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

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Abstract Pima Indians have a high prevalence of hyperinsulinemia, obesity, and diabetes, but they have low plasma cholesterol levels, reduced low density lipoprotein synthesis, and little arteriosclerotic heart disease. To investigate lipoprotein metabolism further in this group, very low density lipoprotein (VLDL) metabolism was studied, using [^{3}H]glycerol as an endogenous precursor of triglyceride (TG) synthesis, in 15 obese Pima nondiabetic males and compared to that of 10 obese and 13 normal weight, normolipidemic, nondiabetic Caucasian males. The resultant kinetic data were analyzed using a multicompartmental model which includes two pathways for VLDL-TG synthesis and a process of stepwise delipidation for VLDL catabolism. As compared to obese Caucasians, the obese Pimas had a lower rate of VLDL-TG synthesis, and a lower proportion of slow pathway for synthesis. The fractional catabolic rate in the Pimas was higher than in either Caucasian group, a larger proportion of VLDL-TG was delipidized at each step, and particle residence time was shorter. When the relation between VLDL-TG metabolism and plasma insulin was examined, plasma insulin levels in the Pima were not correlated with VLDL-TG synthetic rates, catabolic rates, or plasma pools. On the other hand VLDL-TG synthetic rates were correlated with plasma free fatty acid levels. Thus, in this population with low plasma lipids and reduced arteriosclerotic heart disease, VLDL-TG synthesis is low, VLDL-TG catabolism is accelerated, and VLDL pools appear to be insensitive to the influence of body weight and hyperinsulinemia.—**Howard, B. V., L. Zech, M. Davis, L. J. Bennion, P. J. Savage, M. Nagulesparan, D. Bilheimer, P. H. Bennett, and S. M. Grundy.** 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.* 1980. **21:** 1032–1041.

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Mechanisms for the control of VLDL metabolism in man are complex and not well understood. Evidence has been presented that VLDL production may be related to intake of dietary carbohydrates (1, 2), insulin levels (3–6), and free fatty acid (FFA) levels (7–9). VLDL degradation also may be affected by several factors including insulin (10–12), thyroid hormones (13), and caloric intake (14). VLDL levels in plasma are the resultant of synthesis and catabolism, and changes in either process may influence VLDL pool size. VLDL metabolism is also influenced by obesity, and obesity is often associated with hypertriglyceridemia. Obese individuals consume more calories, are hyperinsulinemic (15), and may have increased transport of FFA (16); each or all of these factors may contribute to elevated triglyceride levels in obese subjects.

The Pima Indians are a genetically homogeneous population (17) with no evidence of genetic hyperlipoproteinemia (18). Moreover they have a high prevalence of obesity and hyperinsulinemia (19). On the other hand, the Pimas have low plasma cholesterol levels (20) and a reduced prevalence of arteriosclerotic

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; TG, triglyceride; FFA, free fatty acid; FCR, fractional catabolic rate.

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heart disease (21, 22). They thus constitute a well defined study population in which some of the variables that influence lipid metabolism have been minimized. A study of LDL metabolism has indicated that LDL production is decreased (23). Since LDL is a product of VLDL catabolism in normal humans, the study of VLDL metabolism in Pimas could provide information about mechanisms responsible for their low levels of plasma cholesterol. Furthermore, this population should be ideal for a study of the influence of obesity and plasma insulin levels on VLDL metabolism.

Recently a new method for the study of VLDL transport has been developed by Zech et al (24). This method involves administering tritiated glycerol to label the TG moiety and obtaining the specific activities of VLDL-TG over a 48-hr period. The resultant kinetic data are analyzed using a multicompartmental model. This method has recently been developed and validated from studies in a large number of subjects (14), and it has been useful for the study of mechanisms producing hypertriglyceridemia (14) and the influence of diets (25) and drugs (26) on VLDL metabolism. We have used this technique to investigate VLDL-TG metabolism in a group of Pima Indians. Plasma insulin and FFA levels were also measured during the course of the studies to examine relations among plasma insulin, free fatty acids, and VLDL-TG metabolism. These data on VLDL metabolism have been compared to those obtained in both normal and obese normolipidemic Caucasians studied under identical conditions (24).

