# Analysis of low-density lipoproteins by preparative ultracentrifugation and refractometry

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SUMMARY A simplified method for the analysis of both the glyceride-rich $S_f$ 20-$10^5$ and the cholesterol-rich $S_f$ 0-20 low-density lipoproteins is presented. It consists of serum lipoprotein fractionation by preparative ultracentrifugation and subsequent quantitative analysis by refractometry. Comparison of this technique with the technically more difficult analytic ultracentrifugal methodology reveals comparable results for these two principal low-density lipoprotein groups. The relationships between these lipoproteins and the serum levels of total lipids, glycerides, total cholesterol, and phospholipids are also presented. One of the advantages of this procedure is that it provides a reliable and reproducible means for quantifying the principal glyceride-bearing lipoprotein group—the $S_f$ 20-$10^5$ lipoproteins.

THE CONCENTRATIONS of serum cholesterol and serum glycerides in large part are reflected by the circulating levels of the low-density lipoproteins. Much of the available ultracentrifugal data (1, 2) on these low-density lipoproteins has been given for four lipoprotein groups: the $S_f$ 0-12, $S_f$ 12-20, $S_f$ 20-100, and $S_f$ 100-400. However, from the point of view of metabolic behavior and response to dietary or pharmacological manipulation, these four lipoprotein classes may be appropriately grouped into two categories — the $S_f$ 0-20 and $S_f$ 20-400. On the basis of hydrated density, these lipoprotein classes may be described as low-density (1.006–1.050 g/ml) and very low-density (0.92–1.006 g/ml) lipoproteins, respectively. The former represent the principal cholesterol-bearing lipoproteins and the latter the major glyceride-bearing lipoproteins.

Unfortunately, the complete ultracentrifugal determination (3) of the low-density lipoprotein spectrum, although it provides intrinsically more information than the method presented here, suffers from the fact that such analysis is both expensive and technically difficult. By far the greatest difficulty is in the final analytical ultracentrifugal analysis itself and not in the preparation stage of lipoprotein isolation, which is a comparatively simple procedure. The following method, a refinement of a previous procedure (4), avoids the complexity of analytical ultracentrifugation by employing instead a relatively simple, accurate, and reproducible refractometric analysis of the low-density lipoprotein fractions obtained by preparative ultracentrifugation.

## METHODS

All preparative runs were made at 18° in a Spinco Model LH ultracentrifuge equipped with a "vacuum sentinel" (5) to eliminate the risk of sample loss due to vacuum failure. Isolation of lipoproteins was accomplished after 18 hr centrifugation at 40,000 rpm in a 40.3 rotor. For each lipoprotein-containing sample, two fractions were pipetted from the top of the tube: the top milliliter, containing all of the lipoprotein fraction, and the second milliliter, which provided a protein- and lipoprotein-free salt reference (or "background") solution for that sample. Pipetting was done in a darkened room on a fixture equipped with a focused light beam allowing visualization of the lipoproteins by their Tyndall scattering. Fractions collected in standard 1-ml volumetric vials were transferred for storage into 9-ml air-tight screw-cap vials (No. 60910, Owens-Illinois, Toledo 1, Ohio) fitted with Teflon gaskets. Because of potential evaporation and condensation within each lipoprotein-containing vial, it is essential for optimal accuracy that refractive index determinations be made as soon as possible after the frac-

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tions are collected (within 4 to 6 hr). Storage at room temperature prior to refractometric analysis is recommended. Refractive index measurements were made with a Bausch and Lomb Precision Abbé Refractometer (6), with a range of $n_D$ from 1.203 to 1.508, thermostated to 26.00 ± 0.02° with a circulating water bath temperature controller (Precision Scientific Co., Chicago, Ill.).

