Part D. Section 3: Fatty Acids and Cholesterol

Introduction

Dietary fats, or lipids, are a macronutrient class that includes fatty acids, triglycerides, and cholesterol. Fats supply fuel energy (9 kcal/g) and the essential fatty acids, linoleic and alpha-linolenic acids. Fats, therefore, are a key factor in the maintenance of caloric balance and body weight. Specific fatty acids also serve as precursors for numerous biological pathways that influence inflammation, coagulation, and gene expression among other functions. Fat soluble vitamins (vitamins A, D, E, K) and carotenoids are absorbed and transported with fats.

Fatty acids are bound to glycerol as triglycerides for transport and storage in the human body. Fatty acids are heterogeneous and classified based on their chain length, the number of double bonds, the position of the first double bond from the methyl end, and a *cis* versus *trans* configuration across a double bond. These heterogeneities are important determinants of the significant variation in biological effects of the different fatty acids. Fatty acid quantity and quality also vary by their source, with important differences between meat, fish, and plant sources, as well as natural versus synthetic sources. This heterogeneity allows for food consumption choices to modulate the quantity and quality of fats that, in turn, influence metabolic and health outcomes.

Cholesterol, a sterol, is an important structural component of cell walls of tissues of the human body. Cholesterol is also a precursor for a number of steroid hormones synthesized by the adrenal glands, ovaries, and testes. Bile acids, required for solubilization and absorption of dietary fats, are synthesized from cholesterol in the liver, stored in the gallbladder and secreted into the small intestine after a fat-containing meal. Endogenous hepatic synthesis of cholesterol is adequate to produce all the cholesterol needed for these vital functions. Exogenous, or dietary, cholesterol down-regulates cholesterol synthesis in the liver to maintain cholesterol balance. Pharmacologic agents inhibit the rate-limiting step of cholesterol synthesis, catalyzed by the enzyme HMG-CoA reductase, as a means of reducing endogenous cholesterol synthesis; this also increases receptor-mediated uptake of low-density lipoprotein (LDL) cholesterol by the liver.

A critical health issue related to dietary fat is the quality of fat in the American diet. The consumption of certain fats, such as saturated fatty acids (SFA) and *trans*[1] fatty acids, is associated with a poor lipid/lipoprotein profile and increased risk of cardiovascular disease (CVD). On the other hand, the unsaturated fats, monounsaturated fatty acids (MUFA) and polyunsaturated fatty

[1] Trans fatty acids as used in this Report refers to industrial trans fatty acids and is a term consistent with that defined by the US Food and Drug Administration for use in food labeling as unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in a trans configuration produced by chemical hydrogenation (Federal Register notice. Food Labeling; Trans Fatty Acids in Nutrition Labeling; Final Rule and Proposed Rule. Vol. 68, No. 133, p. 41433-41506, July 11, 2003). Natural (or ruminant) trans fatty acids will be designated as rTFA.

acids (PUFA) have significant metabolic benefits and are health-promoting. Currently, several lines of evidence indicate that the type of fat is more important in decreasing metabolic and CVD risk than the total amount of fat in the diet. Metabolic studies have established that it is the type of fat, rather than total fat intake that affects common intermediate risk factors, such as serum lipid and lipoprotein levels (Hu, 2001). Results from controlled clinical trials and epidemiological studies have shown that replacing SFA with unsaturated fats is more effective in decreasing CVD risk than is reducing total fat intake overall (Smit, 2009). Additionally, prospective cohort studies and secondary prevention trials provide methodologically strong evidence that consumption of *n*-3 fatty acids from seafood and plant sources has a significant cardio-protective effect and decreases cardiovascular mortality (Mozaffarian, 2008; Mozaffarian and Rimm, 2006). Furthermore, dietary fat and intermediate risk factors do not affect CVD risk in a uniform way. Numerous factors influence CVD risk, including fatty acids (*n*-3 fatty acids, specific SFA, MUFA and PUFA, and *trans* fatty acids); carbohydrate quantity, type and quality; intakes of legumes, nuts, fruits, and vegetables; as well as micronutrients. For example, isocaloric substitution of dietary fat with carbohydrate can lead to increased serum triglycerides and decreased serum HDL cholesterol (Smit, 2009; Nordmann, 2006). Additionally, the effects of dietary fat, as well as the other macronutrients, and intermediate risk factors, are diverse and highly dependent on other factors such as physical activity and life style habits, and, importantly, individual genetic predisposition that is based on underlying genetic polymorphisms.

The issue of excess dietary cholesterol is also of public health concern. Traditionally, because dietary cholesterol has been shown to raise LDL cholesterol and high intakes induce atherosclerosis in observational studies, the prevailing recommendation has been to restrict dietary cholesterol intake, including otherwise healthy foods such as eggs. The potential negative effects of dietary cholesterol are relatively small compared to those of SFA and *trans* fatty acids (Clarke, 1997; Howell, 1997). A further important consideration is significant variation in the population in individual responses to cholesterol intake; differences in susceptibility are likely based on well-characterized genetic polymorphisms in several genes encoding enzymes, apolipoproteins, receptors, and transporters involved in lipid metabolism and storage. The underlying genetic polymorphisms are manifested as individuals who are "hyper-responders" and "hypo-responders" referring to those who respond to cholesterol intake with elevated serum LDL cholesterol and those who, at the same level of cholesterol intake, do not exhibit increased serum LDL cholesterol, respectively.

This section of the 2010 DGAC report continues with brief explanations on the types of fats and cholesterol and food sources of these nutrients, a discussion of trends in fat and cholesterol intakes in the American diet, and contextual information on recommended intakes and health outcomes. The chapter then provides NEL systematic evidence-based reviews of 11 questions on a variety of issues related to fats, cholesterol, and health.

Background on Fats and Cholesterol

Types and Food Sources of Fatty Acids and Cholesterol

Fatty acids and cholesterol are a diverse group of compounds that are found across a wide variety of foods consumed by Americans. The following sections provide additional information on the specific fatty acids and common food sources in the diet.

Saturated Fatty Acids (SFA)

Saturated fatty acids are linear carbon chain molecules with each carbon fully saturated with hydrogen atoms and, therefore, containing no double bonds. Like all fatty acids, SFA have a methyl end and a carboxyl end with varying even number of carbons in between. Due to this configuration, their melting point is high and they are solid at room temperature. The major types of SFA in the American diet are lauric (C12), myristic (C14), palmitic (C16) and stearic (C18) acids. Palmitic and stearic acids are major constituents of animal fats, but plant sources, such as coconut, palm, cocoa, and shea nut oils, are also sources of SFA. Cholesterol-raising SFAs, considered SFA minus stearic acid (discussed below), down-regulate the low density lipoprotein (LDL) receptor by increasing intracellular cholesterol pools and decreasing LDL cholesterol uptake by the liver. The foods that contribute the most saturated fat to the diets of Americans are listed in Table D3.1.

Table D3.1. Food sources of saturated fat by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Regular cheese	8.5	8.5
Pizza	5.9	14.4
Grain-based desserts	5.8	20.2
Dairy desserts	5.6	25.8
Chicken and chicken mixed dishes	5.5	31.2
Sausage, franks, bacon, and ribs	4.9	36.2
Burgers	4.4	40.5
Mexican mixed dishes	4.1	44.6
Beef and beef mixed dishes	4.1	48.7
Reduced fat milk	3.9	52.6
Pasta and pasta dishes	3.7	56.3
Whole milk	3.4	59.7
Eggs and egg mixed dishes	3.2	62.9
Candy	3.1	66.0
Butter	2.9	68.9
Potato/corn/other chips	2.4	71.3
Nuts/seeds and nut/seed mixed dishes	2.1	73.4
Fried white potatoes	2.0	75.4

Source: Sources of Saturated Fat Among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Monounsaturated Fatty Acids

MUFA have one site of unsaturation between neighboring carbon atoms, constituting a single double bond; this chemical property lowers their melting point so that MUFA are liquid at room temperature. MUFA are beneficial in that they increase esterification of cholesterol in the liver, thereby reducing the free cholesterol pool and increasing receptor-mediated uptake of LDL cholesterol, resulting in a decrease in blood cholesterol levels. Oleic acid (18:1), a MUFA common in the diet, is a major constituent of certain vegetable oils (e.g., olive, canola) but is present in many other foods such as nuts, meat and poultry. The foods that contribute the most oleic acid to the diets of Americans are listed in Table D3.2.

Table D3.2. Food sources of oleic acid by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Grain-based desserts	8.9	8.9
Chicken and chicken mixed dishes	7.6	16.6
Sausage, franks, bacon, and ribs	5.9	22.5
Nuts/seeds and nut/seed mixed dishes	5.5	27.9
Pizza	5.4	33.3
Fried white potatoes	4.9	38.2
Mexican mixed dishes	4.6	42.8
Burgers	4.1	46.9
Beef and beef mixed dishes	3.9	50.8
Eggs and egg mixed dishes	3.5	54.3
Regular cheese	3.3	57.5
Potato/corn/other chips	3.2	60.7
Pasta and pasta dishes	3.1	63.8
Salad dressing	2.6	66.4
Dairy desserts	2.3	68.7
Yeast breads	2.2	70.9

Source: Sources of Oleic Acid Among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Polyunsaturated Fatty Acids

PUFA, which have two or more sites of unsaturation (double bonds), are a heterogeneous class of fatty acids with chain length and position of the first double bond affecting important metabolic outcomes. The double bonds contribute to the lower melting point, making PUFA liquid at room temperature. Certain PUFA cannot be synthesized by the human body, but are required in small amounts as substrates for biological pathways that generate metabolic products required for structural and functional purposes. These PUFA are referred to as essential fatty acids and must be

attained from the diet. Both linoleic acid (LA) (C18:2), an n-6 PUFA, and alpha-linolenic acid (ALA) (C18:3), an n-3 PUFA, are essential fatty acids in the diet.

The first double bond in n-6 (omega-6) PUFA is at the sixth carbon from the methyl end. These PUFA are largely derived from vegetable oils such as corn, sunflower, safflower, and soybean oils, but are present in other foods as well. The foods that contribute the most *n*-6 PUFA to the diets of Americans are listed in Table D3.3.

Table D3.3. Food sources of total *n*-6 fatty acids (18:2 + 20:4) by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Chicken and chicken mixed dishes	9.5	9.5
Grain-based desserts	7.4	16.9
Salad dressing	7.3	24.3
Potato/corn/other chips	6.9	31.2
Nuts/seeds and nut/seed mixed dishes	6.4	37.6
Pizza	5.3	42.9
Yeast breads	4.5	47.4
Pasta and pasta dishes	3.5	54.4
Fried white potatoes	3.5	50.9
Mexican mixed dishes	3.3	57.7
Mayonnaise	3.1	60.8
Quickbreads	3.0	63.8
Eggs and egg mixed dishes	2.9	66.7
Popcorn	2.6	69.2
Sausage, franks, bacon, and ribs	2.1	71.4

Source: Sources of *n*-6 PUFA Among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

The first double bond in *n*-3 (omega-3) PUFA is at the third carbon from the methyl end. *n*-3 PUFA are often subcategorized based on their plant or marine sources. ALA is an essential fatty acid from plant sources, such as soybean oil, canola oil, flaxseed, and walnuts. The foods that contribute the most ALA to the diets of Americans are listed in Table D3.4. ALA is poorly converted to long-chain *n*-3 PUFA, primarily docosahexaenoic acid (DHA), so increased intake of ALA does not substantially improve levels of DHA. The long-chain *n*-3 PUFA, eicosapentaenoic acid (EPA) and DHA, which are frequently called "marine oils," originate from marine phytoplankton and are found in seafood. Fish species vary considerably in their EPA and DHA content (IOM Seafood Choices, 2006). The cold water, oily fish (e.g., salmon, trout) have the highest levels and EPA and DHA. As described below, these long-chain *n*-3 PUFA have distinct properties, with evidence that EPA and DHA decrease adult CVD risk, and DHA provides benefits for infant neurodevelopment (see

Questions 7 and 9). The foods that contribute the most EPA and DHA to the diets of Americans are listed in Table D3.5.

Table D3.4. Food sources of alpha-linolenic Acid (ALA) by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Salad dressing	10.5	10.5
Grain-based desserts	6.1	16.6
Pizza	5.8	22.4
Chicken and chicken mixed dishes	5.4	27,8
Yeast breads	5.0	33.9
Mayonnaise	4.0	37.9
Pasta and pasta dishes	3.5	41.4
Quickbreads	3.4	44.9
Fried white potatoes	2.8	47.7
Nuts/seeds and nut/seed mixed dishes	2.7	50.4
Mexican mixed dishes	2.7	53.1
Regular cheese	2.6	55.7
Margarine	2.6	58.3
Burgers	2.6	60.8
Eggs and egg mixed dishes	2.2	63.0
Whole Milk	2.2	65.2
Dairy desserts	2.2	67.4
Other fish and fish mixed dishes	2.0	69.4

Source: Sources of ALA the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Table D3.5. Food sources of EPA and DHA by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Other fish and fish mixed dishes	53.1	53.1
Chicken and chicken mixed dishes	13.8	66.9
Shrimp and shrimp mixed dishes	12.9	79.8
Eggs and egg mixed dishes	5.8	85.6
Tuna and tuna mixed dishes	5.3	91.0

Source: Sources of EPA and DHA Among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Trans Fatty Acids

Trans fatty acids are unsaturated fatty acids that contain a double bond that is in the *trans* configuration, produced by a process referred to as hydrogenation. Hydrogenation has been used by food manufacturers to raise the melting point of PUFA to make products that are solid at room temperature and more resistant to spoilage or becoming rancid. Partial hydrogenation adds hydrogen to PUFA double bonds, thereby increasing the degree of saturation. However, this does not result in 100 percent saturation and one or more of the remaining double bonds are isomerized from a *cis* to *trans* configuration. *Trans* fats produced this way are referred to as synthetic or industrial *trans* fatty acids (iTFA) and are used in margarines, snack foods, and prepared desserts. Elaidic acid (t9-C18:1) is the predominant *trans* fatty acid found in processed fats. *Trans* fatty acids also are produced in smaller amounts in the rumen of grazing animals and are termed natural or ruminant *trans* fatty acids (rTFA). Industrial and ruminant *trans* fatty acids vary in the location of the *trans* double bonds, and whether they differ in metabolic effects and health outcomes is a matter of debate (see Question 6). The presence of rTFA makes it difficult to totally eliminate *trans* fatty acids from the diet without eliminating dairy products and red meats.

Dietary Cholesterol and Plant Sterols/Stanols

Cholesterol is a sterol, i.e., a steroid-based alcohol with a hydrocarbon side-chain. Cholesterol has both hydrophilic properties, due to its hydroxyl end, and hydrophobic properties, due to its hydrocarbon side-chain. Therefore, it is commonly found in the lipid bilayer of cell membranes. The major sources of cholesterol in the American diet are egg yolks, dairy products, and meats. The foods that contribute the most cholesterol to the diets of Americans are listed in Table D3.6. Dietary cholesterol, found in cell walls of animal tissues, should be differentiated from plant sterols and stanols that are naturally occurring substances found in plants. These compounds compete with dietary and biliary cholesterol for sites on micelles and transport proteins, resulting in reduced cholesterol absorption. Plant sterols and stanols are absorbed across the epithelial barrier of the intestine but are pumped back into the lumen by ATP-binding cassette transporters. Although plant sterols/stanols are available as dietary supplements (not discussed here), they likely play a role in the cholesterol-lowering effect of plant-based diets.

