Very-Low-Density Lipoprotein Triglyceride Metabolism in Non-insulin-dependent Diabetes Mellitus

Relationship to Plasma Insulin and Free Fatty Acids

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SUMMARY

To investigate the mechanism of elevated plasma triglycerides in non-insulin-dependent (type II) diabetes, very-low-density lipoprotein triglyceride (VLDL-TG) metabolism was studied in 10 untreated male Pima Indian diabetics and compared with that of 15 weight-matched male nondiabetic controls. VLDL-TG metabolism was studied using tritiated glycerol as an endogenous precursor of VLDL-TG, and the resultant kinetic data were analyzed using a multicompartmental model, which includes two pathways for VLDL-TG synthesis and a stepwise delipidation process for VLDL catabolism. Pima diabetics had VLDL-TG concentrations approximately 150% those of nondiabetics. Rates of VLDL-TG production in the diabetics were not significantly different from those of controls. On the other hand, the fractional catabolic rate for VLDL-TG was significantly lower in the diabetics compared with the nondiabetics. Other catabolic parameters, such as the fraction of VLDL-TG delipidized at each step and the stepwise delipidation rate, were also decreased in the diabetics. To determine the relationships between the increased triglycerides and determinants of lipid metabolism that are altered in diabetes, insulin and free fatty acid concentrations were also assessed. Basal C-peptide levels in the diabetics during the metabolic study were slightly but not significantly higher than those of the nondiabetics. There was a highly significant correlation in the diabetics between plasma C-peptides and VLDL-TG production, whereas VLDL production in Pima nondiabetics was not related to insulin levels. Free fatty acid levels were not significantly elevated in the Pima diabetics. The data indicate that (1) the rise in VLDL-TG in Pima diabetics was a result of decreased capacity for clearance and (2) the absence of elevated VLDL-TG production may be attributed to the lack of increase in free fatty acids. DIABETES 32:271–276, March 1983.

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Hypertriglyceridemia is commonly present in patients with diabetes mellitus.[1–3] Several mechanisms have been postulated for the elevated triglyceride (TG); these appear to vary depending on the type of diabetes, the presence of genetic hyperlipemia, and the method of investigation. Insulin-dependent (type I) diabetics are often observed to have a defect in lipoprotein lipase[4,5] and the catabolism of very-low-density lipoproteins (VLDL).[6,7] However, in some instances an increased production has been observed,[6] and underlying genetic hyperlipemia has been postulated to contribute to the hypertriglyceridemia in cases with marked chylomicronemia.[8,9] In non-insulin-dependent (type II) diabetics the etiology of the hypertriglyceridemia may be more complex. Lewis et al.[10] and Brunzell et al.[11] observed a decreased catabolism of VLDL-TG in type II diabetics, and decreases in lipoprotein lipase activity have been reported.[4,11] However, other studies of VLDL-TG metabolism in type II diabetics indicated an increased production. Early studies[6,12,13] used single compartmental analysis of VLDL-TG kinetic data. More recent studies have used multicompartmental modeling techniques. One study of type II diabetics indicated that overproduction was the primary cause of the elevated triglycerides.[14] In a second study of type II diabetics with marked hypertriglyceridemia, both overproduction and decreased clearance were observed; insulin treatment did not restore triglyceride levels to normal, suggesting the presence of underlying hyperlipemia.[15] The presence of underlying genetic hyperlipemias in some type II diabetics has also been stressed by Chait et al.,[9] who postulated that familial hypertriglyceridemia was a necessary occurrence in those diabetics with triglyceride levels greater than 700 mg/dl.

The Pima Indians are a genetically homogeneous population[16] with no evidence of the presence of genetic

hyperlipemias. They have a high prevalence of non-insulin-dependent diabetes; moreover, the diabetics have elevated plasma triglyceride levels.[17] They thus constitute a well-defined study population in which some of the variables that influence lipid metabolism have been minimized. A recent study characterized VLDL-TG metabolism in nondiabetic Pima Indians.[18] The present study examines VLDL-TG metabolism in a group of Pima diabetics, and it compares the kinetic data to those obtained from weight-matched nondiabetic controls. To determine the relationships between the increased triglycerides and determinants of lipid metabolism that are altered in diabetes, insulin, free fatty acids, and glucose levels were also assessed.

