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Does Dietary Cholesterol Matter?

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Abstract An ongoing dispute in the nutrition field is whether dietary cholesterol contributes significantly to elevated serum cholesterol and to atherosclerotic disease. Carefully controlled metabolic studies have shown that high-cholesterol intakes cause moderate increases in serum cholesterol levels. It is been difficult to verify this in population studies because of confounding factors. Nonetheless, meta-analysis of controlled studies documents a cholesterol-raising action of dietary cholesterol. Most of this effect occurs in low-density lipoproteins (LDLs), but the cholesterol content of other lipoproteins can be increased as well. Moreover, population studies strongly suggest that dietary cholesterol is atherogenic beyond any rise in LDL concentrations. It must be emphasized that dietary cholesterol is only one of several dietary factors influencing serum cholesterol levels. Others include saturated fatty acids, trans fatty acids, soluble fiber, and total caloric intake. To achieve substantial serum cholesterol lowering, favorable changes in all of these factors must be combined. To maximize cardiovascular risk reduction, a lifetime of a healthy diet is needed. Reduced cholesterol intake is only one of several factors required to achieve such a diet. In addition, reduction of cholesterol absorption can enhance serum cholesterol lowering. This can be attained by the addition of plant sterols or plant stanols to the diet or by use of ezetimibe, a cholesterol absorption blocker. By combining dietary cholesterol reduction with other cholesterol-lowering modalities, it should be possible to substantially reduce atherosclerosis throughout life short of using cholesterol-lowering drugs that act systemically.

Keywords Dietary cholesterol · Cholesterol absorption · Eggs · Low-density lipoproteins · Plant sterols · Plant stanols · Ezetimibe

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Introduction

For many years, dietary cholesterol was considered to be a significant contributor to elevated serum cholesterol levels and to atherosclerotic cardiovascular disease (ASCVD). This view was supported by studies in animal models which showed that high-cholesterol intakes can produce marked hypercholesterolemia and atherosclerosis [1–5]. Examples include rabbits, pigeons, chickens, pigs, and some non-human primates. Not all species are sensitive to dietary cholesterol; for instance, rats and dogs are normally resistant to high-cholesterol intakes and do not develop hypercholesterolemia.

There has been a long-running debate on whether serum cholesterol levels in humans are responsive to high intakes of cholesterol. It is quite apparent from many studies that humans do not develop severe hypercholesterolemia when fed high-cholesterol diets. But, as will be discussed, several studies have shown that high intakes of cholesterol can raise serum cholesterol levels. In spite of this evidence, a recent national guideline on nutrition did not recommend a reduction in dietary cholesterol (US Department of Health and Human Services and US Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at http://health.gov/dietaryguidelines/2015/guidelines/). The

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rationale for this recommendation has been summarized by McNamara [6, 7]. Whether this is a justified position requires an understanding of several aspects of cholesterol metabolism and its relation to other nutrients. To put the question into perspective, this paper will examine both the history of research on dietary cholesterol as well as its fates in various pathways of cholesterol metabolism.

Dietary Cholesterol and Serum Cholesterol

Two kinds of studies have been carried out to evaluate the influence of dietary cholesterol on serum cholesterol levels. One type includes metabolic studies carried out under highly controlled conditions; these have been done in relatively small numbers of subjects. They have shown a positive relationship between cholesterol intake and serum cholesterol. Other investigations have recruited larger numbers of subjects but under less carefully controlled conditions; these have given ambiguous results, possibly because of confounding factors.

Metabolic studies were carried out by Keys et al. [8], Hegsted et al. [9, 10], and Mattson et al. [11]; their reports documented that increases in dietary cholesterol raise serum cholesterol levels. Hegsted et al. [9, 10] and Mattson et al. [11] found a linear relation between cholesterol intake and serum cholesterol levels up to a cholesterol intake of at least 500 mg per day. Keys et al. [8] observed a curvilinear response over this range. On average, the rise in serum total cholesterol levels rose about 10 mg/dL, for every 100 mg increase in dietary cholesterol per 1000 calories. Cholesterol intake in the American diet averages between 300 and 500 mg per day; within this range, reducing cholesterol intake by 200 mg per day should produce a corresponding decrease in serum total cholesterol of about 10 mg/dL (approximately 5 %). The major sources of dietary cholesterol in the current American diet are shown in Fig. 1. For individuals, the contribution of eggs to dietary cholesterol varies depending on personal eating habits.

