

Mechanisms for Development of Diabetic Hypertriglyceridemia in Streptozotocin-Treated Rats

EFFECT OF DIET AND DURATION OF INSULIN DEFICIENCY

EVE P. REAVEN and GERALD M. REAVEN

*From the Department of Medicine, Stanford University School of Medicine and
Veterans Administration Hospital, Palo Alto, California 94304*

ABSTRACT A combined ultrastructural and functional approach was employed to define the effects of duration of diabetes and of diet on various aspects of lipid metabolism in rats with severe streptozotocin (SZ)-induced insulin deficiency. Plasma triglyceride (TG) levels rose to a mean of 479 mg/100 ml 24 h after SZ administration in rats eating a fat-free, high carbohydrate diet as compared to a mean of 324 mg/100 ml in rats eating a high fat diet. These changes were associated with a commensurate increase in hepatocyte Golgi very low density lipoprotein (VLDL) content, but only a small increase in estimates of VLDL-TG secretion rate (post-Triton WR 1339 increment in plasma TG level). Although these findings are consistent with the thesis that VLDL-TG synthesis and secretion are increased 24 h after administration of SZ, it seemed unlikely that the observed increase in VLDL-TG secretion could entirely account for the severity of the hypertriglyceridemia. Thus, although lipoprotein removal rate was not measured directly, it was necessary to postulate that a defect in VLDL-TG removal was also present at this stage.

Hypertriglyceridemia was still present 7 days later, only in this instance plasma TG levels were higher in rats eating the high fat diet (a mean of 589 mg/100 ml, as compared to 263 mg/100 ml). Rats with diabetes of 7-day duration had a 50% decrease in both TG entry rate and hepatocyte Golgi complex VLDL content, irrespective of diet. Thus, there was no evidence of increased VLDL-TG secretion in chronic insulin deficiency. In this instance, although not assessed directly, it was necessary to postulate that the hypertriglyceridemia in chronically insulin-deficient rats is due entirely

to a defect in lipoprotein removal, involving both dietary and endogenous fat.

INTRODUCTION

Although hypertriglyceridemia is known to occur in association with severe insulin deficiency, questions remain as to the frequency of hypertriglyceridemia, the mechanisms responsible for its development, and the effect of diet on plasma triglyceride (TG)¹ levels (1-20). Some confusion undoubtedly stems from the fact that studies of different experimental models of diabetes have yielded different insights as to the nature of diabetic hypertriglyceridemia. Indeed, one might predict that differences in severity of hyperglycemia, duration of insulin deficiency, composition of diet, etc., modify experimental results. Thus, a conclusion as to the cause of diabetic hypertriglyceridemia based upon study of TG synthesis and secretion by perfused livers from rats with diabetes secondary to anti-insulin serum (19) might differ from one based upon investigation of ketotic-prone diabetic patients (5). Neither conclusion is right nor wrong; both are accurate summations of one facet of the effect of insulin deficiency on TG metabolism. In order to provide a broader view of this problem we have studied rats injected with the same large dose of streptozotocin (SZ) and used a combined ultrastructural and functional approach to define the effects of duration of insulin deficiency and composition of diet on various aspects of lipid and carbohydrate metabolism in these rats. The results indicate that the degree of hypertriglyceridemia produced in severely insulin-deficient rats is a complex function of both duration of diabetes and composition of diet.

¹Abbreviations used in this paper: SZ, streptozotocin; TG, triglyceride; VLDL, very low density lipoprotein.

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METHODS

Animals and diet. Female Sprague-Dawley rats, 175–200 g each, were housed four to a cage in a room in which lights were automatically turned on at 6 a.m. and off at 6 p.m. Rats were switched from standard laboratory chow to special diet A or B 7 days before beginning acute experiments. In chronic experiments, rats were fed standard chow until 24 h after SZ injection, after which they were switched to diet A or B for the 7 days of the experiment. Special diets were prepared by Nutritional Biochemicals (Biological Diets Div. of ICN Nutritional Biochemicals Div., International Chemical and Nuclear Corp., Cleveland, Ohio) and contained the following ingredients:

	Diet A (nonfat, high carbohydrate)	Diet B (high fat, low carbohydrate)
Total calories	350/100 g	350/100 g
Carbohydrate (sucrose)	70% of calories	40% of calories
Protein (casein)	30% of calories	30% of calories
Fat (corn oil)	0% of calories	30% of calories

Experimental protocol. Rats were fasted overnight and injected intravenously between noon and 2 p.m. with either 75 mg/kg body wt of SZ prepared in pH 4.3 citrate buffer or an equal volume of citrate buffer (SZ was a gift from Dr. William Dulin, Upjohn Co., Kalamazoo, Mich.). Food was removed from the rat cages at 8 a.m. on the last day of each study and blood and/or tissue was obtained between 2 and 4 p.m. For the acute studies the rats were killed approximately 24 h after SZ injection. It should be noted that plasma insulin levels rise during the first 7 h after administration of SZ and fall slowly thereafter, dipping below normal levels sometime between the 12th and 18th h (21). Thus, the duration of insulin deficiency in these acute studies is somewhat uncertain, but likely to be only 6–12 h long.

Chemical procedures. Blood was drawn either by cardiac puncture or from the chest of stunned animals and centrifuged immediately, and aliquots of plasma were stored frozen for subsequent glucose, FFA, TG, and insulin measurements. Glucose was measured with a glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.); FFA by the method of Dalton and Kowalski (22); TG concentrations by the procedure of Kessler and Lederer (23); and insulin by a single antibody method (24) with rat insulin used as a standard.

Measurement of TG entry into plasma. TG entry rates were estimated by injecting rats with Triton WR 1339 and measuring TG accumulation in plasma during the following 2 h (25, 26). Base-line blood samples were drawn by cardiac puncture from postabsorptive rats under light ether anesthesia, and 800 mg/kg body wt of Triton WR 1339 was then injected intravenously. (This dose of Triton was selected after several dose-response experiments had been done in which the amounts of Triton necessary to provide maximal inhibition of TG removal from plasma of the various experimental groups was determined.) A second blood sample was obtained 2 h later. Since it is estimated that appropriate doses of Triton inhibit removal of more than 90% of TG from plasma (25), the increment in plasma TG during the 2-h period, when corrected for variations

in plasma volume, provides an estimate of the rate of TG entry into plasma.

$$(\text{TG increment [mg/ml]} \times \text{plasma volume [ml]}) / 120 \text{ min} \\ = \text{TG entry rate [mg/min]}.$$

Plasma volumes of six additional animals from each experimental group were estimated by standard dye dilution techniques after the intravenous injection of Evans blue, and used to calculate very low density lipoprotein (VLDL)-TG entry rates. Since pre-Triton blood samples were small, and post-Triton samples were contaminated with the detergent, it was necessary to obtain blood from additional non-Triton-injected animals for plasma FFA and insulin determinations. Glucose and TG were measured on all blood samples.

