Large Buoyant LDL-like Particles in Hepatic Lipase Deficiency

Johan H. Auwerx, Carol A. Marzetta, John E. Hokanson, and John D. Brunzell

Hepatic lipase (HL) is thought to play a role in processing very low density lipoprotein to low density lipoprotein (LDL). To analyze the relationship between HL and LDL, the density, size, and chemical composition of LDL isolated from 18 normal subjects and from three subjects with reduced or absent levels of HL activity were compared. In an HL-deficient subject, the major peak of apoprotein (apo) B-containing lipoproteins ("LDL") had a density of 1.023 g/ml and a diameter of 28.4 nm compared to male control subjects (1.044±0.006 g/ml and 25.3±0.3 nm). Two half-sisters of the HL-deficient subject with half the normal levels of HL activity had LDL that also were more buoyant and slightly larger than the LDL isolated from female control subjects. The peak density and average diameter of LDL were correlated with HL activity, consistent with the hypothesis that HL influenced formation and physical characteristics of typical LDL. Apo B-100 was the major apoprotein in the "LDL" isolated from the HL-deficient subject and contained a greater proportion of triglyceride compared to the control subjects' LDL. The absence of HL appears to prevent the production of classical LDL. Our data support the hypothesis that HL helps determine normal LDL characteristics.

(Arteriosclerosis 9:319–325, May/June 1989)

The formation of very low density lipoproteins (VLDL) involves both triglyceride (TG) hydrolysis with remodeling of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), as well as potential direct hepatic production of LDL particles (see reviews1,2). A complicated picture of a "delipidation cascade" is emerging in which the factors that determine whether VLDL will be removed from the circulation or undergo conversion into LDL is just being delineated. Several intravascular enzymes are thought to be involved in the formation of LDL, including lipoprotein lipase (LPL), lipid transfer protein, and hepatic triglyceride lipase (HL). Rubinstein et al.3 have hypothesized that HL is involved in the conversion of IDL to LDL; however, direct experimental data describing the mechanisms involved in this conversion are, as yet, undefined.3,4

The conversion of VLDL remnants or IDL to LDL is thought to occur across the splanchic bed where HL is located.5,6 Support for a role of HL among the factors involved in the processing of IDL to LDL has been derived from observations made on humans and animals with HL deficiency: humans with diseases associated with reduced levels of HL activity (hypothyroidism, uremia, chronic liver disease); and in experimentally induced HL deficiency in rats and monkeys.11-14 In these studies, reduced levels of HL were associated with an increase in IDL concentration and TG accumulation in LDL.6-14

The present study was designed to examine the possible relationships between HL activity and LDL physical characteristics in normolipidemic subjects and in subjects with reduced or absent HL activity levels.

Methods

Study Subjects

Eighteen healthy Caucasian subjects (nine women and nine men), with a mean age of 35.4±11.3 years for women and 26.9±5.6 years for men (range, 18 to 48 years), were recruited for this study, and they participated on a voluntary basis. None of these subjects took any medication or had evidence of a lipoprotein disorder. All patients belonged to a single family with HL deficiency (Auwerx et al., unpublished data). The proband (PG) is a 50-year-old man with complete absence of HL activity in postheparin plasma. He was detected as having hypertriglyceridemia and was subsequently characterized as having B-VLDL and an E3/E3 phenotype. On all examinations, this man had no detectable HL activity in postheparin plasma. Two half-sisters, KG and JG, were also studied and had approximately half the normal levels of HL activity. The HL-deficient subject (PG) had been taking clotilde since 1866 (which resulted in a drop of his TG from the 2000 to 4000 mg/dl range to less than 1000

From the Division of Metabolism, Department of Medicine, University of Washington, Seattle, Washington, Johan H. Auwerx is now at the Department of Developmental Biology, LEGENDO (O.N. 4*3), Campus Gasthuisberg B-3000 Leuven, Belgium.

This work was supported by NIH Grants DK 02456 and HL 30068. A portion of this study was performed in the Clinical Research Center at the University Hospital (NIH RR-37). Johan H. Auwerx is a recipient of a travel grant of the NFWO (Belgium) and the North Atlantic Treaty Organization (NATO). Carol A. Marzetta was supported by an institutional National Service Award, HL-07396.

Richard J. Havel, M.D. kindly acted as Guest Editor for this manuscript.

