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RARE DISEASES AND LIPID METABOLISM (JAG LÓPEZ, SECTION EDITOR)

Hyperlipoproteinemia Type 3: The Forgotten Phenotype

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Abstract Hyperlipoproteinemia type 3 (HLP3) is caused by impaired removal of triglyceride-rich lipoproteins (TGRL) leading to accumulation of TGRL remnants with abnormal composition. High levels of these remnants, called β-VLDL, promote lipid deposition in tuberous xanthomas, atherosclerosis, premature coronary artery disease, and early myocardial infarction. Recent genetic and molecular studies suggest more genes than previously appreciated may contribute to the expression of HLP3, both through impaired hepatic TGRL processing or removal and increased TGRL production. HLP3 is often highly amenable to appropriate treatment. Nevertheless, most HLP3 probably goes undiagnosed, in part because of lack of awareness of the relatively high prevalence (about 0.2 % in women and 0.4–0.5 % in men older than 20 years) and largely because of infrequent use of definitive diagnostic methods.

Keywords Hyperlipoproteinemia type 3 · Triglyceride-rich lipoproteins · Cardiovascular disease · Treatment · Diagnosis

Introduction

Triglyceride (TG)-rich lipoproteins (TGRL) are a complex array of particles having divergent associations with atherosclerosis and coronary artery disease (CAD). Among high TGRL phenotypes, hyperlipoproteinemia type 3 (HLP3) carries the highest CAD risk. It is characterized by the presence of abnormal beta-migrating very low density lipoprotein (β-VLDL) particles. HLP3 (also known as type III hyperlipidemia or familial dysbetalipoproteinemia) is an extreme case of TGRL remnant accumulation due to impaired removal or processing of TGRL remnants, often together with excess TGRL production.

Although HLP3 patients have increased concentrations of all TGRL remnants, the excess of β-VLDL alone is diagnostic. The standard or basic lipid panel cannot distinguish HLP3 from other dyslipidemias. Several extended lipid profiles may also have poor ability to identify patients with HLP3. This, together with lack of appreciation for the frequency of HLP3, has likely led to substantial underdiagnosis and inadequate treatment of this important lipid disorder.

Diagnosis of HLP3

Definitive diagnosis of HLP3 has traditionally relied on ultracentrifugation to equilibrium of plasma at its native density (d=1.006 g/ml). The supernatant fraction contains chylomicrons, the full spectrum of VLDL, larger physiologic TGRL remnants, and, if present, abnormal cholesterol-enriched β-VLDL (see Table 1 for composition of lipoprotein). β-VLDL particles are so named because they have "β" mobility on paper or agarose gel electrophoresis rather than the "pre-β" mobility of normal VLDL particles (see Fig. 1). β-VLDL are derived from both the liver [with apolipoprotein B (APOB)-100] and the intestine (with APOB-48) and differ widely in size (22-212 nm), with particles bearing APOB-48 found predominantly in the larger fractions [1]. β-VLDL are the predominant lipoprotein type that accumulates in the plasma of cholesterol-fed dogs and rabbits, as well as in apolipoprotein E (APOE)-deficient mice.

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Table 1 Size, percent particle mass, and lipid mass ratio of major lipoprotein classes. Percent mass contributions from protein can be calculated as the difference of 100 % and the lipid percent mass shown

Particle	Diameter (nm)	Percent particle mass				TG/TC mass ratio
		TG	CE	FC	PL	
Chylomicron	>100	88	3	1	5	29.3
VLDL	30-70	59	12	5	16	4.92
β-VLDL	22-212	41	29	7	17	1.71
IDL	29-33	20	34	9	20	0.69
LDL	24-27	4	41	11	21	0.11
HDL	7-28	4	14	4	27	0.30[a]

Adapted from Hopkins et al. [119]

CE cholesteryl ester, *FC* free cholesterol, *HDL* high-density lipoprotein, *IDL* intermediate-density lipoprotein, *LDL* low-density lipoprotein, *PL* phospholipid, *TC* total cholesterol (as cholesterol mass only), *TG* triglyceride, *VLDL* very low density lipoprotein

[a] Assumes a 2:1 ratio for HDL3 to HDL2

Electrophoresis of whole plasma followed by neutral lipid staining yields bands at the origin (if chylomicrons are present), followed by the β band, which normally represents only low-density lipoprotein (LDL), a pre-β band corresponding to VLDL, and an α band representing high-density lipoproteins (HDL). Classically, HLP3 has been defined by ultracentrifugation followed by electrophoresis and staining of the *d* <1.006 g/ml or "top" fraction. If a β band is visible (representing β-VLDL particles), or a continuous band is present from the β to the pre-β position (a so-called broad-β band), then HLP3 is diagnosed. By definition, intermediate-density lipoproteins (IDL) are not found in the *d* <1.006 g/ml fraction (having density in the range 1.006 g/ml<*d*<1.019 g/ml) but are rather found in the *d* >1.006 g/ml infranatant fraction, together with LDL (1.019 g/ml<*d*< 1.063 g/ml) and HDL (*d*>1.063 g/ml). Electrophoresis of whole plasma is inadequate for identification of β-VLDL since normal LDL is also found in the β position (although a clear broad β band with whole plasma is suggestive).

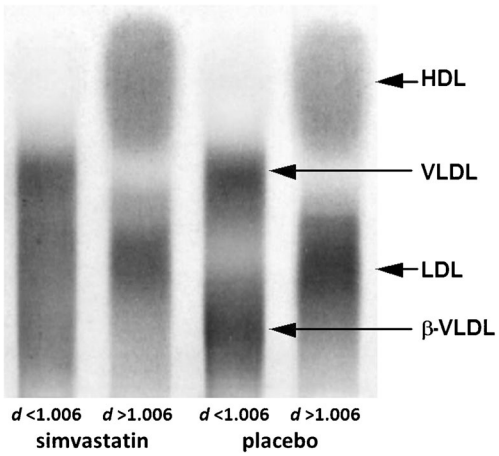


Fig. 1 Agarose gel electrophoresis of the ultracentrifugal top fraction [*d* <1.006 g/ml, very low density lipoprotein (*VLDL*)] and bottom fraction (*d* >1.006 g/ml, containing low-density lipoprotein (*LDL*) and high-density lipoprotein (*HDL*)] followed by Sudan black staining. Shown is a gel prepared from plasma of a patient with hyperlipoproteinemia type 3 (HLP3) before and after treatment with simvastatin at 80 mg. Note the near disappearance of the β-VLDL band after simvastatin treatment

In traditional "beta-quantification," ultracentrifugation is performed on whole plasma and the *d* <1.006 g/ml top fraction is separated (e.g., by tube slicing at the upper one third of the sample). Cholesterol is measured in the isolated top fraction and is called "VLDL-C," recognizing that it actually represents cholesterol from chylomicrons and all other TGRL with *d* <1.006 g/ml. The triglyceride (TG) content of this top fraction can also be measured as VLDL-TG. The ratios of VLDL-C to total plasma TG (VC/TG) or VLDL-C/VLDL-TG (VC/VT) are indices of TGRL composition. This composition is not provided by the NMR lipoprotein quantification (offered by LipoScience, Raleigh, NC, USA), which provides estimated particle concentrations, although lipid compositions of VLDL and other subfractions have been reported by newer NMR methods [2•].

When acted on by lipoprotein lipase (LPL), cholesteryl ester transfer protein, and possibly lecithin–cholesterol acyltransferase, TGRL invariably become cholesterol-enriched during their sojourn through the plasma compartment [3]. In contrast, VC/TG is reduced when there are excess chylomicrons and/or very large VLDL.

Several studies have been performed to establish compositional cutpoints that reliably predicted the presence of β-VLDL particles. These studies found that when VLDL-C is measured by ultracentrifugation, VC/TG≥0.30 or VC/VT≥ 0.35 reliably identified patients with HLP3 when the total TG concentration was 150-1,000 mg/dl [4, 5]. VC/TG or VC/VT ratios do not dependably detect β-VLDL that are present by electrophoresis when the TG concentration is less than 150 mg/dl (relatively nonspecific) or greater than 1,000 mg/dl (insensitive owing to masking by chylomicronemia) [6].

Sniderman et al. [7] suggested using an observed altered relationship between total cholesterol (TC), TG, and APOB as a means to diagnose HLP3. A TC/APOB ratio greater than 6.2, together with a TG/APOB ratio below 10 (with TC and TG expressed as millimoles per liter and APOB in grams per liter) in persons with plasma TG concentration above the 75th percentile (about 150 mg/dl) successfully identified all 38 patients with HLP3 among 1,771 consecutive patients presenting at a tertiary referral lipid clinic. They pointed out that the TC/APOB ratio alone does not distinguish between hyperlipoproteinemia type 1, HPL3, or hyperlipoproteinemia type 5.

