8

Dietary Fats: Total Fat and Fatty Acids

SUMMARY

Fat is a major source of fuel energy for the body and aids in the absorption of fat-soluble vitamins and carotenoids. Neither an Adequate Intake (AI) nor Recommended Dietary Allowance (RDA) is set for total fat because there are insufficient data to determine a defined level of fat intake at which risk of inadequacy or prevention of chronic disease occurs. An Acceptable Macronutrient Distribution Range (AMDR), however, has been estimated for total fat—it is 20 to 35 percent of energy (see Chapter 11). A Tolerable Upper Intake Level (UL) is not set for total fat because there is no defined intake level of fat at which an adverse effect occurs.

Saturated fatty acids are synthesized by the body to provide an adequate level needed for their physiological and structural functions; they have no known role in preventing chronic diseases. Therefore, neither an AI nor RDA is set for saturated fatty acids. There is a positive linear trend between total saturated fatty acid intake and total and low density lipoprotein (LDL) cholesterol concentration and increased risk of coronary heart disease (CHD). A UL is not set for saturated fatty acids because any incremental increase in saturated fatty acid intake increases CHD risk. It is neither possible nor advisable to achieve 0 percent of energy from saturated fatty acids in typical whole-food diets. This is because all fat and oil sources are mixtures of fatty acids, and consuming 0 percent of energy would require extraordinary changes in patterns of dietary intake. Such extraordinary adjustments may introduce undesirable effects (e.g., inadequate intakes of protein and

certain micronutrients) and unknown and unquantifiable health risks. The AMDR for total fat is set at 20 to 35 percent of energy. It is possible to have a diet low in saturated fatty acids by following the dietary guidance provided in Chapter 11.

n-9 *cis* Monounsaturated fatty acids are synthesized by the body and have no known independent beneficial role in human health and are not required in the diet. Therefore, neither an AI nor an RDA is set. There is insufficient evidence to set a UL for *n*-9 *cis* monounsaturated fatty acids.

Linoleic acid is the only *n*-6 polyunsaturated fatty acid that is an essential fatty acid; it serves as a precursor to eicosanoids. A lack of dietary *n*-6 polyunsaturated fatty acids is characterized by rough and scaly skin, dermatitis, and an elevated eicosatrienoic acid:arachidonic acid (triene:tetraene) ratio. The AI for linoleic acid is based on the median intake in the United States where an *n*-6 fatty acid deficiency is nonexistent in healthy individuals. The AI is 17 g/d for young men and 12 g/d for young women. While intake levels much lower than the AI occur in the United States without the presence of a deficiency, the AI can provide the beneficial health effects associated with the consumption of linoleic acid (see Chapter 11). There is insufficient evidence to set a UL for *n*-6 polyunsaturated fatty acids.

n-3 Polyunsaturated fatty acids play an important role as structural membrane lipids, particularly in nerve tissue and the retina, and are precursors to eicosanoids. A lack of α-linolenic acid in the diet can result in clinical symptoms of a deficiency (e.g., scaly dermatitis). An AI is set for α-linolenic acid based on median intakes in the United States where an *n*-3 fatty acid deficiency is nonexistent in healthy individuals. The AI is 1.6 and 1.1 g/d for men and women, respectively. While intake levels much lower than the AI occur in the United States without the presence of a deficiency, the AI can provide the beneficial health effects associated with the consumption of *n*-3 fatty acids (see Chapter 11). There is insufficient evidence to set a UL for *n*-3 fatty acids.

Trans fatty acids are not essential and provide no known benefit to human health. Therefore, no AI or RDA is set. As with saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentration, and therefore increased risk of CHD. A UL is not set for *trans* fatty acids because any incremental increase in *trans* fatty acid intake increases CHD risk. Because *trans* fatty acids are unavoidable in ordinary, nonvegan diets, consuming 0 percent of energy would require significant changes in patterns of dietary intake. As with saturated fatty acids, such adjustments may introduce undesirable effects (e.g., elimina-

tion of commercially prepared foods, dairy products, and meats that contain *trans* fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks. Nevertheless, it is recommended that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet. Dietary guidance in minimizing *trans* fatty acid intake is provided in Chapter 11.

