Insulin Resistance and Pancreatic Insulin Release in the Genetically Obese Zucker Rat[1] (36078)

JUDITH STERN, P. R. JOHNSON, M. R. C. GREENWOOD, L. M. ZUCKER, AND JULES HIRSCH

The Rockefeller University, New York, New York 10021; and Harriet G. Bird Memorial Laboratory, Stow, Massachusetts 01775

Insulin resistance and pancreatic hyperfunction are well documented in human and animal obesity (1). Chlouverakis and White (2) have demonstrated that abdominal muscle and diaphragm of obese-hyperglycemic (*obob*) mice are less responsive to insulin than muscle from normal weight controls. Yen and co-workers (3) have shown that epididymal fat from yellow obese mice is also less responsive to insulin than that from control mice. Both obese strains are hyperinsulinemic (1). Zucker and Antoniades (4) have demonstrated hyperinsulinemia in the genetically obese Zucker rat, but no reports of insulin resistance have been published in this obese animal. If a causal relationship between the peripheral insulin resistance and pancreatic hyperfunction exists, one would expect to find a significant correlation between tissue resistance and pancreatic insulin release in the same animal. Therefore, the present study was designed to explore two questions: (i) Is the Zucker obese rat insulin resistant; and (ii) is it possible to correlate insulin release wth insulin resistance in tissues of the same animal? We measured basal and insulin-stimulated glucose-U-^{14}C uptake into diaphragm glycogen, basal and insulin-stimulated glucose-1-^{14}C conversion to $^{14}CO_2$ in isolated epididymal adipocytes; and (iii) immunoreactive insulin (IRI) release from isolated pancreatic islets.

Methods and Materials. Zucker obese (fa/fa) male rats (5) and their nonobese controls (Fa/—), were fed Purina Laboratory Chow *ad libitum*. All animals were housed in a temperature-controlled room with a 12 hr light–dark cycle. At 6 months of age, the animals were sacrificed by decapitation, blood was collected in heparinized tubes, and diaphragm and epididymal fat pads were removed. Plasma was assayed for IRI as described by Rosselin *et al.* (6). Plasma glucose was determined according to the method of Hoffman (7) with a Technicon AutoAnalyzer.

Pancreatic islets were isolated by collagenase digestion as described by Lacy and Kostianovsky (8) and incubated (5 islets/group) in 1.0 ml of Krebs–Ringer bicarbonate buffer containing 5.0 mg of bovine serum albumin and 5.0 mg of glucose. Islets were incubated for 90 min at 37° in an atmosphere of 95% O_2: 5% CO_2 and buffer was changed at 30 min intervals.

Hemidiaphragms were divided into two pieces and were incubated in 2.0 ml of Krebs–Ringer bicarbonate buffer with, and without, insulin (2000 μU/ml) for 2 hr at 37° in an atmosphere of 95% O_2: 5% CO_2. Each 2.0 ml aliquot of Krebs–Ringer buffer with 0.5% bovine serum albumin contained, 1.0 μCi of glucose-U-^{14}C and 2.0 mg of glucose. Muscle glycogen was isolated as described by Rafaelson *et al.* (9). The glycogen was dissolved in 1.0 ml of water and counted in a Packard Tri-Carb scintillation counter in 18 ml of dioxane/Cab-O-sil (4%) scintillation fluid.

Representative samples of the left epididymal fat pad were taken for adipose cell counting and triglyceride determination (10, 11). Isolated adipocytes were prepared from

[1] This investigation was supported by U.S. Public Health Service Research Grant HD-02761 of the National Institute of Child Health and Human Development and Grant AM-08272 of the National Institutes of Health.

TABLE I. Glucose, Immunoreactive Insulin, and Epididymal Adipose Cellularity in the Zucker Rat.[a]

			Plasma			Epididymal fat pad	
	n	Body wt	Glucose (mg/100 ml)	Insulin (μU/ml)	Insulin (mU/5 islets/90 min)	Cell size (μg TG/cell)	Cell no. ($\times 10^6$)
Zucker obese fa/fa	6	694.5[b] ±36.6	113.1 ±4.0	652.4[c] ±291.9	7.51[b] ±1.28	1.0967[b] ±.0580	7.42 ±1.24
Zucker non-obese Fa/—	7	458.9 ±8.3	108.2 ±10.2	41.4 ±8.8	2.79 ±0.46	0.3419 ±.0578	5.77 ±0.58

[a] Values represent mean ± standard error of mean.
[b] Significantly different from non-obese: $p < .01$; [c] $p < .05$.

the remainder of the left, and also the right fat pad, using the collagenase digestion method described by Rodbell (12). The isolated cells were incubated with glucose-1-^{14}C with and without insulin (2000 μU/ml) and the conversion of glucose-1-^{14}C to $^{14}Co_2$ was determined as previously described (13). Lipids were extracted from the isolated cell suspension with isopropanol:heptane (14) and determined gravimetrically. Student's t test and Pearson correlation coefficients were used to analyze the data; a p value of less than 0.05 was considered significant.

