Lipoprotein metabolism and its derangements

An understanding of lipid metabolism and its dis - orders is increasingly necessary to the GP in this era of sedentary lifestyles and over-indulgence.

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The purpose of this article is to provide the medical practitioner with an understanding of lipids, lipoproteins and their metabolism and disorders. Such an understanding would enhance the assimilation of the sections on clinical assessment and treatment of dyslipoproteinaemia.

Lipids may be defined as organic chemicals that are insoluble in water. In the biological con - text lipids are either car - boxylic acids (fatty acids) or sterols, and their derivatives.

Lipids may be defined as organic chemicals that are insoluble in water. In the biological context lipids are either carboxylic acids (fatty acids) or sterols, and their derivatives. Lipids are less dense than water and will float spontaneously or under centrifugal force. Cholesterol is the principal sterol in the animal kingdom and promotes the impenetrability of the phospholipid bilayer that constitutes the cell membrane. Additionally cholesterol is found in lipoproteins and in bile and is used to synthesise hormones and bile acids. Cholesterol is stored and transported in an esterified form with fatty acids. Salts of fatty acids are detergents that would disrupt associations of lipids such as membranes. For this reason their concentration in the body is low and they are carried on albumin or other proteins for protection. Fatty acids are the building blocks of phospholipids (glycerol with 2 fatty acids, a phosphate and a polar group such as choline, ethanolamine or serine) and triglyceride (glycerol with 3 fatty acids) as well as more complex lipids. Fatty acids serve as energy substrates that are transported and stored as triglycerides. They also have a structural role in cell membranes, as well as recently recognised roles in targeting proteins to cell membranes.

Cells utilise fatty acids for energy through β-oxidation, as structural components in phospholipids and for energy storage as triglycerides. The fatty acids can be taken up from the environment directly or by taking up more complex lipids and digesting them in the lysosomes. The growth of cells demands cholesterol for membrane formation. This demand is met by *de novo* synthesis of cholesterol (in which HMGCoA reductase is the rate-limiting enzyme), expression of low-density lipoprotein (LDL) receptors to take up circulating lipoprotein as a ready source of cholesterol and hydrolysis of cholesterol ester, the storage form of cholesterol. Cells that have high demands for cholesterol utilise the same mechanisms for cholesterol balance when they are mature: hepatocytes, adrenocytes and enterocytes. Mature cells that do not have a demand for cholesterol may accumulate this sterol even if the *de novo* synthesis is down-regulated along with receptor expression. In this setting, recently discovered adenosine cassette-binding proteins are utilised to promote cholesterol efflux, as well as organelles called caveolae.

LIPOPROTEINS

Lipids need to be transported and targeted for specific sites and purposes in the body. Although they have different functions, the lipids will associate with one another owing to their insolubility in an aqueous environment.These associations also involve special proteins, called apolipoproteins or apoproteins. The whole particle, comprising lipid and protein, is called a lipoprotein.The structure of a lipoprotein typically involves the assembly of the more polar lipids, phospholipid, free cholesterol and protein on the exterior of a sphere where they can interface with the aqueous environment, and neutral lipids such as cholesterol ester and triglyceride, in the centre.

The properties of lipoproteins are described in Table I. It can be seen that triglyceride is the major component of the larger lipoproteins which are also the least dense as a result of the low specific gravity of lipids. The large diameters of chylomicrons (CM) and very low-density lipoprotein (VLDL) result in the scattering of light and hence turbidity of plasma.The cholesterol-rich particles contain more protein, increasing the density to LDL which also contain apolipoprotein B (apoB) as it is the final product of VLDL metabolism (Fig. 1), and high-density lipoproteins (HDL) in which the most prominent apoprotein is apoAi. Except for apoB, all other apoproteins can exchange between lipoproteins. Apoproteins serve as a nidus for phospholipids to initiate the formation of lipoproteins, as ligands for receptors and modulators of enzyme activities. The conformation permits apoB to be a ligand for the LDL receptor only on LDL, while apoE is a ligand for this receptor and other LDL receptor-like proteins irrespective of the lipoprotein on which it resides. ApoAi, apoAii and apoAiv are involved in the development of HDL. ApoCii activates and apoCiii inhibits lipoprotein lipase (LPL). Apo(a) is a protein that is linked to apoB on an LDL-like particle. It resembles haemostatic proteins and may have an antifibrinolytic and wound-repair function.