Preparation of tissue for electron microscopy. Samples were taken of the left lobe of the liver, fixed in Millonig's phosphate-buffered osmium tetroxide (pH 7.2, 4°C) for 4 h, and subsequently dehydrated in graded alcohols and embedded in Epon-araldite plastic. Thin sections (400–600 Å) of periportal areas of the liver samples were placed on uncoated grids, stained with Reynold's lead citrate for 60 min, and examined with a JEM 100 B electron microscope (JEOL USA, Electron Optics Div., Medford, Mass.).

Ultrastructural morphometric analysis of tissue sections. In order to obtain a morphological estimate of hepatic synthesis of VLDL, we have measured the number and size of VLDL within hepatocyte Golgi complexes. The use of Golgi VLDL content as an indicator of hepatocyte production appeared justified in view of the fact that Golgi complexes have been described as temporary storage and packaging depots for export VLDL (27–30), and because of preliminary morphometric analyses of the location of VLDL in various compartments of hepatocytes of control cells. These measurements were performed using the point-counting analytic method of Weibel (31) and showed that the volume of VLDL in Golgi complexes is twice ($P < 0.001$) the total volume of VLDL found in any other compartment of the cell. The decision to estimate both number and size of VLDL in Golgi complexes in this current study (rather than relative volume of VLDL as obtained by the Weibel technique) was based on the fact that hepatocytes of animals in different physiological states contain VLDL particles of different sizes, and the likelihood that the relative size of synthesized lipoproteins may be of functional importance.

In order to minimize bias in selecting cells for analysis, the source of the tissue was not known to the microscopist at the time of examination, and the first three cells seen which were nucleated, and of otherwise good technical quality, were analyzed (32). Golgi complexes and associated vesicles of each selected cell were identified at $\times 12,000$ magnification, and the number of related VLDL particles were counted directly from the image produced on the microscope screen. Golgi complexes were identified by their characteristic stacked membranes in close association with vacuoles containing VLDL. Vacuoles which were farther than 1/2 inch (at $\times 12,000$) from identifiable Golgi membranes were arbitrarily omitted from the estimate. The interhepatocyte coefficient of variation of total VLDL associated with Golgi complexes is of the order of 30%. Despite this high level of variation between cells, the inter-animal variation is much lower (see Table II), suggesting that the number of cells and the number of rats sampled permits adequate statistical evaluation.

In addition, electron micrographs of two randomly selected Golgi complexes of each cell were photographically

enlarged to $\times 35,000$ and the diameters of all Golgi-associated VLDL particles were measured with the aid of a $\times 10$ ocular micrometer. Interhepatocyte variation in VLDL diameter is less than 7%. Since the VLDL particles are assumed to be spherical, it is possible to convert values for VLDL diameter to VLDL volume and to measure the average volume of VLDL within hepatocytes. The mean value for VLDL volume multiplied by the mean number of VLDL from hepatocytes of the same animals, provides a measure of total volume of Golgi-associated VLDL within representative hepatocytes. Verification of this method for obtaining Golgi VLDL volume was given by the fact that similar results could be obtained by the Weibel (31) point-counting method. Although the current method estimated absolute volumes and the Weibel method estimated relative volumes, in both cases it was found that Golgi VLDL volume in chronically diabetic rats was reduced by one-half ($P < 0.01$) of the control values (see Table II).

RESULTS

General observations. Control rats, fed either diet A or diet B, maintained their normal daily mean (\pm SE) weight increase of 4 g (± 0.3) regardless of diet consumed. Insulin-deficient rats ate somewhat less than control rats (approximately 12 g as compared to 16 g) and lost an average of 2.4 ± 0.6 g during the 24-h period over which the acute experiments were conducted. Chronically insulin-deficient rats lost a mean (\pm SE) of 19 ± 2 g on diet A (and maintained their original weight on diet B) during the 7-day experimental period, despite the fact that their food and water intake virtually doubled during the last few days of the study. Control rats during this same period gained a mean (\pm SE) of 28 ± 1.8 g.

Effect of duration of insulin deficiency and diet on plasma glucose, FFA, and TG levels. Fig. 1 indicates that control rats fed diet A and diet B have comparable plasma glucose levels, which increase almost threefold 24 h after SZ. After 7 days of insulin deficiency, rats fed diet A had a further increase in plasma glucose values, whereas the plasma glucose values of rats fed diet B did not increase as compared to acutely insulin-deficient rats on the same diet.

Fig. 2 indicates that diet did not affect plasma FFA levels in control rats. Acute and chronic insulin deficiency led to a doubling of plasma FFA concentrations, regardless of diet.

The data in Fig. 3 indicate that dietary composition markedly influences TG levels of all three groups of rats. Thus, TG levels were higher in control rats fed diet A. Acute insulin deficiency resulted in marked elevation of plasma TG levels in animals fed both diets, but the rise was more striking in rats fed diet A. Plasma TG levels continued to remain very high in rats with chronic insulin deficiency, but in this instance rats fed diet B had the highest TG levels.

Effect of duration of insulin deficiency and diet on TG entry rate into plasma. Plasma TG levels before and

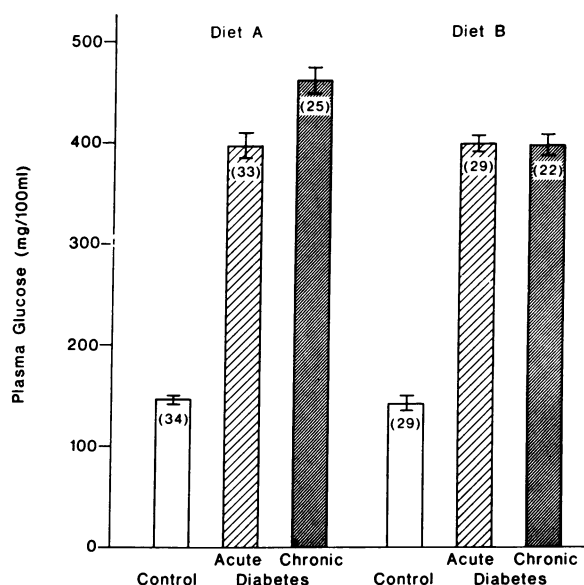


FIGURE 1 Effect of duration of insulin deficiency and diet on plasma glucose levels. The results indicate that acute insulin deficiency results in a three-fold increase ($P < 0.001$) in plasma glucose levels, whether rats are fed a fat-free, high carbohydrate diet (diet A) or a high fat, low carbohydrate diet (diet B). Chronic insulin deficiency results in a further increase ($P < 0.001$) in plasma glucose values in rats fed a high carbohydrate diet (diet A). Bars represent mean (\pm SE) plasma concentrations; number of rats studied indicated by parentheses.