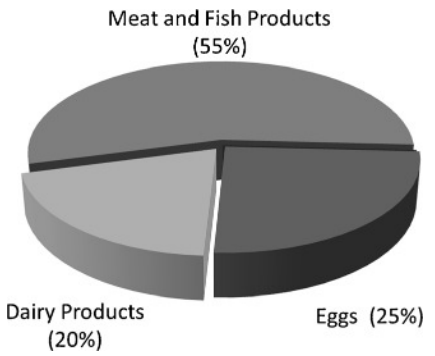

Fig. 1 Major sources of dietary cholesterol and current American diet. In the past, when egg consumption was greater than at present, percentage contribution from eggs was higher. A reduction in egg consumption may be one factor responsible for the fall in cholesterol levels that has occurred in the US population

A sizable number of other metabolic studies have examined the relationship between dietary cholesterol intake and serum cholesterol levels. At least two meta-analyses have incorporated several acceptable investigations [12, 13••]. Both showed that, on average, dietary cholesterol does in fact raise serum cholesterol concentrations. At lower intakes, the rise in cholesterol levels appeared to be linear relative to intake, but above 300–400 mg per day, the response curve became hyperbolic.

Serum Cholesterol Responsiveness

Katan and associates [14–16] have carried out a series of studies to determine response of serum cholesterol levels to dietary cholesterol. In their studies, an increase in dietary cholesterol definitely raised serum total cholesterol. But, the response varied considerably from one person to another. Moreover, even in the same subject, responses were not always consistent from one test study to another. Mechanisms underlying variability in responsiveness to dietary cholesterol have not been explained. A variety of factors could be involved. For example, amounts of dietary fats and types of fat could influence of the solubility of cholesterol in intestinal contents. Other factors will be considered in the below sections.

Dietary Cholesterol and Other Cholesterol-Raising Nutrients

Cholesterol is only one of several nutrients that influence serum cholesterol levels. Among these are saturated fatty acids and trans fatty acids, dietary soluble fiber, and overnutrition, that is exemplified by obesity. Their approximate impact on serum cholesterol levels is shown in Fig. 2. According to available data, the aggregate of these factors, including dietary cholesterol, will on average raise serum cholesterol levels about 25 % [17••]. Each factor alone has a relatively small impact on total serum cholesterol levels, but in aggregate, the

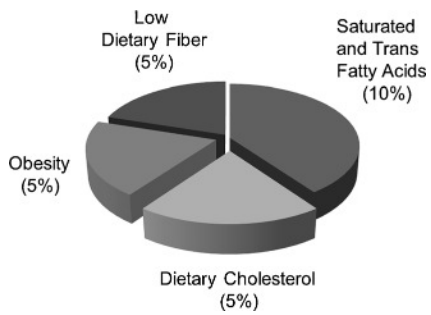

Fig. 2 Contribution of dietary factors to serum cholesterol levels in the US population. Dietary cholesterol and overnutrition (obesity) each account for approximately 5 % higher levels than would otherwise occur. The low intake of soluble dietary fiber can explain another 5 % higher serum cholesterol. Finally, current intakes of saturated fatty acids and trans fatty acids raise serum cholesterol about 10 % higher than would occur with recommended intakes

effect is substantial. This explains why dietary cholesterol should not be eliminated from the mix of other cholesterol-raising nutrients.

Cholesterol Absorption

According to available estimates, about 50 % of dietary cholesterol is absorbed [18, 19]. Several methods have been employed to estimate cholesterol absorption [18, 20–23, 24–27]. Most employed radioactive tracers. Radioactive cholesterol can be incorporated into the diet, and its absorption can be measured either by its appearance into the bloodstream or by its disappearance from the stools. These measurements estimate fractional absorption and not absolute absorption. The latter can be measured by intestinal perfusion methods in which disappearance of a known quantity of cholesterol over a segment of the intestine can be determined [28].

