Relative Contribution of Triglyceride-Rich Lipoprotein Particle Size and Number to Plasma Triglyceride Concentration

Mary Poapst, Michael Reardon, and George Steiner

These studies were undertaken to determine whether differences in plasma triglyceride concentrations reflected differences in the number or in the size of the triglyceride-rich lipoprotein particles in the circulation. A population of 122 men and women, composed of normotriglyceridemic individuals and individuals with endogenous hypertriglyceridemia (plasma triglyceride concentration from 60 mg/dl to 1400 mg/dl), was studied by one of four independent experimental procedures. Rates of triglyceride production and particle (as reflected by apolipoprotein B) production were simultaneously determined. The data suggest that 71% of any increase in triglyceride production was due to an increase in the production of particles, and only 29% was due to an increase in the amount of triglyceride carried per particle. Direct measurement of the triglyceride-rich lipoprotein particle size by electron microscopy demonstrated that for a fivefold difference in triglyceride borne by the Sf 12-400 lipoproteins (from 100 mg to 500 mg triglyceride/dl plasma), the mean particle size rose by only 29%. Hence, 71% of this difference was due to an increase in particle number. The same results were obtained when triglyceride levels were related to the number of triglyceride-rich lipoproteins in the plasma. The number of particles for these studies was either estimated from apo B concentration or by calculations based on the chemical composition of ultracentrifugal subfractions. These studies have used four different approaches to show that changes of plasma triglyceride concentration primarily reflected a change in the number, not the size, of triglyceride-rich lipoprotein particles. Our studies also demonstrated that 75% to 80% of the triglyceride-rich lipoproteins in both normotriglyceridemic and hypertriglyceridemic subjects were in the Sf 12-60 or intermediate density lipoprotein subfraction.

(Arteriosclerosis 5:381-390, July/August 1985)

Until recently, evaluation of the atherogenic risk in various hyperlipidemic states has focused on the concentration of the lipids in whole plasma, or in one or more lipoprotein fractions. This focus has been expanded, and recent studies suggest that the atherogenic potential of plasma lipids may also be related to the size, the composition, or the number of lipoprotein particles transporting these lipids in the circulation. An association between endogenous hypertriglyceridemia (Type IV hyperlipidemia) and atherosclerosis has been documented. However, the mechanisms mediating this link are unclear. In the current literature, investigators have discussed the possibility that the atherogenic risk of endogenous hypertriglyceridemia may be related to the number and composition of circulating triglyceride (TG)-rich lipoproteins or to products of their metabolism.

Endogenous TG is transported primarily in the very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL). Endogenous hypertriglyceridemia reflects an increase in VLDL and IDL TG levels. This could result either from an increase in the mass of TG per particle (seen as an increase in the particle size), from an increase in the number of TG-rich lipoproteins in the circulation, or from a combination of both processes. Because of the possible implications for the atherogenic risk of hypertriglyceridemia, we examined the relationship between plasma TG levels and the size and number of plasma TG-rich lipoprotein (VLDL and IDL) particles. These studies were undertaken in a large group of normolipidemic and hypertriglyceridemic (Type IV) sub-
jects. Plasma TG concentrations for the group ranged from 60 to 1400 mg/dl. Our data suggest that over this range, differences in plasma TG concentration primarily reflect differences in the number, rather than the size of the TG-rich lipoproteins. A preliminary report of some of this work has been made.

Methods
The subjects for these studies were drawn from individuals attending the Ambulatory Care Center of the Toronto General Hospital. They were either normolipidemic or had endogenous hypertriglyceridemia (Type IV hyperlipidemia). In all such subjects, the bulk of plasma TG is transported in VLDL and IDL; that is, in lipoprotein particles falling within the Sf 12-400 spectrum. Subjects were excluded from the study if their fasting plasma showed evidence of floating lipoproteins or of lipoproteins of Sf >400 (which were presumed to be chylomicrons, indicating either that the subject was not fasting or had Type I or V hyperlipoproteinemia). Circumstances did not permit family investigations that were sufficiently detailed to establish the genotypes of the hypertriglyceridemic subjects according to the criteria of Goldstein et al. Apart from elevated TG levels in the hypertriglyceridemic subjects, all were healthy and free from any clinical or biochemical evidence of any disorder that would alter plasma lipids, and none was receiving any food or drug known to influence lipid metabolism. With the exception of one subject who was part of an earlier study (the data of which have been further calculated here), all were within 120% of ideal body weight. Clinical characteristics of the subjects in each of the study groups have been outlined in Table 1. In the course of these studies, no statistically significant differences were found between the values for men and women, and therefore these data were combined.

