PART D: SCIENCE BASE

Section 4: Fats

INTRODUCTION

Fats supply energy and essential fatty acids, and serve as a carrier for the absorption of the fat-soluble vitamins A, D, E, and K, and carotenoids. Fats are a source of antioxidants and numerous bioactive compounds and serve as building blocks of membranes and play a key regulatory role in numerous biological functions. Dietary fat is found in foods derived from both plants and animals.

Fats are composed of triglycerides that consist of fatty acids and glycerol. Individual fatty acids have different biological effects ranging from modulating clinical markers of disease risk to regulating many intracellular biological mechanisms due to changes in intracellular signaling and gene expression (Clarke SD, 2004). Fatty acids modulate lipid metabolism and other physiological systems that affect risk factors for chronic diseases. Whether these effects on health outcomes are beneficial or harmful depend on the specific fatty acids and the mix of fatty acids in the diet and the body. Individual fatty acids are present in foods as mixtures. Different foods are rich sources of specific fatty acids.

Fatty acids are classified on the basis of chain length, degree of saturation (as defined by the number of double bonds in the molecule), and position of the first double bond from the methyl terminus. The fatty acid classes are:

- ***Saturated fatty acids*** **—**Saturated fatty acids have no double bonds. They primarily come from animal products such as meat and dairy products. In general, animal fats are solid at room temperature. *Stearic acid* is a saturated fatty acid that has different biological effects than other saturated fatty acids. Important food sources of stearic acid are beef, hydrogenated/partially hydrogenated vegetable oils, and chocolate.
- ***Monounsaturated fatty acids*** **—**Monounsaturated fatty acids (MUFAs) have one double bond. Plant sources that are rich in MUFAs include vegetable oils (e.g., canola oil, olive oil, high oleic safflower and sunflower oils) that are liquid at room temperature and nuts.
- ***Polyunsaturated fatty acids*****—**Polyunsaturated fatty acids (PUFAs) have two or more double bonds, and may be of two types, based on the position of the first double bond:
  —n-6 PUFAs. Linoleic acid, one of the n-6 fatty acids, is required but cannot be synthesized by humans and, therefore, is considered essential in the diet. A lack of dietary n-6 PUFAs is characterized by rough, scaly skin and dermatitis. Primary sources are liquid vegetable oils including soybean oil, corn oil, and safflower oil.
  —n-3 PUFAs. α-linolenic acid is an n-3 fatty acid that is required because it is not synthesized by humans and, therefore, is considered essential in the diet. A lack of α-linolenic acid in the diet can result in symptoms of a deficiency including scaly and hemorrhagic dermatitis, hemorrhagic folliculitis of the scalp, impaired wound healing, and growth retardation. It is obtained from plant sources including soybean oil, canola oil, walnuts, and flaxseed. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 fatty acids that are contained in all fish and

shellfish. Fish that naturally contain more oil (e.g., salmon, tuna, trout) (which are called fish high in n-3-fatty acids in this report) are higher in EPA and DHA than are lean fish (e.g., cod, haddock, flounder).

- **Trans *fatty acids*—***Trans*** fatty acids are unsaturated fatty acids that contain at least one double bond in the *trans* configuration. The partial hydrogenation of polyunsaturated oils causes isomerization of some of the remaining double bonds and migration of others, resulting in an increase in the *trans* fatty acid content and the hardening of the oil. Elaidic acid (t9-C18:1) is the predominant *trans* fatty acid found in processed fats. Sources of *trans* fatty acids include hydrogenated/partially hydrogenated vegetable oils that are used to make shortening and commercially prepared baked goods, snack foods, fried foods, and margarine. With respect to *trans* fatty acids, the descriptors "hydrogenated" and "partially hydrogenated" are used interchangeably but convey the presence of elaidic acid in the vegetable oil that has been subjected to the hydrogenation process. For the sake of accuracy, in oil that is fully hydrogenated (i.e., the unsaturated fatty acids have been converted to stearic acid), there are no *trans* unsaturated fatty acids. Thus, fats that are hydrogentated/partially hydrogentated have variable amounts of *trans* fatty acids depending on the extent of hydrogenation. *Trans* fatty acids also are present in foods that come from ruminant animals (e.g., cattle and sheep). Such foods include dairy products, beef, and lamb. The predominant naturally occurring *trans* fatty acid is *trans*-vaccenic acid (t11-C18:1). Conjugated linoleic acid (c9, t11-C18:2) is derived from vaccenic acid and is found to a lesser extent in foods from ruminant animals.
- ***Cholesterol*** is a sterol present in all animal tissues. Free cholesterol is a component of cell membranes and serves as a precursor for steroid hormones including estrogen, testosterone, aldosterone, and bile acids. Humans are able to synthesize sufficient cholesterol to meet biologic requirements, and there is no evidence for a dietary requirement for cholesterol.

The Dietary Guidelines Advisory Committee (the Committee) placed a strong focus on fats because of the substantial body of research linking different types of fats to blood lipid values and heart health. Lipids and lipoproteins in the blood historically have attracted much interest because of their functions in biological events that underlie the prevention and progression of cardiovascular disease (see Part B, "Introduction," for further information).

BLOOD LIPIDS

There are different types of lipids circulating in the blood; cholesterol and triglycerides have been most intensively studied because of the diverse mechanisms by which they modulate risk of cardiovascular disease. Cholesterol and triglycerides are packaged into lipoprotein particles for transport in the circulation. The composition and biological properties of the different lipoprotein fractions varies markedly. The predominant lipoprotein particles are: chylomicrons, very-low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

- Cholesterol is transported in the blood primarily by LDL, HDL, and VLDL. Chylomicrons transport dietary cholesterol absorbed from the intestine. Total serum

cholesterol is the sum amount of cholesterol found in lipoporoteins in the blood. A high total cholesterol concentration is a risk factor for coronary heart disease (CHD).

- Triglycerides are a naturally occurring ester of three fatty acids and glycerol. They are the chief constituent of fats and oils and commonly circulate in the blood in the form of lipoproteins, principally in chylomicrons and VLDL. There is a positive relationship between serum triglyceride value and the incidence of CHD. A high triglyceride level is one of the diagnostic criteria for metabolic syndrome, a condition that increases risk of cardiovascular disease. The high, and growing, prevalence of metabolic syndrome (1 in 4 individuals in the United States) has important public health implications (Ford et al., 2002).

Blood Lipoproteins

- Chylomicrons and VLDL are triglyceride-rich lipoproteins that transport dietary and endogenous lipids through the circulation.
- LDL transports about 60 to 70 percent of total serum cholesterol. An increase in LDL cholesterol increases the risk of CHD. Lowering levels of LDL cholesterol reduces the risk for CHD.
- HDL carries approximately 20 to 30 percent of total serum cholesterol. A high level of HDL cholesterol is associated with a reduced risk for CHD, and may help prevent atherosclerosis.

OVERVIEW OF QUESTIONS ADDRESSED

This section addresses seven major questions related to different types of fat and how they are related to health.

1. What are the relationships between total fat intake and health?
2. What are the relationships between saturated fat intake and health?
3. What are the relationships between *trans* fat intake and health?
4. What are the relationships between cholesterol intake and cardiovascular disease?
5. What are the relationships between n-6 PUFA intake and health?
6. What are the relationships between n-3 fatty acids and health?
7. What are the relationships between MUFA intake and health?

The general search strategies used to find the scientific evidence related to each of these questions appears in Part C. Tables summarizing the findings were prepared for Questions 1 (see Appendix G-3) and 5 (see Table D4-2). USDA's Center for Nutrition Policy and Promotion conducted special analyses related to nutritional effects of varying the percentages of total fat and of including more fish in food intake patterns. Those analyses are described briefly under Questions 1 and 6, respectively, and in full in Appendix G-2. The Committee relied on findings in the science-based report Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (IOM, 2002) and considered findings in the 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 [ATP III]) (National Cholesterol Education Program [NCEP], 2002) and the Department of Health and Human Services' Agency for Health Care

Policy and Research (AHRQ) report Effects of Omega-3 Fatty Acids on Cardiovascular Disease (Wang et al., 2004).[1]

QUESTION 1: WHAT ARE THE RELATIONSHIPS BETWEEN TOTAL FAT INTAKE AND HEALTH?

Conclusion

At low intakes of fat (< 20 percent of energy) and high intakes of carbohydrates (>65 percent of energy), risk increases for inadequate intakes of vitamin E, α-linolenic acid, and linoleic acid, and for adverse changes in HDL cholesterol and triglycerides. At high intakes of fat (> 35 percent of energy), the risk increases for obesity and CHD. This is because fat intakes that exceed 35 percent of energy are associated with both increased calorie and saturated fat intakes. Total fat intake of 20 to 35 percent of calories is recommended for adults and 25 to 35 percent for children age 4 to 18 years. A fat intake of 30 to 35 percent of calories is recommended for children age 2 to 3 years.

Rationale

Overview. The conclusion regarding the recommended range of total fat intake is based on the Institute of Medicine's (IOM's) Acceptable Macronutrient Distribution Range (AMDR) of 20 percent to 35 percent of calories from fat (IOM, 2002). As stated in Section 1, the Committee recommends that the food guidance provided aim to achieve the most recent Recommended Dietary Allowances (RDAs), Adequate Intakes (AIs), and AMDRs for all nutrients. Evidence concerning the health effects of low- and high-fat intakes was obtained from the same IOM report and from more recent publications identified by the Committee's literature search.

The lower limit for fat intake is set at 20 percent of calories because serum triacylglycerol concentrations increase and serum HDL cholesterol concentrations decrease when fat intake is low and carbohydrate intake is high. This, in turn, may increase the risk of CHD. Furthermore, it is difficult to achieve recommended intakes of several nutrients when fat intake is below 20 percent of calories.

The upper limit on total fat intake is related to the saturated-fat content of diets that provide more than 35 percent of calories from fat. Practical efforts to create heart-healthy menus that provide more than 35 percent of energy from total fat result in an unacceptably high content of saturated fatty acids. Because saturated fatty acids are present in all fats, higher intakes of total fat are

[1] The Agency for Health Care Policy and Research (AHRQ) was developed to provide evidence-based reports and technology assessments that could be used by Federal and State agencies and private or public healthcare organizations. In 1997, AHRQ, then known as the Agency for Health Care Policy and Research (AHCPR), launched its initiative to promote evidence-based practice in every-day care. AHRQ established 12 Evidence-based Practice Centers (EPCs) by awarding contracts to institutions throughout the United States and Canada. The EPCs review relevant scientific literature on clinical, behavioral, and organizational topics that are then used to develop evidence reports and technical assessments. The EPCs are required to provide detailed documentation of methods, rationale, and assumptions used throughout the process. EPCs also collaborate with other medical and research organizations in order to include a broad range of experts in the developmental process. In March 2004, AHRQ released several evidence-based reviews related to n-3 fatty acids, including an evidence-based review on the effects of n-3 fatty acids on cardiovascular disease.

associated with increased saturated fatty acid intakes. As discussed under Question 2, increasing the saturated fatty acid content of the diet increases the LDL cholesterol concentration, which, in turn, increases the risk of CHD (IOM, 2002). Other reasons for limiting total fat intake have been proposed: (1) diets with more than 35 percent of energy from fat may increase the risk of caloric excess and certain cancers such as breast and colorectal cancer; and (2) high-fat intakes may promote a prothrombotic state, which may increase CHD risk. An association between dietary fat intake and the risk for diabetes has been reported in some epidemiologic studies, but this association may be confounded by various factors, such as obesity (IOM, 2002).

Published Evidence. The IOM report *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (IOM, 2002) includes a systematic, extensive review of the scientific literature regarding total fat and carbohydrate intake in relation to weight change, blood lipid concentrations, and metabolic parameters for glucose and insulin. Documentation relevant to the conclusions above is found in the following tables:

- 11-1: Decreased Fat Intake and Body Weight Change in Non- or Moderately-Obese Subjects
- 11-2: Fat and Carbohydrate Intake and Blood Lipid Concentrations in Healthy Individuals
- 11-8: Interventional Studies on the Effect of Dietary Fat on the Metabolic Parameters for Glucose and Insulin in Healthy Subjects

Evidence in Table 11-1 (IOM, 2002), which includes nine short-term and nine long-term intervention studies, reports small losses in body weight with substantial reductions (greater than 4 percentage points) in the percentage of energy consumed as fat. The IOM report concludes that evidence suggests that low-fat diets (diets with a low percentage of calories from fat) tend to be slightly hypocaloric compared to higher fat diets in outpatient intervention trials. Data in Table 11-2 (IOM, 2002), which covers 14 intervention studies, demonstrate that decreasing fat and increasing carbohydrate intake is associated with an increase in serum triacylglycerol concentration and a decrease in plasma HDL cholesterol. Moreover, the reduction in HDL cholesterol that is associated with a low fat intake results in a higher total:HDL cholesterol ratio, which may increase the risk of CHD. Table 11-8 (IOM, 2002), which covers 13 intervention studies, reports a lack of definitive evidence that higher fat intakes impair insulin sensitivity in humans. Collectively, the evidence in these tables provides the rationale for the lower and upper range for fat in the diet.

The conclusions were substantiated further by more recent publications that reported on relationships between fat intake and the metabolic syndrome: five clinical trials (Berrino et al., 2001; Larsson et al., 1999; Lovejoy et al., 2001; Poppitt et al., 2002; Vessby et al., 2001), two reports from conferences sponsored by the National Heart, Lung, and Blood Institute/American Heart Association (Grundy, et al., 2004a), and the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association (Grundy et al., 2004b), and one review paper (Grundy et al., 2002). The evidence is convincing that better weight control improves metabolic syndrome and that modest reductions in total fat intake may facilitate both decreasing one's caloric intake and controlling calories for weight control. For individuals with metabolic syndrome, an isocaloric diet higher in total fat (30 to 35 percent of calories) with an emphasis on

unsaturated fatty acids has been shown to improve the clinical profile related to the atherogenic dyslipidemia and insulin resistance.