Address for correspondence: John D. Brunzell, Department of Medicine, Division of Metabolism RG-26, University of Washington, Seattle, WA 98195.

Received January 29, 1988; revision accepted December 2, 1988.
mg/dl), while IG and JG were not on lipid-lowering drugs or other medications that affect lipid metabolism.

**Blood Collection**

After an overnight fast, a blood sample was drawn into 0.1% disodium ethylenediaminetetraacetic acid. An intravenous heparin bolus of 60 IU/kg was then administered, and blood was collected in lithium-heparin tubes. Plasma was separated immediately by centrifugation and stored at either 4°C until lipoproteins were analyzed (started on the same day) or at -70°C until lipase activities were determined.

**Density Gradient Ultracentrifugation**

Discontinuous salt gradients were prepared in SW-41 ultracentrifugation tubes (Beckman Instruments, Palo Alto, CA) by underlaying 5.2 ml of saline (1.006 g/ml), 4 ml of a 1.063 g/ml solution, and 2.5 ml of plasma adjusted to a density of 1.21 g/ml as described. After centrifugation (15°C for 24 hours at 41,000 rpm), the samples were eluted from the top of the tube by pumping 1.85 ml of a dense solution (Fluorinert, 3M Company, St. Paul, MN) into the bottom of the tube at a rate of 0.8 ml/min. The effluent was monitored continuously at 280 nm with a flow cell spectrophotometer (LKB Productor, Bromma, Sweden); 36 fractions of 0.31 ml each were collected for each sample. The density of every other fraction was measured at room temperature by pycnometry. The fractions selected to contain the material defining the major peak of LDL were pooled after density gradient ultracentrifugation (DGUC). The density ranges defining these fractions varied among the subjects and were based on the elution profiles of each LDL sample to ensure that the pooled samples contained the entire lipoprotein peak.

**Single Vertical Spin for Apolipoprotein B Containing Particles**

This technique is a modification of the single spin density ultracentrifugation developed by Chung et al., which optimizes the resolution of apo B-containing lipoproteins (SVS-apo B). A discontinuous salt gradient was formed in Sorval TV-855B (DuPont Company, Wilmington, DE) tubes by underlaying 5 ml of plasma adjusted to a density 1.080 g/ml underneath 12 ml of a 1.006 g/ml NaCl solution. Samples were centrifuged at 65,000 rpm for 60 minutes at 10°C (total w of 2.36 x 10^14) and then were fractionated from the bottom of the tube (flow rate 1.7 ml/min). Thirty-eight fractions were collected (0.45 ml/tube), and the total cholesterol was measured in each fraction. The buoyancy of LDL was characterized by its relative floating number (RF) obtained by dividing the fraction number containing the LDL peak by the total number of fractions collected.

**Sequential Flotation Procedures**

Lipoproteins of d<1.063, 1.006 to 1.019 g/ml, or 1.019 to 1.063 g/ml were prepared by sequential ultracentrifugation in a 40.3 rotor (Beckman Instruments, Palo Alto, CA).

**Nondenaturing Gradient Gel Electrophoresis**

Four micrograms of protein from each <1.063 g/ml lipoprotein sample were applied to alternate wells on 2% to 16% polyacrylamide gradient gels (Pharmacia, Uppsala, Sweden) and electrophoresed as described. Latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin, and ferritin (Pharmacia) with diameters of 38.0, 17.0, and 12.2 nm, respectively, were run as standards. Each lane was scanned at 555 nm after staining for protein with Coomassie blue. The diameter of each LDL was determined by using a calibration curve generated from the standards.

**Sodium Dodecyl Sulfate Polyacrylamide Gradient Gel Electrophoresis**

Apo proteins from LDL isolated by DGUC were electrophoresed on 4% to 30% sodium dodecyl sulfate polyacrylamide gradient gel electrophoresis (SDS-PAGE) as described previously. Apo proteins isolated from d<1.006 g/ml lipoproteins from a patient with LPL deficiency were run as standards.

**Lipoprotein and Hepatic Triglyceride Lipase Activity**

LPL and HL activity were measured in postheparin plasma by a method described previously. Aliquots of postheparin plasma were incubated with the substrate containing trioleoylglycerol, glycerol-tri-[1-14C]oleate (Amersham, Arlington Heights, IL), and lecithin for 60 minutes at 37°C. Enzyme activity is expressed as nanomoles of free fatty acids (FFA) released per minute per milliliter of plasma at 37°C. LPL activity was calculated as the lipolytic activity removed from plasma by incubation with a specific monoclonal antibody against LPL, and HL activity was determined as the activity remaining after incubation with the LPL antibody. For each assay, a lipase standard was included to correct for interassay variation, which averaged 7.2%.

