

Understanding Cholesterol Synthesis and Absorption Is the Key to Achieving Cholesterol Targets

a report by

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The importance of plasma cholesterol reduction in the attenuation of cardiovascular (CV) risk has been clearly demonstrated in large clinical trials using statins. However, despite the clear risks of hyperlipidaemia and the proven benefits of lipid-lowering therapies, only a minority of patients currently achieve recommended low-density lipoprotein (LDL) cholesterol treatment goals in clinical practice.^{1,2} More patients are being treated for lipid reduction than ever before, but there remains a substantial degree of undertreatment. This is due to a number of factors, including patient non-compliance, tolerability issues, variable physician follow-up, patients not receiving adequate dosages of the lipid-lowering drugs available and the drugs themselves not being optimal.

Statins are widely prescribed and are established as first-line therapy for the primary and secondary prevention of coronary artery disease. However, the benefit of treatment varies between patients. Genetic variation can contribute to inter-individual variations in the clinical efficacy

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of drug therapy, and significant progress has been made in identifying common genetic polymorphisms that influence responsiveness to statin therapy. To date, more than 30 candidate genes related to the pharmacokinetics and pharmacodynamics of statins have been investigated as potential determinants of drug responsiveness in terms of LDL cholesterol lowering.³

An important link also exists between dietary cholesterol absorption and cholesterol production. Inhibiting cholesterol synthesis with statins increases cholesterol absorption, and decreasing cholesterol absorption increases cholesterol synthesis. This partially explains why it is difficult to achieve LDL targets in many patients. The intestinal pool of cholesterol is also an important source of blood cholesterol and is derived from biliary secretion and the diet. Approximately half of intestinal cholesterol is absorbed into the bloodstream. The absorption of excess cholesterol can increase the amount of cholesterol stored in the liver, resulting in increased very-low-density lipoprotein (VLDL) secretion and LDL cholesterol formation and downregulation of LDL receptor activity, leading to increased plasma LDL cholesterol levels. Genetic variation at gene loci that affect intestinal cholesterol absorption include apolipoprotein (apo) E4; adenosine

triphosphate-binding cassette transporters G5 and G8; cholesterol production such as 3-hydroxy-3-methylglutaryl co-enzyme A (HMGCoA) reductase; and lipoprotein catabolism such as apoB and the LDL receptor. All may play a role in modulating responsiveness as well as genes involved in the metabolism of statins such as cytochrome P450.³

Cholesterol Metabolism with Emphasis on Synthesis and Absorption

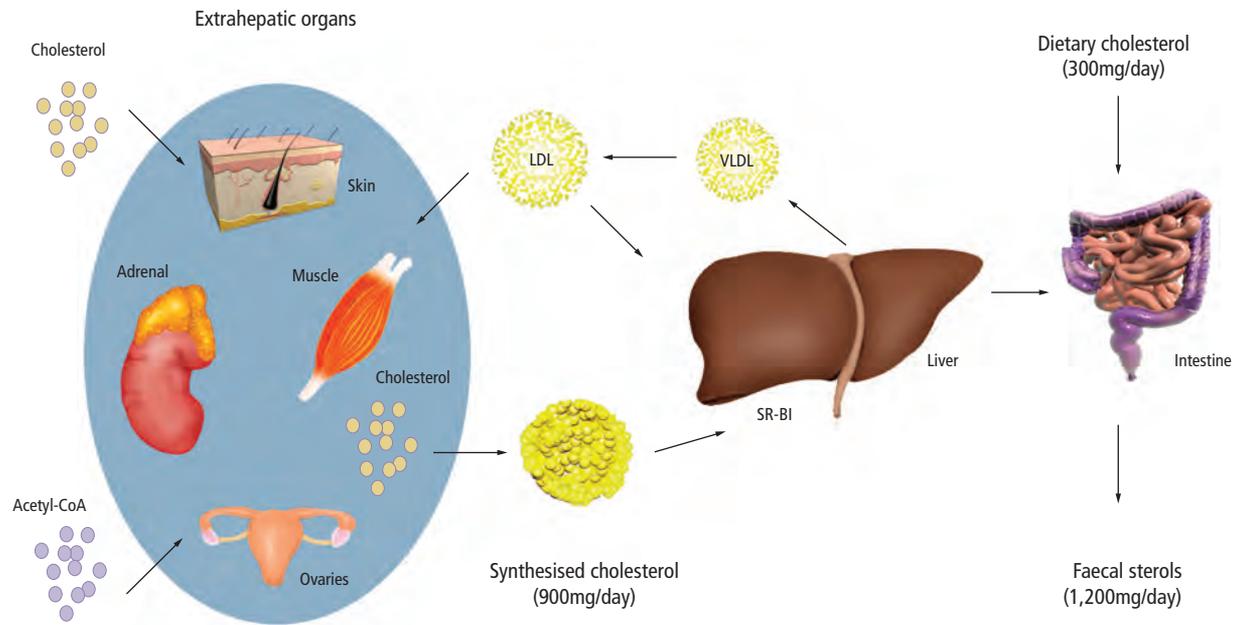
Human cholesterol levels are dependent on several inter-related processes: its synthesis (mainly in the liver, endocrine organs, muscle and skin), absorption from the diet and excretion into bile (see *Figure 1*). The balance between these processes varies between individuals in that some may have a relative large contribution of hepatic synthesis whereas others may have a high dietary absorption. Of the cholesterol absorbed in the intestines, about 75% is from biliary sources undergoing enterohepatic circulation, whereas dietary sources account for about 25% (see *Figures 2 and 3*).⁴ While intestinal absorption of bile acids is essentially complete under normal conditions, cholesterol absorption in healthy adult volunteers is variable, with 29–81% (mean 56%) absorbed in the small intestine. This range of variability has been observed in many studies where cholesterol absorption ranged from 25 to 75%.⁴

