Cholesterol: where science and public health policy intersect

Amy M Brownawell and Michael C Falk

Current US guidelines for cholesterol recommend limiting intake of cholesterol to <300 mg/day for the general population and <200 mg/day for individuals with elevated low-density lipoprotein cholesterol. These recommendations, however, are at odds with international (e.g., Canada, United Kingdom, and Australia) guidelines that provide no specific numerical recommendation, but instead recommend reducing total fat intake and shifting fat consumption away from saturated and trans fats to unsaturated fats. A conference was held on December 3, 2008, to evaluate the data supporting current US nutrition policy recommendations to limit dietary cholesterol and analyze the consequences of this policy on the eating patterns and health of the US population. This review is a summary of the information and perspectives presented by conference speakers and discussed by conference participants.

INTRODUCTION

Current US dietary policy is centered on the tenet that dietary cholesterol intake can alter coronary heart disease (CHD) risk. US guidelines for cholesterol recommend limiting intake of cholesterol to <300 mg/day for the general population and <200 mg/day for individuals with elevated low-density lipoprotein cholesterol. However, since US dietary cholesterol guidelines were initiated, additional research has demonstrated that the dynamics of cholesterol homeostasis and of the development of CHD are extremely complex and multifactorial.[1] In addition to cholesterol, other dietary components affect blood cholesterol levels and CHD risk. These include the intakes of saturated fat, trans fatty acids, and omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), as well as the consumption of fruits, vegetables, legumes, and other sources of soluble dietary fiber. Current US dietary recommendations for cholesterol differ from international guidelines that provide no specific numerical recommendation, but instead recommend reducing total fat intake and shifting fat consumption away from saturated and trans fats to unsaturated fats.

A conference (Cholesterol Conference) organized by the Life Sciences Research Organization (LSRO) was held on December 3, 2008, to evaluate the data supporting current US nutrition policy recommendations to limit dietary cholesterol and analyze the consequences of this policy on the eating patterns and health of the US population. The present report provides a summary of the information and perspectives expressed by Cholesterol Conference speakers and discussed by Cholesterol Conference participants. It is not intended to be a comprehensive review of cholesterol policy. In addition to a discussion of the basis of US dietary cholesterol guidelines and contrasts with international cholesterol guidelines, information is presented about how current US guidelines influence US government-sponsored food programs, food choices, and the adoption of non-US dietary patterns that likely contain differing quantities of potentially important food components. Scientific data discussed and cited by the speakers have been included in this report as well as additional citations required to appropriately convey the information included in the speakers' presentations. Conclusions and comments expressed by Cholesterol Conference participants are

Affiliation: *AM Brownawell* and *MC Falk* are with the Life Sciences Research Organization, Bethesda, Maryland, USA.

Correspondence: *AM Brownawell*, Life Sciences Research Organization, 9650 Rockville Pike, Bethesda, MD 20814-3998, USA. E-mail: brownawella@lsro.org, Phone: +1-301-634-7030, Fax: +1-301-634-7876.

Key words: blood cholesterol, choline, coronary heart disease, dietary cholesterol, Mediterranean diet, omega-3 polyunsaturated fatty acids

doi:10.1111/j.1753-4887.2010.00294.x

summarized in the final section (Conclusion) of this review.

THE LIPID HYPOTHESIS

The lipid hypothesis asserts that hyperlipidemia is a major causative factor in atherosclerosis and coronary heart disease (CHD); other factors such as cigarette smoking, hypertension, diabetes, obesity, physical inactivity, inflammation, and genetics also contribute. Importantly, this hypothesis maintains that blood lipid levels, not dietary lipids, are the causative factor. Blood lipid levels can be measured easily and include measurement of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride levels. The lipid hypothesis states that evaluation of these levels can then be used to determine the risk for CHD and atherosclerosis and that altering blood lipid levels will significantly reduce the burden of disease and its clinical consequences.[2] Results from both the Framingham Heart Study and the Multiple Risk Factor Intervention Trial demonstrated that elevated total plasma cholesterol levels are directly proportional to increased risk of CHD.[3,4] In support of this evidence, randomized clinical trials of plasma cholesterol-lowering therapies (i.e., statin drugs) have proven effective in reducing both blood cholesterol levels and adverse cardiovascular outcomes.[5] Nevertheless, dietary modification remains the first-line therapy for those at risk for CHD, despite the fact that individual response to dietary manipulation is variable. As such, it is essential to distinguish and understand the relationship between dietary lipids and blood lipids and their effect on cardiovascular events. This relationship is essential for rational policy decisions and effective communication with the general public.[2]

BASIS OF CURRENT CHOLESTEROL RECOMMENDATIONS

Current US dietary policy is centered on the tenet that dietary cholesterol intake can alter CHD risk. The National Health and Nutrition Examination Survey (NHANES) 2005–2006 reported mean intakes of 278 mg cholesterol/day in the United States; adult females averaged 237 mg cholesterol/day compared to 358 mg cholesterol/day for adult males.[6]

US guidelines

There are three primary US authoritative guidelines for the intake of cholesterol: 1) the percentage of daily value required by the Food and Drug Administration (FDA) for food labels[7]; 2) *Nutrition and Your Health: Dietary Guidelines for Americans* issued jointly by the Department of Health and Human Services (DHHS) and the US Department of Agriculture (USDA)[8]; and 3) The Institute of Medicine's (IOM) dietary reference intakes.[9]

