Enzyme Studies in the Articular Cartilage of Diabetic Rats and of Rats Bearing Transplanted Pancreatic Islets

R. Silberberg, M.D., G. E. Hirshberg, M.D., and P. Lesker, B.A., St. Louis

SUMMARY

The articular cartilage of normal rats, of rats made diabetic with streptozotocin, and of rats made diabetic with streptozotocin and subsequently transplanted with isologous pancreatic islets was examined for the activities of enzymes engaged in the synthesis and degradation of glycosaminoglycans (mucopolysaccharides). The activities assayed were those of the degrading enzymes B-glucuronidase, B-acetylglucosaminidase, B-acetylgalactosaminidase, B-galactosidase, and those active in synthesis: uridine-diphosphate dehydrogenase, glucose-6-phosphate dehyrogenase, and phosphofructokinase. In the diabetic animals all enzyme activities were increased, those of the degrading enzymes more than those of the others. Implantation of pancreatic islets reversed the changes produced by diabetes, enzyme activities returning to near-normal levels. DIABETES *26*:732-35, August, 1977.

Contrary to the voluminous literature dealing with the effect of diabetes on non-skeletal tissues, data on diabetes-linked changes in bone and cartilage are scanty.[15] Yet, in view of the abundance of glycosaminoglycans, especially in the cartilaginous matrix, such changes are to be expected in both spontaneous and experimental diabetes. As part of an attempt to elucidate the nature of diabetes-linked joint changes and to demonstrate the effectiveness of transplanted pancreatic islands in reversing the effects of diabetes on still another tissue,[17] enzyme activities were assayed in the knee-joint cartilage of rats with streptozotocin-induced diabetes and in rats made diabetic and subsequently transplanted intraportally with homologous pancreatic islets.[3]

From the Departments of Pathology, Surgery, and Orthopedic Surgery, Washington University School of Medicine, St. Louis, Missouri.

Address reprint requests to P. Lesker, B.A., Department of Orthopedic Surgery, Washington University School of Medicine, St. Louis, Missouri.

Accepted for publication February 15, 1977.

MATERIALS AND METHODS

Animals. Fourteen highly inbred male Lewis rats (Simonson Laboratories) 15 weeks of age and weighing about 330 gm. at the beginning of the experiment were used: group 1 (untreated group) consisted of three untreated animals; group 2 (diabetics) included six rats with streptozotocin-induced diabetes;* and group 3 was comprised of five rats made diabetic and subsequently transplanted with approximately 2,500 freshly isolated islets of Langerhans.[6]

Introduction of diabetes. Male rats weighing 330±7 gm. were given 65 mg./kg. streptozotocin intravenously. After reaching a stable diabetic state, animals slated to receive islet implants (group 3) were diabetic for about two weeks prior to transplantation.

Transplantation of islets. Pancreatic islets were harvested from donor Lewis rats (males or females 250-350 gm.) by the collagenase (Sigma, type II) digestion method of Lacy and Kostianovsky[6] as modified by Scharp et al.[13] The islet cells were then collected and rinsed with Hanks' balanced salt solution. Under ether anesthesia, diabetic animals of group 3 were implanted with approximately 2,500 freshly isolated islets by intraportal injection.[3]

The animals were maintained in individual metabolic cages with free access to rat chow and water. Urine volumes and urine glucose were determined every day, and body weights were recorded twice weekly. Blood specimens were taken twice weekly from the tails of rats fasted for four hours.

On the date of killing and following a 16-18-hour fast, femoral arterial blood was collected for glucose and insulin determinations. Glucose estimations were made with the Beckman glucose analyzer (Model ERA

*Streptozotocin was obtained through the courtesy of Dr. W. Dulin, Upjohn Pharmaceutical Company, Kalamazoo, Michigan.

2001). Insulin determinations were performed by double-antibody radioimmunoassay.[11]

The animals were killed by overanesthetizing with dibutal. The dissections were completed between 11:30 a.m. and 12:30 p.m.