**In Vitro Incubation of Low Density Lipoprotein with Hepatic Lipase**

The major peak of apo B-containing particles was isolated from the subject with HL deficiency (PG) and from a normal control by DGUC. Postheparin plasma from an LPL-deficient patient was obtained as a source of HL. The LPL-deficient patient had an LDL cholesterol of 19 mg/dl with an LDL peak density of 1.055 g/ml as determined by DGUC. Chylomicrons from the postheparin plasma were first removed by ultracentrifugation at 50,000 rpm for 4°C in a 60 Ti rotor for 60 minutes. The remaining plasma was used immediately in the incubation studies to minimize loss of HL activity. Two milliliters of postheparin plasma without chylomicrons from the LPL-deficient patient (HL activity = 183 nmol FFA/min/ml), with 550 μg of LDL protein from either PG or the normal subject, and 200 μl 30% human serum albumin (Sigma, St. Louis, MO) were incubated for 1 hour at 37°C or 4°C (for the control). The total incubation volume was 2.45 ml. Incubations were done in duplicate and were stopped by placing the samples on ice. The density of 2 ml of each incubation mixture was then raised to 1.21 g/ml with KBr and was underlay-
Table 1. Lipid and Lipoprotein Characteristics of Study Subjects

| Subjects | Age (yrs) | Total Chol (mg/dl) | TG (mg/dl) | HDL-Chol (mg/dl) | LDL-Chol (mg/dl) | Apo B (nmol FFA/min/ml) | HL activity | LPL activity |
|----------|-----------|------------------|-----------|-----------------|-----------------|------------------------|-------------|--------------|
| Controls |           |                  |           |                 |                 |                        |             |              |
| Men (n=9) | 26.9±5.6  | 161±42           | 71±24    | 51.10           | 103±26         | 88±22                  | 147±53      | 158±62       |
| Women (n=9) | 35.4±11.3 | 175±20           | 56±19    | 64±15           | 102±14         | 84±19                  | 85±51*      | 210±91       |
| PG       | 50        | 196              | 369      | 42              | 107            | 177                    | 0           | 102          |
| IG       | 42        | 183              | 66       | 101             | 72             | 62                     | 39          | 261          |
| JG       | 43        | 166              | 111      | 55              | 90             | 97                     | 31          | 126          |

Values are expressed as means±SD.

Chol = cholesterol, TG = triglyceride, LDL-Chol = LDL cholesterol, HDL-Chol = HDL cholesterol, HL = hepatic lipase, LPL = lipoprotein lipase.

*Statistically different from the control men, p<0.001.

Table 2. Low Density Lipoprotein Composition and Characteristics

| Subjects | LDL diameter (nm) | LDL density (g/ml) | RI-value | Cholesterol ester (%) | Triglyceride (mg/dl) | Free cholesterol (mg/dl) | Phospholipid (mg/dl) |
|----------|------------------|--------------------|----------|-----------------------|----------------------|------------------------|----------------------|
| Controls |                  |                    |          |                       |                      |                        |                      |
| Men (n=9) | 25.3±0.3         | 1.044±0.006        | 0.298±0.002 | 57.7±13.0            | 6.9±3.2              | 11.2±2.3               | 24.2±6.2             |
| Women (n=9) | 25.4±0.2        | 1.042±0.000        | 0.297±0.000 | 58.3±10.6            | 6.0±2.6              | 11.8±1.8               | 23.9±5.1             |
| PG       | 26.4              | 1.023              | 0.405    | 20.6                 | 40.6                 | 6.9                    | 31.9                 |
| IG       | 26.8±26.8         | 1.031±1.035        | 0.324    | 50.9                 | 11.9                 | 14.6                   | 22.6                 |
| JG       | 26.5              | 1.027              | 0.324    | 53.0                 | 14.3                 | 11.3                   | 21.4                 |

*Determined by nondenaturing gradient gel electrophoresis; †determined by density gradient ultracentrifugation; §RI-value = relative flotation value determined by single vertical spin for apoprotein B containing particles; ¶composition data expressed as lipid composition in mass percent. LDL = low density lipoprotein.

erated into a discontinuous salt gradient and was subjected to DGUC. Additional aliquots of the incubation mixture were frozen for HL and LPL activity determination.