Lipoprotein Isolation
After a 14-hour fast, subjects gave blood that was drawn into chilled tubes containing Na₂EDTA at a final concentration of 1 mg/ml. The plasma was separated immediately at 4°C, then stored overnight at 4°C. VLDL (Sf 60-400) and IDL (Sf 12-60) subfractions were isolated by sequential ultracentrifugation, as described by Reardon et al. The reasons for selecting Sf 60-400 as VLDL and Sf 12-60 as IDL have been previously reviewed. Briefly, VLDL (Sf 60-400) was isolated by ultracentrifugation at plasma density at 100,000 g for 2 hours at 18°C, in a Beckman SW 40 Ti rotor. The density of the infranatant was then adjusted to 1.019 with KBr, and IDL (Sf 12-60) was recovered from it by ultracentrifugation at 108,000 g for 18 hours at 12°C in a Beckman 70.1 rotor. Both VLDL and IDL fractions were washed once by recentrifugation at their appropriate densities.

Lipid Analysis
The TG and cholesterol concentrations of plasma and the lipoprotein fractions were determined on extracts of these samples by Technicon Autoanalyzer as described previously. In the electron microscopy study, the particle sizes and triglyceride concentrations were determined directly on whole plasma. For these studies, the TG concentrations in the Sf 12-400 fraction were deduced from the relation between TG concentrations of plasma and Sf 12-400 lipoproteins that had been established for a comparable group of individuals (Figure 1).

Table 1. Clinical Characteristics of the Four Groups Studied

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of subjects</th>
<th>Age (yrs)</th>
<th>Plasma triglyceride (mg/dl)</th>
<th>Plasma cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic</td>
<td>22</td>
<td>24-57</td>
<td>116-510</td>
<td>142-288</td>
</tr>
<tr>
<td>Electron microscopic</td>
<td>30</td>
<td>28-58</td>
<td>60-1400</td>
<td>165-258</td>
</tr>
<tr>
<td>Apo B</td>
<td>50</td>
<td>24-62</td>
<td>105-746</td>
<td>149-278</td>
</tr>
<tr>
<td>Ultracentrifugal</td>
<td>20</td>
<td>21-54</td>
<td>84-1400</td>
<td>146-322</td>
</tr>
</tbody>
</table>
Apo B Quantitation

For all studies other than the apo B turnover study that is described in detail below, apo B concentrations were assayed by an electroimmunoassay procedure that had been developed in our laboratory specifically for the measurement of apo B concentration in TG-rich lipoproteins. It was developed to prevent an underestimation of apo B mass resulting either from the masking of apo B antigenic sites in these larger TG-rich particles or from the limited mobility of larger particles through the agarose gel. The procedure involved pretreatment of the lipoprotein samples with a bacterial lipase before conventional electroimmunoassay, as described by Laurell.

Kinetic Studies

The kinetics of the apo B in the Sf 60-400 fraction and of plasma TG were assessed simultaneously with procedures described earlier by this laboratory. Plasma TG kinetics were determined from the slope of the curve describing TG specific activity vs time for an 8-hour period after a bolus injection of 100 \( \mu Ci \) of \(^3\)H-2-glycerol (New England Nuclear Corporation, Boston, Massachusetts). Methods used to determine TG-specific activities and to analyze the data have been described previously.

Apo B kinetics were determined from the data obtained following a bolus injection of radioiodinated, autologous Sf 60-400 lipoproteins (20 \( \mu Ci \) to 40 \( \mu Ci \)). This was injected at the same time as the radioglycerol. The radioiodinated tracer was prepared from fasting blood drawn 5 days before the study. Under sterile conditions, the Sf 60-400 lipoproteins were isolated from plasma samples that were drawn over the next 48 hours. The apo B was isolated by tetramethyl urea (TMU) precipitation and assayed as described by Le et al. Data obtained from the curve describing log apo B-specific activity vs time were analyzed according to the two-pool model proposed by Gurpide et al.

