

Review Article

Dietary cholesterol: from physiology to cardiovascular risk

Jean-Michel Lecerf^{1*} and Michel de Lorgeril²

¹Nutrition Department, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019 Lille Cedex, France

²CNRS UMR 5525, Medicine University, Faculté de Médecine, Domaine de la Merci, Université de Grenoble, 38700 La Tronche, France

(Received 30 June 2010 – Revised 11 November 2010 – Accepted 10 January 2011 – First published online 9 March 2011)

Abstract

Dietary cholesterol comes exclusively from animal sources, thus it is naturally present in our diet and tissues. It is an important component of cell membranes and a precursor of bile acids, steroid hormones and vitamin D. Contrary to phytosterols (originated from plants), cholesterol is synthesised in the human body in order to maintain a stable pool when dietary intake is low. Given the necessity for cholesterol, very effective intestinal uptake mechanisms and enterohepatic bile acid and cholesterol reabsorption cycles exist; conversely, phytosterols are poorly absorbed and, indeed, rapidly excreted. Dietary cholesterol content does not significantly influence plasma cholesterol values, which are regulated by different genetic and nutritional factors that influence cholesterol absorption or synthesis. Some subjects are hyper-absorbers and others are hyper-responders, which implies new therapeutic issues. Epidemiological data do not support a link between dietary cholesterol and CVD. Recent biological data concerning the effect of dietary cholesterol on LDL receptor-related protein may explain the complexity of the effect of cholesterol on CVD risk.

Key words: Dietary cholesterol: Phytosterols: Cardiovascular risk

Cholesterol owes its name (from the Greek, meaning ‘solid bile alcohol’, coined by Chevreul in 1815) to the fact that it was discovered in gallstones (by Poulletier de la Salle in 1769)⁽¹⁾. The fear inspired by this compound has more to do with its role in atheroma formation than its presence in gallstones. In fact, cholesterol is present in all animal cells, where it functions irreplaceably in membranes. It is absent in plants, which feature other sterols (phytosterols (PS) such as sitosterol, campesterol, stigmasterol, etc.) that the human body cannot synthesise. Cholesterol also performs other roles as a precursor of vitamin D (synthesised in the skin), adrenal and gonadal steroid hormones and bile acids. The body acknowledges this great importance in its capacity of both absorbing dietary cholesterol and synthesising cholesterol *de novo*. In contrast, PS are excreted as soon as they are absorbed and cannot be synthesised. Given that cholesterol is synthesised in the body, there is no absolute need for dietary intake, but regulation of the latter helps maintain a stable pool of cholesterol. Hence, when dietary cholesterol intake is very low (e.g. in vegans), its synthesis and absorption

are up-regulated. Cholesterol cannot be broken down by the body, and thus if dietary intake is too high, biliary and intestinal excretion will also be intensified.

Physiology of the absorption of dietary cholesterol

The small-intestinal absorption of cholesterol⁽²⁾ helps maintain its homeostasis. There are typically two sources of cholesterol entering the intestinal tract: food intake and biliary secretion of cholesterol into the duodenum. Recently, a third source of intestinal cholesterol has been identified in mice: a significant part of excess cholesterol is excreted directly via the intestine upon activation of the liver X receptor, indicating the existence of an important alternative route for cholesterol disposal⁽³⁾. However, the role of this pathway in humans is unclear.

In a typical Western diet, dietary cholesterol intake is approximately 300–450 mg/d and complements the 800–1400 mg of endogenous cholesterol in the bile (bile acids). In total, about 1000–2000 mg of cholesterol reach the lumen

Abbreviations: ABC, ATP-binding cassette; CETP, cholesteryl ester transfer protein; LRP, LDL receptor-related protein; NPC1L1, Niemann–Pick C1-like 1; PS, phytosterol.

* **Corresponding author:** Dr J.-M. Lecerf, fax +33 3 20 87 72 96, email jean-michel.lecerf@pasteur-lille.fr

of the small intestine and can be absorbed. Esterified dietary cholesterol must first be hydrolysed by intestinal pancreatic enzymes, and this hydrolysis leads to the formation of fatty acids and non-esterified cholesterol. However, biliary cholesterol is non-esterified. The role of the bile (which is rich in bile acids, emulsifying phospholipids (lecithin) and cholesterol) is to help form micelles via a detergent effect (with the emulsion enabling the solubilisation of cholesterol). Some evidence suggests that biliary cholesterol, because of its inherent association with bile acids, is absorbed slightly more efficiently than dietary cholesterol⁽⁴⁾, although this difference probably has little impact on overall cholesterol balance⁽⁵⁾. Biliary cholesterol goes to the liver via the portal vein and is also carried by chylomicrons. Bile acids are reabsorbed in the ileum by the apical Na-dependent bile acid transporter. Bile acid-binding agents (such as cholestyramine) limit the resorption of bile acids (which prompt the liver to use more cholesterol for the synthesis of bile acids, as measured by the activity of cholesterol 7- α -hydroxylase); hence, the liver cells increase their synthesis of LDL receptors in order to internalise plasma LDL.

Non-esterified cholesterol is thus incorporated into mixed micelles, an essential step for facilitating its diffusion to the boundary layer and then through the brush border of the intestinal mucosa, mainly in the duodenum and the proximal jejunum.

The scavenger receptor B1 is involved in the brush-border transfer of cholesterol at the apical pole of the enterocyte^(6,7). However, target disruption of the scavenger receptor, class B, type 1 gene (named *Scar-b1*) appears to have little effect on intestinal absorption in mice⁽⁸⁾. Niemann–Pick C1-like 1 (NPC1L1) protein also plays an important role in transporting cholesterol and dietary PS. NPC1L1 expression is enriched in the small intestine and is in the brush-border membrane of enterocytes⁽⁹⁾. Ezetimibe, a hypocholesterolaemic drug, specifically inhibits NPC1L1⁽⁹⁾, which, in turn, results in the

inhibition of both the absorption of dietary and biliary cholesterol and that of PS.