METHODS

Twenty-five male Pima Indians, aged 18–49 yr, were admitted to the Phoenix Clinical Research Center (Table 1). Written informed consent was obtained from all subjects. All patients had normal physical examinations; liver function tests and urinalyses were within normal ranges for this population. None had cardiovascular disease or a family history of hyperlipemia, and none were taking medications known to affect lipid metabolism. The nondiabetic group had a mean age of 27 yr and a mean body weight 167% of ideal. The diabetic group was somewhat older, with a mean age of 36 yr, and was also overweight (mean body weight 153% of ideal). All diabetics had marked fasting hyperglycemia (217 ± 12 mg/dl); the mean 2-h glucose was 280 mg/dl. Duration of diabetes averaged 7.5 yr (6 mo to 20 yr). Seven of the diabetics had previously received insulin or oral hypoglycemic therapy. All medications for diabetes were stopped at least 2 wk before the study.

Patients were admitted at least 7 days before the study and were fed a weight-maintenance diet consisting of 40% fat (P/S ratio = 0.34), 45% carbohydrate, 15% protein, and 500 mg cholesterol/day. Body weight was maintained in all patients during this stabilization period. After 2 days on the diet a routine 100-g oral glucose tolerance test was performed, and plasma samples were obtained at fasting, 30 min, 1, 2, and 3 h for assay of glucose, insulin, and C-peptides. Thirty-six hours before the beginning of the study the patients consumed 60% of their weight-maintenance calories in a formula diet, consisting of 75% carbohydrate (dextrose) and 25% protein (calcium caseinate), which was fed every 3 h. This fat-free regimen eliminated chylomicron production while maintaining constant carbohydrate intake and VLDL levels,[19] and it was continued for the 48 h of the study.

TABLE 1
Characteristics of study groups

	Diabetics	Nondiabetics
N	10	15
Age (yr)	36 (21–49)†	27 (18–49)
Weight (kg)	102 (62–178)	112 (58–182)
% ideal weight	152 ± 16‡	167 ± 12
Fasting glucose (mg/dl)	217 ± 12	88 ± 2
2-h glucose (mg/dl)	280 ± 14	127 ± 6
Basal C-peptide (pmol/ml)*	1.51 ± 0.17	1.28 ± 0.16
Plasma triglyceride (mg/dl)	167 ± 24 (96–318)†	103 ± 10 (47–163)
Plasma cholesterol (mg/dl)	154 ± 11 (103–204)	148 ± 6 (103–193)

*This represents the value before each formula feeding during the metabolic study.
†Range.
‡Mean ± SEM.

The study was initiated just before a formula feeding with a rapid injection via an antecubital vein of 342 μCi of [2-^{3}H]glycerol (New England Nuclear, Boston, Massachusetts; 200 mCi/mmol dissolved in 0.15 M NaCl). Twenty blood samples of 7 ml each were drawn into EDTA from an indwelling needle in the opposite antecubital vein at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 9, 12, 15, 18, 21, 24, 30, 33, 36, 42, 45, and 48 h. Samples that coincided with feeding times were taken just before formula ingestion. The sample at 0.25 h was taken after the 0-time formula was consumed.

Very-low-density lipoprotein triglyceride was isolated by preparative ultracentrifugation from each plasma sample. A known volume (usually 3.5 ml) was overlaid with a solution of sodium chloride of 1.006 g/ml to a total volume of 6 ml. Samples were centrifuged for 18 h at 40,000 rpm in a Beckman preparative ultracentrifuge (40.3 rotor) (Beckman Instruments, Fullerton, California). The top approximately 1 ml from each tube was removed by tube slicing. Triglyceride was measured in an aliquot using an Autoanalyzer II (Technicon Instruments, Tarrytown, New York) by the enzymatic method of Bucolo and Davis.[20] The remainder was extracted with 20 vol of isopropanol, and phospholipids were removed by adsorption onto zeolite (Technicon). A portion of this was used for Autoanalyzer measurement by the Lieberman and Burchard method.[21] The remainder of the extract was evaporated and dissolved into scintillation fluid for analysis of radioactivity in a Packard Liquid Scintillation Spectrometer equipped with an external standard for quench correction. Corrections for recovery of VLDL-TG after centrifugation were made as previously described.[22]

Every hour for the first 6 h (a period covering two formula meals) after injection of labeled glycerol, additional plasma samples were taken for measurement of C-peptides, free fatty acids (FFA), and insulin. Insulin was quantified with the modification of Herbert et al.[23] of the radioimmunoassay method of Berson and Yalow.[24] C-peptides were determined by the method of Faber et al.[25] FFA were measured by a modification of the colorimetric micromethod of Soloni and Sardina[26] as described previously.[27] Glucose was quantified on the Autoanalyzer using the ferricyanide method.[28] Blood samples were also obtained on admission, at initiation of the formula feeding, and immediately before injection of tritiated glycerol for the measurement of individual plasma lipoprotein fractions as described previously.[17]