The subjects consumed a balanced, weight-maintaining diet (approximately 40% fat, 40% carbohydrate, and 20% protein) for a 4-week period preceding the turnover study. During the turnover study period, they ate a fat-free diet (approximately 1% calories as fat) that provided the same mass of protein and carbohydrate as they had consumed in the prestudy period. This dietary regimen maintained apo B and plasma TG concentrations at essentially constant levels (<4% variation from zero time) throughout the turnover study period in these and in previously reported studies. To prevent thyroid uptake of radioiodine, potassium iodide (300 mg/day) was prescribed for a 3-week period starting 3 days before the injection of the radioiodinated tracer.

Electron Microscopy

The size of the lipoprotein particles was determined from electron micrographs of osmium tetroxide-fixed particles, shadow-cast as described by Jones and Price. Grids made up from a suspension of polystyrene latex spheres (mean diameter 0.087 ± 0.0046 \( \mu m \), Dow Chemical Company, Midland, Michigan) were included in each shadow-casting run. Unshadowed grids were also prepared from the same suspension of latex spheres. All grids were examined in a Phillips EM 300 transmission electron microscope and photographed at an instrument magnification of \( \times 10,260 \). Individual particles and latex spheres were measured on micrographs at a final photographic enlargement of \( \times 30,780 \) by using a calibrated micrometer lens that afforded an additional magnification of \( \times 10 \). Diameters of at least 200 lipoprotein particles or latex spheres, measured on two or more grids, were taken as representative of each sample. Comparison of the mean diameters of shadowed and unshadowed latex spheres provided a measure of the thickness of shadowing material applied. The thickness of the shadowing material was subtracted from the measured particle diameters to give the diameters of the lipoprotein particles. Measurement of the unshadowed latex spheres also provided a reference standard of known dimensions to confirm the total magnification factor of the final photographic enlargement.

Calculations

In those studies where particle size was measured by electron microscopy, it was possible to calculate the number of TG-rich lipoprotein particles in the plasma subfractions. This was done by dividing the total TG mass in a subfraction by the average mass of TG per particle in that subfraction. Calculations were done by using the electron microscopic and chemical data in the equations below:

\[
\text{volume of TG in particle core (A}^2) = 0.88 \times [1.33 \pi (r^2 - 21.5)] \tag{1}
\]

\[
\text{mass of core TG (mg/particle) = (volume of TG/particle) \times (partial specific volume of TG)} \tag{2}
\]

\[
\text{particle number/dl plasma = \frac{\text{TG mass in lipoprotein fraction (mg/dl plasma)}}{\text{TG mass per particle}}} \tag{3}
\]

In Equation (1), the value, 0.88, equals the volume of TG as a proportion of the total volume of the neutral lipid core in Sf 60-400 and Sf 12-60 particles as determined by gas-liquid chromatography. The val-
ue, 21.5, is the width of the particles' polar surface shell in Å, and $r$ is the mean particle radius in Å. In Equation (2), the partial specific volume of TG was taken as 1.093.25

## Results

### Relationship between the Rates of Plasma Triglyceride Production and VLDL (Apo B) Production

In both normolipidemic subjects and in those with endogenous hypertriglyceridemia, the newly synthesized endogenous TG-rich lipoproteins enter the circulation as Sf 60-400 particles. These are primarily catabolized to Sf 12-60 particles.12,28 Hence, the rate of Sf 60-400 particle production approximates the production rate for the entire Sf 12-400 spectrum of particles. In this study, we have assumed a production rate of Sf 60-400 apo B to reflect the rate of production of VLDL particles for both normotriglyceridemic and hypertriglyceridemic subjects. The assumption is based on the observation of Redgrave and Carlson27 that the molecular mass of apo B per VLDL particle is constant and not significantly different between the two groups. Newly made TG can enter the circulation across the entire Sf 12-400 spectrum.28 Thus, the ratio of the rate of appearance of new TG in the circulation to the rate of appearance of Sf 60-400 apo B should predict the amount of TG incorporated into each newly synthesized endogenous TG-rich lipoprotein particle.

