Hyperlipoproteinemia in Streptozotocin-treated Rats

Hanoch Bar-On, M.D., Paul S. Roheim, M.D., and Howard A. Eder, M.D., New York

SUMMARY

In order to study hyperlipidemia in diabetes mellitus, rats were made diabetic by administration of streptozotocin and the optimal conditions for production of severe and persistent hyperlipoproteinemia determined. Two groups of rats were compared: rats fed sucrose-rich diets and rats fed laboratory chow. The optimal dose of streptozotocin was 45 mg./kg. body weight for the sucrose-fed rats. With this dose, plasma glucose reached a maximum of over 600 mg./100 ml., and plasma insulin was reduced by 60 per cent. Plasma triglycerides rose in the sucrose-fed rats to over 1,000 mg./100 ml. two days after the streptozotocin was given and then decreased to over 770 mg./100 ml. 12 days after treatment and then to 585 mg./100 ml. 10 weeks after induction of diabetes. With this dose, ketonuria did not occur nor did any of the animals die, as occurred with higher doses. In the chow-fed rats, plasma triglyceride levels were elevated with the induction of diabetes to levels of approximately 300 mg./100 ml.

The concentration of all the plasma lipoproteins increased with the induction of diabetes. The concentration of very-low-density lipoprotein (VLDL) protein in the sucrose-fed diabetic increased fivefold, the low-density lipoprotein (LDL) protein increased, and especially striking was the increase in high-density lipoprotein (HDL) protein concentration, which became more pronounced with the duration of the diabetes. The diabetes produced by streptozotocin administration to sucrose-fed rats, thus, provides a useful model for the study of the hyperlipoproteinemia of diabetes. DIABETES *25*:509-15, June, 1976.

Streptozotocin, a nitroso derivative of glucosamine, has been shown to be a highly effective cytotoxic agent for pancreatic β-cells and causes the development of diabetes when given to rats in doses of 25 mg./kg. body weight. Increasing the dose of streptozotocin results in increased severity of the diabetes.[1] As does alloxan, streptozotocin has been shown to produce hyperlipidemia.[2,3]

The present experiments were designed to compare the effects of a sucrose-rich diet with rat chow diet on the magnitude and type of hyperlipoproteinemia in streptozotocin-treated rats. Plasma glucose, insulin, and triglycerides were measured at intervals following injection of various doses of streptozotocin. Isolated plasma lipoproteins were analyzed in selected groups of diabetic rats fed the two diets. These studies were undertaken in an effort to establish an animal model of diabetic hyperlipoproteinemia.

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From the Departments of Medicine and Physiology, Albert Einstein College of Medicine, New York, New York 10461.

Dr. Bar-On has been on sabbatical leave from the Hebrew University-Hadassah Medical School, Jerusalem, Israel.

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METHODS

Animals and Diets

Male Sprague-Dawley rats (Holtzman Co., Madison, Wisconsin) weighing 150-250 gm. were used. The animals were caged individually and weighed at least twice weekly. They were allowed free access to water and food throughout the experimental period.

Two kinds of diets were used: (1) regular pelleted rat chow diet containing (w/w): 25 per cent protein, 50 per cent carbohydrate (as starch), 4.5 per cent fat, <1 per cent vitamin mixture, 5.0 per cent salt mixture, and 14.5 per cent moisture and fiber (Teklad-Mills, Madison, Wisconsin); (2) a semipurified sucrose-rich diet that contained (w/w): 20 per cent

vitamin-free casein, 60 per cent sucrose, 5 per cent lard, 1 per cent vitamin mixture, 4 per cent salt mixture, and 10 per cent cellulose. The diet was obtained from General Biochemical Division, Chagrin Falls, Ohio.