2 h after the administration of Triton WR 1339 are seen in Table I. Since Triton blocks removal of lipoproteins from plasma, the increment in plasma TG level that occurs is a function of the amount of TG entering the plasma and the size of the plasma pool. The calculated

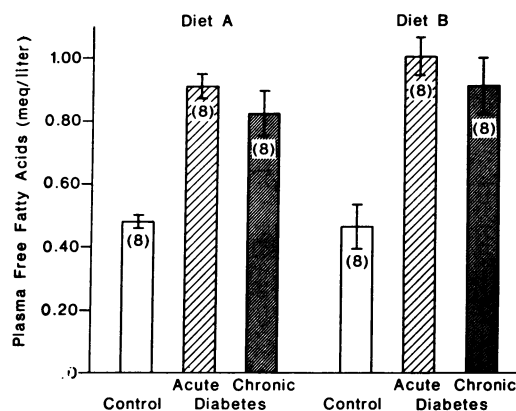


FIGURE 2 Effect of duration of insulin deficiency and diet on plasma FFA levels. The results indicate that FFA doubled as compared to control values ($P < 0.001$) in both acute and chronic diabetes regardless of diet. Bars represent mean (\pm SE) plasma concentrations; numbers of rats studied indicated by parentheses.

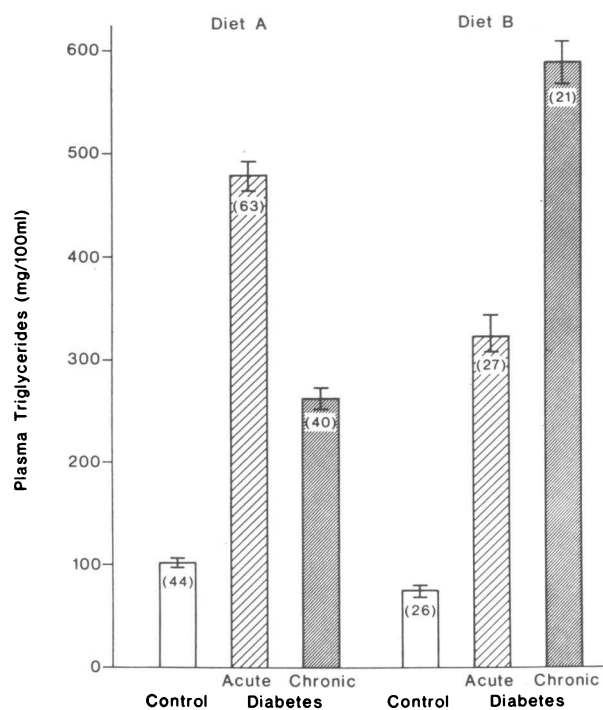


FIGURE 3 Effect of duration of insulin deficiency on plasma TG levels of rats. TG levels of acutely insulin-deficient rats rose 4.7 times control values ($P < 0.0001$) on a fat-free diet (diet A) or 4.3 times ($P < 0.0001$) on a high fat, low carbohydrate diet (diet B). Plasma TG levels of chronically diabetic rats were 2.6 times control values ($P < 0.0001$) on diet A and 7.8 times control values ($P < 0.0001$) on diet B. Within given experimental categories, TG levels were higher ($P < 0.025$) in control rats fed diet A than diet B; in acutely insulin-deficient rats TG levels were higher ($P < 0.01$) in rats fed diet A; in chronic insulin deficiency TG levels were higher ($P < 0.0001$) in rats fed diet B. Bars represent mean (\pm SE) plasma concentrations; number of rats studied indicated by parentheses.

TG entry rates are seen in the last column of Table I and indicate that duration of diabetes had a profound effect on TG entry rate. Thus, acute insulin deficiency resulted in a statistically significant increase in TG entry rate, irrespective of the diet. The basal TG levels of acutely diabetic rats, however, were increased proportionately more than the TG entry rate. For example, in acutely diabetic rats fed diet A, mean basal TG levels have risen to 5.6 mg/ml as compared to mean basal control levels of 1.16 mg/ml. In contrast, mean TG entry rates of the acutely diabetic rats only increase 29% from 1.38 to 1.78 mg/min. These data suggest that the elevations of TG levels that occur in acute insulin deficiency cannot be explained entirely on the basis of an increase in TG entry rate.

The effect of chronic insulin deficiency on TG entry rate was markedly different than that of acute insulin deficiency, and Table I demonstrates that the hypertriglyceridemia of chronic diabetes was associated with a statistically significant decrease in TG entry rate. This fall was seen in rats eating either diet and was of similar magnitude in both groups of rats. These results indicate that the elevated basal levels of plasma TG in rats with chronic diabetes must be due to a decrease in TG removal from plasma. Since base-line TG levels were increased in rats eating a fat-free diet (diet A), the defect in lipoprotein removal involves endogenous lipoproteins. The fact, however, that basal TG levels were even higher in rats eating a high fat diet (diet B) indicates that chylomicron clearance is also decreased in chronic diabetes.

Effect of duration of insulin deficiency and diet on hepatocyte ultrastructure. Fig. 4 shows the typical distribution of Golgi complexes (rectangular areas) found in periportal hepatocytes from a control animal. Hepato-

TABLE I
Effect of Triton WR 1339 on Rate of Entry (Mean \pm SE) of TG into Plasma of Control and Insulin-Deficient Rats

Diet	Experimental group	Number	Weight change	Plasma volume	TG concentration			Estimated rate of TG entry into plasma
					Before Triton	2 h after Triton	Increment in 2 h	
			g	ml	mg/ml	mg/ml	mg/ml	mg/min
A	Control	13	+26 (\pm 2)	10.2 \pm 0.1	1.16 \pm 0.06	17.35 \pm 0.86	16.19 \pm 0.85	1.38 \pm 0.07
A	Acute insulin deficiency	23	-2 (\pm 0.6)	9.9 \pm 0.1	5.60 \pm 0.77	27.17 \pm 1.58	21.57 \pm 0.99	1.78 \pm 0.08*
A	Chronic insulin deficiency	26	-19 (\pm 2)	8.2 \pm 0.1	2.73 \pm 0.35	12.53 \pm 0.78	9.80 \pm 0.52	0.67 \pm 0.03§
B	Control	10	+28 (\pm 2)	9.9 \pm 0.1	0.87 \pm 0.14	12.68 \pm 0.04	11.81 \pm 0.04	0.97 \pm 0.04
B	Acute insulin deficiency	10	-3 (\pm 0.3)	9.7 \pm 0.2	4.19 \pm 0.65	19.08 \pm 1.10	14.89 \pm 0.88	1.20 \pm 0.08†
B	Chronic insulin deficiency	10	+1 (\pm 2)	8.7 \pm 0.1	5.82 \pm 0.52	16.62 \pm 0.50	10.80 \pm 0.37	0.77 \pm 0.03§

* $P < 0.025$ as compared to control on same diet.