Special Analyses. At the Committee's request, U.S. Department of Agriculture's (USDA's) Center for Nutrition Policy and Promotion used a modeling process described in Appendix G-2 to examine how changing the percentage of calories from fat may affect the intake of other nutrients. Of particular concern were intakes of the essential fatty acids (linoleic acid and α-linolenic acid), protein, carbohydrates, added sugars, cholesterol, and vitamin E. The analysis produced food patterns that showed the following:

- At 20 percent of calories from fat, few food patterns met the AIs for both linoleic acid and α-linolenic acid. At 25 percent of calories from fat, most did; and at 30 percent and 35 percent, all did. In most cases, protein, fat and carbohydrate percentages were within the AMDR.
- At calorie levels of 2,600 or more, when only 20 percent of the calories were supplied by fat, 66 to 68 percent of calories were supplied by carbohydrates. To lower the carbohydrate provided by fruits, vegetables, and grains to be consistent with the AMDR of 45 to 65 percent of calories from carbohydrate, the proportion of calories from dietary protein could be increased.
- At 35 percent of calories from fat, the menu modeling resulted in cholesterol levels that were above the standard of 300 mg for energy intakes of 2,800 kcal or higher. This could pose a problem since increases in dietary cholesterol increase LDL cholesterol, which, in turn, increases CHD risk. In a diet that provides more than 30 percent of calories from fat, particular attention must be paid to keeping dietary cholesterol intake at or below the recommended limit (see Cholesterol).
- The amount of vitamin E provided by the patterns consistently increased with increases in the percentage of calories from fat, as well as with increases in the energy content of the pattern. Vitamin E RDAs were met only at the 3,000 and 3,200 calorie levels.

A diet that provides 20 percent of calories from fat could be designed to meet recommended intakes for vitamin E, linoleic, and α-linolenic acid by choosing the foods that are better sources of these nutrients, e.g., certain liquid vegetable oils. Exceptions might occur at the lower calorie levels (i.e., < 1,600 calories).

Positions Taken by Other Expert Groups. Using an evidence-based approach, the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2002) published the following evidence statement and recommendation related to total fat:

Evidence Statement

The percentage of total fat in the diet, independent of caloric intake, has not been documented to be related to body weight or risk for cancer in the general population. Short-term studies suggest that very high fat intakes (>35 percent calories from fat) modify metabolism in ways that could promote obesity. On the other hand, very high carbohydrate intakes (>60 percent calories) aggravate some of the lipid and non-lipid risk factors common in metabolic syndrome.

Recommendations

Dietary fat recommendations should emphasize a reduction in saturated fatty acids. Furthermore, in individuals with lipid disorders or metabolic syndrome, extremes of total fat intake – either high or low – should be avoided. In such persons, total fat intakes should range from 25-35 percent of calories. For some persons with the metabolic syndrome, a total fat intake of 30-35 percent may reduce lipid and nonlipid risk factors.

(National Cholesterol Education Program Expert Panel, 2002, p. V-12)

The evidence of a relationship between total fat intake and certain cancers is suggestive but not conclusive. The Department of Health and Human Services, National Cancer Institute's PDQ® (Physician Data Query), published the following evidence statements:

- *Colorectal cancer*—Epidemiologic, experimental (animal), and clinical investigations suggest that diets high in *total fat* [italics added], protein, calories, alcohol, and meat (both red and white) and low in calcium and folate, are associated with an increased incidence of colorectal cancer.
- *Prostate cancer*—In general, fat of animal origin seems to be associated with the highest risk. In a series of 384 patients with prostate cancer, the risk of cancer progression to an advanced stage was greater in men with a high fat intake. The announcement in 1996 that cancer mortality rates had fallen in the United States prompted the suggestion that this may be due to decreases in dietary fat over the same time period.

(http://www.cancer.gov/cancerinfo/pdq/prevention)

A more recent analysis of nutrition and cancer (Bingham and Riboli, 2004) details the difficulty in assessing whether fat intake is a risk factor for breast cancer. In particular, self-reported dietary assessment instruments may not provide an accurate assessment of dietary fat because of measurement error biases (Prentice and Sheppard, 1990). Based on a summary of the literature, total fat seems not to be associated with breast cancer risk (Kushi and Giovannucci, 2002). This conclusion is consistent with the findings of the *Nurses' Health Study* (Holmes et al., 1999), which reported no association between total fat intake and breast cancer.

An evidence-based technical report of the American Diabetes Association included the following statement for dietary fat and diabetes, "Reduced-fat diets when maintained long-term contribute to modest loss of weight and improvement in dyslipidemia" (Franz et al., 2004).

Comparison of the Committee's Findings With Other Recommendations. Both the Adult Treatment Panel (ATP) III (NCEP, 2002) and this Committee agree on the upper limit for total fat recommendations. The basis for the difference in the lower limit for the total fat recommendations—25 percent of calories made by ATP III and 20 percent of calories made by this Committee—is that ATP III focuses on recommendations for individuals at risk for CHD, such as those seeking health care who present with an atherogenic dyslipidemia that is aggravated by a very-low fat diet. This Committee, by contrast, targets the general public. As stated in Section 1, the Committee is adopting Dietary Reference Intake recommendations from the Institute of Medicine. Thus, consistent with the IOM report (IOM, 2002), which has as a focus on healthy individuals, the 20 percent lower level of total fat in the diet is acceptable.

Total Fat and Children's Health. Total fat intake of 30 to 35 percent of calories is recommended for children age 2 to 3 years. A fat intake of 25 to 35 percent of calories is recommended for children age 4 to 18 years. This is consistent with the AMDR for fat established by the IOM (IOM, 2002). The AMDRs for fat that have been estimated for children are primarily based on a transition from high-fat intakes that occur during infancy to the lower fat recommendations for adults.

Evidence is less clear about whether or not low- or high-fat intakes during childhood can lead to increased risk of chronic diseases later in life. Children can consume fat intakes within the recommended range without compromising intakes of energy and of essential vitamins and minerals (Nicklas and Johnson, 2004). Two large intervention trials successfully reduced children's total fat intake while maintaining vitamin and mineral intakes (Nicklas et al., 1996; Obarzanek et al., 1997). In the Dietary Intervention Study in Children, the treatment group consumed 28 percent of calories from total fat; the children experienced normal growth and development and maintained normal nutritional biochemical values (Obarzanek et al., 1997).

Intake Levels

Data from the *Third National Health and Nutrition Examination Survey* (NHANES III) and from NHANES 1999–2000, indicate

- For all ages of the U.S. population, the daily mean percentage of calories from total fat was 32.7 percent (Briefel and Johnson, 2004)
- For children age 2 to 19 years, mean fat intake was 33.5 percent of energy (Troiano et al., 2000)
- Among males age 12 to 19 years, fat accounted for 35.7 percent of calories for non-Hispanic blacks, compared with 33.2 percent for non-Hispanic whites and 34.1 percent for Mexican Americans. (Troiano et al., 2000)
- For females age 12 to 19 years, fat intake was 36.1 percent of calories for non-Hispanic blacks compared with 33.4 percent for non-Hispanic whites and 34.1 percent for Mexican Americans. (Troiano et al., 2000)

Investigators using data from the *Continuing Survey of Food Intake by Individuals* (CSFII) (1994–1996, 1998) reported the following additional information:

- Fewer than 5 percent of children and adults have intakes below 20 percent of calories from fat. However, approximately 25 percent of children and adults have intakes greater than 35 percent of calories from fat (IOM, 2002).
- Among children age 6 to18 years, intake of total fat was 32 percent of calories (Gleason and Suitor, 2001).

Among adults age 20 to 74, Briefel and Johnson (2004) report that total fat intake decreased from a mean of 36 percent of calories in 1971–1974 to 33 percent of calories in 1999–2000 and ranged from approximately 32 to 36 percent of calories among the different population groups surveyed. However, the absolute level of fat intake has increased: it was 73.4 g in 1989–1991 and 76.4 g in 1994–1996 (Chanmugam et al., 2003). The concurrent increase in total fat intake means that the decrease in the percentage of calories from fat results from an increase in total energy intake coming mainly from carbohydrates**.**

QUESTION 2: WHAT ARE THE RELATIONSHIPS BETWEEN SATURATED FAT INTAKE AND HEALTH?

Conclusion

The relationship between saturated fat intake and LDL cholesterol is direct and progressive, increasing the risk of cardiovascular disease (CVD). Thus, saturated fat consumption by adults should be as low as possible while consuming a diet that provides 20 to 35 percent calories from fat and meets recommendations for α-linolenic acid and linoleic acid. In particular,

- For adults with LDL cholesterol below 130 mg/dL, less than 10 percent of calories from saturated fatty acids is recommended.
- For adults with an elevated LDL cholesterol (≥ 130 mg/dL), less than 7 percent of calories from saturated fatty acids is recommended.[2]

Rationale

This conclusion concurs with the recommendation for saturated fat intake made by the IOM, which is to decrease saturated fat intake as much as possible within the context of a nutritionally adequate diet (IOM, 2002). The IOM recommendation is supported by evidence from a systematic, extensive review of 27 controlled trials. The recommendation that saturated fat be reduced to 10 percent of calories dates back to 1977 with the publication of Dietary Goals for the United States (U.S. Senate, 1977). Since then, the scientific evidence has supported the recommendation that saturated fat intake be further decreased in persons with elevated LDL cholesterol (Dixon and Ernst, 2001). The specific recommendation for less than 7 percent of calories from saturated fat is consistent with the evidence-based recommendation for individuals with an LDL cholesterol ≥130 mg/dl made by the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP, 2002); and the Committee's review of 33 more recent controlled trials on saturated fat intake and health and of a meta-analysis.

Saturated Fat and Blood Lipids. Summaries of evidence for a positive dose-response relationship between saturated fat intake and LDL cholesterol appear in Figures D4-1 through D4-3 shown below and in Table 11-2 of the Institute of Medicine report (IOM, 2002).

Figure D4-1 represents data from a meta-analysis of 395 dietary experiments among 129 groups of individuals and displays the relationship between saturated fat intake and total serum cholesterol concentrations. Figure D4-2 plots regression equations for three meta-analyses to show calculated changes in serum LDL cholesterol concentration in response to the change in the

[2] For persons with known heart disease, medical advice and the use of ATP III Panel Guidelines are indicated.

percentage of energy provided by saturated fatty acids. The figures show that serum total and LDL cholesterol concentrations increase progressively as saturated fatty acid intake increases. Regression analysis of the data reported in Figure D4-2 demonstrates that for each 1 percent increase in energy from saturated fatty acids, serum LDL cholesterol concentrations increase by 1.3 to 1.8 mg/dl (Clarke et al., 1997; Hegsted et al., 1993; Mensink and Katan, 1992). Over the range of saturated fatty acid intake reported in the literature (2 to 33 percent of energy), serum total and LDL cholesterol concentrations continue to increase. In addition, increasing saturated fatty acid intake increases the LDL:HDL cholesterol ratio progressively (Figure D4-3), which increases CHD risk. The saturated fatty acid-induced increase in the LDL:HDL cholesterol ratio is less than that reported for *trans* fatty acid (see Question 3 for more information about *trans* fatty acids).

The conclusions noted above were further substantiated by recent publications examining the impact of saturated fatty acids on components of the metabolic syndrome. Four clinical trials that replaced saturated fatty acids with MUFAs showed that lipid profiles improved, and some beneficial effects on insulin sensitivity were reported (Heilbronn et al., 1999; Lovejoy et al., 2002; Perez-Jimenez et al., 2001; Vessby et al., 2001).

Magnitude of Effect. A reduction of one percentage point in energy from saturated fat decreases serum LDL cholesterol about one to two percent, on average (NCEP, 2002). Thus, decreasing saturated fat intake from 12 percent of calories to less than 7 percent of calories would reduce LDL cholesterol by about 8 to 10 percent. An LDL cholesterol lowering response of 8 to10 percent would be expected to reduce the risk of CHD by 8 to 10 percent, since a 1 percent reduction in LDL cholesterol decreases risk for CHD events by approximately 1 percent. This estimate of the magnitude of effect of decreasing saturated fat intake is derived from a large sample population with inherent variation about the mean. For example, there is evidence that the response is greater in individuals with elevated LDL cholesterol levels and that some individuals, especially those who are overweight or obese, are less responsive to dietary saturated fatty acids than expected (Denke, 1995; Schaefer et al., 1997).

The recommendation to decrease saturated fat from about 12 percent of calories (estimated current intake) to less than 7 percent of calories for adults with an LDL cholesterol level ≥ 130 mg/dl would be expected to decrease CHD risk by about 8 to 10 percent. Likewise, if saturated fat intake were decreased from 12 percent of calories to 9 percent of calories in adults who have an LDL cholesterol < 130 mg/dl, this 3 percentage point reduction in saturated fat would decrease LDL cholesterol about 4 to 6 percent, resulting in an approximate 5 percent reduction in CHD risk.

Saturated Fats and Cancer. In a meta-analysis of dietary fat and breast cancer risk, the summary relative risk for saturated fat was 1.19 (95 percent CI: 1.06,1.35), based on an analysis of 23 case-control studies and 12 cohort studies (Boyd et al., 2003). The Committee identified two case-control studies published after that meta-analysis was completed. In a study of Korean women that included 224 cases and 240 controls, Do et al. (2003) report that higher breast cancer incidence was not observed with higher saturated fatty acid intake (more than 19.5 g per day). However there was a statistically significant trend of increasing breast cancer incidence with increasing total saturated fatty acid intake. In the Norfolk, UK, center of the *European*

Prospective Investigation of Cancer, each of 186 women with breast cancer was matched with four healthy controls (840 total participants). In this study, the risk of breast cancer was strongly associated with the amount of saturated fat consumed. Women who consumed more than 35 g per day of saturated fat had more than twice the risk of developing breast cancer than that of women who consumed 10 g per day or less of saturated fat (Bingham and Riboli, 2004).

