Clinical Significances of Lipoprotein Metabolism

Fortunately, few individuals carry the inherited defects in lipoprotein metabolism that lead to hyper- or hypolipoproteinemias (see Tables below for brief descriptions). Persons suffering from diabetes mellitus, hypothyroidism and kidney disease often exhibit abnormal lipoprotein metabolism as a result of secondary effects of their disorders. For example, because lipoprotein lipase (LPL) synthesis is regulated by insulin, LPL deficiencies leading to Type I hyperlipoproteinemia may occur as a secondary outcome of diabetes mellitus. Additionally, insulin and thyroid hormones positively affect hepatic LDL-receptor interactions; therefore, the hypercholesterolemia and increased risk of atherosclerosis associated with uncontrolled diabetes or hypothyroidism is likely due to decreased hepatic LDL uptake and metabolism.

Of the many disorders of lipoprotein metabolism, familial hypercholesterolemia (FH) may be the most prevalent in the general population. Heterozygosity at the FH locus occurs in 1:500 individuals, whereas, homozygosity is observed in 1:1,000,000 individuals. FH is an inherited disorder comprising four different classes of mutation in the LDL receptor gene. The class 1 defect (the most common) results in a complete loss of receptor synthesis. The class 2 defect results in the synthesis of a receptor protein that is not properly processed in the Golgi apparatus and therefore is not transported to the plasma membrane. The class 3 defect results in an LDL receptor that is incapable of binding LDLs. The class 4 defect results in receptors that bind LDLs but do not cluster in coated pits and are, therefore, not internalized.

FH sufferers may be either heterozygous or homologous for a particular mutation in the receptor gene. Homozygotes exhibit grossly elevated serum cholesterol (primarily in LDLs). The elevated levels of LDLs result in their phagocytosis by macrophages. These lipid-laden phagocytic cells tend to deposit within the skin and tendons, leading to xanthomas. A greater complication results from cholesterol deposition within the arteries, leading to atherosclerosis, the major contributing factor of nearly all cardiovascular diseases.

Lipoprotein(a) and Atherogenesis

Lipoprotein(a) [Lp(a)] was originally described as a new serum lipoprotein particle by Kare Berg in 1963. Lp(a) is composed of a common LDL nucleus linked to a molecule of apolipoprotein(a) [apo(a); encoded by the LPA gene] by disulfide bonds between a cysteine residue in a Kringle-IV (KIV) type 9 domain in apo(a) and a cysteine residue in apolipoprotein B-100 (apoB-100). When attached to apoB-100 the apo(a) protein surrounds the LDL molecule. Synthesis of Lp(a) occurs in the liver. The half-life of Lp(a) in the circulation is approximately 3–4 days. Although Lp(a) was described over 40 years ago its precise physiological function remains unclear. However, numerous epidemiological studies have demonstrated that elevated plasma levels of Lp(a) are a significant risk factor for the development of atherosclerotic disease.

The Kringle domains of apo(a) exhibit 75%-85% similarity to the KIV domains of plasminogen. The Kringle domain is a highly glycosylated domain found in numerous proteins and is so-called because the three dimensional structure resembles a looped Danish pastry. Each Kringle domain is composed of approximately 80 amino acid residues and the structure is stabilized by three internal disulfide bonds. There are 10 distinct sub-classes of KIV domain in apo(a) designated KIV1 through KIV10. The apo(a) KIV1 and KIV3 through KIV10 domains are present as single-copy domains. The KIV2 domain is present in a variable number of repeated copies (from 2–43) and constitutes the molecular basis for the highly variable size of Lp(a) in different individuals. Apo(a) also contains a Kringle V (KV) domain that resembles the catalytic domain of plasminogen. Indeed, the apo(a) gene located on chromosome 6q26 is a member of the plasminogen superfamily and given the similarities between apo(a) and plasminogen it has been hypothesized that apo(a) influences the processes of hemostasis.