However, NPC1L1 knockout mice display substantial but only partial inhibition of cholesterol absorption, suggesting that a small proportion of luminal cholesterol is absorbed independently. Additional proteins, such as caveolin-1, are required to reconstitute an active cholesterol transporter (NPC1L1), which form heterocomplexes and might represent additional Ezetimibe targets that regulate intestinal transport⁽¹⁰⁾. Less than 1% of PS make their entry into the bloodstream, whereas 50–60% of intestinal cholesterol reaches the circulatory system⁽¹¹⁾. In fact, the two ATP-binding cassette (ABC) hemi-transporters G5 and G8 (ABCG5 and ABCG8) excrete PS into the intestinal lumen. Given that PS are not good substrates for acyl CoA cholesterol acyltransferase, these compounds must be eliminated, thus excretion of PS protects the body against its accumulation. Furthermore, PS decrease the absorption of cholesterol by competing with it at the micelle level, and by inducing the expression of another transporter (ABCA1) and/or the ABCG5 and ABCG8 hemi-transporters may probably increase cholesterol efflux from the enterocytes into the intestinal lumen⁽¹²⁾ (Fig. 1). Since the characterisation of mice expressing no ABCA1 revealed no impairment in percentage of cholesterol absorption, even after challenge with a synthetic liver X receptor agonist, and the *ABCA1* gene is regulated by heterodimers of the nuclear liver X receptor⁽¹³⁾, it has been hypothesised that ABCA1 may be involved in the transfer of cholesterol from the enterocytes into the lymph. As with Ezetimibe, PS indirectly lower plasma cholesterol by mimicking a (hepatic) cholesterol deficiency, which induces an increase in LDL receptor synthesis. Mutations of the *ABCA1* gene are responsible for Tangier disease and certain hypoalphalipoproteinaemia, whereas mutations of the *ABCG5* and *ABCG8* genes result in sitosterolaemia (an autosomal recessive condition in which plasma PS levels are considerably elevated, due to a lack of excretion

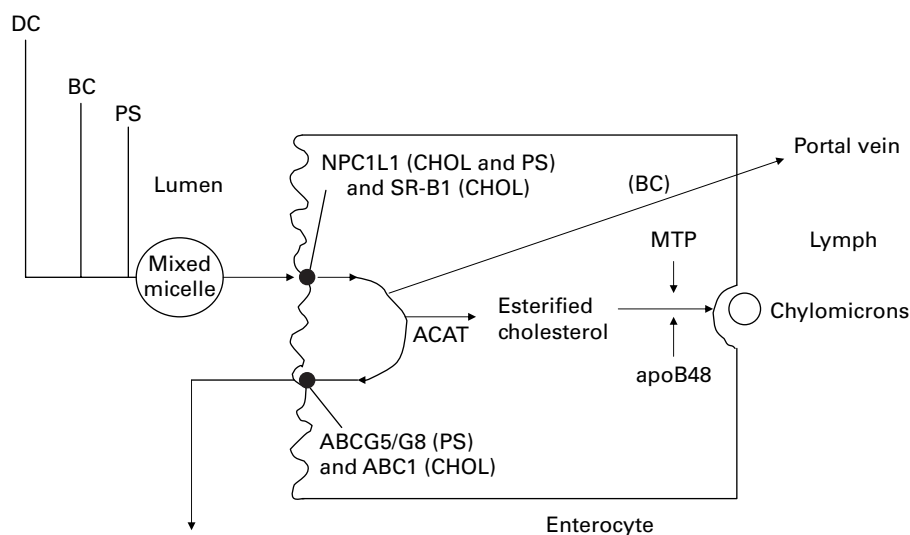


Fig. 1. Intestinal cholesterol and phytosterol absorption. NPC1L1, Niemann–Pick C1-like 1; SR-B1, scavenger receptor B1; ACAT, acyl CoA cholesterol acyltransferase; ABC Gx, ATP-binding cassette hemi-transporter Gx; ABC, ATP-binding cassette; MTP, microsomal transfer protein; DC, dietary cholesterol; BC, biliary cholesterol; PS, phytosterols; CHOL, cholesterol.

into the intestine). Other genetic polymorphisms (such as apoE phenotype) are involved in cholesterol and PS absorption^(14,15). The presence of an E4 allele is associated with an increased absorption of cholesterol and, probably, PS. Moreover, individuals carrying the E4 allele have a significantly lower response to LDL-cholesterol lowering with the statin atorvastatin⁽¹⁶⁾.

Once in the enterocyte, cholesterol is re-esterified by acyl CoA cholesterol acyltransferase. This step prevents non-esterified cholesterol from returning to the intestinal lumen. It also eases the incorporation of esterified cholesterol into chylomicrons, in which it is 'packaged' with microsomal transfer protein, apoB48 and dietary TAG. Next, chylomicrons are excreted from the basal pole of the enterocyte into the mesenteric lymph. During the absorption of cholesterol, there is little increase in the cholesterol content of the small-intestinal wall, indicating that cholesterol can be rapidly processed and exported from the enterocyte into the intestinal lymph, with a peak after 6–8 h⁽¹⁷⁾. They reach the bloodstream via the thoracic duct at the junction of the jugular and subclavian veins. Metabolism of the circulating chylomicrons starts with the hydrolysis of TAG by lipoprotein lipase in the capillary endothelium, which thereby releases NEFA for energy production. The chylomicron remnants are then captured by the liver via apoB or apoE receptors, and, finally, cholesterol enters the liver.