Table D3.6. Food sources of cholesterol by percent contribution to intake based on National Health and Nutrition Examination Survey, 2005-2006

Food Item	Contribution to intake %	Cumulative contribution %
Eggs and egg mixed dishes	24.6	24.6
Chicken and chicken mixed dishes	12.5	37.1
Beef and beef mixed dishes	6.4	43.6
Burgers	4.6	48.2
Regular cheese	4.2	52.4
Sausage, franks, bacon, and ribs	3.9	56.3
Other fish and fish mixed dishes	3.4	59.7
Grain-based desserts	3.3	63.0
Dairy desserts	3.2	66.3
Pasta and pasta dishes	3.1	69.3
Mexican mixed dishes	2.9	75.1
Pizza	2.9	72.2
Cold cuts	2.7	77.8
Reduced fat milk	2.5	80.3
Pork and pork mixed dishes	2.3	82.6
Shrimp and shrimp mixed dishes	2.0	84.6

Source: Sources of Cholesterol Among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Trends in Fat and Cholesterol Intakes in the American Diet in Relation to Previous US Dietary Guidelines Recommendations

The relationship between dietary saturated fat, *trans* fat and cholesterol and deleterious health outcomes at the population level has long been recognized, with recommendations for modification of total fat, SFA, and cholesterol dating back to the 1980 Guidelines (Table D3.7). The recommendation for keeping trans fats as low as possible appeared in the 2005 DGA. As evidence accumulated, the restriction of SFA to less than 10 percent of energy first appeared in the 1990 Guidelines and the restriction of dietary cholesterol to less than 300 mg per day appeared in the 1995 Guidelines. Recommendations related to total fat generally restricted consumption to less than 30 percent of energy. However, in the 2002 IOM report on macronutrient requirements there was the adoption of an AMDR of fat intake of 20-35 percent of calories because there were no clear differences in health outcomes in populations consuming dietary fat within this range. Thus, the 2005 US Dietary Guidelines adopted this range of percent energy from total fat.

Table D3.7. Quantitative advice related to dietary fat, Dietary Guidelines for Americans, 1980-2005

	1980	1985	1990	1995	2000	2005
Total fat	Avoid too much	Avoid too much	≤30%	≤30%	≤30%	20-35%[1]
Saturated fat	Avoid too much	Avoid too much	≤10%	≤10%	≤10%	≤10%
Cholesterol	Avoid too much	Avoid too much	Low	≤300mg	≤300 mg	≤300 mg

Note: [1]30-35% for ages 2-3 years; 25-35% for ages 4-18 years.
Source: DGA 1980-2005.

Despite the consistency of advice, a comparison of the recommendations to trends in the American diet over the same period of time shows no reduction in the intake of total fat, SFA, or cholesterol. Tables D.3.8 and D.3.9 show USDA estimates from large samples of the US population on consumption of fats and cholesterol, beginning with the Nationwide Food Consumption Survey in 1977-78 through the most recent National Health and Nutrition Examination Surveys (NHANES) in 2005-2006.

Sampling methods, data collection methods, dietary survey instruments, and food composition databases can vary from one survey to the next (Guenther, 1994). Especially problematic is detecting changes in macronutrient distributions, that is, the percentages of calories that come from carbohydrate, fat, protein, and alcohol. Nonetheless, trends in the estimates can be informative about US dietary intakes over time. Table D3.8 shows a modest increase in total fat intake reported from the early 1990s, yet there was a decrease in the percent of energy from fat over the three decades covered in the table. Over this same time period there was an increase in total energy intake, driven mostly by an increase in total carbohydrate intake. Given the onset of a national epidemic of obesity over this time period, it is unlikely that total fat alone was an important contributory factor.

Dietary cholesterol intake has been stable over time, reaching and exceeding the Guideline target of less than 300 mg/day for men. It should be noted that cholesterol intake of men and women varied greatly, with average male consumption of cholesterol exceeding recommended levels and virtually unchanged at 350 mg/day since 2000, in contrast to levels of 240 mg/day for women over this period.

Table D3.9 shows the percent of calories from fat as unchanged since 1990, with mean SFA at 11 to 12 percent energy (above recommended 10%) and unchanged for the past 15 years. Similarly, levels of MUFA (12%) and PUFA (7%) have been stable over this time. Sex-specific data show no major differences in SFA, MUFA, and PUFA intake between men and women (for detailed tables, see http://www.ars.usda.gov/ba/bhnrc/fsrg).

Table D3.8. Intake of fats (grams/day) and cholesterol (mg/day), USDA national surveys of all persons in US, 1977-2006

Dietary component	NFCS 1977-78 (n=~30,000)[1] Mean (SE)[4]	CSFII 1989-91 (n=15,128)[1] Mean	CSFII 1994-96 (n=15,968)[2] Mean (SE)	NHANES 2001-02 (n= 9,033)[3] Mean (SE)	NHANES 2003-04 (n=8,273)[3] Mean (SE)	NHANES 2005-06 (n= 8,549)[3] Mean (SE)
Total fat (g)	84.6 (0.83)	71.8	74.4 (0.7)	81.0 (0.54)	82.7 (0.71)	81.9 (1.35)
SFA (g)	NA[6]	25.7	25.6 (0.3)	26.7 (0.25)	27.7 (0.24)	27.8 (0.49)
PUFA (g)	NA	13.8	14.6 (0.2)	16.1 (0.13)	17.2 (0.25)	17.0 (0.31)
MUFA g)	NA	26.7	28.6 (0.3)	30.1 (0.22)	31.0 (0.29)	30.1 (0.48)
Cholesterol (mg)	NA	270	256 (3)	273 (2.7)	273 (4.6)	278 (3.3)

Sources: Published USDA, ARS reports What We Eat In America-National Health and Nutrition Examination Surveys (NHANES), Continuing Surveys of Food Intakes by Individuals (CSFII), and Nationwide Food Consumption Survey (NFCS), 1 day data.
[1]Includes all persons from birth.
[2]Includes all persons from birth; excludes breast-fed children.
[3]Includes persons 2 yrs and over; excludes breast-fed children.
[4]SE= Standard error.
[5]Unpublished data from Food Surveys Research Group, ARS, USDA.
[6]NA = Not available.
This table is available at: http://www.ars.usda.gov/ba/bhnrc/fsrg.

Table D3.9. Intake of fats as percent of energy, USDA national survey of all persons in US, 1977-2006

Dietary component	NFCS 1977-78 (n=~30,000)[1] Mean (SE)[4]	CSFII 1989-91 (n=15,128)[1] Mean	CSFII 1994-96 (n=15,968)[2] Mean (SE)	NHANES 2001-02 (n=9,033)[3] Mean (SE)	NHANES 2003-04 (n=8,273)[3] Mean (SE)	NHANES 2005-06 (n= 8,549)[3] Mean (SE)
Total Fat (%)	40.1 (0.16)	34.4	32.8 (0.1)	33 (0.3)	33.4 (0.25)	33.6 (0.19)
SFA (%)	NA[3]	12.3	11.3 (0.1)	NA[6]	11.2 (0.11)	11.4 (0.09)
PUFA (%)	NA	6.6	6.4 (0.01)	NA	7.0 (0.09)	7.0 (0.08)
MUFA (%)	NA	12.7	12.5 (0.1)	NA	12.5 (0.09)	12.3 (0.07)
Energy (kcal)	1854 (12.9)	1839	2002 (16)	2178 (16.1)	2195 (15.6)	2157 (29.0)

Sources: Published USDA, ARS reports What We Eat In America-National Health and Nutrition Examination Surveys (NHANES), Continuing Surveys of Food Intakes by Individuals (CSFII), and Nationwide Food Consumption Survey (NFCS), 1 day data.
[1]Includes all persons from birth.
[2]Includes all persons from birth; excludes breast-fed children.
[3]Includes persons 2 yrs and over; excludes breast-fed children.
[4]SE= Standard error.
[5]Unpublished data from Food Surveys Research Group, ARS, USDA.
[6]NA = Not available.
This table is available at: http://www.ars.usda.gov/ba/bhnrc/fsrg.

Recommended Intakes and Health Outcomes Related to Dietary Fat and Cholesterol

In the 2002 report *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (IOM, 2002), the Institute of Medicine (IOM) did not establish either an Adequate Intake (AI) or Recommended Dietary Allowance (RDA) for total fat intake. Rather, an Acceptable Macronutrient Distribution Range (AMDR) of 20 - 35 percent of energy was established for total fat consumption for adults. Furthermore, the IOM did not set a tolerable Upper Intake Level (UL) for total fat because available evidence was insufficient to define a level at which adverse

outcomes, such as obesity, occur. However, for SFA, although there is also no UL, the rationale was that there is no incremental level of SFA intake that does not incrementally increase CVD risk.

For dietary cholesterol, because cholesterol can be synthesized endogenously in sufficient amounts for metabolic and structural needs, there is no evidence for a dietary requirement for cholesterol; therefore, there is no AI, RDA, or AMDR for cholesterol. Similar to SFA, there is no UL set for dietary cholesterol. It should be noted, however, that both SFA and cholesterol are unavoidable in omnivorous diets, and attempts to reduce intake completely would require significant changes to dietary patterns and introduce undesirable effects, such as inadequate intakes of micronutrients and protein.

Given the state-of-the-art of our current knowledge regarding dietary fat and health, the DGAC 2010 has addressed the following questions for application to US public health:

List of Questions

THE INFLUENCE OF DIETARY FATS ON CARDIOVASCULAR DISEASE (CVD) AND OTHER HEALTH OUTCOMES

1. What is the effect of saturated fat intake on increased risk of cardiovascular disease or type 2 diabetes, including effects on intermediate markers such as serum lipid and lipoprotein levels?
2. What is the effect of dietary cholesterol intake on risk of cardiovascular disease, including effects on intermediate markers such as serum lipid and lipoprotein levels and inflammation?
3. What is the effect of dietary intake of MUFA when substituted for SFA on increased risk of cardiovascular disease and type 2 diabetes, including intermediate markers such as lipid and lipoprotein levels and inflammation? And what is the effect of replacing a high carbohydrate diet with a high MUFA diet in persons with type 2 diabetes?
4. What is the effect of dietary intake of *n*-6 PUFA on risks of cardiovascular disease and type 2 diabetes, including intermediate markers such as lipid and lipoprotein levels and inflammation?

SPECIFIC FATTY ACIDS THAT AFFECT PLASMA LDL, HDL, AND NON-HDL CHOLESTEROL LEVELS

5. What are the effects of dietary stearic acid on LDL cholesterol?
6. What effect does consuming natural (ruminant) versus synthetic (industrially hydrogenated) *trans* fatty acids have on LDL-, HDL- and non HDL cholesterol levels?

RELATIONSHIPS BETWEEN CONSUMPTION OF *N*-3 FATTY ACIDS AND HEALTH OUTCOMES

7. What is the relationship between consumption of seafood *n*-3 fatty acids and risk of CVD?
8. What is the relationship between consumption of plant *n*-3 fatty acids and risk of CVD?

9. What are the effects of maternal dietary intake of *n*-3 fatty acids from seafood on breast milk composition and health outcomes in infants?

CARDIOVASCULAR HEALTH EFFECTS RELATED TO CONSUMPTION OF SPECIFIC FOODS HIGH IN FATTY ACIDS

10. What are the health effects related to consumption of nuts?
11. What are the health effects related to consumption of chocolate?

Methodology

The DGAC 2010 first reviewed the 2005 DGAC Report to inform their review process. Several lines of evidence indicate that the type of fat is more important in decreasing metabolic and CVD risk than the total amount of fat in the diet; therefore, the committee focused their review on the metabolic effect of specific types of fats and fatty acids. (Questions related to the effect of macronutrient distribution in the diet are found in ***Part D. Section 1: Energy Balance and Weight Management***.) Topics in this section on fatty acids and cholesterol that were considered by the 2005 DGAC include: saturated fat (SFA) (Question 1), cholesterol (Question 2), monounsaturated fatty acids (MUFA) (Question 3), *n*-6 polyunsaturated fatty acids (PUFA) (Question 4), stearic acid (Question 5), *trans* fatty acids (Question 6), *n*-3 fatty acids from seafood (Question 7) and plants (Question 8). New questions considered by the 2010 DGAC examined maternal intake of *n*-3 fatty acids from seafood and the effect on breast milk composition and infant health (Question 9) and health effects related to consumption of nuts (Question 10) and chocolate (Question 11).

Full NEL evidence-based reviews were conducted on Questions 1-6, 9, and 11 whereas, a combination of NEL and American Dietetic Association's (ADA) Evidence Analysis Library reviews were conducted for Questions 7, 8 and 10 (described below). A description of the NEL evidence-based systematic review process is provided in ***Part C: Methodology***. Additional information about the search strategy and articles considered and included for each question can be found at www.nutritionevidencelibrary.com. To address several issues about the feasibility and desirability of potential 2010 DGAC recommendations related to cholesterol (Question 2), stearic acid and cholesterol-raising (CR) fatty acids (Question 5) and seafood (Question 7), the committee conducted several modeling exercises using the USDA food intake patterns. Summaries of these analyses are presented here and a description of the approach used is described in ***Part C: Methodology***. The full modeling analyses reports can be found online at www.dietaryguidelines.gov.

For Question 1 on SFA effects on CVD risk and Questions 3 and 4 on MUFA and *n*-6 PUFA, the conclusions expressed in the 2010 DGAC report are informed by evidence compiled for the 2005 DGAC report, but are based primarily on NEL evidence gathered and reviewed since 2004. As

described in the Review of Evidence section, for some questions, the search was extended back further to capture a larger body of evidence, particularly related to diabetic-risk populations. Conclusions to Question 1 on SFA effects on T2D risk, Question 5 on stearic acid, Question 6 on *trans* fatty acids, Question 9 on maternal *n*-3 fatty acid intake, and Question 11 on chocolate are based on literature published since 2000. Although Questions 3 and 4 on MUFA and *n*-6 PUFA did not go back to 2000, the results from Question 1 on SFA and T2D risk also strengthen the evidence for these questions, as SFA was replaced by MUFA or PUFA. The conclusion to Question 2 on dietary cholesterol is based on literature published since 1999. Results of a NEL search since 2004 for question 7 on seafood are supplemented by the findings of an earlier evidence review conducted by the ADA Evidence Analysis Library on health benefits related to consumption of fish or fish-derived *n*-3 fatty acids, covering the literature published from 2004 to 2007 (http://www.adaevidencelibrary.com). Question 8 on plant-derived *n*-3 fatty acids is also based on this earlier systematic review conducted by the ADA that included health benefits related to consumption of plants or plant-derived *n*-3 fatty acids. The NEL updated this search from 2007 to 2009 for this question. The review for Question 10 on nuts was also informed by a previous review conducted by the ADA on almonds that covered the literature published from 2001 through 2004 (http://www.adaevidencelibrary.com).

Prior DGAC made recommendations about dietary fat consumption targeting atherosclerotic CVD as the primary disease of concern. The 2010 DGAC continues this focus, but considered additional disease outcomes and intermediate markers of these outcomes. Atherosclerotic CVD includes coronary heart disease (with major clinical presentations as angina pectoris, acute myocardial infarction, or sudden cardiac death), atherothrombotic stroke, and peripheral arterial disease. Type 2 diabetes (T2D), as affected by dietary fat, is a new consideration for the 2010 DGAC. In contrast to CVD, T2D is clearly increasing in prevalence and incidence. T2D is a strong risk factor for atherosclerotic disease, but also carries a high burden of disability and healthcare costs, with diabetic nephropathy, retinopathy, and neuropathy as major sequelae. Because of this, T2D and T2D risk were included as disease outcomes related to fatty acid and cholesterol consumption.

The relationships of fatty acids or cholesterol to various cancers were also considered but have very recently been reviewed by the World Cancer Research Fund/American Institute for Cancer Research Report (WCRF/AICR, 2007). The evidence regarding cancer is less conclusive than that related to CVD and T2D. Population-wide recommendations, therefore, have been driven by the public health impact of CVD and T2D.

A series of intermediate markers have been examined because of their strong etiologic association with atherosclerotic CVD and T2D, and their use as outcomes in prospective studies and randomized clinical trials. These measures include blood lipids and lipoproteins, glucose intolerance, insulin resistance, blood pressure, and biomarkers of inflammation. These intermediate markers are

linked to risk of both CVD and T2D, as indicators of altered metabolism. This is manifested most clearly by metabolic syndrome that is clinically characterized by five criteria: blood pressure, waist circumference, fasting triglyceride levels, HDL cholesterol and fasting blood glucose. Metabolic syndrome is considered an intermediate stage in the progression to full-blown T2D.

For each of the NEL review questions in this chapter, the following general criteria applied. Study designs included systematic reviews, meta-analyses, randomized controlled trials, prospective cohort studies and case-control studies. Research was conducted in developed nations and participants were healthy adults and those at elevated risk of chronic disease, including CHD/CVD and T2D, with related conditions including hyperlipidemia, insulin resistance, and associated metabolic disturbances. Study participants with CVD were included in Questions 7 and 8, and individuals with T2D were included in Question 1 to 4. Pregnant and lactating women and infants were included in the review of the literature related to maternal intake of DHA and infant health outcomes.