Apo(a) proteins exhibit a variability in size due to a polymorphism caused by a variable number of the KIV repeats. To date at least seven different isoforms of Lp(a) have been characterized based upon electrophoretic mobilities. These different isoforms are designated F, B, and S1 through S5. The different isoforms are grouped into low molecular weight (LMW) and high molecular weight (HMW) isoforms determined by the number of KIV repeats in the apo(a) protein found in the Lp(a). The level of Lp(a) found in healthy individuals depends upon whether their plasma contains the LMW or HMW isoforms. Individuals with the LMW isoforms have high plasma Lp(a) concentration while those with the HMW isoforms have low concentrations.

When in the circulation Lp(a) particles can be affected by oxidative modification similar to that of the other plasma lipoprotein particles. Lp(a) and oxidized Lp(a) [oxLp(a)] particles interact with macrophages via scavenger receptor uptake leading to cholesterol accumulation and foam cell

formation. Indeed, oxLp(a) are phagocytosed more rapidly than other lipoprotein particles and therefore accumulate in the subendothelial space at high levels. This process can lead to progression of atherogenesis, thus accounting for the direct correlation between the plasma level of Lp(a) and coronary artery disease. In addition to oxidation of Lp(a) leading to increased foam cell production, glycation of the particle also may contribute to atherogenesis. In fact, there is a strong correlation in the level of glycated Lp(a) and the severity of hyperglycemia observed in poorly controlled type 2 diabetes.

Although the precise physiology of Lp(a) is poorly understood, as indicated above, there is a strong correlation between plasma concentration of Lp(a) and atherogenic events that lead to coronary artery disease. For a discussion of the processes of blood coagulation and the role of plasminogen visit the Blood Coagulation page. Because of the high degree of similarity between apo(a) and plasminogen it is suggested that Lp(a) may contribute to the thrombotic aspects of ischemic heart disease. Lp(a) has been shown to competitively inhibit the binding of plasminogen to its receptor on endothelial cells as well as to its binding sites on fibrinogen and fibrin. This interference of plasminogen binding leads to reduced surface-dependent activation of plasminogen to plasmin. The normal function of plasmin is to degrade the fibrin clot that forms as a result of vessel injury. Therefore, high plasma concentrations of Lp(a) may represent a source of antifibrinolytic activity. Of significance to the potential for atherogenesis, the antifibrinolytic potential of Lp(a) particles is related to their size. The LMW isoforms of Lp(a) have been shown to have a higher fibrin-binding capacity than the HMW isoforms. Lp(a) also interferes with other aspects of the normal processes of coagulation in addition to its effects on plasminogen function. Lp(a) stimulates the production of plasminogen activator inhibitor-1 (PAI-1) leading to a reduced ability of t-PA to activate the process of clot dissolution. Increased production of PAI-1 also leads to enhanced proinflammatory events via activation of monocyte adhesion to the vessel wall. Lp(a) has also been shown to modulate platelet activation interfering with the interaction of platelets with exposed collagen fibers in the injured vessel wall. In addition to the role of Lp(a) in inhibiting plasminogen binding, Lp(a) has been shown to inhibit the release of tissue plasminogen activator (t-PA) from endothelial cells. With reduced release of the enzyme (t-PA) that converts plasminogen to plasmin and interference with plasminogen binding to fibrin clots Lp(a) can exert a significant negative effect on the ability to dissolve blood clots.