† $P < 0.05$ as compared to control on same diet.

§ $P < 0.001$ as compared to control on same diet.

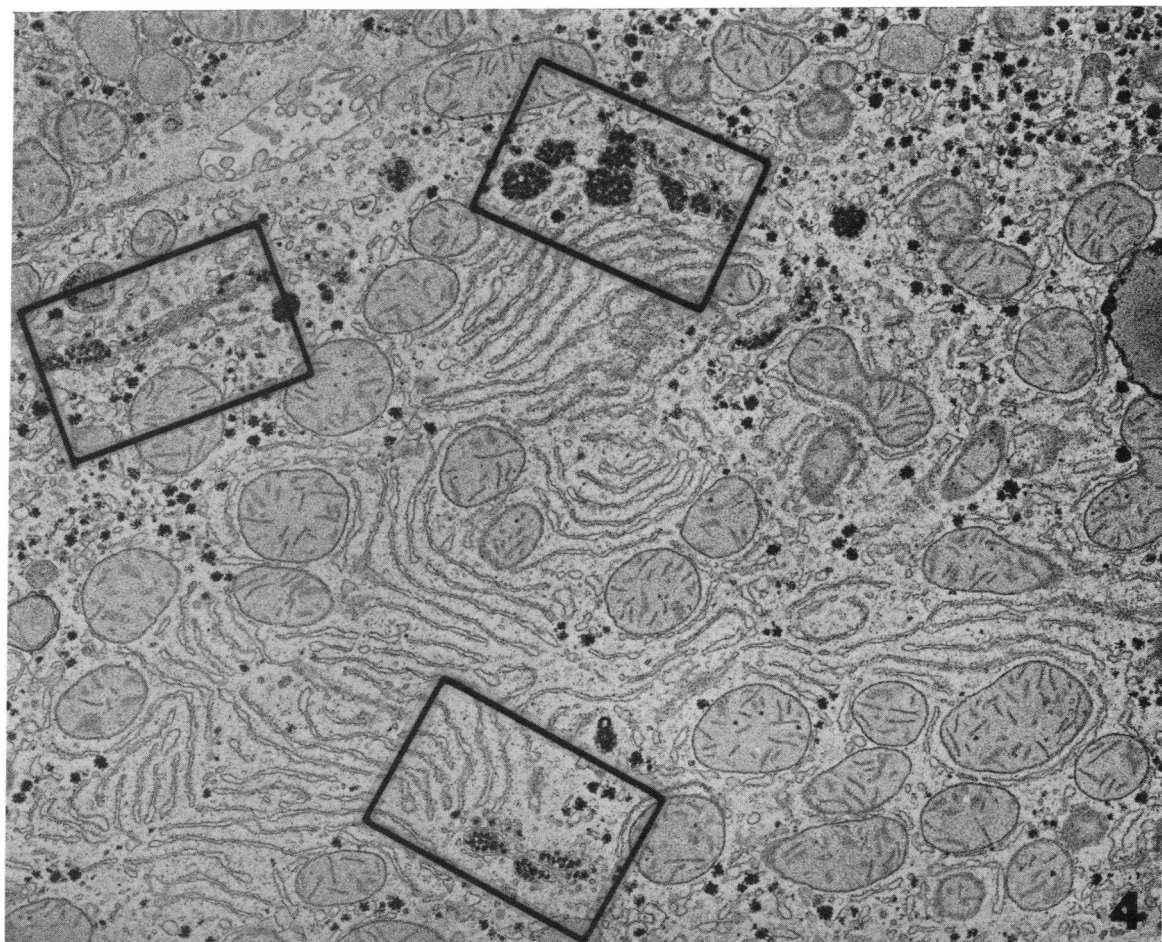


FIGURE 4 Low magnification electron micrograph of portion of periportal hepatocyte from a control rat fed a high fat, low carbohydrate diet (diet B). Micrograph shows one large and two smaller Golgi complexes within rectangular boxes. $\times 14,000$.

cytes from normal animals on diet A or B contain a mean (\pm SE) of 1.13 ± 0.08 profiles of Golgi complexes per 100 cm^2 cytoplasm. Each Golgi complex consists of organized stacks of smooth-surfaced membranes (Golgi cisternae) associated with vacuoles which are generally filled with electron dense VLDL particles (Fig. 5). Although isolated VLDL particles can also be found enmeshed within membranes of the smooth endoplasmic reticulum, and within Golgi vacuoles at considerable distance from Golgi membranes, quantitation of VLDL particles from electron micrographs of randomly selected hepatocytes indicate that the great majority of VLDL particles in normal hepatocytes are located within the vesicles and membranes of the Golgi complexes themselves.

Golgi complexes of periportal hepatocytes obtained from acutely diabetic rats (rectangular areas, Fig. 6) appeared to have more prominent membranes, with relatively fewer VLDL-filled vacuoles than Golgi complexes

in control cells. The individual VLDL particles contained in the vacuoles appeared to be larger than normal. In addition, more smooth endoplasmic reticulum-associated VLDL particles and neutral lipid droplets were noted in these cells than in hepatocytes from control animals.

After 7 days of insulin deficiency, glycogen and neutral lipid was dramatically reduced in periportal hepatocytes. In fact, quantitation (31) of intrahepatocyte neutral lipid indicated that the volume of visible lipid in chronically insulin-deficient rats was only 20% of normal. The rough endoplasmic reticulum was disorganized and greatly reduced in amount. Some of these features, which are illustrated in Fig. 7, have been recently quantitated by morphometric techniques.² In addition, Golgi

² Alford, F., M. Millea, E. Reaven, R. Shorenstein, and G. Reaven. Characteristics of membrane-bound and free ribosomes from liver of diabetic rats. II. Effects of duration of insulin deficiency. Manuscript submitted for publication.