Other Procedures

Total plasma cholesterol, LDL cholesterol, HDL cholesterol, phospholipid, and triglyceride determinations were performed according to the procedures of the Lipid Research Clinic.²⁴ Protein was determined by the method of Lowry et al.²⁵ by using bovine serum albumin as standards. Apo B and apo E were analyzed by radioimmunoassay (Albers J and Adolphson J, unpublished method).

Statistics

The results are expressed as the means±SD. Where appropriate, an unpaired t test was applied.

Results

The lipid and lipoprotein characteristics of the subjects with abnormalities in HL subjects were compared to the control subjects (Table 1). The control subjects had TG, total and LDL cholesterol, and plasma apo B concentrations below the 75th percentile and HDL cholesterol above the 25th percentile for each parameter as defined by the LRPC Population Studies.²⁷ No statistical differences were seen between the male and female subjects in any of these measurements. The control female subjects, however, had significantly lower HL activity than the control male subjects (85±51 vs. 147±53 nmol FFA/min/ml, respectively; p<0.001). In addition, the female control subjects had slightly higher LPL activities compared to the male control subjects, but these differences were not statistically significant (210±91 vs. 156±62, respectively).

In contrast to the control subjects, the HL-deficient subject (PG) had elevated plasma TG and apo B concentrations. IG and JG had approximately 40% of the HL activity compared to the control females (39 and 31, respectively, vs. 85 nmol FFA/min/ml). LPL activity was low normal in PG and JG and high normal in IG, but all were within the 90% confidence limits of normal controls.

The physical characteristics of LDL isolated from control subjects and the patients with HL abnormalities were analyzed using DGUC, SVE-apo B, and nondenaturing gradient gel electrophoresis (GGE) (Table 2). In general, the patients with abnormal HL activity had a larger and more buoyant major lipoprotein fraction compared to the LDL from control subjects. An SVE-apo B lipoprotein cholesterol profile (Figure 1A) and a densitometric scan of d<1.063 lipoproteins separated by GGE from a control male subject (Figure 1B) with the RI of 0.297 and diameter of 25.2 nm are representative of the control group. The HL-deficient subjects, PG (Figures 1C and 1D), had a major peak of lipoproteins (LDL) which was unusually buoyant (RI=0.405) and large (25.4 nm) compared to the control subjects' LDL.

The "LDL" (1.016<d<1.063) isolated from PG had a higher percentage of TG and PL compared to the control LDL (Table 2). Based on SDS-PAGE, 'LDL' isolated
from PG by DGUC contained primarily apo B-100 and very little apo E (Figure 2). The apo E/apo B mass ratio of PG's 'LDL' was similar to the apo E/apo B in the control LDL determined in a subset of nine normal persons with peak densities between 1.036 and 1.049 g/ml (0.01 vs. 0.03±0.01, respectively).

The density and size characteristics of PG's major peak of lipoproteins were intermediate to LDL and IDL; therefore, additional DGUC analyses were done to determine the characteristics of these lipoproteins with reference to classically defined IDL (1.006<d<1.019) and LDL (1.019<d<1.063). The d<1.006 and 1.006<d<1.019 lipoprotein fractions were first isolated by sequential ultracentrifugation and then subjected to DGUC under identical conditions (Figure 3). The peak density of the major population of lipoproteins was 1.023 g/ml compared to 1.044 g/ml for male controls (see Table 2). The peak density of particles isolated within the IDL density range (d<1.019 g/ml) was 1.020 g/ml and contained approximately half of the mass of the original peak of apo B containing particles. The peak of the d>1.019 fraction was 1.027 g/ml. Although the major population of apo B containing lipoproteins isolated at an intermediate position to classically defined IDL and LDL, these particles contained primarily apo B-100 and are referred to as 'LDL' to differentiate them from IDL and LDL of the control subjects.

