Hypersecretion of Pancreatic Somatostatin in the Obese Zucker Rat

Effects of Food Restriction and Age

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SUMMARY

Islets of 5-mo-old obese Zucker rats secreted 50% more somatostatin (SRIF) in response to 8.3 mM or 16.7 mM glucose than did islets from lean controls; the islet SRIF contents of obese and lean rats were similar. As expected, islets of obese rats demonstrated a greater basal and a fivefold greater glucose-induced insulin release and also a twofold greater insulin content than did islets from lean rats. Obese rats were pair fed with lean animals from the age of 9 wk until killed, when 5 mo old. While this curtailed the weight gain of obese rats to that of lean controls, it caused no reduction in the per cent body weight found in the form of lipid. The responses of the pancreatic delta and beta cells to pair feeding were markedly different. Pair feeding caused no alteration in either SRIF content or glucose-induced SRIF release, while the expected reductions in both islet insulin content and glucose-induced insulin secretion were observed.

Islets from older obese Zucker rats (15–18 mo old) had four to five times greater contents of both SRIF and insulin than did islets from age-matched lean controls. The obese rats of this age had a moderate glucose intolerance. SRIF secretion from the islets of such rats was distinctly greater than from those of lean controls at all glucose concentrations tested (range, 1.0–16.7 mM). The delta cells of the older obese rats had lost their sensitivity to glucose, while those of lean controls remained sensitive. Beta cells of islets from both obese and lean 15-mo-old rats remained glucose sensitive. Under all conditions tested, secretion of SRIF and insulin was greater from islets of obese than lean rats.

The results demonstrate a marked difference in pancreatic delta and beta cell responses to pair feeding in the obese Zucker rat. At present, the role played by hypersecretion of pancreatic SRIF in the obesity syndrome of the Zucker rat remains obscure.

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Obesity is associated with hyperinsulinemia, hyperphagia, and an increased incidence of diabetes mellitus. Somatostatin is a potent inhibitor of insulin release (see ref. 1 for review), and there is some evidence that it may play a role in the rate of nutrient absorption from the gut.[2] A preliminary study of pancreatic somatostatin secretion in obesity was made using the spontaneously obese Zucker rat. Rates were studied at two ages: 5 mo and 15 to 18 mo. In addition, the effects of pair feeding on pancreatic hormone content and secretion were investigated in the younger obese rat.

METHODS

The Zucker rats used were from a colony established in Düsseldorf since 1975. The colony descended from three breeding pairs obtained from the outbred colony of Dr. Lois Zucker.[3] A subline of homozygous lean rats was developed within the Düsseldorf colony to obtain genetically defined lean control animals. Back crossings were made at each sixth generation to prevent genetic segregation. Obese animals were the offspring of heterozygous lean parents in a subline where there had been brother-sister matings for four generations. The animals were kept under conventional conditions in a room with a constant temperature (24 ± 1°C) and a 12 h light-dark cycle. They were fed standard rat chow containing 66% of total calories as carbohydrate, 23% as protein, and 11% as fat.

Animals of two age groups were used for studies on insulin and pancreatic somatostatin (SRIF) secretion and islet hormone content.

5-MONTH-OLD RATS

Animals in this group were five months old at the end of the experiment. There were three subgroups, each consisting of five rats: (1) obese, ad libitum–fed rats; (2) obese rats, in which the food intake was restricted to equal that of the lean

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animals; and (3) lean, ad libitum–fed rats. Lean and obese littermates can be distinguished from each other as early as four weeks. However, since genetically defined homozygous lean and obese rats are, by definition, from different litters, an overlap in their mean weights can occur up to 9 or 10 wk, depending on the average litter size. The pair feeding was carried out for 16 wk, from the age of 7–9 wk until the end of the experiment. During the eighth week of this feeding experiment, some animals developed a respiratory infection. All rats were treated by daily doses of chlortetracycline for two separate periods of 10 days, with an interval of one week. The treatment was completed four weeks before the end of the experiment (one ad libitum–fed obese and one lean rat were excluded from the experiment because of the infection).

