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Insulin Secretion by Perfused Islets from the Obese Zucker Rat (38796)

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Two characteristic metabolic abnormalities of the obese state are: (1) plasma hyperinsulinism (1) and (2) resistance to the hypoglycemic action of this hormone (2). The events initiating and maintaining these abnormalities are not known. If the primary defect is pancreatic β cell hypersecretion of insulin, then peripheral insulin resistance may represent a protective mechanism to prevent hypoglycemia. In order to explore the possibility that hypersecretion of insulin is an inherent characteristic of the pancreatic β cell in obesity, insulin secretion from islets of the genetically obese Zucker rat was examined *in vitro* by perfusion of individual islets.

The Zucker strain of rat (3) is an animal model of genetic obesity which is characterized by hyperinsulinemia and mild glucose intolerance (4–6). By use of an *in vitro* perfusion system (7), the dynamics of insulin secretion from islets of the Zucker-obese rat were compared to that of the islets of the following controls: (1) phenotypically normal Zucker-thin littermates of comparable age, (2) phenotypically normal Zucker-thin littermates of comparable weight (but much older), and (3) Sprague–Dawley animals of similar age and weight, but non-obese. Both glucose and L-leucine were used in different experiments as insulinogenic stimuli. By utilization of such a system, we examined the possibility that inappropriate hypersecretion of insulin by β cells from obese rat islets may contribute to the hyperinsulinism characteristic of the obese state.

Methods. A colony of Zucker rats was developed from 12 breeding pairs obtained from Baird Laboratories, Stowe, Vermont. Since the "obesity" gene (fa) in the Zucker-obese rat (fafa) is transmitted as an autosomal recessive, approximately one out of four offspring of heterozygous parents became clinically obese by 6 wk of age. The heterozygote (Fafa) and the homozygote normal (FaFa) are clinically indistinguishable (thin) and used as controls (termed "Zucker-thin" in this manuscript). In addition, Sprague–Dawley rats also served as controls because at 13 wk of age their weight closely approximates that of the Zucker-obese rat.

In each experiment, 25–50 pancreatic islets from a 3-mo-old male Zucker-obese rat were compared to an equal number of islets from a male control animal. The specific age, weight, and strain for each rat in each experiment is given in Table I. Experiments I and II compared insulin secretion from the Zucker-obese rat islets with that of islets from Zucker-thin littermates. In these experiments, the ages of the animals were identical, but the total body weight of the Zucker-obese rat was almost twice that of the control rat. In experiment III, a 14-wk-old Zucker-obese rat was compared with a 34-wk-old Zucker-thin rat since at 34 wk of age, the Zucker-thin attains approximately the same weight as that of a 14-wk-old Zucker-obese rat. In this experiment, therefore, the ages of the animals differed, but the total body weight was similar. In experiments IV and V, the islets of the Zucker-obese rat were compared to those removed from a different strain of rat; i.e., the Sprague–Dawley strain. The Sprague–Dawley grows more rapidly than the Zucker strain of rat and at 13 wk, his weight is similar to that of the 13-wk-old Zucker-obese rat. However, Sprague–Dawley rats do not carry the obesity gene (Fa) and are not clinically obese. In experiments IV and V, therefore, rats of a different strain but with the same age and weight were compared to the Zucker-obese animal. In the final experiment, VI, a 13-wk-old Zucker-obese rat was compared to a thin littermate. This experiment was similar to experiments

TABLE I. THE SIX EXPERIMENTS DESCRIBED IN THIS MANUSCRIPT ARE LISTED BELOW WITH A DESCRIPTION OF THE CHARACTERISTICS OF THE ANIMALS FROM WHICH THE ISLETS WERE OBTAINED.

Expt. no.[a]	Chamber	Strain	Genotype	Phenotype	Wt (g)	Age (wk)	Sex	Insulinogenic stimulus	Rationale for control
I	A	Zucker	fafa	Obese	450	13	M	Glucose	Littermates, same age
	B	Zucker	Fafa or FaFa	Thin	260	13	M		Thin
II	A	Zucker	fafa	Obese	440	13	M	Glucose	Littermates, same age
	B	Zucker	Fafa or FaFa	Thin	240	13	M		Thin
III	A	Zucker	fafa	Obese	410	14	M	Glucose	Older age
	B	Zucker	Fafa or FaFa	Thin	420	34	M		Same wt
IV	A	Zucker	fafa	Obese	410	14	M	Glucose	Different strain, same age
	B	Sprague–Dawley	—	Thin	428	14	M		Same wt
V	A	Zucker	fafa	Obese	415	14	M	Glucose	Different strain, same age
	B	Sprague–Dawley	—	Thin	425	14	M		Same wt
VI	A	Zucker	fafa	Obese	425	13	M	L-Leucine	Littermates, same age
	B	Zucker	Fafa or FaFa	Thin	250	13	M		Thin

[a] Each experiment was performed with simultaneous perfusion of chambers A and B in parallel; chamber A containing the Zucker-obese islets, and Chamber B containing appropriate control islets.