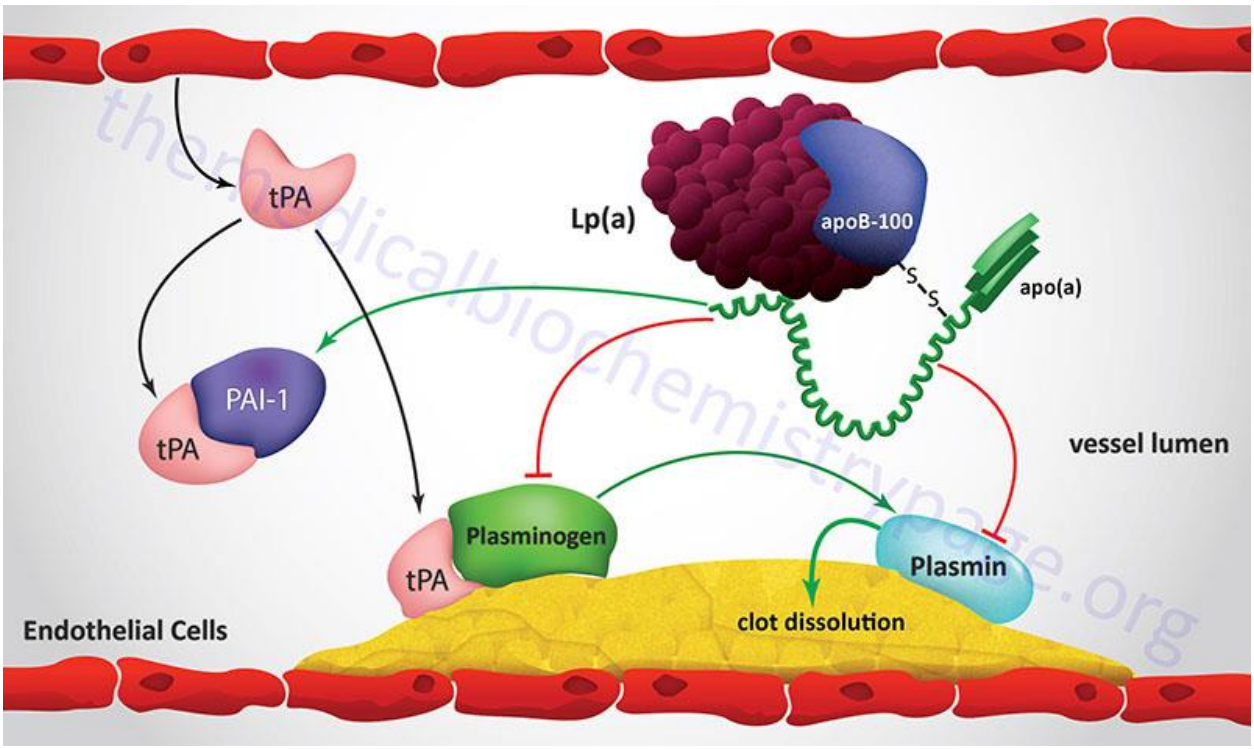

FIGURE 4. **Role of Lp(a) in promotion of atherogenesis.** Lp(a) represents a circulating abnormal variant of LDL. The formation of Lp(a) occurs when apolipoprotein a [apo(a)] forms a disulfide bonded complex with the apoB-100 component of LDL. The apo(a) component of Lp(a) particles promotes the process of atherogenesis, in part, due to its ability to interfere with the normal events of hemostasis. This interference results from apo(a) binding to plasminogen binding sites preventing plaminogen and t-PA from interacting. If t-PA cannot cleave plasminogen to plasmin then fibrin clots cannot be dissolved. Lp(a) also interferes with plamin binding sites on the fibrin clot which also inteferes with the process of clot dissolution all of which leads to enhanced atherogenesis. The green arrows indicated enhanced activity such as the ability of Lp(a) to increase the production and activity of PAI-1. Red T-lines represent inhibitory processes.

In addition to the interactions with plasminogen, leading to enhanced atherogenesis, Lp(a) has been shown to stimulate smooth muscle cell (SMC) growth. This effect of Lp(a) is exerted via an inactivation of transforming growth factor-β (TGF-β). Activated TGF-β inhibits the proliferation and migration of SMC, thus the inhibition of this regulatory effect of TGF-β leads to accelerated blood vessel stenosis with concomitant enhancement of the atherogenic process. oxLp(a) has also been shown to inhibit nitric oxide-dependent vasodilation which will tend to exacerbate the atherogenic process in hypertensive patients.

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Hyperlipoproteinemias

Disorder	Defect	Comments
Type I (familial LPL deficiency, familial hyperchylomicronemia)	(**a**) deficiency of LPL; (**b**) production of abnormal LPL; (**c**) apoC-II deficiency	slow chylomicron clearance, reduced LDL and HDL levels; treated by low fat/complex carbohydrate diet; no increased risk of coronary artery disease
Familial hypercholesterolemia, FH Type IIA hyperlipoproteinemia	5 classes of LDL receptor defect	reduced LDL clearance leads to hypercholesterolemia, resulting in atherosclerosis and coronary artery disease
Type III (familial dysbetalipoproteinemia, remnant removal disease, broad beta disease, apolipoprotein E deficiency)	hepatic remnant clearance impaired due to apoE abnormality; patients only express the $apoE_2$ isoform that interacts poorly with the apoE receptor	causes xanthomas, hypercholesterolemia and atherosclerosis in peripheral and coronary arteries due to elevated levels of chylomicrons and VLDLs
Type IV (familial hypertriglycerideemia)	elevated production of VLDL associated with glucose intolerance and	frequently associated with type-II non-insulin dependent diabetes mellitus, obesity, alcoholism or