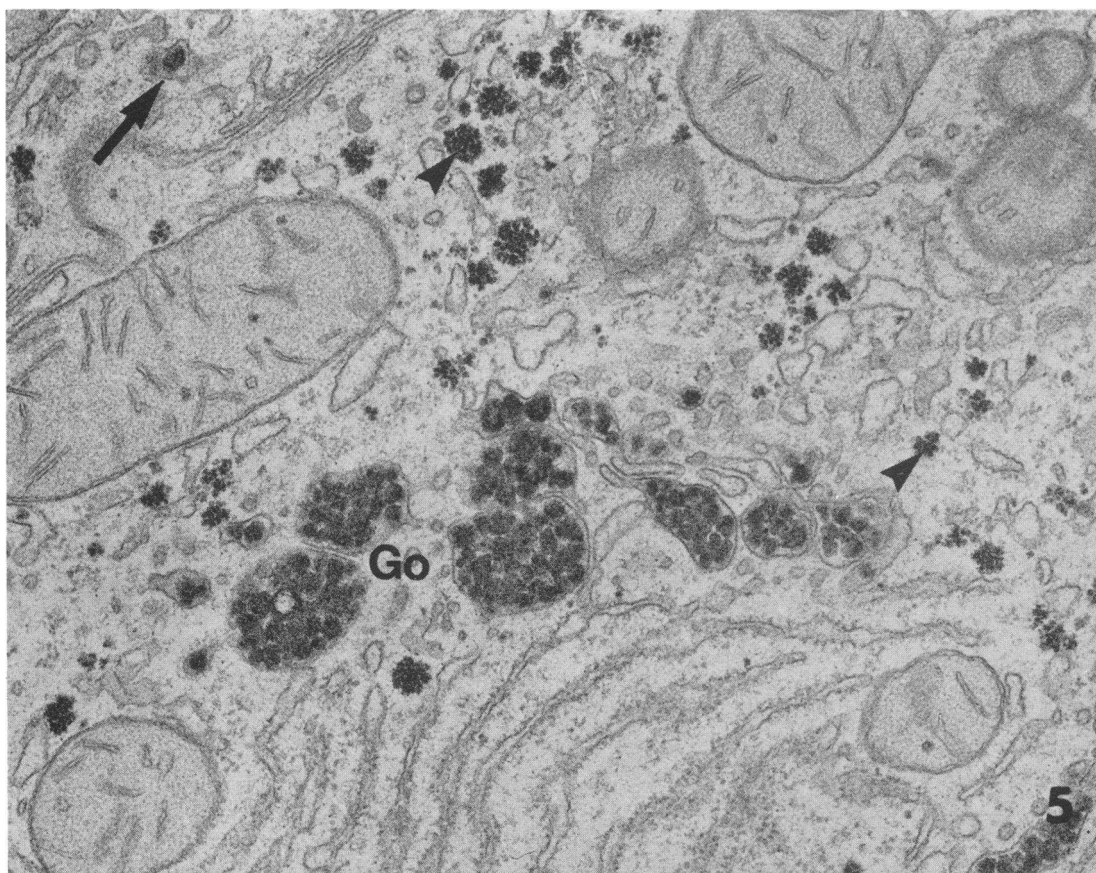


FIGURE 5 Higher magnification view of large Golgi complex (Go) seen in Fig. 4. Note that vacuoles associated with the Golgi complex are filled with electron dense VLDL particles. Arrow points to isolated VLDL particles encircled by smooth endoplasmic reticulum membranes at some distance from the Golgi complex itself. Clusters of glycogen particles are shown by arrowheads. $\times 41,000$.

complexes (rectangular areas, Fig. 7) tended to be longer and flatter than in normal cells, although the mean (\pm SE) number of Golgi profiles (1.05 ± 0.11 Golgi complexes per 100 cm^2 cytoplasm) did not differ significantly from controls. Few VLDL-filled Golgi vacuoles were found in association with the prominent stacked membranes of the complexes (Fig. 8).

These ultrastructural changes were not modified by diet, and they were also unaffected by prior administration of Triton. The only apparent effect of Triton on hepatocyte ultrastructure was to increase the number of VLDL particles visible in the hepatic sinusoids, and this was true of both control and diabetic rats.

Effect of duration of insulin deficiency and diet on measurements of hepatocyte Golgi VLDL number and volume. The results of morphometric measurements of the number and size of VLDL particles within hepatocyte Golgi complexes appear in Table II. It can be seen

that the rise in plasma TG levels that occurred in acutely insulin-deficient rats was not associated with a significant increase in number of VLDL particles in the Golgi, although there was a striking increase in the size of individual VLDL particles. This resulted in a three- to fourfold increase in the total content of VLDL (column III of Table II) associated with Golgi complexes of hepatocytes from acutely insulin-deficient rats.

In contrast, there was an unequivocal decrease in the number of VLDL particles in Golgi from hepatocytes of rats with chronic insulin deficiency in the absence of any change in diameter of VLDL particles. The net effect was a 50% decrease in content of Golgi VLDL particles as a result of chronic insulin deficiency.

Effects of caloric restriction and insulin administration. The combined decrease in VLDL-TG secretion rate and decrease in lipoprotein removal rate that was seen in chronically diabetic rats was associated with

significant weight loss. In order to evaluate the role of weight loss per se, an additional series of experiments were carried out in which the effect of caloric restriction was studied. Six normal rats ate approximately 8 g, or one-half the normal consumption of diet B, per day for 7 days. The mean weight loss was 0.5 g over this period. The results of these studies appear in Table III and indicate that caloric restriction produces neither hyperglycemia nor hypertriglyceridemia. Indeed, a significant fall in plasma TG level occurred, associated with both a fall in VLDL-TG entry rate and in number of VLDL particles seen within Golgi. The fact that plasma TG levels fell in proportion to the fall in VLDL-TG secretion rate suggests that caloric restriction did not inhibit TG removal from plasma. In contrast, marked hypertriglyceridemia occurred in chronically insulin-deficient rats, in spite of the fact that their

TG entry rates and number and size of VLDL particles were similar to those of calorically restricted rats, supporting the notion that the hypertriglyceridemia is secondary to a defect in lipoprotein removal and a specific sequela of insulin deficiency. This suggestion gains further credence from the demonstration in Table III that insulin replacement inhibited the development of hypertriglyceridemia in SZ-treated rats.