METHODS

Written informed consent was obtained from all the subjects in these studies. In addition, the procedures used were approved by the Human Studies Committees of the National Institutes of Health, Bethesda; Indian Medical Center, Phoenix; and Veterans Administration Medical Center, San Diego.

Fifteen male Pima Indians between the ages of 18–49 years were admitted to the Phoenix Clinical Research Center (**Table 1**). The group had a mean age of 27 and a mean body weight 167% of ideal. All patients had normal liver and gastrointestinal function as evaluated by physical examination, blood chemistry tests, and urinalyses. None had evidence of cardiovascular disease or family history of hyperlipemia. None were taking any medications known to affect lipid metabolism, and all were normoglycemic.

Because of the extreme obesity in the Pima population (18), the data obtained from the studies of VLDL-TG metabolism in the Pima group were compared to two control groups (Table 1). The first was a group of 13 normolipidemic Caucasians used to validate the compartmental model (24). A second control group consisted of 10 obese normolipidemic Caucasians (14) whose mean percent desirable weight did not significantly differ from that of the Pimas.

TABLE 1. Characteristics of study groups

	Obese Pima	Obese Caucasian[c]	Normal Caucasian[c]
N	15	10	13
Age (yrs)	27 (18–49)[a]	47 (30–62)	53 (18–61)
Weight (kg)	112 (58–182)	114 (95–127)	68 (59–80)
% Ideal weight	167 ± 12[b]	163 ± 4.3	100 ± 3.6

[a] Range.
[b] Mean ± S.E.M.
[c] These subjects were part of a study group reported by Grundy et al. (14). They were admitted to the Special Diagnostic and Treatment Unit of Veterans Administration Medical Center, San Diego, CA, and they were studied with protocols identical to those described in Materials and Methods.

Patients were admitted at least 7 days before the study and were fed a weight-maintenance diet consisting of 40% fat, 45% carbohydrate and 15% protein (P/S ratio = 0.34, 500 mg cholesterol/day). Body weight was maintained during this period of stabilization in all patients. After 2 days on this diet, a routine 100-gram, 3-hr oral glucose tolerance test was performed, and plasma samples were obtained for assay of glucose and insulin. Thirty-six hours prior to the beginning of the study, the patients consumed 60% of their maintenance calories in a formula diet, consisting of 75% carbohydrate (dextrose) and 25% protein (calcium caseinate), which was fed every 3 hr. This fat-free regimen eliminated chylomicron production while maintaining constant carbohydrate intake and VLDL levels (14), and it was continued for the 48 hr of the study.

The study was initiated with a rapid injection via an antecubital vein of 342 μCi of [2-H^3]glycerol[4] (New England Nuclear, 200 mCi/mM, 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, 39, and 48 hr.

VLDL was isolated by preparative ultracentrifuga-

[4] A few of the Caucasian controls received [1,3-^{14}C]glycerol. Use of this isotope influences the data for the slow component of VLDL-TG synthesis, (24) and only data from Caucasian controls receiving [^{3}H]glycerol were used in the comparison of synthesis pathways in Table 2.

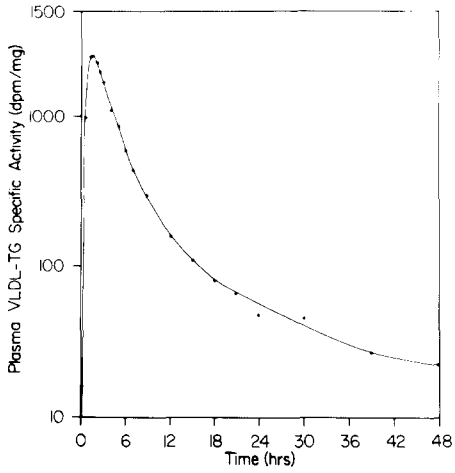

Fig. 1. Typical VLDL-TG specific activity curve obtained in the Pimas after injection of 342 μCi of tritiated glycerol. The curve has been visually fitted.

tion 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 hr at 40,000 rpm in a Beckman preparative ultracentrifuge (40.3 rotor). The top approximate 1 ml from each tube was removed by tube slicing. TG was measured in an aliquot using an Autoanalyzer II (Technicon Instruments, Tarrytown, NY) by the enzymatic method of Bucolo and Davis (27). The remainder was extracted with 20 volumes of isopropanol, and phospholipids were removed by adsorption onto Zeolite (Technicon). A porton of this was used for Autoanalyzer measurement of cholesterol by the Lieberman and Burchard method (28). The remainder of the extract was evaporated and dissolved into LSC scintillation fluid (Yorktown Research) 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 ultracentrifugation were made as previously described (24).