Rats in this group were given intravenous glucose tolerance tests one week before the end of the experiment. At death the pancreas was removed for preparation of isolated islets (see below) and the carcass was stored at −20°C until body lipid content was measured.

15–18-MONTH-OLD RATS

Rats in this group were killed at the age of 15–18 mo; there were obese, ad libitum–fed (N = 6) and lean, ad libitum–fed (N = 4) subgroups. Intravenous glucose tolerance tests were performed, and pancreatic islets were isolated for measurement of hormone content and secretion.

Intravenous glucose tolerance tests were carried out under sodium pentobarbital anesthesia, between 08.30 and 10.00 h, 14 h after food was withdrawn. A catheter was placed in the right jugular vein, and body temperature was maintained at 37 ± 1°C by heating pads. Glucose, 1g/kg as a 40% solution, was given intravenously at 0 min. With respect to the time at which glucose was administered, blood samples were taken at −5, −2, 2, 5, 10, 30, and 60 min.

Pancreatic islet experiments. Rats were killed between 08.30 and 09.00 h in the fed state. Pancreatic islets were isolated by a modification of the collagenase technique.[4] Islet protein content was measured by a micromodification of the Lowry technique.[5] Islets utilized for hormone secretion and content studies were first preincubated for 30 min in a modified Krebs-Ringer bicarbonate buffer (KRB-Hepes) containing 5 mM Na HCO_3, 1 mM $CaCl_2$, 250 kallikrein inhibitory units/ml Trasylol, 0.5% dialyzed bovine serum albumin, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Hepes), pH 7.4, and 2.8 mM glucose. After 30 min of preincubation at 37°C the islets were rinsed twice in KRB-Hepes containing 2.8 mM glucose. The islets were then incubated for 30 min as groups of 10 islets in 1 ml of KRB-Hepes buffer containing the test substances. At the end of the incubation period the medium was removed and stored at −20°C until assayed for insulin and SRIF. Islets were again rinsed twice with KRB-Hepes containing 2.8 mM glucose, and the insulin and SRIF contents were extracted by the acid-ethanol method. Batches of 10 islets were placed in 1 ml acid-ethanol (750:235:15, vol/vol/vol, ethanol-water-concentrated HCl) and left for 20 h at 4°C. The supernatants were stored at −20°C and assayed for insulin and SRIF content.

Body lipid measurement. The lipid content of the carcasses was measured by a previously described method.[6]

Assays. Plasma glucose was measured using the glucose oxidase method.[7] Insulin and SRIF were measured by radioimmunoassay using the charcoal separation technique.[8] Rat insulin and guinea-pig anti-pork insulin serum were used in the insulin assay. Cyclic SRIF was used as standard in the SRIF assay. N-tyrosylated SRIF was iodinated by the chloramine-T method[9] and purified using CM52 cellulose. The SRIF antiserum, raised in rabbits against SRIF conjugated to albumin by the glutaraldehyde method, showed no cross reactivity with insulin, glucagon, pancreatic polypeptide, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, gastrin, motilin, secretin, or cholecystokinin-pancreozymin (the cross reactivity studies were carried out by Dr. J. Ardill). The minimal sensitivity of the assay was 7 pg/ml.

Statistics. Analyses were performed by Student's *t* test for unpaired data. Values are expressed as the mean ± SEM.

MATERIALS

Rat insulin standard was obtained from Novo Research Institute, Bagsvaerd, Denmark; guinea-pig anti-pork insulin serum was a gift from Dr. H. H. Schöne, Farbwerke Hoechst, Frankfurt, F.R.G. Cyclic SRIF and N-tyrosylated SRIF were bought from Serono, Freiburg, F.R.G. SRIF antiserum was a gift from Dr. J. Ardill, Queen's University, Belfast, U.K. Trasylol was kindly provided by Prof. G. L. Haberland, Bayer A. G., Wuppertal, F.R.G.

RESULTS

5-MONTH-OLD RATS

Body weight: total. The weights of animals in this group are shown in Table 1. At the outset (age 9 wk) the weights of the obese and homozygous lean rats were not significantly different. The mean weight gain of the obese pair-fed rats (131 ± 17 g) during the 16 wk period of pair feeding was similar to that of the lean rats (128 ± 4 g). During the same period, obese ad libitum–fed rats gained 298 ± 2 g.