Clinical manifestations of HLP3 can include tuberous xanthomas (see Fig. 2), and yellowish discoloration of the palmar creases (palmar striae), as well as early-onset CAD (37 % of 49 HLP3 patients referred to an NIH

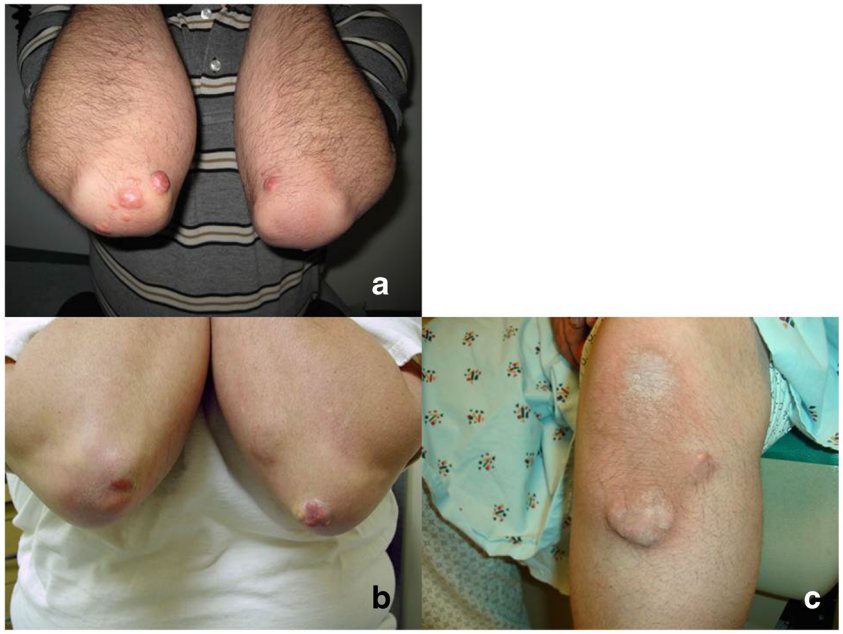


Fig. 2 **a** Tuberous xanthomas on the elbows of a patient with HLP3. **b** Tuberous xanthomas on the elbows on a patient with both familial hypercholesterolemia and HLP3. **c** Tuberous xanthomas on the knee of the patient in **b**

lipid clinic had CAD with mean age of onset of 39 years!) [4, 8]. The frequency of tuberous xanthomas or palmar striae among HLP3 patients was estimated to be only about 20 % in one series [9], but may be considerably lower [6].

Epidemiology of HLP3 and Association with CAD

The Lipid Research Clinics Prevalence Study was the only study to apply classic criteria to define HLP3 in a large, representative general population. HLP3 was defined as the presence of a β-VLDL band on electrophoresis of the *d* <1.006 g/ml fraction separated by initial ultracentrifugation (performed in all participants), with the additional requirement of VC/TG≥0.30 in those with TG concentration of 150-1,000 mg/dl. HLP3 was found in 0.4 % of men aged 20 years and older in the general population, and at half that rate (0.2 %) in similarly aged women not using hormones [6]. In the only other study of which we are aware to implement measurement of lipid fractions by ultracentrifugation in all subjects from a large, representative population (more than 1,700 controls), the prevalence of HLP3 (VC/TG≥0.30, TG concentration above 150 mg/dl) was 0.68 %, with most subjects having TG concentration below 300 mg/dl [10, 11].

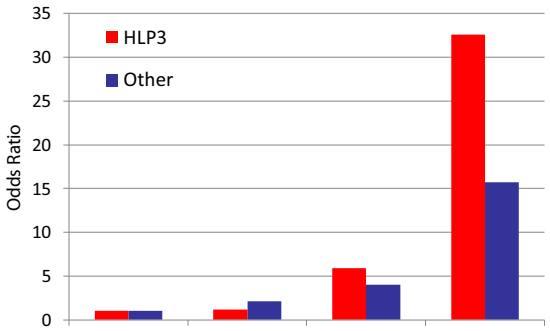
Only after the above-mentioned ultracentrifugation-based diagnostic criteria were established was a role for APOE in HLP3 identified. Several families were identified in which HLP3 subjects had APOE 2-2, whereas other family members without APOE 2-2 frequently had what appeared to be dominantly transmitted familial combined hyperlipidemia [12, 13]. It was hypothesized that HLP3 resulted from two inherited defects, APOE 2-2 causing impaired removal of TGRL remnants, and a second condition which increased production of VLDL. Obesity also clearly contributed to increased expression of HLP3, whereas weight loss could markedly decrease the levels of plasma lipids, often with equally marked elevation in the levels of lipids up on regaining weight [14]. Hypothyroidism and estrogen deficiency, as in menopause, were also seen to greatly increase HLP3 expression or severity [15]. Because the APOE 2-2 genotype occurs at a frequency of about 1 % and familial combined hyperlipidemia has a similar frequency, the prevalence of HLP3 is often calculated to be about one in 10,000 [16]. However, this indirect estimate should not take precedent over the direct observation (Table 1).

A recent study of a large lipid clinic population in Germany (3,272 consecutive patients) identified 350 cases of HLP3 using the criteria of Sniderman et al. mentioned above [17••]. Remarkably, although those with the APOE 2-2 genotype showed the highest likelihood of having HLP3, they accounted for only 16 % of patients with HLP3 (see Table 2). Clearly, HLP3 was not restricted to those with APOE 2-2; in fact, most HLP3 patients did not have the APOE 2-2 genotype. These observations are in accord with our own prior findings [108], and provide an explanation for the large discrepancy between prevalence estimates using classic criteria versus adding a requirement for APOE 2-2. Rare APOE mutations accounted for only a couple of these

Table 2 Apolipoprotein E (*APOE*) genotyping of 3,272 consecutive patients seen at a lipid clinic in Germany. Hyperlipoproteinemia type 3(*HLP3*) was diagnosed in 350 patients using the criteria plasma TC/apolipoprotein B (APOB)>6.2 and TG/APOB<10 (with TC and TG expressed as millimoles per liter and APOB in grams per liter). Surprisingly, only 16 % of patients had the classic APOE 2-2 genotype. Those with HLP3 not related to APOE 2-2 underwent sequencing of APOE in search of possible rare mutants, but only two or three such cases were found to explain non-APOE 2-2 HLP3 [17••]

APOE genotype	No. of patients	HLP3 patients (350)	Percentage of APOE genotype	Percentage of all HLP3 patients
2-2	108	55	50.9	15.7
2-3	338	53	15.7	15.1
2-4	105	20	19.0	5.7
3-3	1,701	141	8.3	40.3
3-4	903	72	8.0	20.6
4-4	110	6	5.5	1.7
Abnormal[a]	7	3	42.9	0.9

[a] Refers to atypical APOE gel pattern



non-HDL-C	<160	160-189	190-219	220+
HLP3 OR (95% CI)	1.0	1.2 (0.4-4.0)	5.9 (1.02-34)	32.6 (4-257)
Other OR (95% CI)	1.0	2.1 (1.6-2.6)	4.0 (2.9-5.5)	15.7 (9.3-26)
HLP3 in cases	0%	0.43%	0.43%	1.88%
HLP3 in controls	0%	0.45%	0.17%	0.06%

Fig. 3 Risk of premature coronary artery disease (CAD; myocardial infarction, coronary artery bypass graft, or percutaneous transluminal coronary angioplasty by the age of 60 years in men or 70 years in women) associated with HLP3 among 1,170 premature CAD cases and 1,759 population-based controls. HLP3 was defined as a measured VLDL cholesterol (*VLDL-C*) to total triglycerides (VC/TG) ratio of 0.30 or higher with a concentration of total triglycerides above 150 mg/dl. Those with HLP3 were further broken down as having mild, moderate, and severe HLP3, defined as shown according to non-HDL cholesterol (*non-HDL-C*) categories. Risks are compared in those with and without HLP3 according to the non-HDL-C category. Logistic regression was performed with adjustment for age, gender, hypertension, diabetes, history of cigarette smoking, HDL-C level, and non-HDL-C category (with or without HLP3). *CI* confidence interval, *OR* odds ratio

German patients with HLP3 but without APOE 2-2 [17••]. A few more instances of rare APOE variants caused HLP3 in a Spanish population, but again explained only a small percentage of cases [18•].

Although markedly increased risk of atherosclerotic disease has long been appreciated for patients with severe HLP3, a population-based estimate of risk was not available until relatively recently [10, 11]. In these studies we reported a fivefold to eightfold increase in CAD risk among HLP3 patients. Considerably higher risk was associated with severer HLP3. The more recent of these two studies included 1,759 population-based controls and 1,170 case patients with onset of clinical CAD by age 60 years in men and age 70 years in women. The prevalence of HLP3 (0.68 %) in the control population was very similar to the Lipid Research Clinics Prevalence Study estimate [6]. The prevalence of HLP3 among CAD case patients was 2.7 %, almost identical to that reported by Hazzard et al. [19].