There are large inter-individual variations in cholesterol absorption; for example, the Tarahumara Indians of Mexico are weak absorbers⁽¹⁸⁾. In most mixed populations or even in pure vegetarians, the average absorption coefficient is 50 (SD 10)% but can be anywhere between 20 and 80% in healthy subjects consuming a moderately low-cholesterol diet^(19,20). Intestinal cholesterol absorption efficiency increases with ageing, because it significantly increases secretion rates of biliary lipids and the cholesterol content of the bile, as well as the size and hydrophobicity index of the bile salt pool⁽²¹⁾. Furthermore, there are sex differences in intestinal cholesterol absorption efficiency⁽¹⁷⁾. Intra-individual variability is lower: when tested several times under standardised conditions, the percentage of cholesterol absorbed is highly reproducible⁽²²⁾. In a given individual, relative absorption is low when dietary cholesterol content is high, and this is even the case for moderate intakes: the absorption coefficient drops from 41% for an intake of 26 mg to 16% for an intake of 421 mg⁽²³⁾. This explains why LDL-cholesterol is more sensitive to low intakes of dietary cholesterol than it is to high intakes^(24,25). But cholesterol feeding increases absolute cholesterol absorption and the serum concentration of total and LDL-cholesterol⁽²⁵⁾. The results regarding the effects of dietary cholesterol on cholesterol absorption are complicated by whether the study is an acute or chronic dietary cholesterol challenge. For instance, the long-term intake of a high-cholesterol diet does not seem to have much effect on fractional absorption rates in humans^(14,26). In animal studies, the presence of long-chain *n*-3 PUFA leads to a reduction in the absorption of cholesterol, together with an increase in the activity of hepatic 7- α -hydroxylase (a marker of bile acids synthesis) and bile salt excretion⁽⁶⁾. These mechanisms

are responsible for a decrease in both non-esterified and esterified cholesterol levels in the liver.

So, it appears that cholesterol absorption is a multistep process that is regulated by multiple genes and occurs solely in the small intestine. The numerous redundant cellular factors influencing intestinal cholesterol absorption indicate that cholesterol absorption must be protected due to its physiological importance.

Regulation of plasma cholesterol levels

Plasma cholesterol depends on many dietary and genetic factors at the same time. Plasma cholesterol level is the net result of intestinal cholesterol absorption and hepatic cholesterol synthesis, on the one hand, and biliary excretion and cellular use, on the other hand. Hence, it is not hard to note that many factors can influence plasma cholesterol.

Intestinal absorption

Dietary cholesterol content has little impact on plasma cholesterol (including LDL-cholesterol). Using literature data, Hegsted calculated that in the range from 0 to 400 mg for 4184 kJ (1000 kcal), the response is usually linear but almost flat⁽²⁷⁾. Indeed, 16 years ago, Apfelbaum⁽²⁸⁾ had already demonstrated that replacement of 50% of the intake of 'normal' butter by cholesterol-free butter only led to a 0.3% decrease in total blood cholesterol levels (i.e. fifteen times less than the physiological weekly variation), prompting him to question the value of this food product (although this effect has not lasted).

A meta-analysis concluded that for most individuals over the range of practical intake (0–400 mg/d), each 100 mg leads to an increase in total cholesterol by 0.056 mmol/l, HDL-cholesterol by 0.008 mmol/l and LDL-cholesterol:HDL-cholesterol by 0.02⁽²⁹⁾. However, several metabolic-ward studies have generated contradictory results concerning the link between dietary cholesterol intake and/or egg consumption, on the one hand, and plasma cholesterol, on the other hand^(29–31); in certain subjects, the massive consumption of eggs has no effect on plasma cholesterol levels⁽³²⁾. In moderately hypercholesterolaemic subjects, the consumption of seven eggs/week (instead of two) only led to very minor changes in total and LDL-cholesterol levels⁽³³⁾. In subjects consuming a low-fat diet with an increased ratio of polyunsaturated:saturated fatty acids, eating seven eggs/week (compared with just two) did not have an impact on total and LDL-cholesterol beyond 4 weeks regardless of whether the subjects were hypercholesterolaemic or normolipidaemic⁽³⁴⁾. However, variation in dietary cholesterol (200–600 mg/d) rather than the proportion of saturated and polyunsaturated fat (polyunsaturated:saturated 0.8 *v.* 0.3) had the most influence on LDL-cholesterol levels⁽³⁵⁾. An increased intake of dietary cholesterol (582 *v.* 278 mg) from the consumption of two eggs/d (*v.* a non-egg diet) does not increase plasma LDL when accompanied by an energy-restricted diet during 12 weeks with moderate weight loss⁽³⁶⁾. In normolipidaemic subjects, eating two eggs/d did not have any effect on total

cholesterol, LDL-cholesterol and apoB (regardless of the composition and quantity of the dietary lipid intake)⁽³⁷⁾, although HDL2-cholesterol levels rose (a positive effect, in principle)^(35,38). However, the rise in HDL-cholesterol with dietary cholesterol must be interpreted with caution; indeed, it merely reflects the main role of HDL in cholesterol transport, but it cannot be taken to imply that this is beneficial. In overweight men consuming a carbohydrate-restricted diet, dietary cholesterol from three large eggs (640 mg) increased HDL-cholesterol, whereas it had no effect on the LDL-cholesterol:HDL-cholesterol ratio⁽³⁹⁾. Similarly, in elderly subjects, the consumption of three large eggs daily increased LDL-cholesterol and HDL-cholesterol and did not change the ratio, but increased the LDL peak diameter⁽⁴⁰⁾. The absence of a negative effect on cardiovascular risk in certain studies could be thus linked to a concurrent elevation of HDL-cholesterol and LDL-cholesterol, although total cholesterol:HDL-cholesterol ratio generally increases⁽²⁹⁾. A decrease in cholesteryl ester transfer protein (CETP) activity has also been mentioned⁽⁴¹⁾, but its meaning is not clear. Indeed, CETP inhibition could have two opposite effects⁽⁴²⁾: blocking the transfer of cholesteryl esters to lipoproteins, such as LDL, containing apoB results in increased levels of HDL-cholesterol and enlarged HDL. Although HDL-cholesterol can be taken up directly by the liver through the HDL scavenger receptor, class B, type 1, the inhibition of CETP may reduce the rate of the return of HDL-cholesterol to the liver, thus impairing reverse cholesterol transport and increasing cardiovascular risk. But CETP inhibition has the potentially beneficial effects of increasing cholesterol efflux from macrophages mediated by *ABCG1* and of increasing the uptake of LDL-cholesterol by the liver.