At every hour for the first 6 hr after injection of labeled glycerol, additional plasma samples were taken for measurement of insulin and FFA. Insulin was quantified by the modification of Herbert et al. (29) of the radioimmunoassay method of Berson and Yalow (30).

FFA was quantified by a modification of the colormetric micromethod of Soloni and Sardina (31) as described previously from this laboratory (32). Glucose was quantified in the Autoanalyzer using the ferricyanide method (33).

Blood samples were also obtained on admission, at initiation of formula feedings, and immediately prior to injection of tritiated glycerol for the quantification of individual plasma lipoproteins. From each sample VLDL, LDL, and HDL were isolated by sequential flotation in the ultracentrifuge using a modification of the method of Havel, Eder, and Bragdon (34) as described previously (35). Triglyceride and cholesterol in plasma and the isolated lipoproteins were quantified on the Autoanalyzer II using the cholesterol and triglyceride methods described above.

The resultant specific activity curves (as shown in **Fig. 1**) were analyzed using a linear first order compartmental model (**Fig. 2**) described by Zech et al (24). 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 of 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 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 delipidized at steps 1, 6, 7, and 8, and for the fraction of TG remaining for intermediate density lipoprotein (IDL) formation. In the analysis of the data we have assumed that all of the "tail" of the curve (Fig. 1, 12–48 hr) was due to a slow synthetic pathway. As previously described (24), 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 as-

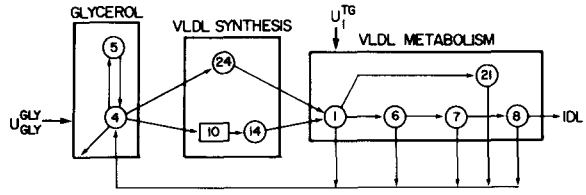

Fig. 2. Multicompartmental model (24) for the analysis of kinetic data on VLDL-TG metabolism. The model employs two pathways for incorporation of plasma glycerol into VLDL-TG; the slow path is through compartment 24 and the fast path is via compartment 10 and 14. There is a stepwise delipidation of VLDL 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^x_j. U^{Tx}_1 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 $R^{TG}_{0,8}/R^{TG}_{VLDL}$. 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 (24).

TABLE 2. VLDL-TG metabolism in Pimas and Caucasians

	Obese (A) Pima	Obese (B) Caucasian	Normal (C) Caucasian	*P* Values A vs B	*P* Values A vs C
		(mean ± S.E.M.)			
VLDL-TG synthesis (R^{TG}_{VLDL})					
mg/hr	803 ± 71	1414 ± 245	726 ± 60	0.01	ns
mg/hr/kg	7.4 ± 0.59	12.3 ± 2.0	10.6 ± 0.95	0.01	ns
mg/hr/kgIW	12.1 ± 0.99	19.8 ± 3.4	10.7 ± 0.92	0.01	ns
mg/hr/dl	23.2 ± 1.9	38.9 ± 6.5	23.8 ± 2.0	0.01	ns
Slowpath/Fastpath	0.32 ± 0.05	0.70 ± 0.26[e]	0.48 ± 0.13[f]	0.05	0.10
FCR^{TG} (hr^{-1})	0.42 ± 0.03	0.32 ± 0.05	0.21 ± 0.02	0.05	0.001
Rate of delipidation (hr^{-1})[a]	0.40 ± 0.03	0.28 ± 0.06	0.20 ± 0.03	0.05	0.001
VLDL residence time (hr)[b]	6.1 ± 0.40	7.1 ± 0.71	7.7 ± 0.52	0.10	0.02
Fraction remaining for IDL[c]	0.023 ± 0.01	0.067 ± 0.04	0.25 ± 0.07	0.10	0.005
Fraction delipidized[d]	0.61 ± 0.05	0.49 ± 0.09	0.37 ± 0.07	0.10	0.005
VLDL-TG (mg/dl)	59 ± 7.7	127 ± 15	117 ± 7.6	0.001	0.001
VLDL (CH/TG)	0.16	0.19	0.24		