Nutrients Provided by Diets Very Low in Saturated Fats. Results of menu modeling activities (IOM, 2002) indicate that diets can be planned to meet nutrient recommendations for linoleic acid and α-linolenic acid while providing very low amounts of saturated fatty acid (3 to 5 percent of calories from saturated fatty acid). ATP III has 10 different menu simulations for different ethnic and gender groups that meet the recommendations of the therapeutic lifestyle changes diet (NCEP, 2002, Diet Appendix B). That diet recommends less than 7 percent of calories from saturated fat, less than 200 mg of cholesterol per day, 1 to 2 g of stanol/sterol esters[3] per day and 10 to 25 g per day of soluble fiber. In addition, weight control and daily physical activity are recommended. In these simulations, the saturated fatty acid content of the diet can be as low as 4 to 6 percent of calories.

Positions Taken by Other Expert Groups. Using an evidence-based approach, the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults published the following evidence statement and recommendation related to saturated fat:

> *Evidence Statement*
> There is a dose response relationship between saturated fatty acids and LDL cholesterol levels. Diets high in saturated fatty acids raise serum LDL cholesterol levels. Reduction in intakes of saturated fatty acids lowers LDL cholesterol levels. High intakes of saturated fatty acids are associated with high population rates of CHD. Reduction in intake of saturated fatty acids will reduce risk for CHD.
>
> *Recommendation*
> The therapeutic diet to maximize LDL cholesterol lowering should contain less than 7 percent of total calories as saturated fatty acids.
>
> (National Cholesterol Education Program Expert Panel, 2002, p. V-4)

In addition, an evidence-based technical report of the American Diabetes Association included the following statement for saturated fat and diabetes:

> Less than 10 percent of energy intake should be derived from saturated fats.
>
> To lower LDL cholesterol, energy derived from saturated fat can be reduced if weight loss is desirable or replaced with either carbohydrate or monounsaturated fat when weight loss is not a goal.
>
> (Franz et al., 2004, p. S39)

ATP III has defined the following categories for LDL cholesterol values (NCEP, 2002):

[3] Plant sterols are isolated from soybean and tall pine-tree oils. Plant sterols can be esterified to unsaturated fatty acids, creating sterol esters. Hydrogenating sterols produces plant stanols and, with esterification, stanol esters (NCEP, 2002).

- Optimal: < 100 mg/dl
- Near optimal/above optimal: 100 to 129 mg/dl
- Borderline high: 130 to 159 mg/dl
- High: 160 to 189 mg/dl
- Very high: ≥ 190 mg/dl

Recently, NCEP revised these recommendations (Grundy et al., 2004c). In high-risk persons, the recommended LDL cholesterol goal is less than 100 mg/dl; but when risk is very high, an LDL cholesterol goal of less than 70 mg/dl is a therapeutic option. When risk is moderately high, the recommended LDL cholesterol goal is less than 130 mg/dl, but an LDL cholesterol goal of less than 100 mg/dl is a therapeutic option. This more rigorous LDL cholesterol goal likely will require pharmacologic therapy in combination with the dietary changes.

For all adults, including those with an LDL cholesterol concentration less than 130 mg/dl, the risk of heart disease continues to decrease progressively as LDL cholesterol decreases. Clinical trials demonstrate the efficacy of LDL-cholesterol lowering as an important means of reducing the risk of CHD. Consequently, risk is decreased the most when LDL is decreased most.

Saturated Fatty Acids and the Health of Children. Research on the impact of saturated fatty acid consumption in healthy children is lacking.

Stearic Acid

Stearic acid has attracted interest as a substitute for *trans* fatty acids in prepared foods that require a solid fat. Stearic acid offers the functional properties needed for these foods, but the question arises of how it affects blood lipid values. Stearic acid is a unique saturated fatty acid with respect to its effects on blood lipids and lipoproteins. Stearic acid has been shown to have a neutral effect on serum total and LDL cholesterol concentrations (Bonanome and Grundy, 1988; Denke, 1994; Hegsted et al., 1965; Keys, 1965; Yu et al., 1995; Zock and Katan, 1992). A meta-analysis of 35 studies suggests that stearic acid has a minimal effect on LDL cholesterol and no effect on HDL cholesterol (Mensink et al., 2003). In contrast, the other long chain saturated fatty acids increase both LDL cholesterol and HDL cholesterol (Mensink et al., 2003).

Because of the growing interest in stearic acid as a substitute for *trans* fatty acids in solid fats, there is a need to assess the effects of this fatty acid on cardiovascular disease risk factors beyond blood lipids and lipoproteins. Only one published study provides evidence about the effects of stearic acid on other cardiovascular disease (CVD) endpoints. In particular, Baer et al. (2004) designed a study to evaluate the effects of individual fatty acids on hemostatic risk factors for CVD. Compared with diets that provided 2 to 3 percent of calories from stearic acid, a diet that provided 8 percent of calories from stearic acid increased fibrinogen concentration by 4.4 percent. This translates to an approximate 7 percent increase in the risk of myocardial infarction. This study also compared the hemostatic effects of a diet that provided 4 percent of calories from stearic acid plus 4 percent of calories from *trans* fats with those of a high-carbohydrate (54.6 percent of calories from carbohydrate) control diet. In this comparison, there was no effect on fibrinogen concentration. Typical consumption of stearic acid in the United States is approximately 3.5 percent of calories. Thus, at intakes of stearic acid that are equal to

or slightly higher than amounts consumed in the United States, no adverse effects on fibrinogen levels would be expected.

Saturated Fat Intake

Based on data from NHANES III and 1999–2000, reported saturated fat intake by Americans is as follows:

- For all ages of the U.S. population over 2 months, the daily mean percentage of calories from saturated fat was 11.2 percent. In 1999–2000, 41 percent of the population age 2 years and older reported intakes of less than 10 percent of calories from saturated fat (Briefel and Johnson, 2004).
- Adult women, persons age 60 and older, Hispanics, and persons with higher household income were more likely than others to have intakes of less than 10 percent of calories from saturated fat (Briefel and Johnson, 2004).
- Among adults ages 20 to 74 years, mean saturated fat intake decreased from 13 percent of calories in 1971–1974 to 11 percent of calories in 1999–2000 (Briefel and Johnson, 2004).
- For persons ages 2 to 19 years, mean saturated fat intake was 12.2 percent of energy (Troiano et al., 2000).
- Mean saturated fat intake ranged from 11.6 percent of energy for females age 12 to 15 years to 12.8 percent for males age 6 to 8 years. Mean saturated fat intake ranged from 11.7 percent of calories for non-Hispanic white females age 12 to 19 years to 12.8 percent for Mexican American males age 6 to 11 years. (Troiano et al., 2000)

Using data from CSFII, 1994–1996, Gleason and Suitor (2001) found that mean usual intake of saturated fat was 12 percent of calories among school-age children.

In summary, current saturated fat intake is approximately 11 to 13 percent of calories. This represents a 1 to 2 percentage point decrease since the early 1970s for the population at large. Some population groups are consuming less than 10 percent of calories from saturated fat.

QUESTION 3: WHAT ARE THE RELATIONSHIPS BETWEEN *TRANS* FAT INTAKE AND HEALTH?

Conclusion

The relationship between *trans* fatty acid intake and LDL cholesterol is direct and progressive, increasing the risk of CHD. *Trans* fatty acid consumption by all population groups should be kept as low as possible, which is about 1 percent of energy intake or less.

Rationale

Overview. This conclusion is supported by a systematic, extensive review of the evidence conducted by the IOM (2002) covering 20 controlled trials and 11 epidemiologic studies; the evidence-based review conducted by the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2002); and the Committee's review of 7 more recent publications.

Summaries of evidence of relationships of *trans* fatty acid intake and health from the IOM report (IOM, 2002) appear in Tables 8-9 through 8-13 of that report and in Figure D4-3 below. Those tables cover the following topics:

- Table 8-9: Dietary *Trans* Fatty Acids and Blood Lipid Concentration: Controlled Feeding Trials
- Table 8-10: Hydrogenated Fat Intake and Blood Lipid Concentrations: Controlled Feeding Trials
- Table 8-11: Dietary *Trans* Fatty Acids, Hydrogenated Fat, and Blood Lipids Concentrations: Free Living Trials
- Table 8-12: *Trans* Fatty Acid Intake and Blood Clotting, Blood Pressure, and Low-Density Lipoprotein Oxidation
- Table 8-13: Dietary *Trans* Fatty Acids: Epidemiologic Studies

Review of the Evidence

Trans *Fatty Acids and Blood Lipids.* The data reported in Tables 8-9 and 8-10 of the IOM report summarize 12 controlled feeding studies, and the data in Table 8-11 summarize 7 trials with subjects consuming self-selected diets (IOM, 2002). These data show that, when compared with unsaturated fatty acids, *trans* fatty acids/hydrogenated fat increase LDL cholesterol concentrations. In addition, when *trans* fatty acids are substituted for saturated fatty acids, HDL cholesterol concentration decreases; and a dose-response effect is observed. There is a progressive dose-dependent relationship between *trans* fatty acid intake and an increase in the LDL:HDL cholesterol ratio (Figure D4-3). This observed relationship is progressive over the range of *trans* fat intake from 0.5 to 10 percent of calories. The magnitude of this effect is greater for *trans* fatty acids than for saturated fatty acids. The saturated fatty acids increase HDL cholesterol, albeit modestly, even when comparisons are made at low levels of saturated fat intake, but the dose-response relationship for *trans* fatty acid intake and the LDL:HDL cholesterol ratio begins to become greater than that observed for saturated fatty acids at about 2.5 percent of energy intake.

Recent clinical studies support the findings described above: both *trans* fat and saturated fat increase LDL cholesterol similarly; however, saturated fat increases HDL cholesterol whereas *trans* fat does not (Judd et al., 2002; Lovejoy et al., 2002). Several of the recent studies have shown that replacing saturated fats with *trans* fat decreases serum HDL cholesterol (de Roos et al., 2001, 2002, 2003). A meta-analysis of 60 controlled trials (Mensink et al., 2003) reported that the consumption of *trans* fat significantly increased the total:HDL cholesterol ratio. Dietary *trans* fatty acids also have been shown to increase small, dense LDL cholesterol proportionately to the amount of dietary *trans* fatty acids (Mauger et al., 2003).

The data reported in Table 8-12 of the IOM report (IOM, 2002) indicate that *trans* fatty acids have little effect on hemostatic factors, susceptibility of LDL cholesterol to oxidation, or blood pressure. Other clinical studies have reported adverse effects of *trans* fatty acids on other cardiovascular disease risk factors including postprandial lipids (Gatto et al., 2003) and impaired endothelial function (de Roos et al., 2002). Recent epidemiologic evidence from the *Nurses' Health Study I and II* indicates that *trans* fatty acid intake is positively associated with the systemic inflammatory markers for cardiovascular disease, with soluble tumor necrosis factor α

receptors 1 and 2 in all women, and with IL-6 and C-reactive protein in women with higher body mass index (Mozaffarian et al., 2004).

Trans *Fatty Acids and Cardiovascular Disease.* Epidemiologic evidence from 6 cohort studies (Table 8-13, IOM, 2002) suggests that a high *trans* fat intake is associated with an increased risk of coronary artery disease. In an analysis of data from the *Seven Countries Study*, Kromhout et al. (1995) reported strong positive associations between 25-year death rates from CHD and the average intake of the *trans* fatty acid elaidic acid ($r = 0.78$, $P < 0.001$), and the average intake of the four major long-chain saturated fatty acids ($r > 0.8$, $P < 0.001$) and of dietary cholesterol ($r = 0.55$, $P < 0.05$). Hu et al (1997) reported that intake of *trans* fat was associated with an increased risk of coronary heart disease in women. Women in the highest quintile of *trans* fat intake (2.9 percent of energy) had a 27 percent greater risk of coronary heart disease than women in the lowest quintile (95 percent CI: 1.03, 1.56, $P = 0.02$ for trend). In comparison, women in the highest quintile of saturated fat intake had a 16 percent greater risk of coronary heart disease than women in the lowest quintile (95 percent CI: 0.93,1.44, $P = 0.04$ for trend). Similar findings were reported by Pietinen et al. (1997). Among men in the top quintile of *trans* fatty acid intake (median = 6.2 g per day), the multivariate relative risk of coronary death was 1.39 (95 percent CI: 1.09,1.78; $P = 0.004$) compared with men in the lowest quintile of intake (median = 1.3 g per day); there was no association between intakes of saturated, monounsaturated, polyunsaturated fatty acids or dietary cholesterol and the risk of coronary death. In addition, case-control studies demonstrate an association between *trans* fat intake and the risk of myocardial infarct. For example, compared with the lowest quintile of intake, the relative risk of acute myocardial infarction for the highest quintile of *trans* fatty acid intake was 2.4 (P for trend < 0.0001) (Ascherio et al., 1994).

