Normal and abnormal lipid and lipoprotein metabolism

There is a range of disorders of lipoprotein metabolism with varied biochemical changes, risk and manifestations depending on the cause.

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This article focuses on lipid and lipoprotein metabolism and introduces a range of genetic disorders that may be encountered in medical practice.

Lipids

Lipids are defined as organic molecules that are poorly soluble in water but dissolve well in organic (non-polar) solvents such as chloroform. This broad definition includes fatty acids and their esterified forms (phospholipids, triglyceride) and sterols and their esters. There are many more complex lipids that are beyond the scope of this article.

Fatty acids are carboxylic acids. Their detergent nature is exemplified by soap, which is made from triglyceride saponified by caustic soda. Free or unesterified forms are therefore generally not found in significant concentrations in the body. In plasma, fatty acids are bound to albumin. Biologically important fatty acids are 16 - 22 carbons long. Their carbon:carbon bonds may be saturated, mono-unsaturated or polyunsaturated. Nomenclature indicating double bonds is variable, but often the methyl carbon serves as the reference point. Using this nomenclature the fatty acids are referred to as n-x or omega-x fatty acids. Typically, unsaturated fatty acids from the sea are n-3, from terrestrial plants n-6 and from mammals n-9.

Fatty acids are oxidised for energy but are transported or stored as triglyceride.

Esterified fatty acids perform an essential role in cell membranes as phospholipids. The fatty acids on the middle carbon of the glycerol backbone of phospholipids are generally unsaturated and their cleavage by phospholipase initiates the eicosanoids and related products that are well known for their action on coagulation and inflammation. In general, the n-3 polyunsaturated fatty acids confer favourable anti-atherogenic properties. Fatty acids are oxidised for energy but are transported or stored as triglyceride. Since these esters of glycerol are large and insoluble molecules, lipases have to break them down into their component fatty acids for entry into cells and transport within cells. Adipocytes assimilate fatty acids and synthesise triglyceride, whereas muscle tissue requires fatty acids for energy.

Cholesterol is an essential component of cell membranes in animals but is also vital for steroid hormone and bile acid synthesis as well as embryonal development. Cholesterol is transported or stored as cholesterol ester (CE). In the plant kingdom 'phytosterols' are used for membranes: sitosterol, campesterol and others. These sterols are specifically excluded from mammalian systems by enteric and hepatic transporter proteins known as adenosine-binding cassette G5 and G8 (ABCG5 and ABCG8).

Lipoproteins

Lipoproteins are associations of proteins and lipids that undergo changes in their passage through the circulation as summarised in Fig. 1. The slightly polar lipids and non-polar lipids associate into spherical structures that are suspended in the plasma and whose fate is dependent on the associated proteins.

The **apoproteins** can be classified into two main groups: hydrophobic and amphipathic (having hydrophobic and hydrophilic aspects). The extremely hydrophobic apolipoprotein B (apoB) is essential for initiating the assembly of triglyceride-rich lipoproteins in enterocytes and hepatocytes. The latter cells translate the entire gene to produce a very high molecular mass protein ($apoB_{100}$) that is crucial for assembly and secretion of very-low-density lipoprotein (VLDL). ApoB remains associated with the particle through its metabolism to low-density lipoprotein (LDL). Enterocytes edit the mRNA for apoB at 48% of the length to produce $apoB_{48}$ for the assembly of the chylomicron (CM). Both the CM and VLDL transport triglyceride and smaller amounts of cholesterol.

The amphipathic apoproteins may exchange between lipoproteins. They include apoAI, apoAII, apoAIV, apoAV, apoCI, apoCII, apoCIII and apoE as well as several lesser-known apoproteins. Apoproteins AI - V are found predominantly on high-density lipoproteins (HDL) where they serve structural roles and also activate the enzyme lecithin:cholesterol acyltransferase (LCAT) that esterifies cholesterol on HDL. The CE moves to the core and provides space for more free cholesterol to occupy the shell of the lipoprotein. About 80% of plasma CE is derived by this route. ApoCII activates lipoprotein lipase (LPL) and apoCIII inhibits it. ApoE is an important apoprotein for the clearance of remnants of the triglyceride-rich lipoproteins. Cholesterol ester transfer protein (CETP) exchanges neutral lipids between lipoproteins, causing a net movement of cholesterol from HDL and LDL to CM and VLDL and a net movement of triglyceride in the reverse direction.