	hyperinsulinemia	administration of progestational hormones; elevated cholesterol as a result of increased VLDLs
Type V familial	elevated chylomicrons and VLDLs due to unknown cause	hypertriglycerideemia and hypercholesterolemia with decreased LDLs and HDLs
Familial hyperalphalipoproteinemia Type II hyperlipoproteinemia	increased level of HDLs	a rare condition that is beneficial for health and longevity
Type II Familial hyperbetalipoproteinemia	increased LDL production and delayed clearance of triglycerides and fatty acids	strongly associated with increased risk of coronary artery disease
Familial ligand-defective apoB	2 different mutations: Gln for Arg (amino acid 3500) or Cys for Arg (amino acid 3531); both lead to reduced affinity of LDL for LDL receptor	dramatic increase in LDL levels; no affect on HDL, VLDL or plasma triglyceride levels; significant cause of hypercholesterolemia and premature coronary artery disease
Familial LCAT deficiency Norum disease Fish-eye disease	absence of LCAT leads to inability of HDLs to take up cholesterol (reverse cholesterol transport)	decreased levels of plasma cholesteryl esters and lysolecithin; abnormal LDLs (Lp-X) and VLDLs; diffuse corneal opacities, target cell hemolytic anemia, and proteinuria with renal failure
Wolman disease (cholesteryl ester storage disease)	defect in lysosomal cholesteryl ester hydrolase; affects metabolism of LDLs	reduced LDL clearance leads to hypercholesterolemia, resulting in atherosclerosis and coronary artery disease

heparin-releasable hepatic triglyceride lipase deficiency	deficiency of the lipase leads to accumulation of triglyceride-rich HDLs and VLDL remnants (IDLs)	causes xanthomas and coronary artery disease

Hypolipoproteinemias

Disorder	Defect	Comments
Abetalipoproteinemia (acanthocytosis, Bassen-Kornzweig syndrome)	no chylomicrons, VLDLs or LDLs due to defect in apoB expression	rare defect; intestine and liver accumulate, malabsorption of fat, retinitis pigmentosa, ataxic neuropathic disease, erythrocytes have thorny appearance
Familial hypobetalipoproteinemia	at least 20 different apoB gene mutations identified, LDL concentrations 10-20% of normal, VLDL slightly lower, HDL normal	mild or no pathological changes
Tangier disease	reduced HDL concentrations, no effect on chylomicron or VLDL production	tendency to hypertriglycerideemia; some elevation in VLDLs; hypertrophic tonsils with orange appearance

Pharmacologic Intervention

Drug treatment to lower plasma lipoproteins and/or cholesterol is primarily aimed at reducing the risk of atherosclerosis and subsequent coronary artery disease that exists in patients with elevated circulating lipids. Drug therapy

usually is considered as an option only if non-pharmacologic interventions (altered diet and exercise) have failed to lower plasma lipids.

Alirocumab (Praluent®), Evolcumab (Repatha®): These drugs are the newest type of anti-hypercholesterolemia drugs recently approved by the FDA for use in the US. Both drugs are injectible antibodies that block the function of proprotein convertase subtilisin/kexin type 9, PCSK9. PCSK9 is serine protease of the subtilisin-like proprotein convertase 2 family. A major function of PCSK9 is the endosomal degradation of the LDL receptor (LDLR), thereby reducing the recyling of the LDLR to the plasma membrane. This effect of PCSK9 leads to a reduced ability of the liver to remove IDL and LDL from the blood contributing to the potential for hypercholesterolemia. The potential for the pharmaceutical benefits of the interference in the activity PCSK9 was recognized by a confluence of several studies. Patients with a specific form of familial hypercholesterolemia not due to mutations in the LDLR gene were shown to have severe hypercholesterolemia due to mutations in the PCSK9 gene resulting in hyperactivity of the enzyme. In addition, it was found that in certain individuals with low serum LDL levels there was an association with the inheritance of nonsense mutations in the PCSK9 gene which result in loss of PCSK9 activity. Hypercholesterolemic patients taking another cholesterol-lowering drug while simultaneously utilizing either of these new PCSK9 inhibitors saw further reductions in serum LDL levels of betweeen 55% and 77%.