[a] Equal to $L_{4,1}$, Fig. 2.
[b] Particle residence time is equal to $4/(L_{4,1} + L_{6,1})$, Fig. 2.
[c] Equal to $R^{TG}_{0,8}/R^{TG}_{VLDL}$, Fig. 2.
[d] Equal to $L_{4,1}/(L_{4,1} + L_{6,1})$, Fig. 2.
[e] N = 5, this parameter could be determined only in those receiving [^{3}H]glycerol.
[f] N = 10, this parameter could be determined only in those receiving [^{3}H]glycerol.

sumed to be zero. The previous work showed that the contribution of small quantities of β-VLDL to the tail would be negligible (24).

Plasma mass of VLDL-TG was calculated from VLDL-TG concentrations and the estimated plasma volumes. Plasma volume was calculated as previously reported (14) using the equation: plasma volume (liters) = ideal weight × (0.045) + excess weight × (0.010). Ideal weight was calculated from Standard Metropolitan Life Insurance tables (36). The data for synthesis were expressed not only in mg/hr, but were also normalized for ideal body weight or plasma volume; these latter two methods of expression attempt to correct for obesity according to the assumption that increases in body weight reflect primarily increases in adipose tissue mass and not increases in liver function or plasma space (14). Statistical analyses, including analysis of variance and simple stepwise correlation analysis were performed utilizing the Statistical Analysis System (37).

RESULTS

Comparison of VLDL-TG metabolism in Pimas and controls

The kinetic data obtained from the multicompartmental analysis of specific activity curves for all three groups of subjects are shown in **Table 2**.[5] The obese Pima subjects had rates of VLDL-TG synthesis significantly lower than obese Caucasians and similar to those of normal Caucasians. This difference was observed when the data were expressed as total body synthesis (mg/hr) or normalized for weight (mg/hr/kg), ideal weight (mg/hr/kg IW), or plasma volume (mg/hr/dl). Thus obesity in the Pima did not cause significantly elevated VLDL synthesis as it did in Caucasians.

Examination of the distribution of slow and fast pathways for VLDL-TG production (Fig. 2) indicated that the slow pathway appeared to contribute little to VLDL-TG synthesis in this population. The proportion of slow/fast pathway in the obese Pimas was lower than both normal and obese Caucasians. This is also shown in Fig. 1, for the specific activity curves for the Pimas had small "tails".

There were also unique aspects of VLDL-TG catabolism in this population (Table 2). The fractional catabolic rate was significantly higher than for both the normal Caucasians and the obese Caucasians, and the particle residence time (time average VLDL remains in plasma before conversion to IDL) was shorter. Pima data showed a high fraction delipidized (proportion of TG removed at compartments 1, 6, 7, or 8, Fig. 2) and a low fraction remaining for IDL formation (Fig. 2). The Pima VLDL also had a low proportion of cholesterol (Table 2). These data are consistent with the concept that the Pimas secrete larger, more TG-rich VLDL which are delipidized very rapidly.

Since the parameters of VLDL-TG synthesis and

[5] Complete kinetic data from all patients available from the authors upon request.

TABLE 3. Subgroups of Pima subjects divided by weight

Group	N	% Ideal Weight	Fasting Insulin	Fasting Glucose	2-Hour Glucose	Fasting FFA	VLDL-TG	Cholesterol
			(μU/ml)	(mg/dl)	(mg/dl)	(μEq/L)	(mg/dl)	(mg/dl)
1	6	123 (97–144)[a]	14.5 ± 2.3[b,c]	85 ± 2.2	131 ± 10	515 ± 77	61.0 ± 18.3	164 ± 11
2	9	199 (162–260)	26.8 ± 3.4[c]	89 ± 3.1	125 ± 7	445 ± 50	57.4 ± 5.8	152 ± 8.6

[a] Mean value with range in parentheses.
[b] Mean ± S.E.M.
[c] Values are significantly different ($P < 0.01$).