Daily values. The most pervasive message about dietary levels of cholesterol is transmitted via the food label. The Nutrition Labeling and Education Act of 1990 required manufacturers to provide nutrition labeling for most foods. The amount of cholesterol in a food product, expressed in milligrams per serving, must be included on the Nutrition Facts panel, and that amount must also be listed as a percentage of the daily value.[7] For cholesterol, the daily reference value is the uppermost limit that the FDA considers desirable in a healthy diet. The FDA set this value at 300 mg to be consistent with recommendations issued in the 1989 National Research Council's report *Diet and Health: Implications for Reducing Chronic Disease Risk*.[10] The Division of Nutrition Programs and Labeling at the FDA is now considering changing the food label; specifically, it is considering whether the current cholesterol cut-off should remain or whether food composition data, menu modeling, and data from dietary surveys might indicate that a new level could be established. As recommended by the IOM, this level would be the lowest possible amount that could be achieved in nutritionally adequate diets. Based on food-modeling exercises, the USDA[11] calculated that the lowest recommended cholesterol intake in a lacto-ovo vegetarian diet that meets essential nutrient recommendations would be approximately 160–212 mg/day, depending on the level of each individual's energy requirements.

Nutrition and your health: dietary guidelines for Americans. In its report, *Nutrition and Your Health: Dietary Guidelines for Americans*,[12] the Committee on Diet and Health (DH Committee) recommends that individuals in the general US population limit their intake of cholesterol to less than 300 mg/day and that those with elevated LDL-C limit their intake to less than 200 mg/day. The DH Committee considered a variety of information types and sources in forming this recommendation.

Evidence from early feeding studies demonstrated that as cholesterol intake increases from approximately 15 mg/day to 600 mg/day, total blood cholesterol increases overall, but the response is variable for any one individual.[13] At intakes greater than 600 mg/day (up to 3,600 mg/day), however, blood values may not respond to further increases in dietary cholesterol.[10] Next, the DH Committee analyzed prediction equations developed by Hegsted et al.[14,15] from early feeding study data. Using Hegsted's equation, each 100 mg increase in dietary cholesterol leads to an estimated 4 mg/dL increase in total blood cholesterol. Based on a feeding study by McNamara et al.,[16] the DH Committee estimated that, at least one-third of the population should respond substantially to increased dietary cholesterol by raising blood TC levels. Of the responders, the DH Committee estimated that about 20% should respond to dietary cholesterol changes (or approximately 7% of the total population was expected to respond). However, no practical method of identifying responders exists. The DH Committee also reviewed data published by Katan[17–19] and surmised that 90% of the changes in TC were due to changes in LDL-C. In 1988, The National Cholesterol Education Program (NCEP) identified LDL-C as the key index for clinical decision making about cholesterol-lowing therapy and as the specific target for therapy.[20] The DH Committee concluded that of the lipoprotein fractions, LDL-C had the strongest and most consistent relationship to cardiovascular disease (CVD) risk. The DH Committee estimated that "on average, 200 mg of dietary cholesterol per 1,000 kcal elevates LDL-C by 8–10 mg/dL." No references were cited, but this range is consistent with Hegsted's equation assuming that most of the change in blood cholesterol is in the LDL-C fraction.[21–23]

The next evidence considered by the DH Committee came from a, then unpublished, meta-analysis authored by Sir Richard Peto, which has since published.[24] By analyzing 18 published and 2 unpublished randomized trials of drug, diet, or drug plus diet to lower blood cholesterol for prevention or treatment, a 10% reduction in TC was found to be associated with an average reduction of CHD if the trial lasted for 4 years (i.e., a 1% reduction in TC is associated with an average 1.6% reduction in CHD risk). In making its final recommendations, the DH Committee rounded Peto's 1:1.6 ratio between change in total blood cholesterol and CHD risk to 1:2. The DH Committee also assumed that if the amount of dietary cholesterol decreased, blood cholesterol would decrease at a similar magnitude. This assumption was made even though feeding studies used to define the relationship between diet and blood cholesterol added cholesterol to the subjects' background diet. Despite the fact that the relationship defined by Peto was based on a combination of treatments that included dietary changes, smoking cessation, and lipid-lowering drugs, the DH Committee anticipated that reducing only dietary cholesterol would lower blood cholesterol, and thus reduce CHD risk.

Given the evidence to date, the DH Committee adopted a public health strategy/population approach designed to shift the distribution of blood cholesterol concentrations in the entire population into a lower range. It was accepted that for any individual, response may be minimal, but that approximately one-third of the population would benefit and lower their blood cholesterol level. The DH Committee projected that for individuals with blood TC levels in the range of 250–300 mg/dL, each 1% reduction in TC would decrease CHD rates by approximately 2% after 5–7 years. The DH Committee preferred to recommend a numerical value as a threshold for cholesterol intake because a specific target for dietary modification would be less susceptible to misinterpretation when translated into food choices rather than just advising people to eat less cholesterol.[10] Therefore, the recommendation was made that adults and children over the age of 2 years limit their intake of dietary cholesterol to 300 mg/day.

