PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 317–321 (1980)

Decreased Insulin Secretion by Isolated Pancreatic Islets from Rats Fed 4% Protein Diet (40869)

RAFI YOUNOSZAI* AND PADMAKAR K. DIXIT[1]

Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Abstract. Male weanling rats were fed either 4 or 16% (control) protein diets for 10 to 12 weeks. The animals were administered an i.v. injection of 30% glucose solution (3 g/kg body wt) and glucose tolerance was determined. The glucose tolerance in rats fed low protein diets was not significantly different from that of the control rats, indicating that clearance of high blood glucose levels in the animals fed low protein diets was not affected.

Using a collagenase digestion technique, islets of Langerhans were isolated from another set of animals fed 4% or control diets. When these islets were perifused with a medium containing glucose, there was a typical biphasic insulin secretory response. The isolated islets from low protein fed rats exhibited considerably low insulin secretory response when exposed to medium containing 16.7 m*M* glucose (300 mg/dl), although no difference was found in the pancreatic insulin content of normal and low protein fed rats. This indicates that decreased insulin secretion could not be due to a decreased available pool of insulin. When glucagon (5 μg/ml medium) or arginine (20 m*M*) was added to a medium containing 8.4 m*M* glucose (150 mg/dl), the *in vitro* insulin release by the islets obtained from protein malnourished and control rats was enhanced. Again the control rat islets secreted much greater amounts of insulin. Insulin secreted by the islets in response to glucagon (5 μg/ml medium) was greater than that secreted in response to 16.7 m*M* glucose. It is suggested that the decreased insulin secretory response of islets from protein malnourished animals may be due to a diminished number of glucose recognition sites on the beta cells.

Protein deficiency (PD) causes atrophy of the exocrine pancreas (1). A more serious effect of protein malnourishment is on the endocrine pancreas, resulting in carbohydrate intolerance in children (2), adults (3), and animals (4). The exact mechanism by which severe protein deficiency leads to carbohydrate intolerance is unknown. Two possibilities may be considered: (a) decreased pancreatic insulin content and (b) decreased capacity of the beta cells to release insulin in response to glucose. A continued persistence of carbohydrate intolerance was believed to be a potential cause of diabetes in protein malnourished subjects, although subsequent studies have not substantiated this conjecture (5, 6).

Protein deficiency in rats can be produced with ease and has many features commonly associated with Kwashiorkor caused by protein–calorie malnutrition (PCM) in children. In the present communication we report our findings on (a) intravenous glucose tolerance, and on (b) *in vitro* insulin secretory response to glucose and glucagon of isolated islets from rats fed low protein diets for long periods of time.

Materials and methods. Male weanling Holtzman rats, about 70 g in weight, were distributed into two groups and fed for 10 to 12 weeks either a 4 or a 16% protein (control) diet (Teklad Diets, Madison, Wis.). The composition of the two diets is given in Table I, from which it would be apparent that in low (4%) protein diet the proportion of sucrose was increased in lieu of protein, thus providing the same caloric contents in the two diets.

Glucose tolerance test. The animals were fasted overnight and a 30% glucose solution in 0.9% saline (1 ml/100 g body wt) was injected by tail vein. The animals were bled by tail vein just before, and 30, 60, and 120 min after glucose administration. Blood glucose was determined on 0.05-ml samples by a modification of Hoffman's ferricyanide

[1] To whom requests for reprints may be addressed.

* Present address: Department of Anatomy, College of Osteopathic Medicine of the Pacific, Pomona, California 91766.

0037-9727/80/070317-05$01.00/0

TABLE I. COMPOSITION OF 4 AND 16% PROTEIN DIETS

	Low protein (4%) diet g/kg	16% protein (control) diet g/kg
Casein, vitamin-free test	40.0	160.0
Corn starch	150.0	150.0
Sucrose	685.0	565.0
Corn oil	60.0	60.0
Nonnutritive fiber (cellulose)	20.0	20.0
Mineral mix, Williams–Briggs modified (Teklad Cat. No. 170911)	35.0	35.0
Vitamin, mix, Teklad (Teklad Cat. No. 40060)	10.0	10.0
	1000.0	1000.0

reduction method (7) using a Technicon Auto-Analyzer.

In vitro insulin secretion by isolated islets obtained from rats fed protein deficient and normal diets. Islets of Langerhans were isolated by the method described by Lacy and Kostianovsky (8). A known number (30–50) of islets were placed on millipore filters (5-μm mesh, Millipore Corp., Bedford, Mass.) in a perifusion assembly, and perifused with a medium gassed with a mixture of O_2 + CO_2 (19 + 1), using the technique of Lacy *et al.* (9). The medium was circulated using a multichannel peristaltic pump (Sage Co.) at a flow rate of 0.9 to 1.1 ml/min.