The resultant specific activity curves (as shown in Figure 1) were analyzed using a linear first-order compartmental model (Figure 2) described by Zech et al.[22] This model proposes two pathways for the incorporation of plasma glycerol into VLDL-TG, one slower than the other. It also utilizes a stepwise delipidation pathway for VLDL in the plasma compartment. The analysis yields data for rates of VLDL-TG synthesis (R^{TG}_{VLDL}), fractional catabolic rate (FCR^{TG}), and a distribution of synthesis between a slow (through compartment 24) and a fast (through compartments 10 and 14) pathway for triglyceride transport. It predicts values for the residence time of VLDL particles in the delipidation chain, for the fraction of VLDL-TG delipidized at steps 1, 6, 7, and 8, and for

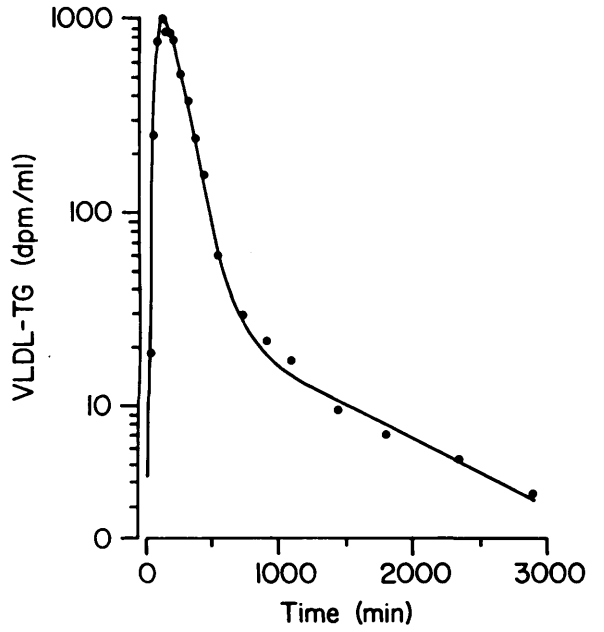

FIGURE 1. Typical VLDL-TG specific activity curve obtained in the Pimas after injecting 342 μCi of tritiated glycerol. Points are individual data. The curve was generated from the model in Figure 2.

the fraction of TG remaining for intermediate-density lipoprotein (IDL) formation or further metabolism. This method is advantageous over a single compartmental analysis (i.e., the estimation of production by a mono-exponential fit of the decay curve) because the model accounts for both the flattening at the top of the curve and the "tail," or multi-exponential portion of the decay. Failure to account for these in both cases leads to overestimation of the production rate. In the analysis of the data we have assumed that all of the "tail" of the curve (Figure 1, 12–48 h) was due to a slow synthetic pathway. As previously described,[22] some of the "tail" might be caused by a slow degradation path (compartment 21, or β-VLDL). However, because the cholesterol:TG ratio in the VLDL fraction was always below 0.21, the quantity of β-VLDL was assumed to be zero.

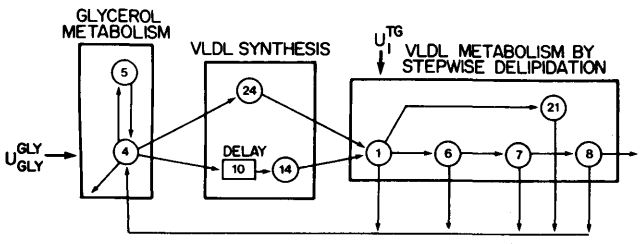

FIGURE 2. Multicompartmental model[22] for the analysis of kinetic data of VLDL-TG metabolism. The model employs two pathways for incorporation of plasma glycerol into VLDL-TG; the slow path is through compartment 24 and the fast path is via compartments 10 and 14. There is a stepwise delipidation of VLDL in the plasma (compartments 1, 6, 7, and 8). Fractional rate of transport to compartment i from compartment j is expressed as $L_{i,j}$. Steady-state transport of substance x into or out of subsystem j is expressed as R_j^x. U_1^{TG} represents VLDL-TG synthesis from nonplasma glycerol sources. $L_{i,j}$ is assumed to be the same for all four steps of the delipidation chain. The fraction delipidized at each step is equal to $L_{4,1}/(L_{4,1} + L_{6,1})$. The proportion of VLDL-TG metabolized to IDL is equal to $R_{0,8}^{TG}/R_{VLDL}^{TG}$. VLDL residence time is equal to $4/(L_{4,1} + L_{6,1})$, since there are four steps in the delipidation chain, and time for each = $1/(L_{4,1} + L_{6,1})$. Sensitivities to various parameters in the model have been previously determined.[22]