The determinants of the effect of dietary cholesterol on plasma cholesterol are essentially the intestinal absorption of cholesterol, the very high simultaneous intakes of SFA, the presence of hypercholesterolaemia and certain genetic factors (such as the presence of the apoE allele and the E4 phenotype, in particular)⁽⁴³⁾. For instance, individuals carrying the apoE4 allele are more sensitive to dietary cholesterol, and a 10% increase in total cholesterol can result from an additional 300 mg cholesterol/d⁽⁴⁴⁾. The effect is attenuated by a high polyunsaturated:saturated fatty acid ratio⁽²⁸⁾. A drop in the intestinal supply of absorbed dietary and biliary cholesterol induces a reduction in hepatic cholesterol levels, which decreases the esterification of non-esterified cellular cholesterol, reduces the excretion of bile acids, and, above all, increases the synthesis of the LDL receptors needed to capture cholesterol in the circulating chylomicrons and thus lower plasma cholesterol levels. In parallel, a fall in hepatic cholesterol leads to an increase in endogenous cholesterol synthesis via 3-hydroxy-3-methyl-glutaryl CoA reductase (of which the precursor lathosterol is a marker); this increase in endogenous cholesterol production attenuates the effect of receptor up-regulation.

Cholesterol synthesis and cholesterol absorption are negatively correlated and thus co-regulated^(25,45).

Other nutritional factors have an impact on cholesterol absorption; this is notably the case for dietary fibres, which

are able to sequester bile acids and thus affect the enterohepatic bile acid cycle⁽²⁾.

Cholesterol synthesis

Most nutritional factors⁽⁴⁶⁾ modulate the synthesis of cholesterol and/or the catabolism of cholesterol-rich lipoproteins (LDL and HDL) in some way. Dietary restriction very rapidly (within 24 h) and very strongly down-regulates cholesterol synthesis: this is related to interruption of the required carbon backbone supply chain (via the acetyl-CoA pool). Likewise (and paradoxically), increasing meal frequency also reduces cholesterol synthesis, which leads to a fall in LDL-cholesterol. Fatty acids are powerful regulators of plasma cholesterol; the maximum response is achieved within the first 2 weeks. SFA reduce LDL receptor activity in the liver, which elevates circulating LDL and lowers cholesterol synthesis⁽⁴⁷⁾. However, direct measures to quantify endogenous cholesterol synthesis (i.e. sterol balance and combined sterol balance/radiolabelled cholesterol turnover studies) in human subjects, hamsters and guinea pigs have indicated no dietary fat saturation effects on endogenous cholesterol synthesis rates and are not in accordance with these indirect measurements. Some authors have shown that dietary cholesterol feeding suppresses human cholesterol synthesis measured by ²H incorporation and urinary mevalonic acid levels^(14,26,48). So, the direct analysis of endogenous cholesterol synthesis rates has consistently shown reductions in cholesterol synthesis in human and animal peripheral tissues with cholesterol feeding, including human mononuclear cells. Miettinen documented an almost equal decrease in synthesis (mg/kg per d) per increase in absorbed cholesterol (mg/kg per d)^(49,50). SFA also strongly enhance HDL-cholesterol⁽⁵¹⁾. *n*-6 PUFA augment cholesterol synthesis, despite a decrease in plasma cholesterol. This could be due to both the increased esterification of hepatic cholesterol and the use of cholesterol by peripheral tissues.

Dietary cholesterol content has only a slight effect on cholesterol synthesis⁽⁴⁵⁾, because adaptation mainly takes place through intestinal absorption. This can be explained by the fact that the liver itself does not use much cholesterol, since most of the latter is synthesised by extrahepatic tissues that do not capture cholesterol-rich chylomicrons. PS lead to a moderate increase in cholesterol synthesis. In passing, it is worth noting that the inverse relationship between PS intake and cholesterol synthesis means that plasma sitosterol concentration can be used as a marker of cholesterol absorption and thus an inverse indicator of cholesterol synthesis.

Genetic factors are heavily involved in regulating cholesterol metabolism and also testify to the existence of many gene–nutrition interactions (thus explaining the variability of individual responses)^(40,52,53). The most notable polymorphisms are for apoE (responsible for 7% of the variation in cholesterol levels), apoA4, apoA1, apoB and apoC3, whereas CETP polymorphisms are not extensively involved⁽⁵⁴⁾.

Lastly, one of the nutritional factors that must be taken into account is overweight, in general, and abdominal obesity in particular^(55,56), since TAG lipolysis in adipocytes generates

NEFA which go straight to the liver via the portal vein and are incorporated into the VLDL precursors of LDL. Obesity and overweight actually have a greater effect on cholesterol synthesis than would be predicted. At an ideal weight, the rate of synthesis is 11–13 mg/kg per d, but with added adiposity, the estimated synthesis rate due to weight excess is 20 mg/kg per d for the excess tissue⁽⁵⁷⁾.

Hyper-absorbers – poor synthesisers

Plasma PS (campesterol/sitosterol) are markers of the intestinal absorption of cholesterol. Their plasma levels are low because of their elimination through ABCG5/ABCG8 excretion. Cholestanol and lathosterol, precursors of cholesterol, are markers of cholesterol synthesis. Overweight, the metabolic syndrome, insulin resistance and type 2 diabetes are linked to the decrease in cholesterol absorption and the increase in cholesterol synthesis as a mimic of ABCG5/ABCG8 gene overexpression⁽⁵⁷⁾. Ezetimibe induces a decrease in cholesterol and PS absorption and a compensatory increase in cholesterol synthesis, which attenuates its LDL-cholesterol-lowering effect. Conversely, statins induce a decrease in the lathosterol:campesterol ratio⁽⁵⁸⁾. The degree of change in this ratio may be subject to genetic variability influenced by genetic polymorphisms at the locus for ABCG5/ABCG8. However, Van Himbergen⁽⁵⁹⁾ did not observe a significant correlation between changes in total cholesterol and changes in plasma campesterol in absolute terms. But the changes in these markers are more informative than baseline levels. When using absolute values, subjects with the greatest reduction in both synthesis and absorption had a significantly greater reduction in total cholesterol than subjects in whom the converse was true⁽⁵⁹⁾. Some subjects are bad responders to statins, and they have high plasma sitosterol and campesterol levels. They are hyper-absorbers and conversely poor synthesisers, and they need higher dosages of statins^(60,61). However, statin resistance cannot be summarised to the relative absorption and synthesis of cholesterol. It could probably be also a function of the pharmacodynamics of statins with genetic variation in statin clearance in the liver. In a Scandinavian Simvastatin Survival Study (4S) trial, subjects with high plasma PS levels had no improvement in cardiovascular events, and those subjects in the highest cholestanol quartile had more coronary events compared with those in the lowest cholestanol quartile^(62,63). Many observational studies have shown that high serum PS concentrations are associated with higher cardiovascular risk. Moreover, dietary PS induce higher plasma and arterial PS levels, while phytosterols decrease serum PS^(64,65). So, in hyper-absorbers, Ezetimibe is well adapted alone or with phytosterols, in order to decrease plasma cholesterol and PS. Statin treatment is especially effective in high synthesisers if individuals are also low absorbers⁽⁶⁰⁾. Conversely, statins are less effective in poor synthesisers (who are hyper-absorbers) and also enhance PS absorption. This leads to increased plasma PS levels and reduces the statins' cardiovascular benefit^(64,65). An association of statins with Ezetimibe would be efficient. In this case,

association of statins with PS should be deleterious despite the synergistic effect on plasma cholesterol level.