The islets were perifused for 35 to 50 min with Hanks' medium containing 2.8 m*M* glucose (50 mg/dl) and the perifusates collected in albumin coated test tubes using a fraction collector. This initial period established the basal insulin levels. After reaching steady state, the islets were perifused with a medium containing 16.7 m*M* glucose (300 mg/dl) for 60 min; this is designated a stimulatory period. In experiments in which the effect of glucagon and arginine on insulin secretion by islets was studied, immediately following the basal period the islets were first exposed to a medium containing 8.4 m*M* glucose (150 mg/dl) for a period of 30 min, which we designate as prestimulatory period. The islets were then challenged for a period of 30 min by media containing 150 mg/dl glucose plus 500 μg/dl (1.7 μM) glucagon or 150 mg/dl glucose plus 20 m*M* arginine (stimulatory period).

Immunoreactive insulin (IRI) was determined in the pooled perifusate samples by the two antibody assay procedure described by Morgan and Lazarow (10), and the results were expressed as μuIRI released per islet per minute. By simultaneously perifusing with the same stock solutions selected islets of about the same size from rats fed protein-deficient and control diets, it was possible to compare the effect of different secretagogues.

Extraction of pancreatic insulin. Pancreas was weighed and homogenized with chilled 10% trichloracetic acid (TCA). After approximately 2 hr, the contents were centrifuged in the cold for 30 min at 1500*g*. The supernatant was discarded and the sediment was repeatedly extracted with small volumes of acid alcohol as described by Scott and Fisher (11). Aliquots were assayed for IRI, and the results expressed as units per gram wet weight of pancreas. Acid–alcohol extracts from the pancreas were also assayed for immunoreactive somatostatin (IRS) by the method described by Peterson *et al.* (12), and the results expressed as μg IRS/g pancreas.

Results. Glucose tolerance. As shown in Fig. 1, the animals fed low protein (4%) diets exhibited no significant difference in i.v. glucose tolerance as compared to those fed the control diet containing 16% protein.

Insulin secretion. The *in vitro* insulin secretory pattern of islets obtained from rats fed low protein and control diets exhibited a typical biphasic response when challenged by medium containing high (300 mg/dl) glu-

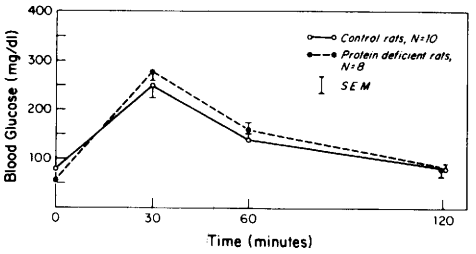

FIG. 1. Intravenous glucose tolerance in rats fed control and protein deficient diets.

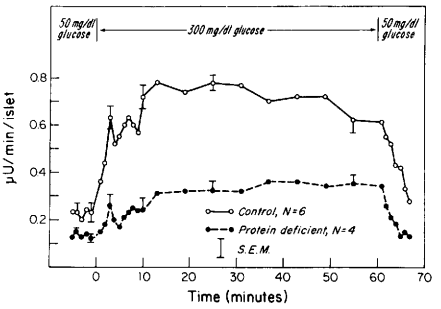

FIG. 2. Insulin secretory patterns of islets from control and protein deficient rats following stimulation with high (300 mg/dl) glucose medium. In the prestimulatory period the islets were perifused with a medium containing basal (50 mg/dl) levels of glucose. Insulin secretion during only the last 5 min of the prestimulatory period is shown.

cose (Fig. 2). In response to various concentrations of glucose the amount of insulin secreted by islets obtained from rats on low protein diets was markedly lower than that secreted by islets obtained from rats on control diets (Table II). When the islets from protein deficient rats were exposed to media containing 150 mg and 300 mg/dl glucose, the amount of insulin released was, respectively, 1.4- and 2.4-fold greater than when the islets were exposed to medium containing 50 mg/dl glucose. The corresponding values for control rat islets were 1.2- and 2.6-fold greater (see Table II). The amount of insulin released by islets from protein deficient rats in response to arginine was not significantly lower than that released by control rat islets.