THE INFLUENCE OF DIETARY FATS ON CARDIOVASCULAR DISEASE (CVD) AND OTHER HEALTH OUTCOMES

The 2005 DGAC addressed the issue of total fat intake as a determinant of major health outcomes, body weight, blood lipid concentrations, and other metabolic parameters, based on the IOM report *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (IOM, 2002). Based on this review, the recommendation was to avoid very low fat diets (<20% of energy from fat) to reduce the risk of inadequate intakes of fat-soluble vitamins and the essential fatty acids, LA and ALA. The 2005 DGAC also recommended avoidance of very high fat diets (>35% of energy from fat), as such diets are associated with increased caloric intake and related weight gain. Therefore, total fat intake of 20 to 35 percent of calories was recommended for adults, 25 to 35 percent for children ages 4 to 18 years, and 30 to 35 percent for children ages 2 to 3 years. Since the 2005 DGAC report, there has been little evidence in adults to contradict this as a healthy range of total fat as percent of calories. The issue of children, ages 2 to 18 years, is more challenging to evaluate because of the limited number of studies and the difficulty in tracking and documenting diet in this age group. Pediatric guidelines are currently under review by the National Heart, Lung, and Blood Institute (NHLBI).

Most studies with higher percentage of energy from fat also include higher levels of SFA both in absolute units and in percent of energy. The 2010 DGAC, therefore, has focused on the quality of fats within the 20 to 35 percent AMDR range. Because there are major etiologic links between dietary consumption of fats or cholesterol and cardiovascular disease, lipids and lipoproteins are important intermediate markers in the study of dietary fats and cholesterol. In keeping with the 2010

DGAC's focus on a broader range of intermediary and disease outcomes, the following questions were considered for evidence-based analysis.

Question 1. What is the Effect of Saturated Fat Intake on Increased Risk of Cardiovascular Disease or Type 2 Diabetes, Including Effects on Intermediate Markers such as Serum Lipid and Lipoprotein Levels?

Conclusion

Strong evidence indicates that intake of dietary SFA is positively associated with intermediate markers and end point health outcomes for two distinct metabolic pathways: 1) increased serum total and LDL cholesterol and increased risk of CVD and 2) increased markers of insulin resistance and increased risk of T2D. Conversely, decreased SFA intake improves measures of both CVD and T2D risk. The evidence shows that 5 percent energy decrease in SFA, replaced by MUFA or PUFA, decreases risk of CVD and T2D in healthy adults and improves insulin responsiveness in insulin resistant and T2D individuals.

Implications

As the evidence indicates that a 5 percent energy decrease in SFA, replaced by MUFA or PUFA, results in meaningful reduction of risk of CVD or T2D, and given that in the US population 11-12 percent of energy from SFA intake has remained unchanged for over 15 years, a reduction of this amount resulting in the goal of less than 7 percent energy from SFA should, if attained, have a significant public health impact. As an interim step toward this less than 7 percent goal, all individuals should immediately consume less than 10 percent of energy as saturated fats. This impact would not only be limited to a reduction in heart disease and stroke, but also in T2D, a disease currently rising in incidence and prevalence. This substitution of MUFA and PUFA for SFA assumes no change in energy intake. The age of onset of T2D is substantially younger than that of CVD and increasingly frequent in adolescence. Reduction in SFA in children and young adults may provide benefits decades earlier than currently appreciated. The growing data to support a risk of T2D from SFA consumption supports the need for fat-modified diets in persons with pre-diabetes, including those with metabolic syndrome, and those with established diabetes. Early signs of atherosclerotic CVD are also seen in children and a number of studies indicate that the atherosclerotic process begins in childhood and is affected by high blood cholesterol levels. Therefore, reduction in SFA in children and young adults may provide benefits decades earlier than currently appreciated relative to both CVD and T2D incidence.

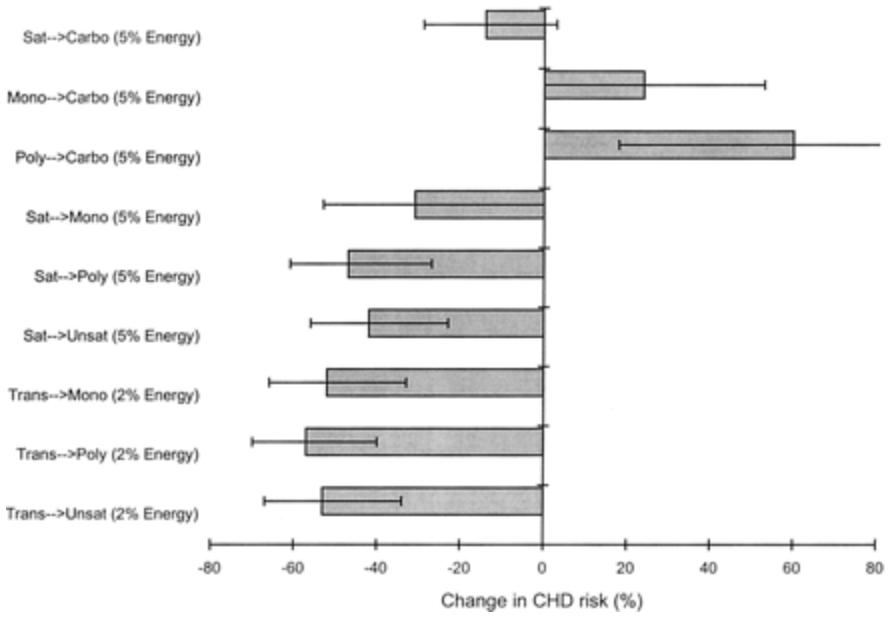
Review of the Evidence

The NEL systematic review of the literature published since 2004 identified 12 studies assessing the relationship between SFA intake and CVD risk in healthy adults or those at elevated chronic disease risk. Studies were conducted in the US, Europe and South America and overall, 10

randomized controlled trials, one non-randomized trial and an analysis of 11 pooled cohorts with meta-analysis were identified. The intervention studies ranged in sample size from 14 to 191 participants and the pooled analysis included 344,696 participants. Of the 12 studies, eight were methodologically strong (Azadbakht, 2007; Berglund, 2007; Chen, 2009; Furtado, 2008; Jakobsen, 2009; Kralova, 2008; Lefevre, 2005; Lichtenstein, 2005), and four were methodologically neutral (Buenacorso, 2007; Bourque, 2007; Chung, 2004; Dabadie, 2005). Most methodologically strong studies were feeding trials with an "average American" diet at baseline, which involved a reduction in SFA through replacement with MUFA, PUFA, or, to a lesser extent, carbohydrates. Dietary SFA replacement (5-7% of energy) with either MUFA (Berglund, 2007; Lichtenstein, 2005) or PUFA (Chung, 2004; Kralova, 2008; Lichtenstein, 2005) significantly decreased total and LDL cholesterol. Replacement of SFA with carbohydrates decreased plasma total and LDL cholesterol. However, compared to MUFA or PUFA, carbohydrate decreased HDL cholesterol and increased serum triglycerides (Berglund, 2007). A study by Lefevre et al. (2005) included two levels of total fat (30% and 25%) and SFA (9% and 6%) in the Step I and Step II diets, respectively, and demonstrated a dose-response effect in lowering LDL cholesterol. However, compared to the average American diet, the Step I and Step II diets also decreased HDL cholesterol levels and raised triglyceride levels in the blood. Furthermore, these authors showed that individuals who were insulin resistant responded less favorably to the STEP II diet than did those with normal insulin sensitivity. A study by Kralova et al. (2008) examined changes in cholesterol efflux to determine whether reduced HDL cholesterol, on a high PUFA/low SFA diet, had a negative effect on reverse cholesterol transport. The study showed no change in cholesterol efflux.

One meta-analysis examined effects of SFA reduction on incident coronary heart disease (CHD) outcomes by estimating the anticipated effects from statistical models where SFA is exchanged for equal energy from MUFA, PUFA, or carbohydrates (Jakobsen, 2009). These authors examined 11American and European cohort studies and found a significant inverse association for PUFA (with 5% substitution for SFA) and coronary events (hazard ratio = 0.87, 95% CI, 0.77-0.97, and coronary death hazard ratio = 0.74, 95% CI, 0.61-0.89). They also found a positive association between substitution of MUFA or carbohydrates for SFA and risk of coronary events, but not risk of coronary deaths. To provide further context for the question of SFA replacement with other healthy fats or carbohydrates and CVD risk, a review by Hu et al. (2001) was helpful. Figure D3.1 shows the estimated changes in risk of coronary heart disease associated with isocaloric substitution of SFA (at 5% energy) with healthy fats such as MUFA or PUFA or carbohydrates, as well as substitution of trans fatty acids (at 2% energy). In all cases of isocaloric SFA or trans fatty acid substitution, there is a decrease in CHD risk. However, it should be noted that when MUFA or PUFA are substituted by any kind of carbohydrates, CHD risk increased.

Figure D3.1. Saturated fatty acid substitution and coronary heart disease risk



Note: Estimated changes (percent with 95% confidence intervals) in risk of coronary heart disease (CHD) associated with isocaloric dietary substitutions. Adjusted for coronary risk factors and total energy intake. Sat=SFA, Carbo=carbohydrate, Mono=MUFA, Poly=PUFA, *Trans*=*trans* fatty acids, Sat-Carbo=substitute carbohydrates for SFA.
Source: Hu et al., 2001 *J Amer Col Nutr* 20,5-19. Used with permission, the American College of Nutrition.

The NEL review of the literature published since 2000 on the association of dietary SFA and T2D identified 12 studies conducted in the US, Europe, Canada, and China that examined the effect of dietary SFA on altered glucose metabolism, markers of insulin resistance, and T2D risk. Two were methodologically strong review articles including one which evaluated 15 trials, 9 trials in 358 non-diabetic participants and six trials in 93 participants with T2D (Galgani, 2008), and one reviewing 14 prospective cohort and 5 cross-sectional studies (Hu, 2001). Nine were randomized clinical trials ranging in size from 11 to 522 participants, including six methodologically strong studies (Han, 2001; Lindstrom, 2006a; Lindstrom, 2006b; Lopez, 2008; Perez-Jimenez, 2001; and Vesby, 2001) and three methodologically neutral studies (Paniagua, 2007; Shah, 2007; and St-Onge, 2003). The one prospective cohort study with 84,204 participants from the Nurses' Health Study was methodologically strong (Salmeron, 2001). The Galgani review of randomized controlled trials indicated that three studies provided evidence that MUFA or PUFA replacement of SFA improved insulin sensitivity, including one high-powered study that indicated a 10 percent decrease in insulin sensitivity on high SFA, versus high MUFA, diets. However, nine studies showed no effect of MUFA or PUFA replacement. The Hu review concluded that higher intake of PUFA (and potentially long-chain *n*-3 PUFA) were beneficial, whereas higher intakes of SFA and *trans* fatty acids impaired glucose metabolism and increased insulin resistance. Four randomized controlled trials

showed MUFA-enriched diets improved glucose uptake and insulin sensitivity: Lopez et al. (2008) showed that increased dietary MUFA improved insulin sensitivity and promoted pancreatic beta cell function; Paniagua et al. (2007) showed a diet high in MUFA improved blood glucose and Homeostatic Model Assessment (HOMA) – Insulin Resistance (IR) (HOMA-IR) scores over both SFA and carbohydrates in insulin resistant individuals; Perez-Jinenez et al. (2001) showed a MUFA-enriched diet improved glucose uptake in peripheral tissues and insulin sensitivity; and Vesby et al. (2001) showed SFA decreased, whereas MUFA did not change, insulin sensitivity. Three studies provided evidence that decreased SFA intake may decrease risk of T2D; two large randomized controlled trials (Lindstrom, 2006a; Lindstrom, 2006b) and one prospective cohort study (Salmeron, 2001). One randomized controlled trial by Shah et al. (2007) showed that insulin responsiveness was improved with either MUFA- or PUFA-enriched diets in individuals with T2D.

Question 2. What is the Effect of Dietary Cholesterol Intake on Risk of Cardiovascular Disease, Including Effects on Intermediate Markers such as Serum Lipid and Lipoprotein Levels and Inflammation?

Conclusion

Moderate evidence from epidemiologic studies relates dietary cholesterol intake to clinical CVD endpoints. Many randomized clinical trials on dietary cholesterol use eggs as the dietary source. Independent of other dietary factors, evidence suggests that consumption of one egg per day is not associated with risk of CHD or stroke in healthy adults, although consumption of more than seven eggs per week has been associated with increased risk. An important distinction is that among individuals with T2D, increased dietary cholesterol intake is associated with CVD risk.

Implications

Overall, the evidence shows that consumption of dietary cholesterol in the amount of one egg per day is not harmful and does not result in negative changes in serum lipoprotein cholesterol and triglyceride levels. Neither does consumption of eggs at this level increase risk of CVD in healthy individuals. Eggs also are a good source of high quality protein and numerous micronutrients. However, in individuals with T2D, egg consumption (at one egg/day) does have negative effects on serum lipids and lipoprotein cholesterol levels and does increase risk of CVD. Furthermore, consumption of more than seven eggs per week is not recommended for the general public. Overall, limiting dietary cholesterol to less than 300 mg per day, with further reductions of dietary cholesterol to less than 200 mg per day for persons with or at high risk for CVD and T2D, is recommended.

Review of the Evidence

The NEL systematic review identified 16 studies published since 1999 that evaluated the effect of dietary cholesterol intake on CVD risk conducted in the US, Europe, Mexico, and Japan. Eight

randomized controlled trials, including two methodologically strong studies (Ballesteros, 2004; Knopp, 2003) and six methodologically neutral studies (Goodrow, 2006 ; Greene, 2005; Harman, 2008; Mutungi, 2008; Reaven, 2001; Tannock, 2005) with sample size ranging from 28 to 201 participants were reviewed. Five prospective cohort studies, including four methodologically strong studies (Djousse, 2008; Hu, 1999; Qureshi, 2007; Tanasescu, 2004) and one methodologically neutral study (Nakamura, 2006) ranging in size from 5,687 to 80,082 participants, were reviewed. And one meta-analysis of 17 studies that was methodologically strong (Weggemans, 2001), and two systematic reviews, one methodologically strong pooled analysis of 167 cholesterol feeding studies in 3,519 participants (McNamara, 2000) and one methodologically neutral review of 8 prospective cohort studies on dietary cholesterol and 6 prospective cohort studies on eggs (Kritchevsky and Kritchevsky, 2000) met the eligibility criteria and were reviewed. The majority of these articles reported on comparisons of egg versus egg substitute or no egg intake. In studies comparing eggs versus egg substitute, one randomized controlled trial (Ballesteros, 2004) and one pooled analysis (McNamara, 2000) showed that LDL cholesterol and HDL cholesterol increased in hyper-responders, but did not change in hypo-responders; overall, the LDL:HDL did not change in hypo- or hyper-responders. Identification of hypo-and hyper-responders showed inter-individual variation to dietary cholesterol that may result in differing health outcomes for individuals with different genetic predispositions.

Harman et al. (2008) found that LDL cholesterol decreased in both egg and egg substitute groups, and two studies in elderly adults (Greene, 2005; Goodrow, 2006) indicated that LDL cholesterol and HDL cholesterol were not affected by egg intake. Two randomized controlled trials showed an increase in LDL diameter in the egg group (Ballesteros, 2004; Greene, 2005). Two randomized controlled trials in 65 insulin-sensitive and 75 insulin-resistant individuals determined that egg consumption was associated with increased LDL cholesterol, but only in insulin-sensitive individuals (Knopp, 2003; Tannock, 2005). However, Reaven et al. (2001) found that high cholesterol intake did not increase LDL cholesterol in either insulin-sensitive or insulin-resistant subgroups. All studies that measured HDL cholesterol found that HDL cholesterol was increased with egg consumption, and one such study was in a carbohydrate-restricted diet background (Mutungi, 2008). One study assessed markers of inflammation and found increased C-reactive protein and serum amyloid A with high egg consumption, but found no difference in circulating cytokines (Tannock, 2005). One meta-analysis of 17 studies indicated that high dietary cholesterol intake increased the total:HDL cholesterol ratio. However, this effect was attenuated in the low SFA subgroup (Weggemans, 2001).