In essence, this method consists in isolating by preparative ultracentrifugation two lipoprotein fractions from each serum sample. The first fraction is the glyceride-rich, very low-density lipoproteins (VLD) less dense than 1.006[1] g/ml (this corresponds approximately to the $S_f$ 20-$10^5$). The VLD lipoproteins are obtained ultracentrifugally without prior density manipulation of serum. For this purpose, 6 ml of serum is directly centrifuged for 18 hr at 40,000 rpm. With regard to density adjustments, serum may be considered to be 94% by volume a salt solution (primarily NaCl) of density 1.0065 g/ml. If 6 ml of serum is unavailable for the VLD fractionation, the difference between the available serum and the recommended 6 ml capacity of the preparative tube is made up with an appropriate volume of a 0.202 molal[2] solution ($\rho_{20}$ = 1.0065 g/ml).

The total low-density lipoprotein fraction ($S_f$ 0-$10^5$) or TLD fraction is obtained by centrifuging for 18 hr at 40,000 rpm a solution consisting of 3 ml serum and 3 ml of a 3.278 molal NaCl salt solution ($\rho_{20}$ = 1.1168 g/ml). The "background" 1.742 molal NaCl solution resulting from the use of the above volumes serves as the reference "background" solution to which all TLD runs must be brought. Thus, if only 2 ml serum is available, a salt solution of the required density is obtained by first adding 0.94 ml of a 0.202 molal NaCl solution to the 2 ml of serum. Then, by adding 3 ml of the 3.278 molal NaCl salt solution to this mixture, the final "background" salt solution will be the same as that resulting from mixing 3 ml serum and 3 ml 3.278 molal NaCl. It should be pointed out that this density, 1.0651 g/ml, is the solution density *before* preparative ultracentrifugation and is equal to 1.0630 g/ml at 26°. Since the TLD preparative tubes weigh approximately 0.4 g more than the VLD preparative tubes, they must be appropriately arranged to achieve rotor balance if run together.

During preparative centrifugation, appreciable salt redistribution occurs from the top to the bottom of the preparative tube. The extent of this redistribution is dependent upon the nature and concentration of the salt as well as the time and conditions of ultracentrifugation. Under the conditions of this method, the redistribution of salt for both the VLD and TLD runs are shown in Fig. 1. Thus, the density of the salt background solution in the top 1 ml of the VLD run falls from approximately 1.0065 g/ml to 1.0061 g/ml and the density of the top 1 ml of the TLD run is reduced from approximately 1.0651 g/ml to 1.0621 g/ml. It is essential to take fully into account this salt distribution if lipoprotein concentrations are to be measured accurately.

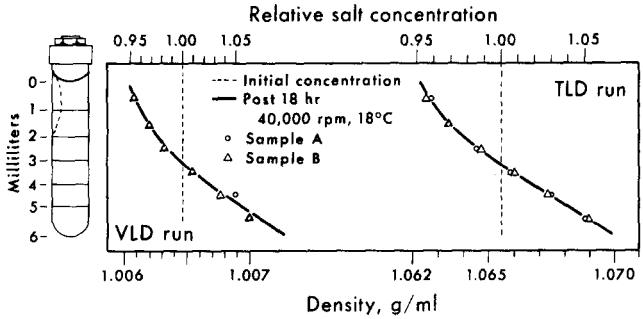


FIG. 1. The ultracentrifugal redistribution of NaCl in both the very low-density (VLD) and total low-density (TLD) preparative runs.

The actual calculations of liproprotein concentrations are made using the following relationship:

$$\text{Lipoprotein concentration, mg\%} = (\Delta S_{TF} - \Delta S_{BG}) \frac{K_1 \times 1{,}000}{C_0 \times S.R.I.}$$

where:

$\Delta S_{TF}$ = Abbé scale reading increment of top fraction above the water reference.[3]

$\Delta S_{BG}$ = Calculated Abbé scale reading increment of top fraction salt "background" solution above the water reference.

$K_1$ = Instrument-dependent conversion factor of scale reading increment to refractive index increment. Our values were:

$$K_1\,(TLD) = 5.30 \times 10^{-3}$$
$$K_1\,(VLD) = 5.48 \times 10^{-3}$$

$C_0$ = Concentration factor of lipoprotein fraction (serum volume/lipoprotein fraction volume).

$S.R.I.$ = Specific refractive increment: 0.00158 for VLD (see Table 1) and 0.00154 for TLD runs. As used here, S.R.I. is the increase in refractive index of the indicated salt solutions resulting from the presence of 1 g lipoprotein/100 ml solution, measured at 26°.