When the islets from rats fed protein deficient and control diets were exposed to glucagon in the presence of 150 mg/dl glucose, there was a marked increase in insulin release, as compared to when the medium contained only 150 mg/dl glucose (Table II). However, again the control rat islets released twofold the amount of insulin. In response to glucagon + 150 mg% glucose the islets from rats fed control and protein deficient diets released about 25 times the amount of insulin released in response to 150 mg/dl glucose in the medium (Table II).

Pancreatic insulin and somatostatin content. The total pancreatic insulin content of protein deficient rats when expressed as μg/g pancreas is almost the same as that of the control rats. However, when expressed on the basis of units insulin/100 g

TABLE II. A COMPARISON OF THE AMOUNT OF INSULIN (IRI) SECRETED IN 30 min PER ISLET FROM PROTEIN DEFICIENT (4% PROTEIN) AND CONTROL RATS WHEN CHALLENGED BY GLUCOSE, ARGININE, AND GLUCAGON[a]

Concentration of secretagogue	Insulin released per islet in 30 min (μm)		Difference between protein deficient and control
	Protein deficient	Control	*P* value
Glucose (mg/dl)			
50	(15) 3.6 ± 0.8[b]	(15) 8.2 ± 0.8	<0.0001
150	(10) 5.0 ± 1.1	(9) 10.4 ± 1.6	<0.01
300	(4) 8.8 ± 1.6	(6) 22.0 ± 1.0	<0.0001
Glucose 150 mg/dl +20 m*M* Arginine	(4) 11.0 ± 2.2	(4) 16.4 ± 3.4	NS
150 mg glucose/dl +5 μg glucagon/ml	(3) 136.0 ± 20.0	(2) 272.0 ± 24.0	<0.0001

[a] Numbers in parentheses denote number of rats.
[b] SEM.

TABLE III. INSULIN AND SOMATOSTATIN CONTENT OF PANCREAS FROM RATS FED LOW (4%) PROTEIN DIETS

	Animals fed 4% protein diet	Animals fed 16% protein diet	*P*
Body weight (g)	(6)[a] 96.6[b] ± 5.8[c]	(6) 258 ± 15.7	<0.001
Wt of pancreas (g)	(6) 0.33 ± 0.03	(6) 1.09 ± 0.09	<0.001
IRI units/g pancreas	(6) 1.31 ± 0.06	(7) 1.48 ± 0.11	NS
IRI units/100 g body wt	(9) 0.45 ± 0.03	(6) 0.65 ± 0.07	<0.01
Somatostatin μg/g pancreas	(4) 6.76 ± 0.74	(4) 3.03 ± 0.57	<0.001

[a] *N*.
[b] Mean.
[c] SEM.

body wt there is a significant difference ($P < 0.01$) (Table III).

In the protein deficient rats the somatostatin content per gram of pancreas is markedly (twofold) greater than that of the control rats (Table III).

Discussion. A reduction of dietary protein from 18 to 11% and also a substitution of sucrose for starch leads to impaired glucose tolerance in rats (13, 14). In view of these observations and because numerous studies suggest decreased insulin secretion in malnourished humans (15, 16) and rats (17), we reduced the dietary protein level to 4% and commensurately increased sucrose content of diet. It was believed that this would enhance stress on the endocrine pancreas of protein deficient animals and produce severe impairment of glucose tolerance as a result of decreased insulin secretion.

When intravenous glucose was injected, the blood glucose levels of rats fed 4% protein diet after 30, 60, and 120 min were not found to be significantly different from those fed the control diet. This is contrary to the reports by other workers who generally administered glucose by oral route (17). On the basis of our studies it would seem that the sudden load of glucose was cleared as efficiently by rats fed low protein diets as did those on control diet. It should be emphasized that immediately following a glucose load, the blood sugar levels are elevated to over 1000 mg/dl. However, we were more interested in the glucose clearance and, hence, the 0- and 30-min blood sugar values were connected in Fig. 1. Two possible explanations may be suggested for the rapid glucose clearance in protein malnourished rats. (a) The islet beta cells of rats on low protein diet release comparable amounts of insulin in response to glucose load as those of the controls. (b) The peripheral tissues of rats on low protein diet due to their status of "chronic starvation" rapidly utilize glucose from circulation.

Our *in vitro* perifusion studies indicate that isolated islets from protein deficient rats do not secrete insulin as efficiently as the normal rat islets in response to glucose and to glucagon. Decreased insulin secretion by islets from protein deficient rats is not due to a decreased available pool of insulin in the pancreas, since the total extractable pancreatic insulin from normal and protein deficient rats was closely similar (Table III). Weinkove *et al.* (18) also had observed that the pancreatic insulin content of the protein deficient rats was normal, which they attributed to a reduction in total pancreatic islet volume due to chronic protein starvation. These workers further observed that plasma insulin levels of protein deficient animals were considerably lower than those of control rats, indicating decreased insulin secretion *in vivo* in response to i.v. glucose load by protein deficient rats (18).