In the prospective cohort studies, Djousse et al. (2001) found that egg consumption up to six eggs per week in the Physicians' Health Study was not associated with risk of all-cause mortality, but consumption of more than seven eggs per week was associated with a 23 percent increased risk of

death. In the Japan Public Health Center study, egg consumption was not associated with CHD incidence (Nakamura, 2006). In NHANES I, no relationship was established between egg consumption (>6 eggs/wk) and risk of stroke or ischemic stroke, and risk of myocardial infarction and all-cause mortality was not different between egg and non-egg consumption groups (Qureshi, 2007). A combined analysis of the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS), found no significant association between egg consumption and risk of CHD or stroke in men or women (Hu, 1999). A review of epidemiological studies (Kritchevsky and Kritchevsky, 2000) showed there was no association between consumption of one egg per day and risk of CVD, but only in non-diabetic men and women. Furthermore, three methodologically strong prospective cohort studies warned that egg consumption was associated with increased CVD risk in individuals with T2D (Djousse, 2001; Hu, 1999; Tanasescu, 2004) and this warrants further investigation.

Dietary Cholesterol Modeling

The USDA food patterns were designated to meet adequacy and reduction goals, and 2005 DGAC recommended cholesterol intakes of less that 300 mg per day for persons not at risk for CVD. A food pattern modeling analysis was carried out to identify nutrient amounts that would change and the nutrient goals that would be met or not met for the patterns at each calorie level when dietary cholesterol is limited to less than 200 mg per day. (See the Cholesterol report, online Appendix E3.8, available at www.dietaryguidelines.gov). To meet the lower criteria of less than 200 mg of cholesterol per day, all patterns were modified as follows. Eggs were limited to less than two per week. The amounts of meat and chicken were decreased by about 20 percent, and nuts and soy products were substituted to maintain the same total amount from the meat and bean group in each pattern. The amounts of solid fats, which include fats in milk products as well as meats and poultry, were capped at 10 grams per day, and oils were substituted isocalorically. With these modifications, dietary cholesterol was reduced 23 to 31 percent. These modified patterns also showed a 3.5 percent reduction in protein, a 10 percent reduction in choline, a 2 - 7 percent reduction in vitamins A and D, a 21 percent reduction in EPA (20:5 *n*-3), and a 3 percent reduction in DHA (22:6 *n*-3). In contrast, vitamin E increased 4 - 25 percent, thiamin increased 13 - 19 percent, linoleic acid increased 3 - 20 percent, and alpha-linolenic acid increased 8 percent. The resulting patterns had adequate protein, but amounts of choline, and vitamin D (which were below adequate intake (AI) levels set by the IOM in the patterns containing 300 mg/dl per day) were even less adequate in the patterns containing less than 200 mg of cholesterol per day. The health implications of a lower choline diet are not well defined.

Diets with less than 200 mg per day of cholesterol can be constructed for those for whom such a diet has a positive benefit-to-cost ratio. This diet can be achieved by reducing eggs, meat, chicken, and solid fats (including fats in milk products), and replacing them with unsalted nuts, soy products, and oils.

Question 3. What is the Effect of Dietary Intake of MUFA when Substituted for SFA on Increased Risk of Cardiovascular Disease and Type 2 Diabetes, Including Intermediate Markers such as Lipid and Lipoprotein Levels and Inflammation? And What is the Effect of Replacing a High Carbohydrate Diet with a High MUFA Diet in Persons with Type 2 Diabetes?

Conclusion

Strong evidence indicates that dietary MUFA are associated with improved blood lipids related to both CVD and T2D, when MUFA is a replacement for dietary SFA. The evidence shows that 5 percent energy replacement of SFA with MUFA decreases intermediate markers and the risk of CVD and T2D in healthy adults and improves insulin responsiveness in insulin resistant and T2D individuals. Moderate evidence indicates that increased MUFA intake, rather than high carbohydrate intake, may be beneficial for persons with T2D. High MUFA intake, when replacing a high carbohydrate intake, results in improved biomarkers of glucose tolerance and diabetic control.

Implications

At the current level of 11 to 12 percent of energy from SFA, healthy American adults would benefit substantially by replacing 5 percent of that total energy with MUFA (e.g., 12 percent SFA reduced to 7 percent SFA, 12 percent MUFA increased to 17 percent MUFA). Beneficial outcomes would include reduced rates of CVD and T2D as well as improved lipids and lipoproteins, inflammatory markers, and measures in insulin resistance. Persons with a predisposition to T2D or established T2D may especially benefit from a high MUFA diet, both as a substitute for SFA and as a substitute for carbohydrates. Given the high prevalence of T2D and the metabolic syndrome in the US, such benefits would have a large public health impact.

Review of the Evidence

Thirteen studies published since 2004 and conducted in the US, Europe, and Australia were reviewed to determine the effect of MUFA on health outcomes. These included one methodologically strong meta-analysis evaluating 11 prospective cohort studies (Jakobsen, 2009) and 11 randomized controlled trials ranging from 14 to 162 participants, including six methodologically strong studies (Appel, 2005; Berglund, 2007; Due, 2008; Lopez, 2008; Thijssen and Mensink, 2005; and Thijssen, 2005), and five methodologically neutral studies (Allman-Farinelli, 2005; Binkoski, 2005; Clifton, 2004; Paniagua, 2007; and Rasmussen, 2006). The reviewed studies also included one

methodologically strong prospective cohort study of 5,672 participants from the Nurses' Health Study who reported a diagnosis of T2D (Tanasescu, 2004). Overall, MUFA replacing SFA in the diet as percent of energy leads to a decrease in LDL cholesterol (Allman-Farinelli, 2005; Appel, 2005; Berglund, 2007), a decrease in serum triglycerides (Allman-Farinelli, 2005), a decrease in markers of inflammation (Allman-Farinelli, 2005), and a decrease in CVD risk (Appel, 2005; Rasmussen, 2006). Increasing MUFA intake, rather than replacing SFA with MUFA, also leads to a decrease in total cholesterol (Haban, 2004), LDL cholesterol (Haban, 2004), LDL:HDL ratio (Due, 2008), serum triglycerides (Brunerova, 2007), inflammatory markers (Brunerova, 2007), and fasting insulin and HOMA-IR scores (Brunerova, 2007; Due, 2008). However, Clifton et al. (2004) found a greater decrease in total cholesterol and HDL cholesterol in women who consumed a very low-fat diet, compared with a high MUFA diet, and no difference in the LDL:HDL ratio between the two diets (Clifton, 2004). Replacing SFA with MUFA, compared to replacement with carbohydrates, decreased serum triglycerides (Appel, 2005) and increased HDL cholesterol (Appel, 2005; Berglund, 2007). Lastly, a prospective cohort study involving a T2D subpopulation within the Nurses' Health Study found that replacing 5 percent energy from SFA with equivalent energy from MUFA was associated with a 27 percent lower risk of CVD. The authors conclude that replacing SFA with MUFA may be more protective against CVD than replacement with carbohydrate (Tanasescu, 2004).

Comparing substitution of SFA with MUFA versus PUFA showed a greater decrease in total and LDL cholesterol with PUFA substitution (Binkoski, 2005). Furthermore, a pooled analysis of 11 prospective cohort studies showed that risk of coronary events and coronary death was lowest with 5 percent energy substitution of SFA with PUFA; PUFA substitution resulted in the greatest decrease, with MUFA showing somewhat less, and carbohydrate showing the least improvement when substituted for SFA (Jakobsen, 2009). In a comparison of individual fatty acids, oleic acid was no different than stearic or linoleic acid in its effect on measures of serum lipids or lipoproteins and markers of inflammation (Thijssen and Mensink, 2005; Thijssen, 2005).

To determine the effects of replacing a high carbohydrate diet with a high MUFA diet in persons with type 2 diabetes, five randomized controlled trials published since 2004 were reviewed. These randomized controlled trials were conducted in the US and Europe and ranged in size from 11 to 95 participants. Two studies were methodologically strong (Brehm, 2009; Gerhard, 2004) and three were methodologically neutral (Brunerova, 2007; Rodriguez-Villar, 2004; and Shah, 2005). In persons with T2D, a high MUFA diet compared to high carbohydrate diet decreased blood LDL cholesterol and triglycerides (Rodriguez-Villar, 2004), increased HDL cholesterol (Brunerova, 2007), and decreased fasting blood glucose and HbA1c (Brunerova, 2007). On the other hand, when high MUFA and carbohydrate diets were also low calorie or weight loss diets, the results were more difficult to interpret. Brehm et al. (2008) found no significant differences in fasting glucose, insulin, hemoglobin A1c, or HDL cholesterol between the MUFA and carbohydrate groups. Both groups

improved compared to baseline due to decreased caloric intake (200-300 kcal/d). Gerhard et al. (2004) did not find any significant difference in blood lipids or glycemic control in a comparison of high MUFA versus high carbohydrate diets in T2D individuals; however, in this case, the two diet interventions were not isocaloric and the MUFA diet was a higher calorie diet. Shah et al. (2005) measured the effects of high MUFA versus carbohydrate on blood pressure in persons with T2D and found that long-term consumption of a high-carbohydrate diet may modestly raise blood pressure in persons with T2D.

Question 4. What is the Effect of Dietary Intake of *n*-6 PUFA on Risks of Cardiovascular Disease and Type 2 Diabetes, Including Intermediate Markers such as Lipid and Lipoprotein Levels and Inflammation?

Conclusion

Strong and consistent evidence indicates that dietary PUFA are associated with improved blood lipids related to CVD, in particular when PUFA is a replacement for dietary SFA or *trans* fatty acids. Evidence shows that energy replacement of SFA with PUFA decreases total cholesterol, LDL cholesterol and triglycerides, as well as numerous markers of inflammation. PUFA intake significantly decreases risk of CVD and has also been shown to decrease risk of T2D.

Implications

All recommendations assume an isocaloric replacement of SFA or *trans* fatty acids with PUFA. In this setting, both CVD and, potentially, T2D may be reduced with PUFA replacement. The mechanisms of CVD reduction, including improvement in serum lipid levels and reduced markers of inflammation, may have additional health benefits. PUFA consumption in the US is lower than that of SFA or MUFA, although the only essential fatty acids are PUFA, so a reduction of SFA from 12 percent to 7 percent of energy through an increase in PUFA alone would increase PUFA from 7 percent to 12 percent of energy. This, or replacing SFA with some combination of PUFA and MUFA, should yield significant public health benefits.

Review of the Evidence

Ten studies published since 2004 were reviewed to determine the effect of PUFA on health outcomes. These studies were conducted in the US, Canada, Europe, and Australia. These included one methodologically strong pooled analysis of 11 prospective cohort studies (Jakobsen, 2009); five randomized controlled trials, including two methodologically strong studies (Thijssen and Mensink, 2005; and Thijssen, 2005) and three methodologically neutral studies (Liou, 2007; St-Onge, 2007; and Zhao, 2004) ranging in size from 23 to 45 participants; and four prospective cohort studies ranging in size from 1,551 to 78,778 participants. Of these cohort studies, three were methodologically strong (Laaksonen, 2005; Mozaffarian, 2005; and Oh, 2005) and one was

methodologically neutral (Hodge, 2007). Randomized controlled trials that investigated the effects on serum lipid and lipoprotein levels of replacing SFA with PUFA showed that PUFA improved serum lipid profiles (St. Onge, 2007; Zhao, 2004). Zhao et al. (2004) found that high LA or high ALA diets compared to the average American diet decreased serum total cholesterol, LDL cholesterol, and triglycerides similarly. St-Onge et al. (2007) reported that replacing snacks high in SFA or *trans* fats with snacks high in PUFA reduced LDL cholesterol concentrations, total cholesterol, and triglycerides. However, varying LA, with SFA held constant, showed that high or low LA did not influence total cholesterol, LDL cholesterol, or HDL cholesterol levels (Liou, 2007). Comparing individual fatty acids, diets providing 7 percent of energy from linoleic acid, stearic acid, or oleic acid showed no significant differences in serum LDL or HDL cholesterol (Thijssen and Mensink, 2005).

Studies that examined markers of inflammation or measures of oxidative stress showed PUFA improved inflammatory marker levels. Zhao et al. (2004) reported that while both high ALA and LA diets decreased C-reactive protein, the finding was significant only for ALA. Additionally, while both high-PUFA diets similarly decreased intercellular cell adhesion molecule-1 (ICAM-1) versus the average American diet, the ALA diet decreased vascular cell adhesion molecule-1 (VCAM-1) and E-selectin more than the LA diet. The comparison of high versus low LA, with SFA constant, showed no difference in C-reactive protein, interleukin-6, or platelet aggregation (Liou, 2007). Comparison of linoleic acid, stearic acid, or oleic acid showed that, in men, platelet aggregation time was favorably prolonged with consumption of LA versus stearic acid, but was not different compared to oleic acid (Thijssen, 2005).

Four prospective cohort studies showed that higher PUFA intake was associated with lower risk of CHD and total mortality (Hodge, 2007; Laaksonen, 2005; Mozaffarian, 2005; and Oh, 2005). A pooled analysis of 11 prospective cohort studies showed that risk of coronary events and coronary death was lowest with 5 percent energy substitution of SFA with PUFA>MUFA> carbohydrate (Jakobsen, 2009).

The NEL review for this question included a prospective study with nested case-cohort analyses on the effects of a dietary PUFA on T2D risk. The authors reported an inverse association between dietary LA and T2D, compared to a positive association for stearic acid and total saturated fatty acids (Hodge, 2007). In addition, the review for this question is supplemented by evidence from question 1 on SFA and T2D risk that reviewed the literature from 2000. This, and the fact that blood lipids are intermediate markers of risk for both CVD and T2D, further supports the association between PUFA intake and decreased T2D risk.

SPECIFIC FATTY ACIDS THAT AFFECT PLASMA LDL, HDL, AND NON-HDL CHOLESTEROL LEVELS

More than 50 years of research has defined the impact of fatty acids on cholesterol metabolism, yet stearic acid is still categorized as a SFA and *trans* fatty acids are categorized as PUFA, based on their respective chemical properties. However, as more evidence becomes available showing that stearic acid has different metabolic effects than other SFA and does not raise blood cholesterol, and that elaidic acid and other *trans* fatty acids do raise blood cholesterol similar to SFA, a better classification of fatty acids with deleterious health effects would be "cholesterol-raising FA." This category would consist of SFA with carbon chain lengths from C12-C16 (i.e. excluding stearic acid and smaller SFA) and *trans* fatty acids. The 2010 DGAC reviewed recent evidence on the effects of these particular fatty acids on blood cholesterol and lipoprotein levels.

Question 5. What are the Effects of Dietary Stearic Acid on LDL Cholesterol?

Conclusion

Moderate evidence from a systematic review indicates that when stearic acid is substituted for other SFA or *trans* fatty acids, plasma LDL cholesterol levels are decreased; when substituted for carbohydrates, LDL cholesterol levels are unchanged; and when substituted for MUFA or PUFA, LDL cholesterol levels are increased. Therefore, the impact of stearic acid replacement of other energy sources is variable regarding LDL cholesterol, and the potential impact of changes in stearic acid intake on cardiovascular disease risk remains unclear.

Implications

Since stearic acid is not known to raise LDL cholesterol, the DGAC is recommending that stearic acid not be categorized with known "cholesterol-raising fats," which include C12, C14, C16 SFA and *trans* fatty acids. Foods that are high in stearic acid, such as dark chocolate and shea nut oil, need not be considered as problematic as foods high in other SFA or *trans* fatty acids. In addition, setting the recommended percent of energy from these cholesterol-raising fats to a less than 5 to 7 percent will help to maintain blood cholesterol at desirable concentrations.

Review of the Evidence

Background

Stearic acid consumption in the US varies considerably between men (mean 8.8g/day) and women (mean 5.9 g/day), with modest increases between 1994 and 2006 (USDA/ARS, 1997-2008). The foods that contribute the most stearic acid to the diets of Americans are listed in Table D3.10.