metabolism in the Pima population resembled more closely those of the Caucasians of normal weight, it appeared that extreme obesity in this population did not result in either elevated rates of TG synthesis or other alterations in VLDL-TG metabolism. When correlation analysis was conducted between the parameters of VLDL-TG metabolism such as synthesis or FCR and body weight, no significant correlations were observed (data not shown). To examine further the influence of body weight within the Pima group on parameters of VLDL-TG metabolism, the 15 Pimas were divided into two subgroups, one below and one above the 150th percentile for ideal weight (**Table 3**). These two subgroups differed in mean plasma insulin levels; however, subgroups were similar in age, plasma glucose levels, and levels of plasma lipids. When VLDL-TG metabolism was compared in these two subgroups (**Fig. 3**), the FCR was similar in the two weight groups. VLDL-TG synthesis per hour was slightly higher ($P < 0.10$) in the higher weight subgroup, but when it was normalized to ideal weight or plasma volume, there were no significant differences in the mean synthetic rates between the two subgroups. The ratio of slow to fast pathways, although low in all Pimas, was significantly higher in the more obese subgroup ($P < 0.05$). This index also was higher in the obese Caucasians as compared to the normal weight Caucasians (Fig. 3 and Table 2).

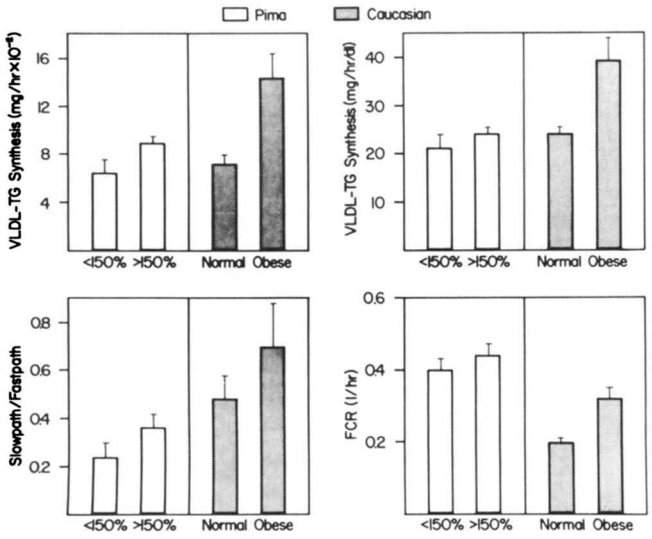

Fig. 3. Effect of body weight on VLDL-TG metabolism in Pimas and Caucasians. Pimas are divided into 2 groups as described in Table 3. Obese and normal weight Caucasians are those described in Tables 1 and 2.

Relationship between plasma lipids and VLDL metabolism

Since plasma cholesterol is low in the Pimas, and low rates of LDL synthesis have been demonstrated (23), the relationships between VLDL metabolism and lipoprotein cholesterol levels were examined. There was no significant relationship between VLDL-TG synthesis and plasma LDL cholesterol levels (**Fig. 4A**). In addition, there was no relationship between HDL cholesterol levels and the FCR for VLDL-TG (Fig. 4B).

Relation between plasma insulin levels and VLDL-TG metabolism

In addition to the glucose tolerance test, plasma insulin levels were monitored over a 6-hr period which encompassed two formula feedings during the study. The fluctuations in plasma insulin levels, which con-

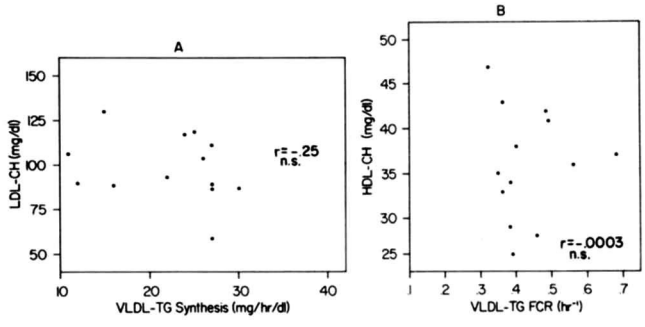

Fig. 4. Relation between VLDL-TG metabolism and plasma lipoprotein concentrations in obese Pimas. A. Relation between LDL cholesterol levels and VLDL-TG synthesis (r = −0.25, n.s.). B. Relation between HDL cholesterol and the fractional catabolic rate of VLDL-TG (r = −0.0003, n.s.).