TABLE 2
VLDL-TG metabolism in diabetics and nondiabetics

	Diabetics (mean ± SEM)	Nondiabetics (mean ± SEM)	P
VLDL-TG synthesis mg/h	890 ± 128	803 ± 71	NS
mg/h/kg IW	13.3 ± 2.0	12.1 ± 1.0	NS
FCR (h^{-1})	0.33 ± 0.02	0.42 ± 0.03	<0.01
Rate of delipidation (h^{-1})*	0.26 ± 0.04	0.40 ± 0.03	0.01
Fraction delipidized†	0.39 ± 0.08	0.61 ± 0.05	<0.05
VLDL-TG residence time (h)‡	3.0 ± 0.2	2.4 ± 0.2	<0.05
VLDL residence time (h)	5.6 ± 0.4	6.1 ± 0.4	NS
Fraction remaining‖	0.26 ± 0.09	0.023 ± 0.01	0.001
Slow path/fast path	0.47 ± 0.17	0.32 ± 0.05	NS

*Rate of stepwise delipidation ($L_{4,1}$) of each step in delipidation chain (Figure 2).
†Fraction of VLDL-TG delipidized at each step of delipidation chain ($L_{4,1}/L_{4,1} + L_{6,1}$) (Figure 2).
‡Equal to $1/FCR^{TG}$.
§Time for passage of VLDL through all steps of the delipidation chain ($4/L_{4,1} + L_{6,1}$) (Figure 2).
‖Fraction of triglyceride remaining for further metabolism after passage through the delipidation chain (Figure 2).

Plasma mass of VLDL-TG was calculated from VLDL-TG concentrations and the estimated plasma volumes. Plasma volume was calculated as previously reported[19] using the equation plasma volume (liters) = 0.045 × ideal weight (kg) + 0.010 × excess weight (kg). Ideal weight was calculated from standard Metropolitan Life Insurance Tables.[29] The data for synthesis were expressed not only in mg/h but were also normalized for ideal body weight or plasma volume. These latter two methods of expression attempt to normalize for obesity on the assumption that increases in body weight primarily reflect increases in adipose tissue mass and not increases in liver function.[19] Statistical analysis was performed using the Statistical Analysis System (Carey, North Carolina).

RESULTS

Comparison of VLDL-TG metabolism in nondiabetics and diabetics. The diabetic Pima subjects had mean plasma TG and VLDL-TG levels almost twice as high as those of the nondiabetics. The range of triglycerides in the diabetic subjects in this study corresponded to that found in diabetics of this age in the Pima population as a whole; in the diabetic Pimas significant hypertriglyceridemia[9] is rarely observed (B. V. Howard, unpublished data). Total cholesterol in the diabetics was elevated, but not significantly (Table 1). The kinetic data obtained from multicompartmental analysis of the specific activity curves are summarized for diabetics and nondiabetics in Table 2. Rates of VLDL-TG production in the diabetics were not significantly different from the nondiabetics, whether expressed as mg/h or normalized for ideal body weight. The FCR for VLDL-TG was significantly lower in the diabetics, as compared with the nondiabetics. In addition, there were significant decreases both in the stepwise delipidation rate and in the fraction of VLDL-TG delipidized at each step of the delipidation chain in the diabetics. These parameters are measures of the rate at which TG is removed from VLDL during its catabolism by lipolytic enzymes. On

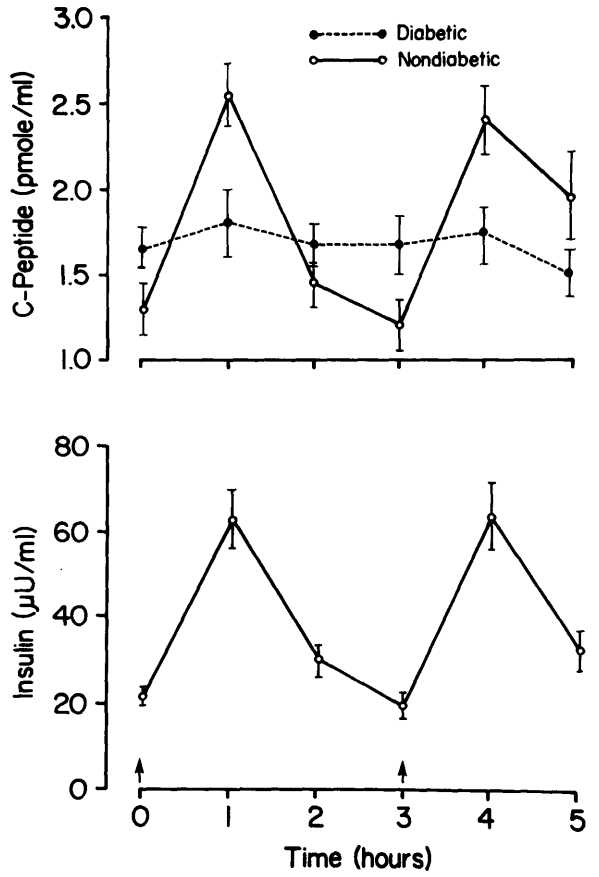

FIGURE 3. Plasma C-peptide and insulin levels during study of VLDL-TG metabolism in diabetics (●----●) and nondiabetics (○——○). Insulin is not shown for the diabetics because several had antibodies to insulin. Values are for a 6-h period, which included two formula feedings (arrows). Bars indicate SEM.