Dietary reference intake. The Food and Nutrition Board of the IOM reviewed data regarding the association of dietary cholesterol and cardiovascular disease. It primarily examined three types of evidence: animal models, epidemiological data, and the effects of dietary cholesterol on blood lipoproteins. IOM used the effects of dietary cholesterol on blood lipoproteins as the basis for its conclusions regarding the association of dietary cholesterol and CVD.[9] The IOM cited Hegsted et al.[25] as suggesting that changes in LDL-C "roughly parallel" and "approximate" changes in blood TC. The IOM further stated that approximately 80% of the increase in blood TC in response to changes in dietary cholesterol is in the LDL-C fraction, an estimate that, although not specifically cited, is consistent with the IOM's discussion of the report of Clark et al.[24]

The IOM reviewed 50 clinical studies examining the lipoprotein response to adding up to 4,800 mg/day cholesterol onto baseline diets and concluded there was a positive linear trend between cholesterol intake and blood cholesterol concentration.[9] Most studies reviewed by the IOM tested responses at extremely high cholesterol intakes; less than one-half included measures of blood cholesterol associated with changing dietary cholesterol by 500 mg/day or less. According to the IOM,[9] none of the studies it reviewed examined the effects of very small incremental changes in dietary cholesterol in sufficiently large samples to permit statistical treatment of the data to define the lowest level of cholesterol intake shown to increase TC or LDL-C concentration (i.e., the lowest-observed-adverse-effect level). The IOM considered that increasing dietary cholesterol would increase blood TC (and LDL-C) "which would be predicted to result in increased risk for CHD."[9] The IOM cited Wells and Bronte-Stewart,[26] who indicated that as little as 17 mg/day of dietary cholesterol might raise TC, although it was acknowledged that only three subjects were examined. The IOM suggested that substantially greater amounts (i.e., 100 mg/day) could lead to an increase in LDL-C that would be associated with a 1–2% increase in CHD. Using the risk assessment model, the

IOM was unable to devise a tolerable upper intake level for cholesterol because neither a no-observed-adverse-effect level nor a lowest-observed-adverse-effect level could be determined. The IOM reasoned that "any incremental increase in cholesterol intake increases CHD risk" and recommended that intake of dietary cholesterol be as low as possible.[9]

International guidelines

In addition to cholesterol, other dietary intakes affect blood cholesterol levels and CHD risk. These include saturated fat, trans fatty acid, and omega-3 and omega-6 polyunsaturated fatty acid (PUFA) intakes, as well as the consumption of fruits, vegetables, legumes, and sources of soluble dietary fiber. In contrast with US policy, the guidelines of Canada[27]; Australia[28]; the Department of Health, London[29]; the Scottish Office[30]; and the World Health Organization[31] place no limit on dietary cholesterol. Instead, they recommend reducing total fat intake and shifting fat consumption away from saturated and trans fats to unsaturated fats. Limiting saturated fat is considered the primary objective by these agencies because they believe it has a stronger association with blood cholesterol levels and CHD risk than dietary cholesterol.[32,33] Furthermore, both total fat and saturated fat intake are significantly correlated with dietary cholesterol. The rationale provided by the European Heart Network[34] is typical of countries that have chosen to omit a specific goal for dietary cholesterol: 1) cholesterol in the diet increases LDL-C levels in the blood, but to a much lesser extent than saturated fat, and the response varies widely among individuals; 2) foods high in cholesterol are usually also high in saturated fat, so reducing intakes of saturated fat should lead to an accompanying fall in cholesterol intakes; and 3) although there is some evidence of a specific relationship between cholesterol consumption and CVD,[35] no population goal is included because dietary cholesterol intakes in Europe tend to be within the usual population goal of less than 300 mg/day specified by expert groups and consensus documents.

US FOOD PROGRAMS AND CONSUMER FOOD CHOICES

The US government administers numerous nutrition programs including the Supplemental Nutrition Assistance Program (SNAP), the Supplemental Feeding Program for Women, Infants, and Children (WIC), the National School Lunch Program (NSLP) and School Breakfast Program (SBP), Farmer's Market Nutrition Programs, and the Nutrition Services Incentive Program for the elderly. In June 2009, the SNAP program provided food assistance to a record 35 million Americans.[36] During that same month, over 9 million women and children participated in WIC programs.[37] During fiscal year 2008, over 40 million children per day participated in the SBP and NSLP.[38,39] Therefore, government-sponsored nutrition programs that are based on nutrition policy, specifically the 2005 *Dietary Guidelines for Americans*,[12] shape the diets of a substantial and growing segment of the US population.

SNAP, NSLP, SBP, and WIC

The foods offered under SNAP are based on the Thrifty Food Plan, which combines *What We Eat in America* data (from NHANES) and food prices from the Economic Research Service. The 2005 Thrifty Food Plan Dietary Standard for cholesterol was ≤300 mg/day. The participants meeting this recommended intake of cholesterol according to NHANES 1999–2004 was greatest for WIC children (ages 1–4 years) and NSLP children and young adults (ages 5–17 years). Adult SNAP program participants had the poorest compliance; approximately 30% consumed >300 mg/day. These individuals represent the lowest income levels, with many earning <70% of poverty-level income.

The NSLP and SBP hold the potential to provide US schoolchildren with access to nutritious, low-cost meals to support their growth, development, and health. The USDA has contracted with the Food and Nutrition Board of the IOM of the National Academies of Science to review and assess the food and nutritional needs of school-aged children in the US using the 2005 *Dietary Guidelines for Americans*[12] and IOM's Dietary Reference Intakes and to use that review as a basis for recommendations to revise NSLP and SBP Nutrition Standards and Meal Requirements. In its 2009 report, *School Meals: Building Blocks for Healthy Children*,[40] the IOM committee recommends that the USDA adopt standards for menu planning, including increasing the amount and variety of fruits, vegetables, and whole grains; setting a minimum and maximum level of calories; and focusing more on reducing saturated fat and sodium. It is expected that FNS will now update NSLP and SBP regulations and incorporate suggested changes into the nutrition standards and meal patterns of the programs. These changes come at a time of intense public scrutiny of school meals. Many cities and school districts are independently working to improve the quality of student meals and to re-emphasize good eating habits. Initiatives include the planting and tending of school gardens by students, partnerships with local farmers to provide fruits, vegetables, and educational opportunities, and the teaching of basic cooking skills.

