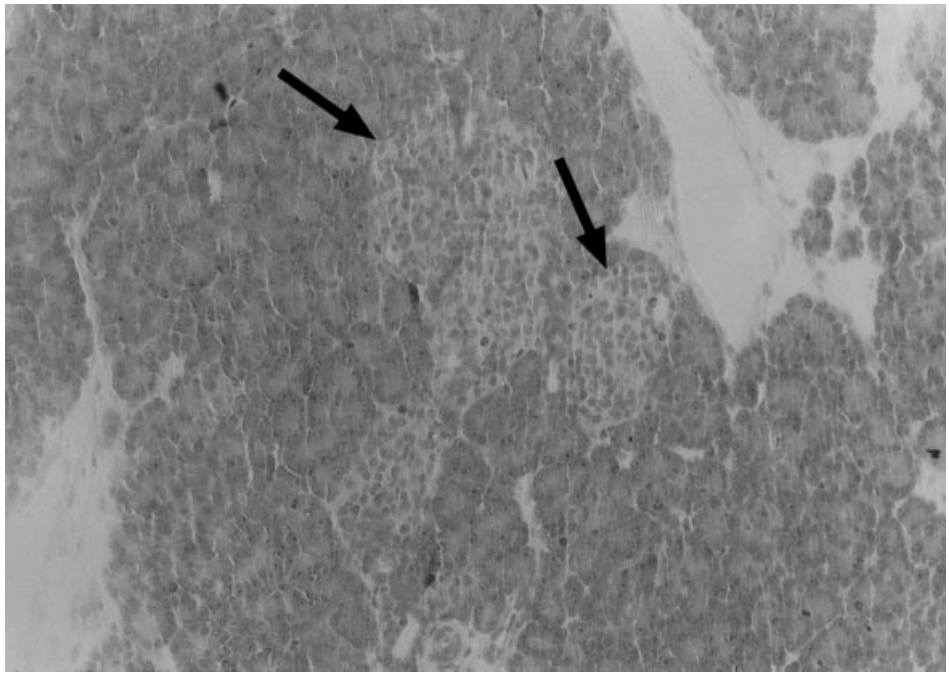

Figure 4. Another section of the pancreas described in Figure 3. Note the deformed and hypertrophic, close-associated islet group possibly prior to degeneration. Compare these with the islet photomicrographs of Gonet *et al*. [1]. Hematoxylin-eosin stain. Magnification 50 ×

3. C57BL6/J mice – the nondiabetic hosts of the *ob* mutation – respond to a high energy diet with glucose intolerance, hyperglycemia followed by hyperinsulinemia and hypertension, but without total loss of pancreatic function [49,50].
4. *Macaca mulatta* rhesus monkeys on *ad libitum* nutrition manifest a process of hyperinsulinemia and marked insulin resistance preceding the hyperglycemia, associated with weight gain. This is spread over 8–10 years, culminating in the loss of insulin secretion and β-cell amyloid deposits [51,52].

The spiny mice described here present another type of lesion on high caloric intake, characterized by marked insulinogenesis, in the face of low insulin secretion response. This is evident from the accumulation of pancreatic insulin, islet hypergranulation, hypertrophy and hyperplasia, occurring both on sucrose- and fat-rich diets. However, this is more pronounced and more lethal after protracted maintenance on a fat-rich diet.

Sucrose diet and insulin resistance

There is only moderate insulin resistance and hyperglycemia during several months of feeding on a sucrose diet. According to the findings of Tobey *et al*. in rats, the resistance in animals on a fructose diet is expressed by nonsuppression of hepatic glucose production rather than peripheral glucose uptake [53]. However, in spiny mice on a fat-rich diet the insulin resistance occurs along with excessive pancreatic insulin accumulation, islet hypertrophy and hyperplasia leading to terminal, often sudden, disintegration of islets, resulting in fatal ketotic diabetes [1,2].

The triglycerides secreted from the liver of spiny mice face a handicap at the site of adipose tissue, since the activity of lipoprotein lipase does not rise adequately [21], most probably because of a lack of insulin stimulation. This triggers the build-up of hypertriglyceridemia because of a redistribution of the sites of carbohydrate metabolism: preference for liver over periphery in fructose metabolism. An aggravation of hyperlipidemia ensues with continuation of the sucrose-rich diet, since the rate of triglyceride removal is inversely related to plasma triglyceride concentration due to the saturation of lipoprotein lipase capacity. Triglyceride storage in muscles has been recorded [22], which may increase the peripheral insulin resistance.

Long-term sucrose diet is associated with other previously reported detrimental effects in rodents, apart from hyperlipidemia and deposition of triglycerides in muscles and other nonadipose tissues. There is decreased fertility and survival of the parents and newborn [54] and fetal malformations [55].