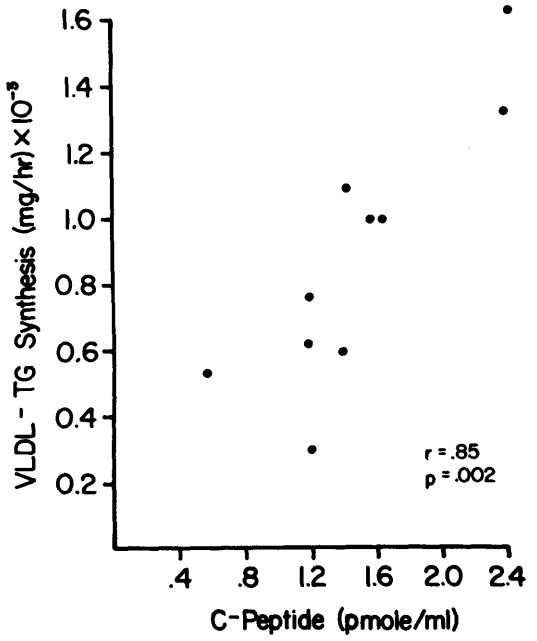

FIGURE 4. Relationship between VLDL-TG synthesis and plasma C-peptide levels in diabetics. C-peptide values are the mean of the time points shown on Figure 3 (r = 0.85, P = 0.002).

the other hand, the VLDL particle residence time in the diabetics was only slightly lower than that in the nondiabetics.

Examination of the distribution of slow and fast pathways for VLDL-TG production suggested that the slow pathway contributes little to VLDL-TG synthesis in the Pima nondiabetics. The proportion of slow to fast pathway was somewhat, although not significantly, higher in the diabetics. The fraction of VLDL-TG remaining for IDL was significantly higher in the diabetics.

Relationship between plasma insulin and FFA levels and VLDL-TG metabolism. Plasma insulin levels were monitored during the study over a 6-h period encompassing two formula feedings (Figure 3). This interval was considered to be representative of the daily plasma insulin, since the formula diet was maintained at 3-h intervals for the course of the metabolic studies. Plasma levels in between formula findings are referred to as "basal," since they are not equivalent to fasting levels. C-peptide levels were also measured as an indicator of insulin secretion, since the presence of insulin antibodies in several diabetics prevented the measurement of insulin. Plasma insulin and C-peptide levels rose in the nondiabetics after each formula feeding. In the diabetics basal plasma C-peptide levels were slightly higher than in nondiabetics, but there were only minimal rises after each formula feeding, reflecting the decrease in insulin secretory capacity in response to carbohydrate stimulus. The sum of the C-peptides over the 6-h period encompassing two formula feedings in the diabetics was 9.01 pmol/ml compared with 10.85 in the nondiabetics. In diabetic subjects whose insulin could be measured (N = 4) basal insulin averaged 42 μU/ml, and there were only small rises after each formula feeding. In the diabetics there was a highly significant correlation between plasma C-peptide levels and VLDL production (Figure 4). In contrast, in the nondiabetics we found no correlation between basal or stimulated C-peptide values and VLDL-TG pools, FCR, or rate of synthesis (data not shown), and, similarly, there was no correlation between any of the parameters of VLDL-TG metabolism and plasma insulin.[18]

FFA levels also were monitored in both groups (Figure 5). FFA levels declined after each feeding in nondiabetics, coin-

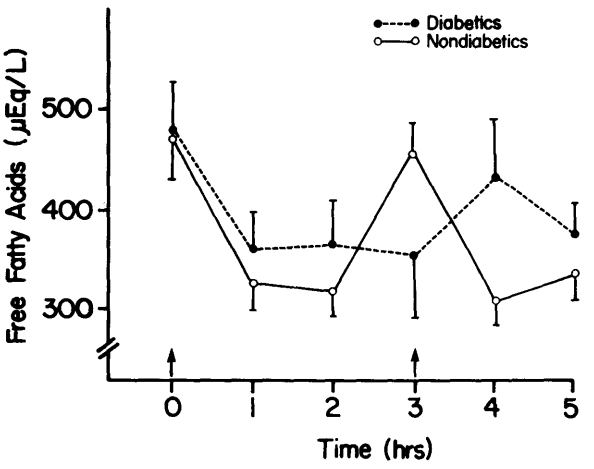

FIGURE 5. Plasma free fatty acid levels during study of VLDL-TG metabolism in diabetics (●----●) and nondiabetics (○——○). Values are for a 6-h period, which included two formula feedings (arrows). Bars indicate SEM.