More recent studies have reported an association between the *trans* fatty acid content of adipose tissue (a biomarker of long-term fatty acid intake) and the risk of nonfatal myocardial infarct (Baylin et al., 2003; Clifton et al., 2004). In the study conducted by Clifton et al., both vegetable and animal *trans* fat contributed to the increased risk. However, other epidemiologic studies report a link only between the intake of hydrogenated vegetable oils and coronary artery disease; the intake of *trans* fatty acids from animal sources had no observed adverse effect (Ascherio et al., 1999; Ascherio et al., 1996; Willett et al., 1993). Recent evidence, however, suggests that the risk of CHD is similar between total ruminant and industrial *trans* fatty acids for intakes up to 2 g per day (Weggemans et al., 2004). In a case control study (Lemaitre et al., 2002) reported that higher total *trans* fatty acids in red blood cell membranes was associated with a modest increase in the risk of primary cardiac arrest (odds ratio for interquintile range, 1.5; 95 percent CI, 1.0 to 2.1). Notably, higher levels of *trans* isomers of linoleic acid were associated with a 3-fold increase in risk, whereas *trans* isomers of oleic acid were not.

Although intakes of saturated fat, *trans* fat, and cholesterol all should be decreased, because saturated fat consumption is proportionately much greater than that of these other fats, saturated fat should be the primary focus of dietary modification.

Positions Taken by Other Expert Groups. Using an evidence-based approach, the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults published the following evidence statement and recommendation related to *trans* fat:

> *Evidence Statement*
> *Trans* fatty acids raise serum LDL cholesterol levels. Consequently, higher intakes of *trans* fatty acids increase risk for CHD. Prospective studies support an association between higher intakes of *trans* fatty acids and CHD incidence. However, *trans* fatty acids are not classified as saturated fatty acids, nor are they included in the quantitative recommendations for saturated fatty acids intake of <7 percent of calories in the TLC [Therapeutic Lifestyle Changes] diet.
>
> *Recommendation*
> Intakes of *trans* fatty acids should be as low as possible. The use of liquid vegetable oil, soft margarine, and *trans* fatty acid-free margarine are encouraged instead of butter, stick margarine, and shortening that contain *trans* fat.
>
> (National Cholesterol Education Program Expert Panel, 2002, p. V-9)

Numerous other expert groups have conducted evidence-based reviews or published consensus statements related to *trans* fatty acids.

> The American Diabetes Association recommends that intake of *trans* unsaturated fatty acids be minimized.
>
> (Franz et al., 2004, p. S39)

> An FDA Food Advisory Committee, Nutrition Committee, recently voted (6 yes, 1 abstain) in favor of the following statement: "Although current scientific evidence does not indicate a specific acceptable daily intake for *trans* fatty acids, it is consistent with reducing *trans* fatty acid intake to a level less than 1 percent of energy (2 g per day for a 2,000 calorie diet)."
> (FDA Food Advisory Committee, Nutrition Subcommittee Transcripts. April 28, 2004 p. 92)

> The AHA [American Heart Association] Dietary Guidelines Revision 2000 recommends that *trans* fatty acid intake be limited, and that the total intake of cholesterol-raising fatty acids not exceed 10 percent of energy.
>
> (Krauss et al., 2000, p. 2288)

> An earlier statement issued by the American Heart Association recommended that naturally occurring unhydrogenated oil be used when possible and attempts made to substitute unhydrogenated oil for hydrogenated oil or saturated fat in processed foods. Also, softer margarines should be substituted for harder margarines and cooking fats.
>
> (Lichtenstein, 1997, p. 2590)

The conclusion to keep *trans* fatty acid consumption by all population groups as low as possible also is supported by the World Health Organization Report (WHO) (2003), which recommends < 1 percent of energy from *trans* fatty acids and the Danish Nutrition Council (Stender and Dyerbery, 2003), which recommends that the use of industrially produced *trans* fatty acids in foodstuffs be ceased as soon as possible.

Trans *Fatty Acid Intake*. Using 1989–1991 CSFII data, the estimated mean *trans* fatty acid intake for the U.S. population ages 3 years and older was 2.6 percent of total energy intake (Allison et al., 1999). For individuals age 20 years and older, the estimated average daily intake of *trans* fat in the U.S. population is about 5.8 g per day, which represents about 2.6 percent of total energy intake. Industrial sources provide approximately 80 percent of *trans* fat in the diet, compared to 20 percent from animal sources. The major food sources of *trans* fat for U.S. adults are shown in Table D4-1.

Most *trans* fat comes from industrial sources of fat. However, even if partially hydrogenated fats were removed from the food supply, the Committee estimates that *trans* fats still would provide about 1 percent of the calories because some *trans* fatty acids are produced in the deodorization of vegetable oils (principally as elaidic acid), and meat and dairy products contain naturally occurring *trans* fatty acids as vaccinic acid and conjugated linoleic acid (CLA). There is emerging evidence that the naturally occurring *trans* fatty acids, vaccinic acid, and conjugated linoleic acid, have unique biological effects. In animal studies, CLA can decrease fat deposition and body lipid content (Wang and Jones, 2004). However, the few human studies conducted to date have not demonstrated a similar effect. There is also evidence from animal studies that CLA protects against the development and progression of atherosclerosis (Toomey et al., 2003). Studies with both animals and cell models demonstrate anti-carcinogenic effects of CLA and vaccinic acid for many types of cancer (Banni et al., 2001; Corl et al., 2003; Ip et al., 1999). According to the Food and Drug Administration (68 Fed. Reg. 41443 (July 11, 2003)), the average *trans* fat intake from animal sources is 1.2 g per day. This is approximately 0.5 percent of calories, of which conjugated linoleic acid contributes a small quantity (151 to 212 mg per day) (IOM, 2002). *Trans* fat from animal products is estimated to provide less than 1 percent of calories in the revised USDA food intake pattern (Table D1-13). Decreased consumption of foods made with industrial sources of *trans* fats (see plant foods in Table D4-1) provides the most effective means of reducing *trans* fat intake.

QUESTION 4: WHAT ARE THE RELATIONSHIPS BETWEEN CHOLESTEROL INTAKE AND CARDIOVASCULAR DISEASE?

Conclusion

The relationship between cholesterol intake and LDL cholesterol concentrations is direct and progressive, increasing the risk of CHD. Thus, cholesterol intake should be kept as low as possible, within a nutritionally adequate diet. In particular,

- For adults with an LDL cholesterol < 130 mg/dL, less than 300 mg of dietary cholesterol per day is recommended.
- For adults with an elevated LDL cholesterol (≥130 mg/dL), less than 200 mg of dietary cholesterol/day is recommended.

Rationale

***Overview*.** This conclusion is supported by evidence from a systematic, extensive review of the scientific literature by the IOM (2002) covering 49 controlled trials and 14 observational studies; the evidence-based review conducted by the NCEP Expert Panel on Detection, Evaluation, and

Treatment of High Blood Cholesterol in Adults (2002); and the Committee's review of 5 more recent controlled trials.

Summaries of evidence of effects of dietary cholesterol on serum cholesterol and CHD from the IOM report (IOM, 2002) appear in Tables 9-2 through 9-4 of that report and Figure D4-4 below. The tables cover the following topics:

- Table 9-2: Effects of Adding Dietary Cholesterol to Defined Diets with Strict Control of Dietary Intake on Serum Cholesterol Concentrations
- Table 9-3: Effects of Adding Dietary Cholesterol to Self-Selected Diets with Strict Control of Dietary Intake on Serum Cholesterol Concentrations
- Table 9-4: Dietary Cholesterol and Coronary Heart Disease

There is a historical basis for the cholesterol recommendation that dates back to 1968 when the American Heart Association recommended about ~300 mg per day to decrease the risk of CHD (American Heart Association, 1968). In 1977 the Dietary Goals for the United States recommended that dietary cholesterol be reduced to 300 mg per day (U.S. Senate, 1977). Since then, the scientific evidence has supported this recommendation and the more contemporary guidance that dietary cholesterol intake be decreased further in persons with elevated LDL cholesterol (Dixon and Ernst, 2001).

Review of the Evidence

Dietary Cholesterol and Serum Cholesterol. The data summarized in the tables cited above show that, in most studies, as dietary cholesterol increases there is a corresponding increase in total serum cholesterol. A meta-analysis (Figure D4-4) of 27 controlled metabolic feeding studies of added dietary cholesterol indicates a relationship with change in serum cholesterol that is steeper in the range from zero to 300 to 400 mg per day of added dietary cholesterol and less steep above this level. However, data summarized in Table 9-4 of the IOM report (IOM, 2002) covering 15 observational studies, show a lack of consistency in epidemiologic observations relating dietary cholesterol to clinical cardiovascular disease and CHD endpoints. The inconsistent findings may be due to limited power to detect effects (e.g., relatively small increases in LDL cholesterol concentration and inaccuracy in dietary intake data), limited power to distinguish the effects of dietary cholesterol independent of other factors (such as saturated fat, energy intake, and fiber intake), or other factors.

This conclusion concurs with the recommendation for cholesterol intake made by the IOM, which is to decrease cholesterol intake as much as possible within the context of a nutritionally adequate diet (IOM, 2002). The IOM recommendation is supported by evidence from a systematic, extensive review of the scientific literature. The specific recommendation for less than 200 mg per day is consistent with the evidence-based recommendation for individuals with an LDL cholesterol greater than 130 mg/dl made by ATP III (NCEP, 2002).

Magnitude of Effect. Based on a meta-analysis of 27 controlled feeding studies, for a baseline cholesterol intake of zero, the estimated increase in serum cholesterol is 5 mg/dl per 100 mg of added dietary cholesterol per day—up to 400 mg of cholesterol per day. In contrast, when baseline cholesterol intake is 300 mg per day, the estimated increase in serum cholesterol is 1.5

mg per day in response to the addition of 100 mg of dietary cholesterol per day (Hopkins, 1992). Equations based on data from numerous studies predict that 100 mg of added dietary cholesterol per day will increase serum cholesterol by 2 to 3 mg/dl (Clarke et al., 1997; Hegsted, 1986; Howell et al., 1997). Of this increase in total serum cholesterol, approximately 80 percent is in the LDL fraction. For an individual with a total serum cholesterol level of 200 mg/dl, a 2 to 3 mg increase represents an approximate 1 to 1.5 percent increase in serum cholesterol level (equivalent to a 0.8 to 1.2 percent increase in LDL cholesterol). This increase would be expected to increase CHD risk about 1 percent (IOM, 2002; NCEP, 2002). Notably, however, the effect of added cholesterol is variable among individuals ranging from essentially no response to a greater response. For example, a recent study has shown that both normal weight and overweight/obese individuals who are insulin resistant seem to have a diminished response to dietary cholesterol compared with insulin sensitive individuals (Knopp et al., 2003). Based on the collective evidence, the magnitude of response to dietary cholesterol is much less than that observed for saturated and *trans* fat intake.

Positions Taken by Other Expert Groups. Using an evidence-based approach, the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults published the following evidence statement and recommendation related to cholesterol:

> *Evidence Statement*
> Higher intakes of dietary cholesterol raise serum LDL cholesterol levels in humans. Through this mechanism, higher intakes of dietary cholesterol should raise the risk for CHD. Reducing cholesterol intakes from high to low decreases serum LDL cholesterol in most persons.
>
> *Recommendation*
> Less than 200 mg per day of cholesterol should be consumed in the TLC [Therapeutic Lifestyle Changes] Diet to maximize the amount of LDL cholesterol lowering that can be achieved through reduction in dietary cholesterol.
>
> (National Cholesterol Education Program Expert Panel, 2002 p. V-4)

Using an evidence-based approach, the American Diabetes Association published the following evidence-based nutrition principle and recommendation related to cholesterol:

> Dietary cholesterol intake should be <300 mg per day. Some individuals (i.e., persons with LDL cholesterol ≥ 100 mg/dl) may benefit from lowering dietary cholesterol to <200 mg per day.
>
> (Franz et al., 2004, p. S39)

Dietary Cholesterol and Children's Health. Research on the impact of dietary cholesterol consumption on LDL cholesterol and other cardiovascular disease risk factors in healthy children is lacking.

Cholesterol Intake. Mean cholesterol intake intake is above the recommended level of 300 mg per day for adult males and below it for adult females. For adults age 20 to 74, age-adjusted mean dietary cholesterol intake was 341 mg in men and 242 mg in women (1999–2000) (Briefel

and Johnson, 2004). Troiano et al. (2000) found an increase in cholesterol intake with age for young males, reaching a peak of 375 mg at age 16 to 19 years. Among males age 12 to 19 years, the mean (but not median) intake exceeded 300 mg regardless of racial/ethnic group. Among females, the highest mean intake (233 mg) occurred at age 9 to 11 years.

QUESTION 5: WHAT ARE THE RELATIONSHIPS BETWEEN n-6 PUFA INTAKE AND HEALTH?

Conclusion

An n-6 PUFA intake between 5 to 10 percent of energy may confer beneficial effects on coronary artery disease mortality.

Rationale

Overview. The conclusion regarding the range of intake of n-6 PUFAs is based on the IOM's AMDR for this fatty acid class (IOM, 2002). Evidence concerning beneficial effects on coronary artery disease mortality was obtained from the same IOM report and a systematic review of 17 published papers.

The n-6 PUFAs include linoleic acid, which accounts for about 85 percent to 90 percent of the total PUFA consumption, and arachadonic acid, which contributes less than 2 percent of the total (IOM, 2002). A dietary source of linoleic acid is essential for life and health. Linoleic acid serves as a precursor to eicosanoids. A lack of dietary n-6 PUFAs is characterized by rough, scaly skin; dermatitis; and an elevated eicosatrienoic acid:arachidonic acid (triene:tetraene) ratio. The IOM (IOM, 2002) set an AI for linoleic acid of 17 g per day for men and 12 g per day for women. It also set an AMDR for linoleic acid of 5 to 10 percent of calories (IOM, 2002). The lower end of the range meets the AI for linoleic acid. The upper end of the range was based on three lines of evidence: (1) individual dietary intakes in a North American/U.S. population rarely exceed 10 percent of energy, (2) epidemiologic evidence for the safety of intakes greater than 10 percent of energy generally are lacking, and (3) high intakes of linoleic acid create a pro-oxidant state that may predispose to several chronic diseases, such as CHD and cancer.