WIC food packages were recently revised and the interim final rule revisions largely reflect recommendations made by the IOM in its report, *WIC Food Packages: Time for a Change*.[41] The maximum monthly allowances for fresh shell eggs were reduced from 2 or 2.5 dozen to 1 dozen for children (Food Package IV) and pregnant, non-breastfeeding, partially breastfeeding, and fully breastfeeding women enrolled in Food Packages V, VI, and VII. The quantities of milk in each package were reduced as well. The revised maximum daily allowance of eggs is consistent with recommendations of the IOM and the *Dietary Guidelines for Americans* to reduce cholesterol. Beans and peanut butter are other protein options included in Food Packages IV–VII, with the addition of canned fish for fully breastfeeding women (Package VII). The changes were made to provide more balanced nutrient intakes (including providing more fresh fruits and vegetables) and a wider variety of foods with greater WIC participant choice while maintaining cost neutrality.[41]

US consumers and food choices

Although dietary therapy remains the first line of treatment for patients with elevated blood cholesterol, a cross-sectional study of 13,777 postmenopausal women with self-reported hypercholesterolemia requiring statin drug therapy reported that only 20% reported total fat, saturated fat, and dietary cholesterol consumption consistent with National Cholesterol Education Program Step II dietary goals (i.e., $\leq$30% calories from fat, <7% of calories from saturated fat, and daily dietary cholesterol $\leq$200 mg).[42]

According to a 2007 survey by the International Food Information Council (IFIC), the top health concern of US consumers is cardiovascular health, defined as heart disease, hypertension, blood cholesterol levels, and stroke.[43] Despite years of advising Americans to minimize cholesterol intake, cholesterol content has continued to decline on consumers' nutrition priority list. In 1997, 20% were concerned about their dietary intake of cholesterol; by 2004, only 14% of consumers were concerned.[44] In 2004, concern over dietary fat, carbohydrate, sugar, calories, and sodium content ranked higher than cholesterol.[44] Sixty-seven percent of Americans are concerned with the amount of fat they consume, and 69% say they are concerned with the type of fat they consume.[45] Consumers continue to pay particular attention to trans fat. Awareness of trans fat remains high at 90%, and 64% say they are trying to reduce trans fat in their diet.[45] However, consumers' understanding of healthful fats, such as unsaturated fats, still appears to be lacking.[45] These marketing studies suggest that US consumers are not basing food choices on dietary cholesterol content, but they may desire additional guidance regarding dietary fats and healthier food choices.[45]

NON-US DIETARY PATTERNS AND POTENTIALLY IMPORTANT FOOD COMPONENTS

Cholesterol Conference speakers and participants discussed the merits of the Mediterranean diet alternative to current US dietary patterns as well as two food components that could prove beneficial if better integrated into US diets, namely omega-3 PUFAs and choline.

Mediterranean diets

Although the healthiest mix of dietary fats and other foods is still debated, a Mediterranean diet, representing the traditional food patterns of Greece and southern Italy,[46] has been reported to be a model of healthy eating for its contribution to a favorable health status and better quality of life.[47,48] As compared with many Western diets, Mediterranean diets are generally characterized by higher intakes of fruits and vegetables, breads, cereals, fish, nuts, seeds, and legumes, with olive oil as the principal source of fat, less red meat consumption, and daily intake of moderate amounts of alcohol. In Greece, cultured dairy products (i.e., yogurt) are typically consumed. The Mediterranean dietary pattern compared to the diet consumed in the US has a higher polyunsaturated/saturated fat ratio, less sodium, and a higher content of dietary fiber, vitamins, and minerals, particularly more vitamin A, vitamin C, folic acid, and magnesium.

The Lyon Diet Health Study illustrates the potential benefits of a diet emphasizing fruits, vegetables, breads, cereals, and fish, but it also implicates risk factors beyond plasma lipids and lipoproteins.[49] The Lyon Diet Heart Study was a prospective, randomized controlled-trial that tested the effect of a Mediterranean diet on coronary recurrence rates after a first myocardial infarction. Subjects in the experimental group were instructed by a research cardiologist and dietitian to adopt a Mediterranean-type diet. This diet emphasized increasing intakes of bread, cereals, fresh fruit, vegetables, legumes, and fish, while reducing intakes of delicatessen foods and meat (beef and pork replaced with poultry), and excluding butter and cream, which were replaced with an experimental canola oil-based margarine rich in oleic and α-linolenic acids.[50] The oils recommended for salad and food preparation were canola and olive oils exclusively. Moderate red wine consumption was allowed at meals. In contrast, control subjects were expected to follow their attending physicians' dietary advice without counseling by a dietician and a diet close to the Step 1 diet of the American Heart Association (characterized as 30% of total energy as fats; 10% saturated, 10% monounsaturated, and 10% polyunsaturated, with a cholesterol intake <300 mg/day).[50] After 46 months of follow-up, 204 control and 219 experimental subjects (93% of original subjects) participated in the final examination. Despite similar coronary risk factor profiles (i.e., plasma lipids and lipoproteins, systolic and diastolic blood pressure, body mass index, and smoking status), subjects following the Mediterranean-type diet had a 50–70% lower risk of recurrent heart disease, as measured by three outcome categories: 1) cardiac death and non-fatal heart attack; 2) the preceding plus unstable angina, stroke, heart failure, and pulmonary or peripheral embolism; and 3) all of these measures plus events that required hospitalization.[49]