cident with the increases in plasma insulin. In diabetics, basal FFA were not significantly different from those of nondiabetics, and the decline after each feeding was not significantly different from nondiabetics, despite the minimal C-peptide secretion. The antilipolytic response to insulin is very sensitive, and it has been established previously in diabetics of this population that free fatty acid declines in response to minimal insulin changes.[27]

DISCUSSION

We examined VLDL-TG metabolism in a group of Pima Indian diabetics and compared the kinetic data with those from a group of weight-matched nondiabetic controls. We sought to minimize variables in population and methodology, which have confounded past analysis of diabetic hypertriglyceridemia, by the study of diabetics from a carefully characterized population and through the use of multicompartmental analysis of the kinetic data. In the Pima diabetics there was no severe hypertriglyceridemia; triglycerides were elevated approximately 150% compared with weight-matched nondiabetic controls. The elevated plasma VLDL-TG appeared to be caused by decreased clearance capacity. The fractional catabolic rate, the rate of stepwise delipidation, and the fraction of VLDL-TG delipidized were all lower in the diabetics. VLDL-TG production rates were not significantly different from controls whether expressed as mg/h or normalized per kg ideal body weight. Since VLDL-TG concentration was in a steady state, the elevated TG levels in the Pima diabetics thus could be attributed to the lower FCR.

Many investigators have reported overproduction of VLDL-TG in type II diabetics. In a study of type II diabetics with moderate hypertriglyceridemia, Nikkila and Kekki[6] found increased production of triglyceride. Greenfield et al.[13] showed increased hepatic VLDL-TG secretion in a group of obese type II diabetics, and Kissebah et al.[30] have shown overproduction of VLDL apo-B in non-insulin-dependent diabetics. Because these studies were done with single exponential analysis of the kinetic data, the validity of these claims of overproduction has been disputed. However, recently Abrams et al.,[14] using multicompartmental analysis, showed increased production of VLDL-TG in a group of type II diabetics; production rates declined after insulin therapy. Dunn et al.[15] also recently observed overproduction in type II diabetics with underlying hyperlipemia.

An explanation for the lack of overproduction of VLDL-TG observed in the present population of diabetics requires an understanding of the mechanisms by which diabetes may lead to overproduction. First, there is evidence in many populations,[12,31,32] including the Pimas,[18] that FFA concentrations may control VLDL production. It is thus possible that overproduction of VLDL-TG in type II diabetics may depend on the extent of free fatty acid elevation. The role of FFA in diabetic hypertriglyceridemia has been recently reviewed by Reaven and Greenfield.[33] In previous studies where overproduction was observed in diabetics,[6,12] the FFA levels were significantly elevated. However, the Pima diabetics did not have significant elevations in FFA. Thus, the difference between our results and the observations of VLDL-TG overproduction may be attributable to differences in free fatty acid levels.

A second possibility proposed by previous investigators[14] is that the elevated plasma glucose levels in diabetics may cause overproduction of VLDL-TG. Our results suggest that elevated glucose does not necessarily lead to overproduction, since hyperglycemia in the Pimas was as severe as in the diabetics in the other studies.

The third possibility is that elevated insulin levels in diabetes lead to VLDL overproduction. However, Abrams et al.[14] reported that VLDL-TG production decreased after insulin therapy in a group of diabetics. In addition, the Pima diabetics had slightly higher basal insulin levels (as indicated by C-peptide) than the nondiabetic controls. These observations suggest that the overproduction of VLDL-TG in diabetics is not caused by elevated insulin. In the Pima nondiabetics VLDL-TG metabolism was not related to insulin levels.[18] It is not clear why there was a significant correlation between VLDL-TG production and plasma C-peptides in the Pima diabetics. It is possible that this relationship is a reflection of the increased insulin resistance that has been demonstrated in both the periphery[34] and in the liver (J. Andrews, unpublished data) of diabetics in this population.