The assembly of chylomicrons and VLDL is by a specialised mechanism in enterocytes and hepatocytes. A large protein, apoB, serves as the template for assembly of triglyceride: in the hepatocyte the whole gene (B_{100}) is employed while in the enterocyte the mRNA of this gene is edited to code for $apoB_{48}$.The intracellular neutral lipid is added to the apoB by microsomal triacylglycerol transfer protein (MTP).

In the circulation, lipoproteins undergo continuous modification. On the vascular endothelium of muscle and adipose tissue, held in place by proteoglycans, is LPL which, in the presence of apoCii, will hydrolyse triglycerides. At the liver, hepatic lipase has a similar action but acts also on smaller lipoproteins that contain triglyceride. Lecithin:cholesterol acyl transferase (LCAT) acts mainly on HDL to convert free cholesterol to cholesterol ester which, upon moving into the core of HDL as a neutral lipid, will create a space for more cholesterol in the phosholipid shell of the particle. Cholesterol ester transfer protein (CETP) exchanges neutral lipid between lipoproteins, but owing to the rapid catabolism and removal of triglyceride-rich lipoproteins, there is a nett transfer of cholesterol from HDL and of triglyceride to HDL. In hypertriglyceridaemia, the enrichment of LDL and HDL with triglyceride makes them susceptible to a reduction in size by hepatic lipase. Paraxonase is an enzyme present on HDL and is involved in the metabolism of oxidatively modified fatty acids. Several other proteins that are associated with lipoproteins are still undergoing investigation.

LIPOPROTEIN METABOLISM

A convenient division of lipoprotein metabolism identifies 5 pathways: (1) an exogenous lipid path-

Table I. Properties of lipoproteins

Lp	Density (g/ml)	Size (nm)	Protein (% mass)	Triglyceride (% mass)	Cholesterol (% mass)	Apoproteins
CM	<0.95	75 - 1200	1 - 2	85 - 90	5 - 8	B_{48}, A, C, E
VLDL	0.95 -1.006	30 - 80	6 - 12	55 - 65	15 - 20	B_{100}, A, C, E
IDL	1.006 -1.019	25 -35	10 - 20	15 - 30	20 - 30	B_{100}, C, E
LDL 1.020 - 1.063		18 - 25	20 - 25	8 - 12	42 - 48	B_{100}
Lp(a)	1.055 - 1.095	27 - 32	25 - 35	3 - 12	35 - 45	B_{100}, (a)
HDL_2	1.063 - 1.112	9 - 12	35 - 45	6 - 10	22 - 28	A, C, E
HDL_3	1.112 -1.210	3 - 5	50 - 60	3 - 5	15 - 18	Ai, Aii

CM = chylomicrons; VLDL = very low-density lipoprotein; IDL = intermediate density lipoprotein consisting mainly of remnants of VLDL; LDL = low-density lipoprotein; Lp(a) = lipoprotein 'little a'; HDL = high-density lipoprotein.