Owing to increasing interest in non-HDL cholesterol (non-HDL-C), we utilized data from the more recent of our CAD case–control studies[10] to examined the CAD risk associated with categories of non-HDL-C in persons with and without HLP3. When we graded the severity of HLP3 by non-HDL-C concentration and compared these risks with those for persons without HLP3 but with similar non-HDL-C concentration, we found widely overlapping risk estimates as shown in Fig. 3 (previously unpublished results). There were no individuals who met the criteria for HLP3 with a non-HDL-C concentration under 160 mg/dl. However, the risk estimate for HLP3 in those with non-HDL-C concentration of 220 mg/dl or higher was approximately twofold higher than in those with similarly elevated non-HDL-C concentration but without HLP3 (although these risks were not significantly different). In another model, if non-HDL-C concentration was simply entered as a continuous variable and allowed to apply to all persons with or without HLP3, then the CAD risk associated with HLP3 became nonsignificant (odds ratio 1.2, $p=0.62$ for all HLP3), even for the severest HLP3 category (odds ratio 3.2, 95 % confidence interval 0.4-27, $p=0.29$). These results suggest that, to a large extent, reduction of non-HDL-C concentration to current treatment goals is a reasonable guide for treatment of HLP3 patients, whether or not HLP3 is recognized. However, the data also suggest that severer HLP3 may carry excess risk beyond what is predicted by non-HDL-C concentration alone.

Several other laboratory methods, such as levels of IDL, remnant-like particle (RLP) cholesterol (RLP-C), and apolipoprotein C (APOC) 3 in VLDL, have been used to assess CAD risk associated with TGRL remnants. These risk estimates can vary substantially, depending on the method or parameter used, but most show significantly increased CAD risk associated with higher levels of TGRL remnants [3, 20•].

Atherogenicity of β-VLDL

Several classes of cholesterol-enriched TGRL remnants appear to be atherogenic [21••]. β-VLDL are arguably the most atherogenic of these given the very high CAD risk of HLP3 patients and the rapid atherosclerosis progression seen in APOE knockout mice fed a Western diet. It appears that it is the cholesteryl ester component of TGRL in these mice that is atherogenic, rather than the TG. This was demonstrated by showing that deficiency of acyl-CoA:cholesterol acyltransferase 2, which resulted in TGRL with normal or high TG levels but very little cholesteryl ester, almost entirely abrogated atherosclerosis in APOE null mice despite similarly high APOB-48 and APOB-100 particle concentration [22].

In now-classic studies, incubation of macrophages with native LDL did not cause foam cell formation owing to downregulation of the LDL receptor (LDLR) [23–25]. However, after LDL were modified by oxidation or acetylation, they were avidly taken up by macrophages, with conversion to foam cells. In these same studies, β-VLDL from cholesterol-fed rabbits or dogs needed no modification to promote foam cell formation. Some uptake of β-VLDL into macrophages was found to be mediated by the LDLR, but also by other pathways such as the SR-B1 scavenger receptor as shown in macrophages from $LDLR^{-/-}$ mice [26].

Endothelial activation, as measured by expression of the chemokines monocyte chemoattractant protein 1, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1, is enhanced by incubation with so-called RLP obtained from hyperlipidemic, diabetic human subjects [27]. Incubation of monocytes with RLP also promotes their adhesion to endothelial cells [28]. RLP can also induce a strong inflammatory response with vigorous NADPH oxidase activation and superoxide formation followed by apoptosis in endothelial cells through activation of lectin-like oxidized-LDL receptor 1 [29].

Although β-VLDL and some other TGRL remnants can promote foam cell formation without a need for modification [21••], they must first enter the subendothelial space to do so. Most TGRL remnants are small enough to enter this space, in which they are avidly retained [30]. Once in the subendothelium, VLDL and IDL can be altered by sphingomyelinase, causing them to aggregate, fuse, and increase in binding affinity to proteoglycans [31]. Not surprisingly, this sphingomyelinase-induced aggregation of TGRL appears to promote avid macrophage uptake and foam cell formation [32]. Importantly, TGRL remnants carry many cholesteryl ester molecules per particle. For example, a chylomicron remnant with a diameter of 100 nm has been estimated to carry 40 times more cholesteryl ester than an LDL particle [33].

In one study, lipoproteins were eluted from thoracic and abdominal aortic tissue at autopsy, and TGRL and LDL fractions were then separated by density gradient ultracentrifugation. When these fractions were incubated with mouse peritoneal macrophages, the TGRL increased incorporation of radioactive oleate into cholesteryl esters by 10-20-fold versus only threefold to fourfold for LDL [34]. Oxidized β-VLDL or VLDL remnants isolated from patients with HLP3 or type IV hyperlipidemia were found to cause greater macrophage cholesteryl ester accumulation than did oxidized LDL [35, 36].

Pathophysiology of β-VLDL Accumulation in HLP3—New Insights from Mouse and Human Genetics

Impaired Hepatic Processing of TGRL is the Hallmark of HLP3

Fundamental to understanding the basis of HLP3 is recognizing the normal, physiological role of the liver in both direct removal from the bloodstream of some TGRL remnants and the conversion of other TGRL remnants to LDL (see Fig. 4). When VLDL or chylomicrons are infused into animals whose liver has been removed or who have had a "functional" hepatectomy, TGRL remnants accumulate and production of true LDL is almost entirely eliminated [37]. In vitro, incubation of TGRL with purified LPL, with or without added hepatic lipase (HL), causes lipolysis but does not generate normal LDL [38]. Probably little or no LDL is generated from TGRL outside the liver. The highly impaired hepatic conversion of small VLDL and IDL to LDL in patients with HLP3 is illustrated in Fig. 5 [39]. This impaired processing of TGRL in HLP3 patients leads both to accumulation of remnants and frequently to reduced LDL cholesterol (LDL-C) levels.

Recognition that LDLR binding by the APOE 2 variant is markedly deficient (less than 2 % of the common APOE 3 variant's affinity) provided a model system for impaired hepatic removal of TGRL remnants in HLP3 [40]. β-VLDL particles from APOE 2-2 HLP3 patients were directly shown to have very poor affinity for the LDLR [41]. In one study, β-VLDL was found to be absent from patients with hyperlipoproteinemia type 5 despite very high total TG and chylomicron levels, but accumulation of β-VLDL could be induced by rapid administration of heparin, which causes activation of LPL and HL, but apparently also led to inhibition of further processing of the remnants produced [41].

Soon after recognition of APOE and its interactions with LDLR, another receptor of the LDLR family, LDLR-related protein 1 (LRP1), was found to play a role in liver-mediated removal of remnants. LRP1 also has somewhat decreased affinity for APOE2 compared with APOE3 [42, 43]. Impaired endocytosis of LRP1 due to a mutation in the intracellular tail of the receptor resulted in increased remnant accumulation in LDLR-deficient mice [44]. In vitro, APOC1 and less so

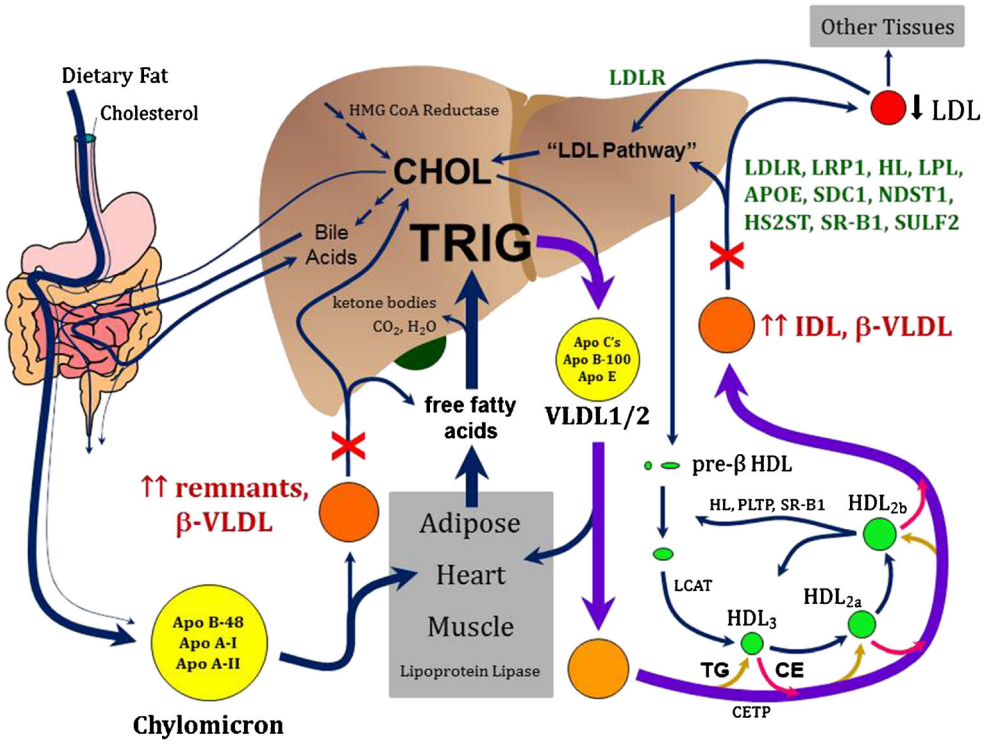


Fig. 4 Kinetic basis of HLP3. *Heavy purple lines* suggest increased production of VLDL, and *red X*'s denote impaired removal or processing of the triglyceride-rich lipoprotein remnants. *apo* apolipoprotein, *CE* cholesteryl ester, *CETP* cholesteryl ester transfer protein, *CHOL* cholesterol, *HMG* high mobility group, *IDL* intermediate-density lipoprotein, *LCAT* lecithin–cholesterol acyltransferase, *LDL* low-density lipoprotein, *LDLR* low-density lipoprotein receptor, *TG* triglycerides, *TRIG* triglycerides, *PLTP* phospholipid transfer protein, *LRP1* LDL receptor-like protein 1, *HL* hepatic lipase, *LPL* lipoprotein lipase, *APOE* apolipoprotein E, *SDC1* syndecan 1, *NDST1* GlcNAc N-deacetylase/N-sulfotransferase 1, *HS2ST* hepatic uronyl 2-O-sulfotransferase, *SR-B1* scavenger receptor B1, *SULF2* heparan sulfate glucosamine-6-O-endosulfatase-2

APOC2 were found to inhibit binding of TGRL remnants to LRP1. However, an abundance of APOE was able to displace APOC1 and allow LRP1 binding [45]. Transgenic overexpression of APOC1 in $APOE^{-/-}$ mice markedly increased hyperlipidemia [46].

