Evidence for a New Cause of Defective Plasma Removal of Very Low Density Lipoproteins in Insulin-Deficient Rats

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SUMMARY

These studies were initiated to see if factors other than reduced lipoprotein lipase activity might contribute to the defect in plasma removal of very low density lipoprotein (VLDL) that is observed in insulin-deficient rats. VLDL-triglyceride (TG) was labeled in vivo with ^{3}H-glycerol in control and diabetic rats, and aliquots of plasma containing ^{3}H-VLDL were injected into normal recipient rats. The half-time ($t_{1/2}$) of removal was almost twice as long when plasma from diabetic rats was injected, and this was true when the diabetic rats were fed either sucrose or regular chow. A comparable increase in $t_{1/2}$ was observed when ^{3}H-VLDL isolated from normal rats was recombined with VLDL-free plasma from control and diabetic rats and injected into normal recipients. As before, the changes observed were not dependent upon antecedent diet. However, no significant difference in $t_{1/2}$ was observed when ^{3}H-VLDL was isolated from control and diabetic rats and injected into normal recipients. Thus, there appears to be a factor present in VLDL-free plasma obtained from diabetic rats that interferes with removal of VLDL from the vascular compartment. Whether this factor is found in diabetic plasma in vivo, or is transferred from diabetic VLDL to diabetic plasma in the isolation procedure, remains to be clarified. In either event, there appears to be a factor, other than reduced LPL activity, that may play a role in the defect of VLDL-TG removal seen in insulin deficiency. DIABETES *30*:496–499, June 1981.

Hypertriglyceridemia develops in insulin-deficient man,[1] dog,[2] and rat,[3] and in all three situations the rise in plasma triglyceride (TG) levels appears to be secondary to a defect in removal of TG-rich lipoproteins from plasma. On the other hand, there is less consensus concerning the cause of this abnormality of lipoprotein catabolism. Earlier studies, based on nonspecific measurement of plasma post-heparin lipolytic activity (PHLA), concluded that the defect in removal of TG-rich lipoproteins from plasma that occurred in subjects with insulin deficiency was due to a decrease in lipoprotein lipase (LPL) activity.[1,4,5] However, the results of more recent studies[6,7] in which LPL activity has been measured by specific methodology, have suggested that insulin deficiency may not lead to a fall in plasma LPL activity. Furthermore, adipose tissue LPL activity in rats with experimentally induced insulin deficiency has been found to be either unchanged,[8] or, if reduced, unrelated to the concomitant rise in plasma TG concentration.[9,10] Given these observations, it seemed reasonable to investigate other possible explanations for the defect in removal of TG-rich lipoproteins that exists in subjects with insulin deficiency. For example, it had been previously noted that the apoprotein composition of very low density lipoprotein (VLDL) from sucrose-fed rats was altered when they were made insulin deficient.[11] These observations raised the possibility that the compositional changes in lipoprotein particles that occur in insulin deficiency could interfere with their normal removal from plasma. Alternatively, insulin deficiency might result in the appearance of plasma factors that could inhibit lipoprotein catabolism. The present experiments were undertaken to evaluate these alternatives, and the results serve to further question the notion that reductions in LPL activity are solely responsible for the development of hypertriglyceridemia in subjects with insulin deficiency.

MATERIALS AND METHODS

Animals and diets. Experiments were carried out on male Sprague-Dawley rats, weighing 200–300 g. Two groups of donor rats were used. One group was fed regular rat chow, which contained (as percent of total calories) 11% fat, 29% protein, and 60% carbohydrate (subsequently referred as "chow-fed" rats). The other group of rats were fed a pelleted

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Received for publication 27 August 1980 and in revised form 5 February 1981.

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sucrose-lard diet, comprising (as percent of total calories) 12% lard, 22% casein, and 66% sucrose (subsequently referred as "sucrose-fed" rats). All animals (donor and recipient rats) had free access to water and food, and were maintained on a 12-h light (0600–1800)/dark (1800–0600) cycle. Insulin-deficient diabetes was induced by the intravenous injection of streptozotocin (42 mg/kg body wt), dissolved in 0.05 M citrate (pH 4.5), and experiments were performed after 7–10 days of insulin deficiency. Food was withdrawn at 0800 on the morning of the experiments, and all studies were conducted 6 h later. At that time it was assumed that the circulating triglyceride (TG) pool was entirely of endogenous origin.