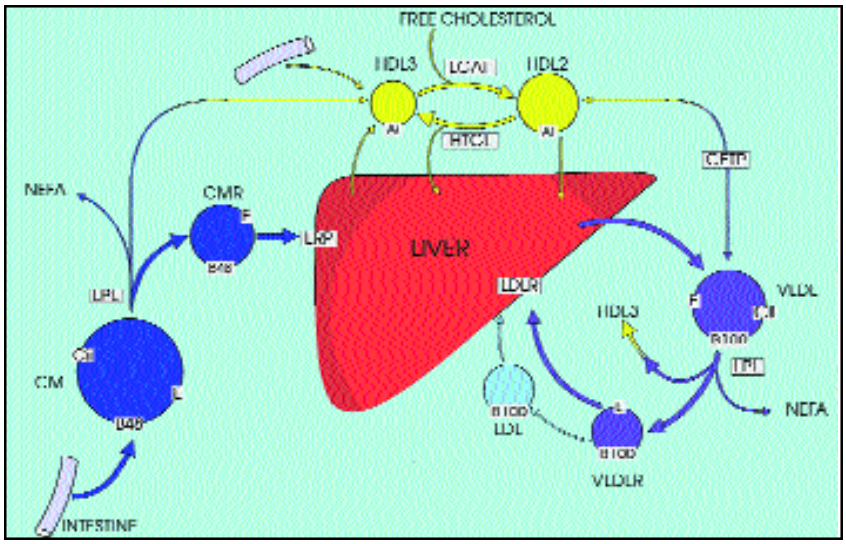

Fig. 1. Lipoprotein metabolism in plasma. The lipoproteins are not drawn to scale.

way for dietary lipids, (2) an endogenous triglyceride pathway which also has a component that yields (3) LDL as a source of cholesterol, (4) reverse cholesterol transport and (5) lipoprotein (a). A sixth consideration may be the complex in plasma of albumin and free fatty acids. The latter two pathways are not considered in detail in this review.

The exogenous pathway distributes dietary cholesterol and fatty acids. Dietary lipids comprise mainly triglyceride and cholesterol but some phospholipid and cholesterol ester will also be present, as well as the plant sterols (phytosterols include sitosterol, campesterol and others) that are not assimilated. The pancreatic enzymes digest these dietary lipids to fatty acids and cholesterol before the enterocyte re-assembles triglyceride and phospholipid to package into the CM along with the absorbed cholesterol from dietary and biliary origin.The CM pass through the thoracic duct to the systemic circulation where most of their apoproteins are obtained through redistribution from HDL.The apoCii thus obtained will activate the LPL on the endothelium of muscle and adipose tissue, as well as in breast tissue under appropriate hormonal stimulation.The fatty acids released by lipolysis serve the following purposes: energy substrate, energy stores and the preparation of milk. In the process during which the core of the CM is reduced in size, the redundant phospholipid on the surface buds off with apoAi to form HDL and 'CM-remnants' form.The apoE on these remnants acts as the ligand for the LDL receptor and the LDL receptor-related protein so that the remaining triglyceride and the dietary cholesterol may be returned to the liver. The digestion and absorption and packaging of dietary lipid delays the appearance of CM in the circulation but a rise in triglyceride concentration and a slight turbidity of plasma can be expected 3 hours after a fatty meal. A 10 - 12-hour fast should allow for complete disposal of CM from the circulation.

The endogenous triglyceride pathway could be viewed as parallel to the exogenous pathway for triglyceride distribution between meals. The VLDL triglyceride also undergoes lipolysis by activated LPL and produces HDL and remnants. The bulk of the VLDL remnants is cleared by virtue of apoE, but a small proportion forms LDL.This pathway sustains the fasting triglyceride concentration.

During the transformation of VLDL remnants to LDL, a process thought to be dependent in part on hepatic lipase, all apoproteins other than apoB are lost and apoB assumes the correct conformation to bind the LDL receptor (also called the B,E receptor).This lipoprotein provides the bulk of the total cholesterol in the plasma of humans and acts as the source of cholesterol for cells which require this substance.

The process that can remove cholesterol from somatic cells and return it to the hepatocyte to satisfy its needs for cholesterol (cholesterol and bile acid synthesis for bile), is called reverse cholesterol transport. Cells which have low demands for cholesterol may still accumulate it in excess from (down-regulated) *de novo* synthesis and nonspecific uptake from the exterior. The cell responds to apoAi on HDL by transporting cholesterol to the surface with an adenosine binding cassette transporter protein, ABCA1. Phospholipid and cholesterol transfer to apoAi to form the nascent lipoprotein that commences the HDL pathway. At this point LCAT will further develop HDL by forming cholesterol ester with a fatty acid from phospholipid, resulting in a transfer to the core. Cholesterol ester in turn, will be exchanged by CETP into the triglyceride-rich lipoproteins which are destined to return to the liver. The cholesterol ester may also be delivered in HDL through scavenger receptor B1 on the liver.