Atorvastatin (Lipotor®), Simvastatin (Zocor®), Lovastatin (Mevacor®): These drugs are fungal HMG-CoA reductase (HMGR) inhibitors and are members of the family of drugs referred to as the **statins**. The net result of treatment is an increased cellular uptake of LDLs, since the intracellular synthesis of cholesterol is inhibited and cells are therefore dependent on extracellular sources of cholesterol. However, since mevalonate (the product of the HMG-CoA reductase reaction) is required for the synthesis of other important isoprenoid compounds besides cholesterol, long-term treatments carry some risk of toxicity. A component of the natural cholesterol lowering supplement, red yeast rice, is in fact a statin-like compound.

The statins have become recognized as a class of drugs capable of more pharmacologic benefits than just lowering blood cholesterol levels via their actions on HMGR. Part of the cardiac benefit of the statins relates to their ability to regulate the production of *S*-nitrosylated COX-2. COX-2 is an inducible enzyme involved in the synthesis of the prostaglandins and thromboxanes as well as the lipoxins and resolvins. The latter two classes of compounds are anti-inflammatory lipids discussed in the Aspirin page. Evidence has shown that statins activate inducible nitric oxide synthase

(iNOS) leading to nitrosylation of COX-2. The *S*-nitrosylated COX-2 enzyme produces the lipid compound 15*R*-hydroxyeicosatetraenoic acid (15*R*-HETE) which is then converted via the action of 5-lipoxygenase (5-LOX) to the epimeric lipoxin, 15-epi-LXA_4. This latter compound is the same as the aspirin-triggered lipoxin (ATL) that results from the aspirin-induced acetylation of COX-2. Therefore, part of the beneficial effects of the statins are exerted via the actions of the lipoxin family of anti-inflammatory lipids.

Additional anti-inflammatory actions of the statins results from a reduction in the prenylation of numerous pro-inflammatory modulators. Prenylation refers to the addition of the 15 carbon farnesyl group or the 20 carbon geranylgeranyl group to acceptor proteins. The isoprenoid groups are attached to cysteine residues at the carboxy terminus of proteins in a thioether linkage (C-S-C). A common consensus sequence at the C-terminus of prenylated proteins has been identified and is composed of CAAX, where C is cysteine, A is any aliphatic amino acid (except alanine) and X is the C-terminal amino acid. In addition to numerous prenylated proteins that contain the CAAX consensus, prenylation is known to occur on proteins of the RAB family of RAS-related G-proteins. There are at least 60 proteins in this family that are prenylated at either a CC or CXC element in their C-termini. The RAB family of proteins are involved in signaling pathways that control intracellular membrane trafficking. The prenylation of proteins allows them to be anchored to cell membranes. In addition to cell membrane attachment, prenylation is known to be important for protein-protein interactions. Thus, inhibition of this post-translational modification by the statins interferes with the important functions of many signaling proteins which is manifest by inhibition of inflammatory responses.

Some of the effects on immune function that have been attributed to the statins are attenuation of autoimmune disease, inhibition of T-cell proliferation, inhibition of inflammatory co-stimulatory molecule expression, decreases in leukocyte infiltration, and promotion of a shift in cytokine profiles of helper T-cell types from Th1 to Th2. Th1 cells are involved in cell-mediated immunity processes, whereas, Th2 cells are involved in humoral immunity process. The cytokines produced by Th2 cells include IL-4, IL-5, IL-10 and IL-13 and these trigger B cells to switch to IgE production and to activate eosinophils.