Preparation of endogenously labeled TG. Diabetic and control rats, which served as donors of the endogenously labeled TG, were injected via the tail vein with 0.4 mCi of ^{3}H-glycerol (sp. act. 10.0 Ci/mmol). Forty minutes later the rats were exsanguinated via the abdominal aorta. Plasma was separated in a refrigerated centrifuge, and dialyzed overnight against 0.15 M of NaCl at 4°C. At this time interval approximately 95% of the incorporated ^{3}H-glycerol was present as TG. Approximately 90% of the 95% of label present in TG was in VLDL, with the remainder being distributed in IDL, LDL, and HDL. Furthermore, the figures were identical for normal and diabetic donors. Thus, subsequent differences in removal rates between control and diabetic donors cannot be due to differences in the distribution of injected radioactivity. VLDL and VLDL-free plasma were prepared after 20-h ultracentrifugation at 200,000 × *g*, using a 40.3 T_1 rotor in a Beckman model L-2 ultracentrifuge.

Experimental protocol. Three subsets of experiments were performed. (1) Dialyzed plasma, containing ^{3}H-VLDL-TG obtained from either diabetic or control donor rats, was injected into normal recipient rats. (2) ^{3}H-VLDL-TG, isolated from plasma of diabetic and control donor rats, was injected into normal recipient rats. (3) ^{3}H-VLDL-TG, isolated from plasma of normal rats, was incubated for 10 min at 37°C with VLDL-free plasma obtained either from diabetic or control rats. Aliquots of the reconstituted plasma were then injected into normal recipient rats.

Determination of VLDL-TG removal rate. Aliquots (1.0–1.5 ml) of dialyzed plasma, or isolated VLDL, or isolated VLDL reconstituted with VLDL-free plasma, each containing 1.2–1.6 mg of TG, were injected into the tail veins of normal chow-fed recipient rats. The tail was amputated proximal to the site of the injection, and 0.5 ml of blood was collected into small plastic (heparin-coated) tubes at 3, 6, 9, and 12 min after the administration of the tritiated test substance. The plasma was separated by centrifugation, and one aliquot was used for determination of plasma TG concentration to attest to the existence of a steady state.[12] The other aliquot was used to extract lipid by the Folch method;[13] polar lipids were then removed by silicic acid, and the TG fraction evaporated to dryness. Radioactivity was measured by liquid scintillation counting, using a standard toluene scintillation liquid. The half-time ($t_{1/2}$) of TG removal was directly determined from the decrease in the specific activity time curve. VLDL-TG kinetics determined with this approach have yielded data comparable to that obtained with Triton WR 1339, but we have found the Triton method less reproducible.

The goodness of fit to a straight line was calculated for each experiment, and the r-value in 95% of the studies was ≥0.995. If the r-value was <0.995, the data were excluded. To assess the reproducibility of the method, aliquots of plasma containing prelabeled VLDL-TG from one diabetic and one control donor were injected into five separate control recipients. These results appear in Figure 1, and demonstrate several points. In the first place, the removal rates are clearly first order, which mean r-values of 0.99. Secondly, the VLDL-TG from one donor was removed at a comparable rate in five different recipient rats. In subsequent studies, we used 2–3 recipient rats per donor, and the mean of these individual measurements was used to calculate the $t_{1/2}$ of each donor VLDL-TG.

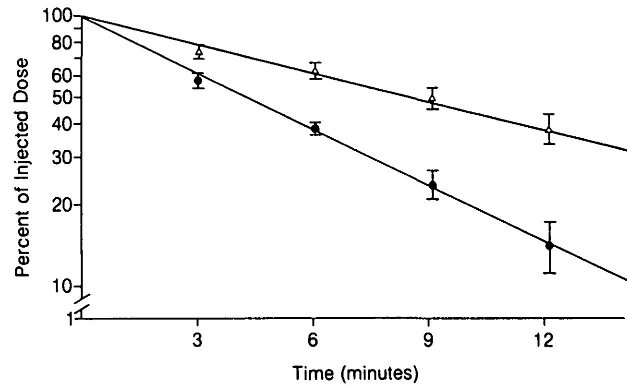

FIGURE 1. Disappearance after injection into normal recipients of plasma containing prelabeled ^{3}H-VLDL-TG obtained from chow-fed control (●) and diabetic (△) rats. In each case aliquots of plasma from the donor rats were injected into five recipient rats, and the data points depict the mean (± SEM) of the individual measurements.

RESULTS

Plasma glucose and TG concentrations. Plasma glucose and TG concentrations of control and diabetic rats are seen in Table 1, which includes values obtained at 0800 (a.m.), as well as 6 h after removal of food (p.m.). Diabetic rats had significantly higher plasma glucose concentrations than control rats at any time, and this was true of both chow-fed and sucrose-fed rats. Plasma TG levels were higher in sucrose-fed rats than in chow-fed rats, and diabetic rats had an approximate twofold increase in plasma TG concentrations irrespective of diet.