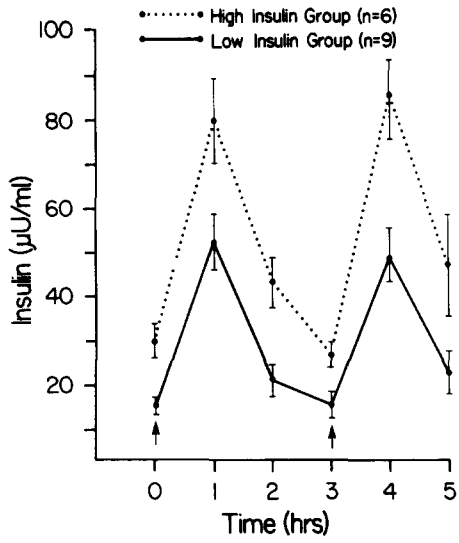

Fig. 5. Plasma insulin levels during study of VLDL metabolism. Values are for a 6-hr period which included two formula feedings (arrows); (●– – –●) subgroup with fasting insulins greater than 20 μU/ml (N = 6) (●——●); subgroup with fasting insulins below 20 μU/ml (n = 9). Bars indicate S.E.M.

sisted of a rise after each formula feeding, are shown in **Fig. 5**. There was a wide range of insulin levels in the Pima group and, for analysis of the effects of insulin, the group was divided into two subgroups with fasting plasma insulin levels either above or below 20 μU/ml (**Table 4**). Mean insulin responses during the study and insulin responses to an oral glucose load were different between the two subgroups. The mean age, and both the fasting and the 2-hr glucose values did not differ between the subgroups, but the hyperinsulinemic group was more obese. These two subgroups did not differ significantly in any of the kinetic parameters describing VLDL-TG metabolism (Table 4), including synthesis, FCR, or concentration of VLDL-TG. Moreover, there was no significant correlation between plasma insulin levels and any of the parameters describing VLDL-TG metabolism. **Fig. 6** shows the lack of correlation between baseline insulin values and VLDL-TG pools (Fig. 6A), FCR (Fig. 6B), and synthesis (Fig. 6C) normalized for plasma volume. Furthermore, no significant relationship was observed between VLDL-TG synthesis and peak insulin values (r = 0.31, n.s.) or between baseline or peak insulin levels and synthesis expressed as mg/hr/kg (r = −0.005, n.s. and r = 0.10, n.s., respectively).

Relation between plasma FFA levels and VLDL-TG metabolism

In order to investigate further the factors that determine VLDL-TG production and catabolism in this population, FFA levels were monitored in 13 of the 15 individuals during the VLDL-TG studies. FFA levels declined after each formula feeding (**Fig. 7**). VLDL-TG synthesis was not significantly related to fasting FFA levels, but postprandial FFA levels were directly and significantly correlated with rates of VLDL-TG synthesis (**Fig. 8**). There was also an inverse correlation between VLDL-TG synthesis and the magnitdue of the postprandial FFA decrements ($r = -0.56, P < 0.05$). Fasting FFA levels did not correlate with fasting plasma insulin (r = 0.02, data not shown), and there was no significant difference between insulin subgroups in their plasma FFA levels at any time during the study (Fig. 7).

DISCUSSION

This study was undertaken to investigate plasma lipoprotein metabolism in the Pima Indians. This population is interesting because its members have low plasma cholesterol levels (20) and little arteriosclerotic heart disease (21, 22) despite their high prevalence of obesity and diabetes. The data have revealed several unique aspects of VLDL-TG metabolism in this population.

Despite their marked obesity, Pimas had very low levels of VLDL-TG. VLDL-TG production appeared to be decreased as compared to weight-matched Caucasians, and levels of VLDL-TG synthesis were similar to those of normal weight controls. Al-

TABLE 4. Effect of insulin on VLDL-TG metabolism in subgroups of Pima subjects divided by insulin levels

N	(kg)	Insulins during OGTT[a]		Mean Insulin During Study	VLDL-TG Synthesis	FCR	Slow/Fast Pathway
		Fasting	2-Hour				
				(μU/ml)	(mg/hr/dl)	(hr^{-1})	
9	98 ± 11	15.4 ± 0.97	149 ± 23	29.8 ± 3.1	22.1 ± 2.6	0.405 ± 0.03	0.31 ± 0.07
6	132 ± 11	35.5 ± 4.1	243 ± 52	51.7 ± 7.0	24.1 ± 2.6	0.452 ± 0.05	0.33 ± 0.06

[a] Oral glucose tolerance test.
Means of age, fasting, and 2-hr glucose did not differ between the two groups.

