Biochemical and morphologic studies on diabetic rats: Effects of sucrose-enriched diet in rats with pancreatic islet transplants

(diabetes/liver/triacylglycerol secretion/Golgi apparatus/GERL)

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ABSTRACT Isolated pancreatic islets were administered to Lewis rats with streptozotocin-induced diabetes. Then the rats were fed either a semisynthetic diet containing 60% (wt/wt) sucrose for 3 weeks or were continued on chow. Transplantation resulted in a decrease in serum glucose, an increase in serum insulin, and a marked decrease in serum triacylglycerol, particularly in the sucrose-fed diabetic rats. In these rats, in demarcated areas of hepatocytes surrounding portal vein termini, lipid was deposited in the cytosol and large lipoprotein particles engorged the Golgi apparatus, Golgi-derived secretory vacuoles, and GERL. This model permits observation of the effects of pancreatic islet hormones on lipogenesis by hepatocytes *in situ*.

Lacy and his collaborators (1-4) have developed procedures for isolating pancreatic islets from rats and have demonstrated the advantages of injection into the portal vein. In diabetic recipients, serum glucose levels decreased and serum insulin levels returned to normal (3). Islet cell transplantation has also been shown to decrease the elevated triacylglycerol (TG) levels found in diabetic rats (5).

It has been shown that feeding a semipurified high-sucrose diet produces hypertriglyceridemia in normal rats (6) and more severe hypertriglyceridemia in diabetic rats (7). In this report, we describe the effects of this diet on rats bearing intraportal transplants of isologous pancreatic islets.

MATERIALS AND METHODS

Male Lewis rats weighing 125-150 g (Microbiological Associates, Bethesda, MD) were used for donors, recipients, and controls. The rats were made diabetic by injection of streptozotocin (65 mg/kg of body weight). One to 2 months after induction of diabetes, the rats received intraportal injections of islets isolated by the collagenase procedure and separated on a Ficoll gradient (8).

There were eight groups of rats: nondiabetic and diabetic, with and without islet transplants, fed either chow or a sucrose-enriched diet. Each group consisted of three or four rats. The sucrose-enriched diet was a semipurified diet containing 20% (wt/wt) vitamin-free casein, 60% sucrose, and 5% lard (6). After 3 weeks on this diet, the rats (nonfasting) were anesthetized with ether and exsanguinated via the abdominal aorta.

Serum glucose was measured by using the Statzyme kit (Worthington) and insulin was measured by radioimmunoassay (9) with rat insulin as the standard. The lipids were extracted from the serum and livers, and TG was measured by a modification (10) of the method of Van Handel and Zilversmit (11). Cholesterol in serum and liver was determined by the method of Abell *et al.* (12).

The morphological procedures are detailed elsewhere (13, 14). Electron microscopic comparison of the Golgi zones of periportal and centrolobular hepatocytes from the same lobule required examination of toludine blue-stained Epon sections, approximately 2 μ m in thickness.

RESULTS

Serum glucose concentrations (Table 1) were similar in all groups of nondiabetic rats but were 4-fold higher in diabetic rats without transplants (control). In diabetic rats with transplants, the glucose concentrations were lower than in diabetic rats without transplants but were slightly higher than those in nondiabetic rats. Serum insulin levels were low in diabetic rats without transplants and were markedly increased after transplantation. In nondiabetic rats or diabetic rats with transplants, serum insulin levels were consistently higher in the sucrose-fed rats.

Serum TG concentrations were increased in the diabetic rats, more so in those fed the sucrose diet than in those fed the chow. Transplantation resulted in a decrease in serum TG to the concentrations found in nondiabetic rats. In all groups, sucrose-fed animals had higher TG concentrations than chow-fed rats. Mean serum cholesterol concentrations were similar in all groups, except that they were higher in the sucrose-fed diabetics; transplantation resulted in restoration of serum cholesterol to normal.

Gross examination of the liver from diabetic rats with transplants and fed the sucrose diet for 3 weeks revealed numerous white spots in all lobes (Fig. 1A). Those fed chow showed either no white spots or very few spots and these were very small (Fig. 1A).

Oil Red O staining of frozen sections (Fig. 1 B and C) showed that the white areas were composed of hepatocytes filled with cytosolic lipid spheres. Use of the Wachstein-Meisel phosphatase procedure, with ATP as substrate, prior to Oil Red O staining (13, 14) visualized the plasma membranes of the islet cells as well as of other cells. This made it possible to find the transplants. The transplants were not seen in central veins but were lodged in termini of portal veins. Thus, the areas of lipid-filled hepatocytes were always periportal. A few lipidfilled periportal hepatocytes surrounded the transplants in both the nondiabetic rats fed the sucrose-enriched diet and the di-

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Abbreviation: TG, triacylglycerol.



