Correlation Between Morphology and Function in Isolated Islets of the Zucker Rat

ALBERTO HAYEK AND WILLIAM WOODSIDE

SUMMARY

Obesity in the Zucker rat is accompanied by hyperlipemia, hyperinsulinism, insulin resistance, pancreatic hyperplasia, and islet hypertrophy. This study correlates the morphologic heterogeneity of isolated pancreatic islets with secretion of insulin and glucagon in a perifusion system. Islet size was arbitrarily defined as large (>0.45 mm) or small (<0.12 mm). Protein content and volume ($V = \frac{4}{3}\pi r^3$) were calculated for groups and individual islets, respectively. Islets from obese rats secreted more insulin in response to glucose and aminophylline than islets from lean rats (peak 7.8 ± 2.4 vs. 1.5 ± 0.37 μU/islet/min, $P < 0.005$). Insulin release was related directly to islet size and protein content. Small islets from lean and obese animals produced less insulin per islet than large islets ($P < 0.005$). In terms of islet volume, however, large islets were inefficient insulin releasers as compared to small islets ($P < 0.005$). Stimulation with Br-cAMP released glucagon from islets of lean but not from large islets of obese animals (peak 11 ± 3.3 vs. 4.1 ± 0.3 pg/μg protein per minute, $P < 0.05$). Arginine produced the same effect on glucagon release ($P < 0.05$) as stimulation with Br-cAMP. The observed increased insulin release rates and the blunted glucagon response are related to islet size in the pancreas of the Zucker rat. **DIABETES 28:565–569, June 1979.**

The Zucker rat is characterized by obesity, hyperlipemia, and hyperinsulinism in the homozygous state (*fa/fa*).[1] The homozygous normal and the heterozygote (*Fa/?*) are both lean and phenotypically indistinguishable. Increased pancreatic insulin content, hyperinsulinemia, and concomitant insulin resistance have been well-defined in the Zucker rat;[1–3] also, isolated pancreatic islets from *fa/fa* release more insulin than those from lean rats.[4,5] However, there is disagreement concerning the role of other pancreatic hormones in the initiation and maintenance of the metabolic abnormalities seen in the obese animal. In this context, circulating levels of glucagon have been reported to be both decreased[6] and normal,[7] and metabolic effects of glucagon on liver both blunted[8,9] and similar to normal rats.[10,11]

In many strains of rodents, morphologic and structural abnormalities of the pancreatic islets are seen in association with obesity, including hypertrophy, hyperplasia, degranulation of B cells, and fibrosis. These observations, documented in *db/db* mice,[12] *ob/ob* mice,[13] sand rats,[14] and spiny mice[15] have been extended to the islets of the Zucker rat as described in two recent reports.[16,17]

The studies reported here were carried out to assess the functional significance of the morphologic changes observed in isolated islets of the Zucker rat with respect to insulin and glucagon release in a perifusion system.

MATERIALS AND METHODS

The Zucker animals used in this study came from a colony established previously at the University of New Mexico School of Medicine. The colony was originally derived from 12 heterozygous breeding pairs obtained from the H. G. B. Memorial Laboratory, Stow, Massachusetts. The rats were fed, ad libitum, a diet (LAB-BLOX-8604-00, Allied Mills, Chicago, Illinois), containing a minimum of 24% protein, 4% fat, 4.5% crude fiber, and at least 65% grain carbohydrate up to the time they were killed. Both male and female rats were used, ages ranging between 20 and 24 wk.