Omega-3 polyunsaturated fatty acids

Specific components of the non-Western diets have also been scrutinized extensively. The omega-3 PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) provided by fish and other foods may beneficially affect cardiovascular health. EPA and DHA can lower plasma triglyceride levels,[51,52] and evidence is mounting, from observational studies and clinical trials, that supplementation with EPA and DHA leads to a reduced risk of CHD and sudden cardiac death.[53] Two studies, INTERLIPID[54] and INTERMAP,[55] evaluated the eating patterns of Japanese subjects in Japan and compared them to first-generation Japanese subjects residing in the United States or the United Kingdom. In Japan, those aged 40 years and older consume more than 100 g fish/day,[55] and whole egg intake has increased in recent decades, reflecting the encouragement of egg consumption to improve protein nutrition.[54] Thus, the Japanese diet is typically high in both omega-3 PUFAs and cholesterol (446 mg/day among men and 359 mg/day among women), yet it is lower in total and saturated fat and has a higher polyunsaturated/saturated fat ratio than diets in Western industrialized countries.[54,55] The Japanese subjects in Japan had lower body weight, LDL-C levels, and risk of CHD than comparable US and UK populations, but they also had a higher risk of stroke.[54,55]

Choline

The exclusion or strict limitations of some foods in the US diet that are considered to be high in cholesterol have raised concerns that certain nutritional deficiencies could be created. Many individuals limit or avoid egg yolks and liver (beef and chicken) due to high dietary cholesterol levels, even though both are rich in the nutrient choline. Most excellent-to-good sources of choline are animal in origin and are often limited (e.g., liver, pork, beef, chicken, fish), although broccoli, brussels sprouts, and soybeans are also good sources.[56] Choline is required to maintain the structural integrity and signaling functions of cell membranes for neurotransmission and for the transport of lipids. Choline and its derivative, betaine, are also methyl donors involved in the metabolism of vitamin B_{12}, folate, and methionine. Choline is essential during human fetal development, where it plays critical roles in neural tube development, stem cell proliferation and apoptosis, brain and spinal cord structure and function, and lifelong memory function.[57] Recently, Shaw et al.[58] investigated the role of nutrients involved in one-carbon metabolism in neural tube defects (NTDs) and structural malformations. Over 180,000 mid-pregnancy (week 15–18 of pregnancy) blood samples were obtained from a population of folate-fortified women in California between 2003 and 2005. Of this sample, there were 80 NTD-affected pregnancies, 31 of which had spina bifida and 49 of which had anencephaly. Controls ($N = 409$) were randomly selected from a group of women with pregnancies not affected by NTDs. Serum samples were analyzed for methylmalonic acid, total homocysteine, cysteine, methionine, total choline, betaine, cystathionine, pyridoxal phosphate, pyridoxal, pyridoxic acid, folate, cobalamin, riboflavin, and creatinine. The most striking finding was a significantly lower mean total choline level in cases than controls (standard deviation at least one-half). After comparing serum levels by quartile, total choline still yielded the strongest associations. Odds ratios were 1.8 for women in the lowest quartile of total choline, versus 0.4 in the highest quartile ($P = 0.0003$). No association was observed between serum folate levels and NTD incidence, which was a result expected by the authors because the population was exposed to a folic acid-fortified food supply. The authors speculate that the choline-NTD association may be linked to the interrelationship between choline, folate, and methionine in one-carbon metabolism and that a deficiency in choline might affect folate and homocysteine metabolism.[58]

Choline is a required nutrient, and the IOM of the National Academy of Sciences set an adequate intake level for choline of 550 mg/day for men and 425 mg/day for women.[59] Choline can be synthesized de novo, where its production is estrogen responsive, but recent data suggest de novo synthesis is inadequate to meet the requirements of humans.[60] When deprived of adequate dietary choline, humans develop a deficiency syndrome characterized by fat deposition within the liver that can progress to liver or muscle necrosis.[60] Polymorphisms of the gene encoding phosphatidylethanolamine N-methyltransferase (PEMT), a protein that catalyzes de novo synthesis of phosphatidylcholine in the liver that can then be converted to choline, may confer susceptibility to nonalcoholic fatty liver disease.[61] However, fatty liver is often non-symptomatic and may only be detectable by biopsy or magnetic resonance imaging-based techniques.[62] As such, the prevalence of any choline deficiency syndrome in the US population is unknown.

Using data collected in 24-h dietary recalls during NHANES 2003–2004 and the USDA's database for the choline content of common foods,[56] Jensen et al.[63] determined that the prevalence of inadequate choline intake is common for all age groups except young children (1–4 years and 5–8 years). The usual mean US intake is substantially below the adequate intake (AI) levels for choline established by the IOM for older children (9–13 years and 14–18 years), adult males, and adult females, including pregnant women.[63] If choline intake in the United States was increased by 125 mg/day, the equivalent of one large egg, a 4-oz serving of meat, or a 1-oz serving of liver, the percentage of adults with estimated usual intakes of total choline greater than the AI would increase substantially. However, egg yolks and liver have relatively high cholesterol contents and individuals with high choline intakes have high cholesterol intakes,[64] so US dietary recommendations to increase choline intake would also need to consider the effect of increased cholesterol intake.