[1] Unless otherwise indicated, all densities are given at 20°.

[2] Solutions are given in molal concentrations (moles/1000 g $H_2O$) to avoid temperature dependence.

[3] A water reference reading is essential before and after each set of refractometric readings, and it is further recommended that additional water readings be taken after every tenth sample reading.

TABLE 1 SPECIFIC REFRACTIVE INCREMENTS OF VLD LIPOPROTEINS IN 0.192 MOLAL NaCl

| Serum | Concentration of Lipoprotein Fractions | | Serum Concentration VLD Lipoproteins | Specific Refractive Increment | |
|---|---|---|---|---|---|
| | Sample A | Sample B | | Sample A | Sample B |
| | *mg/ml* | | *mg/100 ml* | $\Delta n/g/100\ ml$ | |
| 1 | 19.52 | 19.43 | 404 | $0.00158_4$ | $0.00159_2$ |
| 2 | 32.65 | 32.61 | 638 | $0.00154_3$ | $0.00154_4$ |
| 3 | 15.11 | 15.35 | 305 | $0.00159_4$ | $0.00156_9$ |
| 4 | 6.27 | 6.57 | 132 | $0.00165_9$ | $0.00158_3$ |
| 5 | 4.88 | 5.07 | 81 | $0.00157_1$ | $0.00151_2$ |

The contribution of the salt solution present in the centrifuged top fraction is evaluated from measurements on the second milliliter fraction from both the VLD and TLD runs. Refractometry on these fractions allows calculation of the "background" refractive index of the top milliliter aliquots from each individual VLD and TLD run.

Experimentally, the ratio of the refractive index increment (above the water reference) of the first milliliter to the second milliliter, $V_1°/V_2^1$, for both the TLD and VLD salt reference runs as described here, was 0.985. Thus, multiplying the refractive index increment of the respective second milliliter reference fractions by 0.985 yields, in each case, the refractive increment of the "background" salt solution of the lipoprotein-containing fraction. Further, if pipetting difficulties occur, as when large amounts of lipoproteins are present, a pooled first and second milliliter lipoprotein fraction can be taken, together with a third milliliter reference fraction. In this case, the ratio of $V_2^0/V_3^2$ for the salt background solution refractive increment is approximately 0.975 for both the TLD and VLD runs. In all cases, the concentration of lipoproteins is obtained from the difference in refractive index increment (above the water reference) of the lipoprotein-containing fractions and the calculated refractive increment for its appropriate salt "background."

Probably the most convenient and accurate refractometric analysis of small quantities of solution can be made with a precision Abbé refractometer, temperature-controlled to at least 0.1°. The readings should be made at constant temperature somewhat above that of room temperature, in this procedure at 26.0°. In this method, readings should be made in a manner so as to take full advantage of the accuracy of the precision Abbé, with scale readings estimated to the nearest 0.005 units. Over the range of measurements, relative accuracy of refractive index measurements,[4] with proper care, can be made to within approximately ±0.00003 refractive index units. An ordinary Abbé refractometer cannot give sufficient accuracy for this method. A Pulfrich refractometer (7), of course, can provide somewhat greater accuracy of measurement than the Abbé. Because of its convenience and because it is adequate for the purpose, the precision Abbé is recommended.

Refractometry should be done using only one drop of the lipoprotein fraction by taking the "reflection" reading. We have found this to be the most favorable technique for the analysis of turbid lipoprotein fractions as obtained from lipemic sera. Also, nearly all the fraction is then available, if desired, for other lipid analyses. With a capillary pipette, the drop of lipoprotein solution is placed slightly above the center of the outer ground-glass prism and the prism closed immediately. Thereafter, a time delay of exactly 1 min between application of the sample and taking the refractometric reading is recommended. This time is sufficient for approximate temperature equilibration but insufficient to allow any significant evaporation. After each reading, the surfaces of the opposing prisms are thoroughly washed by directing a stream of about 50 ml of distilled $H_2O$ against them and wiping unidirectionally with a nonabrasive wiper (Type 900-S, Kimberly Clark Corp., Neenah, Wisconsin). The use of a polyethylene washing bottle is recommended to avoid scratching the prism surfaces. To further insure completely dry prism surfaces, an unheated air stream is directed for 5 sec into each prism surface using a hair dryer (Model 202, Oster Mfg. Co., Milwaukee, Wisconsin).