In subjects consuming a consistent diet, both fractional and absolute absorption of cholesterol is negatively associated with cholesterol synthesis.⁵ This dynamic process responds to diet. A typical North American diet contains approximately 450mg of cholesterol per day (of which 55% is absorbed), while cholesterol synthesis on such a modest cholesterol diet is 11–13mg/kg/day.⁶ Reduced absorption efficiency and reduced cholesterol synthesis, which has been mechanistically tied to reduced HMGCoA reductase activity, are the major compensatory mechanisms for increased dietary intake. Other mechanisms, such as increased biliary re-excretion of cholesterol or increased faecal bile acids, play minor roles in the compensatory process,⁶ and an increase in bile acid synthesis is variable. McNamara and colleagues investigated the effects of altering dietary cholesterol and the quality of the fat from polyunsaturated to saturated in diets to determine which is the most important determinant of serum cholesterol. When dietary cholesterol is increased from 250mg per day

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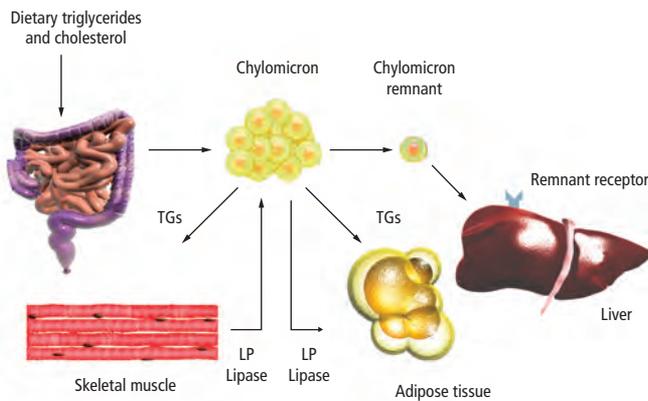
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Figure 1: Cholesterol Balance



Cholesterol homeostasis is maintained by balancing dietary intake of cholesterol and de novo synthesis with elimination (conversion to bile acids and faecal excretion). The intestine absorbs exogenous cholesterol from dietary and biliary sources and transports it to the liver. Approximately 30–40% of dietary cholesterol is absorbed. The liver, intestine and extrahepatic tissues all help regulate the cholesterol balance. LDL cholesterol carries cholesterol synthesised in the liver to extrahepatic tissues, and high-density lipoprotein (HDL) cholesterol carries cholesterol from peripheral tissues back to the liver. Suppression of cholesterol synthesis resulting from high plasma levels involves LDL-cholesterol-bound cholesterol that undergoes endocytosis by means of specific LDL receptors whose levels are regulated by sterol regulatory-binding element proteins. The class B type 1 scavenger receptor (SR-B1) also facilitates uptake of cholesterol from the circulation.