Even though there are some individuals who hypo-respond to dietary cholesterol and others who hyper-respond, people also differ in terms of the intensity of cholesterol synthesis. Finally, this observation has prompted efforts to better define the therapeutic indications of hypolipaeic agents: Ezetimibe for hyper-absorbers (with high sitosterol levels) and statins for strong synthesisers (with high lathosterol levels). A recent study has confirmed the effect of Ezetimibe on surrogate markers for cholesterol absorption (campesterol) and synthesis (lathosterol) and the effect of simvastatin on lathosterol but not on campesterol; however, baseline cholesterol absorption and synthesis did not predict responsiveness to LDL-lowering drugs⁽⁶⁶⁾.

Dietary cholesterol and CVD

Epidemiological data

Generally, dietary cholesterol has little effect on the regulation of plasma cholesterol, as discussed earlier. By way of an example, we have shown (in a randomised study on healthy subjects) that a difference of 800 mg/d in dietary cholesterol results in just a 6% variation in total cholesterol⁽⁶⁷⁾. It is thus legitimate to question whether dietary cholesterol itself (independently of plasma cholesterol levels) has a relationship (either a simple, statistical association or a causal relationship) with the risk of CVD.

Unfortunately, there are a few solid data on which an answer can be based. In fact, this hypothesis has never been tested in a clinical trial, thus depriving us of a decisive argument. In observational epidemiological studies, it is difficult to dissociate the presumed effect of dietary cholesterol from that (also presumed) of other dietary lipids (saturated or unsaturated fats) for a given individual. However, two studies from the 1970s^(68,69) and a published meta-analysis⁽⁷⁰⁾ have suggested that dietary cholesterol could be associated with the risk of CVD (independently of plasma cholesterol).

One way of circumventing the potentially confounding effects of cholesterol and other lipids is to focus one's analysis on a particular food, which is emblematic of cholesterol intake (e.g. eggs), rather than cholesterol itself. Of course, egg contains lipids other than cholesterol (and which could modulate the risk of CVD, notably depending on how the laying hens are fed), but in view of its very high cholesterol content (approximately 250 mg per egg), one can accept a sort of parallelism between the risk due to eggs and that due to dietary cholesterol. What do the few studies on eggs and the risk of CVD tell us? The Framingham study (with fewer than 1000 subjects) concluded as to the absence of the relationship between egg consumption and the risk of CVD⁽⁷¹⁾. The Harvard study on a cohort of 38 000 men and 80 000 women has shown that eating up to one egg/d (or seven eggs/week) did not increase the risk of CVD⁽⁷²⁾. Similarly, in the prospective cohort study of 21 327 men from the Physicians' Health Study⁽⁷³⁾ during an average follow-up of 20 years, egg consumption was not associated with incident myocardial

infarction or stroke. The only exception concerned diabetic women, with a slight increase in risk for those who ate more than one egg/d relative to those who ate less than one egg/week⁽⁷²⁾. In the Physicians' Health Study, all-cause mortality was increased for the consumption of \geq seven eggs/week, and this was stronger among diabetic subjects⁽⁷³⁾. In a study concerning a representative cohort of 9734 adults aged 25–74 years over a 20-year follow-up, consumption of $>$ six eggs/week does not increase the risk of stroke and ischaemic stroke or coronary artery disease. However, in subgroup analysis among diabetics, consumption of $>$ six eggs/week was associated with a significant increased risk of coronary artery disease⁽⁷⁴⁾.

It is noticeable that cholesterol absorption has been shown to be higher in patients with type 1 diabetes⁽⁷⁵⁾ but not in patients with type 2 diabetes⁽⁷⁶⁾. However, it is known from earlier studies that in type 2 diabetic patients, cholesterol synthesis is high⁽⁵⁷⁾. Lastly, there may be ethnic variations, because a Japanese study (of almost 10 000 subjects monitored over 14 years) suggested that eating one egg/d is associated with a slight (but statistically non-significant) risk of cardiovascular death, when compared with eating one egg/week⁽⁷⁷⁾. However, this was only observed in female subjects. The same authors have conducted a prospective study in Japan in a cohort of 90 735 subjects followed during 17–21 years⁽⁷⁸⁾: eating eggs more frequently, up to almost daily, was not associated with an increase in CHD incidence for middle-aged Japanese men and women; moreover, subjects with hypercholesterolaemia were less frequently in frequent egg consumption groups, probably because they avoided eating eggs.

On the basis of these data, one can conclude that if dietary cholesterol does lead to an increased risk of CVD, that risk is low. The clinical implication is thus negligible.

Recent biochemical data

Given the absence of clinical trial data and the methodological limitations of the epidemiological approach, identification of a biological mechanism by which dietary cholesterol may influence the risk of CVD could help resolve this question. In addition to the very minor involvement of dietary cholesterol in the regulation of plasma cholesterol (sometimes naively presented as a factor that obstructs the arterial lumen) and the fact that certain researchers question the importance of plasma cholesterol as a causal factor in CVD^(79,80), the question arises: does dietary cholesterol have a significant role in the pathophysiology of CVD?