In the normal subject who has fasted, the liver sustains a triglyceride concentration of 1.5 mmol/l that is for a large part supported by the influx of fatty acids from adipose tissue. Consider the course of

events after consuming 200 g of chocolate that represents 80 g of triglyceride (or 100 mmol). The CM will enter the plasma from about half an hour after the meal. The concentration rises to a peak after about 3 hours when it becomes turbid, and finally all dietary triglyceride will leave the circulation by catabolism and clearance. If no triglyceride is cleared, the 100 mmol of triglyceride would change the plasma concentration by 33 mmol/l. Plasma of a normal fasting subject eating a low-cholesterol diet will have an LDLC concentration of about 3 mmol/l. When the diet is enriched with cholesterol, the cholesterol meal of 2 egg yolks (approximately 1.2 mmol) will not be detectable even at the peak concentration of CM but the delivery of cholesterol to the liver will load the hepatocyte with cholesterol and will down-regulate the LDL receptors, leading to the establishment of a new, higher, steady-state concentration of LDL — on average, by about 1.5 mmol/l higher. The plasma HDLC is about 1.2 mmol/l and that in VLDL is about 0.8 mmol/l. The sum of these figures is 5 mmol/l.

DISORDERS OF LIPOPROTEIN METABOLISM

Hypercholesterolaemia of apoB-containing lipoproteins and HDL hypocholesterolaemia are the result of an interplay of environmental and genetic factors and are correlated with atherosclerosis. Atherosclerosis is a complex process in arteries, involving inflammation and lipid accumulation and has the potential of becoming complicated by clinically manifest vascular disease. In severe derangements of lipoprotein metabolism, generally characterised as autosomal dominant or recessive illnesses, the physical signs and the high risk of complication make for easy clinical and biochemical recognition. Pancreatitis is the consequence of hypertriglyceridaemia which may also be a clinically recognisable phenotype.

A convenient classification of dyslipidaemia, as described in Table II, is based on the commonly measured total cholesterol and triglycerides and concerns hyperlipidaemias of apoB-containing lipoproteins. It is important to consider the contributions of HDL and LDL separately to better understand the risk of atherosclerosis. Mixed dyslipidaemia and hypertriglyceridaemia may be due to accumulation of single lipoprotein species or combinations of lipoproteins. LDL that has modulated to a small size by triglyceride enrichment is pro-atherogenic.

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A lipoprotein species changes its concentration as a result of a different production and/or clearance rate — these may be primary or secondary. The primary causes for dyslipoproteinaemia are indicated in Table III. The classification given here describes the range of disorders that can be determined by performing cholesterol and triglyceride concentrations. The complete lipid profile is best assessed after full lifestyle modification. The fasting profile is important for providing the triglyceride concentration

Table II. Clinical classification of primary causes of dyslipoproteinaemia

	Hypercholesterolaemia			Mixed	Hyperglyceridaemia	
	Severe	Moderate	Mild	hyperlipidaemia	Moderate	Severe
TG	←	< 1.5	→	1.5 - 5	5 - 15	> 15
TC	> 7.5	> 5.0	> 5.0	Variable	Variable	
LDLC	> 5.0	> 4.0	> 3.0	Variable	Variable	Little to none
IDLC	Negligible	contribution		Small dense LDL in all hypertriglyceridaemias		
Lp(a)	Negligible	contribution		Dysbetalipoproteinaemia		
HDLC		>1.6			Low HDLC with hypertriglyceridaemia	
LpX	Cholestasis					

TG = triglyceride; TC = total cholesterol; IDLC = intermediate-density lipoprotein cholesterol; LDLC = low-density lipoprotein cholesterol; HDLC = high-density lipoprotein cholesterol; LpX = lipoprotein X.
It is important to consider HDL as a significant contributor to moderate and severe hypercholesterolaemia as it is usually a negative risk factor. IDLC usually contributes negligibly to pure hypercholesterolaemia while IDLC increases in dysbetalipoproteinaemia which can also cause severe hyperglyceridaemia. Lipoprotein X occurs with high concentrations of bile acids.