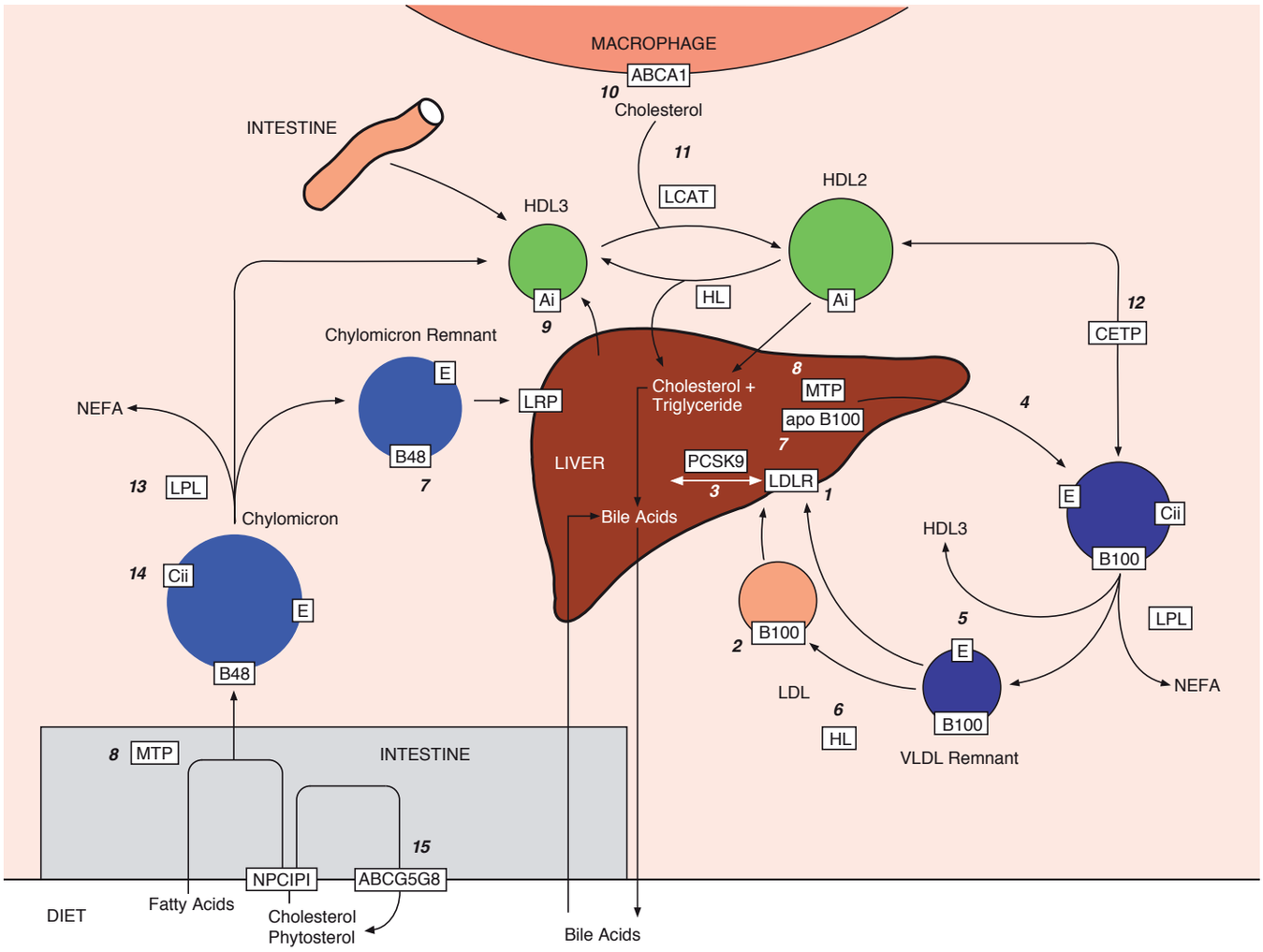

Fig. 1. Lipoprotein pathways and monogenic disorders. ***Apoproteins****: Ai – apolipoprotein Ai; B100 – apolipoprotein B100; B48 – apolipoprotein B48; Cii – apolipoprotein Cii; E – apolipoprotein E.* ***Lipoproteins****: CM – chylomicron; HDL – high-density lipoprotein; LDL – low-density lipoprotein; VLDL – very-low-density lipoprotein.* ***Enzymes and other proteins****: ABCA1 – adenosine-binding cassette transporter A1; ABCG5G8 – adenosine-binding cassette transporter G5 and G8; CETP – cholesterol ester transfer protein; HL – hepatic lipase; LCAT – lecithin:cholesterol acyltransferase; LPL – lipoprotein lipase; MTP – microsomal triglyceride transport protein; NPC1P1 – Niemann-Pick C1-like protein 1; NEFA – non-esterified fatty acid.*