Figure 2: Exogenous Pathway of Lipid Metabolism



Dietary fat is mainly in the form of triglycerides (TGs), although a small amount of cholesterol is also present, most of which is unesterified. The ingested fat undergoes:

- emulsification with bile acids and phospholipids to form micelles in the small intestine;
- subsequent hydrolysis by pancreatic enzymes (lipase and enterase) to form cholesterol, free fatty acids and monoglycerides; and
- absorption from the small intestine into mucosal cells.

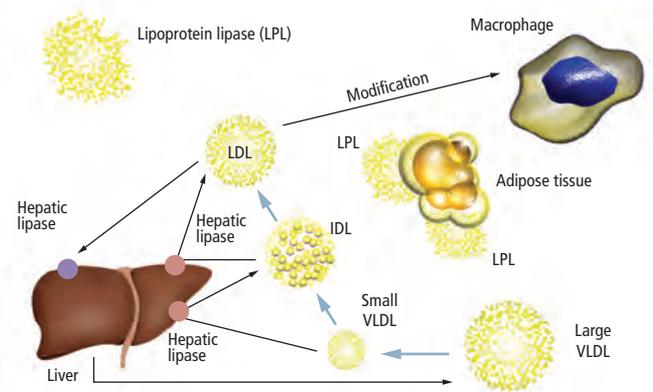
Once resorbed, the lipid is re-esterified to form cholesterol esters (CEs) and TGs. These are combined with free cholesterol, several apoproteins (A, B-48) and phospholipids to form chylomicrons, which are secreted into the lymphatic system and thence via the thoracic duct to the blood. Once in the bloodstream, additional apoproteins are added (E, C-III) from HDL. Lipoprotein lipase (LPL) (on the surface of the endothelium) hydrolyses chylomicrons forming:

- surface remnants (pre-HDL), which join the HDL pool; and
- chylomicron remnants (with apo B-48), which are transported via the blood to the liver. Chylomicron remnant receptors recognise apo E4 on the surface of the remnant, resulting in rapid absorption by hepatocytes.

Note: chylomicron remnants are atherogenic.

to 800mg per day, reduced efficiency of cholesterol absorption and reduced hepatic synthesis were found. However, the main determinant of plasma cholesterol levels was the fat quality of meals.⁷ The plasma cholesterol response from increasing dietary cholesterol by 100mg/day is on average only 2.2mg/dl (see Figure 4).

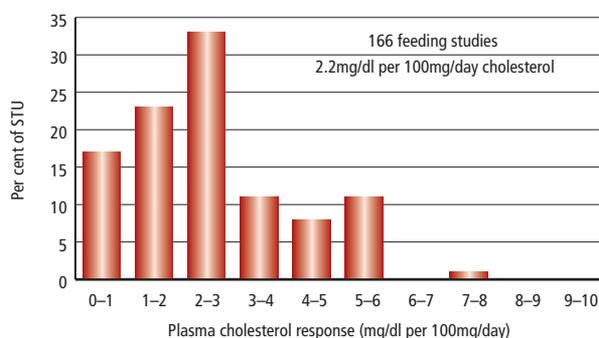
Figure 3: Endogenous Pathway of Lipid Metabolism



After absorption from the small intestine, dietary and biliary cholesterol is packaged into chylomicrons that enter the bloodstream and are unloaded at peripheral sites such as muscle and adipose tissue. LDL receptors take the now smaller chylomicron remnants into hepatic cells where enzymes degrade the remnants, releasing cholesterol and other lipids. Both exogenous and endogenous lipids are packaged into very-low-density lipoprotein (VLDL) cholesterol that transports cholesterol and triglycerides to other body tissues. Loss of triglycerides from VLDL cholesterol results in cholesterol-rich intermediate-density lipoprotein (IDL) cholesterol. IDL cholesterol can return for uptake by the liver by way of LDL receptors or it can remain in the circulation. LDL cholesterol, formed from circulating IDL cholesterol, transports cholesterol from the liver to peripheral tissues. Most LDL cholesterol is taken up by the liver and other cells by means of LDL receptors. A smaller portion is degraded by the LDL receptor-independent or scavenger pathway. HDL produced in extrahepatic tissues takes up free cholesterol and returns it to the liver.