We simultaneously determined the rates of production of plasma TG and VLDL (Sf 60-400) apo B in 22 subjects. Plasma TG concentrations for the group ranged from 116 mg/dl to 510 mg/dl. The rates for both TG and apo B production were significantly higher in the hypertriglyceridemic group as compared with the normotriglyceridemic group, a finding consistent with many previous reports.12,13,29–30 Regardless of the subject's plasma TG concentration, there was a constant relationship between TG and VLDL apo B production rates (correlation $r < 0.001$) (Figure 2). This finding implied that, over the range of apo B production rates, the amount of TG incorporated per particle was relatively constant. Closer examination of the slope of the regression line in Figure 2 indicated that a 100% increase in TG production was accompanied by a 71% increase in VLDL apo B production. In other words, these data predicted that 71% of any increase in TG input into the circulation would be due to an increase in the production of VLDL particles and that only 29% would be due to an increase in the amount of TG carried per particle.

### Relationship between Plasma Triglyceride Concentration and Sf 12-400 Lipoprotein Particle Size

We next undertook to determine by electron microscopy, whether the difference in the amount of TG carried in the TG-rich lipoprotein fraction (Sf 12-400) of normal and hypertriglyceridemic subjects could be accounted for by differences in the size of the lipoproteins. We measured the sizes of the Sf 12-400 lipoproteins in the plasmas of 30 individuals whose plasma TG concentrations ranged from 60 mg/dl to 1400 mg/dl. The TG concentration in the Sf 12-400 fraction for each subject was deduced from his plasma TG concentration as described above, and for this group of subjects the value ranged from 40 mg/dl to 1270 mg/dl.

The diameter of spherical lipoprotein particles can be calculated on the basis of their hydrated density.

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**Figure 2.** The relationship between the production rates for plasma triglyceride (TG) and VLDL apolipoprotein B. The two rates were determined simultaneously for each subject. Fasting plasma TG concentration for each subject is indicated.
and flotation properties on ultracentrifugation. Based on chemical composition, density, and flotation properties, Lossow et al. have calculated that Sf 12 particles should have a diameter of 231 Å, and Sf 400 particles, a diameter of 757 Å. Direct electron microscopic measurement of particle diameters in subfractions of the TG-rich lipoproteins have been found to correspond to, or marginally exceed, these calculated particle diameters. Direct measurement showed us that the Sf 12-400 fraction contained lipoproteins with diameters ranging from 255 Å to 750 Å.

In conducting these studies, we wished to avoid any distortion of particle morphology or size distribution that might be introduced by isolating the Sf 12-400 lipoproteins ultracentrifugally. Therefore, we determined the sizes of the TG-rich particles on whole plasma samples, measuring only those particles within the size range for Sf 12-400 particles as determined above (i.e., particles that were larger than Sf 12 and smaller than Sf 400 lipoproteins). The size distribution spectrums of all lipoprotein particles that lay between 250 Å and 750 Å were plotted for each plasma sample.

The size distribution histograms for each individual with a plasma TG below 160 mg/dl were averaged and were compared to those for individuals with a plasma TG level above 300 mg/dl (Figure 3). As can be seen, the shapes of the histograms were similar, and there was only a slight shift to the right in the hypertriglyceridemic subjects. The modal size distribution in both groups of individuals was between 250 Å and 350 Å. This corresponds to the size of Sf 12-60 particles, the fraction designated IDL.

Similarities in the shape of the size distribution histograms of the Sf 12-400 lipoproteins enabled us to compare the mean particle diameters of the different study subjects. The mean particle diameter was considered in relation to the Sf 12-400 triglyceride concentration in each subject, as shown in Figure 4. There was a linear relationship between the two parameters. Translation of the diameter into the volume of TG in the particle core (see calculations) showed that a 1.45-fold increase in this volume was accompanied by a fivefold increase in Sf 12-400 TG. These data indicated that only 29% of a TG concentration increase could be accounted for by an increase in the mass of TG carried per particle. Therefore, the remaining 71% of the increase in TG concentration reflected an increase in the number of Sf 12-400 particles.

Figure 3. The relationship between the mean particle diameter of the Sf 12-400 lipoproteins in subjects with plasma triglyceride (TG) concentrations of less than 160 mg TG/dl plasma (solid bars) and greater than 300 mg TG/dl plasma (open bars).

