substance in nephrotic syndrome and other renal diseases. This substance may play a critical role in maintaining the association of VLDL with lipoprotein lipase on the capillary-endothelial surface.

The hepatic-lipase deficient patient did not suffer from hyperlipidaemia but had a distinct abnormality in his lipoprotein profile. He showed no reduction in the VLDL₁ to VLDL₂ conversion and the catabolic mechanisms for the removal of VLDL₁, VLDL₂, IDL and LDL were unaffected. There was a substantial decrease in the VLDL₁ to IDL₁ conversion and virtually no transfer to LDL was occurring (Figure 2). A similar phenomenon was seen in the hypothyroid patients who appeared to have a secondary hepatic-lipase deficiency that resulted in raised IDL levels, due to a failure to convert IDL to LDL efficiently. Thyroxine treatment increased hepatic-lipase activity, promoted the conversion of IDL to LDL and significantly reduced the circulating IDL level. The other changes in the IDL and LDL metabolism in thyroid disease may be attributed to decreased activity of the LDL receptor as reported earlier by Thompson et al. [13]. This was corrected by therapy also.

These kinetic studies demonstrate that lipoprotein lipase and hepatic lipase operate at different ends of the VLDL to LDL delipidation sequence. Their roles are complementary and overlap only in the conversion of VLDL₂ to IDL₁.

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via a remnant receptor [3], while the remnants resulting from VLDL hydrolysis are processed in part to form low-density lipoproteins (LDL). The surface remnants of these triglyceride-rich lipoproteins contribute greatly to the lipid components of high-density lipoprotein (HDL).

**Molecular genetics of lipoprotein-lipase deficiency**

The first mutations that were described in the LPL gene occurred in a patient with undetectable LPL mass and activity in plasma and were a major deletion and a duplication [4]. Since this report in 1989, many different DNA-sequence alterations have been identified that cause LPL deficiency [5]. A summary of the sequence alterations that have been reported from our laboratories is shown in Figure 1. As can be seen the majority of these were missense mutations, mostly in exons 4, 5 and 6. In most instances different mutations segregate in unrelated families and in distinct population groups.

An exception to this is the substitution of leucine for proline at amino acid 207 that constitutes approx. 73% of the mutant alleles in patients with LPL deficiency from Quebec, Canada [6]. The prevalence, spatial distribution and genealogy of this mutation in the Quebec population have been determined [7]. Genealogical reconstruction traced this mutation to 16 founders, all of whom migrated to Quebec in the early 17th century from the northwestern part of France, especially from the regions of Perche.

Another mutation found in higher frequency in different populations is the substitution in exon 5 of glycine for glutamic acid at residue 188. This mutation has been found in unrelated affected persons of different ancestries, including those of French-Canadian, British, Polish, Dutch, German and East-Indian descent [8, 9]. This mutation has been found in all probands of Indian descent in Cape Town, South Africa as well [10]. These affected Indian kindreds have been traced independently to immigrants who arrived in South Africa during the first half of this century. No definite genetic relationship between these particular families has been established, although they came from four villages very close to the town of Khed, approx. 150 km from Bombay. A common ancestral origin for the mutation at position 188 has been postulated that may predate the spread of the Caucasian populations [8].

Most of the mutations shown in Figure 1 represent missense mutations and their functional significance has been demonstrated clearly by *in vitro* site-directed mutagenesis studies [4, 6, 8, 10–14, 16–18] (T. Bruin, S. Tuzgol, D. E. van Diermen, N. Hoogerbrugge-van der Linden, J. O. Brunzell, M. R. Hayden and J. J. P. Kastelein, unpublished results; T. Bruin, S. Tuzgol, W. J. Mulder, A. E. van der Ende, M. R. Hayden and J. J. P. Kastelein, unpublished results; Y. Ma, M.-S. Liu, D. Chitayat, U. Beisiegel, P. Benliane, I. Forsythe, J. D. Brunzell and M. R. Hayden, unpublished results; H. E. Henderson, Y. Ma, M.-S. Liu, I. Clark-Lewis, J. J. P. Kastelein, J. D. Brunzell and M. R. Hayden, unpublished results). Other mechanisms that produce a catalytically defective protein include insertions resulting in frameshifts. A frameshift mutation...
resulting from an insertion of 5'-GGGCT-3' at residue 102 results in a premature stop codon in exon 4 and a truncated protein is formed [16]. This truncated LPL protein is not detectable with the 5D2 monoclonal antibody that has epitope specificity for residue 400 in the C-terminal portion of the LPL protein [23]. Another frameshift mutation at the 3' end of exon 5 is due to the insertion of 5'-TAAATATT-3' after the second nucleotide of codon 229. This mutation results in a frameshift as well that is expected to produce a truncated protein of 238 residues, including nine foreign residues at its C terminal [23, 24].