Pancreatic islets from obese and lean animals were isolated with collagenase, using the digestion-filtration method described by Shibata et al.[18] Large islets (largest diameter > 0.5 mm) usually remained in the digestion chamber. After a 2-min centrifugation, the precipitate containing the islets was resuspended in Hanks' solution and placed in petri dishes. The digest remaining in the chamber was flushed into the same petri dishes to recover large islets. Islet size was determined with an eyepiece micrometer graduated in tenths of a millimeter and placed at the bottom of the petri dish. The largest diameter of each islet, when placed on the micrometer, was used as the index of islet size. Islet

From the Departments of Pediatrics and Pharmacology, University of New Mexico School of Medicine, Albuquerque, New Mexico.
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volume was calculated according to the formula, $V = \frac{4}{3}\pi r^3$. Comparable calculations have been used for islets in the *ob/ob* mouse.[13]

The islets were selected individually with a micropipette under a dissecting microscope and transferred immediately to Swinney perifusion chambers until each contained 10–20 islets. The islets were classified arbitrarily as large (diameter > 0.45 mm) or small (diameter < 0.12 mm). Islets from lean animals rarely exceeded the latter measurement.

For each experiment, sets of two chambers were run simultaneously in a perifusion system used previously in this laboratory,[4] based on the original methodology of Lacy et al.[30] While the islets in one chamber were subjected to the different experimental conditions, the other containing similar islets served as a control using buffer only as perfusate. At the end of a series for each specific experiment, the results were pooled and analyzed statistically. The perfusing fluid was Hanks' solution, adjusted to a pH of 7.4 and gassed continuously with 95% O_2 and 5% CO_2. Flow rate was maintained at 1 ml/min for each chamber. Prior to stimulation, the islets were perifused for 20–30 min in order to achieve steady-state secretion of insulin and glucagon. A series of stimulations, e.g., high glucose (5 mg/ml) and aminophylline (10 mM) on the one hand, and 8-Br-cAMP (2 mM) and arginine (10 mM) on the other hand, were used to induce maximal release rates for insulin and glucagon, respectively. Insulin and glucagon were assayed in samples collected from the effluent perfusate fluid by double antibody radioimmunoassay, as reported previously in rats.[6] For determination of glucagon, 250 μl of the perfusate was transferred to plastic tubes containing 100 μl of Trasylol (200,000 IU/ml). The tubes were frozen and assayed subsequently with 30 K antibody obtained from Dr. Roger Unger, Dallas, Texas. At the conclusion of the perifusions, the Nitex filter[19] containing the islets was removed and placed in a tube containing 100 μl of 1 N NaOH and processed for the measurement of total protein according to the microanalysis of Lowry et al.[20]

Statistical analyses were carried out using a nonparametric one-way analysis of variance (Kruskal-Wallis test) and the nonparametric multiple comparison test.[21]

The sequence of experiments was as follows.

Experiment 1. An initial study was conducted in order to assess the magnitude of insulin and glucagon release from islets derived from *fa/fa* and *Fa/?* rats, in response to low (0.5 mg/ml) and high (5 mg/ml) concentrations of glucose and aminophylline (10 mM). Samples of 1 ml were collected every 10 min during basal and stimulated situations, except immediately after the introduction of the stimulant, where, in addition, two more samples were collected in the initial 2 min. No attempt was made in this experiment to separate islets according to size.

Experiment 2. While performing the above described study, it was evident that there was a striking heterogeneity in the size of islets derived from the pancreas of the obese rat. Accordingly, a single experiment was planned with large and small islets obtained from the same obese animal and put under the same experimental conditions as described above. Each chamber contained 10 large (diameter 0.8 to 1 mm) or 10 small islets (diameter <0.12 mm). Insulin release was measured in the two groups of islets and compared with the release of normal islets derived from a lean animal (diameter <0.12 mm).

To validate statistically the above study, large and small islets were obtained from *fa/fa* as well as from lean rats with normal islets. Each chamber contained either five large islets (diameter 0.45–1.1 mm) or 10 small islets (diameter <0.12 mm) and they were all maximally stimulated for 30 min with high glucose (5 mg/ml) and aminophylline (10 mM). A 1-ml sample for insulin measurement was collected at 0 time (basal) and 5, 10, 20, and 30 min during stimulation. Insulin release was expressed in terms of individual islet, according to size, protein content, and volume.