Although APOC3 on VLDL may inhibit LPL, a more important effect may be to delay VLDL uptake and catabolism, thus further enhancing cholesteryl ester enrichment of VLDL [47, 48]. Transgenic overexpression of APOC3 increases plasma TG levels and atherosclerosis in $LDLR^{-/-}$ mice [49]. More importantly, excess APOC3 in LDL and/or VLDL is associated with increased CAD risk in humans [50, 51].

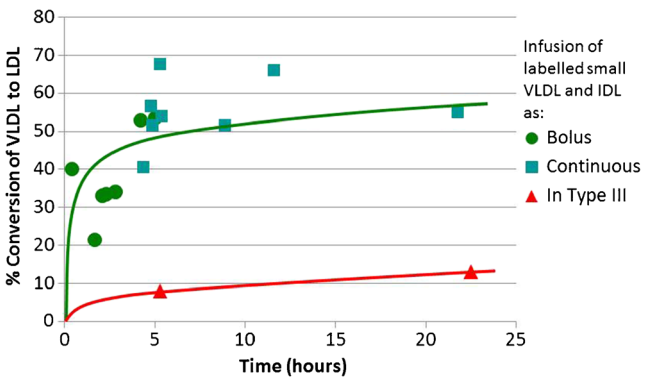


Fig. 5 Human hepatic arteries and veins were cannulated to measure conversion of infused, radiolabelled, small VLDL and IDL to LDL in liver. (Adapted from Turner et al. [39])

although this is mitigated somewhat by the presence of APOE on particles with APOC3 [20•]. The presence of APOE on VLDL appears to promote their removal by the liver, thus overcoming the inhibitory effect of APOC3 [52].

Another receptor of the LDLR family is the VLDL receptor. Unlike LRP1, however, it is principally found in peripheral tissues active in lipolysis and fatty acid uptake, such as adipose, heart, and skeletal muscle. Its deficiency promotes accumulation of VLDL remnants in knockout mice when the flux of TGRL is high [53]. The scavenger receptor SR-B1 is also expressed on hepatocytes and may mediate uptake of β-VLDL, particularly in the absence of the LDLR [26].

Heparan sulfate proteoglycans (HSPG) also bind APOE on TGRL remnants, with different APOE variants having different affinities. APOE2 has low binding affinity for HSPG, and APOE Leiden apparently shows no binding to HSPG at all [54]. A "secretion–capture" mechanism has been reported in which hepatocytes secrete APOE at high concentrations into the space of Disse, causing localized enrichment of TGRL remnants with APOE (and perhaps displacement of APOC apoproteins) and capture of these TGRL by HSPG as well as by LRP1 and the LDLR [55].

Syndecan 1 (SDC1) is the main backbone protein of HSPG found on basal microvilli of hepatocytes which project into the space of Disse. SDC1 is decorated by three heparan sulfate chains with specific sulfation patterns required for normal binding of TGRL remnants. In addition, these HSPG bind HL and LPL. Knockout of SDC1 in mice leads to TGRL

remnant accumulation [56]. Furthermore, heparin was shown to compete with HSPG for remnant binding, possibly explaining the above observation that heparinization of hyperlipoproteinemia type 5 patients could induce the appearance of β-VLDL. Liver-specific deletion of glucosoaminyl *N*-deacetylase/*N*-sulfotransferase 1, one of the enzymes required for normal sulfation of HSPG, also led to remnant accumulation in mice. The phenotype was exacerbated in the face of background LDLR deficiency [57]. Similarly, hepatic uronyl 2-*O*-sulfotransferase was required for normal remnant processing, whereas glucosaminyl 6-*O*-sulfotransferase 1 was not, suggesting a critical role of 2-O sulfation of heparan sulfate [58]. Nevertheless, 6-*O*-sulfate removal by heparan sulfate glucosamine-6-*O*-endosulfatase 2 (SULF2) apparently leads to HSPG degradation. SULF2 activity is markedly upregulated in diabetic db/db obese mice, resulting in impaired remnant removal. Notably, SULF2 is induced tenfold in hepatocytes by advanced glycosylation end products [59].

LPL can be separated from its anchor glycosylphosphatidylinositol-anchored HDL-binding protein 1 on the surface of endothelial cells and become bound instead to TGRL. LPL thus bound to TGRL remnants can then promote interaction with hepatic HSPG. HL may be similarly loosed, bound to TGRL, and promote binding of TGRL remnants to hepatic HSPG. Both LPL and HL thus appear to promote TGRL uptake and clearance from plasma. Rare homozygous HL deficiency with absence of HL protein results in accumulation of β-VLDL and probably increased CAD risk [60], whereas mutations that cause loss of HL activity with preservation of HL protein result only in TG enrichment of most lipoproteins but not accumulation of β-VLDL [61, 62].

A number of other APOE variants other than APOE-2, mostly rare, were identified that can cause HLP3, and many do so in a dominant fashion (see Table 3) [63]. All these mutations have in common impaired binding to the LDLR, LRP1, and/or HSPG. Several of the less common APOE variants appear to predominate in certain racial or ethnic groups [64, 65]. For example, the R136S APOE variant, commoner in a Spanish population and among certain Africans, was shown to cause an incomplete dominant transmission, with more frequent expression of VC/TG>0.30 in those with higher BMI and in males [66].

Table 3 APOE variants associated with HLP3 besides APOE 2

E3K	R136S	K146Q	R224Q in E2
E3K + E13K	R136C	K146E	V236E in E2
G13K + R145C	R142L	K146N, R147W	R251G in E4
L28P in E4	R142C in E4	R147W	
C112R, R142C	R145C	K164E	Dup121-127
G127D, R158C	R145H	E244K + E245K	(APOE Leiden)

From OMIM and Matsunaga et al. [63]

E2 is (112C, 158C), *E3* is (112C, 158R), and *E4* is (112R, 158R)

Why and how some TGRL remnants undergo hepatocellular uptake and degradation whereas others are processed to LDL remains unanswered. Apparently, HSPG is more actively involved in uptake or processing of smaller particles, whereas LRP1 and LDLR mediate processing of larger TGRL remnants [67]. Inducible deficiency of LRP1 in mice primarily led to the appearance of chylomicron remnants if the mice were also LDLR deficient; however, in the presence of a functioning LDLR, LRP1 deficiency led to LDLR upregulation and little phenotypic effect [42]. Nevertheless, internalization of the LDLR is not necessary for it to function in TGRL processing since murine deficiency of LDLR adaptor protein 1, an intracellular adapter required for internalization of LDLR into clathrin-coated pits, causes impaired catabolism of LDL but not accumulation of β-VLDL [68]. About 25 % of heterozygous familial hypercholesterolemia (FH) patients with a single APOE2 allele expressed HLP3, whereas nearly all those with APOE 2-2 have HLP3 [69]. Surprisingly, these FH patients do not appear to be at greater risk of CAD than other FH patients, presumably because of a concomitant reduction in LDL-C levels [70, 71].

Increased VLDL Production Aggravates HLP3

Both the quantity and the nature of VLDL produced by the liver may greatly affect HLP3 expression. For example, obesity is associated with markedly increased VLDL production (particularly APOC3-containing VLDL1)[47] and severer HLP3.

APOB is constitutively synthesized, inserted into the lumen of the endoplasmic reticulum (ER) as it is translated, and then almost immediately acquires lipid through a poorly understood process mediated by microsomal TG transport protein. Without lipid, the highly hydrophobic APOB protein rapidly misfolds and is degraded by ER-associated degradation, a proteasome dependent process. Lipid droplets in hepatocytes, which are found near the ER and Golgi apparatus, are not composed simply of lipid, but contain numerous proteins, including APOA5 and APOC3. They behave as complex organelles with machinery akin to vesicles for fusion and transport [72, 73]. After emerging from the ER, the newly formed VLDL has the approximate TG content of a smaller VLDL2 particle. These nascent VLDL2 particles are transported to the Golgi apparatus prior to secretion. In persons with normal or low plasma TG levels, little or no additional TG appears to be added in the Golgi apparatus and primarily VLDL2 particles are secreted.