Figure 4. The relationship between the triglyceride concentration and the mean particle diameter of Sf 12-400 lipoproteins in the fasting plasma of normal and hypertriglyceridemic subjects, as determined by electron microscopy.
Relationship between the Triglyceride Concentration and Particle Number of 
Sf-400 Lipoproteins as Reflected by 
Apo B Concentration

Since the mass of apo B per particle is equal for all particles in the Sf 12-400 range, the total apo B concentration of this fraction provides an index of the number of Sf 12-400 lipoprotein particles in the plasma. The apo B concentration and the TG concentration of the Sf 12-400 fraction were determined in samples taken from 50 subjects whose plasma TG ranged from 105 mg/dl to 746 mg/dl. The data were plotted in Figure 5. The two parameters were again positively correlated (p < 0.001). Figure 5 demonstrates that for a fivefold increase in the Sf 12-400 TG concentration (from 100 mg/dl to 500 mg/dl plasma), there was a 3.5-fold increase in the apo B concentration. These data indicated that 70% of an increase in the TG concentration could be accounted for by an increase in the number of Sf 12-400 particles which left only 30% to be accounted for by an increase in the TG content per particle. These figures were derived by using apo B concentration as an index of Sf 12-400 particle numbers and were similar to those obtained by the other independent approaches described in this report.

Figure 5. The relationship between the triglyceride concentration and the apolipoprotein B concentration of Sf 12-400 lipoproteins in the fasting plasma of normal and hypertriglyceridemic subjects.

Relationship between the Triglyceride Concentration and Particle Number In 
Sf 20-400 Lipoproteins

The three independent approaches described above suggested that changes in plasma TG concentration were associated more with changes in the number than with changes in the size of the TG-rich lipoproteins. To examine this suggestion with yet another approach, we calculated the changes in particle number in relation to changes in TG concentration from data previously reported from this laboratory. That study included 20 subjects whose plasma TG concentrations ranged from 84 mg/dl to 1400 mg/dl. The TG-rich plasma lipoproteins had been subfractionated by a cumulative, density gradient ultracentrifugation, into three different VLDL subfractions: VLDL₁ (Sf 100-400), VLDL₂ (Sf 60-100), and VLDL₃ (Sf 20-60). The ultracentrifugal subfractionation procedure was evaluated at that time by electron microscopy. The VLDL₁, VLDL₂, and VLDL₃ subfractions were found to have mean diameters of 545 Å, 358 Å and 291 Å, respectively. These mean particle diameters for each subfraction were consistent with those calculated by Lossow et al on the basis of the flotation properties. We have now used this lipoprotein size data together with the TG concentrations.
content of each fraction to estimate the numbers of lipoprotein particles that would be found in the VLDL₁, VLDL₂, and VLDL₃ for each of the 20 subjects in that study population. The sum of these values has given us the particle number per deciliter of plasma for each individual. These, in turn, have now been related to each individual's Sf 20-400 TG concentration (Figure 6). The two parameters (particle numbers vs Sf 20-400 TG) were again found to be positively correlated (p < 0.001). Figure 6 indicates that for a fivefold increase in the Sf 20-400 TG concentration (from 100 mg/dl to 500 mg/dl plasma), there was a 3.6-fold increase in the calculated particle number. These data implied that 72% of the increase in the TG concentration could be accounted for by an increase in lipoprotein particle number. Hence, only 28% of the increase in TG concentration within the Sf 20-400 fraction could be accounted for by an increase in the TG content of the lipoprotein particles. Even though these values were obtained from the Sf 20-400 fraction, they agreed with those obtained from the Sf 12-400 fraction by the other three approaches.

Proportion of Sf 12-60 vs Sf 60-400 Particles

The electron microscopy study showed that the majority of Sf 12-400 particles were in a size range that would describe 12-60 lipoproteins (Figure 3). We therefore sought to determine the actual proportion of Sf 12-400 particles in the Sf 12-60 fraction. This was done by estimating the apo B concentration in the Sf 12-60 fraction and that in the Sf 60-400 fraction. Plasmas from the 50 individuals described in Figure 5 were examined. Their Sf 12-400 lipoproteins were separated into Sf 12-60 and Sf 60-400 subfractions by ultracentrifugation. The apo B concentrations in each subfraction increased linearly as the Sf 12-400 TG concentration rose (Figure 7). There was a small change in the proportion of the particles found in each subfraction at different TG levels. At Sf 12-400 TG concentrations of 100 mg/dl, 300 mg/dl, and 500 mg/dl plasma, the Sf 60-400 subfraction contained 20%, 23%, and 25% of the total particle population respectively. Thus, over this broad range in plasma TG concentrations, 75% to 80% of the TG-rich lipoproteins were present in the smaller or IDL (Sf 12-60) subpopulation.