Induction of Diabetes Mellitus

After the animals were kept for 21 days on the respective diets, the rats were injected intravenously with streptozotocin (kindly supplied by Dr. William Dulin, Upjohn Co., Kalamazoo, Michigan) in doses ranging from 25 to 85 mg./kg. body weight. The streptozotocin was freshly dissolved in 0.05 M citric acid, pH 4.5, and was injected within five minutes of its preparation. The volume of administered solution did not exceed 0.5 ml. for each rat; control rats were injected with 0.5 ml. 0.05 M citric acid. During the first 24 hours following injection, the rats were supplied with 5 per cent glucose in their drinking water, to combat the hypoglycemia that occurs 7-12 hours after administration of streptozotocin.[2] The urine was collected and monitored for the presence of glycosuria and ketonuria throughout the study.

Two days following administration of streptozotocin, 1.5 ml. blood was withdrawn from the subclavian venous plexus, and the animals were killed 12 days after the administration of streptozotocin by exsanguination from the abdominal aorta. In each instance, the blood was obtained without prior fasting. From each group of rats, epididymal fat pads were removed, pooled, and weighed.

In order to study long-term effects of streptozotocin, rats were killed 10 weeks after the administration of the drug. Male adult rats were divided into four groups, each comprising 8-10 rats. Two groups were fed chow and two were fed the sucrose-rich diet. The rats (one group on each diet) were injected with streptozotocin following 21 days of basal diet. These rats received 45 mg./kg. body weight of streptozotocin.

Chemical Methods

Lipoprotein separation was carried out by the method of Havel, Eder, and Bragdon,[4] using the SW 41 Rotor in the Beckman Model L-2 65B ultracentrifuge at 15° C. Chylomicrons were removed after centrifugation at 10,000 × *g* for 30 minutes. Very-low-density lipoproteins (VLDL), d< 1.006, and low-density lipoproteins (LDL), d 1.006-1.063, were separated by 20 hours' ultracentrifugation at 200,000 × *g*, while high-density lipoproteins (HDL), d 1.063-1.21, were floated by 40 hours of ultracentrifugation at 200,000 × *g*. Densities above 1.006 were adjusted by addition of KBr solutions or solid KBr.

The isolated fractions were separated with a Spinco tube slicer; they were washed once by resuspending them at their respective density and then repeating the ultracentrifugal separations.

Glucose was determined by the Glucostat method (Glucostat Reagent Kit, Worthington Biochemical Corp. Freehold, New Jersey), and immunoreactive insulin was determined by the double-antibody technic of Hales and Randle,[5] with human insulin used as standards. Lipids in plasma or lipoprotein fractions were extracted according to the method of Folch, Lees, and Sloane-Stanley.[6] Triglycerides were determined according to Van Handel's modification[7] of the method of Van Handel and Zilversmit.[8] Cholesterol was measured by the method of Abell, Levy, Brodie, and Kendall.[9] Phospholipids were determined by the method of Bartlett,[10] a factor of 25 being used to convert lipid P to phospholipid. Protein content of the lipoprotein fractions was determined by the modification by Sata, Havel, and Jones[11] of the method of Lowry, Rosenbough, Farr, and Randall.[12]

RESULTS

Glycosuria occurred in both chow-fed and sucrose-fed rats within 24-36 hours following the administration of streptozotocin at the dose of 35 mg./kg. Polyuria, polydypsia, and polyphagia were also apparent. Ketonuria was not observed in the rats that received 25-55 mg./kg. streptozotocin but was present in the sucrose-fed rats receiving 65 mg. or 85 mg./kg. streptozotocin. All the latter rats died 72 hours after the administration of the drug. At a dose of 55 mg./kg., 40 per cent of the rats on the sucrose-rich diet died within 12 days after the injection of the drug. One out of six of the chow-fed rats died in the same period. None of the rats receiving 45 mg./kg. died (table 1).

The rate of weight gain was similar in the chow-fed and sucrose-fed rats during the three weeks prior to the injection of streptozotocin. The rats that received 25 mg. and 35 mg./kg. of streptozotocin continued to gain weight over a 12-day period. However, rats receiving 55 mg./kg. streptozotocin lost weight and appeared sick. In figure 1 are shown the weight curves of rats that received 45 mg./kg. streptozotocin over a 10-week period. The nondiabetic control rats continued to gain weight. In the sucrose-fed diabetic rats, no weight gain was apparent, while the chow-fed diabetic rats showed some weight gain.