1 and 2 except that L-leucine was utilized as the insulinogenic stimulus.

Islets were isolated by the method of Lacy *et al.* (7). After isolation, islets were transferred immediately to a Swinney perfusion chamber and placed in a 13-mm Millipore filter (pore size 8.0 μm). Each experiment consisted of two chambers connected in parallel, one containing the islets of the Zucker-obese rat, and the other chamber, the control islets. The perfusing fluid consisted of a buffered bicarbonate solution containing 30 mg/dl glucose and 0.5 mg/ml aminophylline as previously described by Johnson *et al.* (9). Fifteen minutes prior to and during perfusion of the islets, the buffer was gassed with 95% O_2, 5% CO_2. Flow rate through the chambers was controlled with a single peristaltic pump (utilizing two pumping cassettes) and adjusted to a rate of 1 cc/min/chamber. Samples were collected for assay at 2- or 4-min intervals. After 70 min of perfusion, a steady-state secretion of insulin had been reached and either glucose or leucine was added to the buffer to give a final concentration of 330 mg/100 ml glucose or 10.0 m*M* L-leucine.

Statistical analysis was performed according to Bahn (10). Significance of the difference between the means of the "glucose" experiments was determined by means of Student's *t* test at 8 min prior to stimulation, and 12 min and 30 min poststimulation.

Results. Glucose as the insulinogenic stimuli (*Fig. 1*). As has been previously reported, the islets from the Zucker-obese rat appeared much larger in size and were more easily isolated than control islets. When insulin secretion is expressed as μU/islet/min, the Zucker-obese rat islets hypersecrete insulin as compared to all control rat islets. Eight minutes prior to glucose stimulation, the obese rat islets secreted excessive amounts of insulin (mean 1.2 $\pm$ 0.3 μU/islet/min) compared to the mean 0.3 $\pm$ 0.4 μU/islet/min of the controls ($P < 0.01$). With glucose stimulation, the typical biphasic insulin response was observed at 12 min poststimulation in both the obese and control rat islets (obese mean 6.5 $\pm$ 2.4 μU/islet/min compared to control mean 1.4 $\pm$ 0.1 μU/islet/min; $P < 0.001$). Excessive insulin secretion from Zucker-obese islets was observed at all time points monitored. For

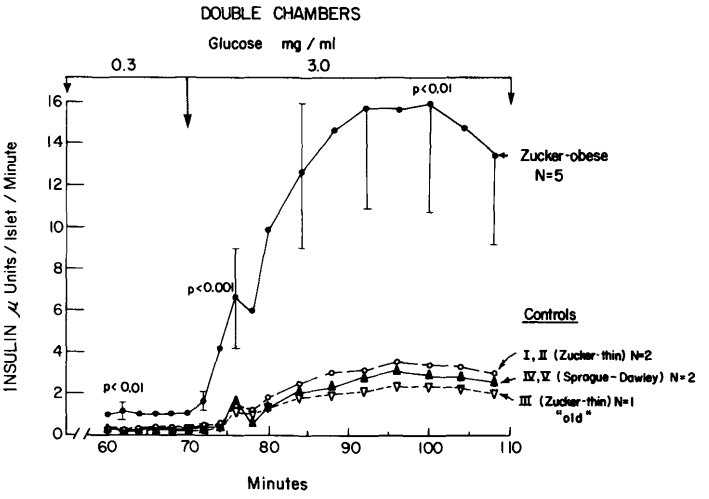

FIG. 1. Comparison of insulin secretion of islets from Zucker-obese rats to the insulin secretion of islets from different nonobese controls utilizing 300 mg/dl glucose as the insulinogenic stimulus.