Normally, absorption of ingested cholesterol ranges from 40 to 60 % [18]. This variability may contribute in part to how much dietary cholesterol affects serum cholesterol levels. The key mediator of absorption is a protein named Neiman-Pick C1-like protein-1 (NPC1L1) [29••, 30]. This protein binds cholesterol at the surface of the enterocyte. The resulting complex is internalized into lysosomes via clathrin-dependent endocytosis. Additional adaptor proteins appear to play a role in internalization of cholesterol. Non-esterified cholesterol that is transferred into the enterocyte is esterified with a fatty acid and is incorporated into chylomicrons. Polymorphisms in NPC1L1 have been shown to modify amounts of cholesterol being absorbed [31].

Biliary Cholesterol

If we assume a cholesterol intake of 500 mg per day and a 50 % absorption, approximately 250 mg of dietary cholesterol is absorbed. It must be noted, however, that a larger amount of cholesterol enters the intestine through the bile. In the average adult, about 1000 mg of cholesterol enters through the biliary tree [23, 32]. This proportion thus is at least twice the dietary intake and even more if the diet contains less than 500 mg per day. Dietary cholesterol can be called exogenous cholesterol, whereas that from the bile can be called endogenous cholesterol. According to available evidence, about 50 % of endogenous cholesterol is reabsorbed [28]. Hence, a total of about 750 mg of cholesterol reenters the body each day and is carried into the systemic circulation by chylomicrons.

Fates of Newly Absorbed Cholesterol

Before dietary cholesterol can be incorporated into chylomicrons, it must be esterified with a fatty acid. Only about 1 % of lipid carried in chylomicrons is cholesterol ester; the remainder is triglyceride. In the circulation, the triglyceride of chylomicrons is hydrolyzed by lipoprotein lipase. Removal of triglycerides leaves cholesterol-rich lipoproteins called chylomicron remnants. Almost all of newly reabsorbed cholesterol ester is taken up by the liver with chylomicron remnants [33]. In the lysosomes of liver cells, cholesterol esters are hydrolyzed, and non-esterified cholesterol enters liver pools of cholesterol. The compartmentalization of cholesterol in the liver cell is beyond the scope of this review. But in general, total amounts of cholesterol in the liver regulate several pathways: cholesterol synthesis, conversion of cholesterol into bile acids, secretion of cholesterol into bile, esterification and storage of cholesterol esters, incorporation of cholesterol into newly synthesized lipoproteins, and hepatic uptake of cholesterol-enriched lipoproteins.

Role of the Liver in Protection Against Dietary Cholesterol-Induced Hypercholesterolemia

Much has been learned about hepatic cholesterol metabolism through animal studies. All of the pathways identified in animals exist in humans, although magnitudes for each pathway vary according to species. Although dietary cholesterol can raise serum cholesterol levels in humans, several mechanisms protect against marked hypercholesterolemia. These can be briefly discussed.

Cholesterol Synthesis The steps in cholesterol synthesis have been elucidated in detail [34]. First is the condensation of three acetyl coenzyme A (CoA) molecules to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). This molecule is converted to mevalonate by HMG-CoA reductase. Mevalonate is converted to the isopentenyl pyrophosphate (IPP), which is then condensed through several steps to squalene. Through another series of steps, squalene is transformed into cholesterol. HMG-CoA reductase is a major rate-limiting step in cholesterol synthesis, but overall control of cholesterol synthesis is exceedingly complex, and multiple enzymes appear to modulate various synthetic steps [35].

It has been known for more than 40 years ago that diets high in cholesterol suppress cholesterol synthesis in the livers of dogs and other animals [36, 37]. This is one way to protect against hypercholesterolemia. Suppression of cholesterol synthesis is due to coordinate changes in mRNA levels encoding multiple enzymes in the cholesterol biosynthetic pathway [38]. Research shows that high-cholesterol diets reduce the levels of the nuclear NH2-terminal domains of sterol regulatory element binding proteins (SREBPs), which activate transcription of sterol-regulated genes [39]. Conversely, removal of cholesterol from the diet increases cholesterol synthesis in the liver.