McNamara and colleagues showed that while about two-thirds of subjects can compensate for increased cholesterol intake, the more important and more consistent determinant of plasma total cholesterol (TC) and LDL cholesterol levels was the dietary fat quality (saturated versus unsaturated) than the cholesterol content *per se*.⁷ An extreme example of the tight regulation of these processes is the case report of a man who eats 25 eggs (5g of cholesterol) per day but has a normal plasma cholesterol. This man absorbed only 18% of dietary cholesterol compared with 55% in controls

Figure 4: Plasma Cholesterol Response to Increased Dietary Cholesterol



The plasma cholesterol response from increasing dietary cholesterol by 100mg/day is on average 2.2mg/dl, as can be seen in this graph derived from 166 feeding studies.

consuming a mean 220mg of cholesterol per day.⁸ Indeed, endogenous cholesterol synthesis decreases with increased dietary consumption; this is a graded response within the normal range of daily cholesterol consumption from 26–650mg.⁹ Highly responsive suppression of endogenous cholesterol synthesis is observed in the Masai of East Africa, who have low serum cholesterol and low prevalence of atherosclerosis assessed by histology at necropsy despite a high-fat, high-cholesterol diet (composed chiefly of Zebu cattle milk, cow blood and occasional meat, providing 66% of calories from fat and 600–2,000mg of cholesterol/day) and high dietary cholesterol absorption.¹⁰ It has been estimated that 15–25% of the population are hyper-responders to dietary cholesterol. Hyper-responders to dietary cholesterol experience an almost three-fold greater response to dietary cholesterol compared with the rest of the population (see *Table 1*).

Whether a given patient mainly absorbs cholesterol ('absorber'), synthesises cholesterol ('synthesiser') or shows an intermediate phenotype ('mixed') may be important for lipid-lowering therapy. Individuals who are hyperabsorbers of cholesterol may not only have markedly different lipid and lipoprotein levels from those who have a synthesiser phenotype; their response to statin therapy may also be suboptimal. The Scandinavian Simvastatin Survival Study (4S) trial protocol pre-specified an up-titration of the simvastatin dose from 20 to 40mg/day in patients who failed to reach treatment TC below 5.2mmol/l at six weeks.¹¹

A similar titration was also employed in the Incremental Decrease in End-points Through Aggressive Lipid-lowering Therapy (IDEAL) trial.¹² When the patients who required the up-titration of dose (poor responders) are compared with a subgroup of those who did not (good responders), differences in cholesterol metabolism emerge. The good responders had higher baseline levels of cholesterol synthesis markers and lower levels of absorption markers than those with a poor response. In another substudy of the 4S trial, cholestanol was determined in 867 patients at baseline before randomisation to placebo or simvastatin and the population was stratified into quartiles of cholestanol:cholesterol ratio.¹³ Those in the lowest quartile, representing patients with more synthesis of cholesterol and less absorption of cholesterol, had the greatest responses in serum cholesterol to simvastatin and the greatest reduction in the precursor sterols; this is consistent with the greater inhibition of cholesterol synthesis in patients who primarily synthesise cholesterol despite more patients who are poor synthesisers of cholesterol having the dose of simvastatin increased.

Table 1: Hypo- and Hyper-responders to Dietary Cholesterol

	Hypo	Hyper
Per cent of population	80	20
Response (mg/dl per 100mg/day)	1.4±0.2	3.9±0.6
(N)	13	10
95% confidence interval	1.0–1.9	2.5–5.3
P	0.0002	

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Similar data have been reported with atorvastatin, where 20 or 40mg/day (average 29mg/day) for one year increased campesterol by about 80% and reduced lathosterol by 50%. This reduction in cholesterol precursors correlated with the reduction in TC. Interestingly, as with simvastatin, patients who had higher baseline levels of cholesterol absorption markers had a poorer LDL cholesterol response to atorvastatin.¹⁴ These results, showing changes in markers of cholesterol synthesis and absorption, have been confirmed in statin intervention trials that used sterol balance and fractional absorption.¹⁵ This rebound increase in cholesterol absorption with statin use may explain why a small proportion of treated patients have diminished response to statins on long-term follow-up.¹⁶

Likewise, inhibition of cholesterol absorption has also been shown to produce rebound increases in cholesterol synthesis. Patients with inhibited cholesterol absorption, e.g. those with gut resections or coeliac disease, have increased cholesterol synthesis as determined by sterol balance and increased levels of synthesis markers.¹⁷ This effect is also seen with pharmacotherapy. Ezetimibe reduced fractional cholesterol absorption from 50 to 23%, a 54% (p<0.001) reduction, and this effect was also confirmed by reductions in campesterol and sitosterol:cholesterol ratios of 41 and 34%, respectively. Conversely, ezetimibe 10mg/day increased synthesis by 89% (p<0.001) by sterol balance and also increased the validate surrogate of cholesterol synthesis, the lathosterol:cholesterol ratio, by 72% (p<0.001).¹⁸