Effects of stimulated thyroid hormone production by sucrose diet

The elevation in circulating T_3 and its generation was observed by Danforth *et al*. [56] after 3 weeks overnutrition in humans. This was especially effective with carbohydrate. The elevation of T_3 may represent a general response to dietary affluence, while its reduction might be characteristic of inanition. In spiny and albino mice only the effect of sucrose was notable. Such a

result of sucrose feeding is most probably the outcome of the priority of liver metabolism of the sucrose-derived fructose, because of the predominant fructokinase location and the necessity of hepatic disposal of the excess of this dietary carbohydrate [37]. The metabolic expression of an inordinate fructose load forces upon the liver a high-reaching adaptive induction of glycolytic and lipogenic enzymes with resultant hyperlipidemia.

The sucrose-elicited elevation of T_3 concentration and activity of mitochondrial FAD-glycerolphosphate oxidase and the membrane ATPase appear to exert a protective response against the substrate surplus, involving a thermogenic energy dissipation as evident from the increase in the body temperature of spiny mice. The T_3-induced thermogenesis [41–43] through the elevation of Na^+/K^+ ATPase and FAD-glycerolphosphate oxidase [38–40,57,58], accompanied by other 'futile' metabolic cycles in opposing enzyme couples and metabolic pathways, may be instrumental in the dissipation of the extra energy made available to an animal accustomed to thrifty metabolism. Among the energy wasting cycles increased by T_3 excess are hexokinase-glucose-6-phosphatase, phosphofructokinase-fructose-bis phosphatase [59–62] and malate shuttling by the simultaneous rise in pyruvate carboxylase and NADP-malate dehydrogenase activities [63–65]. The coincident amplification by T_3 of opposing pathways in fat metabolism: fatty acid synthesis versus fatty acid oxidation and T_3-enhanced adipose tissue lipolysis compromises the gain in ATP through fatty acid oxidation by reconsumption in the process of fat synthesis. This represents a mitochondrial–cytoplasmic shuttle with ATPase-like activity [30]. Cycling at various metabolic sites may limit the gain in adipose tissue and body weight in spiny mice on sucrose, compared with regular chow.

These energy-consuming reactions on a sucrose-rich diet may also have a protective effect on the preservation of pancreatic islet function. It is plausible that these changes may diminish the substrate and energy availability for exaggerated insulinogenesis in the endocrine pancreas, thus restraining the processes leading to to islet expansion through the critical β-cell hypergranulation. No such protective mechanism seems to exist on the high fat diet preventing the destructive islet hyperinsulinogenesis. However, it must be emphasized that this argument assumes expansion susceptible islets which may be specific to spiny mice and kindred species [66] and should not be generalized as a mechanism of islet breakdown in other species prone to nutritional diabetes. However, Del Zotto *et al*. [67] have recently reported increased β-cell replication, islet neogenesis and elevated pancreatic insulin content in young male Syrian hamsters fed sucrose as a 10% solution in drinking water for 5–21 weeks. Although the pancreatic insulin content rose as much as tenfold, glucose stimulated insulin secretion was also increased, unlike the spiny mice, and no islet disruption causing overt diabetes was observed. On the other hand, Koyama *et al*. [68] reported that sucrose feeding of Goto–Kakizaki rats, with an inborn defect in β-cell proliferation, induced a severe hyperglycemia, 50% shrinking in β-cell mass with apoptosis and oxidative stress.

T_3-sucrose metabolic synergism

A synergism between the T_3 elevation and sucrose consumption in effecting the hepatic enzyme changes has been proposed by Moriash *et al*. [69]. Also, an interrelationship between the T_3-induced lipogenesis, lipolysis and other pathways was postulated by Oppenheimer *et al*. [70], with T_3 acting at least in part as a coordinate inducer of the responsible enzyme genes. While T_3 alone was shown here to induce the elevation in hepatic lipogenic enzymes (Table 6), the effect of sucrose feeding was several-fold higher than the T_3 treatment. Therefore, it is highly likely that sucrose was chiefly responsible for the rises in glycolytic and lipogenic enzymes. Direct effects of sucrose-derived fructose on translatable enzyme mRNA synthesis were reported for pyruvate kinase [71], malate enzyme [72,73], FAD-glycerolphosphate oxidase, acetyl CoA carboxylase [74] as well as of Na^+/K^+ATPase [75]. The associated elevation in the T_3 concentration might have provided a potentiating or ancillary role.

The studies presented here indicate that spiny mice present a model of diabetes characterized by excessive insulinogenesis in response to nutritional energy surplus, in conjunction with a low response to insulin secretagogues, thus leading to islet cell expansion and disruption.

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