FIG. 1. (A) Abdominal cavities of diabetic rats bearing islet transplants. Rat at left was maintained on chow; rat at right was fed the sucrose-enriched diet for 3 weeks. All lobes of the liver of the sucrose-fed rat show white spots; the number of spots varies from one lobe to another, and variability is also evident in different areas of the same lobe. White spots are not present on the liver of the chow-fed rat. (B) Frozen section of liver from sucrose-diet-fed diabetic rat bearing islet transplant. The white spots, which in this section are stained red with Oil Red O, are composed of hepatocytes filled with cytosolic lipid spheres. The two boxed areas show islet transplants (the lower right one is shown at higher magnification in C). Note that the hepatocytes surrounding the central veins (C) possess little or no cytosolic lipid spheres. ($\times 25$.) (C) The lower boxed area of B, at higher magnification. The plasma membrane of the islet cells are stained brown. Such membrane staining is more intense in the bile canaliculi (B) and even more so in the walls of arterioles, one of which courses from the top right toward the islet. A transverse section of an arteriole is seen to the left of the islet. Between it and the islet is a clear area, the lumen of a portal vein terminus. Arrowheads indicate three of the many cytosolic lipid spheres. ($\times 250$.)

abetic rats fed chow. Such hepatocytes were not seen at all around the transplants in nondiabetic rats fed chow or in nondiabetic chow-fed rats without transplants.

Table 2 shows the TG concentrations in the livers of the eight groups. Two values are given for the diabetic rats with transplants, fed sucrose: from portions of liver (a) with a greater number of macroscopically visible white spots and (b) with fewer white spots. The TG concentration was nearly 2-fold higher in the portions with more white spots; this correlates with the staining results. In all instances, sucrose-fed animals had higher TG concentrations and greater absolute amounts of TG (data not shown) than did corresponding chow-fed rats. Islet transplantation in nondiabetic rats and in chow-fed diabetic rats had no significant effect on TG concentrations. Moreover, the portions of the livers from nondiabetic sucrose-fed transplanted rats with few small white spots had TG concentrations

	Table 1. Serum concentrations (mean \pm SD)				
Animal	Diet*	Glucose, mg/dl	Insulin, µunits/ml	TG, mg/dl	Cholesterol, mg/dl
Nondiabetic control	Chow (4)* Sucrose (4)	154 ± 8 155 ± 8	52 ± 14 117 ± 51	76 ± 28 176 ± 13	57 ± 4 69 ± 8
Nondiabetic transplant	Chow (4) Sucrose (4)	164 ± 13 169 ± 18	58 ± 18 96 ± 31	82 ± 20 218 ± 66	55 ± 5 71 \pm 8
Diabetic control	Chow (3) Sucrose (3)	566 ± 47 669 ± 41	$\begin{array}{rrrr} 12 \pm & 9 \\ 8 \pm & 5 \end{array}$	241 ± 87 1499 ± 989	60 ± 9 164 ± 91
Diabetic transplant	Chow (3) Sucrose (4)	227 ± 21 212 ± 20	52 ± 28 72 ± 52	84 ± 35 154 ± 122	60 ± 11 82 ± 11

* Number in parentheses is number of rats in each group.

that did not differ significantly from the values found in sucrose-fed nondiabetic rats. Mean liver cholesterol concentrations ranged from 158 to 217 mg/100 g (wet weight) and did not differ significantly among the various groups.

In the diabetic rats with transplants and fed the sucrose diet, the hepatocytes that surrounded the transplant had much enlarged Golgi-derived secretory vacuoles containing large lipoprotein particles, up to 115 nm in diameter. GERL was also much enlarged with large particles. Numerous cytosolic lipid spheres were present in the cytoplasm of these hepatocytes (Figs. 1C and 2A). The situation was strikingly different in the hepatocytes surrounding the central veins. Away from higher concentrations of insulin and other islet hormones (16), these hepatocytes had few cytosolic lipid spheres (Fig. 1B) and the lipoprotein particles were in the normal size range (Fig. 2B).