Hypercholesterolaemia of apoB-containing lipoproteins*: 1 – LDLR mutations; 2 – familial binding-defective apolipoprotein B100; 3 – proconvertase subtilin/kexin type 9 (gain of function mutations destroys LDL receptors, loss of function mutations decreases LDL); 4 – familial combined hyperlipidaemia putatively from overproduction of lipoproteins.* ***Mixed hyperlipidaemias:*** *4 – familial combined hyperlipidaemia; 5 – dysbetalipoproteinaemia from apolipoprotein E mutations; 6 – hepatic lipase deficiency.* ***Hypocholesterolaemia of apoB-containing lipoproteins****: 7 – truncations of apoB; 8 – MTP deficiency.* ***Hypoalphalipoproteinaemias:*** *9 – apoAi mutations/deletion; 10 – ABCA1 deficiency; 11 – LCAT deficiency.* ***Hyperalphalipoproteinaemias:*** *6 – hepatic lipase deficiency; 12 – CETP deficiency.* ***Hypertriglyceridaemias:*** *4 – familial combined hyperlipidaemia; 13 – lipoprotein lipase deficiency; 14 – apoCii deficiency; 15 – phytosterolaemia results in hypercholesterolaemia when ABCG5G8 is defective.*

The only mature cells that have a persistent demand for cholesterol are those that produce lipoproteins, bile acids or corticosteroid hormones.

Other enzymes include paraoxonase, which is thought to protect against peroxidation, and phospholipase A2 that may incite inflammation. Newer technology has revealed HDL to be extremely complex with as many as 48 different proteins present, including proteins viewed as part of immune responses and enzyme inhibitors.

Lipoproteins were originally classified as α-, β- and pre-β-lipoproteins according to their migration during electrophoresis. These represent HDL, LDL and VLDL, respectively. Lipoprotein characteristics are listed in Table I. As density increases progressively from lipid → water → protein, the density of lipoproteins varies according to their composition. HDL is rich in protein and the lipoparticles are small and dense relative to other lipoproteins. The largest and least-dense lipoproteins are the very triglyceride-rich CM that, like the larger VLDL, can scatter light to render plasma turbid. The buoyancy of CM permits it to rise to the surface overnight in the refrigerator. The remnants of CM and VLDL, after LPL has hydrolysed triglycerides, are smaller and denser and eventually enter the intermediate-density lipoprotein (IDL) category. Particles containing $apoB_{100}$ can be metabolised from this category to LDL. The amount of triglyceride in lipoproteins destined to form LDL, or that can be exchanged into LDL in the circulation by CETP, influences the size of the LDL particle because hepatic triglyceride lipase (HL) can hydrolyse the triglyceride to form smaller particles. The larger LDL is known as LDL_A and the smaller, denser LDL that is more readily oxidisable as LDL_B. The latter is typically associated with mild hypertriglyceridaemia (>2.5 mmol/l) in the metabolic syndrome and diabetes mellitus and with higher cardiovascular risk.

Table I. Chief characteristics of common lipoprotein species

	Lipoproteins				
	CM	**VLDL**	**IDL**	**LDL**	**HDL**
Size (nm)	75 - 1 200	30 - 80	25 - 35	18 - 26	3 - 12
Density (g/ml)	<0.95	0.95 - 1.006	1.006 - 1.019	1.020 - 1.063	1.063 - 1.210
Electrophoresis	Origin	preβ	broadβ	β	α
Apoproteins	B48, CII, CIII, E, AIV	B100, CII, E, CIII, AI	B100, E	B100	AI, AII, AIV, CI, CII, CIII
Core lipid (mass)	TG>>CE	TG>CE	TG=CE	CE>>TG	CE
Subspeciation		Large (TG-rich)		Large (A)	HDL2 (larger)
		Small (CE-rich)		Small (B)	HDL3 (smaller)
Modulation	LPL, CETP	LPL, CETP	HL, CETP	LCAT, HL, CETP	LCAT, HL, CETP
Receptors	LRP	LRP	LRP, LDLR	LDLR	SRB1
Cholesterol concentration – in fasting (mmol/l)	0	<0.8	0	3	1.2

CM – chylomicron; VLDL – very-low-density lipoprotein; IDL – intermediate-density lipoprotein; LDL – low-density lipoprotein; HDL – high-density lipoprotein; TG – triglyceride; CE – cholesterol ester; LPL – lipoprotein lipase; HL – hepatic lipase; LCAT – lecithin:cholesterol acyltransferase; LRP – low-density lipoprotein receptor-related protein; LDLR – low-density lipoprotein receptor; SRB1 – scavenger receptor B1; CETP – cholesterol ester transfer protein.