The extent to which dietary cholesterol suppresses hepatic cholesterol synthesis in humans is not known with certainty. This is because it is not possible to measure cholesterol

synthesis in the human liver. Nonetheless, it has been shown through cholesterol balance measurements that high intakes of cholesterol decrease whole body cholesterol synthesis [23]. Most of this suppression most likely occurs in the liver. In this study, Quintão et al. [23] found that high-cholesterol intakes had a small effect on serum cholesterol concentrations in spite of high-cholesterol absorption. The small response appeared to be due, at least in part, to feedback inhibition of endogenous cholesterol synthesis.

Bile Acid Synthesis Another potential mechanism for protection against diet-induced hypercholesterolemia may be enhanced conversion of cholesterol into bile acids. Such has been reported in dogs and rodents [40, 41]. In humans, in contrast, high-cholesterol intakes seemingly do not increase bile acid synthesis [23]; hence, this mechanism does not explain why high-cholesterol diets produce only small increments in serum cholesterol in humans.

Biliary Cholesterol Output Another potential mechanism for protecting against hypercholesterolemia during high-cholesterol feeding is to re-secrete any excess hepatic cholesterol into bile. In fact, Quintão et al. [23] demonstrated that high-cholesterol intakes enhance biliary cholesterol output. This response appears to play an important role in avoiding hypercholesterolemia on high-cholesterol diets. Whether it pertains in other animal models is unclear, but induction of cholesterol gallstones in hamsters upon feeding high cholesterol in diet suggests a greater secretion of cholesterol into bile [42].

Storage of Cholesterol Esters in Liver In several animal species, feeding dietary cholesterol expands the pool of cholesterol ester in the liver. In the rat, expansion of the cholesterol ester pool in the liver may protect against hypercholesterolemia, but in rabbits and monkeys, high-cholesterol diets appear to overwhelm cholesterol ester storage capacity and produce hypercholesterolemia. In cholesterol-fed monkeys, LDL particles become enriched in cholesterol ester derived from hepatic pools [43]. This phenomenon has not been reported in humans, but it is certainly possible that some of the increase in LDL cholesterol resulting from high-cholesterol diets could be secondary to enrichment of LDL particles with liver-derived cholesterol esters.

Suppression of Hepatic LDL Receptor Expression Finally, in diet-responsive species, excess dietary cholesterol seemingly suppresses hepatic LDL receptor expression and thereby raises serum LDL-cholesterol levels [44, 45]. This mechanism likely accounts for much of the rise in serum LDL-cholesterol levels in humans fed high-cholesterol diets. The molecular pathways for this action have been elucidated in detail by Goldstein and Brown [46].

Atherogenicity of Dietary Cholesterol

LDL-Cholesterol (LDL-C) Most of the effect of dietary cholesterol on serum total cholesterol levels can be explained by an increase in LDL-C. Randomized controlled trials indicate that over a 5-year period, a 1 % change in LDL-C produces a corresponding 1 % change in risk for ASCVD events [47••]. Since a high-cholesterol intake raises LDL-C levels on average by about 5 %, it is plausible that risk for ASCVD is raised, at least by a similar percentage. But, it must be kept in mind that genetic epidemiology strongly suggests that over a lifetime, a 1 % higher LDL-C will raise long-term risk by approximately 3 % [48••]. If this observation can be applied to the dietary cholesterol question, then a lifetime of high-cholesterol intake could raise risk for ASCVD up to 15 %.

As mentioned before, to expect dietary habits to significantly lower LDL-C levels, several dietary factors must be simultaneously modified. Besides decreasing dietary cholesterol, cholesterol-raising fatty acids should be reduced along with weight control. Adding soluble fiber to the diet will further lower cholesterol levels.