Table III. Mechanisms and manifestations of primary dyslipoproteinaemia phenotypes

Pattern	Primary cause(s)	Clinical manifestations
CM	LPL deficiency	Recessive; childhood to young adulthood; severe hypertriglyceridaemia, pancreatitis; rare but present in South Africa
	ApoCii deficiency	
CM, VLDL	FHTG	Dominant; poorly understood
	FCH	Dominant; overproduction of VLDL but dietary fat or genes in lipolytic cascade may increase CM; sometimes eruptive xanthomata
VLDL	FHTG, FCH	No physical signs
IDL	ApoE mutations	Dyslipidaemia only in adult; some have cutaneous, palmar & tendinous xanthomata; mostly apoE2/2 that requires additional trigger but also dominant forms; uncommon
LDL	LDL receptor (FH)	Dominantly inherited; adults commonly display tendon xanthomata, corneal arcus, premature ischaemic heart disease; LDL clearance limited; world-wide prevalence about 0.2% but founder effects in South Africa: 1% Afrikaners; homozygous FH displays cutaneous & tendinous xanthomata with highly premature ischaemic heart disease; very rarely a recessive phenocopy is due to deficiency of adaptor protein for LDLR
	ApoB (FDB)	Phenocopy of FH but rare in South Africa
	FCH	Dominant, variable pattern in family; no physical signs, about 2% of population
	Small dense LDL	Poorly understood causes, about 20% adults, typical of insulin resistance
	Phytosterolaemia	Very rare, recessive; phenocopy of hoFH with moderate hypercholesterolaemia only
Absent apoB	Hypobetalipoproteinaemia	Dominant, uncommon; low cholesterol and triglyceride in heterozygote; homozygous state has extremely low cholesterol, stunted development, retinitis pigmentosa and neuromuscular degeneration
	Abetalipoproteinaemia	Recessive, rare; defect of MTP required to assemble lipoproteins; phenocopy of homozygous hypobetalipoproteinaemia
Low HDL	ApoAi defects	Rare; some confer low HDL and heart disease
	LCAT deficiency	Rare, dominant; renal disease or clouded cornea (fish eye disease)
	Tangier disease	Rare, dominant; adenosine binding cassette transporter defect disrupts reverse cholesterol transport; homozygote may display hepatosplenomegaly and tonsillar enlargement
High HDL	CETP deficiency	Rare, dominant; atherogenic as reverse cholesterol transport may be disrupted; mainly reported from the Orient

CM = chylomicrons; VLDL = very low-density lipoproteins; IDL = intermediate-density lipoproteins (remnants); HDL = high-density lipoproteins; LPL = lipoprotein lipase; FHTG = familial hypertriglyceridaemia; FCH = familial combined hyperlipidaemia.

from which LDL cholesterol is calculated but LDL cholesterol can also be measured by direct means. High LDL concentration as a result of decreased clearance may be due to mutations in the LDLR gene (familial hypercholesterolaemia (FH) or apoB (familial binding defective B_{100}, (FBD)). This pattern is called type II hyperlipidaemia in the Fredrickson classification. Overproduction of VLDL is thought to be the cause of familial combined hyperlipidaemia (FCH) and the lipoprotein phenotype within the family varies considerably according to the presence of relatively minor additional genes: increases of LDL, and/or VLDL (type IIb or when VLDL is predominant, type IV).These disorders are dominantly inherited. Dysfunctional apoE delays the clearance of remnant lipoproteins

which accumulate as dysbetalipoproteinaemia (type III hyperlipidaemia).There are recessive and dominant forms of dysbetalipoproteinaemia.The commonest setting for dysbetalipoproteinaemia is the homozygous state of apoE2, which delays remnant clearance, together with some other genetic or environmental factor that stresses production of VLDL or clearance by LDLR. The accumulation of CM alone, as a result of deficiency of LPL or its activator apoCii, is type I hyperlipidaemia and is recessively inherited.The accumulation of VLDL and CM, known as type V hyperlipidaemia, is usually the result of overproduction and underclearance of lipoproteins. It represents an interplay of dietary fat, lipase activity and other apoprotein variations.