DISCUSSION

The results of this study demonstrate that hypertriglyceridemia is a common occurrence in severely insulin-deficient rats, with the relative degree of hypertriglyceridemia being a function of both duration of insulin deficiency and of dietary composition. Theoretically, the rise in plasma TG levels could result from decreased removal of circulating lipoproteins or from increased

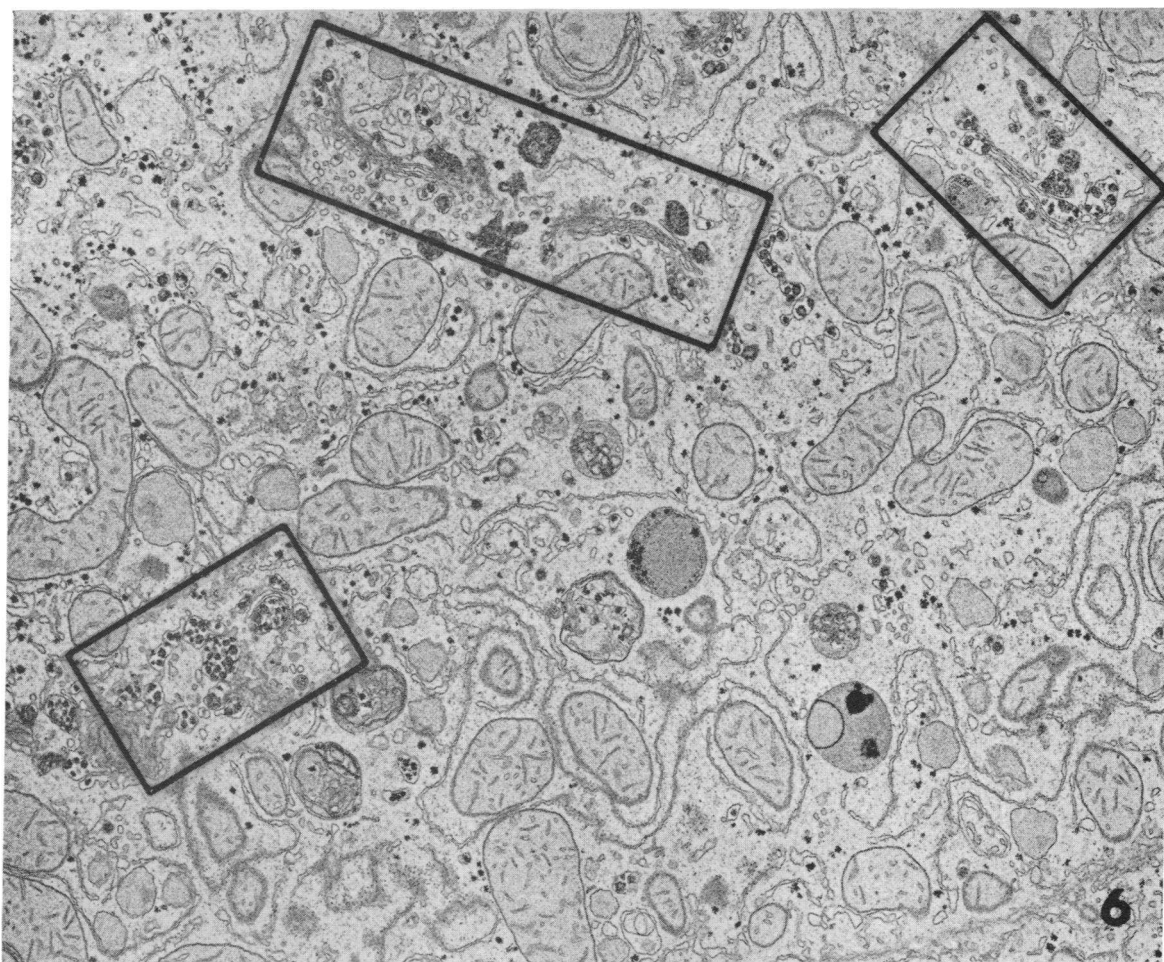


FIGURE 6 Low magnification electron micrograph of portion of periportal hepatocyte from an acutely insulin-deficient rat fed a high fat, low carbohydrate diet (diet B). Micrograph shows several Golgi-associated vacuoles (within rectangular areas) in which VLDL particles are somewhat larger than in the hepatocyte from control cell depicted in Fig. 4. $\times 14,000$.

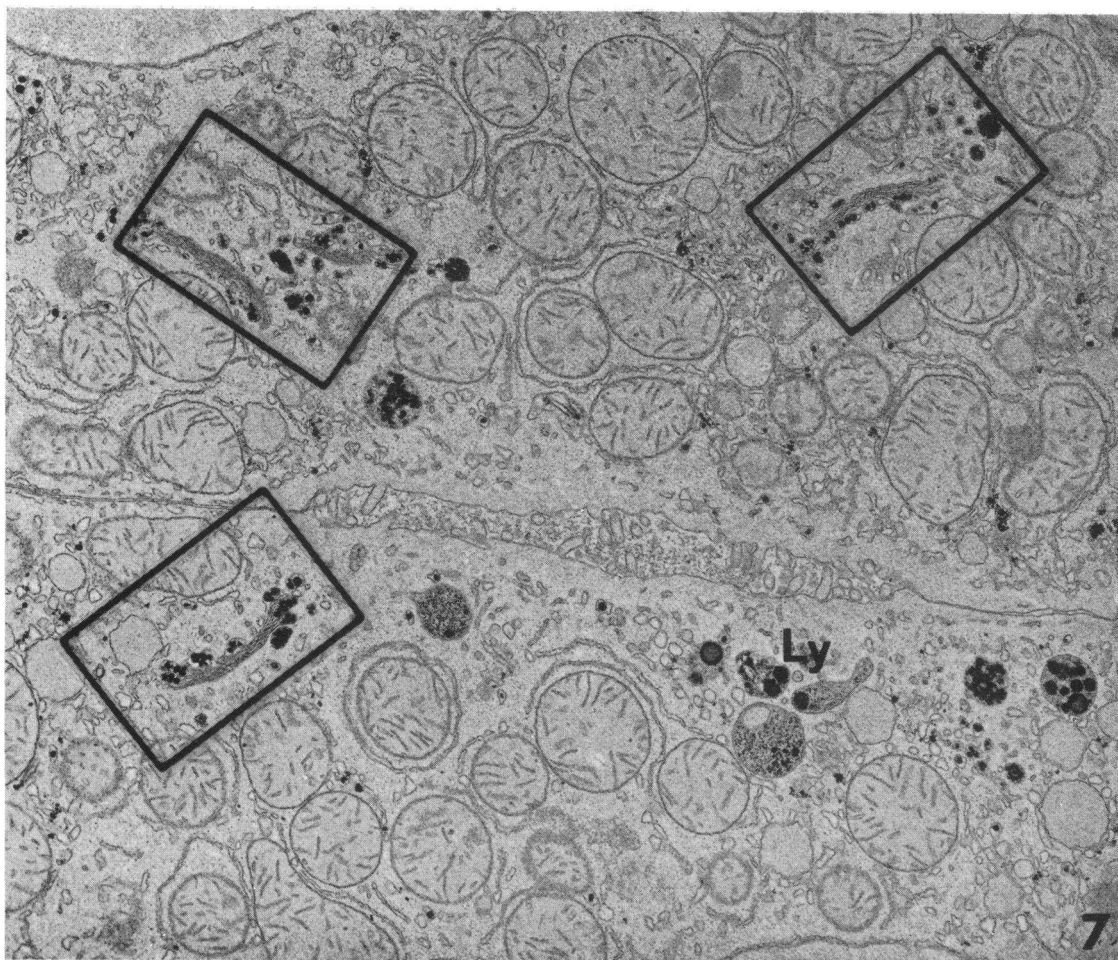


FIGURE 7 Low magnification electron micrograph of portions of two periportal hepatocytes from a chronically insulin-deficient rat fed a high fat, low carbohydrate diet (diet B). Rectangular boxes show several Golgi complexes in which the stacked membranes of the Golgi complexes are especially prominent. Note that there are relatively fewer Golgi-associated vacuoles and VLDL particles (within vacuoles) in this cell as compared to hepatocyte from control animal shown in Fig. 4. Note the relative absence of glycogen. Lysosomes (Ly) are well stained. $\times 14,000$.