Since no data were available on the specific refractive increment (S.R.I.) of the VLD lipoproteins in 0.192 molal NaCl, it was considered necessary to evaluate this S.R.I. experimentally. VLD lipoprotein fractions were prepared from five normal non-fasting males as previously described. Refractive index measurements were made on these lipoprotein samples and their corresponding second milliliter of salt "background" solutions. Duplicate samples, approximately 0.5 ml each, of the lipoprotein fractions and their salt "background" solutions were weighed, blown down under $N_2$ for 30 min at 60° to remove nearly all $H_2O$, and dried to constant weight over $P_2O_5$ in a vacuum oven at 80° (8). Samples were weighed to approximately ±0.05 mg using a Mettler H-16 semimicro analytical balance. In determining the actual weight of the lipoproteins, the salt "background" present in each lipoprotein sample was corrected for sedimentation of the salt and for volume occupied by the lipoproteins. A specific refractive increment of 0.00154 units (9, 10) is used for the TLD

[4] A photoelectric sensing device can be used more effectively to set (with a servo-mechanism) the quadrant of the refractometer to the critical angle characteristic of each sample. Further, with this provision, a direct scale readout is possible.

TABLE 2 COMPARISON OF ANALYTICAL ULTRACENTRIFUGAL (U.C.) RESULTS WITH REFRACTOMETRIC ($\Delta n$) RESULTS (VALUES EXPRESSED IN MG/100 ML)

| Serum No. | $S_f$ 0-20 (U. C.) | TLD − VLD ($\Delta n$) | $S_f$ 20-400 (U. C.) | VLD* ($\Delta n$) | $S_f$ 0-400 (U. C.) | TLD† ($\Delta n$) |
|---|---|---|---|---|---|---|
| 1 | 407 | 375 | 11 | 64 | 418 | 439 |
| 2 | 431 | 356 | 73 | 118 | 504 | 474 |
| 3 | 405 | 367 | 39 | 61 | 444 | 428 |
| 4 | 448 | 429 | 95 | 188 | 543 | 617 |
| 5 | 505 | 472 | 95 | 197 | 600 | 669 |
| 6 | 456 | 403 | 90 | 168 | 546 | 571 |
| 7 | 482 | 421 | 101 | 185 | 583 | 606 |
| 8 | 356 | 347 | 53 | 69 | 409 | 416 |
| 9 | 283 | 241 | 21 | 49 | 304 | 290 |
| 10 | 340 | 353 | 18 | 40 | 358 | 393 |
| 11 | 388 | 347 | 6 | 29 | 394 | 376 |
| 12 | 365 | 338 | 38 | 78 | 403 | 416 |
| 13 | 432 | 370 | 54 | 64 | 486 | 434 |
| 14 | 352 | 304 | 63 | 95 | 415 | 399 |
| 15 | 582 | 550 | 61 | 101 | 643 | 651 |
| 16 | 268 | 270 | 2 | 14 | 270 | 284 |
| 17 | 344 | 279 | 48 | 40 | 392 | 319 |
| 18 | 347 | 235 | 627 | 803 | 974 | 1,038‡ |
| 19 | 320 | 312 | 12 | 41 | 332 | 353 |
| 20 | 571 | 524 | 141 | 150 | 712 | 674 |
| 21 | 277 | 258 | 5 | 9 | 282 | 267 |
| 22 | 329 | 327 | 2 | 9 | 331 | 336 |
| 23 | 498 | 481 | 109 | 165 | 607 | 646 |
| 24 | 269 | 227 | 203 | 258 | 472 | 485 |
| 25 | 447 | 360 | 216 | 286 | 663 | 646 |
| 26 | 188 | 159 | 238 | 292 | 426 | 451 |
| 27 | 579 | 465 | 99 | 113 | 678 | 578‡ |
| 28 | 1,283 | 1,058 | 756 | 793 | 2,039 | 1,851§ |
| 29 | 225 | 229 | 82 | 142 | 307 | 371 |
| 30 | 499 | 411 | 117 | 216 | 616 | 627 |
| Mean values..... | 423 | 376 | 116 | 161 | 538 | 537 |

* All VLD lipoprotein fractions were analyzed at 6 $C_0$.
† Unless otherwise noted, TLD lipoproteins were analyzed at 3 $C_0$.
‡ Analyzed at 2 $C_0$.
§ Analyzed at 1.5 $C_0$.