DISCUSSION

In 1971, Shiff et al. (6) reported that rats fed a sucrose-enriched diet had liver TG concentrations 30% higher than did chow-fed rats. Most likely this effect is due to the fructose derived from sucrose. Hepatic activities of enzymes involved in lipogenesis (NADP-malate dehydrogenase, citrate cleavage enzyme, and acetyl-CoA carboxylase) are higher in rats fed fructose or glucose than in rats fed chow (17). Fructose is superior to glucose as a fatty acid precursor because of its more rapid rate of catabolism to pyruvate and the subsequent formation of acetyl-CoA by pyruvate dehydrogenase (18). Lakshmanan et al. (19) have reported that fatty acid synthetase activity is negligible in acutely diabetic rats. However, Bar-On et al. (7) have shown that insulin levels increase slowly in rats with chronic streptozotocin diabetes. In the present study, the rats had been diabetic for 1-2 months; their insulin levels were low but presumably adequate to sustain fatty acid synthesis. Moreover, entry of fructose into the glycolytic pathway is not insulin-dependent. and this probably compensated for the decreased fatty acid

Table 2. Liver TG concentrations

	TG, mg/100 g wet*			
Animal	Control	Transplant		
Nondiabetic:				
Chow	$407 \pm 56(4)$	$1302 \pm 62(4)$		
Sucrose	968 ± 331 (4)	871 ± 325 (4)		
Diabetic:				
Chow	339 ± 183 (3)	$352 \pm 57 (3)$		
Sucrose	727 ± 198 (3)	$1902 \pm 775 (4) (a)^{\dagger}$		
		$1053 \pm 756 \ (b)^{\dagger}$		

* Mean \pm SD; number of rats in each group is shown in parentheses.

[†] See text.

synthetase activity. The hypertriglyceridemia in these diabetic rats was probably a result of a defect in lipoprotein removal due to decreased activity of lipoprotein lipase.

Lakshmanan *et al.* (19) also demonstrated that treatment of diabetic rats with insulin rapidly resulted in a 20-fold increase in the relative rate of synthesis of fatty acid synthetase. The high concentrations of insulin in the areas of liver around the islet cells probably result in increased levels of fatty acid synthetase around the islets. This, in addition to increased amounts of acetyl-CoA derived from fructose would result in accelerated fatty acid synthesis. Although pancreatic islet cell transplants were found in livers of sucrose-fed nondiabetic rats, the paucity of fatty areas may have been due to repression, by the normal pancreas, of insulin secretion in the transplanted islets.

Marked lipid accumulation occurred only in the periportal hepatocytes surrounding the transplanted pancreatic islets in diabetic rats fed the sucrose diet. Accumulation of lipid could be the result of increased synthesis or decreased removal. The finding of enlarged Golgi-derived secretory vacuoles containing the large lipoprotein particles suggests that secretion of lipoprotein by the hepatocytes is occurring actively. The failure of serum TG to increase in these rats may be due, in part, to the fact that these areas constitute a relatively small portion of the liver lobules and, in part, to restoration of lipoprotein lipase activity.

A strikingly similar situation occurs in the homozygous Zucker fatty rat which has hyperinsulinemia (20). Feeding the sucrose diet to this rat for as little as 1 week results in the production of a markedly fatty liver with TG concentrations of 10-32 g/100 g of liver. All hepatocytes are similar in appearance to the hepatocytes surrounding the pancreatic islet transplants described in this publication: numerous cytosolic lipid spheres are present, and large lipoprotein particles are found in the Golgi apparatus, Golgi-derived vacuoles, and GERL (13).

The studies with the transplanted diabetic rats are relevant to the studies by Reaven *et al.* (21) who observed increased TG secretion and increased serum insulin in rats fed the high-sucrose diet.

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FIG. 2. (A) Portion of the same liver shown in Fig. 1 B and C. Three alpha cells (A) are seen; an erythrocyte (E) is present. Numerous cytosolic lipid spheres (L) are seen in the three hepatocytes included in the field. (\times 3400.) (B) Portion of a centrolobular hepatocyte from the liver shown in Fig. 1 B and C. Arrow points to a very low density lipoprotein (VLDL) particle within a dilatation of a Golgi element. Two arrowheads indicate lipoprotein particles in GERL. Note that tubules of GERL or smooth endoplasmic reticulum course through a passageway (15) in the Golgi stack. Also labeled are an autophagic vacuole (A), a peroxisome (P), and endoplasmic reticulum (ER). (\times 43,500.) (C) Portion of a periportal hepatocyte from the same hepatic lobule as in B. Note the much-increased size of the very low density lipoprotein particles (arrows) in dilatations of what are probably portions of GERL or vacuoles derived from them. Also labeled is part of a cytosolic lipid sphere. (\times 43,500.)

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