To determine whether HL could convert PG's 'LDL' to particles more typical of control LDL, an in vitro incubation study was done with postheparin plasma isolated from an LPL-deficient subject as the source of HL. Incubation of PG's 'LDL' with HL resulted in an increase in the peak density of the 'LDL' from 1.024 to 1.033 g/ml (Table 3). The control LDL incubated under identical conditions had only a slight increase in peak density from 1.036 to 1.038 g/ml. 'LDL' from PG and the normal subject incubated with purified LPL did not change the densities of these particles (data not shown).
Figure 3. Lipoprotein elution profiles by density gradient ultracentrifugation of d < 1.006 fraction (--), 1.006-< d < 1.019 fraction (---), and d > 1.019 fraction (- -) first isolated by sequential ultracentrifugation from the hepatic lipase-deficient patient. Peak density in g/ml is given for each profile.

Table 3. Low Density Lipoprotein Incubation Study

|                  | Before incubation | After incubation | Difference (peak density in g/ml) |
|------------------|-------------------|------------------|-----------------------------------|
| PG (LDL)         | 1.024             | 1.033            | 0.009                             |
| Normal LDL       | 1.038             | 1.036            | 0.002                             |

* Determined by density gradient ultracentrifugation and pycnometry.

The diameter of LDL correlated with its density in the control and abnormal subjects (Figure 4). Absolute HL deficiency (PG) was associated with the most buoyant 'LDL' particles, while half-normal levels of HL (IG and JG) were associated with 'LDL' intermediate to the buoyant particle in HL deficiency and normal LDL.

Discussion

The physical and chemical characteristics of the major apo B containing particles in the subjects with varying degrees of reduced HL activity were distinctive. The patient with HL deficiency had an accumulation of a TG-enriched, large and buoyant apo B containing lipoproteins as determined by DGUC, SVS-apo B, SDS-PAGE, and nondenaturing GGE. These observations correspond with previous studies that have shown the accumulation of TG-rich lipoproteins and slightly more buoyant 'LDL' in both familial and acquired forms of HL deficiency as described by analytical ultracentrifugation. Triglyceride-enriched 'LDL' from HL-deficient subjects differs dramatically from dense LDL isolated from subjects with hypertriglyceridemia. 29,30 Deckelbaum et al. 29,30 have shown that lipid transfer protein can exchange VLDL-TG with LDL cholesteryl ester, resulting in net transfer and an enrichment of LDL with TG in vitro. The subsequent action of lipoprotein lipase can then hydrolyze the TG in the in vitro modified LDL and result in smaller more dense LDL. 29,30 Thus, the buoyant, TG-enriched, large 'LDL' seen in the HL-deficient subject (PG) are especially striking when compared to LDL found in hypotriglyceridemic subjects. The patients with intermediate levels of HL (IG and JG) also accumulated moderately TG-enriched large and buoyant LDL when compared to LDL isolated from the control subjects. It is unclear why buoyant 'LDL' persist in subjects with reduced or absent levels of HL activity when LPL is available. Perhaps these lipoproteins are poor substrates for LPL, similar to normal LDL. 31,32,33

The major apo B containing lipoproteins isolated from the HL-deficient subject (PG) have physical characteristics between classically defined IDL and LDL and contain primarily apo B-100. These lipoproteins probably represent the final product of VLDL and IDL metabolism in this subject. As illustrated in the subject with HL deficiency, the assumed final or terminal product of VLDL and IDL metabolism may not be completely isolated within the traditionally defined density or flotation intervals of LDL.

The abnormalities in the composition of 'LDL' from the HL-deficient subject may be due to a complex interplay between lipoprotein production and modification of the varying apo B containing particles and HL. HL, an enzyme synthesized by the liver, has been implicated in helping to determine the physical characteristics of LDL. 3,4 Although there was no correlation between LDL size or density and HL activity among the normal subjects, this may be due to the small variation in LDL size and density, the large variability in HL activity (see Table 2), or the inability to 'normalize' these parameters to account for differences in age, body weight, and gender. However, an association between LDL size or density and HL activity existed when control subjects and those with HL deficiency were analyzed together.

Complete HL deficiency as in PG or partial deficiencies in HL activity seen in his half-sisters (IG and JG) are...
associated with a more buoyant and larger 'LDL'. The effect of HL deficiency can help explain the alterations in lipid composition of the LDL particle. That is, HL catalyzes the hydrolysis of mono-, di-, triacylglycerol, and phospholipid, resulting in a decrease in TG/ protein ratio,4,35,36 consistent with the TG and PL enrichment seen in the HL-deficient subject. Apoprotein composition was not unusual in the subjects with HL abnormalities, with apo B-100 being the major apoprotein in LDL from the normal subjects as well as in the 'LDL' from PG, IG, and JG. Although the LDL isolated for these studies were prepared by ultracentrifugation, which can lead to loss of apo E from the lipoproteins,4,36 all samples were subjected to only 24 hours of centrifugation (compared to >70 hours when using conventional sequential ultracentrifugation methods), and the samples were processed by the same techniques. Small amounts of apo E were detected by SDS-PAGE and radioimmunoadsorbent in both LDL from the control subjects and the 'LDL' from the HL patient. However, no apo E enrichment of the 'LDL' fraction was detected.