Under conditions of nutrient abundance (particularly high fructose or sucrose consumption), elevated plasma glucose concentration, or impaired insulin signaling, and especially with excess intrahepatic TG, VLDL2 particles in the Golgi

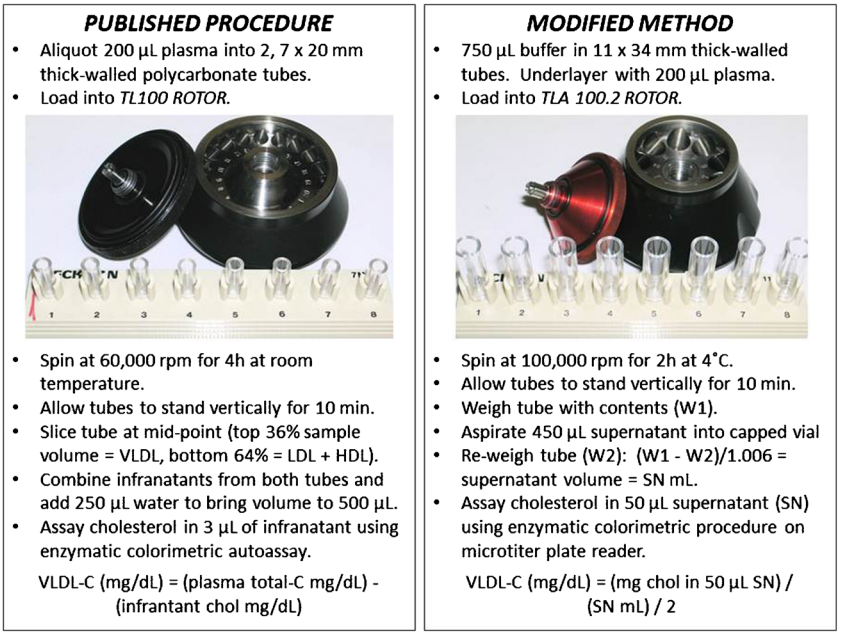


Fig. 6 Comparison of our previously published ultracentrifugation micromethod for VLDL-C ("no-wash", tube-slicing, and lipid analysis in the d >1.006 g/ml fraction) with our improved procedure (underlayering, supernatant aspiration followed by weighing and lipid analysis of the d <1.006 g/ml fraction). The buffer used for the 750-µl overlayer contains 150 mM NaCl, Na_2EDTA at 1 mg/mL, NaN_3 at 1 mg/mL, pH 7.4, d=1.006 g/ml

apparatus acquire additional TG and apoproteins, including APOC3, to become larger VLDL1 particles prior to secretion [72, 73, 74•]. APOC3 appears to promote the formation of both cytoplasmic and luminal lipid droplets and transfer of this lipid to nascent VLDL particles, leading to increased secretion of the larger, more TG-rich VLDL1 [73, 75•]. APOC3 synthesis can be induced in cultured hepatocytes by high glucose content in the medium, suggesting a possible mechanism for the increased plasma APOC3 and VLDL1 levels commonly seen in diabetic dyslipidemia [76•]. In HLP3, increased APOC3 production and impaired hepatic removal of VLDL-associated APOC3 leads to plasma levels of VLDL APOC3 nearly ten times greater than in normal individuals and more than two times higher than those of subjects with hyperlipoproteinemia type 2b [77, 78].

Intrahepatic APOA4 appears to have a role similar to that of APOC3 in promoting VLDL1 production and secretion [79•]. In contrast, APOA5 has an inhibitory effect on VLDL1 production within hepatocytes [80]. Overexpression of human APOA5 in APOE-deficient mice reduced both plasma lipid levels and atherosclerosis [81•].

Oxidation of fatty acids in nascent VLDL in the Golgi apparatus leads to misfolding and autophagy-dependent degradation of these deformed VLDL1 particles, a process termed post-ER, presecretory proteolysis. Post-ER, presecretory proteolysis of VLDL is enhanced by intake of omega-3 fatty acids (which are particularly prone to oxidation) and also by normal acute insulin signaling through activation of a type II phosphatidylinositol kinase [82•, 83•]. VLDL1 levels are increased by insulin resistance even in the absence of frank TG level elevation [84]. In addition, VLDL1 synthesis is directly stimulated by increasing glucose levels in cell culture [85, 86]. Statins appear to specifically decrease VLDL1 production by as yet unclear mechanisms [87].

Larger VLDL1 particles have more TG, a lower VC/VT ratio, and more APOC3 than VLDL2 particles [88]. Only about 10 % of large VLDL1 particles (100–400 Svedberg floatation units) are converted to LDL, even in normal individuals, whereas more than 40 % of smaller VLDL2 particles (20–100 Svedberg floatation units) are converted to LDL in normal subjects [89]. Thus, VLDL1 might be expected to be more likely precursors of β-VLDL in HLP3. Furthermore,

Table 4 Agreement between our published "no-wash" beta-quantification micromethod [101] and our improved underlayering technique among 272 subjects, 112 having HLP3 diagnosed by the older method. The sensitivity of the "wash" method with a VLDL cholesterol (*VLDL-C*)/VLDL-TG ratio cutpoint of 0.30 or greater rose to 90 % among subjects with a plasma TC concentration of 250 mg/dl or higher and a TG concentration of 250 mg/dl or higher. We calculated sensitivity and specificity for the "wash" method using the "no-wash" method as the standard. However, we believe the VLDL-C/VLDL-TG ratio method utilizing data generated by floating the triglyceride-rich lipoprotein remnants (TGRL) through the saline layer provides more specific results, representing a more accurate assessment of TGRL free from contamination from denser lipoproteins

Improved "wash" method VLDL-C/ VLDL-TG	Compared with standard "no-wash" beta quantification		κ (95 % CI)
	Sensitivity (%)	Specificity (%)	
≥0.30	82.1	94.4	0.78 (0.70-0.85)
≥0.35	67.9	97.5	0.68 (0.60-0.77)
≥0.40	46.4	99.4	0.50 (0.40-0.59)

CI confidence interval

larger, TG-rich VLDL1 particles appear to be more atherogenic than the smaller VLDL2 particles in that they are taken up more avidly by macrophages [90] and fibroblasts [91], and may activate endothelial cells [92] more than VLDL2 particles. The greater APOC3 content of VLDL1 may cause this greater uptake and/or activation [92]. Indeed, APOC3 was shown to directly bind Toll-like receptor 2 on human monocytoid THP-1 cells, resulting in increased production of nuclear factor κB and activation with greater adhesion to endothelial cells [93].

Direct evidence of increased VLDL1 production in HLP3 is limited. In two subjects with APOE 2-2 and mild HLP3, VLDL1 production was apparently not increased, but conversion of VLDL2 to LDL was impaired [94]. Other studies reported no clear increase in VLDL APOB production in patients with HLP3, although secretion of VLDL containing APOC3 appeared to be increased in one study [95], and increased VLDL production seemed to correlate with greater severity among HLP3 patients in another study [96]. Nevertheless, in HLP3, VLDL1 particles undergo rapid, normal lipolysis, but then their remnants undergo hepatic uptake and catabolism only very slowly, a kinetic feature that has no parallel in normolipidemic individuals or persons with type IV hyperlipidemia (characterized by excess accumulation of VLDL of relatively normal size and composition). At least a portion of the smaller VLDL2 particles in HLP3 patients appear to be converted relatively rapidly to LDL; however, some VLDL2 particles have prolonged plasma residence [89]. Intestinally derived TGRL remnants also have delayed catabolism from plasma and accumulate in HLP3. Even after a 14-h fast, β-VLDL of both intestinal and hepatic origin remain in HLP3 [1].

Lipolysis generally appears to be normal in HLP3 patients. Thus, loss-of-function mutations of LPL are relatively common in hypertriglyceridemia types IV and V but are rare in HLP3 [97]. Concordantly, LPL activity measured in post-heparin plasma of Japanese HLP3 patients was normal, although HL activity was moderately reduced [98].

The above observations are generally consistent with the findings of recent human genetic studies. Comparing HLP3 patients with APOE 2-2 with normolipidemic APOE 2-2 carriers, Henneman et al. [99] found increased frequency of *APOC3* 3238 G>C and *APOA5* -1131 T>C mutations (which are in linkage disequilibrium with each other) among HLP3 patients, with some evidence of an *APOA5* variant and an *LPL* rare variant being associated as well. Johansen et al. [100••] examined association of recently identified lipid gene markers with specific lipid phenotypes. Markers associated with HLP3 in 37 patients included *APOE* (with the 2 allele explaining 34.6 % of the total variance), *APOA5* (with the second strongest association), *KLHL8* (part of an E3 ubiquitin ligase complex), *COBLL1* (unknown function), and *NAT2* (encoding an *N*-acetyl transferase, involved in drug metabolism). Interestingly, variants of *LRP1* were not associated. Given the complexity of lipoprotein secretion and processing, involvement of unfamiliar genes in HLP3 should not be surprising.