Body weight: lipid content. The percent body weight found as lipid at the end of the experiment is shown in Table 1. Obese ad libitum–fed rats had approximately 40% of their total body weight in the form of lipid, while lipid accounted for only 9% of body weight in lean rats. Obese rats, pair fed with lean rats for 16 wk, had approximately the same proportion of body weight in the form of lipid as had obese ad libitum–fed rats.

Intravenous glucose tolerance test. The plasma glucose results during IVGTT are shown in Table 2a. Both obese groups showed a slight delay in the return of the plasma glucose towards the basal concentration when compared with the lean group.

Hormone content, hormone secretion, and protein content of isolated pancreatic islets. The SRIF content of islets of 5-mo-old obese rats was similar to that of the lean (Table 3). Glucose stimulated SRIF secretion from islets of both obese and lean rats of this age. A significant response above basal SRIF release (that occurring with 2.8 mM glucose) occurred at 5.5 mM glucose for islets of lean rats ($P < 0.05$) and at 8.3 mM glucose for islets of obese rats ($P < 0.005$ for both ad libitum–fed and pair-fed rats). While SRIF secretion by islets of lean and obese ad libitum–fed

TABLE 1
Total body weight and percent body weight lipid in five-month-old rats (pair-feeding experiment)
Mean ± SEM

		Weight at start of study (g) (age, 9 wk)	Weight at end of study (g) (age, 23–25 wk)	Weight gain (g)	% body weight as lipid at end of study
Lean	(n = 4)	268 ± 2	396 ± 4	128 ± 4	9.3 ± 1.4
Obese, pair fed	(n = 4)	292 ± 23	423 ± 20	131 ± 17	42.4 ± 1.8
Obese, ad libitum fed	(n = 3)	245 ± 30	543 ± 32	298 ± 2	39.9 ± 2.1

Animals entered the study at age 9 wk. Lean ad libitum–fed, obese pair-fed, and obese ad libitum–fed rats were killed in groups 14–16 wk later, when they were 23–25 wk old.

rats was similar at 2.8 mM and 5.5 mM glucose, at higher glucose concentrations (8.3 mM and 16.7 mM), approximately 50% more SRIF was secreted by islets from obese ad libitum–fed rats than by those of lean rats (Figure 1). As expected, islets of obese ad libitum–fed rats had higher insulin and total protein contents than did those of the lean (Table 3), and, in addition, insulin secretion from islets of obese ad libitum–fed rats was greater than that from islets of lean rats at all glucose concentrations tested (Figure 2).

The beta and delta cells of obese rats showed a markedly different response to pair feeding. As expected, the insulin content (Table 3) and glucose-induced insulin release (Figure 2) from islets of obese rats were significantly reduced by pair feeding, although, even after pair feeding, both insulin content and insulin release rates remained higher than those of islets from lean rats. By contrast, pair feeding had no significant effect on either islet somatostatin content

TABLE 2
Plasma glucose (mg/dl) during i.v. GTT (1g/kg)
time (minutes)

	Basal‡	2	5	10	30	60
5-mo-old rats*						
Lean (4)	144 ± 7	981 ± 249	624 ± 41	470 ± 39	224 ± 19	172 ± 22
Obese, pair fed (4)	164 ± 35	1054 ± 108	936 ±141	595 ± 33	305 ± 48	216 ± 33
Obese, ad libitum fed (3)	155 ± 6	925 ± 78	672 ± 17	534 ± 26	312 ± 33	246 ± 35
15- to 18-mo-old rats†						
Lean (2)	135	702	567	382	217	151
Obese, ad libitum fed (2)	178	1040	866	697	421	331

* Results are expressed as mean ± SEM.
† Results are expressed as mean value.
‡ Basal value is the mean of values at −5 and −2 min.