Review of the Evidence

n-6 Fatty Acid Intake and Blood Lipids. Evidence from six intervention studies was provided in the IOM report (IOM, 2002, see Table 11-9: *Interventional Studies on n-6 Fatty Acid Intake and Blood Lipid Concentrations*). The studies included in the table demonstrate that higher n-6 polyunsaturated fatty acid intake generally is associated with a more favorable CHD lipid risk profile.

n-6 *Fatty Acid Intake and CVD.* A number of epidemiologic studies have examined the association between n-6 PUFA intake and CVD. In two population studies (Artaud-Wild et al., 1993; Hegsted and Ausman, 1988), PUFA intake was negatively associated with CVD mortality after adjusting for dietary saturated fat. Several prospective studies (Ascherio et al., 1996; Garcia-Palmieri et al., 1980; Gordon et al., 1981; Hu et al., 1997; Shekelle et al., 1981), two

longitudinal studies (Joossens et al., 1989; Tell et al., 1994), and one cross-sectional study (Djousse et al., 2001) reported a beneficial association of dietary PUFAs with CVD morbidity and mortality (Table D4-2). In contrast, no significant association was found between dietary PUFAs and CVD in the *Seven Countries Study* (Keys, 1997, Kromhout et al., 1995). Similarly, other epidemiologic studies did not find a beneficial association between PUFAs and CVD (Kark et al., 2003; Kushi et al., 1985; Posner et al., 1991).

Adverse Effects of PUFA. In a systematic review of research, the Committee found no studies that reported adverse effects, even in the *Jerusalem Study* in which 25 percent of the population had PUFA intakes that exceeded 12 percent of calories (Kark et al., 2003). However, as noted previously, the upper end of the AMDR took into account that the epidemiologic evidence for the safety of intakes greater than 10 percent of energy generally are lacking and that high intakes of linoleic acid may create a pro-oxidant state (IOM, 2002).

Positions Taken by Other Expert Groups. This conclusion also was supported by the evidence-based *Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.*

> *Evidence Statements*
> Linoleic acid, a polyunsaturated fatty acid, reduces LDL cholesterol levels when substituted for saturated fatty acids in the diet. Polyunsaturated fatty acids also can cause small reductions in HDL cholesterol when compared with monounsaturated fatty acids, especially when present in high amounts in the diet. Controlled clinical trials indicate that substitution of polyunsaturated fatty acids for saturated fatty acids reduces risk for CHD.
>
> *Recommendation*
> Polyunsaturated fatty acids are one form of unsaturated fatty acids that can replace saturated fat. Most polyunsaturated fatty acids should be derived from liquid vegetable oils, semi-liquid margarines, and other margarines low in *trans* fatty acids. Intake of polyunsaturated fat can be as high as 10 percent of total calories.
>
> (National Cholesterol Education Program Expert Panel, 2002, p. V-11)

Using an evidence-based approach, the American Diabetes Association published the following evidence-based nutrition principle and recommendation related to PUFAs:

> Polyunsaturated fat intake should be ≈ 10 percent of energy intake.
>
> (Franz et al., 2004, p. S39)

n-6 PUFA Intake. Mean n-6 PUFA intakes by Americans fall within the AMDR. Based on CSFIII 1989-91 data, mean intakes by adults are approximately 5 to 6 percent of total energy intake (Allison et al., 1999). Using NHANES III data, mean intakes by children and adolescents ranged from about 6 to nearly 8 percent, depending on the age and ethnic group (Troiano et al., 2000).

QUESTION 6: WHAT ARE THE RELATIONSHIPS BETWEEN n-3 FATTY ACID INTAKE AND HEALTH?

Conclusion

An α-linolenic acid intake between 0.6 to 1.2 percent of calories will meet requirements for this fatty acid and may afford some protection against CVD outcomes.

The consumption of two servings (approximately 8 ounces) per week of fish high in EPA and DHA is associated with reduced risk of both sudden death and CHD death in adults. To benefit from the potential cardioprotective effects of EPA and DHA, the weekly consumption of two servings of fish, particularly fish rich in EPA and DHA, is suggested. Other sources of EPA and DHA may provide similar benefits; however, further research is warranted.

Rationale

α-*Linolenic Acid*

Overview. The conclusion regarding the range of intake of α-linolenic acid is based on the IOM's AMDR for this fatty acid (IOM, 2002). Evidence concerning protection against CVD outcomes was obtained from the same IOM report, several more recent studies, and data from the evidence-based report from the HHS Agency for Health Care Policy and Research (AHRQ) entitled *Effects of Omega-3 Fatty Acids on Cardiovascular Disease* (Wang et al., 2004).

A dietary source of α-linolenic acid is essential for life and health. The IOM (IOM, 2002) set an AI for α-linolenic acid of 1.6 g per day for men and 1.1 g per day for women. This represents approximately 0.6 percent of energy intake for sedentary adults. The AMDR for α-linolenic acid is 0.6 percent to 1.2 percent of calories. The lower boundary of the recommended range meets the AI for α-linolenic acid. The AI for α-linolenic acid is based on the median intakes in the United States and Canada—countries in which an α-linolenic acid deficiency is nonexistent in healthy individuals. The upper boundary corresponds to the highest reported α-linolenic acid intake from foods consumed by individuals in the United States and Canada.

Evidence Relating to Cardiovascular Disease. The IOM (2002, pp 11-1 to 11-2) stated, "A growing body of literature suggests that higher intakes of α-linolenic acid, EPA, and DHA may afford some degree of protection against CHD." In addition, the recently released AHRQ report (Wang et al., 2004) also supports the conclusion that α-linolenic acid intakes within the AMDR range of 0.6 percent to 1.2 percent of calories may afford some protection against cardiovascular disease outcomes. Both reports summarized the three epidemiologic studies conducted in the United States that demonstrated that an α-linolenic acid intake of 0.53 to 2.8 g per day reduced the risk of cardiovascular disease events (Djousse et al., 2001), fatal ischemic heart disease (Hu et al., 1999), and all-cause mortality (Dolecek, 1992). In addition, both reports summarized two secondary prevention randomized controlled clinical trials (de Lorgeril et al., 1999; Singh et al., 1997) that demonstrated a beneficial effect of α-linolenic acid on cardiovascular events in post-myocardial infarct patients. These studies reported that increased α-linolenic acid intake (2.0 g per day and 2.9 g per day, respectively) reduced the risk of recurrent coronary events. These α-linolenic acid intake values correspond to approximately 0.8

and 1.2 to 1.3 percent of calories, respectively—values that fall within and slightly above the upper range of the AMDR for α-linolenic acid. In these two studies, the control group consumed 0.27 percent of energy and 0.8 g per day as α-linolenic acid, respectively.

In a primary prevention study on cardiovascular disease outcomes in a population with a high habitual fish intake conducted in Norway more than 30 years ago, a diet that provided 5.5. g per day of α-linolenic acid from linseed oil did not improve outcomes compared with a diet that provided 0.14 g per day of α-linolenic acid from sunflower seed oil (Natvig et al., 1968). Notably, the two diets differed in other ways related to the unique fatty acid profiles of linseed oil and sunflower oil. Another primary prevention trial in subjects with multiple CVD risk factors (Bemelmans et al., 2002) determined the 10-year estimated ischemic heart disease risk in subjects followed for 2 years. The trial found no effect of a diet that provided 6.3 g per day of α-linolenic acid compared with a diet that provided 1 g per day of α-linolenic acid. Possible reasons to explain a lack of effect of these primary prevention studies may relate to study duration and sample size, neither of which may have been sufficient to test the hypothesis adequately. In addition, in the Natvig et al. study, the high habitual fish intake of the Norwegian population could have blunted an α-linolenic acid effect. In the Bemelmans et al. study (2002), the "low" α-linolenic acid intake group may have consumed a level of α-linolenic acid sufficient to achieve a beneficial response that was comparable to the response of the high-intake group.

There is no evidence of a beneficial effect of α-linolenic acid intake on the incidence of stroke. Collectively, the evidence supports the hypothesis that the consumption of α-linolenic acid reduces all-cause mortality and various cardiovascular disease events. However, the evidence is strongest for fish or fish oil supplements, as discussed below.

Evidence Relating to Cancer. A meta-analysis of 9 cohort and case-control studies evaluated the relationship between α-linolenic acid and prostate cancer (Brouwer et al., 2004). In this analysis, the intake of α-linolenic acid or the concentration of α-linolenic acid in the blood was used to assess the relative risk of prostate cancer. The results of the individual studies were variable. Based on meta-analysis, however, the relative risk of prostate cancer was higher in the men with the highest intakes or highest blood concentrations of α-linolenic acid than in men with the lowest intakes. The mean α-linolenic acid intake in the highest intake group was 2.0 g per day, and it was 0.8 g per day in the lowest intake group. Of the four prospective studies evaluated in the meta-analysis, two assessed the intake of α-linolenic acid, and two evaluated blood values of α-linolenic acid. In the two that assessed intake, one reported a slight protective effect of α-linolenic acid intake for prostate cancer incidence (RR 0.76; 95 percent CI: 0.66, 1.04) (Schuurman et al., 1999). In contrast, the *U.S. Health Professionals' Follow-Up Study* reported a slightly increased risk of prostate cancer with increasing α-linolenic acid intake (RR 1.25; 95 percent CI: 0.82, 1.92) (Giovannucci et al., 1993).

In a followup to the Giovannucci et al. study, α-linolenic acid intake was unrelated to the risk of total prostate cancer among 2965 new documented cases of total prostate cancer, of which 448 were advanced prostate cancer (Leitzmann et al., 2004). However, the multivariate relative risks of advanced prostate cancer from the extreme quintiles of α-linolenic acid intake from nonanimal sources were 2.02 (95 percent CI: 1.35, 3.03, P for trend 0.0004); and from meat and dairy products, the relative risks were 1.53 (95 percent CI: 0.88, 2.66, P for trend 0.06). In this study,

the lower and upper quintiles of total α-linolenic acid intake were less than 0.37 percent and more than 0.58 percent of energy, respectively. Of note, the upper quintile of α-linolenic acid intake in the study by Leitzmann et al. is comparable to the mean α-linolenic acid intake of the U.S. population using 24-hour recall data (NHANES III), suggesting that the food frequency data reported are underestimates of actual intake. If there is an association between α-linolenic acid intake and prostate cancer risk, it likely would be seen at higher intake levels than those reported. At this time, there are insufficient data to reach a conclusion about an association between α-linolenic acid intake and risk of prostate cancer (Astorg, 2004; Attar-Bashi et al., 2004). Thus, further research is warranted to resolve this question.

Positions Taken by Other Expert Groups. WHO (2003) recommends 1 to 2 percent of energy from n-3 PUFAs. The EuroDiet Core Report, *Nutrition and Diet for Healthy Lifestyles in Europe* (2000) recommends 2 g of linolenic acid per day plus 200 mg of very long chain n-3 fatty acids per day.

EPA, DHA, and Fish

Overview. The conclusion regarding fish was reached and supported by evidence from an analysis of epidemiologic studies of the cardioprotective effects of fish consumption among healthy populations (Dolecek, 1992; Siscovick et al., 1995; Hu et al., 2002; Mozaffarian et al., 2003) and information from the evidence-based AHRQ Report *Effects of Omega-3 Fatty Acids on Cardiovascular Disease* (Wang et al., 2004). Information in *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (IOM, 2002) provided the starting point of the examination of evidence. Although α-linolenic acid can be elongated to form EPA and DHA, this conversion occurs slowly in humans and the conversion rates are incompletely understood. Thus an important source of EPA and DHA is fish that is high in these fatty acids. This is of significance because the evidence indicates that EPA and DHA are responsible for the cardioprotective effects of fish consumption. In addition, there is evidence that the nonmarine n-3 fatty acid, α-linolenic acid, also plays a cardioprotective role.

Because the biological potency of EPA and DHA is much greater than that for α-linolenic acid, the IOM (2002) did not recommend one AMDR to for the entire n-3 fatty acid class. Instead, the IOM recommended that up to 10 percent of the AMDR for α-linolenic acid can be consumed as EPA and/or DHA (0.06 to 0.12 percent of energy). No Upper Level (UL) was established for α-linolenic acid (or for the sum of EPA and DHA) mainly because of insufficient data to use the model of risk assessment to set this value. With respect to health benefits of EPA and DHA, the IOM report notes the following:

> "a growing body of literature suggests that diets higher in EPA and DHA may afford some degree of protection against CHD" (IOM, 2002, p S-6)
>
> "n-3 polyunsaturated fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) have been shown to reduce the risk of CHD and stroke by a multitude of mechanisms" (IOM, 2002, Chapter 3, pp 3–5)

After the release of the IOM Report, new evidence was published demonstrating benefits of fish consumption on CVD among U.S. populations (Hu et al., 2002; Mozaffarian et al., 2003).

Review of the Evidence. The AHRQ report *Effects of Omega-3 Fatty Acids on Cardiovascular Disease* (Wang et al., 2004) summarizes 22 prospective cohort studies that were conducted in many parts of the world including the United States, China, Japan, and countries in the Mediterranean and Northern Europe. Most of the cohorts had several thousand subjects; the range was 272 to 223,170 subjects, with most subjects at least age 40. The background diets of the study populations from other parts of the world differed from those of the U.S. population. Several of the large population studies in the United States included only males or only females, with the study duration ranging from 4 to 30 years. Most of the studies used food frequency questionnaires to estimate the dietary fish intake. Most studies provided quantitative estimates of the amount of fish consumed (many also quantified the sum of EPA and DHA intake) and categorized them into various quantiles (e.g., tertiles, quartiles, quintiles). Other studies reported only the frequency of fish consumption or simply whether fish was consumed. Despite some limitations, if viewed together, these studies provide evidence that is highly applicable to the U.S. population. Overall the evidence from the primary and secondary prevention studies supports the hypothesis that the consumption of n-3 fatty acids (EPA, DHA, α-linolenic acid), fish, and fish oil reduces all-cause mortality and various CVD outcomes. These outcomes include sudden death and cardiac death (coronary or myocardial infarct (MI) death).