VLDL-TG removal. Figure 2 displays the half-time of removal ($t_{1/2}$) of VLDL-TG when plasma from control and diabetic rats is injected into normal recipient rats. The data from sucrose-fed donor rats are seen in the left panel, and

TABLE 1
Plasma glucose and triglyceride levels in donor rats

Rats and diets	Glucose (mg/dl)		TG (mg/dl)	
	a.m.	p.m.	a.m.	p.m.
Chow-fed				
Control	153 ± 6	153 ± 4	125 ± 12	97 ± 11
Diabetic	485 ± 19	507 ± 20	229 ± 46	204 ± 35
Sucrose-fed				
Control	148 ± 2	155 ± 2	221 ± 23	163 ± 15
Diabetic	443 ± 25	376 ± 19	364 ± 32	305 ± 41

Numbers represent mean (± SEM) of morning (a.m.) and afternoon (p.m.) plasma concentrations obtained from 12–15 donor rats.

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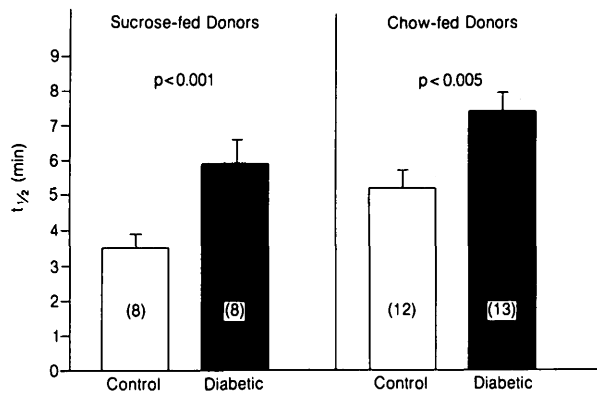


FIGURE 2. Removal rate ($t_{1/2}$) after injection into normal recipients of plasma containing prelabeled ^{3}H-VLDL obtained from sucrose-fed and chow-fed control and diabetic rats. (N) refers to number of rats studied.

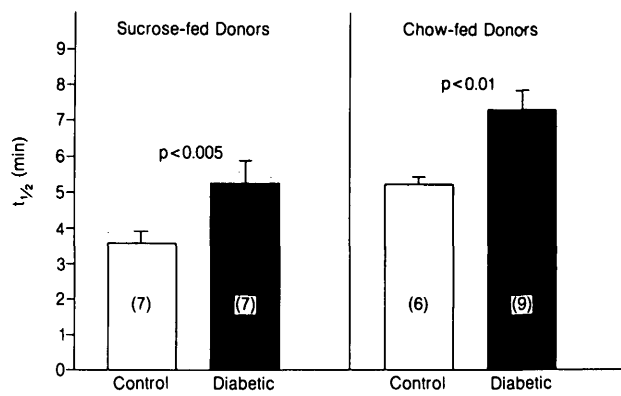


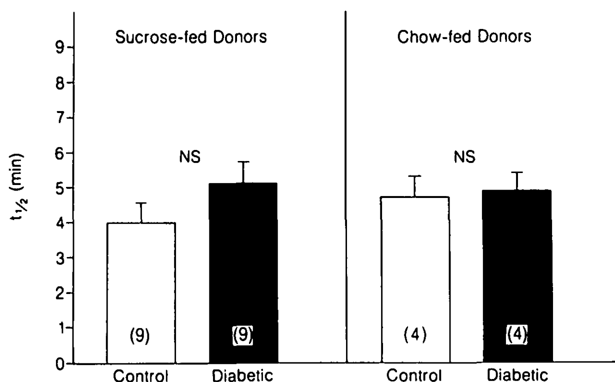
FIGURE 4. Removal rate ($t_{1/2}$) after injection into normal recipients of prelabeled ^{3}H-VLDL isolated from normal donor rats and recombined with VLDL-free plasma from sucrose-fed and chow-fed diabetic rats. (N) refers to number of rats studied.

demonstrate that the $t_{1/2}$ of VLDL-TG removal was significantly longer when plasma from diabetic rats was injected. To assess the relative importance of diet, we also studied chow-fed control and diabetic donor rats. These results are seen in the right panel of Figure 2, and demonstrate that TG from diabetic donors was also removed more slowly. However, the difference between control and diabetic plasma is less dramatic when donor rats are chow-fed, and the $t_{1/2}$ of both experimental groups was somewhat longer when donors were chow-fed.