Experiment 3. Large islets (diameter 0.45 to 1 mm) obtained from obese animals and normal islets from lean animals were stimulated with 8-Br-cAMP (2 mM) in the presence of high and low glucose in order to assess the release of glucagon. Samples were collected every minute for the first 5 min after the introduction of the stimulant and every 5 min thereafter. Glucagon release was calculated in terms of individual islets and protein content. Subsequently, a more physiologic stimulation was carried out, using arginine as the secretagogue for a period of 3 min[22] after a ½-h incubation in Hank's solution. In the latter experiment, small islets from obese rats were also included to better define glucagon release in the two populations of islets from *fa/fa* as compared to *Fa/?*.

Experiment 4. To define the distribution of islet size in *fa/fa*, four animals were killed and the first 100 isolated islets from each rat were characterized in terms of size, protein content, and peak insulin release. For this study, three groups of islets were easily identified: one, with diameters ranging between 0.07 and 0.12 mm, a second group with diameters between 0.45 and 0.60 mm, and a third group with diameters between 1 and 1.2 mm. From each of these populations, a group of islets was individually stimulated with glucose (5 mg/ml) and aminophylline (10 mM) to determine peak insulin release during a 30-min period. None of the 200 islets isolated from two lean animals exceeded 0.15 mm in its maximum diameter.

RESULTS

Experiment 1. Insulin release was greater in islets from homozygous obese animals (*fa/fa*) either under basal conditions or with high concentrations of glucose and aminophylline (Figure 1). Peak concentration of insulin release was obtained at 20 min poststimulation in *fa/fa*, reaching a value of 7.8 ± 2.4 μU/islet/min, while in lean animals it was only 1.5 ± 0.37 μU/islet/min ($P < 0.005$).

The release of glucagon into the same perifusate was, however, not significantly different in the basal (*fa/fa* 6.8 ± 1.1; *Fa/?* 6.5 ± 1.8 pg/islet/min) or stimulated (*fa/fa* 6.4 ± 1.4; *Fa/?* 6.6 ± 1.7 pg/islet/min) state.

Experiment 2. The results of this single study, carried out on large and small islets obtained from the same obese rat and compared with islets of a normal rat, demonstrate that small islets from obese rats are similar to islets from lean rats in their capacity to release insulin (peak, 2.2 vs. 2.3 μU/islet/min). Large islets, on the other hand, release much more insulin (peak, 13 μU/islet/min) when maximally stimulated and compared with small islets from the same animal and to islets of the lean rat.

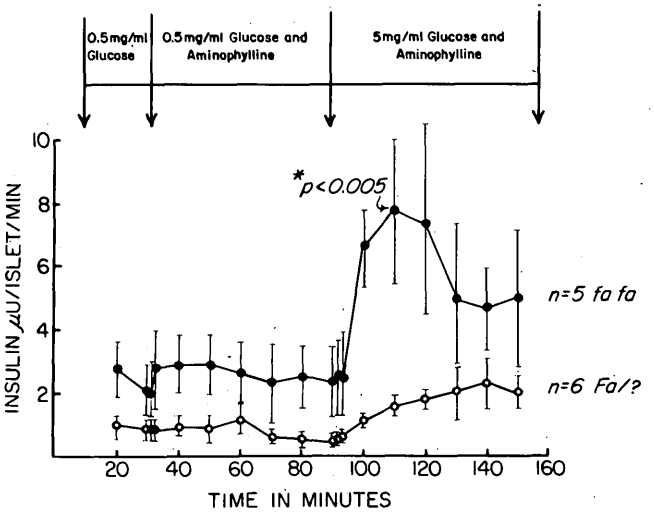

FIGURE 1. Insulin release in response to glucose and aminophylline (10 mM). Each point represents mean ± SE.