In certain cells, dietary cholesterol stimulates the expression of a key membrane receptor in cellular physiology: the LDL receptor-related protein (LRP). This protein was first identified through its role in the metabolism of lipoproteins in chylomicron remnants but is also involved in the fibrinolysis system and the risk of thrombosis⁽⁶⁷⁾. LRP may decrease fibrinolytic activity by accelerating the degradation of tissue plasminogen activator – a protein that plays a major role in the activation of the fibrinolysis system. A dysfunctional fibrinolysis system promotes thrombotic complications, and thus high cholesterol

consumption could (via the LRP) promote CVD. This is conceivable but remains a hypothesis.

Another possibility, which goes against the aforementioned mechanism, is that LRP is essential for maintaining a stable arterial wall and preventing the development of atherosclerotic damage – independently of plasma cholesterol^(81,82). LRP appears to accelerate the degradation of proteins (metalloproteinases) that weaken the arterial structure. The absence of LRP (e.g. in GM mice) or low LRP levels (in patients with a low cholesterol intake) could thus promote the development of arterial damage. Hence, LRP may protect against CVD. In other words, dietary cholesterol appears to modulate a membrane protein that is essential for normal arterial function (LRP) and which, depending on the circumstances (e.g. as a function of age or other associated factors such as arterial hypertension and diabetes), may either increase or decrease the risk of CVD. This ambivalence of LRP undoubtedly explains the fact that the epidemiological data on dietary cholesterol are just as ambiguous.

Conclusion

Cholesterol is a vital component of the human body. This may explain that its availability is highly regulated in terms of both absorption and synthesis. Since cholesterol absorption is controlled by several biological systems, an alteration of one of these systems does not totally disturb absorption. This mechanism could explain the inter-individual absorption variability in the current human population. Moreover, a low dietary cholesterol intake is compensated by an increase in absorption, suggesting that the balance between absorption and synthesis is also modulated. Contrary to cholesterol, PS absorption is extremely low, and the human body owns a natural clearance system. In the human population, there exist low absorbers and high synthesizers and, inversely, this could explain a variable and complementary efficiency of drugs, which inhibit absorption or synthesis. However, it is not possible to predict these effects on the basal levels of plasma PS or cholesterol precursors (lathosterol). Due to these adaptive mechanisms, although dietary cholesterol enhances plasma cholesterol (LDL-cholesterol and HDL-cholesterol), this rise is low, and the interpretation of an HDL-cholesterol increase is difficult. Recent epidemiological studies have not shown any relationship between cardiovascular risk and dietary cholesterol intake and/or egg consumption, or else have shown a very low effect, except in diabetic subjects. This could be due to a high dietary cholesterol absorption rate in type 1 diabetes and a high synthesis in type 2 diabetes. So knowledge about dietary cholesterol physiology is an important practical and scientific concern.

Acknowledgements

The authors would like to thank Coralie Schnebelen, Elise Clerc and Marie-Claude Berti ere for their helpful comments, and Yvette Soustre and Helen Bishop MacDonald for their kind re-reading. No grant supported the present study, and neither of the authors had personal or financial conflict of

interest related to the present study. J.-M. L. coordinated the study and wrote the manuscript, and M. d. L. was involved in the manuscript writing.