TABLE 3
Hormone and protein content of pancreatic islets (mean ± SEM)

	Insulin (ng/10 islets)	Somatostatin (ng/10 islets)	Protein (μg/10 islets)
5-mo-old rats (pair-feeding experiment)			
Lean	580 ± 20 (148)	2.3 ± 0.2 (19)	8.0 ± 0.9 (8)
Obese, pair fed	900†‡ ± 62 (111)	2.6 ± 0.2 (20)	23.8† ± 2.1 (11)
Obese, ad libitum fed	1101† ± 67 (70)	2.4 ± 0.2 (20)	20.2† ± 1.8 (7)
15- to 18-mo-old rats			
Lean	755 ± 46 (71)	3.7 ± 0.3 (72)	19.4 ± 2.2 (5)
Obese, ad libitum fed	4086† ± 201 (86)	13.1† ± 1.2 (42)	95.5* ±11.6 (5)

P values: * <0.005, † <0.001 vs lean; ‡ <0.05 vs obese, ad libitum fed
Parentheses denote the number of observations.

(Table 3) or glucose-induced SRIF release rates (Figure 1). The protein contents of islets of obese ad libitum–fed and obese pair-fed rats were similar (Table 3).

15–18-MONTH-OLD RATS

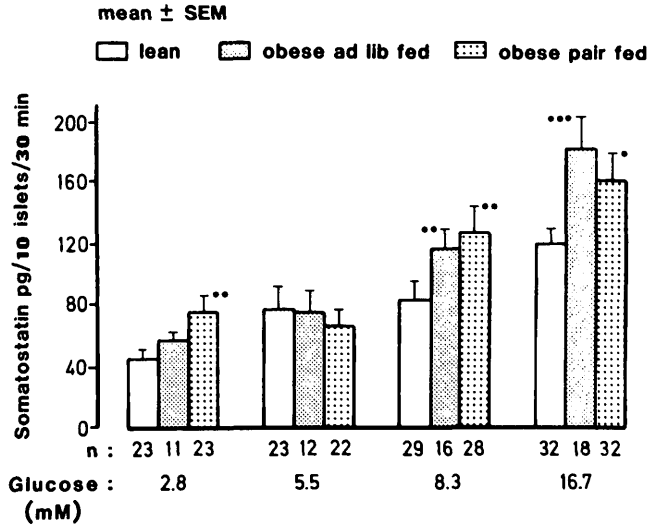
Body weight. A total of six obese ad libitum–fed and four lean rats of this age was used. The mean weight of the obese rats was 697 ± 49 g and of the lean, 538 ± 24 g.

The intravenous glucose tolerance test was performed on two obese and two lean rats; the mean plasma glucose values are shown in Table 2. The older obese rats had a

FIGURE 1. Somatostatin secretion from isolated pancreatic islets of 5-mo-old Zucker rats.

P values: * <0.05, ** <0.02, *** <0.005 **vs lean**

At each glucose concentration the amount of somatostatin secreted from islets of obese pair-fed rats did not differ significantly from that secreted by islets of obese ad libitum–fed rats.



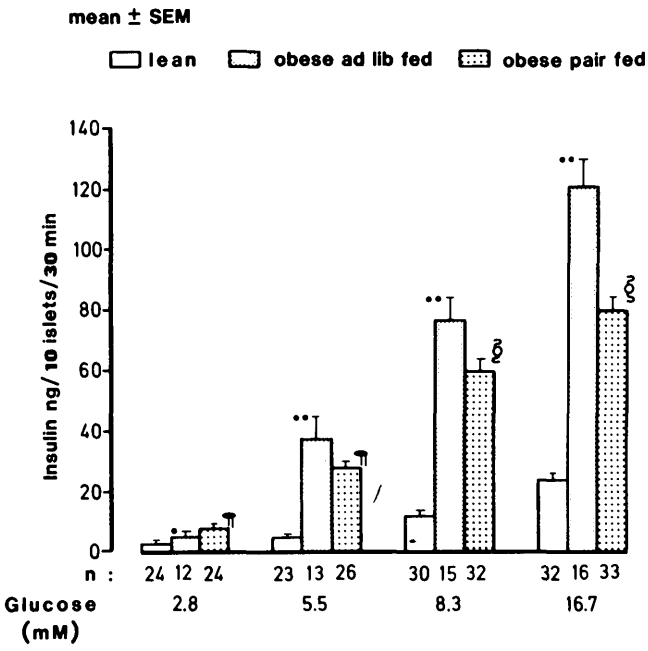


FIGURE 2. Insulin secretion from isolated pancreatic islets of 5-mo-old Zucker rats.