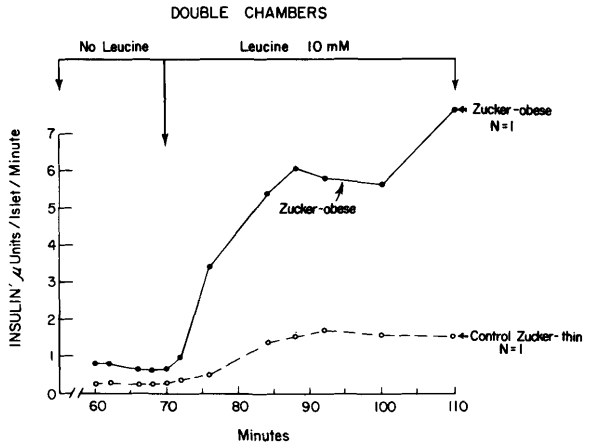

FIG. 2. Comparison of insulin secretion of islets from a Zucker-obese rat to the insulin secretion of islets from a Zucker-thin littermate utilizing 10 m*M* L-leucine as the insulinogenic stimulus.

example, at 30 min poststimulation Zucker-obese mean 16 ± 5.2 μU/islet/min was significantly greater than nonobese rat control mean 2.9 ± 0.2 μU/islet/min; $P < 0.01$.

Leucine as the insulinogenic stimulus (*Fig. 2*). Leucine is an amino acid which is reported to stimulate insulin secretion without requiring the presence of glucose (11). The results of experiment 6 are given in Fig. 2 and demonstrate that the islets from the Zucker-obese rat hypersecrete insulin in response to this amino acid stimulus when compared to a thin littermate.

Discussion. Our results demonstrate that the pancreatic islet from the Zucker-obese rat hypersecretes insulin *in vitro* in response to both glucose and leucine stimuli. This observation extends previous reports (4–6) suggesting that *in vivo* insulin hypersecretion is characteristic of these rats. The insulin hypersecretion is not simply the result of animal weight, age, or strain since control rats with similar characteristics, but without the excessive body adipose tissue (Table I) do not demonstrate this hypersecretion.

The mechanisms involved in this hypersecretion of insulin may relate to the large

size of the islet characteristic of the Zucker-obese rat, the number of β cells within the islet, or to altered molecular biology of insulin secretion in these islets. Nevertheless, our results do suggest that hypersecretion of insulin by pancreatic islets contributes to the hyperinsulinism characteristic of the obese state. Since the hypersecretion of insulin occurred in the absence of the peripheral insulin resistance characteristic of the obese state, we conclude that at the time and under the conditions of this study, an inherent defect in regulation of insulin secretion is present in the Zucker-obese β cells. Whether this inherent hypersecretion is induced by previous *in vivo* exposure to "insulin resistance" or results from a genetically transmitted characteristic of the Zucker-obese strain is not resolved by this study. Further studies utilizing tissue culture of islets or examination of these islets removed prior to the development of insulin resistance are required to resolve this question. In any case, if this inherent hypersecretion of insulin by the Zucker-obese β cells is also present *in vivo*, then the insulin resistance present may protect the animal from insulin-induced hypoglycemia.

Summary. The dynamics of insulin secretion from pancreatic islets of the Zucker-obese rat were studied by *in vitro* perfusion of individual islets. Glucose and L-leucine were used as insulinogenic stimuli. Control pancreatic islets were obtained from both normal weight Zucker-thin littermates and equivalent weight Sprague–Dawley rats.

Our results demonstrate that pancreatic islets from 13-wk-old Zucker-obese rats hypersecrete insulin in the basal state and in response to both glucose and amino acid (L-leucine) stimuli. Neither pancreatic islets from control Zucker-thin littermate animals (matched with the Zucker-obese animals for age or for total body weight), nor islets from Sprague–Dawley rats of comparable age and weight demonstrate comparable hypersecretion of insulin.

These findings; i.e., hypersecretion of insulin from obese pancreatic islets, suggest that the plasma hyperinsulinism characteristic of the obese state is maintained, at least in part, by an inherent abnormality of β cell secretion. Whether this abnormality in β cell secretion results from a genetic trait in the Zucker-obese strain or is induced by the insulin resistance of the obese animal is not resolved by this study. In any case, the observed *in vitro* hyperinsulin secretion from these islets supports the postulation that *in vivo* peripheral insulin resistance characteristic of obesity may be a physiological response that protects the animal from insulin-induced hypoglycemia.

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