Table D3.10. Top food sources of stearic acid among US population, 2005-2006 NHANES

Food Item	Contribution to intake %	Cumulative contribution %
Grain-based desserts	8.3	8.3
Regular cheese	6.1	14.4
Sausage, franks, bacon, and ribs	6.0	20.4
Chicken and chicken mixed dishes	5.7	26.1
Pizza	5.7	31.8
Burgers	5.1	36.9
Beef and beef mixed dishes	4.8	41.7
Mexican mixed dishes	4.4	46.1
Dairy desserts	4.3	50.4
Candy	4.2	54.5
Pasta and pasta dishes	3.3	57.8
Fried white potatoes	3.2	61.1
Eggs and egg mixed dishes	3.2	64.2
Reduced fat milk	3.0	67.2
Whole milk	2.6	69.9
Yeast breads	2.5	72.3
Cold cuts	2.2	74.5
Butter	2.2	76.7

Source: Sources of Saturated Fat, Stearic Acid, and Cholesterol Raising Fat among the US Population, 2005-2006. Risk Factor Monitoring and Methods Branch Website. Applied Research Program. National Cancer Institute. http://riskfactor.cancer.gov/diet/foodsources Updated November 9, 2009. Accessed April 16, 2010.

Evidence Summary

A NEL review of the evidence since 2000 resulted in one systematic review with univariate and multivariate regression analysis of all selected studies. This review examined the effect of stearic acid on blood LDL cholesterol when substituted for SFA, MUFA, PUFA, carbohydrate, or *trans* fatty acids (Hunter, 2010). Although this systematic review provided broad qualitative and quantitative analysis, it was scored as methodologically neutral based on one limitation: the selected studies included in the review were not individually graded. However, this review provided the most updated evidence and covered all aspects of stearic acid replacements and risk/benefit outcomes related to LDL cholesterol and CVD risk. Overall, this review covered three epidemiologic studies that examined stearic acid specifically, and 20 randomized controlled trials that examined high stearic acid intake as a replacement of other dietary fats or carbohydrate. The randomized controlled trials were grouped according to comparisons with 1) high SFA (palmitic acid, myristic acid, or butterfat) (Aro, 1997; Becker, 1999; Bonanome and Grundy, 1988; Denke and Grundy, 1991; Dougherty, 1995; Judd, 2002; Kelly, 2001, 2002; Kris-Etherton, 1993; Nestel, 1998; Snook, 1999l; Sundram, 2007; Schwab, 1996; Tholstrup, 1994, 1995); 2) high carbohydrate (Nestel, 1998; Judd, 2002; Kris-Etherton, 1994); 3) high unsaturated fat (oleic acid or linoleic acid) (Bonanome and Grundy, 1988;

Denke and Grundy, 1991; Dougherty, 1995; Hunter, 2000; Zock and Katan, 1992; Mensink, 1992; Kris-Etherton, 1993; Judd, 2002; Thijssen and Mensink, 2005; Berry, 2007; Louheranta, 1998); and 4) baseline (or habitual) diet (Snook, 1999; Schwab, 1996; Kelly, 2001, 2002). Four studies assessed the effect of substituting stearic acid for *trans* fatty acids in the diet (Aro, 1997; Judd, 2002; Sundram, 2007; Zock and Katan, 1992).

Overall, the results showed that in comparison with SFA, stearic acid lowered LDL cholesterol, was neutral with respect to HDL cholesterol and lowered the ratio of total to HDL cholesterol. In comparison with unsaturated fatty acids, MUFA and PUFA, stearic acid tended to raise LDL cholesterol, lower HDL cholesterol and increase the ratio of total to HDL cholesterol. Univariate regression analysis of the data substituting stearic acid for cholesterol-raising SFA indicated that the LDL cholesterol concentration deceases as dietary stearic acid increases. The univariate regression coefficient for this relation was -0.036 ($p=0.034$). The regression coefficient suggests that for each 1 percent of energy increase in stearic acid, when substituted for cholesterol-raising SFA, the LDL-cholesterol concentration could decrease by 0.036 mmol/L. When multivariate regression analysis was done, with adjustments for both between-study, and within-study, variation, the multivariate regression coefficient for this relation was 0.043 ($p<0.001$), suggesting that for each 1 percent energy increase in cholesterol-raising SFA, when substituted for stearic acid, the LDL cholesterol concentration would increase by 0.043 mmol/L.

A one-to-one substitution of stearic acid for *trans* fatty acids showed a decrease or no effect on LDL cholesterol, an increase or no effect on HDL cholesterol, and a decrease in the ratio of total to HDL cholesterol. Replacing industrial *trans* fatty acids with stearic acid could increase stearic acid intake from 3 percent to 4 to 5 percent of energy in the US population.

Although not part of the formal NEL review, the 2002 IOM report is consistent with Hunter et al. (2010). The IOM report emphasized that stearic acid has been shown to have a neutral effect on LDL cholesterol levels (Bonanome and Grundy, 1988; Denke, 1994; Hegsted, 1965; Keys, 1965, Yu, 1995; Zock and Katan, 1992), in comparison to palmitic, lauric, and myristic acids that increase LDL cholesterol levels (Mensink, 1994). Stearic acid was indicated to be similar to oleic acid in its effects (Kris-Etherton, 1993).

Cholesterol-raising Fatty Acids Modeling

Food pattern modeling analyses were carried out to answer the question, "What would the impact be on food choices and overall nutrient adequacy if the cholesterol-raising fatty acids were limited to (a) less than 7 percent of total calories and (b) less than 5 percent of total calories." (See the Reducing Cholesterol-raising Fatty Acids report, online Appendix E3.9, available at

www.dietaryguidelines.gov). Cholesterol-raising fatty acids were defined as total SFA minus stearic acid. *Trans* fatty acids are not available in the USDA food composition databases because levels in foods have been rapidly changing, however, they are captured in the solid fat values.

Changes in the base food patterns needed to bring cholesterol-raising fats to less than 7 percent and less than 5 percent of calories were identified, and the impact on food selections and other nutritional goals was assessed. In the base patterns, stearic acid constitutes 2.2 to 2.6 percent of calories, and cholesterol-raising fatty acids provide 6.0 to- 6.8 percent of calories, so no changes were needed to achieve the goal of less than 7 percent. If all solid fats were removed and isocalorically replaced with oils, total SFA would be decreased to 7.0 to 7.5 percent of calories and cholesterol-raising fatty acids would be decreased to 5.0 to 5.5 percent of calories.

Question 6. What Effect does Consuming Natural (Ruminant) Versus Synthetic (Industrially Hydrogenated) Trans Fatty Acids have on LDL-, HDL- and Non HDL Cholesterol Levels?

Conclusion

Limited evidence is available to support a substantial biological difference in the detrimental effects of industrial *trans* fatty acids (iTFA) and ruminant *trans* fatty acids (rTFA) on health when rTFA is consumed at 7-10 times the normal level of consumption.

Implication

The level of daily intake of rTFA is quite small with the US adult population's average daily intake approximating 1.2 g (1.5g for men and 0.9 g for women). This represents less than 2 percent of total daily energy intake. This is a relatively minor exposure in the diet regardless of its metabolic effect.

The very limited data available provide insufficient evidence to suggest rTFA and iTFA be considered differently in their metabolic effects. Total *trans* fatty acid intake should be considered the target for dietary change. Total elimination of rTFA would require elimination of red meat and dairy products from the diet. Although total elimination of iTFA may be desirable, the elimination of rTFA would have wider implications for dietary adequacy and is not recommended. It is best to avoid iTFA while leaving small amounts of rTFA in the diet. Overall, *trans* fatty acid levels in the US food supply have decreased dramatically following mandatory *trans* fatty acids labeling regulations, which went into effect in 2006. Continued reductions in iTFA are to be encouraged.

Review of the Evidence

Based on the 2002 IOM review covering 20 controlled trials and 11 epidemiologic studies, as well as the NCEP Adult Treatment Panel Review (NCEP 2002) and seven additional publications, the 2005 DGAC concluded that the relationship between *trans* fatty acid intake and LDL cholesterol

is positive and HDL cholesterol is inverse, increasing the risk of CHD. The 2005 DGAC's recommendation was that *trans* fatty acids consumption should be kept as low as possible, defined as less than 1 percent of energy. An obstacle to removing *trans* fatty acids altogether has been its dual source in the food supply. The great majority comes from hydrogenation of unsaturated fats industrially, but about 1 to 2 percent is found naturally in the gastrointestinal tracts of ruminant animals, ending up in meats and dairy products. The 2010 DGAC therefore considered the question of whether rTFA, which are structurally different from iTFA, have different effects from iTFA on serum lipid and lipoprotein levels.

A NEL review of the evidence from 2000 found two methodologically strong randomized controlled cross-over trials (Motard-Belanger, 2008; Chardigny, 2008) and one methodologically neutral review (Jakobsen, 2006) that compared the effects of iTFA and rTFA on plasma lipid concentrations and CVD risk. Chardigny et al. (2008) compared experimental diets containing 11 to 12g per day (about 5% of daily energy) of rTFA and iTFA in 40 healthy normolipidemic individuals in France and found no difference in effect in men and that *trans* fatty acids from natural sources significantly increased HDL cholesterol and LDL cholesterol in women. This level of intake of rTFA is far above current US rTFA consumption, which is small compared to iTFA consumption (IOM Report, 2002). Motard-Belanger et al. (2008) evaluated four isocaloric experimental diets in 38 normolipidemic men: 1) high rTFA (10.2 g/2500 kcal); 2) moderate rTFA (4.2 g/2500kcal); 3) high iTFA (10.2 g/2500kcal); 4) low TFA from any source (control) (2.2 g/2500kcal). The investigators found plasma LDL cholesterol was significantly higher after the high iTFA diet as compared to the moderate rTFA diet, and after the high rTFA diet compared to moderate rTFA or control diets. Plasma HDL cholesterol concentrations were significantly lower after the high rTFA diet compared to the moderate rTFA diet. These results indicate that moderate rTFA intake has neutral effects on plasma lipids related to CVD risk.

One methodologically neutral review (Jakobsen, 2008) evaluated results from three prospective cohort studies and one case-control study which assessed the effect of consumption of rTFA on CHD outcomes and reported no statistically significant association. A prospective cohort study included in the Jakobsen review (Oomen, 2001) assessed the association between *trans* fatty acid intake and CHD in 667 Dutch men between the ages of 64 and 84 years with no history of CHD. These investigators found a non-significant association between rTFA or iTFA and risk of CHD. Relative risks of CHD for an increase of 0.5 percent energy from rTFA and iTFA were 1.17 (95% CI 0.69-1.98) and 1.05 (95% CI 0.99-1.15), respectively.

The risk of CVD associated with *trans* fatty acids is due, in part, to *trans* fatty acid effects on LDL and HDL cholesterol, inflammatory processes, as well as interference with fat metabolism. In countries like Denmark, dramatic declines in CVD of about 60 percent have been attributed to progress made in lowering the intake of *trans* fatty acids from commercial sources (Leth, 2006;

Stender, 2008), culminating in the passage of legislation limiting their use. Although simultaneous advances in the prevention and treatment of CVD have played a role, the importance of eliminating iTFA cannot be overlooked. Mozaffarian et al. (2006) estimated that reducing commercial *trans* fatty acid intake from 2.1 percent of energy to 1.1 percent or 0.1 percent of energy could have prevented 72,000 or 228,000 CVD deaths per year, respectively. The FDA suggested that removal of *trans* fatty acids in just 3 percent of breads and cakes and 15 percent of cookies and crackers would save up to $59 billion in health care costs in the next 20 years.

Accordingly, a number of US companies are taking innovative steps to reduce *trans* fatty acids in their food products (Table D3.11).

Table D3.11. Mean *trans* fatty acid levels in certain foods from Food Label and Package Surveys (FLAPS) 2006–2007 and mean *trans* fatty acid levels of comparable food products

Food	2004[a]	FLAPS 2006-2007[a]
Cakes		
Number of samples	n = 10	n = 11
Mean TFA levels g/100 g (SE)[b]	2.85 (1.03)	0.98 (0.47)
Biscuits		
Number of samples	n = 5	n = 5
Mean TFA levels g/100 g (SE)	4.40 (0.25)	5.41 (0.70)[d]
Margarines and spreads		
Number of samples	n = 7	n = 9
Mean TFA levels g/100 g (SE)	12.24 (1.06)	4.37 (2.36)[c]
Cookies		
Number of samples	n = 12	n = 14
Mean TFA levels g/100 g (SE)	4.5 (0.62)	1.9 (0.84)
Crackers		
Number of samples	n = 11	n = 17
Mean TFA levels g/100 g (SE)	5.20 (0.51)	0.71 (0.39)[c]
Potato chips		
Number of samples	n = 8	n = 10
Mean TFA levels g/100 g (SE)	0.45 (0.45)	0.0 (0) NS[e]
Tortilla chips		
Number of samples	n = 8	n = 9
Mean TFA levels g/100 g (SE)	1.76 (0.6)	0.0 (0)[c]
Frozen potato products		
Number of samples	n = 6	n = 7
Mean TFA levels g/100 g (SE)	1.97 (0.48)	0.74 (0.24)[c]
Cereal and granola		
Number of samples	n = 8	n = 9
Mean TFA levels g/100 g (SE)	1.70 (0.8)	0.0 (0)[c]
Tortillas		
Number of samples	n = 6	n = 7
Mean TFA levels g/100 g (SE)	0.76 (0.39)	0.22 (0.22)[f]

[a] *Trans* fat levels for 2004 are from Satchithanandam et al. 2004a, and were analyzed from food products. The levels from FLAPS are values from food labels.
[b] SE = Standard error.
[c] Significant decrease at $p \leq 0.05$.
[d] Significant increase at $p \leq 0.05$.
[e] NS = Not significant.
[f] Mean is NS, but median is significant decrease at $p \leq 0.05$.
Source: Mossoba et al. (2009) *J. of AOAC International*, 92 (5), 1284-1300. Used with permission, AOAC International.

RELATIONSHIPS BETWEEN CONSUMPTION OF *N*-3 FATTY ACIDS AND HEALTH OUTCOMES

This question had been reviewed extensively by several expert panels and the 2005 DGAC. As *n*-3 PUFA are derived from two sources, plant and marine, the 2010 DGAC examined both sources for benefits impacting primary and secondary prevention of CVD. Although most expert panels have focused on *n*-3 supplements, this review examined the consumption of *n*-3 PUFA in whole foods (dietary supplement interventions were excluded) in individuals with and without CVD. In addition to the potential beneficial effects of *n*-3 PUFA on CVD risk in adults, significant findings have emerged on the benefits of maternal long-chain *n*-3 PUFA intake during pregnancy and lactation related to improved neurodevelopment in the infant and child.

Question 7. What is the Relationship Between Consumption of Seafood *n*-3 Fatty Acids and Risk of CVD?

Conclusion

Moderate evidence shows that consumption of two servings of seafood per week (4 oz per serving), which provide an average of 250 mg per day of long-chain *n*-3 fatty acids, is associated with reduced cardiac mortality from CHD or sudden death in persons with and without CVD.

Implications

An increase in seafood intake to two servings per week at 4 oz per serving, is advised for high-risk (those with CVD) and average-risk persons, especially as the first presentation of CVD (myocardial infarction, stroke) is frequently fatal or disabling. The quantity and frequency of seafood consumption is important, but the type of seafood (those providing at least 250 mg of long-chain *n*-3 fatty acids per day) also is critical. Increased consumption of seafood will require efficient and ecologically friendly strategies be developed to allow for greater consumption of seafood that is high in EPA and DHA, and low in environmental pollutants such as methyl mercury. (See ***Part D.8: Food Safety and Technology*** for a detailed discussion of the risks and benefits of seafood consumption).