Consumption of foods containing choline and its derivative betaine may have other health benefits, including protective cardiovascular effects and breast cancer prevention. Lower levels of inflammatory markers for CVD risk were associated with greater daily dietary intake of choline and betaine by the more than 3,000 Greek citizens enrolled in the cross-sectional ATTICA study.[62] Individuals who consumed >310 mg/day choline had significantly lower C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-α (TNF-α) concentrations than did persons who consumed <250 mg/day. ATTICA choline intake levels, however, were still well below the US AI level of 425 mg/day for women and 550 mg/day for men set by the IOM.[65] One important consideration is that the traditional Greek diet is more plant-based than the typical American diet, resulting in greater betaine intake. ATTICA participants who consumed >360 mg betaine/day had lower concentrations of homocysteine, CRP, and TNF-α than did those who consumed <260 mg/day.[66] In the United States, the average betaine intake is 110–190 mg/day.[67–69] Data suggests that women who consume higher amounts of dietary choline appear to be at lower risk of developing breast cancer. A case-controlled study examined the diets of 3,000 women who were enrolled in the Long Island Breast Cancer Study Project and found that the risk of developing breast cancer was 24% lower among women with the highest intake of choline (>455 mg/day) compared to women with the lowest intake (<196 mg/day).[70] More recently, higher intakes of choline and betaine were shown to reduce breast cancer mortality in the same study group.[71]

CONCLUSION

Elevated TC and LDL-C levels negatively impact cardiovascular health. The effect of dietary cholesterol on cardiovascular health is less definitive, especially because fat intake in various forms is interrelated. Increased consumption of omega-3 PUFAs may reduce triglyceride levels; substitution of polyunsaturated fats for saturated fats may reduce TC levels; and reduced consumption of trans fats may help to increase HDL-C levels. US recommendations to limit intake of cholesterol to <300 mg/day are based on the following: 1) studies that show inter-individual response to changes in cholesterol; 2) early feeding studies that added (not removed) cholesterol to the diet; 3) a majority of studies that examined very high cholesterol intake (>500 mg/day); and 4) meta-analyses that linked blood cholesterol levels and CHD based on changes not only in the diet, but also smoking cessation and lipid-lowering drugs. In contrast, many international guidelines recommend reducing saturated fat intake in order to reduce cholesterol intake without setting specific numerical targets. It was the opinion of some Cholesterol Conference participants that recommendations based on attempts to isolate the effects of dietary cholesterol from complex dietary patterns and extrapolations from calculated models are flawed and may lead to unintended negative consequences. It is, perhaps, more effective to recommend broader dietary patterns where there is more direct evidence for an effect on health outcomes.

Cholesterol Conference participants addressed the point that dietary recommendations stated in scientific language are potentially confusing to the general US population. Numerical recommendations (e.g., ≤300 mg cholesterol/day) might cause individuals to focus intently on one component, leading to the strict limitation or exclusion of certain foods. Messages based on FDA, USDA/DHHS, and/or IOM recommendations but expressed instead as directives to eat a greater variety of foods (i.e., "a rainbow on your plate" or "eat a rainbow every day"), increase fruit and vegetable consumption (e.g., "5 a day"), eat two servings of fish (preferably fatty fish) a week, choose polyunsaturated fats for cooking and food preparation, and eat fewer processed foods, may be more tangible guidelines for the US population. Improved access to healthy foods (i.e., the ability to use SNAP and WIC benefits at farmers' markets), and more choices for those receiving food assistance may also improve food quality. Further education may also broaden awareness among US consumers that additional benefits often accompany better eating habits, such as greater satiety and, perhaps, weight loss; improvement of medical conditions, such as diabetes and high blood pressure; and greater energy levels with potential quality-of-life improvements.

Acknowledgments

Funding. The LSRO-organized Cholesterol Conference was held on December 3, 2008, in Bethesda, Maryland. This conference followed the publication of the LSRO report *The Scientific Evidence and Approach Taken to Establish Guidelines for Cholesterol Intake in Australia, Canada, the United Kingdom, and the United States*[72] that served as an organizing principle. Funding from the Egg Nutrition Center supported both the Cholesterol Conference and the manuscript. Conference cosponsors included the USDA Agricultural Research Service and the American Society for Nutrition. Cholesterol Conference details and speaker presentations can be found at http://www.LSRO.org.

Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. Jones PJ. Dietary cholesterol and the risk of cardiovascular disease in patients: a review of the Harvard Egg Study and other data. Int J Clin Pract Suppl. 2009;63:1–36.
2. Steinberg D. *The Cholesterol Wars*. San Diego: Academic Press/Elsevier; 2007.
3. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA. 1986;256:2823–2828.
4. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. Ann Intern Med. 1979;90:85–91.
5. Choy PC, Siow YL, Mymin D, O K. Lipids and atherosclerosis. Biochem Cell Biol. 2004;82:212–224.
6. USDA Agricultural Research Service. Tables of nutrient intakes from food: mean amounts consumed per individual, one day, 2005–2006. 2008; Available at: http://www.ars.usda.gov/SP2UserFiles/Place/12355000/pdf/0506/Table_1_NIF_05.pdf. Accessed 6 April 2009.
7. US Food and Drug Administration. *The Food Label*. US Food and Drug Administration 1999; Available at: http://www.fda.gov/opacom/backgrounders/foodlabel/newlabel.html. Accessed 10 March 2006.
8. US Department of Agriculture and US Department of Health and Human Services. *Nutrition and Your Health: Dietary Guidelines for Americans, 2000*. 2000; Available at: http://www.health.gov/dietaryguidelines/dga2000/DIETGD.PDF. Accessed 28 April 2010.
9. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: The National Academies Press; 2005.
10. National Research Council. *Diet and Health. Implications for Reducing Chronic Disease Risk*. Washington, DC: National Academies Press; 1989.
11. US Department of Agriculture. Report on the varying levels of fats in the food patterns. 2005; Available at: http://www.health.gov/dietaryguidelines/dga2005/report/HTML/G2_Analyses.htm#lactoovo. Accessed 7 July 2006.
12. US Department of Health and Human Services, US Department of Agriculture. *Dietary Guidelines for Americans*. 2005; Available at: http://www.healthierus.gov/dietaryguidelines/. Accessed 21 March 2006.
13. Beveridge JM, Connell, WF, Mayer, GA, Haust, HL. The response of man to dietary cholesterol. J Nutr. 1960;71:61–65.
14. Hegsted DM. Serum-cholesterol response to dietary cholesterol: a re-evaluation. Am J Clin Nutr. 1986;44:299–305.
15. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr. 1965;17:281–295.
16. McNamara DJ. Effects of fat-modified diets on cholesterol and lipoprotein metabolism. Annu Rev Nutr. 1987;7:273–290.
17. Katan MB, Beynen AC, de Vries JH, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol. 1986;123:221–234.
18. Katan MB, van Gastel AC, de Rover CM, van Montfort MA, Knuiman JT. Differences in individual responsiveness of serum cholesterol to fat-modified diets in man. Eur J Clin Invest. 1988;18:644–647.
19. Katan MB, Berns MA, Glatz JF, Knuiman JT, Nobels A, de Vries JH. Congruence of individual responsiveness to dietary cholesterol and to saturated fat in humans. J Lipid Res. 1988;29:883–892.
20. National Cholesterol Education Program. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2001; Available at: http://www.guideline.gov. Accessed 6 February 2006.
21. National Institutes of Health. Lowering blood cholesterol to prevent heart disease. NIH Consensus Statement. 1984; Available at: http://consensus.nih.gov/1984/1984Cholesterol047html.htm. Accessed 6 Febrary 2006.
22. National Institutes of Health. Lowering blood cholesterol to prevent heart disease. NIH Consensus Development Conference Statement. Nutr Rev. 1985;43:283–291.
23. Office of Medical Applications of Research of the National Institutes of Health. Consensus conference. Lowering blood cholesterol to prevent heart disease. JAMA. 1985;253:2080–2086.
24. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ. 1997;314:112–117.
25. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serum lipids: an evaluation of the experimental data. Am J Clin Nutr. 1993;57:875–883.
26. Wells VM, Bronte-Stewart B. Egg yolk and serum cholesterol levels: importance of dietary cholesterol intake. Br Med J. 1963;5330:577–581.
27. Health Canada. Eating well with Canada's Food Guide. 2007; Available at: http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php. Accessed 7 April 2009.
28. National Health and Medical Research Council. Nutrient reference values for Australia and New Zealand. Including recommended dietary intakes. 2006; Available at: http://www.nhmrc.gov.au/publications/_files/n35.pdf. Accessed 26 June 2006.
29. Department of Health: London. Choosing a better diet: a food and health action plan. 2005; Available at: http://www.dh.gov.uk/assetRoot/04/10/57/09/04105709.pdf. Accessed 19 June 2006.