Decreased catabolism of VLDL-TG has been observed previously in type II diabetics by Lewis et al.[10] and Brunzell et al.[11] It is possible that type II diabetics often have a defect in clearance of VLDL-TG, although in many cases it may be masked by marked overproduction, since clearance appears to rise in response to overproduction.[19] Although lipolytic activities were not measured in the Pima subjects, the most likely mechanism for the lowered clearance capacity is a decreased lipoprotein lipase activity related to their relative insulin deficiency. Decreased postheparin lipolytic activity has been reported in type II diabetics by Nikkila and Kekki[6] and Brunzell et al.,[11] and Taskinen et al. have recently shown decreases in adipose tissue lipoprotein lipase[35] in type II diabetic men. Another possible mechanism for the decreased clearance of VLDL-TG is that physiologic perturbations that accompany the hyperglycemic state may alter VLDL catabolism, as has been observed previously in experimental animals.[36]

It is important to ask how relevant these observations in the Pima may be to other groups of diabetics, since the TG levels were not markedly elevated in this group. Recent population studies on triglycerides in diabetics in a Lipid Research Clinic's prevalence group[37] suggest that many diabetics have only mild elevations in plasma triglycerides. These smaller elevations may well be important in the etiology of atherosclerosis, however, since there is no evidence that only those diabetics with severely elevated triglycerides are at increased risk for atherosclerosis. A decrease in TG removal in diabetics could be a possible mechanism for the development of atherosclerosis, since it could result in delayed clearance of the potentially atherogenic VLDL remnants.

Finally, the diabetics in this study were observed to have a significantly increased fraction of VLDL-TG remaining for further metabolism. This increased remaining TG may explain the increased TG content in LDL reported in diabetics by Schonfeld et al.[38] and also observed in Pima diabetics.[17] The increased fraction remaining for further metabolism together with the decreased catabolic parameters suggest that the diabetics may secrete a larger, more TG-rich VLDL particle, which is catabolized less efficiently.

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REFERENCES

[1] Joslin, S., and Bradley, R. F.: Cardiovascular Disease. Philadelphia, Lea & Febiger, 1971.

[2] Bierman, E. L.: Hypertriglyceridemia in early diabetes. Adv. Metab. Disord. (Suppl. 2) II:67–72, 1973.

[3] Goldberg, R. B.: Lipid disorders in diabetes. Diabetes Care *4*:561–71, 1981.

[4] Nikkila, E. A., Huttunen, J. K., and Ehnholm, C.: Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Relationship to plasma triglyceride metabolism. Diabetes *26*:11–21, 1977.

[5] Jones, D. P., Stolken, G. R., and Arky, R. A.: Lipoprotein lipase activity in patients with diabetes mellitus with and without hyperlipemia. Diabetes *15*:565–70, 1966.

[6] Nikkila, E. A., and Kekki, M.: Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism *22*:1–21, 1973.

[7] Bagdade, J. D., Porte, D., and Bierman, E. L.: Diabetic lipemia. N. Engl. J. Med. *276*:427–33, 1967.

[8] Brunzell, J. D., Hazzard, W. R., Motulsky, A. G., and Bierman, E. L.: Evidence for diabetes mellitus and genetic forms of hypertriglyceridemias as independent entities. Metabolism *24*:1115–21, 1975.

[9] Chait, A., Robertson, H. T., and Brunzell, J. D.: Chylomicronemia syndrome in diabetes mellitus. Diabetes Care *4*:343–48, 1981.

[10] Lewis, B., Marcini, M., Mattock, M., Chait, A., and Frazier, T. R.: Plasma triglycerides and fatty acid metabolism in Diabetes Mellitus. Eur. J. Clin. Invest. *2*:445–53, 1972.

[11] Brunzell, J. D., Porte, D., Jr., and Bierman, E. L.: Abnormal lipoprotein-lipase-mediated plasma triglyceride removal in untreated diabetes mellitus associated with hypertriglyceridemia. Metabolism *28*:901–907, 1979.

[12] Kissebah, A. H., Alfarsi, S., Adams, P. W., and Wynn, V.: Role of insulin resistance in adipose tissue and liver in the pathogenesis of endogeneous hypertriglyceridaemia in man. Diabetologia *12*:563–71, 1976.

[13] Greenfield, M., Kolterman, O., Olefsky, J., and Reaven, G. M.: Mechanism of hypertriglyceridaemia in diabetic patients with fasting hyperglycaemia. Diabetologia *18*:441–46, 1980.

[14] Abrams, J. J., Ginsberg, H., and Grundy, S. M.: Metabolism of cholesterol and plasma triglycerides in nonketotic diabetes mellitus. Diabetes *31*:903–10, 1982.

[15] Dunn, F. L., Bilheimer, D. W., Raskin, P., and Grundy, S. M.: Pathogenesis of hypertriglyceridemia in diabetes mellitus. The effects of improved glucose regulation. Clin. Res. *28II*:319A, 1980.

[16] Matson, G. A., Burch, T. A., and Polesky, H. F.: Distribution of hereditary factors in the blood of Indians of the Gila River, Arizona. Am. J. Phys. Anthropol. *29*:311–38, 1968.