P values: * <0.01, ** <0.001 vs lean; ¶ <0.05, § <0.01 vs obese, ad libitum fed

For the sake of simplicity, P values of obese pair-fed vs lean rats are not shown in the figure. At all glucose concentrations, the islets from obese pair-fed rats secreted more insulin than islets from lean rats (P < 0.001 at all glucose concentrations).

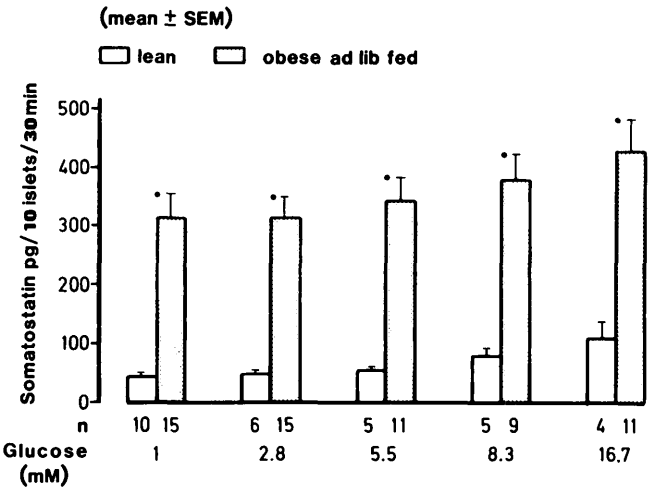


FIGURE 3. Somatostatin secretion from isolated pancreatic islets of 15- to 18-mo-old Zucker rats.

P value: * <0.001 vs lean

At 8.3 and 16.7 mM glucose, somatostatin secretion from islets of lean rats was stimulated above basal (1 and 2.8 mM) (P < 0.01 for both 8.3 and 16.7 mM glucose). Increasing glucose concentrations above basal (1 and 2.8 mM) did not cause significant stimulation of somatostatin secretion from islets of obese rats. (Please note the difference in scale between Figures 1 and 3).

more marked glucose intolerance with respect to the lean controls than did the younger obese rats. Although glucose tolerance tests were performed on only a limited number of 15- to 18-mo-old rats in this study, the results reflect the usual findings in rats from this colony, i.e., 15- to 18-mo-old obese rats have a greater glucose intolerance than younger obese rats (L.H., unpublished observations).

Hormone content, hormone secretion, and protein content of isolated pancreatic islets. The islet SRIF content of 15-mo-old lean rats was 50% greater than that of 5-mo-old lean rats, while for obese rats there was a fivefold increase in SRIF content when islets of the older obese rats were compared with those of the younger rats. Contrary to the findings in the 5-mo-old rats, where islet SRIF content was similar in the obese and lean, in 15-mo-old obese rats the islet content was four times that of 15-mo-old lean rats.

SRIF release at all glucose concentrations was greater from islets of 15-mo-old obese rats than from islets of age-matched lean rats. In lean rats the pancreatic SRIF secretion was stimulated by glucose concentrations of 8.3 and 16.7 mM (Figure 3), the amount secreted being in the range of that secreted from islets of younger lean rats (Figure 1). In contrast with islets from younger obese rats, SRIF secretion from those of the older obese animals showed no significant increase with increasing glucose concentrations. In addition, the amount of SRIF secreted by islets of older obese animals was greater at all glucose concentrations than that secreted by islets of younger obese rats. At 16.7 mM glucose, for example, islets from the older obese rats secreted approximately twice as much SRIF as did the islets of 5-mo-old obese rats.