Review of the Evidence

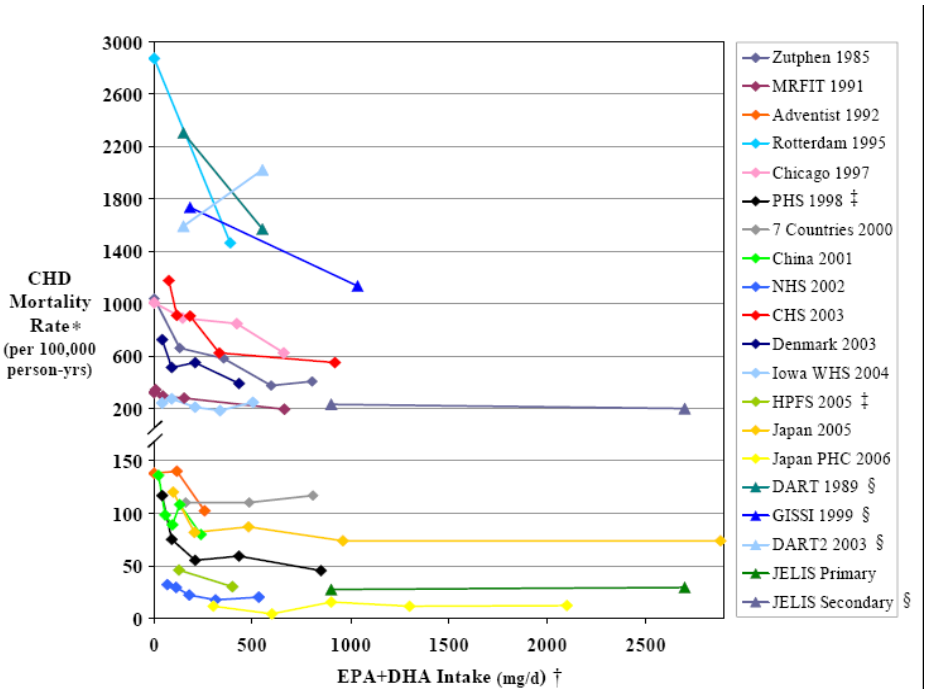
The 2010 DGAC conducted a full NEL search of the literature from 2004 to evaluate the association of seafood consumption and CVD risk. Results of this review were supplemented by an earlier evidence review of the literature from 2004 to 2007 conducted by the American Dietetics Association on health benefits related to consumption of fish or fish-derived *n*-3 fatty acids in individuals without or with CVD. Taken together, the NEL and ADA evidence reviews identified 25 studies published since 2004 assessing the health benefits of seafood consumption in persons

without CVD. These included six systematic reviews/meta-analyses, including four methodologically strong reviews with meta-analyses of randomized controlled trials and prospective cohort studies (He, 2004; Konig, 2005; Mozaffarian 2008; Mozaffarian and Rimm, 2007), one methodologically strong systematic review of 14 randomized controlled trials, 25 prospective cohort studies, and 7 case-control studies (Wang, 2006) and one methodologically neutral meta-analysis of 14 cohort and 5 case-control studies (Whelton, 2004). These also included four randomized controlled trials ranging in size from 33 to 48 participants conducted in the US and Finland, including two methodologically strong study (Lara, 2007; Seierstad, 2005) and two methodologically neutral studies (Lindqvist, 2009; Lankinen, 2009). Lastly, this included 15 prospective cohort studies conducted in the US, Europe, Japan, and China, ranging in size from 300 to 57,972 participants, including eight methodologically strong (Brouwer, 2006; Frost and Vestergaard, 2005; Iso, 2006 ; Järvinen, 2006; Mozaffarian, 2004; Mozaffarian, 2005; Virtanen, 2008; Virtanen, 2009) and seven methodologically neutral studies (Albert, 2002; Folsom & Demissie, 2005; Levitan, 2009; Pangiotakos, 2007; Streppel, 2008; Turunen, 2008; Yamagishi, 2008).

Three of the systematic reviews assessed both fish and long-chain *n-3* FAs (Mozaffarian 2008; Mozaffarian & Rimm, 2007; Wang, 2006) and three meta-analyses covered only fish (Konig, 2005; Whelton, 2004; and He, 2004). The systematic reviews and meta-analyses were consistent in showing that fatty fish consumption at about two servings per week (about 250mg EPA+DHA/d) decreases risk of CVD events. Intakes above this level appeared to result in no significant additional decreases in risk of CVD events, as shown in Figure D3.2a and D.3.2b.

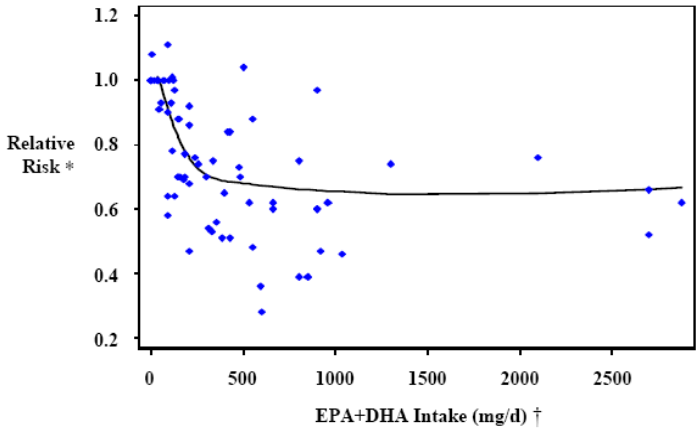
The randomized controlled trial evidence showed an inverse protective association between fish intake and intermediate markers of CVD risk and CVD health outcomes. The interventions were fish-specific and included the following: one study that showed herring significantly increased serum HDL levels (Lindqvist, 2009); two studies on salmon that showed salmon versus no fish intake improved serum lipids and blood pressure (Lara, 2006) and intake of salmon with different levels of EPA + DHA showed the high EPA + DHA salmon improved serum lipids and markers of inflammation (Seierstad, 2005); and one study comparing fatty versus lean fish showed that fatty fish consumption improved serum lipid profiles and markers of insulin resistance and inflammation (Lankinen, 2006).

Figure D3.2a. Relationship between intake of fish or fish oil and relative risks of CHD death in prospective cohort studies and randomized clinical trials



Note: Absolute coronary heart disease (CHD) mortality rates vary more than 100-fold across different populations (due to differences in age, prior CHD, and other risk factors), but the relative effects of intake of fish or fish oil are consistent, whether for primary or secondary prevention, for cohort studies or randomized trials, or for comparing populations at higher or lower absolute risk. Compared with little or no fish intake, modest consumption (~250-500 mg/d eicosapentaenoic acid [EPA] plus docosahexaenoic acid [DHA]) is associated with lower risk of CHD death, while at higher levels of intake, rates of CHD death are already low and are not substantially further reduced by greater intake.
Source: Mozaffarian and Rimm, *JAMA* 2006;296:1885-1899. Used with permission, American Medical Association, Chicago, IL.

Figure D3.2b. Relative risk of coronary heart disease death by dose of EPA+DHA



Note: The relationship between intake of fish or fish oil and relative risk of coronary heart disease (CHD) death in a pooled analysis of the prospective studies and randomized trials show that fatty fish consumption at about two servings per week (about 250 mg EPA+DHA/d) decreases risk of CVD events. Intakes above this level appeared to result in no significant additional decreases in risk CVD events.
Source: Mozaffarian and Rimm, *JAMA* 2006;296:1885-1899. Used with permission, American Medical Association, Chicago, IL.

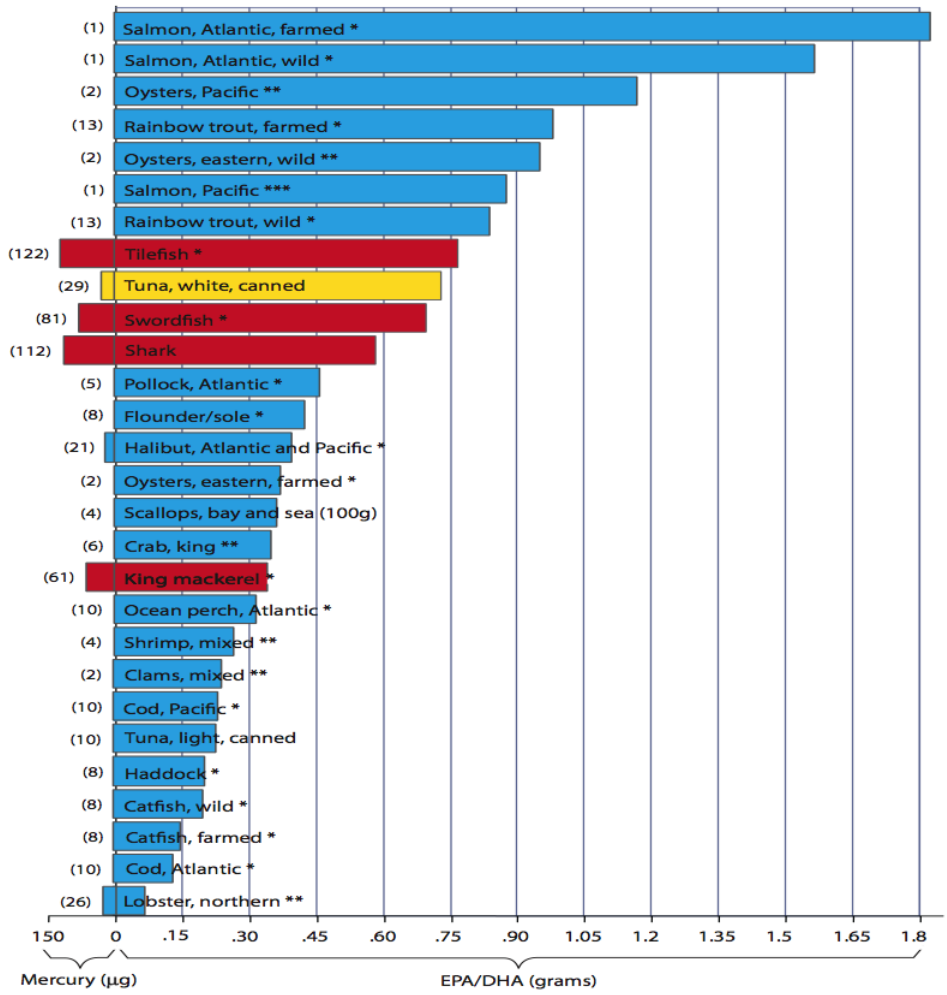
Evidence from prospective cohort studies was substantial and focused on primary CVD prevention in healthy adults. Ten prospective cohort studies examined the association between fatty fish and CVD outcomes and found a positive association between seafood and seafood-derived *n*-3 fatty acid consumption and decreased CVD incidence/risk (Levitan, 2009; Virtanen, 2008; Yamagishi, 2008; Streppel, 2008; Turunen, 2008; Järvinen, 2006; Iso, 2006; Mozaffarian, 2005; Lemaitre, 2003; Albert, 2002). Three prospective cohort studies examined fish and fish-derived fatty acid consumption and atrial fibrillation and found either no association between fish *n*-3 fatty acid intake and reduced risk of atrial fibrillation (Brouwer, 2006; Frost and Vestergaard, 2005) or a inverse association between consumption of tuna or other broiled or baked fish (but not fried fish) and incidence of atrial fibrillation (Mozaffarian, 2004). Virtanen et al. (2009) reported *n*-3 fatty acids (especially DHA) to be effective in reducing atrial fibrillation in men. One prospective cohort study examined the association between fatty fish intake and intermediate markers of CVD risk and found moderate intake of fatty fish was inversely associated with serum lipids and blood pressure (Panagiotakos, 2007). One prospective cohort study assessed fish *n*-3 FA intake on CVD and CHD mortality and found no independent association with CHD or stroke mortality (Folsom and Demissie, 2005). One prospective cohort study found a positive association between fish intake and increased incidence of T2D (Kaushik, 2009). This is the only observational evidence regarding risk of T2D, but the randomized controlled trial on fatty vs. lean fish by Lankinen et al. (2009) examined markers of insulin resistance and can be added to the evidence regarding T2D.

The 2005 DGA indicated there was sufficient evidence to suggest that *n*-3 PUFA consumption provided protection for persons with existing CVD. For the current 2010 review, conclusions related to persons with CVD relied on the ADA evidence-based review referred to above, as a NEL search did not yield additional studies that met the inclusion criteria. Four studies were reviewed by the ADA that addressed the relationship between consumption of fish-derived *n*-3 fatty acids and risk of CVD events in persons with CVD. One was a methodologically strong meta-analysis covering 11 randomized controlled trials (Bucher, 2002) and three studies were methodologically strong prospective cohort studies conducted in the US with cohort size ranging from 228 to 415 participants (Erkkila, 2003, 2004, 2006*)*. All of these articles provided evidence of the protective effects of consuming long-chain *n*-3 fatty acids on risk of CVD events in persons with known CVD. Erkkila et al. (2003) found blood levels of ALA, EPA and DHA were associated with a reduction in risk of all-cause mortality, but associations with combined fatal and non-fatal CVD events specifically were not significant, suggesting a totally different mechanism. Erkkila et al. (2004) and Erkkila et al. (2006) found fish-derived *n*-3 fatty acids exerted protective effects against progression of coronary artery arteriosclerosis. Women who ate two or more servings of fish per week had significantly fewer new lesions, and women with plasma DHA levels above the median exhibited less atherosclerosis progression than those below the median. A meta-analysis that included two diet

intervention trials (Bucher, 2002) assessed the effect of a diet high in long-chain *n*-3 fatty acids from fish (compared to control) and found long-chain *n*-3 fatty acids decreased the relative risk of myocardial infarction, sudden death, and overall mortality in persons with coronary artery disease.

Figure D3.3 shows examples of seafood and their respective content of EPA and DHA and methyl mercury. (See ***Part D.8: Food Safety and Technology*** for a detailed discussion of the risks and benefits of seafood consumption.)

Figure D3.3. Estimated EPA/DHA content and methyl mercury content of 3 oz. portions of seafood



* = cooked, dry heat.
** = cooked, moist heat.
*** = EPA and DHA content in Pacific salmon is a composite of chum, coho, and sockeye.
Source: Institute of Medicine (IOM). Seafood Choices, 2006. Used with permission, National Academies Press, Washington, DC.

Seafood Modeling

The implications for nutrient adequacy of increasing seafood in the USDA food patterns was studied by modeling three scenarios of differing levels of seafood consumption, using the reference 2000 calorie per day food intake pattern:

- Scenario 1: 4 oz. per week of seafood high in *n*-3 fatty acids.
- Scenario 2: 8 oz. per week of seafood, including seafood both low and high in *n*-3 fatty acids in proportions to those currently consumed by Americans.
- Scenario 3: 12 oz. per week of seafood low in *n*-3 fatty acids.

One goal of this modeling analysis was to quantify seafood consumption recommendations for the general public—something not done previously because of a lack of strong evidence on the role of seafood consumption in population health. The three scenarios were modeled to determine the amounts of foods to include in the Meat and Beans group so as to meet nutrient recommendations without altering the calorie level of the patterns. (See the Seafoods report, online Appendix E3.10, available at www.dietaryguidelines.gov). The analysis showed that the amounts of seafood in the base USDA food patterns could be increased to 8 ounces per week without any negative impact on nutrient adequacy. The total amounts of EPA and DHA for the three seafood scenarios modeled were 292 mg per day for 4 oz. of high n-3 seafood (Scenario 1); 253 mg per day for 8 oz. of the current mixture of low and high n-3 seafood (Scenario 2); and 201 mg per day for 12 oz. of low n-3 seafood (Scenario 3). This analysis did not incorporate the methyl mercury content of fish included in the patterns; however, the amounts of methyl mercury found in the seafood varieties used in the patterns are zero to minimal. (See Part D.8: Food Safety and Technology for a detailed discussion of the risks and benefits of seafood consumption.)

Question 8. What is the Relationship between Consumption of Plant *n*-3 Fatty Acids and Risk of CVD?

Conclusion

Alpha-linolenic acid (ALA) intake of 0.6 - 1.2 percent of total calories will meet current recommendations and may lower CVD risk, but new evidence is insufficient to warrant greater intake beyond this level. Limited but supportive evidence suggests that higher intake of n-3 fatty acids from plant sources may reduce mortality among persons with existing CVD.

Implications

Evidence is currently insufficient to make a formal guideline to increase n-3 intake from plant sources without additional evidence from randomized clinical trials and prospective observational

studies among participants with a broad range of n-3 intake. As relatively little ALA converts to EPA and DHA, evidence is lacking that plant-derived n-3 fatty acids alone will provide the same cardioprotective effects as EPA and DHA consumed at the recommended level discussed above. This increases the need for efficient and ecologically friendly strategies to allow for greater consumption of seafood n-3 fatty acids, unless plant-derived sources of EPA or DHA can be developed.