The phenotype of lipoprotein-lipase deficiency

In the search for naturally occurring mutations in the LPL gene, the phenotype chosen for study has been chylomicronaemia. Therefore it is not surprising that patients who were shown to have mutations causing LPL deficiency presented with the classical phenotype of chylomicronaemia, often presented in infancy with abdominal pain, hepatosplenomegaly or failure to thrive. Some patients also develop eruptive xanthomas and lipaemia retinalis. The preliminary assessment of mutations in the LPL gene revealed that these mutations were almost always associated with a significant clinical phenotype.

However, we and others have described a mutation at residue 447 that is a C-G transversion that results in a premature termination codon at residue Ser447 [11, 25]. As a result the two C-terminal amino acids (Ser-Gly) are deleted from the mature protein. Despite this LPL retains most of its catalytic activity towards triacylglycerol. Therefore DNA changes in the LPL gene may be associated with completely defective LPL activity or with only a minor impairment of LPL catalytic activity. This reinforces the importance of performing in-vitro mutagenesis studies on any exonic DNA alteration in the LPL gene to try to determine whether these DNA changes represent functional defects or are DNA polymorphisms.

Mutations in the LPL gene may be associated also with partial LPL activity and a milder clinical phenotype. We have reported recently a mutation in the LPL gene that results in a defective LPL with partial residual activity [14]. Interestingly this patient is a 30-year-old woman who was not diagnosed with LPL deficiency until she developed pancreatitis during pregnancy. In the non-pregnant state the patient had only modestly elevated plasma-triglyceride levels. DNA analysis revealed that this patient was homozygous for a C-G transversion at nucleotide 770 in exon 5 that resulted in an amino-acid substitution of cysteine for serine at residue 172. In-vitro mutagenesis showed that this mutation had approx. 6% of the normal LPL enzymatic activity, which was significantly higher than that seen in other LPL-gene mutations previously identified in our laboratory. This mutation occurs in a residue highly conserved in LPL, pancreatic lipase and hepatic lipase. Nevertheless data reported suggest that this mutation in the LPL gene may result in partial activity that could manifest with only mild hypertriglyceridaemia. Different environments that either increase VLDL secretion and/or further decrease LPL activity may precipitate chylomicronaemia in these patients. These environmental triggers could include pregnancy, oestrogen therapy, or possibly alcohol intake, diabetes or β-adrenergic blocking agents.

Summary

We have described a large number of different mutations in the LPL gene that result in completely catalytically defective LPL protein. More recently exonic polymorphisms in the LPL gene have been described that do not result in the catalytic activity of LPL being significantly impaired. Furthermore we have recently described a patient who is homozygous for a mutation in the LPL gene in a conserved region of exon 5 that results only in partial residual activity and a very mild clinical phenotype. This may suggest that the frequency of mutations in the LPL gene is greater than has been previously recognized.

Recognition and selection of patients for analysis was based on the phenotype of chylomicronaemia. However, the existence of the Ser172>Cys mutation in the LPL gene that results in only moderate hypertriglyceridaemia in the absence of environmental factors might suggest that mutations in this gene are more frequent and could be seen in patients with a milder clinical phenotype. The clue to detecting these changes in the LPL gene might be to investigate patients who present with chylomicronaemia due to different environmental triggers while, in the absence of these environmental factors, they have only moderate hypertriglyceridaemia.

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Lipoprotein Metabolism in Health and Disease

Department of Medical Biochemistry and Biophysics, University of Umeå, S-901 87 Umeå, Sweden

Lipoprotein lipase (LPL) hydrolyses triglycerides and some phospholipids in chylomicrons and in very-low-density lipoproteins [1]. The products of the lipolysis, free fatty acids and monoacylglycerols, move readily along and across cell membranes and aqueous spaces in the tissue [2]. Hence the result of the lipase reaction is to make lipids available for the lipolysis, free fatty acids and monoacylglycerols, LPL activity in some tissues, notably adipose tissue and muscle, changes rapidly in response to physiological and pathophysiological conditions [1, 3]. These changes in LPL activity are associated with parallel changes in the tissue uptake of fatty acids from plasma lipoproteins [3]. We will review here studies on the mechanisms involved in the acute changes of LPL activity that occur in response to feeding/fasting.

LPL acts at, but is not made in, vascular-endothelial cells. Other cells produce and release the lipase for transfer to binding sites at the endothelium. Quantitatively the dominant tissues in LPL production are adipose and muscle [1]. During lactation there is high LPL activity in the mammary gland; this directs plasma-triglyceride fatty acids to the synthesis of milk lipids [4]. Other sites of LPL synthesis include macrophages [5], hormone-pro-