Results. As previously reported by Johnson *et al.* (5), Zucker obese rats have greatly enlarged epididymal adipose cells (Table I). We have confirmed the results of Zucker and Antoniades (4) that Zucker obese are normoglycemic, while plasma IRI levels range from 3 to more than 20 times those of nonobese controls (Table I).

In addition, the release of IRI from pancreatic islets of obese rats was two to three times greater than controls (Table I). Significant correlations were obtained between: (i) adipose cell size and plasma IRI ($r = +.639$, $p < .02$); and (ii) adipose cell size and pancreatic IRI release ($r = +.652$, $p < .05$).

The importance of considering the method of data expression when reporting metabolic activities of adipose tissue has been discussed by a number of authors (13, 16, 17). To summarize briefly, none of these (cpm/mg of TG; cpm/mg of total lipid; cpm/mg of wet wt; cpm/mg of N; cpm/mg of DNA) adequately relate metabolic activity to the adipocyte. Our data clearly indicate that adipocytes from Zucker obese rats metabolize glucose at the same rate as do cells from lean Zucker controls when the data are expressed on a per cell basis (Fig. 1). The stimulatory effect of insulin on glucose conversion to CO_2 in adipocytes, however, was significantly less in the obese than in the control rats ($p<.05$) (Table II). While the basal incorporation of glucose-U-^{14}C into glycogen was similar in the obese and the controls, the effect of insulin on glucose incorporation into diaphragm glycogen was less in the obese than in the controls (Table II). There was no significant correlation between the insulin resistance of the muscle and the adipocytes in the same animal ($r = +.233$, $p>.40$), however, small

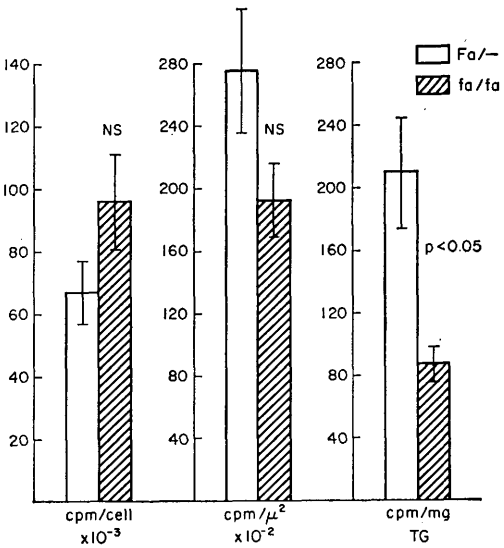

FIG. 1. Glucose-1-^{14}C conversion to $^{14}CO_2$ in adipocytes: Values are mean ± standard error of mean (obese: $n = 5$, controls: $n = 7$).

TABLE II. Effects of Crystalline Insulin (2000 μU/ml) on *in Vitro* Glucose Metabolism by Rat Diaphragm and Adipocytes.[a]

	n	Glucose-U-^{14}C → Glycogen-^{14}C (epm/mg of tissue) (−) Insulin	(+) Insulin	% Basal
1. Diaphragm				
Zucker obese fa/fa	6	372.5 ± 76.6	693.7 ± 152.4	183.0 ± 18.8[b]
Zucker nonobese Fa/—	6	320.5 ± 42.4	805.8 ± 98.2	257.7 ± 22.2
		Glucose-1-^{14}C → $^{14}CO_2$ (epm/adipocyte)		
2. Adipocytes				
Zucker obese fa/fa	5	96.4 ± 14.7	102.0 ± 16.8	107.0 ± 8.6[b]
Zucker nonobese Fa/—	6	64.9 ± 12.8	90.1 ± 14.3	146.7 ± 12.4

[a] Values represent mean ± standard error of mean.
[b] Significantly different from nonobese, $p < .05$.

numbers of animals were used and variation between animals was large.