Laboratory Diagnosis of HLP3

Approximately 25 years ago, our lipid laboratory developed and standardized a micro method for ultracentrifugation to

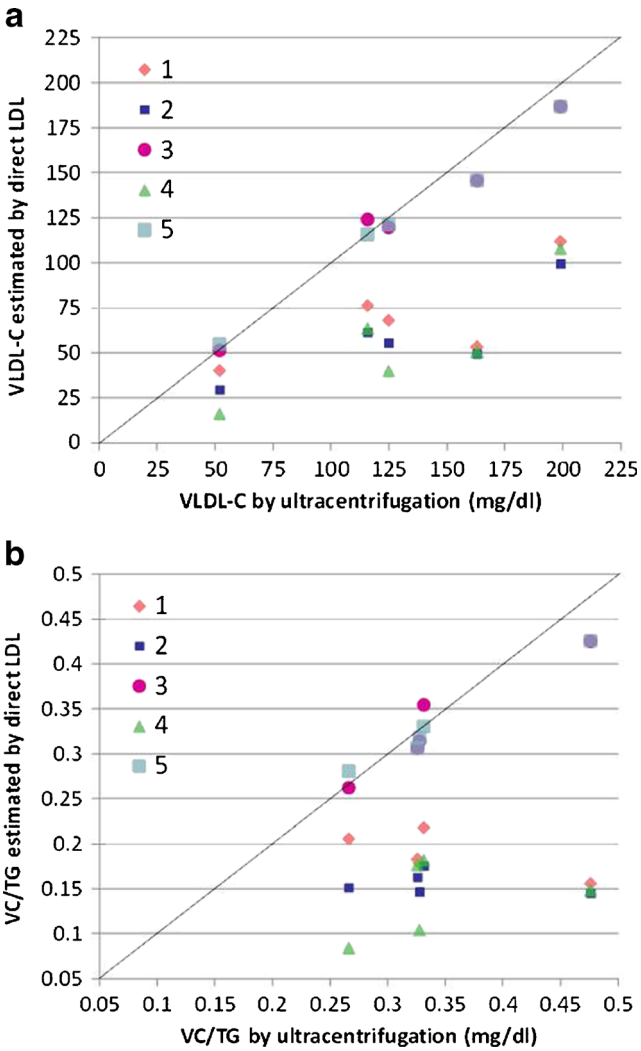


Fig. 7 **a** Comparison of measured VLDL-C concentration obtained by ultracentrifugation (older tube-slicing method) with the concentrations obtained with five different direct LDL methods. Note the widely differing results. Most direct methods underestimated VLDL-C concentration, although methods 3 and 5 were close. The values for the highest two VLDL-C concentrations were virtually superimposable for methods 3 and 5. The methods were as follows: *1* Roche/Kyowa first generation, *2* Roche/Kyowa second generation, *3* Genzyme/Daiichi, *4* Sigma/Wako, and *5* Polymedco/Denka. Analyses were performed by Pacific Biometrics in January 2002. **b** Again there was general underestimation of the VC/TG ratio with several direct LDL methods but good approximation with methods 3 and 5

measure lipid levels [101]. This method involved ultracentrifugation of untreated whole plasma, tube slicing to separate the top one third of the sample from the bottom two thirds, and quantitation of cholesterol in the d >1.006 g/ml and d <1.006 g/ml fractions. Approximately 8 years ago we changed to a method that involves layering a 200-μl sample of plasma beneath 750 μl of buffered saline solution of d= 1.006 g/ml. After ultracentrifugation, the top layer is removed by aspiration, weighed (for accurate volume determination), and lipids are quantified using intensely chromogenic enzymatic reactions. The protocols are compared in Fig. 6. The rationale for the underlayering involves recognition that (1) lower-density lipoproteins generally take less time to float than the time it takes denser lipoproteins (including IDL, LDL, and HDL) to sink [102], (2) as TGRL float through the saline overlayer, they are "washed," providing a cleaner TGRL preparation, and (3) blanking for endogenous free glycerol is unnecessary in order to obtain accurate TG concentrations in the d <1.006 g/ml fraction.

In previously unpublished work, we compared results from the two ultracentrifugation methods in a series of 272 subjects, 112 having HLP3 on the basis of the older, standard beta-quantification method with tube slicing. Correlation between the two methods for VLDL-C was high (r=0.97, p<0.0001), but with a slope different from 1, namely,

$$\text{VLDL–C}(\text{“wash”}) = 0.748(\text{VLDL “no–wash”}) + 1.18.$$

The intercept was not significantly different from 0. These results are consistent with the concept of less nonspecific contamination of the VLDL layer using the improved underlayering method. The concordance of HLP3 diagnosis between the two methods is shown in Table 4.

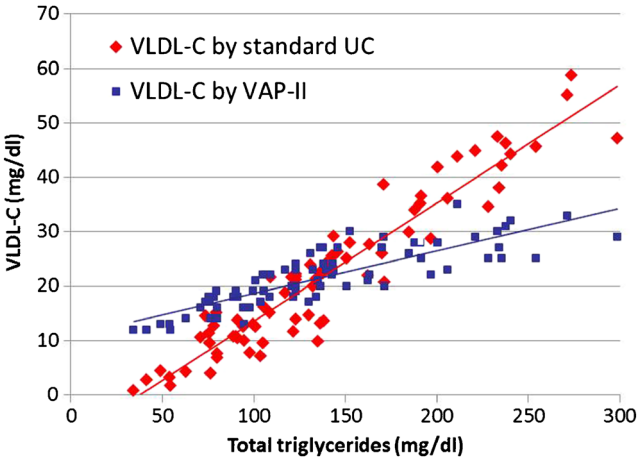


Fig. 8 VLDL-C concentration obtained by our improved micromethod of ultracentrifugation with saline overlayering compared with VLDL-C concentration from VAP-II as a function of th concentration of total triglycerides

Homogeneous assays of LDL (generally referred to as "direct LDL") are often used instead of the Friedewald calculation for LDL-C in hypertriglyceridemia. However, these direct LDL assays can lack specificity towards abnormal lipoproteins and often provide divergent results from "gold-standard" ultracentrifugation methods, particularly among hypertriglyceridemic patients (including HLP3 patients) [103]. β-VLDL from HLP3 patients were found to directly interfere with direct HDL assays [104]. We sent 50 plasma samples to a reference lipid laboratory (Pacific Biometrics,

Table 5 Comparison of several commercial advanced lipid methods with ultracentrifugation for diagnosis of HLP3 (unpublished data). Shown are the means (SD). The HLP3 patients had similar age and gender distributions when compared with the other patients

	HLP3 patients (n=7)	Others (n=37)	p	r vs ultracentrifugation
"No-wash" ultracentrifugation				
TC (mg/dl)	278 (137)	192 (44)	0.15	-
Total TG (mg/dl)	452 (435)	194 (237)	0.17	-
VLDL-C (mg/dl)	185 (146)	29 (21)	0.030	-
VLDL-C/total TG	0.45 (0.08)	0.16 (0.06)	<0.0001	-
Estimated β-VLDL-C (mg/dl)	184 (147)	6.0 (10.0)	0.018	-
Vertical spin auto-profile (Atherotech, VAP)				
TC (mg/dl)	186 (33)	181 (32)	0.69	0.48
TG (mg/dl)	433 (445)	184 (220)	0.19	0.76
VLDL-C (mg/dl)	50 (12)	25 (16)	0.0003	0.97
IDL1 cholesterol (mg/dl)	14.5 (3.5)	3.5 (3.4)	<0.0001	-
VLDL-C/total TG	0.17 (0.09)	0.17 (0.04)	0.86	-
Nuclear magnetic resonance (LipoScience)				
Total TG (mg/dl)	215 (47)	141 (71)	0.011	0.72
VLDL-C (mg/dl)	123 (30)	77 (33)	0.0017	
Polyacrylamide gradient gel electrophoresis (Lipoprint)				
VLDL (relative absorbance)	13.7 (2.7)	3.9 (3.9)	<0.0001	0.88

Seattle, WA, USA) which measured "direct" LDL by five different homogenous methods. Among these samples were samples from four definite HLP3 patients and one subject with borderline HLP3. As shown in Fig. 7 (previously unpublished results), the results from the direct LDL methods differed widely, with only two of the five methods (methods 3 and 5) providing reasonable estimates of VLDL-C concentration and VC/TG ratio determined by ultracentrifugation. Thus, not all direct LDL methods are useful for diagnosis of HLP3.

We have compared our original beta-quantification ultracentrifugation method with several other commercially available lipid tests in 44 patient samples, including seven patients with HLP3. As shown in Table 5, although all the methods showed differences between HLP3 patients and other patients in some measures, there were clear and often marked differences. In particular, we were surprised to find much lower reported VLDL-C concentration when using the vertical spin auto-profile (VAP; provided by Atherotech, Birmingham, AL, USA). VAP showed no difference in the composition of VLDL in patients with and without HLP3 and would have missed all seven patients in this small cohort using the VC/TG ratio. Examination of the literature discloses a clear limitation of VAP if chylomicrons and possibly other large TGRL are present owing to adherence of these lipoproteins to uncoated ultracentrifuge tube walls [105].

To examine this issue further, we performed our "underlayering" ultracentrifugation method on plasma samples from 75 consecutive participants who also had VAP-II measurements (none had HLP3). We found a markedly different slope between VLDL-C concentration obtained by the two methods and plasma TG concentration, with VAP-II producing higher VLDL-C levels at low TG concentrations and lower VLDL-C levels at high TG concentrations (see Fig. 8). These differences led to a relatively low correlation between the two methods for VC/TG (r=0.47, p<0.001) and almost no correlation between our improved VC/VT and the VAP-II VC/TG (r=0.07, p=0.56). Among the 25 subjects with TG concentration above 150 mg/dl, the correlation between our VC/VT and VAP-II VC/TG was much improved (r=0.70, p<0.0001) although correlation for VC/TG between the two methods remained poor (r=0.35, p=0.082). Certainly, further investigation into this issue is warranted.