Nicotinic acid (Niacor® and Niaspan®): Nicotinic acid reduces the plasma levels of both VLDLs and LDLs by inhibiting hepatic VLDL secretion, as well as suppressing the flux of FFA release from adipose tissue by inhibiting lipolysis. In addition, nicotinic administration strongly increases the circulating levels of HDLs. Patient compliance with nicotinic acid administration is sometimes compromised because of the unpleasant side-

effect of flushing (strong cutaneous vasodilation). Recent evidence has shown that nicotinic acid binds to and activates the G-protein coupled receptor identified as GPR109A (also called HM74A or PUMA-G). For more detailed information on the normal biological function of GPR109A go to the Bioactive Lipids page. The identity of a receptor to which nicotinic acid binds allows for the development of new drug therapies that activate the same receptor but that may lack the negative side-effect of flushing associated with nicotinic acid. Because of its ability to cause large reductions in circulating levels of cholesterol, nicotinic acid is used to treat Type II, III, IV and V hyperlipoproteinemias.

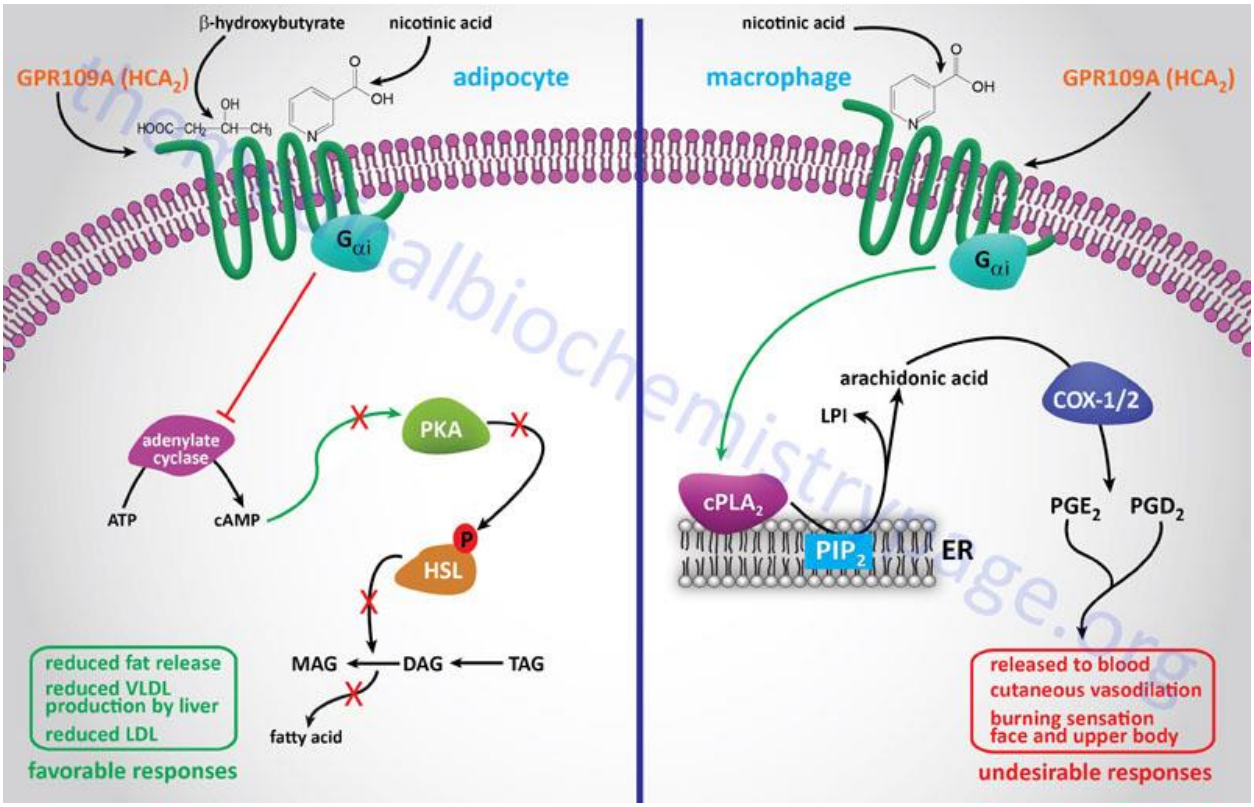

FIGURE 5. Signaling events initiated in response to β-hydroxybutyrate or nicotinic acid binding to GPR109A on adipocytes or macrophages. During periods of fasting, hepatic ketone synthesis increases and the released β-butyrate binds to GPR109A on adipocytes triggering activation of the receptor-associated G_i-type G-protein which then inhibits the activity of adenylate cyclase (AC). Inhibition of AC leads to reduced HSL-mediated release of fatty acids from diacylglycerides. Nicotinic acid binding to GPR109A on adipocytes also leads to reduced fatty acid release. The reduced release of adipose tissue fatty acids leads to decreased synthesis and release of VLDL by the liver. It is this effect of nicotinic acid that contributes to the antidyslipidemic action of this drug. The GPR109A receptor on macrophages is also activated by nicotinic acid but this effect contributes to the undesired side-effets of nicotinic acid therapy.

Within macrophages, GPR109A activation results in increased activation of PLA_2 leading to increased arachidonic acid delivery to COX and increased production of the pro-inflammatory eicosanoids PGE_2 and PGD_2. The release of these eicosanoids causes increased cutaneous vasodilation resulting in the typical flushing and burning pain response to nicotinic acid therapy.

Gemfibrozil (Lopid®), Fenofibrate (TriCor®): These compounds (called fibrates) are derivatives of fibric acid and although used clinically since the 1930's were only recently discovered to exert some of their lipid-lowering effects via the activation of peroxisome proliferation. Specifically, the fibrates were found to be activators of the peroxisome proliferator-activated receptor-α (PPAR-α) class of proteins that are classified as co-activators. The