Review of the Evidence

The NEL conducted an evidence review to determine the relationship between consuming plant-derived *n*-3 PUFA and the risk of CVD events. This review relied upon an evidence-based review conducted by the ADA on the relationship between n-3 fatty acids and CVD, covering the literature from 2004 to 2007 (ADA, 2008). Overall, five studies were reviewed by ADA that addressed this question. These included two methodologically strong case control studies (Lemaitre, 2003, Rastogi, 2004), and three prospective cohort studies (two were methodologically strong [Albert, 2005; Mozaffarian, 2005] and one was methodologically neutral [Folsom and Demissie, 2005]). In addition, the NEL reviewed three studies since 2008, including one methodologically strong case-control study conducted in the US (Lemaitre, 2009), one methodologically strong prospective cohort study covering 2,682 men in Finland (Virtanen, 2009), and one methodologically strong systematic review of 14 randomized controlled trials, 25 prospective cohort studies, and 7 case-control studies (Wang, 2006).

Lemaitre et al. (2009) reported that an increase in red blood cell membrane ALA corresponding to 1 standard deviation was associated with 32 percent higher risk of sudden cardiac arrest (odds ratio = 1.32, 95% confidence interval: 1.07 - 1.63) after adjusting for confounding variables. Virtanen et al. (2009) found that red blood cell membrane ALA and intermediate chain *n*-3 PUFA did not have any association with atrial fibrillation. Wang et al. (2006) conclude from their systematic review that increased intake of *n*-3 fatty acids from fish or fish-oil supplements, but not of ALA, reduces the rates of all-cause mortality, cardiac and sudden death.

Two studies of persons with CVD were part of the 2008 ADA review. One methodologically neutral randomized controlled trial (Baylin, 2003) and one methodologically neutral case control study (De Lorgeril, 1999) found a diet high in plant-derived *n*-3 fatty acids protective against recurrence of myocardial infarction. Both studies used biomarkers. Baylin et al. (2003) found an inverse relationship between adipose tissue ALA and risk of nonfatal acute myocardial infarction. The greatest protection was found in those individuals who also had low total *trans* fatty acids in adipose tissue. Study participants in the top quintiles of adipose tissue ALA (0.72% of fatty acids) had a lower risk of myocardial infarction than those in the lowest quintile (0.35% of fatty acids). The difference in adipose tissue ALA corresponds to approximately 0.3 g per day of dietary intake. De

Lorgeril et al. (1999) found a decreased rate of cardiac death and nonfatal myocardial infarction in those following a Mediterranean diet versus a Western diet (1.24 vs. 4.07 per hundred patients per year). The experimental group had a significantly lower intake of total lipids and SFA, and increased intake of oleic acid, LA and ALA. The plasma concentration of ALA and DHA tended to be inversely associated with recurrence of myocardial infarction.

Question 9. What are the Effects of Maternal Dietary Intake of *n*-3 Fatty Acids from Seafood on Breast Milk Composition and Health Outcomes in Infants?

Conclusion

Moderate evidence indicates that increased maternal dietary intake of long chain *n*-3 PUFA, in particular docosahexaenoic acid (DHA) from at least 2 servings of seafood per week, during pregnancy and lactation is associated with increased DHA levels in breast milk and improved infant health outcomes, such as visual acuity and cognitive development.

Implications

There has been controversy and concern over the consumption of fish during pregnancy and lactation with regard to exposure of the fetus and infant to heavy metals during the most sensitive period of neurodevelopment. The current evidence, however, favors consumption of fish for pregnant and lactating women, particularly in the context of women making educated choices to consume seafood that is high in *n*-3 fatty acids and low in environmental pollutants. The benefits of fish consumption are maximized with fatty fish high in EPA and DHA but low in methyl mercury. These conclusions are consistent with those found in the discussion of seafood benefits and risks in ***Part D.8: Food Safety and Technology***. The previously described modeling analysis of seafood identified scenarios of type and quantity of fish that provide 250 mg per day of EPA + DHA.

Review of the Evidence

Since the 2005 DGAC report, a number of organizations have rendered expert opinions on the subject of *n*-3 PUFA supplements during pregnancy and lactation, including a Cochrane Database Systematic Review (Makrides, 2009), ADA Evidence Analysis Library review (Kaiser, 2008), and the European Union Perinatal Lipid Intake Working Group assessment (Koletzko, 2007). The 2010 DGAC reviewed these reports as well as a background paper by Brenna and Lapillonne (2009), which provided context on the effects of supplemental long-chain *n*-3 PUFA during pregnancy and lactation. This background paper covered 23 randomized controlled trials on supplemental DHA at physiological and pharmacologic levels, and highlighted the benefits of maternal DHA consumption on infant/child intelligence scores, among other positive outcomes.

For the purposes of this review, the DGAC excluded studies with long chain *n*-3 PUFA given in "supplement" form (e.g. fish oil, cod liver oil, fish oil capsules). This removed most randomized clinical trials during pregnancy and lactation from consideration. Also not included were breast feeding versus infant formula feeding studies (before DHA addition), and studies of pre-term versus full-term infants.

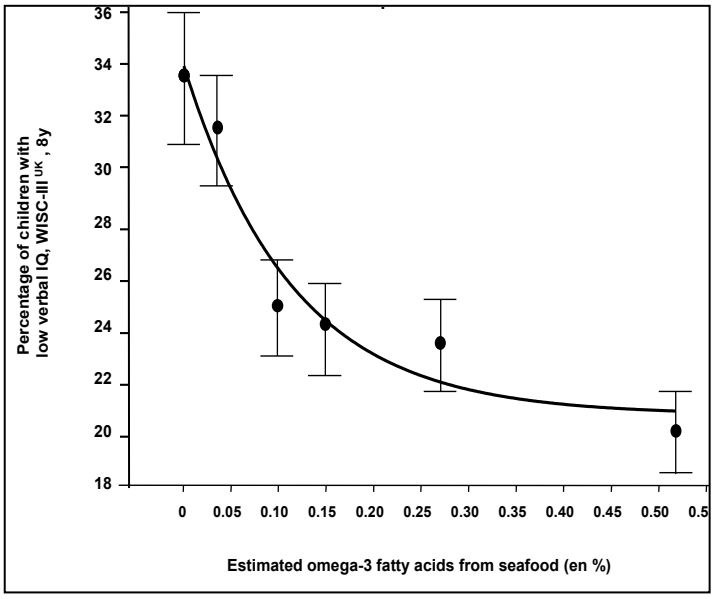
Overall, nine articles were reviewed since 2000 to determine the effect of *n*-3 fatty acids on breast milk composition and infant health outcomes. There were seven methodologically strong prospective cohort studies conducted in the US, Europe, and Canada in healthy women with low-risk pregnancies, healthy mother/infant pairs, or healthy children up to 8 yrs in cohort sizes ranging from 211 to 50,276 participants (Drouillet, 2009; Hibbeln, 2007; Innis, 2001; Oken, 2005; Oken , 2008a; Oken, 2008b; Olsen, 2006). In addition, the evidence included one methodologically strong randomized controlled trial of 350 mother/infant pairs in the US (Colombo, 2004) and one methodologically strong meta-analysis of 65 international studies (Brenna, 2007).

The prospective cohort studies focused on maternal DHA consumption during pregnancy and, overall, the evidence for benefits from maternal DHA consumption during pregnancy was strong. Because randomized controlled trials with DHA supplements were excluded, there were fewer studies on maternal DHA intake during lactation. However, one study examined both pregnancy and duration of breastfeeding with improved infant cognitive outcomes (Oken, 2008b) and another measured breastfeeding with associated DHA biomarkers in infants with improved cognitive outcomes (Innis, 2001).

One prospective cohort study showed that low maternal fish intake was associated with increased risk of children being in the lowest quartile for verbal intelligence quotient (IQ), and increased risk of suboptimal outcomes for fine motor skills and communication/social development scores (Hibbeln, 2007). Hibbeln et al. (2007) estimated incidence of suboptimal verbal IQ in children eight years of age as a function of maternal seafood consumption during pregnancy in 11875 women. The study was conducted in British women and analysis controlled for 28 potentially confounding variables, such as birth weight, alcohol use during pregnancy and smoking. Children of mothers reporting the highest seafood consumption, estimated using a food frequency questionnaire and estimated *n*-3 intake, were significantly less likely to score in the lowest quartile for verbal IQ compared to women who reported no seafood consumption during pregnancy (Figure D3.4).

Figure D3.4. Effect on children's verbal IQ of maternal seafood consumption during pregnancy

Figure D3.4 shows prevalence of children with low verbal IQ according to mothers' consumption of *n*-3 fatty acids from seafood. Estimated maternal consumption of long chain *n*-3 fatty acids is expressed as proportion of total calories (en %). Maternal seafood consumption was grouped into six categories: mothers with no reported consumption plus five equal groups of the remaining population. Means and 95% CI for proportion of children in the lowest quartile for verbal IQ.



Source: Hibbeln et al., 2007 *Lancet*. Feb 17;369(9561):578-85. Used with permission from Elsevier, publisher of *The Lancet*, Oxford, UK.

Two reports from Project VIVA on maternal seafood intake and infant cognition showed that higher fish consumption in pregnancy was associated with better infant cognition, but if the fish consumed resulted in higher mercury levels, this was associated with lower cognition. The visual recognition memory scores were highest among infants of women who consumed more than two weekly fish servings, but had mercury levels less than 1.2 ppm (Oken, 2005). No benefit was associated with fish consumption of less than two servings per week (Oken, 2008a).

The effect of maternal fish consumption during pregnancy and duration of infant breastfeeding on child developmental milestones in participants of the Danish National Birth Cohort showed that higher maternal fish intake and greater duration of breastfeeding were associated with higher child developmental scores at ages 6 and18 months (Oken, 2008b). Related to maternal fish consumption and biomarkers during lactation, increased red blood cell phosphatidylethanolamine DHA in infants was associated with improved visual acuity and speech perception (Innis, 2001).

Maternal fish consumption was also associated with improved perinatal outcomes. A prospective cohort study in Denmark showed that mean gestation length was shorter and odds of preterm delivery were increased in subjects who never consumed fish, compared with those who

consumed fish at least once per week (Olsen, 2006). A study of the EDEN mother-child cohort in France showed that high fish intake during pregnancy was not associated with increased fetal growth, but in a sub-population of overweight women, high fish intake was associated with increased fetal growth and head circumference (Drouillet, 2009).

One randomized controlled trial using high DHA eggs (133 mg DHA/d) fed during pregnancy showed infants with improved measures of visual habituation and attention span, compared to mothers on low DHA eggs (Colombo, 2004).

One meta-analysis of 65 international studies measured distribution of DHA and arachidonic acid (AA) concentrations in breast milk. Brenna et al. (2007) found that in mothers worldwide, DHA concentrations were lower and more variable than AA concentrations in breast milk. The highest DHA concentrations were found in coastal populations and associated with seafood consumption. Overall, compared to AA, breast milk DHA content was more sensitive to dietary intake.

CARDIOVASCULAR HEALTH EFFECTS RELATED TO CONSUMPTION OF SPECIFIC FOODS HIGH IN FATTY ACIDS

Specific whole foods high in fat content were examined for effects on cardiovascular health. The two foods selected for inclusion are nuts and chocolate. The health effects of consuming other high-fat, high-calorie foods, such as full-fat dairy products and meats are discussed in other chapters (see, for example, ***Part D.2. Nutrient Adequacy***).

Question 10. What are the Health Effects Related to Consumption of Nuts?

Conclusion

There is moderate evidence that consumption of unsalted peanuts and tree nuts, specifically walnuts, almonds, and pistachios, in the context of a nutritionally adequate diet and when total calorie intake is held constant, has a favorable impact on cardiovascular disease risk factors, particularly serum lipid levels.

Implications

Most nut consumption is in the form of peanuts, though tree nuts (walnuts, almonds, pecans, pistachios) are frequently used in cooking and as snack foods. Peanuts are also an important source of plant protein. Many nuts (e.g. peanuts, almonds, cashews) are sold with added salt as snack foods; thus, the recommendations for consumption are limited to unsalted nuts as a means to reduce sodium intake. It also is important to note that nuts should be consumed in small portions, as they are high in calories and can contribute to weight gain.

Review of the Evidence

Background

Nuts are a commonly consumed food in the US, and certain varieties, such as peanuts, walnuts, almonds, pecans, and pistachios, are often used in cooking and as snack foods (Table D3.12). Peanuts and other nuts also are an important source of plant protein (Table D3.13). See ***Part D. Section 4: Protein*** for additional information on the contribution of plant sources of protein to the diet.

In recent years, investigators have examined the potential cardiovascular benefits associated with certain foods high in fat. Nuts are a primary example of these foods. Because nuts, especially peanuts, are so frequently consumed in the US, the 2010 DGAC decided to review the evidence on this issue.

Table D3.12. Estimated mean daily intakes of tree nuts and peanuts[1] by adults 20 years and over, US 2005-2006

Gender	Sample size	Mean[2] intake of nuts (grams)	Mean[2] energy from nuts (kcal)	Mean energy from nuts (%)
Men	2163	9.7±0.87	57±5.2	2.2
Women	2357	5.6±0.51	34±3.1	1.9
All adults	4520	7.5±0.46	45±2.7	2.1

[1]Includes tree nuts and peanuts eaten out of hand, either alone or in nuts mixtures containing dried fruits and/or seeds, and peanut butter eaten alone or in sandwiches. Nuts in baked products, such as muffins and cakes, and nuts in candies are *not* included.
[2]Mean±standard error.
Source: USDA, Agricultural Research Service, Food Surveys Research Group.2010. Tree nuts and peanuts. Available at http://www.cnpp.usda.gov/Publications/DietaryGuidelines . Accessed May 5, 2010.

Table D3.13. Nutrient composition of nuts per 1.5 ounces (43 g)

Type	Energy (kcal)	Total fat (g)	Saturated fatty acids (g)	Monounsaturated fatty acids (g)	Polyunsaturated fatty acids (g)	Protein (g)
Almonds	254	22.5	1.7	14.3	5.4	9.4
Brazil nuts	279	28.2	6.4	10.4	8.8	6.1
Cashews	244	19.7	3.9	11.6	3.3	6.5
Hazelnuts	275	26.5	1.9	19.8	3.6	6.4
Macadamias	305	32.4	5.1	25.2	0.6	3.3
Peanuts	249	21.1	2.9	10.5	6.7	10.1
Pecans	302	31.6	2.7	18.7	8.7	4.0
Pistachios	243	19.6	2.4	10.3	5.9	9.1
Walnuts, English	278	27.7	2.6	3.8	20.1	6.5

Source: USDA, Agricultural Research Service, USDA Nutrient Data Laboratory. 2009. USDA National Nutrient Database for Standard Reference, Release 22. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl.

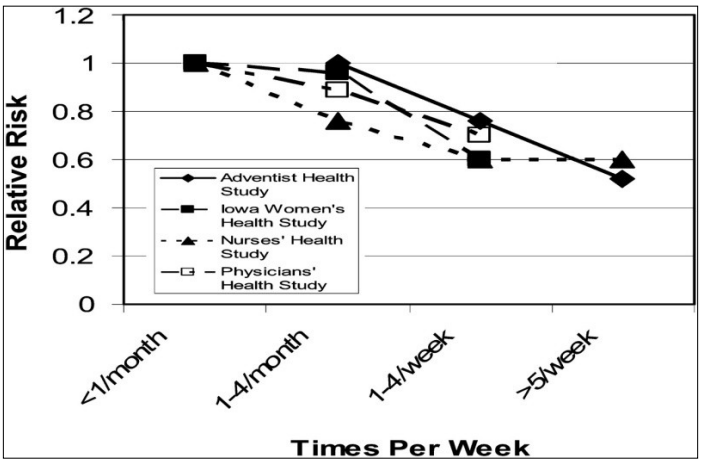
Evidence Summary

The NEL reviewed the literature from 2000 and was informed by studies from a previous systematic review on almonds conducted by the ADA's Evidence Analysis Library. Overall, 17 studies were identified since 2000. These studies included four methodologically strong prospective cohort studies conducted in the US and Europe ranging in cohort size from 6,309 to 51,118 participants (Bes-Rastrollo, 2007; Bes-Rastrollo, 2009; Djousse, 2009; Li, 2009); 10 randomized controlled trials conducted in the US ranging from 15 to 1,224 participants (four methodologically strong (Sabate, 2005; Salas-Salvado, 2008a, 2008b; Wien, 2003) and six methodologically neutral (Gebauer, 2008; Griel, 2008; Kurlandsky and Stote, 2006; Olmedilla-Alonso, 2008; Rajaram, 2009; Sheridan, 2007); and three methodologically strong reviews covering international randomized controlled trials (Banel and Hu, 2009; Mukuddem-Petersen, 2005; Phung, 2009). These 17 studies were further subdivided based on studies of nuts in general (including peanuts) and studies of specific types of nuts in particular and are listed below. Overall, this review provided evidence that consumption of nuts collectively and walnuts, almonds, and pistachio nuts individually, in the context of a healthy diet and when calorie intake is constant, has a favorable impact on CVD risk factors, particularly serum lipid levels. The evidence was strongest for walnuts. Insufficient evidence was available to address the health effects of macadamia nuts or cashews.