The compositional differences between normal LDL and HL-deficient 'LDL' suggest that HL is responsible, in part, for VLDL to LDL processing. We therefore tested the potential of HL to convert the abnormally buoyant and large 'LDL' seen in the HL-deficient subject particles more typical of normal LDL. PG's 'LDL' were incubated with a source of HL from postheparin plasma isolated from an LPL-deficient subject. After incubation with HL, the mean density of the 'LDL' increased from 1.024 g/ml to 1.033 g/ml, consistent with this suggested role for HL.

In summary, we have described studies of the unique characteristics of the major population of apo B containing lipoproteins isolated from a subject with HL deficiency and his two half-sisters with approximately half the normal levels of HL activity. These lipoproteins were in a density and size range between classically defined LDL and IDL; this emphasizes the problem with using traditional density cuts to define lipoprotein classes. The possible relationship between the physical characteristics of LDL and HL activity, as seen in the subjects with abnormal HL activity, is suggestive of a role for HL in partly determining LDL characteristics. The absence of HL appears to prevent the appearance of classical LDL and results in an accumulation of larger, more buoyant particles.

Acknowledgments

The technical assistance of M. Kimura and the word processing by Janetta Shepard and Marilyn Gorder are kindly acknowledged. We thank the members of the G family for their participation and support in this study.