Both distal femurs were dissected out quickly, placed in liquid nitrogen, and stored at −80° C. After dehydration at −30° C., the tissues were brought up to room temperature under vacuum. The articular cartilage was dissected off the inner condyles and weighed. About 0.5 mg. of dry tissue was obtained from each knee joint. Assays were carried out on each sample individually. The samples were homogenized in 0.04 M imidozole–0.04 glycyl-glycine at pH 7.2 —with 0.4 M sucrose and 0.5 per cent triton X-100 for the determination of various enzyme activities and of noncollagen in the supernatant.[1,7] A trichloracetic acid precipitate was prepared for the measurement of DNA.[4]

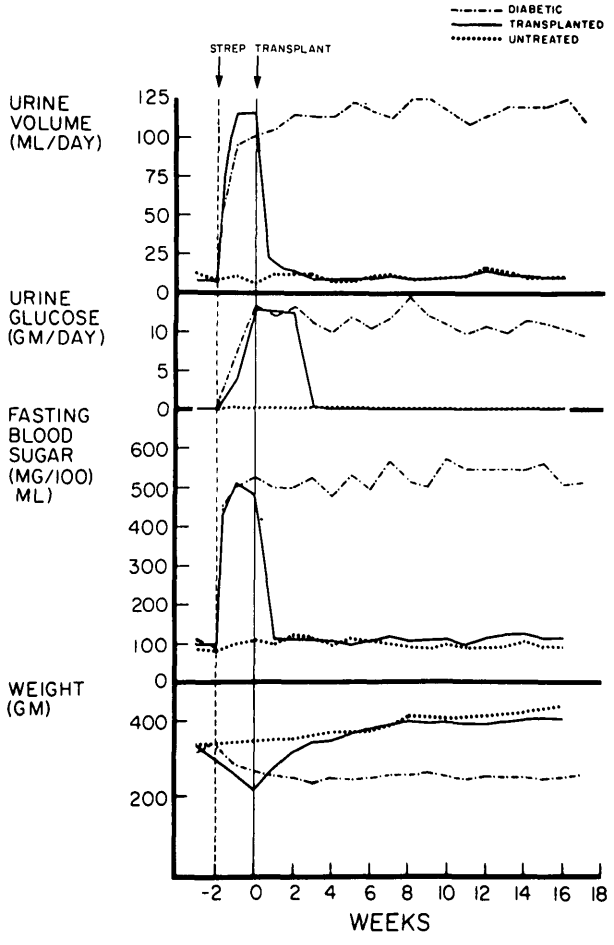

FIG. 1. Twenty-four-hour urine volume and glucose, fasting blood glucose, and weight curves for untreated, diabetic, and transplanted rats.

TABLE 1

Terminal values (means) for body weight, fasting blood glucose, and serum insulin for rats of all groups

	Untreated rats	Diabetic rats	Transplanted rats
No. of animals.	3	6	5
Weight (gm.)	411±21	216±6	385±8
Fasting glucose (mg./100-ml.)	107±6.7	393±41	85±3.2
Insulin (μU./ml.)	20.6±6.1	1.8±0.3	24.5±5.1

The activities of the following enzymes were assayed by methods described fully in an earlier publication:[16] beta-glucuronidase, beta-acetylgalactosaminidase, beta-acetylglucosaminidase, beta-galactosidase—enzymes that take part in the degradation of glycosaminoglycans;[1,7] uridine diphosphate glucose dehydrogenase (UDPGDH), glucose-6-phosphate dehydrogenase (G-6-PDH), phosphofructokinase (PFK), enzymes operative in synthesis of glycosaminoglycans.

In addition to these enzymes and to DNA, noncollagen protein was assayed.[8]

RESULTS

Observations during life. A stable diabetic state was reached an average of seven days after the administration of streptozotocin with no return to normal in any of the animals. Transplants began to correct the diabetic state within two days following transplantation, and normal urine and blood parameters continued throughout the subsequent period of observation (figure 1). Terminal body weights and levels of blood glucose and serum insulin are given in table 1. All these parameters were altered in the diabetic animals and returned to normal in rats bearing transplanted islets.