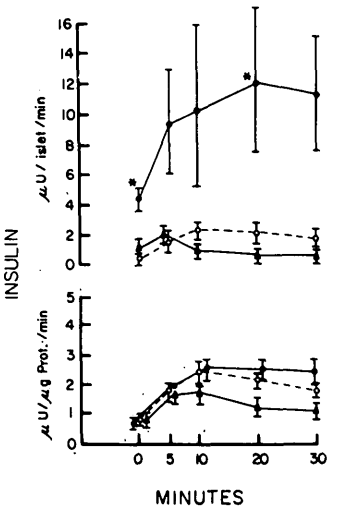

FIGURE 2. Insulin release in response to glucose (5 mg/ml) and aminophylline (10 mM) during a 30-min stimulation period. Each point represents the mean ± SE of six observations. Each chamber contains five large islets (diameter 0.45–1.1 mm) or 10 small islets (diameter <0.12 mm). *Upper portion*: Insulin secretion, expressed as microunits per islet per minute, is significantly greater in large than small islets (*P < 0.005). *Lower portion*: Insulin secretion, expressed as microunits per microgram protein per minute, is not significantly different in the three groups of islets. ● *fa/fa* (large islets); ○ Fa/? (small islets); ▲ *fa/fa* (small islets).

Figure 2 defines the relationship between islet size, protein content, and insulin release in groups of islets isolated from six obese and six lean animals. These studies demonstrate differing rates of insulin secretion in basal as compared to poststimulated state. When results are expressed as microunits of insulin secreted per islet, large islets from obese rats secrete more insulin during both basal (4.2 ± 0.4 μU/islet/min) and stimulated conditions (12 ± 4.2 μU/islet/min) than small islets. On the other hand, at basal and peak readings, small islets from lean animals do not differ significantly from small islets of fat animals. When results are expressed as microunits of insulin secreted per microgram of islet protein, no statistical difference on insulin secretion is discernible in either the basal or stimulated state between large and small islets.

Results expressed as insulin release per unit of islet volume are shown in Table 1. At basal readings, small islets from lean animals do not differ significantly from small islets obtained from obese animals (1.46 ± 0.002 vs. 1.69 ± 0.003 mU/islet volume per minute). Small islets, on the other hand, from both lean and obese rats differ significantly from large islets from fat animals (0.01 ± 0.0002 mU/islet volume per minute, $P < 0.005$). At peak readings, the conclusions are the same as those for basal readings. Thus, small islets produce more insulin per unit volume than large islets from fat animals. The insulin release between the basal and stimulated state in the three groups of islets was significant at P values of <0.02.

Experiment 3. In accord with the data best describing patterns of insulin release by isolated islets, data defining glucagon secretion are expressed as picograms per microgram of islet protein. During basal conditions, the release of glucagon is similar for islets obtained from *fa/fa* and lean rats. However, 8-Br-cAMP produces significantly greater rates of glucagon release by small islets from lean animals at low glucose concentrations; glucagon release is not stimulated at all by the cyclic nucleotide in large islets from obese rats (Figure 3) and, if anything, glucagon secretion is decreased markedly. When exposed to cAMP at high glucose concentrations, the secretion of glucagon is blunted even more in large *fa/fa* than small *Fa/?* islets. In response to arginine as secretagogue, glucagon release is again increased in islets from lean and small islets from *fa/fa*, while secretion is unaffected in large islets from obese animals (Figure 4).

Experiment 4. Table 2 defines the size distribution of islets in obese rats, correlating protein content and peak insulin release for individual groups of islets. It can be seen in the table, that at least 75% of the islets examined are larger than those of lean animals which never exceeded

TABLE 1
Mean volume, volume ranges, and insulin release per islet volume

Type of islet	Volume (mm^3)		Insulin (mU/islet vol/min)	
	Mean	Range	Basal	Peak
Small (*Fa/?*)	0.00058	0.00027–0.00091	1.46 ± 0.002	3.9 ± 1.2
Small (*fa/fa*)	0.00058	0.00027–0.00091	1.69 ± 0.003	2.7 ± 1.1
Large (*fa/fa*)	0.37230	0.04770–0.69690	0.01 ± 0.0002*	0.03 ± 0.001*

For each type, a total of 50 to 60 islets was examined.
* $P < 0.005$.