References

1. Havel RJ. The formation of LDL. Mechanisms and regulation. J Lipid Res 1984;25:1570–1576
2. Rudel LL, Parke JS, Johnson FL, Babuk J. Low density lipoprotein in atherosclerosis. J Lipid Res 1986;27:465–474
3. Rubinstein A, Gibson JC, Pernetti JR, et al. Effect of heparrin-induced lipolysis on the distribution of apolipoprotein E in hepatic triglyceride lipase and lipoprotein lipase. J Clin Invest 1985;75:710–721
4. Kinnunen PK, Virtanen JA, Valno P. Lipoprotein lipase and hepatic endothelial lipase. Their role in plasma lipoprotein metabolism. Atheroscler Rev 1983;11:65–105
5. Turner PH, Miller NE, Cortsas G, Hazzard W, Cottart J, Lewis B. Splanchnic metabolism of apolipoprotein B. Studies of artery-hepatic vein differences of mass and radioactivity in fasted human subjects. J Clin Invest 1981;67:1678–1686
6. Yamada N, Murase T, Akanuma Y, Rakura H, Kosaka K. A selective deficiency of hepatic triacylglycerol lipase in guinea pigs. Biochim Biophys Acta 1979;575:129–134
7. Etienne J, Noe L, Rosalimn M, Doane A-M, Debray J. Post-heparin lipolytic activity with no hepatic triacylglycerol lipase involved in a mammalian species. Biochim Biophys Acta 1981;683:516–523
8. Murase T, Rakura H. Accumulation of intermediate density lipoprotein in plasma after intravenous administration of hepatic triglyceride lipase antibody in rats. Atherosclerosis 1981;39:293–300
9. Goldberg LG, Le N-A, Pernetti JR, Ginsberg HN, Lindgren F, Brown FT. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. J Clin Invest 1982;70:1184–1192
10. Breckenridge WC, Litto JS, Alapovits P, et al. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. Atherosclerosis 1982;45:161–179
11. Carlson LA, Holmquist L, Nilsson-Ehle P. Deficiency of hepatic lipase activity in post-heparin plasma in familial hyper-a-1-glycerideemia. Acta Med Scand 1985;28:435–442
12. Abrams JJ, Grundy SM, Ginsberg HN. Metabolism of plasma triglycerides in hypertriglyceridemia and hyperhydromed in man. J Lipid Res 1981;22:307–322
13. Goldberg AP, Applebaum-Bowden DM, Blumen EL, et al. Increase in lipoprotein lipase during eileate fatigue of hypertriglyceridemia in patients on hemodialysis. N Engl J Med 1979;301:1073–1076
14. Muller P, Folline R, Lambrecht J, et al. Hypertriglyceridemia secondary to liver disease. Eur J Clin Invest 1974; 4:419–428
15. Goldberg LG, Mazlan RG, Rubenstein A, et al. Plasma lipoprotein abnormalities associated with acquired hepatic triglyceride lipase deficiency. Metabolism 1985;34:832–835
16. Brunzel JD, Iverius P-H, Scheibel MS, et al. Primary lipoprotein lipase deficiency. In: Angeli A, Frainich J, eds. Lipoprotein deficiency syndromes. New York: Plenum Publishing, 1986:227–239
17. Marzetta CA. Structural and metabolic heterogeneity of plasma low density lipoproteins in non-human primates. Ph.D. Dissertation, Bowman Gray School of Medicine, Wake Forest University, 1985
18. Chung BH, Wilkinson T, Geer JC, Segrest JP. Preparative and quantitative isolation of plasma lipoproteins: Rapid, single, discontinuous density gradient ultracentrifugation in a vertical rotor. J Lipid Res 1980;21:284–291
19. Havel RJ, Eder HA, Dragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345–1353
20. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982:23:97–104
21. Marzetta CA, Rudal LL. A species comparison of low density lipoprotein heterogeneity in nonhuman primates fed atherogenic diets. J Lipid Res 1986;27:753–762
22. Iverius P-H, Brunzel JD. Human adipose tissue lipoprotein lipase: Changes with feeding and relationship to posthepatic plasma enzymes. Am J Physiol 1985;249:E70–E714
23. Babirak SP, Iverius P-H, Fujimoto WY, Brunzel JD. The detection and characterization of the heterogeneous state for lipoprotein lipase deficiency. Arteriosclerosis 1989;326–334
24. Lipid Research Clinics Program. Manual of laboratory operations, vol 1. Lipid and lipoprotein analysis. DHEW publication no NIH 75-628. Bethesda, MD: National Institutes of Health, 1974
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275
26. Albers JJ, Cabana VG, Haarman WR. Immunoassay of human plasma apolipoprotein B. Metabolism 1975;24:1339–1351
27. Lipid Research Clinic Program. Population studies data book, vol I. The prevalence study. NIH Publication no. 80-1527. Bethesda, MD: National Institutes of Health, 1980
28. Fallor RA, Hokanson JE, Brunzell JD. Low density lipoproteins in familial hyperlipidemias. In: Fidge NH, Nestel PJ, eds. Atherosclerosis. Amsterdam: Elsevier Science Publishers, 1986:159–163
29. Deckelbaum RJ, Eisenberg S, Oshry Y, Butbul E, Sharon I, Olvanira T. Reversible modification of human low density lipoproteins toward triglyceride-rich precursor. A mechanism for losing excess cholesterol esters. J Biol Chem 1982;257:6509–6517
30. Deckelbaum RJ, Granot E, Oshry Y, Rosa L, Eisenberg S. Plasma triglyceride determines structure-composition in low and high density lipoproteins. Atherosclerosis 1994; 4:225–231
31. Fielding CJ. Human lipoprotein lipase. I. Purification and substrate specificity. Biochim Biophys Acta 1970;206:109–117
32. Fielding CJ. Human lipoprotein lipase. II. Inhibition of enzyme activity by plasma low density lipoproteins. Biochim Biophys Acta 1970;206:118–124
33. Blar DM, Havel RJ. Activation of lipoprotein lipase by lipoprotein fractions of human serum. J Lipid Res 1970; 11:565–570
34. Davila RA, Hartman AD, Dory L, van Lenten BJ, Rohein PS. Metabolic fate of VLDL apolipoproteins B and E in hepalactomized rats. Biochim Biophys Acta 1981; 665:154–154
35. Jansen H, Van Tol A, Hulskam WC. On the metabolic function of heparen-releasable liver lipase. Biochim Biophys Acta 1980;102:33–59
36. Rao SN, Cortese C, Miller NE, Levy Y,涟wa B. Effects of heparin infusion on plasma proteins in subjects with lipoprotein lipase deficiency. Evidence for a role of hepatic endothelial lipase in the metabolism of high density lipoprotein subfractions in man. FEBS Lett 1982;150:255–259

Index Terms: low density lipoproteins • hepatic lipase • lipoprotein lipase