Biochemical assays. Calculated on the basis of dry weight of tissue, all enzyme activities were increased in the diabetic animals, the increase being about sevenfold for beta-glucuronidase, about tripled for G-6-PDH, doubled for beta-acetylgalactosaminidase, beta-glucosaminidase, and beta-galactosidase, and raised about 24 per cent for UDGPDH and 19 per cent for PFK. Since enzymes in articular cartilage are presumed to be synthesized by the chondrocytes, it seemed of interest to calculate enzyme activities on the basis of DNA content and thus to obtain an indication of the functional potential of the individual cell. DNA was decreased from a mean of 2.18 in untreated to 1.5

mg./gm. dry weight in diabetic animals. The differences in enzyme activities were therefore more marked, if expressed in terms of DNA rather than on the basis of dry weight: increases varied from a maximum of 10 times for beta-glucuronidase to a minimum of twice the normal value for PFK (table 2).

Folowing transplantation of islets into previously diabetic animals, enzyme activities returned to levels slightly above normal; the differences between the two groups were, however, not significant for any of the enzymes.

Noncollagen protein was not significantly changed in the diabetic animals.

DISCUSSION

In the knee-joint cartilage of young adult male Lewis rats made diabetic with streptozotocin, the activities of enzymes in both degradation and synthesis of glycosaminoglycans were significantly increased. This increase was reversed after implantation of pancreatic islets into diabetic animals. Degrading enzymes were more severely affected than those active in synthesis. This apparent lack of uniformity in the response of enzyme activity is not unusual and may be related to transitory phases in the degrading process.[16] The reversal of the increase seen after islet transplantation was partially complete, the difference between transplanted diabetic rats and untreated controls being nonsignificant.

No significant differences were present in noncollagen protein; in cartilage the latter comprises cellular proteins and the protein core of the glycosaminoglycans; with the associated decrease, in the diabetic animals, of DNA and, accordingly, in the number of chondrocytes, the noncollagen protein values must have been kept high by an increase in cell size and in proteinic cell content.

The amount of tissue available from each joint did not permit the quantitative determination of glycosaminoglycans present in the samples; a separate set of experiments will have to be conducted for this purpose. However, the present results indicate a predominance of degradative over synthetic processes in the diabetic animals, a dysequilibrium that would eventually result in a deficiency of glycosaminoglycans. Our data are thus consistent with those obtained with nonskeletal tissues, such as skin, eyes, and aorta of diabetic animals. In these tissues, diabetes was associated with a decrease in the amounts of sulfated glycosaminoglycans.[2,5,9,12,14] Moreover, in the serum of diabetic patients, levels of chondroitin sulfates A and C were subnormal.[10]

The return of the enzyme activities to nearly normal levels after transplantation of pancreatic islets is an illustration of the effectiveness of this procedure in counteracting the skeletal effects of diabetes established by the investigations reported herein.

TABLE 2

Showing enzyme activities calculated per gm. DNA in articular cartilage of diabetic rats and of rats given streptozotocin and subsequently transplanted with pancreatic islets

	Untreated (6)		Diabetic (12)		Transplanted (13)
β-glucuronidase (mM/gm. DNA/hr.)	0.361±0.063	(P<0.001)	3.818±0.628	(P<0.001)	0.774±0.254*
β-acetyl galactosaminidase (μM/gm. DNA/hr.)	97±11	(P<0.001)	341±47	(P<0.001)	123±22*
β-acetyl glucosaminidase (mM/gm. DNA/hr.)	0.480±0.062	(P<0.001)	1.941±0.253	(P<0.001)	0.702±0.142*
β-galactosidase (μM/gm. DNA/hr.)	120±36	(P<0.001)	366±37	(P<0.001)	124±22*
UDPGDH (mM/gm. DNA/hr.)	10.7±1.2	(P<0.05)	26.8±7.3	(P<0.05)	10.6±1.4*
G-6-PDH (mM/gm. DNA/hr.)	13.9±2.0	(P<0.001)	76.0±11.4	(P<0.001)	23.0±5.1*
PFK (mM/gm. DNA/hr.)	120±14	(P<0.05)	271±67	(P<0.05)	114±10*

( ) indicates no. of samples.
± indicates standard error.
* No significant difference between transplanted and untreated rats.