Discussion. The insulin, glucose profile seen in Zucker obese rats is like that seen in many obese animals (1). Zucker obese are insulin resistant in that very high plasma immunoreactive insulin levels are necessary to maintain normal plasma glucose. In muscle and in isolated adipocytes, basal glucose uptake was similar in obese and controls. Insulin-stimulated uptake of glucose by diaphragm and adipocytes was decreased in the obese compared to controls. These results are similar to those found by others in *in vitro* muscle preparations from *obob* mice (2), and our data provide evidence for the insulin resistance of the Zucker obese rat. It is not clear why normal plasma glucose and high plasma insulin levels coexist in many obese species. There is little evidence that insulin secreted by the obese is biologically inactive, although this possibility should be explored with regard to the Zucker obese rat. We have shown that in the Zucker obese rat there is an impairment of insulin action in muscle and adipose cells. This impairment could be a partial explanation for the hyperinsulinemia and normal blood sugar levels seen in these animals.

How the pancreas is signaled to produce an excess of insulin to maintain basal glucose uptake remains puzzling. Felig *et al.* (18) have shown that the level of branched chain amino acids is directly correlated with high insulin levels; and thus, propose branched chain amino acids originating from muscle as a signal to the pancreas. We find no correlation between the degree of insulin resistance of muscle and pancreatic insulin release ($r = -0.264$, $p > .40$). The primary role of muscle in the production of hyperinsulinemia cannot be ruled out however, on the basis of our data alone, since small numbers of animals were used and we did not study amino acid metabolism. On the other hand, it is possible that a primary signal may come from the adipose cell itself. We do find that the degree of adipocyte insulin resistance correlates well with pancreatic insulin release ($r = -0.707$, $p < .05$) and furthermore with adipose cell size ($r = -.724$ $p < .02$). Preliminary work in our laboratory with a small number of obese and normal adult humans showing various degrees of adipose cellular enlargement also demonstrates a significant correlation between cell size and fasting plasma insulin (19). Furthermore, Salans *et al.* (20) have reported that insulin response in adipocytes returns to normal when cell size is reduced. These findings all suggest a possible relationship between the degree of caloric storage (*i.e.*, adipose cell size) and pancreatic

function.

Summary. Zucker obese rats are normoglycemic, hyperinsulinemic, and release more insulin from isolated pancreatic islets than do their nonobese controls. Basal glucose conversion to glycogen by muscle and to CO_2 by isolated adipocytes is similar in Zucker obese and nonobese; insulin resistance is present in both tissues in the obese. There is a significant correlation between adipocyte response to insulin and pancreatic insulin release in obese and nonobese rats. The authors suggest a role for adipocytes in determining pancreatic function.

We thank Dr. I. A. Macchi, Boston University, for his advice on the islet isolation technique and Mrs. M. Compans, Miss F. Oetjen, and Miss M. Teaman for their technical assistance.

1. Stern, J., and Hirsch, J., Handb. Physiol. Vol. 1, Section 8, Waverly Press, Inc., Baltimore, Md., The Endocrine Pancreas, in press, 1971.

2. Chlouverakis, C., and White, P. A., Metabolism **18**, 998 (1969).

3. Yen, T. T. T., Lowry, L., Steinmetz, J., and Wolff, G. L., Horm. Metab. Res. **2**, 161 (1970).

4. Zucker, L. M., and Antoniades, H. N., Fed. Proc, Fed. Amer. Soc. Exp. Biol. **29**, 379 (1970).

5. Zucker, L. M., and Zucker, T. F., J. Hered. **52**, 275 (1961).

6. Rosselin, G., Assan, R., Yalow, R. S., and Berson, S. A., Nature (London) **212**, 356 (1966).

7. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).

8. Lacy, P. E., and Kostianovsky, M., Diabetes **16**, 35 (1967).

9. Rafaelson, O. J., Laures, V., and Renold, A. E., Diabetes **14**, 19 (1965).

10. Hirsch, J., and Gallian, E., J. Lipid Res. **9**, 110 (1968).

11. Folch, L., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

12. Rodbell, M., J. Biol. Chem. **239**, 375 (1964).

13. Greenwood, M. R. C., Johnson, P. R., and Hirsch, J., Proc. Soc. Exp. Biol. Med. **133**, 944 (1970).

14. Dole, V. P., J. Clin. Invest. **35**, 150 (1956).

15. Johnson, P. R., Zucker, L. M., Cruce, J. A. F., and Hirsch, J., J. Lipid Res. **12**, 706 (1971).

16. Zinder, O., Arad, R., and Shapiro, B., Isr. J. Med. Sci. **3**, 787 (1967).

17. Hellman, B, Ann. N.Y. Acad. Sci. **131**, 151 (1964).

18. Felig, P., Marliss, E., and Cahill, G. F., N. Engl. J. Med. **281**, 811 (1969).

19. Stern, J., Hollander, N., and Hirsch, J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30**, 194 (1971).

20. Salans, L. B., Knittle, J. L., and Hirsch, J., J. Clin. Invest. **46**, 112 (1967).

Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.