Likewise, stanol ester feeding to reduce cholesterol absorption decreases markers of absorption (cholestanol and plant sterols), while increasing cholesterol precursor sterols and the increase in the ratio of a precursor sterol:plant sterol correlated negatively with the LDL cholesterol response to stanol ester feeding.¹⁹ From a 4S subgroup, patients on statin therapy selected for a high baseline cholestanol:cholesterol ratio (indicative of significant cholesterol absorption) had a 7% reduction in TC and a 12% reduction in LDL cholesterol after being treated with sitostanol ester margarine, whereas those with a low cholestanol:cholesterol ratio had no significant reduction in TC or LDL cholesterol.²⁰ The clinical significance of inhibiting cholesterol absorption should not be underestimated, as near-complete inhibition of this pathway in type 2 diabetics with a combination of neomycin and stanol ester margarine decreased LDL cholesterol by 37%.²¹

In an 868 subgroup analysis of patients enrolled in the 4S study, those in the highest quartile of cholestanol:cholesterol had no clinical benefit from simvastatin therapy and had a 2.2-fold increased risk of major cardiac events compared with patients in the lowest quartile.²² Thus, the lack of serum lipid response in patients who have an 'absorber' phenotype does translate into an increased risk of having a

hard clinical end-point. Conversely, patients who are statin hyporesponders, i.e. absorbers, would be expected to have enhanced responses to antiresorptive agents. In a group of hypercholesterolaemic patients receiving statin therapy, hyporesponders to statin therapy were hyper-responders to ezetimibe. This is consistent with the need to treat individual patients with different therapeutic modalities.²³ Indeed, the individual responses to ezetimibe ranged from a 6 to a 60% reduction in LDL cholesterol; this contrasts with the average of 20% reported in population studies where no analysis of inter-individual variation is undertaken.

An understanding of cholesterol metabolism can also be useful in guiding the treatment of patients with familial hypercholesterolaemia in addition to 'regular' patients. In patients heterozygous for familial hypercholesterolaemia, higher plasma mevalonic acid (a surrogate for cholesterol synthesis)²⁴ predicted a good response to statin therapy and those with a good response showed greater reduction in plasma mevalonic acid concentrations on treatment. Furthermore, the E4 genotype, which has been associated with relatively greater absorption of cholesterol, was more common in poor responders than good responders to statin therapy in heterozygous familial hypercholesterolaemia patients. The severity of the underlying mutation in the LDL receptor had no predictive value.²⁵

The higher prevalence of metabolic syndrome and cardiovascular disease observed in South Asians, African-Americans and Hispanics highlights the need to research cholesterol metabolism in different ethnic groups. This increasing prevalence of cardiovascular disease in these populations is partially related to urban living and the adoption of Western lifestyles and diet. Although little formal evaluation of cholesterol metabolism in different ethnic groups has been undertaken recently, extrapolation from available data in other ethnic groups suggests that as diabetes-prone populations gain weight and acquire a metabolic syndrome phenotype, they shift cholesterol metabolism from an 'absorber' to a 'synthesiser' phenotype. Several overdue statin trials in these populations, such as the

Investigation of Rosuvastatin in South-Asian subjects (IRIS), will provide valuable information on cholesterol metabolism in these populations. An understanding of shifting cholesterol metabolism is also important when we give dietary advice to our patients. It is now recognised that reducing cholesterol in the diet is not as important as reducing saturated fat and weight loss.

Implications and Conclusion

The importance of recognising that genetic and environmental factors lead to differences in cholesterol metabolism is of clinical importance for the treatment of hyperlipidaemia. Even though statins will remain the gold standard for treating hypercholesterolaemia for many years to

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come, many high-risk patients fail to reach LDL targets or do not tolerate statins. As we have the therapeutic options to inhibit cholesterol absorption as well as cholesterol synthesis, we are able to individually tailor LDL therapy. LDL cholesterol reductions of more than 65% are possible when combining a statin and ezetimibe. As we aim for lower LDL cholesterol targets, we will need to use combination drugs more often. However, knowledge of the patient's cholesterol metabolism and thus the optimal way to treat a given patient may reduce treatment cost, minimise side effects due to the unnecessary prescription of medications and, most importantly, increase the number of high-risk patients at their target LDL cholesterol of <1.8mmol/l (70mg/dl). ■

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