Notes: IDL is only observed in agarose electrophoresis in dysbetalipoproteinaemia. Apoproteins can exchange between lipoproteins except for apoB. CETP can act on all lipoproteins.

Lipids, cells and drug treatment

The only mature cells that have a persistent demand for cholesterol are those that produce lipoproteins, bile acids or corticosteroid hormones. Growing cells acquire cholesterol for membrane synthesis through *de novo* synthesis in which the rate-limiting enzyme, hydroxymethylglutaryl coenzyme A reductase (HMGCoAR), is tightly regulated. In concert with HMGCoAR, LDL receptors (LDLRs) are upregulated to import cholesterol by binding apoB-containing lipoproteins. As part of the cellular cholesterol homeostasis, upregulation of HMGCoAR and LDLRs is accompanied by the upregulation of proconvertase subtilin/kexin type 9 (PCSK9) to degrade LDLRs and limit the importation of cholesterol. Mutations that diminish the impact of this enzyme render plasma LDL lower and prolong lifespan, while mutations that increase the action of this enzyme raise plasma LDL concentration as much or more than LDLR defects.

Statins inhibit HMGCoAR. Statins have their major impact in the liver by lowering cholesterol in hepatocytes, which leads to upregulation of the LDLR and increased removal of LDL from the plasma. Some limit may also be placed on the production of lipoproteins in the liver as statins can lower LDL when LDLR function is absent.

A similar upregulation of LDLRs is noted in the liver when more cholesterol is diverted to bile acid synthesis after the disruption of enterohepatic cycling of bile acids by soluble fibre or cholestyramine. Enterocytes absorb cholesterol from the micelles in the lumen of the gut by the Niemann-Pick type C1-like protein 1. Ezetimibe interferes with this process. The resultant decrease in delivery of cholesterol to the liver upregulates the LDLRs and a reduction in plasma LDL follows. Phytosterols compete with cholesterol for absorption and similarly reduce plasma LDL concentrations. The small proportion of phytosterols that is absorbed is eliminated from the body.

Macrophages scavenge cellular debris and oxidised lipoproteins through a range of receptors. Macrophages accumulate vast amounts of cholesterol but esterify it to avoid harm from excessive cholesterol in cell membranes. AcylcoA:cholesterol acyltransferase (ACAT) is essential for this purpose and protection against cholesterol toxicity is further gained by exportation through the adenosine-binding cassette transporter A1 (ABCA1) to lipid-poor HDL. Other mechanisms of exporting cholesterol also exist, including ABCG1 that appears to transfer selectively to larger HDL particles.

Lipoprotein pathways

The pathways illustrated in Fig. 1 could be simplified into:

- the post-prandial pathway of CM for dietary lipid
- the provision of triglyceride between meals by VLDL that also yields a cholesterol supply in its final product – LDL
- a reverse cholesterol transport system involving HDL.

The usual range of daily intake of dietary lipid in humans is 20 - 200 g triglyceride and 0 - 500 mg cholesterol. After digestion enterocytes absorb the fatty acids and cholesterol from the lumen of the gut, re-esterify them to glycerol and secrete them in CM. Having travelled through the lacteals and thoracic duct, the CM enter the venous circulation at the subclavian vein. In the systemic circulation the CM acquire many apoproteins from HDL. Muscle and adipose tissue synthesise LPL that attaches to proteoglycans on the endothelial cells. ApoCII on the CM activates LPL in these vascular beds and it digests triglyceride to fatty acids. The core of the CM diminishes in volume, and excessive surface area of phospholipids can depart from the remnant lipoprotein together with apoAI, forming HDL. The CM remnants bind to hepatic receptors (LDLR as well as LDLR-like lipoprotein) by virtue of apoE. A meal delivers CM to the plasma over about 8 hours, elevating the triglyceride in normal persons to a peak of about 4 mmol/l at about 3 hours.