30. The Scottish Office. Report of the Working Group on Monitoring Scottish Dietary Targets. 2005; Available at: http://www.scotland.gov.uk/Publications/2005/01/20577/50669. Accessed 31 July 2006.
31. World Health Organization. Global strategy on diet, physical activity and health [Fifty-Seventh World Health Assembly. Provisional agenda item 12.6]. 2004; Available at: http://www.who.int/gb/ebwha/pdf_files/WHA57/A57-9-en.pdf. Accessed 19 June 2006.
32. Hooper L, Summerbell CD, Higgins JP, et al. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev. 2001;CD002137.
33. Hooper L, Summerbell CD, Higgins JP, et al. Dietary fat intake and prevention of cardiovascular disease: systematic review. BMJ. 2001;322:757–763.
34. European Heart Network. Food, nutrition and cardiovascular disease prevention in the European region: Challenges for the new millennium. 2002 Available at: http://www.ehnheart.org/files/millenium-143851A.pdf. Accessed 26 June 2006.
35. Weggemans RM, Zock PL, Katan MB. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. Am J Clin Nutr. 2001;73:885–891.
36. USDA Food and Nutrition Service. Supplemental Nutrition Assistance Program (Data as of 28 September 2009). 2009; Available at: http://www.fns.usda.gov/pd/34SNAPmonthly.htm. Accessed 30 September 2009.
37. USDA Food and Nutrition Service. Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) (Data as of 28 September 2009). 2009; Available at: http://www.fns.usda.gov/pd/37WIC_Monthly.htm. Accessed 30 September 2009.
38. USDA Food and Nutrition Service. National School Breakfast Program fact sheet. 2009; Available at: http://www.fns.usda.gov/cnd/breakfast/AboutBFast/SBPFactSheet.pdf. Accessed 30 September 2009.
39. USDA Food and Nutrition Service. National School Lunch Program fact sheet. 2009; Available at: http://www.fns.usda.gov/cnd/Lunch/AboutLunch/NSLPFactSheet.pdf. Accessed 30 September 2009.
40. Institute of Medicine. *School Meals: Building Blocks for Healthy Children*. Washington, DC: National Academies Press; 2009.
41. Committee to Review the WIC Food Packages. *WIC Food Packages: Time for A Change*. Washington, DC: National Academies Press; 2005.
42. Hsia J, Rodabough R, Rosal MC, et al. Cholesterol Education Program dietary and lifestyle guidelines among older women with self-reported hypercholesterolemia. The Women's Health Initiative. Am J Med. 2002;113:384–392.
43. International Food Information Council Foundation. 2007 Consumer Attitudes toward Functional Foods/Foods for Health – Executive Summary. 2007; Available at: http://www.ific.org/research/funcfoodsres07.cfm. Accessed 19 March 2009.
44. Food Marketing Institute. *Trends in The United States – Consumer Attitudes and the Supermarket, 2004*. Arlington, VA: Food Marketing Institute; 2004.
45. International Food Information Council Foundation. 2008 Food & Health Survey: Consumer Attitudes toward Food, Nutrition & Health. 2008 August 4; Available at: http://www.ific.org/research/foodandhealthsurvey.cfm. Accessed 19 March 2009.
46. Keys A, Aravanis C, Blackburn H, et al. *A Multivariate Analysis of Death and Coronary Heart Disease*. Cambridge, MA: Harvard University Press; 1980.
47. Willett WC, Sacks F, Trichopoulou A, et al. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr. 1995;61(Suppl):S1402–S1406.
48. Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. Nutr Rev. 2006;64(Suppl):S27–S47.
49. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation. 1999;99:779–785.
50. de Lorgeril M, Salen P, Martin JL, Monjaud I, Boucher P, Mamelle N. Mediterranean dietary pattern in a randomized trial: prolonged survival and possible reduced cancer rate. Arch Intern Med. 1998;158:1181–1187.
51. Berge RK, Madsen L, Vaagenes H, Tronstad KJ, Gottlicher M, Rustan AC. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. Biochem J. 1999;343(Pt 1):191–197.
52. Wong S, Nestel PJ. Eicosapentaenoic acid inhibits the secretion of triacylglycerol and of apoprotein B and the binding of LDL in Hep G2 cells. Atherosclerosis. 1987;64:139–146.
53. Mozaffarian D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. Am J Clin Nutr. 2008;87(Suppl):S1991–S1996.
54. Ueshima H, Okayama A, Saitoh S, et al. Differences in cardiovascular disease risk factors between Japanese in Japan and Japanese-Americans in Hawaii: the INTERLIPID study. J Hum Hypertens. 2003;17:631–639.
55. Zhou BF, Stamler J, Dennis B, et al. Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom, and United States in the late 1990s: the INTERMAP study. J Hum Hypertens. 2003;17:623–630.
56. Patterson KY, Bhagwat SA, Williams JR, et al. *USDA Database for the Choline Content of Common Foods. Release Two*. Beltsville, MD: US Department of Agriculture, Agricultural Research Service; 2008.
57. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. Annu Rev Nutr. 2006;26:229–250.
58. Shaw GM, Finnell RH, Blom HJ, et al. Choline and risk of neural tube defects in a folate-fortified population. Epidemiology. 2009;20:714–719.
59. Institute of Medicine. Choline. In: *Dietary Reference Intakes for Folate, Thiamin, Riboflavin, Niacin, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academies Press; 1998:390–422.
60. Fischer LM, daCosta KA, Kwock L, et al. Sex and menopausal status influence human dietary requirements for the nutrient choline. Am J Clin Nutr. 2007;85:1275–1285.
61. Song J, da Costa KA, Fischer LM, et al. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). FASEB J. 2005;19:1266–1271.
62. Cassidy FH, Yokoo T, Aganovic L, et al. Fatty liver disease: MR imaging techniques for the detection and quantification of liver steatosis. Radiographics. 2009;29:231–260.
63. Jensen HH. Choline intake and the effects of US cholesterol guidelines. [Presented at the Cholesterol Conference, Decem-

ber 3, 2008, Bethesda, Maryland]. 2008; Available at: http://www.lsro.org/csph/presentations.html. Acccessed 19 March 2009.
64. Konstantinova SV, Tell GS, Vollset SE, Ulvik A, Drevon CA, Ueland PM. Dietary patterns, food groups, and nutrients as predictors of plasma choline and betaine in middle-aged and elderly men and women. Am J Clin Nutr. 2008;88:1663–1669.
65. Institute of Medicine. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academies Press; 2000.
66. Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. Am J Clin Nutr. 2008; 87:424–430.
67. Cho E, Zeisel SH, Jacques P, et al. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. Am J Clin Nutr. 2006;83:905–911.
68. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Usual choline and betaine dietary intake and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. BMC Cardiovasc Disord. 2007;7:20.
69. Cho E, Willett WC, Colditz GA, et al. Dietary choline and betaine and the risk of distal colorectal adenoma in women. J Natl Cancer Inst. 2007;99:1224–1231.
70. Xu X, Gammon MD, Zeisel SH, et al. Choline metabolism and risk of breast cancer in a population-based study. FASEB J. 2008;22:2045–2052.
71. Xu X, Gammon MD, Zeisel SH, et al. High intakes of choline and betaine reduce breast cancer mortality in a population-based study. FASEB J. 2009;23:4022–4028.
72. Life Sciences Research Office. *The Scientific Evidence and Approach Taken to Establish Guidelines for Cholesterol Intake in Australia, Canada, the United Kingdom, and the United States*. Bethesda, MD: Life Sciences Research Office; 2006.