($S_f$ 0–$10^5$) fraction. This assumes as a first approximation equivalence in S.R.I. for $S_f$ 0–20, $S_f$ 20–100, and $S_f$ 100–$10^5$ lipoproteins.

Total serum lipids were determined by gravimetric analysis, following extraction patterned after Sperry and Brand (11). Phospholipids were determined from the total lipid infrared spectrogram (12). Cholesterol esters and glycerides were simultaneously determined (13) in the neutral lipid fraction after phospholipid adsorption on silicic acid. Total serum cholesterol was calculated from total cholesterol esters using a factor of 0.791. This calculation assumes a mean molecular weight of 650 for serum cholesterol esters and that 75% of the total serum cholesterol is present as cholesterol esters. All infrared analyses were made using a Perkin-Elmer Model 421 grating spectrophotometer.

## RESULTS

Table 1 presents the results of five duplicate determinations of specific refractive increments as determined with $Na_D$ light for the VLD lipoproteins in 0.192 molal NaCl. The mean S.R.I. and standard error of measurement (five duplicate analyses) was 0.00158 ± 0.00003 units ($\Delta n$/g/100 ml). The range of lipoprotein concentrations studied was from 4.88 mg/ml to 32.61 mg/ml, corresponding to a range in serum concentration of from 81 mg% to 638 mg%, respectively. For comparison, the mean and standard deviation of this lipoprotein class for a series of 32 normal non-fasting males, ages 35 to 49, was found to be 173 ± 124 mg%. It should be emphasized that lipoprotein abnormalities, such as may be found in certain pathological sera, might alter the lipoprotein specific refractive increment.

Table 2 shows a comparison of 30 serum analyses by both analytic ultracentrifugation and refractometric determinations. The mean total low-density values (TLD) obtained by the two methods agree closely, which suggests that there are minimal optical dispersion effects and differences between the methods. Also, these data indicate that in the sera studied the concentrations of $S_f$ 400-$10^5$ lipoproteins were low. The higher VLD lipo-

TABLE 3 ANALYSIS OF VLD LIPOPROTEIN FRACTIONS BY ANALYTIC ULTRACENTRIFUGATION AND REFRACTOMETRY (mg/100 ml)

| Serum No. | $C_0$ | VLD (U.C.)* | VLD ($\Delta n$) |
|---|---|---|---|
| 23 | 6 | 150 | 165 |
| 24 | 6 | 211 | 258 |
| 25 | 6 | 260 | 286 |
| 26 | 6 | 258 | 292 |
| 27 | 3 | 105 | 113 |
| 28 | 6 | 836 | 793 |
| 30 | 6 | 176 | 216 |
| | Mean values . . . | 285 | 303 |

* The ultracentrifugal analysis here measures primarily $S_f$ 20-400 lipoproteins but includes, on the average, approximately 10 mg/100 ml lipoproteins of flotation rates less than $S_f$ 20.

TABLE 4 REPRODUCIBILITY OF REFRACTOMETRIC TLD AND VLD LIPOPROTEIN ANALYSES ON A SINGLE SERUM SAMPLE

| TLD Lipoproteins | | VLD Lipoproteins | |
|---|---|---|---|
| Concentration Factor $C_0$ | Serum Concentration | Concentration Factor $C_0$ | Serum Concentration |
| | *mg/100 ml* | | *mg/100 ml* |
| 3 | 945 | 6 | 529 |
| 3 | 928 | 6 | 512 |
| 3 | 934 | 5 | 510 |
| 2 | 917 | 4 | 495 |
| 1 | 888 | 3 | 475 |