Discussion

This study was undertaken to assess the relative contributions of the number, as compared to the size, of the TG-rich lipoprotein particles in determining the concentration of TG in fasting plasma. The particle size and number were estimated by one of four independent approaches in a total population of 122 individuals whose plasma TG concentrations ranged between 60 mg/dl and 1400 mg/dl.

The first approach was an examination of the
relationship between rates of TG production and of apo B production for the TG-rich lipoproteins in a group of normo- and hypertriglyceridemic subjects. Our interpretation of the experimental data was based on the premise that the apo B production rate reflected the rate of production of the lipoprotein particles in all of the normotriglyceridemic and hypertriglyceridemic subjects studied.27 All subjects showed a constant relationship between TG and apo B production rates. This suggested that even when TG production increased, there was little change in the amount of TG transported per particle. Thus in the group, 71% of any increase in TG production could be accounted for by an increase in apo B (i.e., particle production). Hence, only 29% of the TG production went into the production of larger (i.e., TG-enriched) particles. Although many reports28, 30, 33, 34 indicate that apo B production increases in hypertriglyceridemia, few researchers have investigated the parallel change in TG production. Using an experimental design different from ours, Chait et al.29 and Kissebah et al.30 examined VLDL TG and apo B production rates in two genetically different forms of hypertriglyceridemia, familial combined hyperlipoproteinemia and familial hypertriglyceridemia. Data from both studies are consistent with increased production of VLDL particles in each disorder. Both investigations further suggested that the VLDL particles in patients with familial hypertriglyceridemia were TG-enriched relative to those in patients with familial combined hyperlipidemia or in normal control subjects. However, calculations made from data in the Kissebah report30 indicate that for a 100% increase in the circulating mass of VLDL TG, the corresponding increase in apo B circulating mass was 70% in subjects with familial combined hyperlipidemia and 65% in subjects with familial hypertriglyceridemia. These data provide further support for our findings, indicating that even in familial hypertriglyceridemia, the differences in TG concentration are primarily attributable to an increased number of TG-rich particles in the circulation. The turnover data presented above had predicted that 70% of any increase in TG transported in the plasma of individuals such as those studied could be accounted for by an increase in the numbers of particles produced and only 30% by an increase in the mass of TG transported per particle. These kinetic studies reflected the production of particles. Three other approaches were subsequently taken to examine this prediction. These examined the characteristics of particles already in the circulation. Although such particles reflected both synthetic and catabolic processes, the other three independent approaches all yielded data that closely corresponded to the predictions from the kinetic studies.

One of these other approaches was to directly measure the size of the circulating TG-rich lipoproteins by electron microscopy. As determined by our techniques, the particle-size spectrums for the SI 12-400 lipoproteins corresponded well with those reported by other laboratories.25, 27, 36 Others have also shown25, 27 that the shape of the particle-size distribution spectrums for normotrighlyceridemic and hyper-

Figure 7. Comparison of the relationship between the triglyceride concentration of SI 12-400 lipoproteins of fasting plasma and apolipoprotein B concentration of SI 60-400 (●) and of SI 12-60 (○) subfractions.
triglyceridemic subjects were similar (Figure 3). Although some shift in the spectrums toward the larger particles was evident in samples from hypertriglyceridemic subjects, 75% to 80% of the total particle population remained in the 250 Å to 350 Å (IDL) range. Since in our population the shapes of the size distribution spectrums were similar for lower and higher plasma TG concentrations, the calculated average particle diameter provided a semiquantitative basis upon which to compare the particle size in individuals with differing plasma TG concentrations. The data from this approach indicated that 71% of any difference in TG concentration could be accounted for by an increase in particle number, and only 29% by an increase in the mass of TG within a particle.

Yet another approach taken in this study utilized knowledge of the lipid composition of different subfractions of VLDL to calculate the number of particles within ultracentrifugally separated subfractions. Specific conditions for density gradient ultracentrifugation designed by Lossow et al. were used to subfractionate the TG-rich lipoproteins on the basis of their flotation properties. By direct electron microscopic measurement, we found that the mean diameter of VLDL was 545 Å, of VLDL₃, 358 Å, and of VLDL₄, 291 Å. These values agreed with the mean diameters originally suggested by Lossow et al. The particle numbers of each VLDL subfraction were calculated on the basis of the TG mass contained in the particle core and the TG concentration of each of these subfractions. The sum of these for each individual (i.e., the number of Sf 20-400 particles in the plasma) was then related to that individual's plasma TG concentration. This approach led us to the same conclusion as in the other studies. In this population, we could account for 72% of any increase in TG concentration by an increase in particle number, and only 28% by an increase in their size.