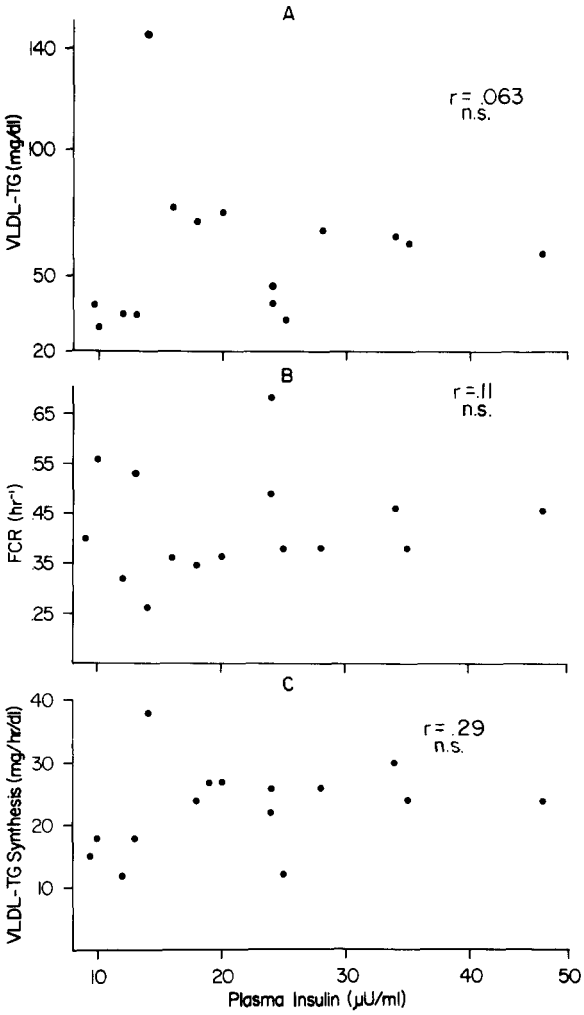

Fig. 6. Relation between insulin levels and VLDL-TG metabolism in obese Pimas. Insulin levels are the mean of times 0 and 3 on Fig. 3. A. Relation between VLDL-TG pools and plasma insulin levels (r = 0.063, n.s.) B. Relation between fractional catabolic rates and plasma insulin levels (r = 0.11, n.s.) C. Relation between VLDL-TG synthesis and plasma insulin levels (r = 0.29, n.s.)

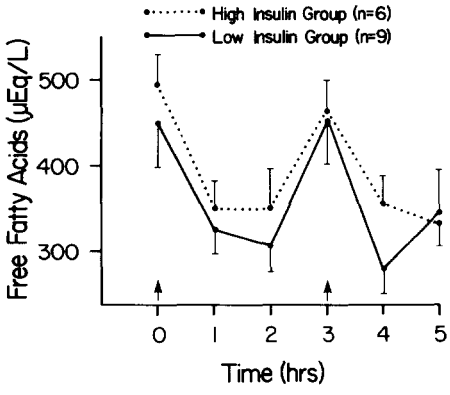

Fig. 7. Plasma free fatty acid levels during study of VLDL metabolism. Notations correspond to Fig. 3.

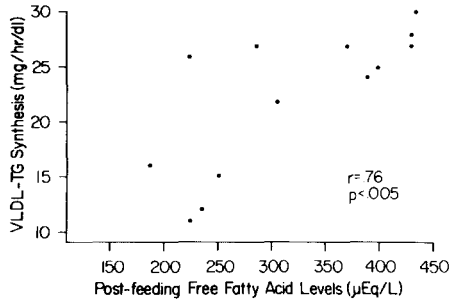

Fig. 8. Relation between VLDL-TG synthesis and free fatty acid levels. Fatty acid levels are the mean of the values at 2 and 5 hr on Fig. 5 (r = 0.76, $P < .005$).

though few normal weight Pimas were available, when the group was divided by degrees of obesity, there was little difference in VLDL production between the subgroups. Thus obesity in the Pima, in contrast to Caucasians, resulted in almost no increase in VLDL production or plasma levels. This finding is consistent with population data in the Pima which did not show a significant correlation of plasma cholesterol and triglyceride levels with body weight or age above age 25 (18).