naturally occurring ligands for PPAR-α are leukotriene B_4 (LTB_4, see the Lipid Synthesis page), unsaturated fatty acids and oxidized components of VLDLs and LDLs. The PPARs interact with another receptor family called the retinoid X receptors (RXRs) that bind 9-*cis*-retinoic acid. Activation of PPARs results in modulation of the expression of genes involved in lipid metabolism. In addition the PPARs modulate carbohydrate metabolism and adipose tissue differentiation. Fibrates result in the activation of PPAR-α in liver and muscle. In the liver this leads to increased β-oxidation of fatty acids, thereby decreasing the liver's secretion of triglyceride- and cholesterol-rich VLDLs, as well as increased clearance of chylomicron remnants, increased levels of HDLs and increased lipoprotein lipase activity which in turn promotes rapid VLDL turnover.

Cholestyramine or colestipol (resins): These compounds are nonabsorbable resins that bind bile acids which are then not reabsorbed by the liver but excreted. The drop in hepatic reabsorption of bile acids releases a feedback inhibitory mechanism that had been inhibiting bile acid synthesis. As a result, a greater amount of cholesterol is converted to bile acids to maintain a steady level in circulation. Additionally, the synthesis of LDL receptors increases to allow increased cholesterol uptake for bile acid synthesis, and the overall effect is a reduction in plasma cholesterol. This treatment is ineffective in homozygous FH patients, since they are completely deficient in LDL receptors.

Ezetimibe: This drug is sold under the trade names Zetia® or Ezetrol® and is also combined with the statin drug simvastatin and sold as Vytorin® or Inegy®. Ezetimibe functions to reduce intestinal absorption of cholesterol, thus effecting a reduction in circulating cholesterol. The drug functions by inhibiting the intestinal brush border transporter involved in absorption of cholesterol. This transporter is known as Niemann-Pick type C1-like 1 (NPC1L1). NPC1L1 is also highly expressed in human liver. The hepatic function of NPC1L1 is presumed to limit excessive biliary cholesterol loss. NPC1L1-dependent sterol uptake is regulated by cellular cholesterol content.

In addition to the cholesterol lowering effects that result from inhibition of NPC1L1, its inhibition has been shown to have beneficial effects on components of the metabolic syndrome, such as obesity, insulin resistance, and fatty liver, in addition to atherosclerosis. Ezetimibe is usually prescribed for patients who cannot tolerate a statin drug or a high dose statin regimen. There is some controversy as to the efficacy of ezetimibe at lowering serum cholesterol and reducing the production of fatty plaques on arterial walls. The combination drug of ezetimibe and simvastatin has shown efficacy equal to or slightly greater than atorvastatin (Lipitor®) alone at reducing circulating cholesterol levels.