[17] Howard, B. V., Savage, P. J., Bennion, L. J., and Bennett, P. H.: Lipoprotein composition in diabetes mellitus. Atherosclerosis *30*:153–62, 1978.

[18] Howard, B. V., Zech, L., Davis, M., Bennion, L. J., Savage, P. J., Nagulesparan, M., Bilheimer, D., Bennett, P. H., and Grundy, S. M.: Studies of very low density lipoprotein triglyceride metabolism in an obese population with low plasma lipids: lack of influence of body weight or plasma insulin. J. Lipid Res. *21*:1032–41, 1980.

[19] Grundy, S. M., Mok, Y. H. I., Zech, L., Steinberg, D., and Berman, M.: Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. J. Clin. Invest. *63*:1274–83, 1979.

[20] Bucolo, G., and Davis, H.: Quantitative determination of serum triglycerides by use of enzymes. Clin. Chem. *39*:475–82, 1973.

[21] Rush, R. L., Leon, L., and Turrell, J.: Automated simultaneous cholesterol and triglyceride determination on the Auto Analyzer instrument. *In* Advances in Automated Analyses. Miami, Florida, Thurman, pp. 503–11, 1970.

[22] Zech, L. A., Grundy, S. M., Steinberg, D., and Berman, M.: Kinetic model for production and metabolism of very low density lipoprotein triglycerides. J. Clin. Invest. *63*:1262–73, 1979.

[23] Herbert, V., Law, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. *25*:1375–84, 1965.

[24] Berson, S. A., and Yalow, R. S.: Quantitative aspects of the reaction between insulin and insulin binding antibody. J. Clin. Invest. *38*:1996–2016, 1959.

[25] Faber, O. K., Binder, C., Markussen, J., Heding, L. G., Naithani, V. K., Kuzuya, H., Blix, P., Horwitz, D. L., and Rubenstein, A. H.: Characterization of seven C-peptide antisera. Diabetes *27* (Suppl. 1):170–77, 1978.

[26] Soloni, F. G., and Sardina, L. C.: Colorimetric microdetermination of free fatty acids. Clin. Chem. *19*:419–24, 1973.

[27] Howard, B. V., Savage, P. J., Nagulesparan, M., Bennion, L. J., Unger, R. H., and Bennett, P. H.: Evidence for marked sensitivity to the antilipolytic action of insulin in obese maturity-onset diabetics. Metabolism *28*:744–50, 1979.

[28] Technicon Methodology File N-26. Tarrytown, N.Y., Technicon Instruments Corp., 1965.

[29] National Research Council Food and Nutrition Board: Recommended Dietary Allowances: A Report. 6th rev. edit. Washington, D.C., National Academy of Sciences Publication #1146, 1964.

[30] Kissebah, A. H., Alfarsi, S., Evans, D. H., and Adams, P. W.: Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in non-insulin-dependent diabetes mellitus. Diabetes *31*:217–25, 1982.

[31] Havel, R. J., Kane, J. P., Balasse, J. O., Segel, N., and Basso, L. V.: Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. J. Clin. Invest. *49*:2017–35, 1970.

[32] Reaven, G., Greenfield, M., Kolterman, O., and Olefsky, J.: An enquiry into the etiology of diabetic hypertriglyceridemia. Excerpta Medicus *481*:191, 1979.

[33] Reaven, G. M., and Greenfield, M. S.: Diabetic hypertriglyceridemia. Evidence for three clinical syndromes. Diabetes *30* (Suppl. 2):66–75, 1981.

[34] Nagulesparan, M., Savage, P. J., Mott, D. M., Johnson, G. C., Unger, R. H., and Bennett, P. H.: Increased insulin resistance in obese, glucose-intolerant southwestern American Indians: evidence for a defect not explained by obesity. J. Clin. Endocrinol. Metab. *51*:739–43, 1980.

[35] Taskinen, M. R., Nikkila, E. A., Kuusi, T., and Harno, K.: Lipoprotein lipase activity and serum lipoproteins in untreated type 2 (insulin-independent) diabetes associated with obesity. Diabetologia *22*:46–50, 1982.

[36] Bar-On, H., Chen, Y. I., and Reaven, G. M.: Evidence for a new cause of defective plasma removal of very low density lipoproteins in insulin-deficient rats. Diabetes *30*:496–99, 1981.

[37] Barrett-Connor, E., Grundy, S. M., and Holdbrook, M. J.: Plasma lipids and diabetes mellitus in an adult community. Am. J. Epidemiol. *115*:657–63, 1982.

[38] Schonfeld, G., Birge, C., Miller, J. P., Kessler, G., and Santiago, J.: Apolipoprotein B levels and altered lipoprotein composition in diabetes. Diabetes *23*:827–34, 1974.