Islets of both obese and lean rats showed an increase in insulin and total protein content with age from 5 to 15 months (Table 3). Islets from obese rats secreted more insulin than those from lean rats at all glucose concentrations (range, 1 mM–16.7 mM). The beta cells of islets from both obese and lean rats remained sensitive to glucose (cf delta cells of 15-mo-old obese rats, see above).

DISCUSSION

It is well known that isolated islets of obese Zucker rats are hypertrophied[10,11] and secrete more insulin than do those of lean controls.[11–15] The present findings are in keeping with these results. The increased size of the islet is mainly a result of beta cell hypertrophy and hyperplasia. The numbers of somatostatin-containing cells (A_1 or delta) and glucagon-containing cells per islet remain unchanged. While under ad libitum–feeding conditions there is a reduction in the number of cells which stain with antipancreatic polypeptide serum, this can be normalized by diet.[16] Since the number of somatostatin-containing cells in islets from obese and lean Zucker rats are, at present, thought to be similar, it seemed relevant to make a comparison of somatostatin secretion rates using the whole islet as the reference point.

Under normal conditions, pancreatic delta cells are glucose sensitive. In this report, in accordance with previous publications,[17–20] we show that the fold increases in somatostatin secretion induced by glucose concentrations of 8.3 mM and 16.7 mM are much less than those for insulin. Although islets from 5-mo-old obese and lean rats had a similar somatostatin content, glucose caused a greater amount of somatostatin to be released from the islets of obese than from the islets of lean rats. Among many other possibilities this could be related to the obesity, the increased insulin levels found in this syndrome, or to the increased food in-

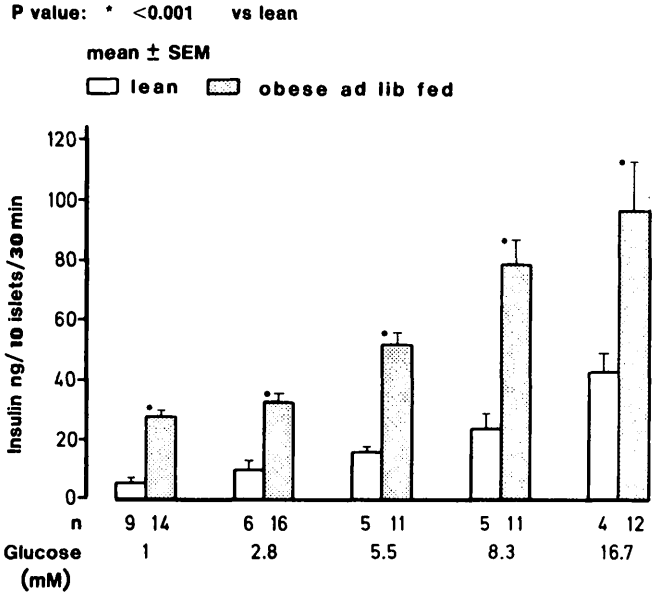
take of obese rats. Pair feeding curtailed the weight increase of obese rats to that of the lean but had no effect on the percentage of total weight found in the form of lipid, i.e. it did not reduce the degree of obesity, a finding noted in a previous study.[21] The results indicate that the hyperphagia had no effect on subsequent glucose-induced somatostatin release, which is in marked contrast to the reduction of glucose-induced insulin release that accompanied the pair feeding. However, since pair feeding did not reduce the degree of obesity and since high insulin levels remained, it is not clear to what extent either factor contributed to the changes of pancreatic delta cell function found in 5-mo-old obese rats.

The islets of obese Zucker rats have an abnormal architecture from a fairly early age.[16] Delta cells, which normally have a peripheral location in the islet, lose this position and become intermixed with the other endocrine cells. The factors that cause these changes and the effects of these changes on delta cell function are unknown. Similar changes do occur in other obesity syndromes. In the obese C57BL/KsJ-*db/db* mouse, changes in the morphology of pancreatic delta cells occur in the presence of increases in pancreatic insulin and hyperinsulinemia, and before the development of hyperglycemia.[22] At a later stage in this syndrome, when hyperglycemia is also present, delta cells leave their peripheral position in the islet and become intermixed with the other endocrine cells.