The liver assembles fatty acids into triglycerides for export in VLDL. Triglyceride, CE, phospholipids and unesterified cholesterol are blended into a VLDL particle before secretion into the venous circulation. The normal fasting triglyceride (about 1.5 mmol/l) reflects hepatic output of VLDL. Circulating VLDL undergoes similar metabolism to CM forming VLDL remnants under the action of LPL. These remnants are cleared from the circulation by virtue of apoE-binding hepatic receptors. However, almost half of the VLDL remnants are further metabolised by HL to LDL. The LDL particles contain only apoB_{100} and this is now in the appropriate conformation for binding to the LDLR. In healthy normal subjects on a low-fat diet, the removal of LDL is balanced by the production rate with a resultant plasma LDLC of about 3 mmol/l.

Table II. Spectrum of primary dyslipidaemias

	Causes	TG	TC	HDLC	LDLC	IDLC
Extreme hypercholesterolaemia (hoFH phenotype)	LDLR, ARH	<2.5	>15	N	>13	0
Severe hypercholesterolaemia	LDLR defect	<2.5	>7.5	N	>5	0
	apoB defect					
	PCSK9 defect					
	FCH					
Moderate hypercholesterolaemia	Polygenic, diet	<2.5	>5	N	>2.5	0
Hyperalphalipoproteinaemia		<2.5	>5	>2.0	N	0
Ideal (normal)		<1.5	<5	>1.2	<2.5	0
Mixed hyperlipidaemia	Metabolic syndrome	1.5 - 5	>5	Low	N (small)	Trace
	FCH	1.5 - 5	>5	Low	High (small)	Trace
	Dysbetalipoproteinaemia	1.5 - 5	>5	Low	Low	High
Moderate hyperTG	FCH	5 - 15	>5	Low	Low (small)	Present
	Hereditary hyperTG					
Severe hyperTG	FCH	>15	>5	Low	Low	Trace
	LPL deficiency					0
	apoCII deficiency					0
Severe hypocholesterolaemia	Heterozygous truncated apoB	<1.5	<2.5	N	<1.5	0
	Homozygous truncated apoB	<1.0	<1.5	N	<0.5	0
	MTP deficiency	<1.0	<1.5	N	<0.5	0

TG – triglyceride; TC – total cholesterol; HDLC – high-density lipoprotein cholesterol; LDLC – low-density lipoprotein cholesterol; IDL – intermediate-density lipoprotein; LDLR – low-density lipoprotein receptor; MTP – microsomal triglyceride transport protein; N – normal; FCH – familial combined hyperlipidaemia.

Note: The ranges given are loosely defined in mmol/l.

HDL can be generated by lipolysis of triglyceride-rich lipoproteins or can be directly secreted from the liver and gut. HDL can accept cholesterol from cells through ABCA1 and ABCG1. The newly formed small HDL particles increase their lipid content because LCAT forms CE from the free cholesterol in the shell. Such CE could be exchanged for triglyceride in the triglyceride-rich lipoproteins by CETP. Now the larger HDL_2 can be recycled to smaller particles by HL. Additionally, phospholipid transfer protein can result in the remodelling of larger particles to recycle some smaller species of HDL. A more direct delivery of HDL cholesterol is by uptake of HDL through receptors such as the scavenger receptor (SR-B1) that has been shown to promote excretion into the bile.

Lipoprotein (a) is a highly variable lipoprotein comprising a LDL particle covalently bound to apoprotein (a), which resembles plasminogen but is catalytically inactive.

Derangements in metabolism

The lipids and proteins in lipoproteins generally flow as described above but there is considerable variability in composition of particles secreted by the enterocyte and hepatocyte, along with variation in apoproteins. The variation may reflect the genetic makeup, lifestyle and responses to disease and could influence both the metabolism of the lipoproteins as well as atherosclerosis.

Table II suggests a simple classification of dyslipidaemia and the attendant genetic causes.

Very **low total plasma cholesterol** concentrations result from disorders of apoB-containing lipoproteins as these account for the bulk of plasma cholesterol. An inability to synthesise apoB-containing lipoproteins, either as a result of no functional apoB (homozygous hypobetalipoproteinaemia) or lack of microsomal triacylglycerol transfer protein (abetalipoproteinaemia – a recessive condition), leaves HDL as the only lipoprotein in the circulation. Triglyceride concentrations are low and do not increase after a meal.