References

- Turley SD (1999) Dietary cholesterol and the mechanism of cholesterol absorption. *Eur Heart J* **1**, Supp., S29–S35.
- Carr TP & Jesch FD (2006) Food components that reduce cholesterol absorption. *Adv Food Nutr Res* **51**, 165–204.
- Kruit JK, Ploscht J, Havinga R, *et al.* (2005) Increased fecal neutral sterol loss upon liver X receptor activation is independent of biliary sterol secretion in mice. *Gastroenterology* **128**, 147–156.
- Wilson MD & Rudel LL (1994) Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. *J Lip Res* **35**, 943–955.
- Samuel P & Mc Namara DJ (1983) Differential absorption of exogenous and endogenous cholesterol in man. *J Lipid Res* **24**, 265–276.
- Dawson PA & Rudel LL (1999) Intestinal cholesterol absorption. *Curr Opin Lipidol* **10**, 315–320.
- Hauser H, Dyer JH, Nandy A, *et al.* (1998) Identification of a receptor mediating absorption of dietary cholesterol in the intestine. *Biochemistry* **37**, 17843–17850.
- Wang DQ & Carey MC (2002) Susceptibility to murine cholesterol gallstone formation is not affected by partial disruption of the HDL receptor SR-B1. *Biochim Biophys Acta* **1583**, 141–150.
- Altmann SW, Davis HR, Zhu LJ, *et al.* (2004) Niemann-Pick C1 like protein is critical for intestinal cholesterol absorption. *Science* **303**, 1201–1204.
- Smart EJ, Derosé RA & Farber SA (2004) Annexin 2 caveolin 1 complex is a target of ezetimibe and regulates intestinal cholesterol transport. *Proc Natl Acad Sci USA* **101**, 3450–3455.
- Ostlund RE (2007) Phytosterols, cholesterol absorption and healthy diets. *Lipids* **42**, 41–45.
- Plat J & Mensink RP (2005) Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am J Cardiol* **96**, Suppl., 15D–22D.
- Plösch T, Kok T, Bloks VW, *et al.* (2002) Increased hepatobiliary and fecal cholesterol excretion upon activation of the liver X receptor is independent of ABCA1. *J Biol Chem* **277**, 33870–33877.
- Gylling H & Miettinen TA (1992) Cholesterol absorption, and synthesis related to low density lipoprotein metabolism during LDL kinetics during varying cholesterol intake in men with different apo E phenotypes. *J Lipid Res* **33**, 1361–1371.
- Kesaniemi YA, Ehnholm C & Miettinen TA (1987) Intestinal cholesterol efficiency in man is related to apoprotein E polymorphism. *J Lip Res* **80**, 578–581.
- Pedro-Botet J, Schaefer EJ, Bakker-Arrema RG, *et al.* (2001) Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. *Atherosclerosis* **158**, 183–193.
- Lammert F & Wang DQ (2005) New insights into the genetic regulation on intestinal cholesterol absorption. *Gastroenterology* **129**, 718–734.
- Mac Murray MP, Connor WE, Lin DS, *et al.* (1985) The absorption of cholesterol and the sterol balance in the Tarahumara Indians of Mexico fed cholesterol-free and high cholesterol diets. *Am J Clin Nutr* **41**, 128–138.
- Sudhop T, Lütjohann D, Kodal A, *et al.* (2002) Inhibition of intestinal absorption by ezetimibe in humans. *Circulation* **106**, 1943–1948.
- Clarenbach JJ, Reber M, Lütjohann D, *et al.* (2006) The lipid lowering effect of ezetimibe in pure vegetarians. *J Lipid Res* **47**, 2820–2824.
- Wang DQH (2002) Aging *per se* is an independent risk factor for gallstone formation in gallstone susceptible mice. *J Lipid Res* **43**, 1950–1959.
- Bosner MS, Ostlund RE, Osofisan O, *et al.* (1993) Assessment of percent cholesterol absorption in humans with stable isotopes. *J Lipid Res* **34**, 1047–1053.
- Ostlund RE, Bosner MS & Stenson NF (1999) Cholesterol absorption efficiency declines at moderate dietary doses in normal human subjects. *J Lipid Res* **40**, 1453–1458.
- Sachs FM, Salazar J, Miller L, *et al.* (1984) Ingestion of egg raises plasma low density lipoproteins in free-living subjects. *Lancet* **1**, 647–649.
- Vuoristo M & Miettinen TA (1994) Absorption modulation and serum concentrations of cholesterol in vegetarians: effects of cholesterol feeding. *Am J Clin Nutr* **59**, 1325–1331.
- Mac Namara DJ, Kolb R, Parker TS, *et al.* (1987) Heterogeneity of cholesterol homeostasis in man. *J Clin Invest* **79**, 1729–1739.
- Hegsted DM (1986) Serum cholesterol response to dietary cholesterol: a re-evaluation. *Am J Clin Nutr* **44**, 299–305.
- Apfelbaum M (1992) Which is the nutritional advantage of the consumption of a free-cholesterol butter? *Rev Prat* **42**, 1925–1926.
- Weggemans RM, Zock PL & Katan MB (2001) Dietary cholesterol from eggs increases the ratio of total cholesterol to high density lipoprotein cholesterol in humans: a meta-analysis. *Am J Clin Nutr* **73**, 885–891.
- Vorster HH, Benade AJ, Barnard HC, *et al.* (1992) Egg does not change plasma lipoprotein and coagulation profiles. *Am J Clin Nutr* **55**, 400–410.
- Tillotson JL, Bartsch GE, Gorder D, *et al.* (1997) Food group and nutrient intakes at baseline in the Multiple Risk Factor Intervention Trial. *Am J Clin Nutr* **65**, Suppl. 1, 228S–257S.
- Kern F (1991) Normal plasma cholesterol of an 88 year old man who eats 25 eggs a day. Mechanisms of adaptation. *N Engl J Med* **324**, 896–897.
- Simons LA, Von Konigsmark M, Simons J, *et al.* (1993) Effect of an egg per day on plasma cholesterol levels in moderate hypercholesterolemia. *Nutr Metab Cardiovasc Dis* **3**, 78–82.
- Edington J, Geekie R, Carter R, *et al.* (1987) Effect of dietary cholesterol on plasma cholesterol concentration in subjects following reduced fat, high fibre diet. *Br Med J* **294**, 333–336.
- Fielding CJ, Havel RJ, Todd KM, *et al.* (1995) Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. *J Clin Invest* **95**, 61–68.
- Harman NL, Leeds AR & Griffin BA (2008) Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss. *Eur J Clin* **47**, 287–293.
- Kestin M, Clifton PM, Rouse IK, *et al.* (1989) Effect of dietary cholesterol in normolipidemic subjects is not modified by nature and amount of dietary fat. *Am J Clin Nutr* **50**, 528–532.
- Ginsberg HN, Karmally W, Siddiqui M, *et al.* (1995) Increases in dietary cholesterol are associated with modest increases in both LDL and HDL cholesterol in healthy young women. *Arterioscler Thromb Vasc Biol* **15**, 169–178.