In the older obese Zucker rats, where hyperinsulinemia and insulin resistance were associated with moderate glucose intolerance, there was also an increase in the islet somatostatin content compared with that of age-matched lean controls. Two groups of workers reported independently that C57BL/6J-*ob/ob* mice exhibiting obesity, hyperinsulinemia, and peripheral insulin resistance have increased amounts of somatostatin in the pancreas,[23,24] although this could not be confirmed by a third group.[25,26] Since increases in islet[27] and total pancreatic[28] content of somatostatin also occur in streptozotocin-induced insulinopenic diabetes, it is difficult, at the present time, to draw any conclusions about the possible respective roles of obesity, hyperinsulinemia, and mild glucose intolerance in the changes of somatostatin content and secretion observed in the obese Zucker rat. High secretion rates[29] and glucose insensitivity[30] of pancreatic delta cells have been reported in experimental insulinopenic diabetes. Although it is possible that similar abnormalities of somatostatin secretion found in islets of 15- to 18-mo-old obese Zucker rats may be due to the glucose intolerance found in these animals, it should be noted that this intolerance is relatively mild and in no way compares with that of experimental insulinopenic diabetes. In addition, the changes of somatostatin physiology noted in the older obese Zucker rat occur while there are high concentrations of insulin within the pancreas (Figure 4).

For the present, it must remain a matter for speculation whether a higher release rate of pancreatic somatostatin in obesity plays a local role in dampening the insulin response to glucose or whether it slows the absorption of nutrients from the gut, a role it may play under normal physiologic conditions.[31,32]

FIGURE 4. Insulin secretion from isolated pancreatic islets of 15- to 18-mo-old Zucker rats.



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REFERENCES

[1] Gerich, J. E., Charles, M. A., and Grodsky, G. M.: Regulation of pancreatic insulin and glucagon secretion. Annu. Rev. Physiol. *38*:353–88, 1976.

[2] Schusdziarra, V., Rouiller, D., and Unger, R. H.: Role of pancreatic somatostatin in nutrient homeostasis. Clin. Res. *26*:533A, 1978 (abstract).

[3] Zucker, L. M., and Zucker, T. F.: Fatty, a new mutation in the rat. J. Hered. *52*:275–78, 1961.

[4] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35–39, 1967.

[5] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[6] Bell, G. E., and Stern, J. S.: Evaluation of body composition of young obese and lean Zucker rats. Growth *41*:63–80, 1977.

[7] Bergmeyer, H. U., and Bernt, E.: *In* Methoden der enzymatischen Analyse. Weinheim/Bergstrasse, Verlag Chemie, 1970, p. 1172.

[8] Herbert, V., Lau, K.-S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. *25*:1375–84, 1965.

[9] Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (Lond) *194*:495–96, 1962.

[10] Shino, A., Matsuo, T., Iwatsuka, H., and Suzuoki, Z.: Structural changes of pancreatic islets in genetically obese rats. Diabetologia *9*:413–21, 1973.

[11] Hayek, A., and Woodside, W.: Correlation between morphology and function in isolated islets of the Zucker rat. Diabetes *28*:565–69, 1979.

[12] Stern, J., Johnson, P. R., Greenwood, M. R. C., Zucker, L. M., and Hirsch, J.: Insulin resistance and pancreatic insulin release in the genetically obese Zucker rat. Proc. Soc. Exp. Biol. Med. *139*:66–69, 1972.

[13] Schade, D., and Eaton, R. P.: Insulin secretion by perfused islets from the obese Zucker rat. Proc. Soc. Exp. Biol. Med. *149*:311–14, 1975.

[14] Stern, J. S., Johnson, P. R., Batchelor, B. R., Zucker, L. M., and Hirsch, J.: Pancreatic insulin release and peripheral tissue resistance in Zucker obese rats fed high- and low-carbohydrate diets. Am. J. Physiol. *228*:543–48, 1975.

[15] Bryce, G. F., Johnson, P. R., Sullivan, A. C., and Stern, J. S.: Insulin and glucagon: plasma levels and pancreatic release in the genetically obese Zucker rat. Horm. Metab. Res. *9*:366–70, 1977.