TABLE 1

Effect of dose of streptozotocin on the survival of rats fed chow or a sucrose-rich diet

Diet	Streptozotocin mg./kg. body weight	Number of rats	Number of rats dead by the 12th day
Chow	0	6	0
	25	6	0
	35	6	0
	45	6	0
	55	6	1
Sucrose-rich	0	10	0
	25	10	0
	35	10	0
	45	10	0
	55	10	4
	65	5	5
	85	5	5

Table 2 shows the weights of the epididymal fat pads obtained from the chow-fed and sucrose-fed rats, control and diabetic, *12 days* after receiving streptozotocin in increasing amounts. It is apparent that up to the 35 mg./kg. dosage level the values do not differ appreciably from the control; at 45 mg./kg. epididymal fat pads decreased in weight to a similar extent in both groups.

One of the prominent characteristics of the chronically diabetic rats that received 45 mg./kg. streptozotocin and were examined *10 weeks* after the injection was a total absence of visceral fat depots. Many of the diabetic rats had advanced lenticular opacities and demonstrated dilatations of the urinary collecting systems with hydronephrosis. Gastric dilatation and ballooning of many loops of the gastrointestinal tract was observed in some of the rats.

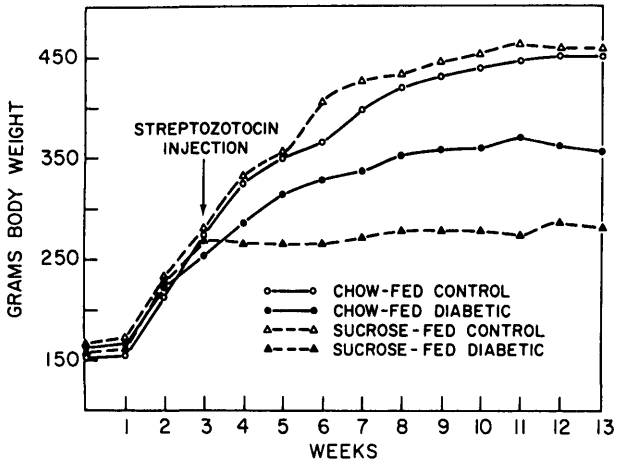

FIG. 1. Mean body weight of diabetic and nondiabetic rats. Both the chow-fed rats and the rats on the sucrose-rich diet received 45 mg./kg. of streptozotocin in the third week of the diet and were followed for an additional 10 weeks.

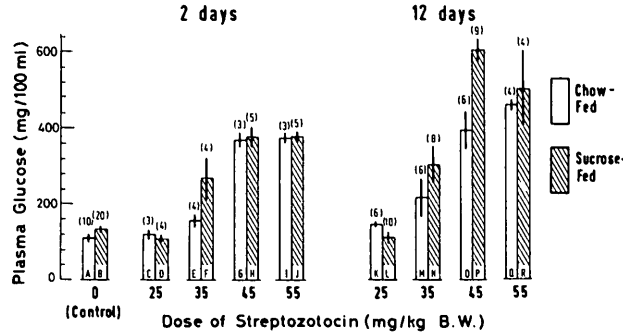

FIG. 2. Plasma glucose concentrations in rats on chow and on sucrose-rich diets receiving increasing doses of streptozotocin. Rats were studied two and 12 days after the administration of streptozotocin. Each value represents the mean ± S.E.M.

Glucose and Insulin Levels

In figure 2 are shown the plasma glucose levels of rats maintained on both diets two and 12 days after receiving increasing doses of streptozotocin. No increase was apparent in either group at 25 mg./kg. dose at both time intervals, but the levels increased progressively with the higher doses and were maximal at the 45 mg./kg. dose.