The central question is, "How much fish consumption in these studies was necessary to elicit a cardioprotective effect?" Collectively, evidence from five U.S. epidemiologic studies (Albert et al., 1998; Dolocek et al., 1992; Hu et al., 2002; Siscovick et al., 2000**;** Mozaffarian et al., 2003) found that the average intake of EPA and DHA (estimated from fish consumption) associated with the lowest risk of coronary events (including CHD death, primary cardiac arrest and ischemic heart disease death) was 496 mg per day. The range of EPA and DHA intake in the studies that conferred the lowest risk was 246 mg per day to 919 mg per day. Because these estimates were derived from fish consumption, it is important to put the average of 496 mg per day of EPA and DHA per day in the context of the amount of fish consumed to achieve this level of intake. A daily intake of 496 mg of EPA and DHA is equivalent to about 3.5 g per week. This is approximately equivalent to the amount of EPA and DHA in two 4-oz. servings of high n-3 fish per week, based on an average EPA and DHA content of high n-3 fish of 1.6 g per serving (values derived from USDA database). These data provide the rationale for the recommendation for 2 servings of high n-3 fish per week.

There is some evidence that consuming more than two servings of fish per week may confer further cardioprotective effects. This was observed in the Mozaffarian et al. (2003) study that found that more than two servings of fish per week (which contributed 919 mg per day of EPA and DHA) was associated with the lowest risk for CHD. In addition, two recent meta-analyses report that fish consumption five or more times per week is associated with lower CHD mortality (He et al., 2004a) and lower incidence of stroke (He et al., 2004b). Compared with those who never consumed fish or ate fish less than once per month, the relative risks for CHD mortality were 0.89 for fish intake 1 to 3 times per month; 0.85 for once per week; 0.77 for 2 to 4 times per week; and 0.62 for 5 or more times per week (He et al., 2004a). The authors reported that for each 20 g per day increase in fish intake there was a corresponding 7 percent lower risk of CHD

mortality. Compared with no fish intake or intake less than once per month, the relative risks for total stroke were very slightly higher than those for CHD mortality at each level of fish intake (He et al., 2004b). The relative risks for ischemic stroke were lower than for total stroke: 0.69 for fish intake 1 to 3 times per month; 0.68 for once per week; 0.66 for 2 to 4 times per week; and 0.65 for 5 or more times per week (P for trend = 0.24). Thus, the optimal quantity of fish consume is not yet clear. Similarly, the AHRQ report (Wang et al., 2004) did not define the optimal quantity of n-3 fatty acids to consume because of the lack of sufficient evidence.

The AHRQ report did conclude, however, that the consumption of n-3 fatty acids from fish or from supplements of fish oil reduces all-cause mortality and various CVD outcomes. The available evidence indicates that the active dietary factor in fish is EPA and DHA. The *DART* study (Burr et al., 1989) showed that male MI survivors who consumed 200 to 400 g of n-3 rich fish per week, which provided an additional 500 to 800 mg per day of n-3 fatty acids to current intake, had the same reduction in recurrent events as did patients receiving fish oil capsules containing 900 mg per day of EPA and DHA. In addition, the *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico* (GISSI) *Prevention Study* (GISSI-Prevenzione Investigators, 1999), the largest prospective clinical trial to test the efficacy of n-3 fatty acids for secondary prevention of CHD, showed that subjects randomized to the EPA + DHA supplement group (850 mg per day of omega-3 fatty acid ethyl esters) with and without 300 mg of vitamin E per day) experienced a 15 percent reduction in the primary endpoint of death, nonfatal MI and nonfatal stroke ($P < 0.02$). In addition, all-cause mortality was reduced by 20 percent ($P = 0.01$) and sudden death was reduced by 45 percent ($P < 0.001$) compared with the control group (vitamin E provided no benefit). Further evidence to support the importance of EPA and DHA comes from the Indian Experiment of Infarct Survival (Singh et al., 1997). MI survivors who were treated with either fish oil capsules (1.8 g per day EPA + DHA) or mustard oil (2.9 g per day α-linolenic acid) for one year had fewer total cardiac events, nonfatal infarctions, arrhythmias and less left ventricular enlargement and angina pectoris than did the placebo group. Only the group treated with fish oil experienced a decrease in cardiac deaths (Singh et al., 1997). Collectively, the available evidence from the controlled clinical trials demonstrates that EPA and DHA are the bioactive compounds that elicit cardioprotective benefits. Thus, these results provide an explanation for the cardioprotective effects of fish consumption reported in the epidemiologic studies.

Although the preponderance of evidence supports a beneficial effect of fish consumption, two studies found no association between fish consumption and health outcomes (Kromhout et al., 1996). Differences in study findings likely relate to differences in the definitions of endpoints and residual confounding of reference groups with less healthy lifestyles (Guallar et al., 1999; Kromhout, 1998); variability in the endpoints studied, the experimental design, the method of estimating fish intake, and differences in the study populations (Sheard, 1998); or a small fraction of the study population reporting little or no fish consumption (Albert et al., 1998).

Fish is a good source of nutrients including protein, the B-vitamins and minerals such as potassium, phosphorous, and selenium and also is low in calories. Since fish is low in saturated fat, it provides a means to reduce saturated fat intake when substituted for foods such as red meats and full-fat dairy products.

Special Analysis. At the Committee's request, USDA's Center for Nutrition Policy and Promotion used a modeling process described in Appendix G-2 to examine how incorporating 8 ounces per week of fish (approximately twice that of current consumption) and/or fish high in n-3 fatty acids in the food intake patterns would affect the nutrient profiles of patterns ranging from 1,000 to 3,200 calories per day. First, all fish items were separated into low n-3 (LO-3) or high n-3 (HI-3) subgroups. The cutoff value specified for placement into the LO-3 or HI-3 group was 500 mg of EPA plus DHA in a 3-ounce serving of the fish. Using this approach, on average, 1 ounce of HI-3 fish (e.g. mackerel, salmon, trout) contains 407 mg of EPA+DHA, and 1 ounce of LO-3 fish (e.g. cod, haddock, snapper) contains 105 mg of EPA+DHA.

Substituting either more fish or HI-3 fish for some meat and poultry in the food intake pattern had little impact on the amounts of other nutrients provided by the food pattern. For most nutrients, no change was evident when expressed as a percentage of the RDA or AI. For iron, a decrease of 2 to 4 percent was seen in the patterns with the 8 ounces of HI-3 fish. For other nutrients, the change was only 0 to 2 percent. The change in total fat was 0 to 1 percent of calories, depending on the calorie level of the pattern.

Using the estimates from USDA's special analysis (see above), 8 ounces of fish that is high in n-3 fatty acids would provide approximately 3250 mg of EPA+DHA a week—an average of slightly less than 500 mg per day, which is about a two-fold increase over current intake (see below). Adverse effects are not observed until intake exceeds 3 g per day (62 Fed. Reg. 30,751 (June 5, 1997)).

Positions Taken by Other Expert Groups.

The American Heart Association—two servings of fish (preferably fatty) per week (Krauss et al., 2000; Kris-Etherton et al., 2002).

National Cholesterol Education Program—recommends fish as a food item for people to choose more often (NCEP, 2002; Table V.2–6).

World Health Organization—regular fish consumption (one to two servings per week; each serving should provide the equivalent of 200 to 500 mg of EPA+DHA) (WHO *Technical Report*, 2003).

European Society for Cardiology—oil fish and n-3 fatty acids have particular protective properties for primary CVD prevention (De Backer et al 2003; Priori et al. 2003; Van de Werf et al., 2003).

United Kingdom Scientific Advisory Committee on Nutrition—consume at least two portions of fish per week, of which one should be oily, and provide 450 mg per day of EPA+DHA (Scientific Advisory Committee on Nutrition, 2004).

American Diabetes Association—two to three servings of fish per week provide dietary n-3 polyunsaturated fats and can be recommended (Franz et al., 2004).

Summary

Collectively, the evidence presented above provided the basis for recommending two servings of fish per week to decrease risk of heart disease. A conservative estimate is that two servings of fish high in n-3 fatty acids per week may reduce the risk of coronary death, primarily sudden death, by as much as 30 percent (Hu et al., 2002) among adults. Fish is recommended rather than supplements because epidemiologic and some RCT data demonstrate benefits of fish; it is a good source of n-3 fatty acids and many other nutrients; and it is low in calories and saturated fatty acids (see Table D4-3, EPA+DHA content of selected fish).

n-3 Fatty Acid Intake

Based on intake data from CSFII (1994–1996, 1998), the total median n-3 fatty acid intake for men and women ranged from 1.3 to 1.8 g per day and 1.0 to 1.2 g per day, respectively (IOM, 2002, Appendix Table E-10).

Depending on age, the median intake of α-linolenic acid ranged from 1.2-1.6 g per day for men and 0.9 to 1.1 g per day for women. Estimated mean intake of α-linolenic acid, based on over 29,000 NHANES III respondents, was 1.33 g per day. This was equivalent to 0.55 percent of total energy intake per day (Wang et al., 2004, Table 3.4).

For all adults, the median intakes of EPA and DHA range from 0.004 to 0.007 g per day and 0.052 to 0.093 g per day, respectively (IOM, 2002, Appendix Tables E-12 and E-14).

Mean intake of EPA and DHA, based on analyses of a single 24-hour recall of NHANES III data, were 0.04 and 0.07 g per day, respectively. Distributions for EPA and DHA were very skewed and data on intakes should be used and interpreted with caution (Wang et al., 2004, Table 1.1).

Based on NHANES 1999–2000 data, mean intake of fish is 2.92 ounces per week (CNPP analysis, Appendix G-2). The majority of the fish consumed (63 percent) is finfish and shellfish that contain less than 500 mg of n-3 fatty acids per 3-ounce serving. The most commonly consumed single fish is tuna (representing 22 percent of total fish consumption, with shrimp (16 percent), salmon (9 percent), mixed fish (8 percent), and crab (7 percent) also commonly reported. Emphasis will need to be placed on fish high in n-fatty acids to achieve the recommendation for fish consumption.

Other sources of long chain n-3 fatty acids are currently on the market. Some are fortified with deodorized fish oil or contain algae as the source of EPA + DHA. With the pending availability of agronomic crops such as corn and soybeans that have been genetically enhanced to contain EPA and DHA, it is conceivable that vegetable oils rich in these n-3 fatty acids will become an important plant source of these fatty acids. EPA + DHA supplements may provide variable amounts of these fatty acids (Consumer Reports, 2003). The α-linolenic acid from plant sources including canola and soybean oils, walnuts and flaxseed can be converted to a limited extent (approximately 10 percent) to EPA + DHA in the body.

Supplementary Information

See the section, "Methylmercury in Fish" in Section 9, "Food Safety" for cautions regarding types of fish to avoid or to eat in limited amounts.

QUESTION 7: WHAT ARE RELATIONSHIPS BETWEEN MUFA INTAKE AND HEALTH?

Conclusion

There is an inverse relationship between the intake of MUFAs and the total cholesterol (TC):HDL cholesterol (HDL-C) concentration ratio. If equal amounts of MUFAs are substituted for saturated fatty acids, LDL-C decreases.

Rationale

Overview. This conclusion was supported by evidence from the IOM (2002) review of 19 clinical trials; the evidence-based review conducted by the Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2002); and the Committee's review of 18 more recent controlled trials.

Since humans can synthesize MUFAs from other fats and from carbohydrates, MUFAs are not required in the diet. However, MUFAs are present in virtually all fat-containing foods, and the dietary intake of MUFAs benefits human health by providing a vehicle to achieve total fat recommendations within the context of recommendations for the intakes of saturated fatty acids and PUFAs.

Implicit to a discussion of monounsaturated fats is how the level of MUFA intake affects various biological endpoints relative to intakes of other fatty acid classes. Table D4-4 illustrates some different ways that MUFAs can vary in the diet. In one scenario, MUFAs could be held constant within a constant amount of total fat, and the amount of saturated fatty acids and PUFAs would vary. Alternatively, MUFAs could vary within a constant amount of total fat, while saturated fatty acids and PUFAs vary. Or, MUFAs could be held constant while total fat and other fatty acids vary. Lastly, MUFAs and total fat could vary while saturated fatty acids and PUFAs are held constant. As shown, the MUFA content of the diet, expressed as a percentage of total calories, can vary with the percentage of energy provided by other fatty acids, the percentage of energy provided by total fat, and a combination of the two. The carbohydrate and protein as a percentage of calories can vary as well. The examples shown in the table are only a few of the many possible combinations. Thus, the biological effects of MUFAs must be studied in the context of the level of total fat (and other macronutrients) and the other fatty acid classes.

Review of the Evidence

MUFAs and Blood Lipids. Figure D4-5, below, demonstrates that an increase in MUFA intake as a percentage of total energy intake results in a decrease in the total cholesterol:HDL-cholesterol ratio (IOM, 2002). A meta-analysis of feeding studies estimated that the regression coefficients for the effects of MUFAs on LDL and HDL cholesterol concentrations were -0.008 and +0.006, respectively, suggesting a slight positive benefit (Clarke et al., 1997).