lipoprotein production, and both mechanisms seem to be implicated in the genesis of the hypertriglyceridemia that occurs 24 h after SZ administration. Thus, the marked hypertriglyceridemia (Fig. 3) that occurs at this time is associated with an increase in plasma TG entry rate (Table I). Hepatocyte Golgi VLDL content (Table II) is also increased, although it is primarily the size of the individual particles,⁸ which accounts for the increase in VLDL. The combination of increased

⁸ VLDL particles within Golgi complexes of many cells were of chylomicron size, and uncertainty has existed as to whether such VLDL particles can be released from the liver (33). However, a recent study (34) gives evidence of hepatic synthesis and secretion of VLDL particles which resemble chylomicrons morphologically and chemically.

TG entry rate and increased hepatocyte Golgi-VLDL content suggests that increased VLDL production and secretion occurs in the acutely diabetic rat, and this is consistent with an earlier *in vivo* study which documented acute increase in TG production after anti-insulin serum injection in dogs (16). Furthermore, the data are consistent with the suggestion of Woodside and Heimberg (19) that livers from rats made acutely insulin deficient with anti-insulin serum can respond to an increased influx of FFA by increasing TG synthesis and secretion. Indeed, in the current study the livers of the acutely deficient rats have a generally good ultrastructural appearance, and it is not surprising that they respond to increased FFA influx with an increase in

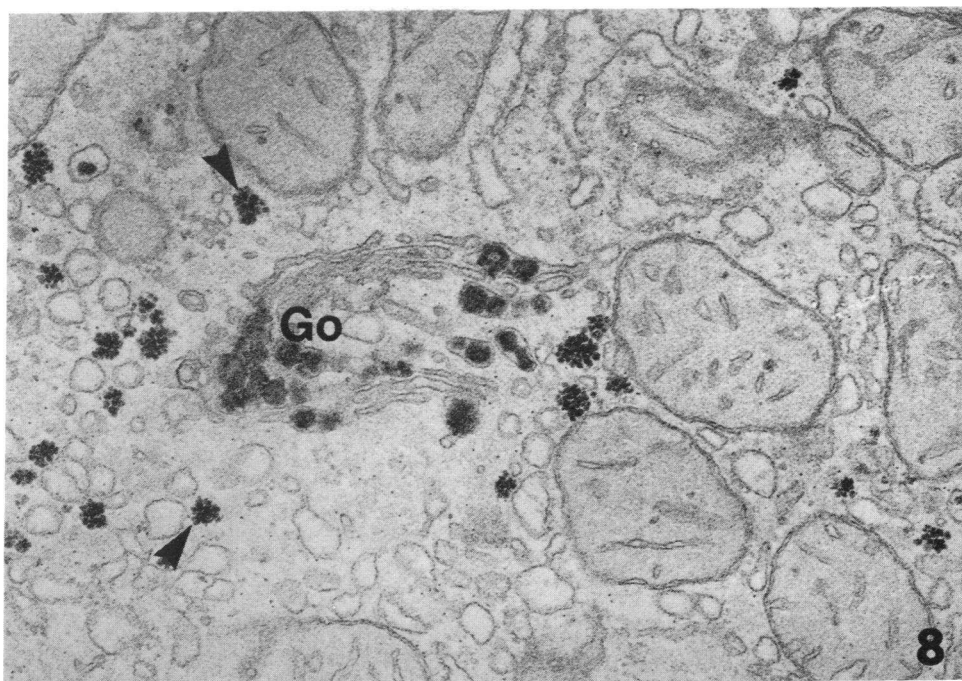


FIGURE 8 Higher magnification electron micrograph of a typical Golgi complex (Go) from a chronically insulin-deficient rat. Note that the prominent Golgi membranes are associated with few vacuoles and VLDL particles. Occasional clusters of glycogen are shown at arrowheads. $\times 41,000$.

TABLE II
Mean (\pm SE) Size and Number of VLDL Associated with Hepatocyte Golgi Complexes

Diet	Experimental group ($n = 4$)	I Measured number of VLDL	II Measured diameter of VLDL	III Estimated average volume of VLDL	IV Volume index (column I \times column III) of total hepatocyte VLDL associated with Golgi complexes
					$\times 10^{-3} \mu m^3$
A	Control	279 ± 27	0.055 ± 0.001	8.55 ± 0.13	2.38 ± 0.21
A	Acute insulin deficiency	162 ± 25	0.098 ± 0.004	51.00 ± 6.60	$8.77 \pm 2.19^*$
A	Chronic insulin deficiency	99 ± 21	0.057 ± 0.001	9.15 ± 0.85	$0.92 \pm 0.26^\dagger$
B	Control	214 ± 23	0.056 ± 0.001	8.97 ± 0.60	1.90 ± 0.45
B	Acute insulin deficiency	246 ± 25	0.076 ± 0.001	22.60 ± 1.80	$5.51 \pm 0.45^\dagger$
B	Chronic insulin deficiency	109 ± 24	0.054 ± 0.002	8.30 ± 0.80	$0.89 \pm 0.27^\S$

* $P < 0.025$ as compared to control on same diet.

$^\dagger P < 0.0005$.

$^\S P < 0.05$.

TABLE III
Metabolic Changes Resulting from Caloric Restriction, Chronic Insulin Deficiency, or Insulin Replacement (Mean \pm SE)*

Treatment	Weight change (n = 6)	Glucose (n = 6)	TG concentration (n = 6)	Estimated rate of TG entry into plasma (n = 6)	Measured number of VLDL in Golgi (n = 4)	Measured diameter of VLDL in Golgi (n = 4)
	g	mg/100 ml	mg/100 ml	mg/min		μ m
Control	+25 (\pm 2)†	143 \pm 2	80 \pm 3†	1.01 \pm 0.05†	220 \pm 10†	0.055 \pm 0.001
Caloric restriction	-0.5 (\pm 1)§	147 \pm 3	37 \pm 7§	0.56 \pm 0.03§	128 \pm 20§	0.053 \pm 0.003
Chronic insulin deficiency	-6.0 (\pm 2)§	422 \pm 11§	561 \pm 65§†	0.69 \pm 0.06§	117 \pm 19§	0.054 \pm 0.004
Insulin replacement	+23 (\pm 4)†	143 \pm 29	82 \pm 8†	0.98 \pm 0.06†	231 \pm 14†	0.053 \pm 0.002

* All rats were consuming a high fat, low carbohydrate diet (diet B).