The final approach used in these studies was to estimate the number of Sf 12-400 particles on the basis of the apo B content of this fraction. The interpretation of these data rested on the earlier demonstrations that apo B concentration provided a valid reflection of the number of particles in this fraction for both normo- and hypertriglyceridemic subjects. It was also based on the assumption that there was no major difference in the distribution of particles between the larger and smaller particles in plasma of normo- and hypertriglyceridemic subjects. Electron microscopy had already proven the latter assumption. In addition, we determined the relative amounts of apo B in the Sf 60-400 and Sf 12-60 fractions. These studies showed that although there was some difference in the distribution of apo B between the fractions, this shift was minor in those with lower plasma TG concentrations compared to those with higher plasma TG levels. At plasma TG concentrations of 500 mg/dl, 75% of the apo B was found in the Sf 12-60 fraction. With apo B used to reflect particle numbers, the findings of these studies agreed with those of the other approaches. Over a plasma TG range from 105 mg/dl to 746 mg/dl, the number of Sf 12-400 particles accounted for 72% of the difference in plasma TG concentration, and hence only 28% of the difference could be accounted for by a difference in particle size.

Thus, the four quite different experimental procedures used in this investigation indicated that, in individuals who were normal or had moderate (up to 1400 mg/dl) endogenous hypertriglyceridemia, the differences in the TG transported by the TG-rich lipoproteins, and hence the plasma TG concentration, is associated primarily with differences in the number of these particles in the circulation and much less with differences in the mass of TG carried per particle. The data indicate that approximately 71% (71%, 71%, 70%, and 72% in the four studies) of any difference in the amount of TG in the TG-rich lipoprotein fraction of the plasma can be accounted for by a difference in the number of those particles in the circulation, and that the remaining 28% to 30% of the difference is attributable to a difference in the TG mass transported per particle.

The agreement between the findings of the four studies, each of which was based on different assumptions and experimental procedures, provides circumstantial support for the approaches that were used in this investigation. The value of these findings lies in three areas. The first is the remarkable consistency of the results. The second is the clear demonstration that the differences in plasma TG concentrations are associated predominantly with a difference in the number, rather than the size of the TG-rich particles in the circulation. The third is that 70% to 75% of the TG-rich lipoproteins have the size and density characteristics of IDL. Although there is ample evidence in the literature of an association between increased apo B production and hypertriglyceridemia, our studies made no assumptions as to whether the increased numbers of apo B-containing, TG-rich lipoproteins associated with hypertriglyceridemia in the present study was due to overproduction of these particles, due to impaired removal of the particles, or due to some combination of these processes.

There is increasing evidence to support the suggestion that intermediate density lipoproteins are atherogenic. The atherogenic importance of increased numbers of particles, even in the presence of normal lipid concentrations, has been documented for LDL in the recent descriptions of hyperapoB-IVa lipoproteinemia. By analogy, it is possible that increased numbers of IDL particles in an individual's plasma could present an atherogenic risk. The present studies demonstrate that 75% of the TG-rich lipoproteins have the size and density characteristics of IDL, and that increases in plasma TG concentrations predominantly reflect increases in the number of these particles. These findings may have important implications for the atherogenic risk of hypertriglyceridemia.
Acknowledgments

We are very grateful to Amnis Kuksis for determining the lipid composition of the lipoprotein fractions by gas liquid chromatography and to Kristine Weltman, Mary Ann Honig, and Anna Josefowicz for their skilled technical assistance. We also thank the nursing and dietary staff of the Clinical Investigation Unit at the Toronto General Hospital for their valuable cooperation in these studies.

References


Index Terms: atherosclerosis • very low density lipoproteins • lipoprotein remnants • apolipoprotein B
Relative contribution of triglyceride-rich lipoprotein particle size and number to plasma triglyceride concentration.
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Arterioscler Thromb Vasc Biol. 1985;5:381-390
doi: 10.1161/01.ATV.5.4.381

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