MUFAs and the Metabolic Syndrome. Recent publications reported the following effects of MUFAs on components of the metabolic syndrome:

- Ten clinical trials that replaced carbohydrates with MUFAs found that MUFAs may have benefical effects on some aspects of glycemic control (Brynes et al., 2003; Campbell et al., 1994; Garg et al., 1994, 1992; Heilbronn et al., 1999; Parillo et al., 1992; Rasmussen et al., 1993; Scott et al., 2003; Straznicky et al., 1999; Wien et al., 2003).
- Four clinical trials that replaced saturated fatty acids with MUFAs showed improvement in lipid profiles and some beneficial effects on insulin sensitivity (Heilbronn et al., 1999; Lovejoy et al., 2002; Perez-Jimenez et al., 2001; Vessby et al., 2001).

A summary of the available evidence demonstrates that, compared with a high-carbohydrate diet (greater than 65 percent of calories from carbohydrate), a diet that provides approximately 20 percent of total calories from MUFA and 35 percent from total fat improves glycemic control in individuals with type 2 diabetes mellitus who maintain their body weight. Specifically, such a diet may decrease triglyceride and increase HDL cholesterol concentrations. Individuals with elevated triglycerides or insulin levels may benefit from increasing MUFAs in the diet (by replacing some carbohydrate calories with a comparable number of calories from MUFAs). In addition, Krauss (2001) has shown that a moderate-fat diet that emphasizes MUFAs may decrease the risk of expression of the atherogenic lipoprotein phenotype (characterized by high triglycerides; low HDL cholesterol; high small-dense LDL)(Reaven, 2001). A review of 18 well-controlled clinical studies compared the effects of substituting either MUFAs or carbohydrate for saturated fat in a blood cholesterol-lowering diet (Kris-Etherton et al., 2000). Replacing saturated fatty acids with MUFAs was found to reduce total and LDL cholesterol values. Compared to baseline values, the range of serum total cholesterol concentration change was -17 to +3 percent on the low-fat/high-carbohydrate diet, whereas the range was -20 to -3 percent on the high-MUFA diet. The range of decrease in plasma LDL cholesterol concentration was similar (-22 to +1 percent) among subjects on the two diets. The change in serum triacylglycerol concentrations ranged from -23 to +37 percent for subjects consuming the low-fat/high-carbohydrate diets and from -43 to +12 percent for diets high in MUFAs. Changes in HDL cholesterol concentrations ranged from -25 to +2 percent for subjects on the low-fat/high-carbohydrate diets compared to a -9 to +6 percent change for subjects on diets high in MUFAs. These data indicate that in weight-stable individuals, a high MUFA-low saturated fatty acid diet results in a more favorable metabolic profile with respect to total cholesterol, HDL cholesterol, and triacylglycerol concentrations than the baseline diet or a low-fat/high-carbohydrate diet. The evidence is clear that replacing saturated fatty acid calories with MUFAs lowers total and LDL cholesterol levels.

Positions Taken by Other Expert Groups. Using an evidence-based approach, the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults published the following evidence statement and recommendation related to MUFAs:

> *Evidence Statement*
> Monounsaturated fatty acids lower LDL cholesterol relative to saturated fatty acids. Monounsaturated fatty acids do not lower HDL cholesterol nor raise triglycerides. Dietary patterns that are rich in monounsaturated fatty acids provided by plant sources and rich in fruits, vegetables, and whole grains and low in saturated fatty acids are associated with

decreased CHD risk. However, the benefits of replacement of saturated fatty acids with monounsaturated fatty acids has not been adequately tested in controlled clinical trials.

Recommendation

Monounsaturated fatty acids are one form of unsaturated fatty acid that can replace saturated fatty acids. Intake of monounsaturated fatty acids can range up to 20 percent of total calories. Most monounsaturated fatty acids should be derived from vegetable sources, including plant oils and nuts.

(National Cholesterol Education Program ATP III Expert Panel, 2002, p. V-10)

In addition, an evidence-based technical report of the American Diabetes Association included the following statements for MUFAs and diabetes:

> For persons with elevated plasma triglycerides, reduced HDL cholesterol, and small-dense LDL cholesterol (the metabolic syndrome), improved glycemic control, modest weight loss, dietary saturated fat restriction, increased physical activity, and incorporation of MUFAs may be beneficial.
>
> Carbohydrate and MUFA together should provide 60 to 70 percent of energy intake. However, the metabolic profile and need for weight loss should be considered when determining the monounsaturated fat content of the diet.
>
> To lower LDL cholesterol, energy derived from saturated fat can be reduced if weight loss is desirable or replaced with either carbohydrate or monounsaturated fat when weight loss is not a goal.
>
> (Franz et al., 2004, p. S39)

MUFA Intake

Based on dietary intake data from CFSII (1994-96), median MUFA intake ranged from 25 to 39 g per day for men and 18 to 24 g per day for women (IOM, 2002, Appendix Table E-8). Data from the 1987-1988 Nationwide Food Consumption Survey indicated that mean intakes of MUFAs for different age-gender groups were 13.6-14.3 percent of energy (Ganji and Betts, 1995).

In children and adolescents, MUFA intake ranged from 12.1 percent of energy for males age 2 to 3 years and 4 to 5 years to 12.9 percent of energy for males age 16 to 19 years. Among males age 12 to19 years, MUFAs accounted for 12.7 percent of calories for non-Hispanic blacks compared with 12.6 percent and 12.8 percent for non-Hispanic whites and Mexican Americans. For females age 12 to 19 years, monounsaturated fatty acid intake was 13.5 percent for non-Hispanic blacks, compared with 12.4 percent for non-Hispanic whites and 12.7 percent for Mexican Americans (Troiano et al., 2000). Thus, the collective evidence from studies that have assessed the diet of persons in the United States indicate that MUFA intake is approximately 12 to 14 percent of calories.

Supplementary Information

Most MUFAs should be derived from plant sources rather than animal sources: plant sources of MUFAs are lower in saturated fatty acids than are animal sources, and plant sources contain no

cholesterol. Also, some plant sources of MUFAs provide vitamins and other compounds that may confer health benefits.

SUMMARY

To reduce the risks of elevated serum LDL cholesterol and of CHD, the Committee recommends three measures:

1. Limiting saturated fat intake to less than 10 percent of calories
2. Limiting *trans* fat intake as much as possible
3. Limiting dietary cholesterol intake to less than 300 mg per day

To promote recommended intakes of vitamin E and essential fatty acids and to decrease the risk of adverse changes in certain blood lipids, the Committee recommends a total fat intake of at least 20 percent of calories. To help reduce the risk of obesity and CHD, the Committee recommends keeping total fat intake at or below 35 percent of calories. Current mean intakes of n-6 PUFAs are within the recommended range for essential fatty acid intake and for obtaining beneficial effects on mortality from coronary artery disease. To reduce the risk of sudden death and CHD death, the Committee recommends the consumption of fish twice weekly, especially fish that are good sources of EPA and DHA. Other sources of EPA and DHA may provide similar benefits; however, further research is warranted.

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Table D4-1. Major Food Sources of *Trans* Fat for U.S. Adults

Food Source	Percent of *Trans* Fat Supplied by the Food
• Cakes, cookies, crackers, pies, bread, etc	40
• Animal products	21
• Margarine	17
• Fried potatoes	8
• Potato chips, corn chips, popcorn	5
• Household shortening	4
• Salad dressing	3[a]
• Breakfast cereal	1
• Candy	1

Source: Based on the Food and Drug Administration's economic analysis for the final *trans* fatty acid labeling rule, *Trans* Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims (68 Fed. Reg. 41443 (July 11, 2003)).

[a] USDA analysis reported 0 grams of trans fat in salad dressing.

Table D4-2. Correlation Coefficients Between Dietary Polyunsaturated Fat (PUFA) Intake and Coronary Artery Disease Mortality and Relative Risk or Cases

Study	Years of Followup	Diet Assessment Methods	Number of Subjects	Number of Subjects With Coronary Artery Disease	PUFA Intakes Percent Energy	PUFA Correlation Coefficients	Relative Risk or cases
CROSS-POPULATION STUDIES							
Seven Countries, Study, Keys, 1970	1958–1964 (5 year)	7-day weighed record, composite analysis (subsample)	12,770	---	3 to 7 percent	NS	---
Eighteen Countries Study, Hegsted and Ausman, 1988	1954–1965, 1973	Food disappearance statistics	---	---	1.4 to 10.9 percent	-0.34	CHD mortality predicted by SFA and PUFA intake (r=0.79)
Forty Countries Study, Artraud-Wild et al., 1993	1957–1977, 1977	Food disappearance statistics	---	---	---	---	-0.33[2] After adjustment for cholesterol and SFA
Seven Countries Study, Kromhout et al., 1995	25 year	Retrospectively constructed food composites (n=498)	12,763	~1,900 deaths	3.4 to 8.6 percent	n-6: 0.0 n-3: -0.36	---
WITHIN-POPULATION STUDIES							
Puerto Rico Heart Health Program, Garcia-Palmieri et al., 1980	Prospective 6 year	24-hour recall	8,218 men	73 (rural) 213 (urban)	4 percent (rural) 6.6 percent (urban)	---	11 vs 10[9] cases 16 vs 17 cases
Three Populations Study, Gordon et al., 1981	Prospective 4year Framingham	24-hour recall	859 men	79	5.7 percent	---	5.4 vs 5.8[10]
	Prospective 6 year Honolulu Heart	24-hour recall	7,272 men	264	5.6 percent	---	6.0 vs 6.7[3]
	Prospective 6 year Puerto Rico Heart Health	24-hour recall	8,218 men	286	6.4 percent	---	5.3 vs 6.0[3]
Western Electric Study, Shekelle et al., 1981[6]	Prospective 19 year	Diet history	1,900 men	---	3.9 percent	-0.258[3]	Low—13.5[8] Mid ——10.4 High ——10.1
Honolulu Heart Prgm., McGee et al., 1984	Prospective 10 year	24-hour recall	7,088 men	456	6 percent	---	0.093[11]
Ireland-Boston Diet-Heart Study, Kushi et al., 1985	Prospective 20 y	Diet history	1,001 men	102 deaths	2.1-3.4 percent	-0.0695	---
Belgium Study, Joossens et	Longitudinal	24-hour recall	21,500 men	---	14 to 27 g/d	Men –0.73[4]	---

al., 1989	(1980–1984)		and women			Women –0.41[3]	
Framingham Study, Posner et al., 1991	Prospective 16 y	24-hour recall	420 men (44-55 y)	99	5.5 percent	0.065	RR = 1.34
Framingham Study, Posner et al., 1991	Prospective 16y	24-hour recall	393 men (≥55 y)	114	5.4 percent	0.051	RR = 1.27
ARIC Study, Tell et al., 1994	Longitudinal (1987-1989)	Semiquantitative food frequency	2,095 BF 5,146 WF 1,319 BM 4,589 WM	(Carotid artery thickness)	5 percent BF 5.1 percent WF 4.8 percent BM 5.1 percent WM	---	-0.003BF[5] -0.007 WF -0.012 BM -0.011 WM
Health Professionals Follow-up Study, Ascherio et al., 1996	Prospective 6y	Food frequency questionnaire	43,757	734 coronary events	7.6 to 15.4 g/day	---	MI = NS Fatal CHD = 0.58[12]
Nurses' Health Study, Hu et al., 1997	Prospective 14y	Semiquantitative food frequency	80,082	939 nonfatal MI or CHD death	2.9 percent 3.9 percent 4.6 percent 5.3 percent 6.4 percent	---	1.0[13] 0.94 0.88 0.81 0.68
NHLBI Family Heart Study, Djousse et al., 2001	Cross-sectional	Semiquantitative food frequency	4,584	566 were at high risk of CAD	3.97 g/d 6.76 g/d 11.68 g/d	---	1.0[14] 0.6 0.61
Jerusalem Acute MI Registry, Kark et al., 2003	Cross-sectional	Diet food frequency instrument	672	180	10 percent 90 percent had > 6 percent 25 percent had > 12 percent	NS	OR = 0.96

[1]ARIC, Atherosclerosis Risk in Communities; BF, black females; WF, white females; BM, black males; WM, white males.
[2] P < 0.05.
[3] P < 0.01.
[4] P < 0.001.
[5]Scaled difference in carotid wall thickness; p+0.056 WM.
[6]Correlation of Keys score: 0.027 (P<0.001); correlation for Hegsted equation: 0.029 (P<0.01).
[7]Correlation of Keys score: 0.025 (P<0.05); correlation for Hegsted equation: 0.01 (P<0.05).
[8]Percent of coronary death according to tertile of PUFA intakes.
[9]Non-cases vs cases.
[10]PUFA intake for noncases vs cases.
[11]Multivariate logistic coefficient for CHD.
[12]P < 0.05 for multivariate adjusted relative risk.
[13]P for trend = 0.003
[14]Prevalence odds ratio for CAD.
-Source: adapted from Cagguila and Mustad, 1997.