The data in Figure 2 are consistent with the idea that insulin deficiency leads to a change in VLDL and/or plasma, which, in turn, decreases the catabolism of VLDL-TG. The first of these two alternatives was evaluated by determining the rate at which isolated (prelabeled) VLDL from control and diabetic rats was removed from the plasma of normal recipient rats. The results of these studies are seen in Figure 3, and it appears that isolated VLDL obtained from sucrose-fed diabetic donors (left panel) is removed somewhat more slowly. However, this difference failed to reach statistical significance, and the results of chow-fed donors (right panel) also show similar values for $t_{1/2}$ of VLDL from control and diabetic donors.

These results suggest that something present in the plasma of diabetic rats is responsible for the prolongation of VLDL removal seen in Figure 2, and support for this hypothesis is presented in Figure 4. Thus, the $t_{1/2}$ of VLDL obtained

FIGURE 3. Removal rate ($t_{1/2}$) after injection into normal recipients of prelabeled ^{3}H-VLDL isolated from plasma of sucrose-fed and chow-fed control and diabetic rats. (N) refers to number of rats studied.



from normal rats is prolonged when combined with VLDL-free plasma from diabetic rats. The ability of VLDL-free plasma from diabetic rats to prolong removal of normal VLDL is somewhat greater when the plasma is obtained from sucrose-fed donors (left panel), but qualitatively similar results were obtained with VLDL-free plasma from chow-fed control and diabetic donor rats.

DISCUSSION

The experimental strategy used in these studies has been to compare the rate at which normal rats remove injected VLDL obtained from diabetic and control rats. In other words, we have attempted to use normal rats as a bioassay system. Since the normal recipient rats are selected randomly from a group of inbred rats, it is assumed that any differences in the rate at which recipient rats remove VLDL obtained from donor rats will be a function of differences in the donors. However, it is not possible to directly compare the $t_{1/2}$ of VLDL obtained from different donor rats unless the VLDL pool size of the recipient is similar. To satisfy this requirement, we were careful to always inject identical amounts of VLDL obtained from control and diabetic rats into the normal recipient rats. Under these conditions, determination of the $t_{1/2}$ of VLDL obtained from control and diabetic donor rats provides a direct comparison of the efficiency with which these particles are removed from the plasma of normal recipient rats. With this approach, we have presented evidence that insulin deficiency leads to an abnormality in the VLDL and/or the plasma of diabetic rats, which interferes with VLDL catabolism. The crucial observation is that the removal of VLDL obtained from diabetic rat donors is prolonged when it is injected into normal rats in combination with plasma from diabetic donors. Since this removal defect was demonstrated in normal rats, it cannot be due to any change in LPL activity.

The data presented provide strong support for the idea that there are factors, other than a reduction in tissue LPL activity, which contribute to the defect in removal of VLDL seen in subjects with insulin deficiency. We began the study thinking that the changes in VLDL composition that have been reported to occur in association with insulin deficiency might render these particles more difficult to remove.[11] At first blush, this alternative would seem to have been ruled out by finding that VLDL isolated from control

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and diabetic rats were removed at the same rate after their injection into normal rats (Figure 3). On the other hand, it is possible that this result is artifactual, resulting from the loss of certain crucial components (apoproteins) from the VLDL during their isolation by ultracentrifugation. If this were the case, such factors should appear in the VLDL-free plasma, and could be responsible for the prolongation in $t_{1/2}$ when VLDL isolated from normal rats are recombined with VLDL-free plasma from diabetic rats (Figure 4). Alternatively, it is certainly possible that the compositional changes that occur in VLDL secondary to insulin deficiency do not affect VLDL catabolism, and that the ability of diabetic VLDL-free plasma to interfere with catabolism of normal VLDL is due to the appearance in plasma of some unidentified inhibitor of VLDL removal. Such a factor, for example, has been postulated to account for the defect in VLDL removal from plasma in subjects with chronic renal failure.[14]

In conclusion, we have presented evidence that something in the plasma of insulin-deficient rats interferes with the removal of VLDL from the vascular compartment. Whether or not this is due to a change in the VLDL and/or the plasma remains to be defined. Finally, the role played by this phenomenon in the development of diabetic hypertriglyceridemia is not clear. However, it does seem clear that we must seriously consider the possibility that factors other than decreased LPL activity contribute significantly to the defect in VLDL catabolism that occurs in association with insulin deficiency.

ACKNOWLEDGMENTS

Dr. Bar-On performed this work while on sabbatical leave from the Department of Medicine B, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

This work was supported, in part, by research grants from the National Institutes of Health (HL-20397) and from the Research Services of the Veterans Administration.

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