Six studies on nuts in general, including peanuts, were reviewed to determine their health benefits. Overall, the studies indicated beneficial effects of nut consumption on intermediate markers and CVD risk. These studies included one systematic review with meta-analysis (Mukuddem-Petersen, 2005) covering 13 randomized controlled trials that showed decreased total and LDL cholesterol in study participants consuming nuts compared to participants consuming control diets. In two prospective cohort studies in high risk populations, one found that consumption of at least 5 servings per week of nuts or peanut butter was significantly associated with lower total, LDL, non-HDL cholesterol and apoB-100 concentrations, as well as a lower risk of CVD (Li, 2009), and one showed that a Mediterranean diet high in nuts resulted in the most significant improvement in inflammatory markers related to endothelial function (Salas-Salvado, 2008). Two prospective cohort studies indicated that nut consumption (= or > 2 servings/week) was associated with decreased incidence of weight gain and obesity (Bes-Rastrollo, 2007; Bes-Rastrollo, 2009). Djousse and colleagues found an inverse relationship between nut consumption and hypertension in lean participants, but not in overweight or obese participants in the Physicians' Health Study (Djousse, 2009).

For additional context regarding nuts in general, two meta-analyses demonstrated consistent and dose-responsive changes in coronary disease risk with increasing doses of nuts per month for four prospective studies (Kris-Etherton, 2008; Sabate, 2009) (Figure D3.5).

Figure D3.5. Frequency of nut consumption and coronary heart disease risk reduction in a dose-response manner



Note: Results are from four epidemiologic studies.
Source: Sabaté J, Ang Y. *Am J Clin Nutr* 2009;89:1643S-1648S. Used with permission, American Society for Nutrition.

Evidence analysis was also conducted on specific types of nuts including almonds, walnuts, macadamia nuts, and pistachios. Overall, studies showed that almond consumption improved total cholesterol (Phung, 2009; Wein, 2003), decreased LDL cholesterol and the LDL:HDL cholesterol ratio (Wein, 2003) or was neutral regarding LDL and LDL:HDL cholesterol ratio (Phung, 2009; Kurlandsky and Stote, 2006). Regarding walnuts, studies showed that walnut consumption improved total cholesterol, LDL cholesterol and the LDL:HDL cholesterol ratio (Banal and Hu, 2009; Rajaram, 2009; Olmedilla-Alonso, 2008). Olmedilla-Alonso et al. (2008) found that meat products with walnuts decreased body weight. However, one randomized crossover trial found that a walnut supplemented diet (12% energy from walnuts) provided more calories per day and increased body weight and BMI (Sabate, 2005). Energy-adjusted results were not significant, indicating that care must be taken to accommodate the caloric content of nuts in the diet. Lastly, studies focused on macadamia nuts (Griel, 2008) or pistachios (Sheridan, 2007; Gebauer, 2008) showed that both decreased total cholesterol, LDL cholesterol, and the LDL:HDL cholesterol ratio.

Question 11. What are the Health Effects Related to Consumption of Chocolate?

Conclusion

Moderate evidence suggests that modest consumption of dark chocolate or cocoa is associated with health benefits in the form of reduced CVD risk. Potential health benefits need to be balanced with caloric intake.

Implications

Chocolate as currently consumed is a small component of the total diet, and benefits or risks will likely be minimal. Potential health effects need to be balanced with caloric intake, as chocolate is a calorie dense product. The predominant fat in chocolate is stearic acid, which has been shown to not raise blood cholesterol. Different formulations of chocolate vary in their content of dairy fat, with darker chocolate containing less dairy fat. Beneficial effects of chocolate have been attributed to polyphenolic compounds, in particular flavonoids. Many plant-based foods contain polyphenolic compounds and chocolate is a minor source. Formulations of chocolate are known to have different polyphenolic profiles, and, if this is the mechanism of chocolate's beneficial actions, different forms of chocolate may confer different benefits.

Review of the Evidence

The current evidence regarding chocolate and health outcomes primarily focuses on flavonoids as bioactive constituents of chocolate and their relation to CVD risk. Flavonoids are a subgroup of polyphenols and within the flavonoid chemical hierarchy the flavan-3-ols (flavanols) are particularly high in dark chocolate and cocoa. The flavan-3-ols in dark chocolate and cocoa are primarily catechins, epicatechins (monomers) and procyanidins (polymers).

A NEL search of the literature since 2000 identified a total of 13 studies that addressed the question on health effects of chocolate consumption. Three methodologically strong systematic reviews of international randomized controlled trials and prospective cohort studies (Desch, 2010; Ding, 2006; Hooper, 2008) were identified. Eight randomized controlled trials conducted in the US, Europe, Australia, and Japan, covering from 25 to 297 participants, that were methodologically strong (Allen, 2008) and methodologically neutral (Baba, 2007; Crews, 2008; Davidson, 2008; Farouque, 2006; Kurlandsky and Stote, 2006; Monagas, 2009; Tuabert, 2007) were identified. And one methodologically strong prospective cohort study of 876 males in the Netherlands (Buijsse, 2006) and one methodologically neutral population-based case-control study conducted in Sweden (Janszky, 2009) were included to address this question.

The systematic review and meta-analysis by Desch et al. (2010) covered 10 randomized controlled trials and showed that high-flavanol chocolate or cocoa significantly lowered systolic and diastolic BP (Desch, 2010). Hooper et al. (2008) included 6 randomized controlled trials in their meta-analysis and showed that dark chocolate or cocoa improved flow mediated dilation both acutely and chronically. Ding et al. (2006) included 21 randomized controlled trials and 11 prospective cohort studies and both flavonoids and stearic acid were examined for association with intermediate markers and CVD outcomes. Overall, the randomized controlled trials suggested that cocoa and chocolate have beneficial effects on blood pressure, inflammatory markers, anti-platelet function, serum HDL, and LDL oxidation. The prospective cohort studies showed that flavonoids in chocolate were positively associated with decreased risk of CHD and myocardial infarction

mortality. Overall, the evidence from these systematic reviews and meta-analyses was strengthened by the consistency of findings across studies.

The randomized controlled trials in this evidence analysis were focused on flavonoids and intermediate markers of CVD risk. Studies showed that dark chocolate or cocoa consumption decreased serum total cholesterol and LDL cholesterol, increased HDL cholesterol, delayed LDL oxidation (Baba, 2007), decreased serum triglycerides, and improved inflammation markers (Kurlandsky and Stote, 2006). However, one study found no effect of dark chocolate consumption on serum cholesterol levels (Kurlandsky and Stote, 2006). Regarding BP, dark chocolate or cocoa consumption decreased systolic blood pressure (Allen, 2008; Tuabert, 2007), diastolic blood pressure (Davidson, 2008), and decreased prevalence of hypertension (Tuabert, 2007). However, one randomized controlled trial found no effect of dark chocolate or cocoa consumption on blood pressure (Crews, 2008). A more detailed analysis of inflammation markers showed that cocoa consumption decreased monocyte expression of numerous cell adhesion molecules (Monagas, 2009). Additionally, high-flavonol cocoa (versus low flavonol cocoa) increased flow-mediated dilation, both acutely and chronically, and reduced insulin resistance (Davidson, 2008). High-flavonol cocoa was also tested in individuals with coronary artery disease and did not improve any markers of arterial blood flow or inflammation (Farouque, 2006).

The evidence regarding chocolate and CVD health outcomes contains relatively few epidemiologic studies. Overall, this evidence included populations in the US, Europe, Japan, and Australia, participating in both primary prevention and, to a lesser extent, secondary prevention studies. Sample sizes ranged from relatively small randomized controlled trials to 470 participants in the Zutphen Elderly Study and 1,169 participants in the SHEEP study.

A prospective cohort study in the Netherlands examined cocoa intake and found it inversely associated with blood pressure and CVD mortality in male participants from the Zutphen Elderly Study (Buijsse, 2006). A population-based case-control study assessed the effects of chocolate consumption in patients with established CHD in the Stockholm Heart Epidemiology Program (SHEEP) where people who had had myocardial infarctions were followed for 8 years. In this study, chocolate consumption had a significant inverse association with cardiac mortality (Janszky, 2009).

Chapter Summary

Dietary fatty acids and cholesterol are major determinants of two major causes of morbidity and mortality in Americans, namely CVD and T2D. The health impacts of dietary fats and cholesterol are mediated through levels of serum lipids, lipoproteins, and other intermediary factors. The consumption of harmful types and amounts of fatty acids and cholesterol has not changed

appreciably since 1990. In order to reduce the population's burden from CVD and T2D, and their risk factors, the preponderance of the evidence indicates beneficial health effects associated with:

1. Limiting saturated fatty acid intake to less than 7 percent of calories, replacing these calories with those from mono- or polyunsaturated fatty acids, rather than carbohydrates. As an interim step toward this less than 7 percent goal, all individuals should immediately consume less than 10 percent of energy as saturated fats.
2. Limiting dietary cholesterol to less than 300 mg per day with further reductions of dietary cholesterol to less than 200 mg per day in persons with or at high risk for CVD or T2D.
3. Avoiding *trans* fatty acids from industrial sources in the American diet, leaving small amounts from *trans* fatty acids from natural (ruminant) sources.
4. Redefining cholesterol-raising fats as saturated fats (exclusive of stearic acid) and *trans* fatty acids, with a recommended daily intake of less than 5 percent of energy.
5. Consuming two servings of seafood per week (4 oz. cooked, edible seafood per serving) which provide an average of 250 mg/day of *n*-3 fatty acids from marine sources.
6. Ensuring maternal dietary intake of long chain *n*-3 fatty acids, in particular DHA, during pregnancy and lactation through two or more servings of seafood per week, with emphasis on types of seafood high in *n*-3 fatty acids and with low methyl mercury content.

Needs for Future Research

Saturated Fatty acids

1. Determine the benefits and risks of MUFA vs. PUFA as an isocaloric substitute for SFA (see below). Confirm the metabolic pathways through which dietary SFA affect serum lipids, especially as some SFA (e.g. stearic acid) do not appear to affect blood lipid levels.

   Rationale: The growing data to support a risk of T2D from SFA consumption indicates the need for fat-modified diets in persons with pre-diabetes, including those with metabolic syndrome, and with established diabetes. Since the ages of onset of T2D now include childhood, studies from adolescence through middle age would be useful to define when SFA-reduced diets would be most effective.

2. Conduct feeding studies using cholesterol from sources other than eggs and funded by non-industry sponsors. Conduct research on low and high risk consumers of dietary cholesterol and determine a better definition of hypo- and hyper-responders to dietary cholesterol, with respective underlying genetic polymorphisms. Identify additional subgroups in which dietary cholesterol appears especially harmful with regard to cardiovascular risk. .

Rationale: Most of the feeding studies with serum lipid and lipoprotein endpoints used eggs as the primary source of cholesterol, and many of the studies were funded by industry. Since the proportion of dietary cholesterol in the US diet supplied by eggs has declined to less than 25 percent, feeding trials on other dietary sources of cholesterol would be useful. Persons with T2D appear to be a subgroup in which dietary cholesterol is particularly harmful and better understanding of the mechanisms and magnitude of risk would be essential, as eggs are an important, low fat source of protein in T2D patients.

3. Determine the mechanism by which dietary MUFA improve serum lipids, glucose metabolism, insulin levels, Homeostatic Model Assessment (HOMA) scores, inflammatory markers, and blood pressure in both healthy persons and in persons with T2D. Studies of replacing carbohydrates or other dietary fat with MUFA should include isocaloric substitutions, so as not to be confounded by differences in energy.

   Rationale: Understanding the mechanism by which MUFA improve risk of CVD and T2D will enhance our ability to make specific recommendations for MUFA consumption in healthy and at-risk individuals.

4. Determine the mechanism by which dietary PUFA improve serum lipids, glucose metabolism, insulin levels, HOMA scores, inflammatory markers, and blood pressure in both healthy persons and in persons with T2D. Studies of replacing carbohydrates or other dietary fat with PUFA should include isocaloric substitutions, so as not to be confounded by differences in energy.

   Rationale: Understanding the mechanism by which PUFA improve risk of CVD and T2D will enhance our ability to make specific recommendations for PUFA consumption in healthy and at-risk individuals. PUFA and MUFA have similar benefits as substitutes for SFA and *trans* fatty acids. Additional isocaloric comparisons of MUFA versus PUFA on metabolic intermediates and especially on clinical outcomes are needed to differentiate these two classes of fatty acids.

5. Examine stearic acid for its benefits as a solid fat, in contrast to liquid oils high in MUFA and PUFA; include other potential metabolic effects of stearic acid, such as inflammation and coagulation.

   Rationale: The benefit of stearic acid is that it has a high melting point and therefore is solid at room temperature, unlike other FAs which do not raise blood cholesterol (e.g. MUFA, PUFA). Comparisons of intermediate markers and other effects of stearic acid versus MUFA and PUFA would clarify ways that it could be best used in a calorie and nutrient-balanced diets.

6. Characterize the difference in metabolic effects and intermediate markers between industrial and ruminant *trans* fatty acids.

   Rationale: Since ruminant and industrial *trans* fatty acids have different chemical structures, better characterization of their metabolic effects though further feeding studies would be warranted.

7. Conduct randomized controlled trials and prospective observational studies in persons with and without CVD on plant compared to marine *n*-3 fatty acids. Examine diets rich in plant *n*-3 fatty

acids in individuals with and without adequate intake of *n*-3 fatty acids from marine sources. Examine the mechanism of action of marine vs. plant *n*-3 fatty acids for synergies and/or inhibition.

Rationale: Although there are consistent data on the benefits of *n*-3 fatty acids from seafood consumption, there is no research on comparing marine versus plant n-3 fatty acids on intermediate markers and CVD outcomes.

8. Investigate further the opposing interactions of high EPA and DHA versus high methyl mercury, especially in dietary patterns in which these consumptions coexist. Investigate high versus low DHA-consuming mothers and infants and the long-term effects on intelligence and other cognitive outcomes.

   Rationale: All aspects of the risk to benefit ratio of consumption of EPA + DHA and methyl mercury, both of which can be present in varying amounts in different types of seafood, should be further elucidated. DHA appears to be the active nutrient in seafood that provides benefits in infant development. Further studies of the role of DHA in neurodevelopment and dose-response relationships between DHA and health/development outcomes would be useful.

9. Conduct randomized controlled trials comparing different types of nuts on intermediate markers, such as serum lipids, and classify each specific type of nut as more or less associated with CVD risk reduction.

   Rationale: Additional randomized trials will be required over longer periods of time to determine if nuts confer long-term benefits. It is difficult to distinguish benefits to health and to intermediate metabolites between different types of nuts.

10. Elucidate further the role of polyphenolic compounds as major active ingredients in the health benefits of chocolate. Test different chocolate formulations that are commonly consumed by the general public.

    Rationale: Many chocolate and cocoa studies used formulations of chocolate that are not readily available to the consumer and were sponsored by industry. In order to determine the real health benefits of chocolate consumption, chocolate formulations that are available to, and consumed by, the general public need to be tested.

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