† Values significantly different ($P < 0.05$) from calorically restricted rats.

§ Values significantly different ($P < 0.05$) from controls.

|| Insulin replacement began 18 h after SZ and rats received 4-6 U lente insulin (s.c.) every 12 h throughout the experimental period.

VLDL-TG secretion. The fact that plasma TG levels and TG entry rates are highest when the acutely insulin-deficient rats are eating a high carbohydrate diet suggests that the liver is even healthy enough to be "carbohydrate induced" (35) at this stage. However, this period of increased TG production appears to be transitory, as suggested by the relatively "inactive" appearance of the Golgi complexes themselves. Despite the fact that the content of VLDL particles associated with these Golgi complexes is actually increased, decreased numbers of Golgi vacuoles are associated with the secretory side of the complexes, and those which are present tend to contain a reduced number of VLDL particles. In contrast, in other situations in which VLDL-TG production has been stimulated, such as in the high carbohydrate-fed control rats of this study, or in corticosteroid-treated rats of an earlier study (32), hepatocyte Golgi complexes are associated with more VLDL-filled vacuoles, as well as more VLDL particles per vacuole. In order to account for the apparent paradox of increased Golgi VLDL content combined with "inactive" Golgi, it is necessary to postulate an initial period of increased hepatocyte VLDL-TG synthesis and secretion, which is already decreasing 24 h after treatment with SZ. Furthermore, it would appear that VLDL-TG synthesis has fallen off less than VLDL-TG secretion, accounting for the larger size of the VLDL particles. This interpretation is consistent with the observation of Heimberg, Van Harken, and Brown (10), who indicated that incorporation of labeled FFA into hepatic TG can occur at a normal rate in perfused livers from rats with severe alloxan diabetes at the same time that there was a marked inhibition of VLDL-TG secretion. The suggestion that VLDL-TG secretion is de-

clining 24 h after SZ treatment is consistent with the observed relationship between plasma TG level and entry rate. For example, plasma TG levels of acutely insulin-deficient rats ingesting diet A are elevated fivefold over control values, whereas the TG entry rate is only increased 29%. This discrepancy makes it difficult to explain the hypertriglyceridemia of acute insulin deficiency entirely on the basis of increased VLDL secretion. Therefore, although VLDL removal from plasma was not assessed directly, it seems reasonable to postulate that these rats have also developed problems in lipoprotein removal. A similar conclusion was reached by Gross and Carlson (13), in their study of the effects of nicotinic acid on TG levels in anti-insulin serum (AIS)-treated rats. Thus, nicotinic acid administration completely inhibited the rise in TG levels 6 h after the administration of AIS, but not after 24 h of insulin deficiency. Finally, this hypothesis is also consistent with measurements of plasma insulin levels in these rats. Mean (\pm SE) postabsorptive plasma insulin levels of 49 (\pm 9) μ U/ml, which rose twofold 6 h after SZ administration, had fallen to 3 (\pm 0.5) μ U/ml 24 h later. Such levels are comparable to those seen in our chronically insulin-deficient rats, and demonstrate that both hyper- and hypoinsulinemia occur in the 24 h after SZ administration. These fluctuations in insulin levels are obviously compatible with an initial period of increased VLDL-TG synthesis and secretion, followed by a decrease in both processes. Thus, it appears that there is an initial period of increased VLDL-TG synthesis and secretion which is followed by a decrease in VLDL-TG secretion and appearance of a defect in lipoprotein removal. The combined effects of these various factors results in the marked elevation of

plasma TG levels that were observed in acute insulin deficiency.

The situation is infinitely simpler in rats which have been insulin deficient for several days. Entry rates of TG into the plasma are significantly decreased, and associated with a decrease in VLDL content of the Golgi complexes. A similar defect in VLDL secretion has been noted by Heimberg, Dunkerley, and Brown (8) in perfused livers from rats with severe alloxan diabetes. However, in spite of the fall in TG entry rate, plasma TG levels of chronically diabetic rats are significantly elevated. This relationship between TG entry rate and plasma TG concentration makes it necessary to postulate that a defect in lipoprotein removal from plasma is responsible for hypertriglyceridemia. Bierman, Bagdade, and Porte have earlier provided evidence that this mechanism is responsible for hypertriglyceridemia in insulin-deficient diabetic patients (5) and in rats with severe alloxan diabetes (6), and Basso and Havel (16) have recently demonstrated that dogs with chronic diabetes secondary to pancreatectomy have hypertriglyceridemia as a result of a defect in lipoprotein removal. More specifically, Bierman, Amaral, and Belknap have suggested that the hypertriglyceridemia is due to a defect in clearance of chylomicrons (6), and this is consistent with the current studies which indicate that hypertriglyceridemia in chronically insulin-deficient rats is accentuated by eating a high fat diet. On the other hand, the fact that hypertriglyceridemia also occurred in rats eating a fat-free diet demonstrates that chronic diabetes also results in a defect in removal of endogenous VLDL.

In conclusion, it appears that a decrease in lipoprotein removal from plasma occurs relatively early in the course of insulin-deficient diabetes, and the intensity of this defect increases with duration of insulin deficiency. In the earliest stages of insulin deficiency, there also seems to be an increase in hepatic TG synthesis and VLDL-TG secretion, apparently due to the fact that the liver is still capable of making both apoprotein and TG, and of responding to the increased FFA flux with increased VLDL-TG synthesis and secretion. At this stage, the higher the proportion of carbohydrate in the diet, the greater the hypertriglyceridemia. As duration of insulin deficiency increases there is a decrease in lipoprotein removal from plasma and the liver loses its ability to secrete VLDL-TG. However, the severity of the defect in lipoprotein removal is greater than the severity of the fall in TG secretion, and hypertriglyceridemia occurs primarily as a result of the removal defect. In this instance, the greater the amount of dietary fat, the greater the hypertriglyceridemia. This formulation is consistent with the majority of observations as to the etiology of insulin-deficient hypertriglyceridemia, ap-

pears to reconcile some of the apparent conflicts in the literature, and emphasizes that levels to which plasma triglyceride rise is the result of the complex interplay between duration of insulin deficiency and composition of diet.

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