protein values determined by refractometry, compared with the $S_f$ 20-400 values determined by analytic ultracentrifugation, suggest that the actual fractionation of the VLD run ($S_f$ 20-$10^5$) probably yields some lipoproteins of flotation rates lower than $S_f$ 20. The actual flotation rate of a lipoprotein of 1.006 g/ml hydrated density is approximately $S_f$ 16 (14). Further, the values of the $S_f$ 0-20 and $S_f$ 20-400 reported here have not been corrected for $S_f$ versus c and Johnson-Ogston effects (15, 3). These corrections, though relatively small for the two major lipoprotein groups (2), would nonetheless tend to increase the value of $S_f$ 20-400 and decrease the value of the $S_f$ 0-20 lipoproteins. However, the primary effect under consideration is that of the self-slowing ($S_f$ versus c effect) of the lipoproteins upon each other. At the mean $S_f$ 0-20 concentrations studied here, the average $S_f$ 20 lipoprotein (present in a medium of approximately 1.4% $S_f$ 0-20 lipoproteins) would exhibit an actual flotation rate of approximately $S_f$ 17.

Table 3 compares the results of seven VLD runs analyzed by both analytic ultracentrifugation and refractometry. For such analyses, a specific refractive increment (S.R.I.) of 0.00158 is used for refractometry and a S.R.I. of 0.00154 employed for analytic ultracentrifugation. For the latter analysis, solid NaCl was added in the amount of 87.2 mg for each milliliter of VLD fraction. This amount, assuming the lipoprotein fraction consists of 1% by volume lipoprotein, is sufficient to bring the small-molecule background density to 1.0638 g/ml. Of course, introducing this amount of salt into each VLD fraction increases the volume and, hence, dilutes the original VLD fraction by a factor of approximately 0.973. Taking this into account, the actual results obtained by each method compare favorably with one another, suggesting again that optical dispersion differences and significant concentration of $S_f$ 400-$10^5$ lipoproteins are apparently not a problem in evaluating the VLD lipoproteins by refractometry.

A measure of the reproducibility of this method, particularly when different amounts of serum are used, is shown in Table 4. Here, for a single serum sample, a reproducibility of approximately ±5% is obtained for both the VLD and TLD runs. An additional measure of anticipated error may be calculated from duplicate analysis to give a standard error of measurement. For six duplicate analyses of the VLD lipoproteins and nine duplicate analyses of the TLD lipoproteins, a $\sigma_{S.E.M.}$ = 5 mg% and a $\sigma_{S.E.M.}$ = 27 mg% were obtained, respectively, for the VLD and TLD lipoproteins. Further, in another group of 16 duplicate VLD samples analyzed at both 3 $C_0$ and 6 $C_0$, the standard error of measurement was 6 mg%. The mean value in both series of analyses was 174 mg%. This accuracy and reproducibility compares favorably with that observed for lipoproteins determined by analytic ultracentrifugal analysis (2).

In a series of 32 non-fasting normal males, ages 35 to 49, the relationships between concentrations of the various total serum lipid classes and the low-density lipoproteins determined by refractometry are shown in Table 5. For this same series, Table 6 shows the mean levels, standard deviations, and standard errors (of the mean) for all the lipid and lipoprotein measurements. It should be pointed out that differences in methodology between chemical procedures commonly used and our infrared technique may slightly alter the levels of each lipid constituent analyzed but would not be expected to alter the relationships observed between the lipoproteins and the various serum lipid classes.

## DISCUSSION

It is evident that the refractometric determination of the low-density lipoproteins is not entirely equivalent to an analytical ultracentrifugal determination. In the first place, lipoproteins above $S_f$ 400, although usually present at relatively low abundance, are measured by refractometry but are not customarily measured by analytic ultracentrifugation. On the other hand, the