Severe hypocholesterolaemia (< 2.5 mmol/l) is the result of an inability to produce apoB-containing lipoproteins. In hypobetalipoproteinaemia, the heterozygotes have very low LDL concentrations and may have offspring who have no detectable LDL,VLDL and CM.This causes malabsorption and oxidation of lipids in the eye and neuromuscular dysfunction. Vitamin E can only be assimilated through CM.The same phenotype, abetalipoproteinaemia, may be the result of the recessively inherited defects in MTP. Hypocholesterolaemia due to an inability to synthesise cholesterol is associated with severe phenotypic sequelae.

Very low HDL cholesterol concentrations may be seen as a recessive trait with defects in the adenosine binding cassette transporters that enable cholesterol to efflux into HDL, while there are also dominantly inherited diseases as a result of mutations of apoAi and LCAT. High HDL concentrations may be seen in CETP deficiency, a condition that may be atherogenic as a result of impaired reverse transport.

Rare, recessively inherited metabolic errors of sterol metabolism include phytosterolaemia (overabsorption of plant sterols resulting in xanthomata and atherosclerosis) and cerebrotendinous xanthomatosis (impaired cholesterol oxidation resulting in accumulation of cholestanol resulting in xanthomata, cataracts and leucodystrophy). These disorders are associated with normal plasma cholesterol concentrations.

IN A NUTSHELL

Fatty acids are important sources of energy and are esterified onto glycerol to form triglycerides and phospholipids. The former serve as a transport and storage mechanism for energy in the body while the latter are structurally crucial for cell membranes. Fatty acids also form esters with cholesterol which can be stored in cells or be transported in lipoproteins.

Dietary fat is digested to the component fatty acids and cholesterol before assimilation by the enterocyte which metabolises them to triglyceride and cholesterol ester before incorporating these into CM. MTP assembles the lipids on $apoB_{48}$. LPL and Cii are crucial for processing CM and apoE is necessary to clear CM remnants. Postprandially, triglycerides in CM are processed rapidly and efficiently. The contribution from dietary cholesterol to the total plasma cholesterol is small immediately after the meal but regulation of the LDLR results in significant changes over weeks.

The liver sustains plasma triglycerides by the production of VLDL, using $apoB_{100}$ and MTP for assembly. VLDL is metabolised in a similar manner to CM; the VLDL remnants clearing by virtue of apoE. In contrast to CM, a proportion of the VLDL remnants is metabolised to LDL. LDL carries most of the plasma cholesterol in humans and is cleared by the binding of apoB to the LDLR. It is atherogenic, especially when smaller and denser, a modulation that can happen with mild hypertriglyceridaemia.

Reverse cholesterol transport begins intracellularly with ABC A1 which permits the export onto apoAi and phospholipid complexes. LCAT allows more cholesterol assimilation onto HDL. CETP transfers cholesterol ester to other lipoproteins for delivery to the liver but cholesterol ester can also be transferred to the liver directly.

Familial hypercholesterolaemia (FH) is a common metabolic error world-wide and is even more common in several South African communities. It is due to mutations in the LDLR. Heterozygous FH is characterised by an increase in LDLC (>5 mmol/l), tendon xanthomata and premature heart disease. Familial binding defective apoB is phenotypically similar. Familial combined hyperlipidaemia (FCH) is also characterised by dominant inheritance but there may be family members with different hyperlipidaemia patterns and tendon xanthomata are absent.

Dysbetalipoproteinaemia is a mixed hyperlipidaemia due to dysfunction of apoE. Severe hypertriglyceridaemia due to CM accumulation is seen with homozygous LPL or homozygous apoCii deficiency. When apoB is truncated to the extent that it cannot assimilate lipid, or when MTP is absent, the failure of CM production causes malabsorption and low postprandial triglycerides and the failure of VLDL production results in very low cholesterol concentrations.