Ten weeks after induction of diabetes, the plasma glucose on the 45-mg./kg. dose remained elevated, with the levels being higher in the sucrose-fed rats (table 3). In figure 3 are shown the plasma insulin levels in the two groups of rats studied two days after administration of graded doses of streptozotocin. Plasma insulin levels fell progressively as the dose of streptozotocin was increased, with the lowest levels of insulin occurring at 45 mg./kg. Twelve days after streptozotocin administration, the plasma insulin returned to normal levels with the 25- and 35-mg./kg. doses. However, in rats that received 45 and 55 mg./kg., the insulin values decreased progressively. In both groups, 10 weeks after 45 mg./kg. of strep-

TABLE 2

Weight of epididymal fat pads in diabetic rats 12 days after streptozotocin treatment

Dose of streptozotocin	Chow-fed	Sucrose-fed
mg./kg. body weight	gm./100 gm. body weight	
0	1.20*	1.28
25	1.12	1.30
35	1.13	1.12
45	0.80	0.85

*Mean of values from samples obtained from pools of five to 10 rats.

TABLE 3

Plasma glucose, insulin, and triglyceride concentrations in rats studied 10 weeks following the administration of 45 mg./kg. of streptozotocin

Diet	No. of Rats	Glucose mg./100 ml.	Insulin μU./ml.	Triglycerides mg./100 ml.
Chow-fed control	8	122.0 ± 7.0*	48.3 ± 9.3	93.8 ± 11.3
Chow-fed diabetic	8	351.5 ± 89.0	42.3 ± 5.1	150.0 ± 13.0
Sucrose-fed control	10	125.0 ± 10.4	50.5 ± 4.2	300.0 ± 88.0
Sucrose-fed diabetic	10	513.5 ± 56.1	44.5 ± 4.2	585.5 ± 139.5

*Mean ± S.E.M.

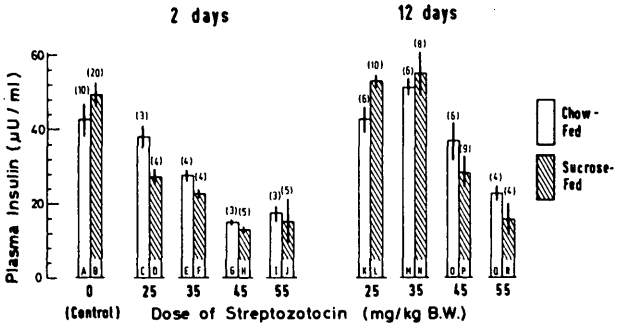

FIG. 3. Plasma insulin concentrations in rats on chow and on sucrose-rich diets receiving increasing doses of streptozotocin. Rats were studied two and 12 days after the administration of streptozotocin. Each value represents the mean ± S.E.M.

tozotocin injection, plasma insulin levels returned to the control levels despite the fact that the plasma glucose remained elevated (table 3).

Plasma Triglyceride Concentrations

In figure 4 are shown the changes in plasma triglyceride levels occurring after two days in both groups of rats receiving progressively larger doses of streptozotocin. The control values in sucrose-fed rats are higher than those of the chow-fed rats. The maximum increase in triglyceride levels was seen when the dose of streptozotocin was increased to 45 mg./kg., and the levels were higher in the sucrose-fed rats.

Ten weeks after the administration of 45 mg./kg. streptozotocin, the sucrose-fed nondiabetic rats continued to have higher triglyceride levels than did the chow-fed diabetic rats (table 3). However, in both groups, the plasma triglyceride concentrations were lower than those found two or 12 days after the administration of the drug.

Serum cholesterol and phospholipid concentrations were measured in 20 sucrose-fed rats studied 12 days after the administration of 45 mg./kg. of streptozotocin and in 22 sucrose-fed control rats. The serum cholesterol level rose from 85 ± 3.0 mg./100 ml. in the nondiabetic controls to 180 ± 15.1 mg./100 ml. in the diabetic rats. The serum phospholipids rose from 139 ± 4.5 mg./100 ml. in the nondiabetic rats to 324 ± 17.8 mg./100 ml. in the diabetic rats.