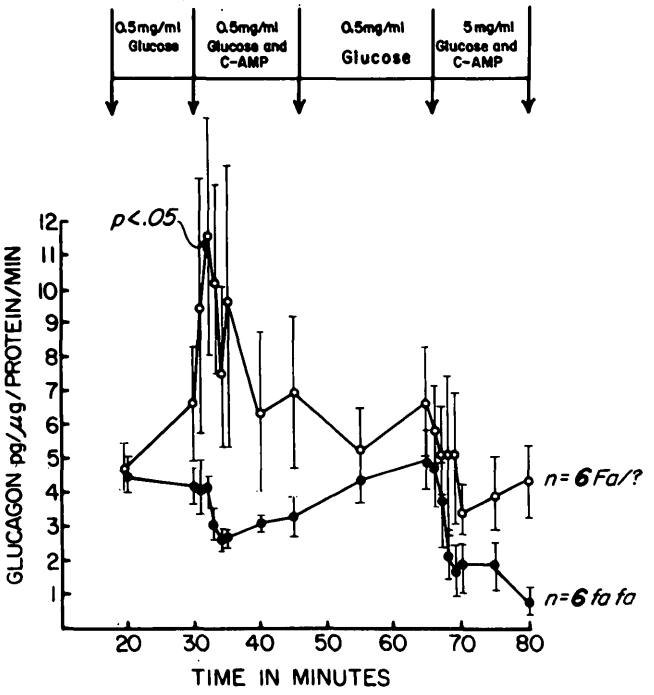

FIGURE 3. Glucagon release in response to glucose and 8-Br-cAMP (2 mM). Each point represents the mean ± SE. Islet protein content in *fa/fa* 26.7 ± 4.7 μg and in *Fa/?* 9.7 ± 1,5 μg.

0.15 mm in diameter. There is, also, a clear relationship between protein content and peak insulin release for the groups of islets studied.

DISCUSSION

The results of these studies indicate that size of the pancreatic islets in Zucker rats markedly determines the capactiy of isolated islets to release insulin under appropriate stimulation. Enlarged islets appear to be solely responsible for the hypersecretion of insulin while small islets similar in size to those of lean animals secrete insulin at normal rates. Although, theoretically, a larger than normal number of normal-sized islets may account for the hyperinsulemia, recent studies on the morphologic alterations in islets of the Zucker rat do not support this contention. Shino et al.[17] noted moderate islet hypertrophy as early as the 5th week of age, increasing as the animal became older and becoming maximal at 24 wk of age, when a good correlation was found between the degree of islet hypertrophy, obesity, and plasma

FIGURE 4. Glucagon release in response to arginine (mean ± SE). Small islets from *fa/fa* and *Fa/?* as compared to large islets from *fa/fa*.

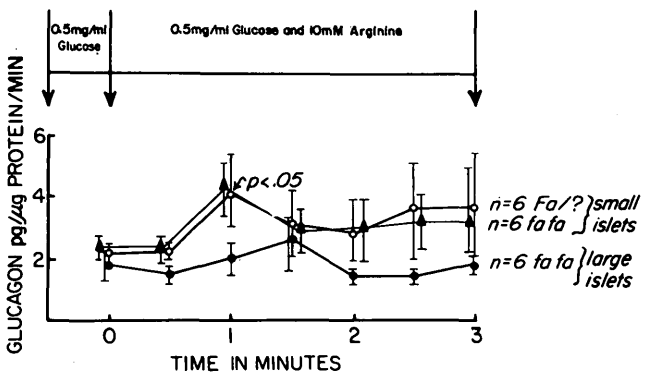

TABLE 2
Size distribution of islets, protein content, and peak insulin release in obese rats

	Range		
Size (mm)	0.07–0.12	0.45–0.60	1–1.2
Protein (μg)	3–8	12–18	28–36
Peak insulin (μU/islet/min)	1.9–3.4	5.8–11.1	16.2–21.5
Percentage*	18–23	21–28	23–26

* The remaining islets have intermediate sizes among the three groups.