39. Mutungi G, Ratliff J & Puglisi M (2008) Dietary cholesterol from eggs increases plasma cholesterol in overweight men consuming a carbohydrate restricted diet. *J Nutr* **138**, 272–276.
40. Green CM, Zern TL & Wood RJ (2005) Maintenance of the LDL cholesterol/HDL cholesterol ratio in an elderly population given a dietary cholesterol challenge. *J Nutr* **135**, 2793–2798.
41. Sutherland WHF, Ball MJ & Walker H (1997) The effect of increased egg consumption on plasma cholesteryl ester transfer activity in healthy subjects. *Eur J Clin Nutr* **51**, 172–176.
42. Rader DJ (2007) Illuminating HDL – is it still a viable therapeutic target? *N Engl J Med* **357**, 2180–2183.
43. Ordovas JM & Schaeffer EJ (1999) Genes, variation of cholesterol and fat intake and serum lipids. *Curr Opin Lipidol* **10**, 15–22.
44. Sarkkinen E, Kurhonen M, Erkkila A, *et al.* (1998) Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and dietary cholesterol. *Am J Clin Nutr* **68**, 1215–1222.
45. Gylling H, Strandberg T & Tilvis R (1994) Regulation of serum cholesterol level in middle-aged and elderly men. Relation of cholesterol absorption and synthesis to lipoprotein metabolism. *Arterioscler Thromb* **14**, 694–700.
46. Jones PJ (1997) Regulation of cholesterol biosynthesis by diet in humans. *Am J Clin Nutr* **66**, 438–446.
47. Mustad VA, Ellsworth JF & Cooper AD (1996) Dietary linoleic acid increases and palmitic acid decreases hepatic LDL receptor protein and mRNA abundance in young pigs. *J Lipid Res* **37**, 2310–2323.
48. Jones PJH, Pappu AS, Hatcher L, *et al.* (1996) Dietary cholesterol feeding suppresses human cholesterol synthesis measured by deuterium incorporation and urinary mevalonic acid levels. *Arterioscler Thromb Vasc Dis* **16**, 1222–1228.
49. Miettinen TA & Kesaniemi YA (1989) Cholesterol absorption: regulation of cholesterol synthesis and elimination and within population variations of serum cholesterol levels. *Am J Clin Nutr* **49**, 629–635.
50. Gylling H & Miettinen TA (2002) Inheritance of cholesterol metabolism of probands with high or low cholesterol absorption. *J Lipid Res* **43**, 1472–1476.
51. Asztalos B, Lefebvre M, Wong L, *et al.* (2000) Differential response to low-fat diet between low and normal HDL cholesterol subjects. *J Lipid Res* **41**, 321–328.
52. Rantala M, Rantala TT & Savolainen MJ (2000) Apolipoprotein B gene polymorphisms and serum lipids: meta analysis of the role of genetic variation to diet. *Am J Clin Nutr* **71**, 713–724.
53. Masson LF, Mc Neill G & Avenell A (2003) Genetic variation and the lipid response to dietary intervention: a systematic review. *Am J Clin Nutr* **77**, 1098–1111.
54. Aitken WAE, Chisolma WAH, Duncan AW, *et al.* (2006) Variation in the cholesteryl ester transfer protein (CETP) gene does not influence individual plasma cholesterol response to changes in the nature of dietary fat. *Nutr Metab Cardiovasc Dis* **16**, 353–363.
55. Tchernof A (2007) Visceral adipocytes and the metabolic syndrome. *Nutr Rev* **95**, Suppl., S24–S29.
56. Ritchie SA & Connell JMC (2007) The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutr Metab Cardiovasc Dis* **17**, 319–326.
57. Gylling H & Miettinen TA (2005) Cholesterol absorption: influence of body weight and the role of plant sterols. *Curr Atherosclerosis Rep* **7**, 466–467.
58. Lamon-Fava S, Diffenderfer MR, Barrett PH, *et al.* (2007) Effects of different doses of atorvastatin on human apolipoprotein B-100, B48, and A1 metabolism. *J Lipid Res* **48**, 1748–1753.
59. Van Himbergen T, Matthan NR, Resteghini NA, *et al.* (2009) Comparison of the effects of maximal dose atorvastatin and rosuvastatin therapy on cholesterol synthesis and absorption markers. *J Lipid Res* **30**, 730–739.
60. Hedman M, Miettinen TA, Gylling H, *et al.* (2006) Serum noncholesterol sterols in children with heterozygous familial hypercholesterolemia undergoing pravastatin therapy. *J Pediatr* **148**, 241–246.
61. Miettinen TA & Gylling H (2002) Ineffective decrease cholesterol by simvastatin in a subgroup of hypercholesterolemic coronary patients. *Atherosclerosis* **164**, 147–152.
62. Miettinen TA, Strandberg TE & Gylling H (2000) Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. *Arterioscler Thromb Vasc Biol* **20**, 1340–1346.
63. Miettinen TA, Gylling H, Strandberg T, *et al.* (1998) Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. *BMJ* **316**, 1127–1130.
64. Kajinami K, Brousseau ME, Nartsupha C, *et al.* (2004) ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. *J Lipid Res* **45**, 653–656.
65. Lecerf JM (2007) Phytosterols and cardiovascular risk. *Nutr Clin Metab* **21**, 17–27.
66. Lakoski SG, Xu F, Vega GL, *et al.* (2010) Indices of cholesterol metabolism and relative responsiveness to ezetimibe and simvastatin. *J Clin Endocrinol Metab* **95**, 800–809.
67. Boucher P, de Lorgeril M, Salen P, *et al.* (1998) Effect of dietary cholesterol on LDL-receptor, HMGCoA reductase and LRP mRNA expression in healthy humans. *Lipids* **33**, 1177–1186.
68. Shekelle RB, Mac Millan Shryock A, Paul O, *et al.* (1981) Diet, serum cholesterol and death from CHD – The Western Electric Study. *N Engl J Med* **304**, 65–70.
69. Mc Gee DL, Reed DM, Yano K, *et al.* (1984) Ten-year incidence of coronary heart disease in the Honolulu Heart Program. *Am J Epidemiol* **119**, 667–676.
70. Stamler JS & Shekelle RB (1988) Dietary cholesterol and human coronary heart disease. *Arch Pathol Lab Med* **112**, 1032–1040.
71. Dawber TR, Nickerson RJ, Brand FN, *et al.* (1982) Eggs, serum cholesterol and coronary heart disease. *Am J Clin Nutr* **36**, 617–625.
72. Hu FB, Stampfer MJ, Rimm EB, *et al.* (1999) A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA* **281**, 1387–1394.
73. Djousse L & Gaziano JM (2008) Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. *Am J Clin Nutr* **87**, 964–969.
74. Qureshi AI, Suri FK, Ahmed S, *et al.* (2007) Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. *Med Sci Monit* **13**, CR1–CR8.
75. Gyllin GH, Laaksonen DE, Atalay M, *et al.* (2007) Markers of absorption and synthesis of cholesterol in men with type 1 diabetes. *Diabetes Metab Res Rev* **23**, 372–377.
76. Miettinen TA, Gylling H, Tuominen J, *et al.* (2004) Low synthesis and high absorption of cholesterol characterize type 1 diabetes. *Diabetes Care* **27**, 53–58.
77. Nakamura Y, Okamura T, Tamaki S, *et al.* (2004) Egg consumption, serum cholesterol and cause-specific and all-cause mortality: the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged. *Am J Clin Nutr* **80**, 58–63.

78. Nakamura Y, Iso H, Kita Y, *et al.* (2006) Egg consumption serum total cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based Prospective Study. *Br J Nutr* **96**, 921–928.
79. Ravnskov U, Rosch PJ, Sutter MC, *et al.* (2006) Should we lower cholesterol as much as possible? *BMJ* **332**, 1330–1332.
80. Okuyama H, Ichikawa Y, Sun Y, *et al.* (2007) Association of high total cholesterol with coronary heart disease mortality differs among subject populations. Familial hypercholesterolemia as a key concept. *World Rev Nutr Diet* **96**, 19–37.
81. Boucher P, Gotthardt M, Li WP, *et al.* (2003) LRP: role in vascular wall integrity and protection from atherosclerosis. *Science* **300**, 329–332.
82. Espirito Santo SM, Pires NM, Boesten LS, *et al.* (2004) Hepatic LRP deficiency in mice increases atherosclerosis independent of plasma cholesterol. *Blood* **103**, 3777–3782.