This population was distinct in having a very small proportion of VLDL-TG synthesis by the slow synthetic pathway. Although the slow/fast pathway ratio was shown to increase with obesity as in Caucasians, it never reached the level found in normal Caucasian controls. The significance of this low proportion of slow pathway and its relevance to the ultimate metabolic fate of VLDL await further studies of the biochemical routes and anatomic location of these two proposed pathways of VLDL-TG synthesis.

The Pima, furthermore, are distinguished by a very rapid clearance (FCR) of VLDL-TG and by a short particle residence time. Analysis of the kinetic data indicated that a high proportion of TG was removed at each delipidation step, and a very low fraction of TG remained for IDL production. Although no direct measurements of particle size were made, the Pima VLDL had a low proportion of cholesterol/TG. All of these factors suggest that large, TG-rich particles are produced and rapidly catabolized. Composition and size of VLDL may be important in determining its rate of clearance (38, 39) and, thus, its ultimate fate.

We explored the relation between plasma insulin and VLDL-TG metabolism in the Pimas, who have a high prevalence of hyperinsulinemia (40) and well documented "insulin resistance" (41). No significant correlation was found between plasma insulin levels and either the VLDL-TG pool size, synthetic rate, or

FCR. These findings contrast with those of Reaven et al (3), Bagdade, Bierman, and Porte (4), and Olefsky, Farquhar, and Reaven (5), who postulated hyperinsulinemia and insulin resistance as causative factors in hypertriglyceridemia. However, other investigators have failed to demonstrate any significant correlation between plasma insulin levels and VLDL-TG synthesis or concentrations (42–44), and, therefore, the role of plasma insulin concentrations in the regulation of VLDL synthesis remains in question.

On the other hand, our data revealed a direct relationship between plasma free fatty acid levels and VLDL-TG synthetic rates. The most significant correlation was between VLDL-TG synthetic rates and either the levels of postprandial free fatty acids or the magnitude of post-prandial decrements. Plasma FFA are a major carbon source for VLDL-TG (7), and relationships between plasma free fatty acids and VLDL-TG production have been observed previously (8, 9, 45). Although measurements of fasting FFA levels in large numbers of the Pima population have not been made, a study of 12 obese nondiabetic Pimas (avg. 166% desirable weight) showed mean fasting FFA levels of 464 μEq/L (32). Thus FFA were not elevated in this obese group, and it was observed, moreover, that they were markedly sensitive to the antilipolytic action of insulin (32). It is possible that the lower VLDL-TG production may be related to a lower availability of FFA to the liver, and further studies on the relationship between VLDL-TG production and FFA transport in this population are warranted.

The Pima data showed no correlation between VLDL-TG catabolism and plasma HDL levels. It has been demonstrated previously that plasma triglyceride levels correlate significantly with adipose tissue lipoprotein lipase activity and inversely with plasma HDL cholesterol levels (46, 47). In the populations used for those studies there was significant hypertriglyceridemia, possibly because TG catabolism by lipoprotein lipase was rate-limiting. In the Pima population, where the FCR for VLDL-TG is higher than normal, other factors may influence HDL cholesterol levels.

In conclusion, the present study has revealed that the Pimas have both a lowered production of VLDL-TG and a more rapid rate of catabolism of VLDL-TG as compared to Caucasians. It is not clear how these changes may be related to the lowered rates of LDL apoB synthesis previously reported (23). A relatively low synthesis of TG-rich particles might provide less apolipoprotein B (apoB) for LDL formation. Based on the mean value obtained for IDL production ($R^{TG}_{0,8}$, Fig. 2) and published values for IDL composition (48), the data predict approximately 900 mg/day of apoB are available for LDL synthesis. This is somewhat more than the 677 mg/day observed for apoB synthesis (23). Alternatively, all of the VLDL-apoB may not be passed on to LDL as in normolipidemic Caucasians (49, 50) and LDL "remnants" may be removed by pathways other than conversion to LDL in the Pimas, as suggested previously by Berman et al. (50), Siggurdson et al. (49), and Reardon, Fidge, and Nestel (51). Further investigation of apoB metabolism will be required to test these possibilities.

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