Chylomicron Remnants One theory holds that cholesterol-enriched chylomicron remnants are atherogenic [49]. Certainly, the cholesterol content of chylomicrons and their remnants will be higher on diets enriched in cholesterol. If these remnants make their way into the arterial wall, they could promote atherogenesis [50, 51].

Cholesterol-Enriched LDL and VLDL Remnants Studies in primate models indicate that high-cholesterol diets lead to formation of large, cholesterol-enriched LDL [52]. To date, such lipoproteins have not been identified in humans in response to dietary cholesterol. In other animal models, such as rabbits, high-cholesterol diets produce cholesterol-enriched very-low-density lipoproteins (VLDL), called beta VLDL [53]. The latter appears to be the atherogenic lipoproteins in rabbits. Similar lipoproteins have not been reported in human-fed high-cholesterol diets.

Reverse Cholesterol Transport It has been reported that cholesterol-enriched chylomicron remnants may inhibit reverse cholesterol transport [54]. This possibility is difficult to prove but represents one mechanism whereby a high-cholesterol diet may promote atherogenesis. It has been noted that high-cholesterol diets can also raise high-density lipoprotein (HDL) cholesterol [55]. This seems paradoxical because of the inverse relation between HDL cholesterol and risk for cardiovascular disease. On the other hand, Weggemans et al. [56] reported that high-cholesterol intakes increase the ratio of total cholesterol-to-HDL cholesterol, which suggests a detrimental pattern for ASCVD risk.

Epidemiologic Evidence for Atherogenicity of High-Cholesterol Diets

One line of evidence supporting a relation between dietary cholesterol and coronary heart disease (CHD) comes from epidemiology. For example, Shekelle and Stamler [57] found a strong relationship between cholesterol intake and CHD risk. This study did not uncover a specific mechanism for the link. Instead, it subsumes all possible mechanisms; perhaps, it is the best available evidence for a significant connection between dietary cholesterol and ASCVD.

Interventions on Dietary Cholesterol and Cholesterol Absorption

One strategy for treatment of hypercholesterolemia is to interfere with the absorption of dietary cholesterol. Of course, any approach of this type will also block the absorption of biliary cholesterol absorption.

Plant Sterols and Stanols These sterols are derived from plant oils. Two types are available: sitosterol and sitostanol. Their structures differ only slightly from cholesterol. They block absorption of cholesterol but are not themselves absorbed [58]. They can be dissolved in margarines and are best administered in this form. Two grams per day of either sitosterol or sitostanol will reduce serum LDL-C levels by approximately 10 % [17••, 58]. At present, they are not widely used in clinical practice for treatment of elevated LDL-C, but they offer potential for primary prevention in middle-aged persons with mildly elevated cholesterol in whom drugs are not indicated.

Ezetimibe A more effective blocker of cholesterol absorption is ezetimibe. This molecule inhibits NPC1L1 in the intestine [59]. It reduces cholesterol absorption by about 50 % [60], and it lowers LDL-C by 15–20 % [61]. This response demonstrates the importance of absorption of intestinal cholesterol in the regulation of serum cholesterol levels. Support for the importance of NPC1L1 in regulation of cholesterol metabolism comes from a major study in which the incidence of ASCVD events was determined in persons with heterozygous inactivating mutations in *NPC1L1* [62••]; in such persons, the risk for cardiovascular events was 47 % lower than in those without the mutation.

Summary

Available evidence from population and genetic studies show that a 5 % lower level of serum cholesterol over a lifetime will result in an approximate 15 % lower incidence of ASCVD. This lower risk theoretically should be achieved by minimizing dietary cholesterol. Therefore, a low-cholesterol intake should be one component of diet for prevention of atherosclerotic disease. An even greater risk reduction is possible by adding plant sterols or stanols that block cholesterol absorption [17••]. Not only is it important to keep dietary intakes of cholesterol low, but consideration should be given to enhancing serum cholesterol reduction by inhibiting the absorption of cholesterol. For instance, when maximal lifestyle intervention is employed, consideration should be given to using ezetimibe as an additional cholesterol-lowering agent.

Compliance with Ethical Standards

Conflict of Interest Scott M. Grundy declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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