Plasma Lipoproteins

In rats receiving 45 mg./kg. of streptozotocin, plasma lipoprotein concentrations were measured 12 days and 10 weeks after administration of streptozotocin. Since the rats were not fasting prior to sacrifice, the plasma was subjected to 30 minutes' centrifugation at 10,000 × *g* in order to remove the chylomicrons. Three major lipoprotein fractions (VLDL, LDL, and HDL) were separated. As seen in table 4, the induction of diabetes in chow-fed rats resulted in a doubling of the amount of protein in VLDL. In the sucrose-fed rats, the induction of diabetes resulted in a fivefold increase in VLDL protein. The protein and cholesterol concentrations of LDL and HDL of the sucrose-fed nondiabetic and diabetic rats were determined also 12 days after the administration of streptozotocin. As seen in table 4, in the LDL of diabetic

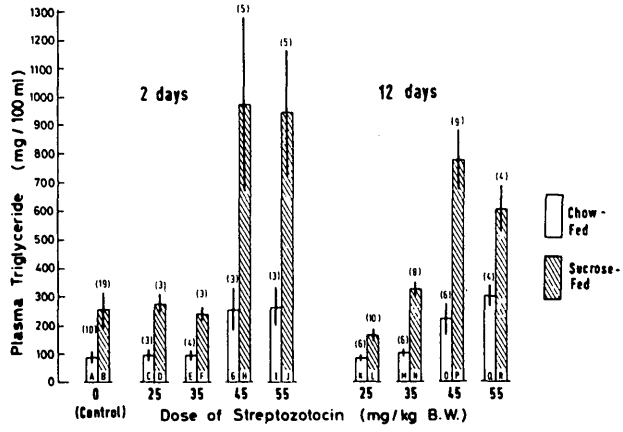

FIG. 4. Plasma triglyceride concentrations in rats on chow and on sucrose-rich diets receiving increasing doses of streptozotocin. Rats were studied two and 12 days after the administration of streptozotocin. Each value represents the mean ± S.E.M.

TABLE 4

Protein and cholesterol in lipoproteins of diabetic and nondiabetic rats studied 12 days after administration of streptozotocin (45 mg./kg.)

		Protein	Cholesterol
		mg./100 ml. plasma	
Chow-fed control	VLDL	8.7	7.5
Chow-fed diabetic	VLDL	15.0	6.6
Sucrose-fed control	VLDL	10.7	7.6
	LDL	1.5	2.6
	HDL	29.7	32.0
Sucrose-fed diabetic	VLDL	53.7	56.7
	LDL	12.4	32.9
	HDL	54.8	54.4

All values are mean of 3-5 pools.

rats, there was an eightfold increase in the concentration of protein, with a tenfold increase in cholesterol. The concentration of HDL protein and cholesterol almost doubled.

Analysis of the various lipoprotein fractions of the rats studied 10 weeks after administration of streptozotocin and their controls is shown in table 5. The VLDL protein was somewhat lower in comparison to that determined after 12 days. However, there was a marked increase in the HDL protein in the sucrose-fed diabetic rats.

DISCUSSION

The objective of the present investigation was to develop a model for the study of diabetic hyperlipoproteinemia. We have attempted to establish the optimal conditions for the induction of hyperlipidemia and hyperlipoproteinemia, i.e. diabetes that would be accompanied by a marked hyperlipemia and hyperlipoproteinemia but at the same time would not cause a marked weight loss, ketosis, and a high rate of mortality. Earlier studies from this laboratory[13] have demonstrated that feeding of sucrose-rich diets to normal rats results in moderate hyperlipidemia and hyperliproproteinemia. Hence, in an effort to enhance the effects of diabetes in the induction of hyperlipidemia, the present studies were carried out in rats kept on a sucrose-rich diet.

We were able to demonstrate clearly that rats made diabetic when given 45 mg./kg. of streptozotocin develop considerably greater hyperlipidemia when prefed and maintained on a sucrose-rich diet throughout the study.

The increase of serum triglycerides in diabetic rats has been reported by a number of investigators.[2,3,14,15] The extent of hyperlipidemia was variable and could be due to differences in diet and dose of streptozotocin. In this study, the role of diet in determining the degree of hyperlipidemia was clearly demonstrated.

Bierman, Amaral, and Belknap[16] carried out studies with alloxan-injected rats that were fed purina chow supplemented with fat or a sucrose-rich, fat-free diet. Four to six weeks and 12-16 weeks after injection of the drug, they found elevated levels of plasma triglycerides only in rats fed a high fat diet. In our long-term studies, triglyceride levels were increased in the sucrose-fed diabetic rats over those of the sucrose-fed controls.