TABLE 5 CORRELATIONS BETWEEN LOW-DENSITY LIPOPROTEIN AND SERUM LIPID VALUES IN 32 NORMAL NON-FASTING MALES, AGES 35–49. PEARSON PRODUCT-MOMENT CORRELATION COEFFICIENTS (16)*

| | TLD | VLD | TLD – VLD | Total Lipid | Triglycerides | Total Cholesterol | Phospholipids |
|---|---|---|---|---|---|---|---|
| VLD | 0.82 | | | | | | |
| TLD – VLD | 0.63 | 0.07† | | | | | |
| Total lipid | 0.96 | 0.80 | 0.58 | | | | |
| Triglycerides | 0.82 | 0.99 | 0.08† | 0.82 | | | |
| Total Cholesterol | 0.73 | 0.30† | 0.86 | 0.77 | 0.32† | | |
| Phospholipids | 0.82 | 0.54 | 0.68 | 0.90 | 0.57 | 0.90 | |
| $A.I._{\Delta n}$‡ | 0.97 | 0.94 | 0.41§ | 0.94 | 0.94 | 0.57 | 0.73 |

* All values significant below the 1% level unless otherwise indicated.
† Not significant.
‡ A refractometric atherogenic index (A.I.) approximately equivalent to the analytic ultracentrifugal A.I. (17) is given by: $A.I._{\Delta n}$ = 0.100 (TLD – VLD, concentration in mg/100 ml) + 0.195 (VLD, concentration in mg/100 ml).
§ Significant at the 5% level.

more subtle features of the entire low-density lipoprotein distribution, revealed in detail with an analytic ultracentrifuge determination, are not obtained with refractometry. Further, certain discrepancies still exist in the determination of the low-density lipoproteins by refractometry and by analytic ultracentrifugation. Such small discrepancies may be due in part to the presence of non-migrating lipoproteins in each of the lipoprotein fractions, thus giving an apparently lower concentration of lipoproteins detected by the Schlieren optical system. Further, some small dispersion effects may be anticipated in that refractive index measurements with the precision Abbé are made with the $Na_{D_{1,2}}$ lines (5890A and 5896A), whereas the analytic Schlieren diagram giving $dn/dx$ (and by integration total lipoprotein $\Delta n$) is obtained primarily with the green line of the Hg arc (5461A). Despite these small discrepancies, however, the principal low-density lipoproteins may be measured refractometrically and compared to analytic ultracentrifuge values even in the absence of a full theoretical explanation of these differences.

The comparison of lipoprotein values with the serum levels of glycerides, cholesterol, phospholipids, and total gravimetric lipid provides additional insight into the interrelationships between the serum lipids and the two major low-density lipoprotein groups. Several of these relationships are worthy of comment. Of particular interest is the very high correlation coefficient (r = 0.99) between the VLD lipoproteins and the total level of serum glycerides. Although, in the normal males studied, approximately 70% of serum glycerides are carried by this lipoprotein group, the correlation seems unusually high. For comparison, approximately 70% of the serum cholesterol is carried by the TLD lipoproteins, yet the correlation between these lipoproteins and serum cholesterol is only 0.73. Further, the correlation between the major cholesterol-bearing $S_f$ 0-20 class (TLD – VLD lipoproteins) and the total serum cholesterol is 0.86. Therefore, it would appear that with the exception of serum glycerides, the relationships between serum lipid values and the low-density lipoproteins are not simple ones.

TABLE 6 TOTAL SERUM LIPID VALUES AND LOW-DENSITY LIPOPROTEIN VALUES IN 32 NORMAL NON-FASTING MALES, AGES 35–49

| Measurement | Mean | Standard Deviation | Standard Error |
|---|---|---|---|
| | *mg/100 ml* | *mg/100 ml* | *mg/100 ml* |
| TLD ($S_f$ 0–$10^5$) | 590 | 158.3 | 28.8 |
| VLD ($S_f$ 20–$10^5$) | 173 | 123.5 | 22.5 |
| TLD – VLD ($S_f$ 0–20) | 417 | 91.3 | 16.6 |
| Total lipid | 796 | 157.9 | 28.7 |
| Triglycerides | 172 | 97.4 | 17.7 |
| Total cholesterol | 251 | 45.2 | 8.2 |
| Phospholipids | 267 | 39.3 | 7.1 |
| $A.I._{\Delta n}$ | 75 | 26.2 | 4.8 |

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