ACKNOWLEDGMENTS

The technical assistance of Mary Raizman, Mary Clair Meyer, Kathy Haid, Sandy Nunnelley, Elizabeth Ruwe, and Christopher Davis is gratefully acknowledged.

These investigations have been carried out with the aid of NIH Research grants AM 18235 and AM 17188, a Kroc Foundation grant, and NIH Training grant GM 00371.

Insulin assays were done in the Washington University Diabetes and Endocrine Centers under NIH grant AM 17904.

REFERENCES

[1]Balduini, C., Brovelli, A., DeLuca, G., Galligani, L., and Castellani, A. A.: Uridine diphosphate glucose dehydrogenase from cornea and epiphyseal-plate cartilage. Biochem. J. *133*:243-49, 1973.

[2]Cohen, M., and Foglia, V.: Aortic mucopolysaccharides in experimental diabetes. Diabetes *19*:639-43, 1970.

[3]Kemp, C. B., Knight, M. J., Scharp, D. W., Ballinger, W. F., and Lacy, P. E.: Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia *9*:486-91, 1973.

[4]Kissane, J. M., and Robins, E.: The fluorometric measurement of DNA in animal tissues with special references to the central nervous system. J. Biol. Chem. *233*:184-89, 1958.

[5]Kofoed, J. A., Bozzini, C. E., and Alippi, R. M.: Skin acidic glycosaminoglycans in alloxan diabetic rats. Diabetes *19*:732-33, 1970.

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

[7]Leabeck, D. H.: Properties of some derivatives of 4-methylumbelliferone. Biochem. J. *78*:22-23, 1961.

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

[9]Malathy, K., and Kurup, P. A.: Metabolism of glycosaminoglycans in alloxan diabetic rats. Diabetes *21*:1162-67, 1972.

[10]Malathy, K., and Kurup, P. A.: Levels of glycosaminoglycans in the serum of diabetic patients. Indian J. Biochem. Biophys. *10*:132-33, 1973.

[11]Morgan, C. R., and Lazarus, A.: Immunoassay of insulin. Two antibody system. Diabetes *12*:115-20, 1963.

[12]Romquin, N., Laurent, M., and Regnault, F.: Membrane and connective tissue glycosaminoglycans during aging and diabetes. Exp. Eye Res. *20*:183 (abstr.), 1975.

[13]Scharp, D. W., Murphy, J. J., Newton, W. T., Ballinger, W. F., and Lacy, P. E.: Application of an improved isolation technique for islet transplantation in primates and rats. Transplant. Proc. *7*:739-41, 1975.

[14]Schiller, S., and Dorfman, A.: The distribution of acid mucopolysaccharides in the skin of diabetic rats. Biochim. Biophys. Acta *78*:371-73, 1963.

[15]Silberberg, R.: The pituitary and skeletal aging. Chapter 12. *In* Hypothalamus, Pituitary and Aging. Everitt, A. V., and Burgess, J. A., Eds. Springfield, Ill., C. C Thomas, 1976.

[16]Silberberg, R., Stamp, W. G., Lesker, P. A., and Hasler, M. J.: Aging changes in ultrastructure and enzymatic activity of articular cartilage of guinea pigs. Geront, *25*:184-98, 1970.

[17]Sutherland, D. E. R., Steffes, M. W., and Mauer, S. M.: Reversal of secondary lesions of diabetes in rats by pancreatic islet transplantation. Chicago, American College of Surgeons. Gardner, M. R., Ed. Surg. Forum *25*:309-11, 1974.