Table D4-3. EPA and DHA Content of Selected Types of Fish

Fish and Description	EPA+DHA per 3 oz of fish
Cod, Atlantic, cooked, dry heat	0.134
Crab, Alaska king, cooked, moist heat	0.351
Flounder, cooked, dry heat	0.426
Haddock, cooked, dry heat	0.202
Mackerel, Pacific and jack, mixed species, cooked, dry heat	1.571
Pollock, Atlantic, cooked, dry heat	0.461
Salmon, Atlantic, farmed, cooked, dry heat	1.825
Shrimp, mixed species, cooked, moist heat	0.268
Snapper, mixed species, cooked, dry heat	0.273
Trout, mixed species, cooked, dry heat	0.796
Tuna, fresh, bluefin, cooked, dry heat	1.278
Tuna, light, canned in water, drained solids	0.230
Tuna, white, canned in water, drained solids	0.733

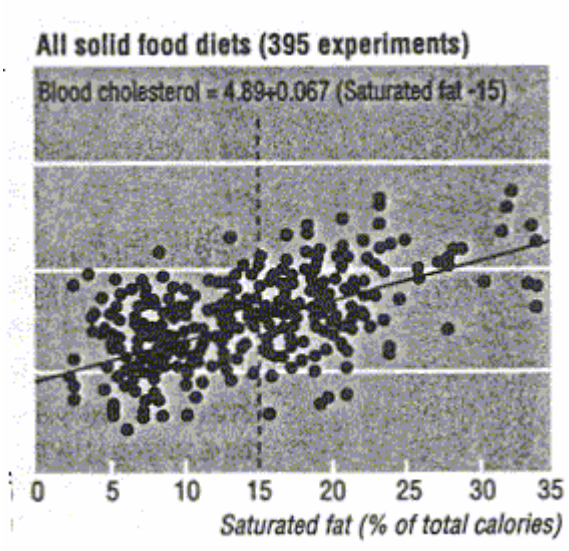
Note: For information on methylmercury in fish, refer to Section 9, “Food Safety”
Source: USDA, Center for Nutrition Policy and Promotion Analysis (Appendix G-2)

Table D4-4. Examples of the Many Varying patterns of Fats, Fatty Acids, and Carbohydrates Possible
Protein level can be determined by difference [total energy percent – (total fat percent + carbohydrate percent) = protein percent]

	Total Fat	SFA	MUFA	PUFA	CHO*
	Percent of Total Energy				
MUFA Constant; Total Fat Constant					
MUFA constant, total fat constant at 35 percent; other fatty acids vary	35	9	20	6	50
	35	5	20	10	50
MUFA constant, total fat constant at 20 percent; other fatty acids vary	20	5	10	5	65
	20	4	10	6	65
MUFA Varies; Total Fat Constant					
Total fat constant at 35 percent, MUFA vary; other fatty acids vary	35	7	23	5	50
	35	8	18	9	50
Total fat constant at 20 percent, MUFA vary; other fatty acids vary	20	7	8	5	65
	20	5	10	5	65
MUFA Constant, Total Fat Varies					
Total fat varies, MUFA constant; other fatty acids vary	33	9	14	10	52
	22	3	14	5	52
MUFA Varies, Total Fat Varies					
Total fat, CHO, & MUFA vary; other fatty acids constant	30	5	18	7	55
	20	5	8	7	65

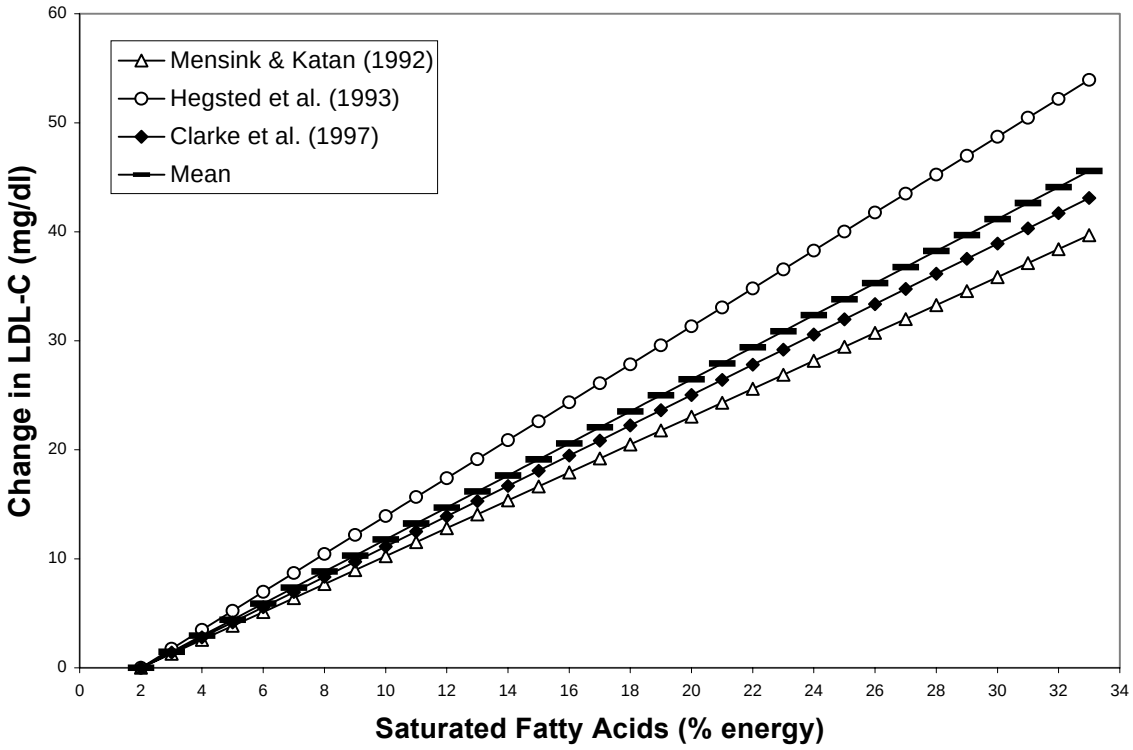
SFA = saturated fatty acids
MUFA = MUFAs
PUFA = PUFAs
CHO = carbohydrate
* Protein held constant at 15 percent of energy for these calculations. Variations in the percentage of energy from protein would change the percentage of energy from carbohydrates.

Figure D4-1. IOM Figure 8-2: Relationship Between Serum Total Cholesterol Concentrations and Saturated Fatty Acid Intake



Source: IOM (Institutes of Medicine). Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2002.

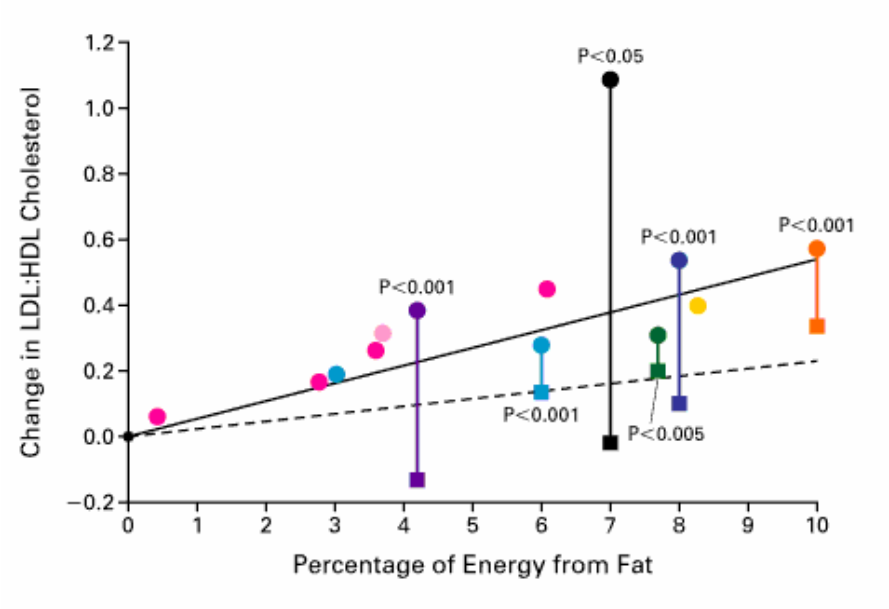
Figure D4-2. IOM Figure 8-3: Calculated Changes in Serum LDL Cholesterol Concentration in Response to Percent Change Dietary Saturated Fatty Acids



Three regression equations were used to establish the response curves. The range in saturated fatty acid intake was 2.2 to 33 percent of energy.

Source: IOM (Institutes of Medicine). Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2002.

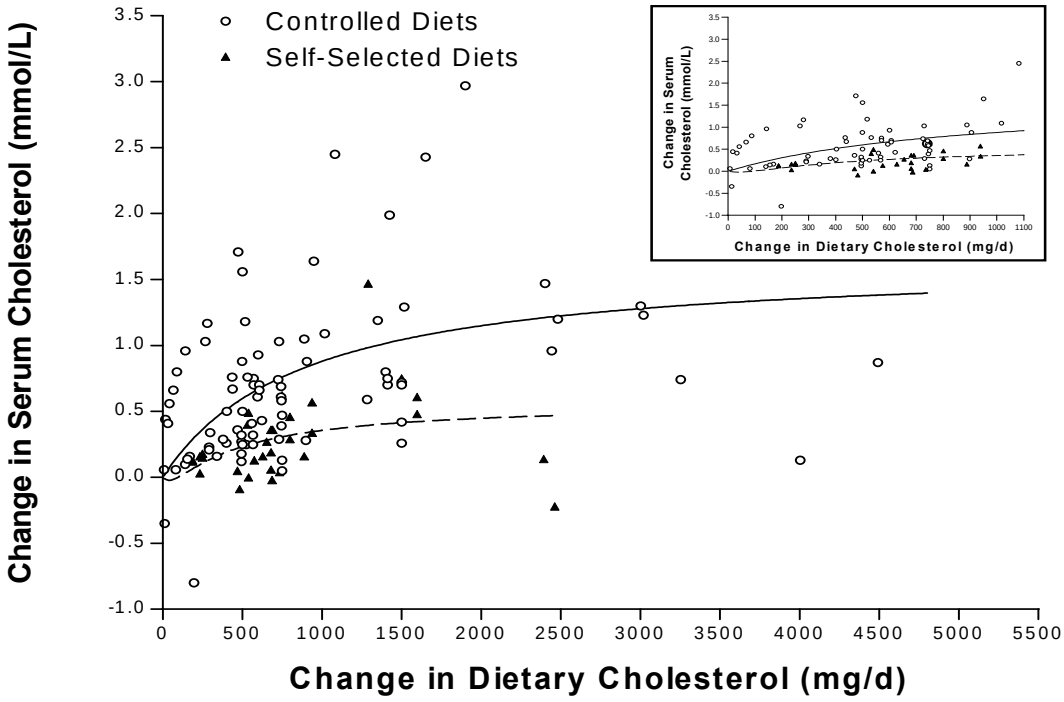
Figure D4-3. IOM Figure 8-4: Change in the LDL:HDL Cholesterol Concentrations With Increasing Energy Intake From Saturated and *Trans* Fatty Acids



Solid line represents the best-fit regression for *trans* fatty acids. Dotted line represents the best-fit regression for saturated fatty acids.

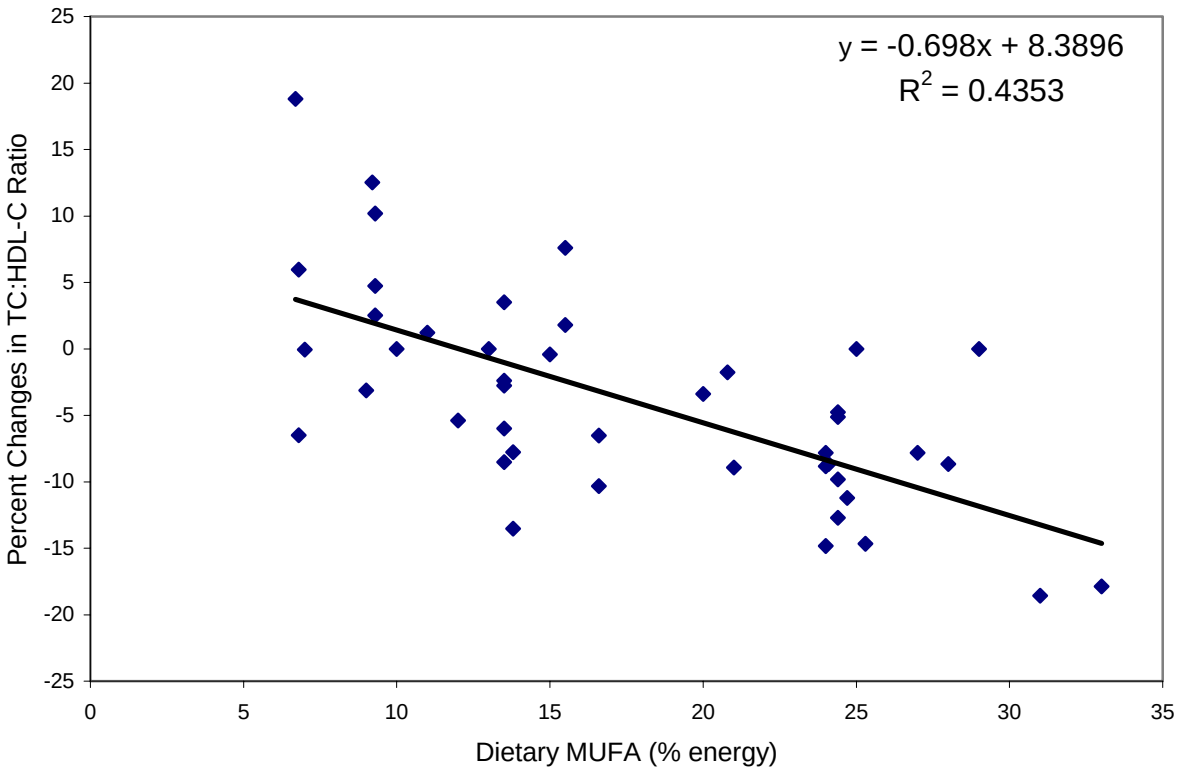
Source: IOM (Institutes of Medicine). Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2002.

Figure D4-4. IOM Figure 9-2: Relationship Between Change in Dietary Cholesterol (0 to 4500 mg/day) and Change in Serum Cholesterol Concentration.



Source: IOM (Institutes of Medicine). Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2002.

Figure D4-5. IOM Figure11-4: Relationship Between Monounsaturated Fatty Acid Intake and Total Cholesterol (TC):HDL Cholesterol (HDL-C) Concentration Ratio



Weighted least-squares regression analyses were performed using the mixed procedure to test for differences in lipid concentrations (SAS Statistical package, version 8.00, SAS Institute, Inc., 1999).

Source: IOM (Institutes of Medicine). Dietary Reference Intakes: Energy, Carbohydrates, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2002.