To assess the severity of HLP3, we have developed an algebraic estimate of β-VLDL-C [69]. Starting with two equations,

$$\text{Total TG} = \text{"real" VLDL–TG} + \beta\text{–VLDL–TG} + \text{"measured LDL–TG"} + \text{HDL–TG}$$

and

$$\text{Measured VLDL–C} = \beta\text{–VLDL–C} + \text{"real" VLDL–C}$$

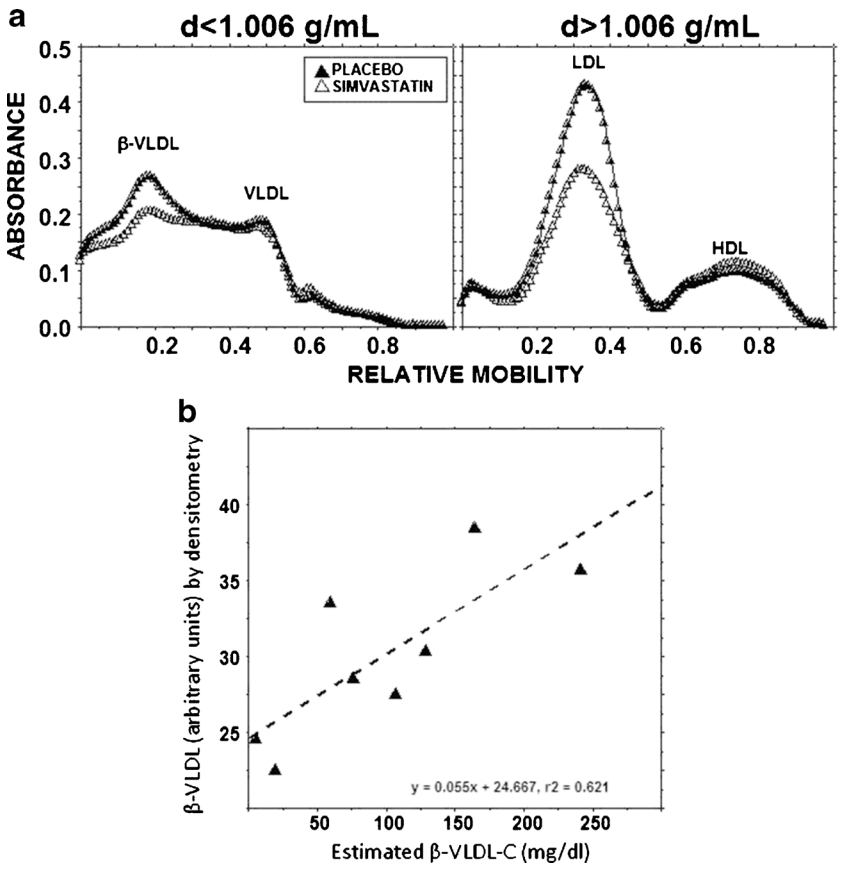


Fig. 9 **a** Effect of simvastatin at 80 mg daily in eight HLP3 patients as measured by densitometric scanning of agarose gels after electrophoresis of the ultracentrifugally isolated *d* <1.006 g/ml fraction (VLDL) and *d* >1.006 g/ml fraction (LDL and HDL) and staining with Sudan black. **b** β-VLDL-C concentration estimated by an algebraically derived equation (see the text) compared with densitometric scanning of the β band after agarose gel electrophoresis (same subjects as in **a**)

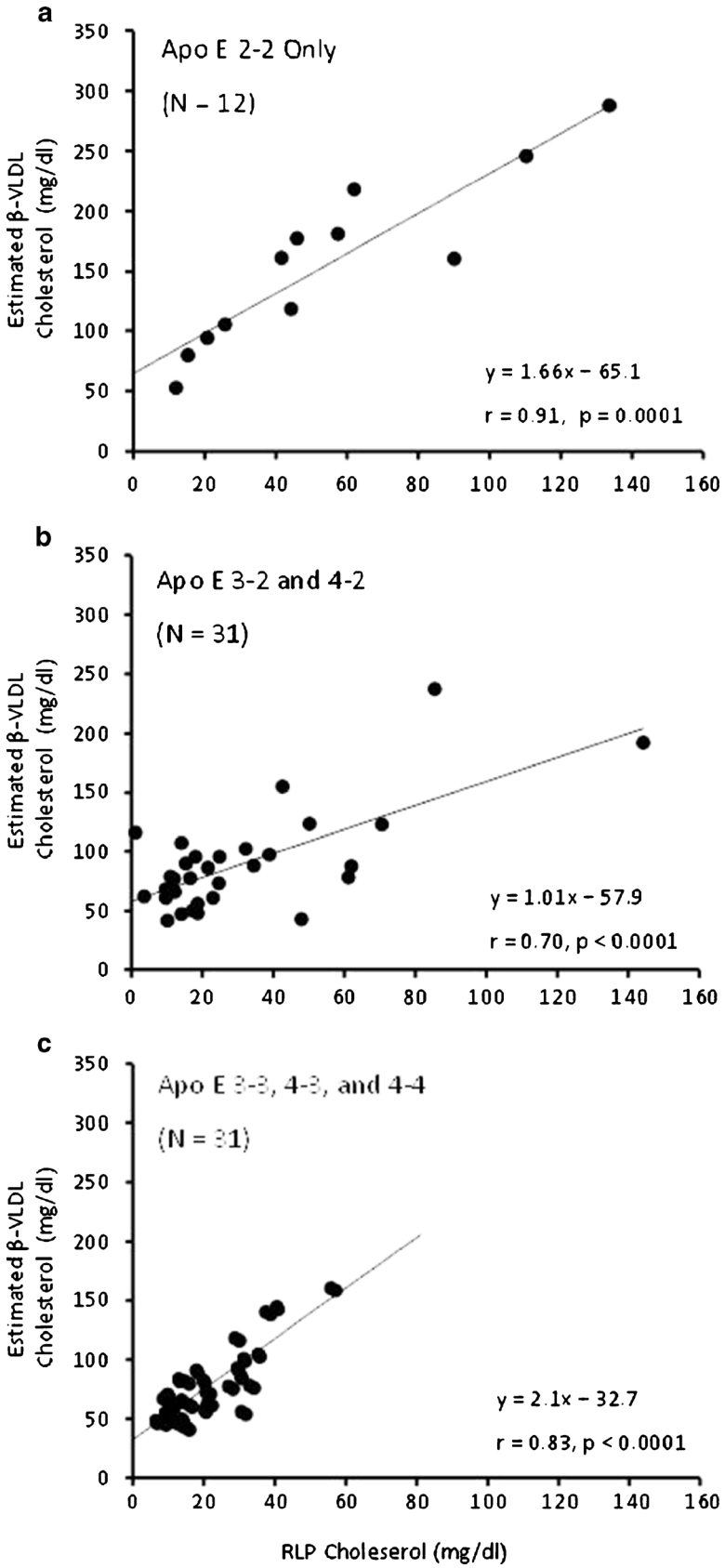


Fig. 10 Estimated β-VLDL-C concentration by algebraic calculation (see the text) versus remnant-like particle (*RLP*) cholesterol in subjects with APOE 2-2 (*top*), APOE 3-2 and 4-2 (*middle*), and APOE 3-3, 4-3, and 4-4 (*bottom*)

Table 6 Case history 1: Caucasian male with coronary artery disease related to "classic" HLP3. All lipids are mg/dl

Age (years)	Cholesterol	TG	HDL	Comments
31	215	328	34	1st MI
40	461	688	28	Still on no diet or lipid medications. MIs at 38 and 40 years, CABG at 40 years
40.5	377	395	33	Low-fat diet
41	371	503	30	Low-fat diet
42	256	418	35	Taking lovastatin 20 mg twice daily
43.1[a]	233	333	32	Taking lovastatin 20 mg twice daily. (outside laboratory)
43.4	176	209	35	APOE 2-2, measured LDL-C 45, VLDL-C 93, VLDL-C/TG 0.44. Lost 20 lb, and taking lovastatin 20 mg every night at bedtime
43.6	142	119	40	Gradually uptitrated niacin to 2,000 mg/day. Subsequently lipid concentrations remained as low or lower

CABG coronary artery bypass graft, *LDL-C* LDL cholesterol, *MI* myocardial infarction

[a] Time of initial referral. Prior values are for historical lipids.

together with the compositional ratios in Table 1 and the assumption that there is no chylomicron contribution in fasting plasma, we can derive the equation

$$\beta\text{-VLDL-C} = 1.53(\text{measured VLDL-C}) + 0.034(\text{mLDL-C}) + 0.093(\text{HDL-C}) - 0.312(\text{total TG}),$$

where mLDL-C refers to LDL-C concentration measured by ultracentrifugation. Alternatively, if VLDL-TG is available, the above equation simplifies to

$$\beta\text{-VLDL-C} = 1.53(\text{measured VLDL-C}) - 0.312(\text{measured VLDL-TG}).$$