[16] Larsson, L.-I., Boder, G. B., and Shaw, W. N.: Changes in the islets of Langerhans in the obese Zucker rat. Lab. Invest. *36*:593–98, 1977.

[17] Schauder, P., McIntosh, C., Arends, J., Arnold, R., Frerichs, H., and Creutzfeldt, W.: Somatostatin and insulin release from isolated rat pancreatic islets stimulated by glucose. FEBS Lett. *68*:225–27, 1976.

[18] Efendić, S., Enzmann, F., Nylen, A., Uvnäs-Wallensten, K., and Luft, R.: Effect of glucose/sulfonylurea interaction on release of insulin, glucagon

and somatostatin from isolated perfused rat pancreas. Proc. Natl. Acad. Sci. USA *76*:5901–04, 1979.

[19] Ipp, E., Dobbs, R. E., Arimura, A., Vale, W., Harris, V., and Unger, R. H.: Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. J. Clin. Invest. *60*:760–65, 1977.

[20] Dolais-Kitabgi, J., Kitabgi, P., Brazeau, P., and Freychet, P.: Effect of neurotensin on insulin, glucagon and somatostatin release from isolated pancreatic islets. Endocrinology *105*:256–60, 1979.

[21] Bray, G. A., York, D. A., and Swerloff, R. S.: Genetic obesity in rats. I. The effects of food restriction on body composition and hypothalamic function. Metabolism *22*:435–42, 1973.

[22] Leiter, E. H., Gapp, D. A., Eppig, J. J., and Coleman, D. L.: Ultrastructural and morphometric studies of delta cells in pancreatic islets from C57BL/Ks diabetes mice. Diabetologia *17*:297–309, 1979.

[23] Makino, H., Matsushima, Y., Kanatsuka, A., Yamomoto, M., Kumagai, A., and Nishimura, M.: Changes in pancreatic somatostatin content in spontaneously diabetic mice, as determined by radioimmunoassay and immunohistochemical methods. Endocrinology *104*:243–47, 1979.

[24] Dolais-Kitabgi, J., LeMarchand-Brustel, Y., and Freychet, P.: Somatostatin in the pancreas and hypothalamus of obese mice. Diabetologia *17*:257–61, 1979.

[25] Patel, Y. C., Orci, L., Bankier, A., and Cameron, D. P.: Decreased pancreatic somatostatin (SRIF) concentration in spontaneously diabetic mice. Endocrinology *99*:1415–18, 1976.

[26] Patel, Y. C., Cameron, D. P., Stefan, Y., Malaisse-Lagae, F., and Orci, L.: Somatostatin: widespread abnormality in tissues of spontaneously diabetic mice. Science *198*:930–31, 1977.

[27] Patel, Y. C., and Weir, G. C.: Increased somatostatin content of islets from streptozotocin-diabetic rats. Clin. Endocrinol. *5*:191–94, 1976.

[28] Patel, Y. C., Cameron, D. P., Bankier, A., Malaisse-Lagae, F., Ravazzola, M., Studer, P., and Orci, L.: Changes in somatostatin concentration in pancreas and other tissues of streptozotocin diabetic rats. Endocrinology *103*:917–23, 1978.

[29] Hara, M., Patton, G., and Gerich, J.: Increased somatostatin release from pancreases of alloxan diabetic rats perfused in vitro. Life Sci. *24*:625–28, 1979.

[30] Hermansen, K., Ørskov, H., and Christensen, S. E.: Streptozotocin diabetes: a glucoreceptor dysfunction affecting D cells as well as B and A cells. Diabetologia *17*:385–89, 1979.

[31] Unger, R. H., Dobbs, R. E., and Orci, L.: Insulin, glucagon and somatostatin secretion in the regulation of metabolism. Annu. Rev. Physiol. *40*: 307–43, 1978.

[32] Schusdziarra, V., Zyznar, E., Rouiller, D., Boden, G., Arimura, A., and Unger, R. H.: Splanchnic somatostatin: a hormonal regulator of nutrient homeostasis. Science *207*:530–32, 1980.