Weinkove *et al.* (19) found a distinct difference in the ultrastructure of insulin granules in beta cells of protein deficient rats, in that the core of the granules was less dense compared to that of the control rats. They believed that the decrease in granular density is indicative of greater insulin concentrations.

The decreased insulin release by the is-

lets from protein deficient rats could be attributed to a decreased number of glucose recognition sites on the beta cells. There is reason to believe that glucose and alloxan compete for a common site on the beta cell membrane (20). It is therefore, not surprising that a diabetogenic dose of alloxan was found ineffective in producing diabetes in animals fed low protein diet (21).

In the protein deficient rats the amount of pancreatic somatostatin content per unit weight of tissue is greater than in the controls (Table III). Whether this has an inhibitory effect on insulin release in protein deficiency is not clear.

According to Grodski's hypothesis of insulin compartmentalization, approximately 2% of the total insulin in the beta cell is in a readily available pool (22). It may be argued that in the protein deficient rats this pool is markedly decreased. However, when islets of protein malnourished animals are exposed to glucagon (5 μg/ml), considerable amounts of insulin are released which are far in excess of that released in response to 300 mg/dl glucose. This indicates that decreased insulin secretion by islets from animals fed low protein diets could not be due to decreased available insulin pool.

No plausible explanation is forthcoming for the decreased insulin secretory action of islets obtained from protein malnourished animals. Diminished number of glucose recognition sites seems to be an attractive hypothesis. Further studies are planned along these lines.

This project was partially supported by grants from The Graduate School, University of Minnesota, and American Diabetes Association of Minnesota (ADAM).

1. Barbezat, G. O., and Hansen, J. D. L., Pediatrics **42**, 77 (1968).
2. Milner, R. D. G., Pediat. Res. **5**, 33 (1971).
3. Smith, S. R., Edgar, P. J., Pozefsky, T., Chhetri, M. K., and Prout, T. E., Metabolism **24**, 1073 (1975).
4. Heard, C. R. C., Diabetes **15**, 78 (1966).
5. Kajubi, S. K., Amer. J. Clin. Nut. **25**, 1140 (1972).
6. Pimstone, B. L., Becker, D. J., Weinkove, C., and Mann, M., *in* "Endocrine Aspects of Malnutrition," (L. I. Gardner and P. Amacher, eds.). Kroc Foundation Symposia No. 1, Kroc Foundation, Santa Ynez, Calif. (1973).
7. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).
8. Lacy, P. E., and Kostianovsky, M., Diabetes **16**, 35 (1967).
9. Lacy, P. E., Walker, M. M., and Fink, C. J., Diabetes **21**, 987 (1972).
10. Morgan, C. R., and Lazarow, A., Diabetes **14**, 669 (1965).
11. Scott, D. A., and Fisher, A. M., J. Clin. Invest. **17**, 725 (1938).
12. Peterson, R., Elde, R., Efendic, S., Hokfelt, T., Johannson, O., Luft, R., Cerasi, E., and Hellerstrom, C., Diabetologia **13**, 463 (1977).
13. Cohen, A. M., and Tietelbaum, T., Metabolism **21**, 235 (1972).
14. Cohen, A. M., and Tietelbaum, A., Metabolism **15**, 1034 (1966).
15. Milner, R. D. G., Proc. Nutr. Soc. **31**, 219 (1972).
16. Becker, D. J., Pimstone, B. L., Hansen, J. D. L., MacHutchon, B., and Drysdale, A., Amer. J. Clin. Nutr. **25**, 499 (1972).
17. Kirsch, R. E., Brock, J. F., and Saunders, S. J., Amer. J. Clin. Nutr. **21**, 820 (1968).
18. Weinkove, C. Weinkove, E. A., and Pimstone, B. L., Clin. Sci. Molec. Med. **50**, 153 (1976).
19. Weinkove, C., Weinkove, E. A., Timme, M., and Pimstone, B. L., Arch. Path. Lab. Med. **101**, 266 (1977).
20. Tomita, T., FEBS Lett. **65**, 140 (1976).
21. Young, J. K., and Dixit, P. K., J. Nutr. **110**, 703 (1980).
22. Grodsky, G. M., J. Clin. Invest. **51**, 2047 (1972).

Received September 7, 1979. P.S.E.B.M. 1980, Vol. 164.