We compared this estimated β-VLDL-C concentration with that obtained by densitometric scanning of the β band from agarose gels stained with Sudan black after electrophoresis of the d <1.006 g/ml fraction in a small series of HLP3 subjects before and after treatment with simvastatin a 80 mg. The results are shown in Fig. 9. We also used the RLP-C assay (marketed by Japan Immunoresearch Laboratories) to quantify remnant particles among HLP3 patients [106, 107]. We found a strong correlation between our estimated β-VLDL-C concentration and RLP-C concentration not only among HLP3 patients with APOE 2-2 but also in HLP3 patients with other APOE genotypes (see Fig. 10), providing evidence for both the validity of our semiquantitative estimate of β-VLDL-C and the

Table 7 Case history 2: Caucasian male with premature MIs and cardiovascular death. All lipids are mg/dl

Age (years)	Cholesterol	TG	HDL	Comments
28.9	354	447	34	Historical lipids. All untreated. Intolerant to simvastatin. BMI 33–36 kg/m^2
31.4	341	468	23	
34.6	370	550	36	
35.1	478	508	45	
37.8	309	371	28	MI
38.0	333	503	40	Ezetimibe 10 mg every day
38.7[a]	329	634	31	LDL 171 by calculation, measured LDL 39, VLDL 259, VLDL-C/TG 0.41. No APOE genotype
38.9	167	255	38	Ezetimibe 10 mg, fish oil 9 g, niacin 1,000 mg
39.9	121	167	35	Ezetimibe 10 mg, fish oil 1 g, niacin 2,500 mg, rosuvastatin 5 mg
42.3	290	309	39	Stopped therapy
42.5	240	101	46	1 day after major MI
42.8	108	116	38	Receiving therapy again. CHF death 3 months later

CHF congestive heart failure

[a] Time of initial referral. Prior values are for historical lipids.

Table 8 Case history 3. Caucasian fastidious male, no history of CAD, BMI 23 kg/m^2, and APOE 3-3. All lipids are mg/dl

Age (years)	Cholesterol	TG	HDL	LDL	VLDL-C	VLDL-C/TG	Therapy, daily doses
45.2	270	276	31	104	134	0.49	No therapy
45.4	128	181	37	35	57	0.31	Simvastatin 80 mg
45.5	150	177	38	44	68	0.38	Simvastatin 40 mg
45.7	190	224	29	67	94	0.42	Placebo
45.8	152	133	38	58	54	0.41	Fenofibrate 200 mg
48.7	123	66	54	55	14	0.21	Niacin 1500 mg, fenofibrate 160 mg

VLDL-C and LDL-C values were all measured by ultracentrifugation

frequent occurrence of HLP3 in persons other than those with APOE 2-2 [108]. The RLP-C assay does not, however, directly measure β-VLDL particles and can instead correlate strongly with total plasma TG concentration in persons with other lipid phenotypes [108, 109].

Finally, we have begun to compare a traditional definition of HLP3 using our current "wash" ultracentrifugation method (namely, VC/VT≥0.35, TG level above 150 mg/dl) with the definition of Sniderman et al. [7] (TC/APOB>6.2 and TG/APOB<10 with units of millimoles per liter for TC and TG and grams er liter for APOB, together with TG concentration above the 75t percentile, equivalent to 160 mg/dl in our population). Among 3,695 consecutive individuals, we found 16 with HLP3 using the ultracentrifugation-based definition (0.43 % prevalence) compared with 53 (1.43 % prevalence) using the method of Sniderman et al. This high prevalence suggests that the method of Sniderman et al. is somewhat nonspecific. There were 13 concordant cases (κ=0.37 with 81 % sensitivity for the approach of Sniderman et al.). There was considerably better agreement between the two methods using a TG cutpoint of 200 mg/dl, with the κ coefficient rising to 0.58; 13 cases remaining concordant, with 14 total cases identified by the ultracentrifugation method and 31 by the method of Sniderman et al. (93 % sensitivity, 99.5 % specificity versus ultracentrifugation).

Treatment of HLP3

Initial hygienic measures are of key importance in treatment of HLP3. These include treatment of hypothyroidism if present and discontinuation of offending drugs (which may include isotretinoin, excess alcohol intake, or oral estrogens or estrogen-like agonists). Ironically, estrogen deficiency also tends to cause or aggravate HLP3, and menopausal replacement hormone therapy may be considered for appropriate candidates (particularly women near menopause with symptoms of estrogen withdrawal such as

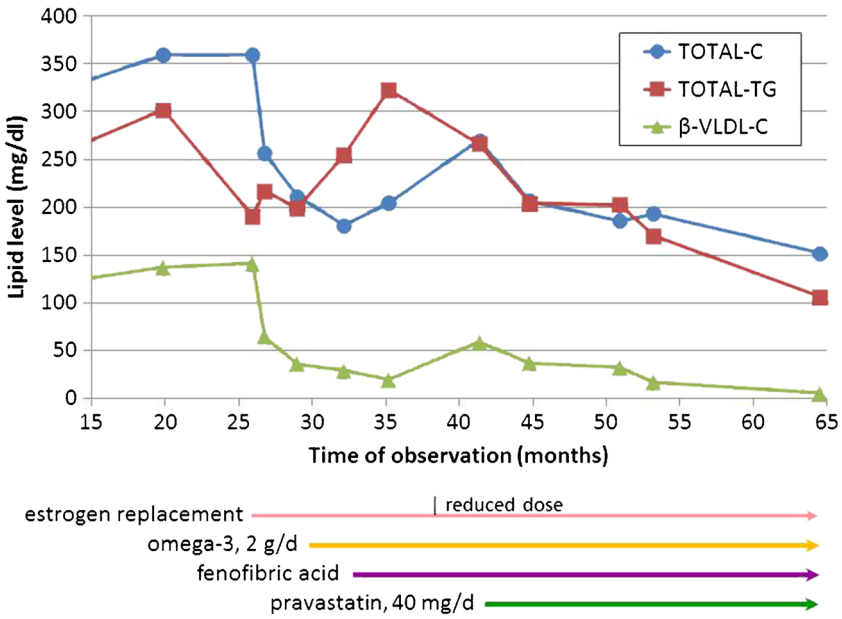


Fig. 11 Case history 4. White woman with HLP3, aged 56 years at the 25th month of observation. Shown are the plasma total cholesterol concentration, the concentration of total triglycerides, and β-VLDL-C concentration estimated by our improved "wash" method of ultracentrifugation including measured VLDL-C concentration and measured concentration of VLDL triglycerides

hot flashes). Weight loss is often central to HLP3 management, and long-term use of appetite suppressants (such as phentermine) may be considered.

Traditionally, a fibrate is the drug of first choice in treating patients with HLP3 [110, 111•]. Niacin has similar efficacy at a total dosage of at least 2,000 mg/day (as extended release once daily, or as immediate release 1,000 mg twice daily), but is generally harder to administer. Both drugs may suppress transcription of APOC3 and reduce VLDL production, particularly VLDL1 [112, 113]. Statins are also effective in treating HLP3, both by reducing VLDL1 production and by upregulation of the LDLR [87, 110, 111•, 114–116]. High-dose fish oil (6 g of omega-3 per day) improved HLP3 (50 % reductions in both TC and TG levels) but did not eliminate β-VLDL [117].

One of the hallmarks of HLP3 is the dramatic responsiveness of both plasma TC and total TG levels to appropriate treatment. Indeed, a history of marked and parallel increases and/or decreases in plasma cholesterol and TG levels (with similar absolute levels when expressed in milligrams per deciliter) is a strong indicator of HLP3, as illustrated by all the cases (Tables 6, 7 and 8, Fig. 11). Combination lipid therapy is often needed.

The most important goal in treating HLP3 is to prevent atherosclerotic disease. HLP3 patients may have CAD events at a young age, as shown by cases 1 and 2 (see Tables 6 and 7). Aggressive treatment of HLP3 can also reduce xanthomas and other clinical manifestations of the excess cholesterol deposition. For example, after 1 year of treatment with fenofibrate and atorvastatin, a Korean patient with HLP3 showed near complete regression of xanthomas, including palmar striae, dramatic regression of stenotic coronary atherosclerosis with no lesions seen on CT angiography after follow-up, and concurrent relief of angina pectoris [118•].

Conclusion

HLP3 is a discrete dyslipidemia phenotype caused by impaired TGRL remnant processing. It is much commoner than is often appreciated. CAD risk can be dramatically increased, especially in more severe cases. Not only a basic lipid profile but also newer "advanced" lipoprotein testing modalities appear to be surprisingly insensitive to the presence of HLP3 and, as a result, most HLP3 patients are probably undiagnosed, and therefore inadequately treated. In the future, simpler, less costly, and more widely available methods of accurate detection of HLP3 should be developed and validated. In the meantime, traditional methods, including ultracentrifugation, will continue to be needed to appropriately diagnose and manage this frequently unrecognized, but often severe disease.

Compliance with Ethics Guidelines All procedures performed in human subjects were approved by the University of Utah Institutional Review Board.

Human and Animal Rights and Informed Consent All human subjects provided informed consent. This article does not contain any studies using animals performed by any of the authors.

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- • Of importance
- •• Of major importance

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