Heterozygous hypobetalipoproteinaemia results in low LDL concentrations, but these subjects have a postprandial increase in plasma triglyceride concentration. While neonates with abetalipoproteinaemia develop normally *in utero*, they may fail to thrive as they malabsorb fat. Later in life they may have neuromuscular dysfunction owing to vitamin E deficiency. In contrast, neonates born with low plasma cholesterol owing to very rare sterol synthetic errors have significant malformations and mental handicap, highlighting the importance of cholesterol in cell and tissue development.

Low HDL cholesterol concentrations in the general population associate with obesity, lesser LPL activity and hypertriglyceridaemia. Some rare disorders of apoAI, LCAT and ABCA1 result in very low concentrations, typically an HDL cholesterol <0.6 mmol/l.

High HDL cholesterol concentrations can result from dysfunctional CETP. There appears to be some increase in atherosclerosis in this condition but also with other causes of HDL cholesterol concentrations >2.5 mmol/l.

Hypertriglyceridaemia results from decreased removal of the CM and/or VLDL, but overproduction of VLDL may occur in certain cases such as patients with diabetes mellitus. In partial lipase deficiency with a high-fat diet, especially with overproduction of VLDL by diabetes or insulin resistance, there is an accumulation of VLDL and eventually also impaired CM clearance, generally signified by fasting triglyceride of >15 mmol/l. Homozygous LPL or apoCII deficiency will result in extreme disruption

of CM clearance, diminished VLDL production because of reduced hepatic triglyceride delivery from the gut, and increased clearance of LDL by upregulation of LDLRs.

Mixed hyperlipidaemias result from accumulation of remnants when apoE is defective (dysbetalipoproteinaemia – usually recessively inherited) or when increases in LDL and VLDL co-exist, such as may be the case in familial combined hyperlipidaemia (usually autosomally dominantly inherited but with variable penetrance of cholesterol or triglyceride elevation in the family).

Hypercholesterolaemia due to reduced clearance of LDL accumulation is generally recognised as the heterozygous familial hypercholesterolaemia (FH) phenotype: tendon xanthomata with arcus cornealis, LDL cholesterol >5 mmol/l and a personal or family history of coronary disease before the age of 55 years. The genes whose mutations cause the heterozygous FH phenotype are the LDL receptor, apoB_{100} and PCSK9. The homozygous FH phenotype produces cutaneous and tendinous xanthomata before adolescence, LDL cholesterol >14 mmol/l, very premature coronary disease, and aortic stenosis. While the homozygous FH is usually due to two LDLR defects, an adaptor protein for the LDLR may be deficient as a recessive cause of the same phenotype. The homozygous FH phenotype is mimicked in phytosterolaemia. In phytosterolaemia the deficiency in ABC transporters G5 and G8 results in the accumulation of phytosterols in lipoproteins and atherosclerosis. Similarly severe physical signs but modest elevations in LDL are seen in cerebrotendinous xanthomatosis. This rare recessive disorder is due to a defect in bile acid synthesis (26 hydroxylase deficiency) and in addition to xanthomata that commence later in childhood, it results in cataracts and central nervous system degeneration.

Further reading

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In a nutshell

- Lipoproteins transport lipids in plasma in spherical particles in which phospholipids and cholesterol form a shell around a core of triglyceride and CE.
- The proteins associated with lipoproteins play roles in the assembly, modulation and clearance of lipoproteins.
- Atherosclerosis is multifactorial, but increases of apoB-containing lipoproteins and decreases of apoAI-containing lipoproteins are crudely predictive for this process.
- ApoB resides permanently in the lipoprotein and almost all plasma apoB is in LDL.
- The best-known genetic atherogenic lipoprotein metabolic error is the accumulation of LDL in FH.
- FH can be caused by mutations in the LDL receptor, apoB (the ligand for this receptor) or PCSK9 (which degrades the LDLR).
- Defects in apoE result in remnant accumulation that is also atherogenic.

Single Suture

Green tea blocks cancer drug

Green tea is usually thought of as preventing cancer, but green tea capsules may have the opposite effect on people taking certain anti-cancer drugs.

A team led by Axel Schönthal of the University of Southern California gave mice with human multiple myeloma tumours the drug bortezomib or EEGC, an antioxidant found in green tea, or both together. Bortezomib alone shrank the tumour, but the mixture did not.

In vitro studies showed that the drug was inactivated by ECGC levels similar to those in a person who regularly takes capsules of green tea extract, which are higher than the levels in a person who drinks moderate amounts of green tea.

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