insulin levels. Our studies in vitro correlate well with their findings, since at least 75% of the islet population in the obese rat appeared to be morphologically abnormal. Larsson et al.[16] also corroborated these observations using histologic and immunocytochemical techniques. They demonstrated the pronounced insulin cell hyperplasia and altered islet architecture and remarked that in an obese animal "normal sized islets were found beside gigantic ones." In addition, a marked diminution of the pancreatic polypeptide cells was observed, especially at ages where islet hypertrophy was maximal. The relationship between insulin release and islet size is strengthened by our data on insulin release; when insulin secretion is expressed in terms of microunits of insulin per microgram of islet protein, all islets, at both basal and stimulated states, showed no differences in their rates of insulin secretion. When data are expressed in terms of insulin release in relation to islet volume, the results are similar to those reported by Lavine et al.[13] in the *ob/ob* mouse, as they also found an inverse relationship between the above parameters.

These apparently paradoxical results may be explained if one assumes that cells of differing size within the islet exhibit differing biologic capacities for hormone synthesis, storage, and/or release. Degranulation of beta cells has, for example, been observed in the *ob/ob* mouse[23] and Zucker rat.[16,17] It is reasonable to assume that, for their "calculated" volume, large islets from obese Zucker rats release insulin less efficiently when compared to small islets from obese or lean animals. Thus, enlarged islets appear to account for the hyperinsulinemia of the Zucker rats, even though they may be less efficient in terms of release rates compared with small islets. An alternative possibility to be considered is that the enlarged islets simply contain more B cells than do small islets and thus secrete more insulin. The fact that the differences between large and small islets disappear when the data are expressed in microunits of insulin per microgram of protein would tend to support our conclusions. This may be true even though some percentage of the B cell population is degranulated, thus influencing the release rate when expressed per unit of islet volume.

The controversy surrounding the possible contribution of glucagon to the hyperlipemia of the genetically obese rodent has not been resolved. Eaton and Schade suggested that the endogenous hyperlipemia might be the result of reduced glucagon secretion[9] or glucagon resistance.[24] Laburthe et al.[10] extracted insulin and glucagon from the pancreas of lean and obese Zucker animals and found no difference in their immunoreactivity and biological potency. Pancreatic glucagon content was similar between lean and obese rats.

Bryce et al.[7] found normal glucagon levels in the obese rats, yet they also demonstrated that islets isolated from obese rats secreted less glucagon after incubation than those from lean Zucker rats. Our perifusion studies with isolated islets support this report and thus lead us to conclude that glucagon secretion in response to stimulation is impaired in islets from the obese animal. Furthermore, this defect appears to be present only in large islets (Figure 4), perhaps partially explaining the discrepant results obtained by other investigators. Stimulation by cyclic AMP of rates of insulin[25] and somatostatin[25] has been recently reported in isolated rat islets of Langerhans. Our data, documenting the release of glucagon by cyclic AMP-dependent mechanisms, suggest that this nucleotide may be a common secretatogue for most of the pancreatic hormones in normal islets. Similar results on glucagon secretion have been achieved following perfusion of the rat pancreas in situ with arginine.[22]

Data on other products of islet secretion—somatostatin and the pancreatic polypeptide—have yet to be reported in the Zucker rat. In other strains, like the diabetic *ob/ob* mice, somatostatin content of whole pancreas and isolated islets has been found to be decreased when compared to normal.[27] If the findings by immunohistochemistry of decreased pancreatic polypeptide cells in the islets of obese Zucker rats[16] are confirmed by demonstration of decreased content and/or release of their product of secretion, further evidence will be added to the theory that the genetic abnormality leading to obesity is expressed in the islet. The strongest argument in favor of this theory resides in the experiments of Gates et al.[28] who reported normalization of blood glucose, plasma insulin, and weight gain in the New Zealand obese mice after peritoneal implantation of normal islets. The results of these experiments, however, have not been duplicated.[29]

In conclusion, islet size in the obese Zucker rat is closely associated with excess insulin secretion, both during basal and stimulated conditions, while the blunted glucagon release is seen only during stimulation. Data from other rodents indicate that morphologically abnormal islets exhibit defects in content and/or release of all their normal secretory products.

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