There is a considerable disagreement in the literature as to the mechanism of the elevation of serum VLDL in diabetes. In most studies of diabetic rats, elevations in serum free fatty acids (FFA) were found,[3,15] and this is in accord with our findings of decreased weight of epididymal fat pads when streptozotocin in doses of 45 mg./kg. was given. It should be noted that the decrease in weight of the fat pads was similar to the chow-fed and sucrose-fed groups, suggesting that FFA mobilization was similar in both groups. It has been suggested that the increased flux of FFA causes increased triglyceride synthesis and, hence, increased formation of VLDL-TG. This has been observed early in experimentally induced diabetes by Balasse, Bier, and Havel[17] and by Woodside and Heimberg.[18] This early increase in VLDL-TG

TABLE 5

Lipoproteins of control and diabetic rats studied 10 weeks following the administration of streptozotocin (45 mg./kg.)

		Protein	Cholesterol
		mg./100 ml. plasma	
Chow-fed Control	VLDL	8.7	7.5
	LDL	5.3	3.1
	HDL	35.3	29.5
Chow-fed diabetic	VLDL	23.2	15.5
	LDL	10.2	7.3
	HDL	46.5	42.5
Sucrose-fed control	VLDL	17.6	7.0
	LDL	4.0	8.1
	HDL	36.1	26.0
Sucrose-fed diabetic	VLDL	44.2	36.5
	LDL	8.1	28.5
	HDL	97.0	62.5

All values are mean of 2 pooled sera.

formation was also demonstrated by Reaven and Reaven.[15] However, in dogs with chronic diabetes, Basso and Havel[19] have demonstrated decreased formation of VLDL-TG, and in rats treated with Triton WR 1339, Reaven and Reaven[15] found a reduced rate of entry of triglyceride into the plasma of rats studied seven days after the induction of diabetes. It is suggested by the latter workers that in most diabetic rats, there is decreased removal of VLDL-TG from the circulation, and our studies of lipoprotein lipase activation and inhibition by serum from diabetic rats are compatible with this hypothesis.[20] We have previously reported that during induction of diabetes in sucrose-fed rats marked changes occur in the subunit protein (apolipoprotein) composition of the various lipoproteins.[21]

The differences in serum VLDL-TG between chow-fed and sucrose-fed diabetic rats are consistent with findings in nondiabetic rats, where the feeding of sucrose- or fructose-rich diets results in moderate elevation of serum VLDL-TG. Fructose feeding, when compared to that of glucose, resulted in increased synthesis of triglyceride by the rat liver.[22] In this study, it was also shown that glucose feeding increased lipoprotein lipase activity, while fructose feeding did not.[22] Subsequently, Waddell and Fallon[23] found that rats fed 75 per cent fructose developed hypertriglyceridemia, in contrast to rats fed 75 per cent glucose. Triglyceride formation from glycerol-3-phosphate was increased in both diets,[23] and the differences in serum triglyceride levels were attributed to a decreased removal of triglyceride in the fructose-fed rats.

In diabetic rats, the effects of feeding diets high in sucrose may result in an exaggeration of the effects of fructose feeding observed in nondiabetic animals. Thus, there may be increased synthesis of serum VLDL-TG, especially early after the induction of diabetes and, subsequently, impaired removal. Recently, Chlouverakis and Schnatz[24] have administered labeled triolein to sucrose-fed rats after discontinuing treatment with insulin. In the sucrose-fed rats, they found high levels of radioactivity in the plasma and a reduced amount of radioactivity in the adipose tissue, and they concluded that the feeding of the high sucrose diet has its primary effect on the removal mechanism of VLDL-TG.

The induction of diabetes produced marked increases in the concentrations of the various lipoproteins, especially VLDL, but also LDL and HDL. These increases were considerably exaggerated in the sucrose-fed rats. It is of considerable interest that in the rats studied 10 weeks after they were made diabetic, VLDL protein decreased, whereas the HDL protein increased markedly.

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