BACKGROUND INFORMATION

Total Fat

Fat is a major source of fuel energy for the body. It also aids in the absorption of the fat-soluble vitamins A, D, E, and K and carotenoids. Dietary fat consists primarily (98 percent) of triacylglycerol, which is composed of one glycerol molecule esterified with three fatty acid molecules, and smaller amounts of phospholipids and sterols. Fatty acids are hydrocarbon chains that contain a methyl (CH_3-) and a carboxyl (-COOH) end. The fatty acids vary in carbon chain length and degree of unsaturation (number of double bonds in the carbon chain). The fatty acids can be classified into the following categories:

- Saturated fatty acids
- *Cis* monounsaturated fatty acids
- *Cis* polyunsaturated fatty acids
  — *n*-6 fatty acids
  — *n*-3 fatty acids
- *Trans* fatty acids

Dietary fat derives from both animal and plant products. In general, animal fats have higher melting points and are solid at room temperature, which is a reflection of their high content of saturated fatty acids. Plant fats (oils) tend to have lower melting points and are liquid at room temperature (oils); this is explained by their high content of unsaturated fatty acids. Exceptions to this rule are the seed oils (e.g., coconut oil and palm kernel oil), which are high in saturated fat and solid at room temperature. *Trans* fatty acids have physical properties generally resembling saturated fatty acids and their presence tends to harden fats. In the discussion below, total fat intake refers to the intake of all forms of triacylglycerol, regardless of fatty acid composition, in terms of percentage of total energy intake.

In addition to the functions of fat and fatty acids described above, fatty acids also function in cell signaling and alter expression of specific genes

involved in lipid and carbohydrate metabolism (Jump and Clarke, 1999; Sessler and Ntambi, 1998). Fatty acids may themselves be ligands for, or serve as precursors for, the synthesis of unknown endogenous ligands for nuclear peroxisome proliferator activating receptors (Kliewer et al., 1997; Latruffe and Vamecq, 1997). These receptors are important regulators of adipogenesis, inflammation, insulin action, and neurological function.

Phospholipids

Phospholipids are a form of fat that contains one glycerol molecule that is esterified with two fatty acids and either inositol, choline, serine, or ethanolamine. Phospholipids are primarily located in the membranes of cells in the body and the globule membranes in milk. A very small amount of dietary fat occurs as phospholipid. The metabolism of phospholipids is described below for total fat. The various fatty acids that are contained in phospholipids are the same as those present in triglycerides.

Saturated Fatty Acids

The majority of dietary saturated fatty acids come from animal products such as meat and dairy products (USDA, 1996). The remaining comes from plant sources. These sources provide a series of saturated fatty acids for which the major dietary fatty acids range in chain length from 8 to 18 carbon atoms. These are:

- 8:0 Caprylic acid
- 10:0 Caproic acid
- 12:0 Lauric acid
- 14:0 Myristic acid
- 16:0 Palmitic acid
- 18:0 Stearic acid

The saturated fatty acids are not only a source of body fuel, but are also structural components of cell membranes. Various saturated fatty acids are also associated with proteins and are necessary for their normal function. Saturated fatty acids can be synthesized by the body.

Fats in general, including saturated fatty acids, play a role in providing desirable texture and palatability to foods used in the diet. Palmitic acid is particularly useful for enhancing the organoleptic properties of fats used in commercial products. Stearic acid, in contrast, has physical properties that limit the amount that can be incorporated into dietary fat.

Cis *Monounsaturated Fatty Acids*

Cis monounsaturated fatty acids are characterized by having one double bond with the hydrogen atoms present on the same side of the double bond. Typically, plant sources rich in *cis* monounsaturated fatty acids (e.g., canola oil, olive oil, and the high oleic safflower and sunflower oils) are liquid at room temperature. Monounsaturated fatty acids are present in foods with a double bond located at 7 (*n*-7) or 9 (*n*-9) carbon atoms from the methyl end. Monounsaturated fatty acids that are present in the diet include:

- 18:1*n*-9 Oleic acid
- 14:1*n*-7 Myristoleic acid
- 16:1*n*-7 Palmitoleic acid
- 18:1*n*-7 Vaccenic acid
- 20:1*n*-9 Eicosenoic acid
- 22:1*n*-9 Erucic acid

Oleic acid accounts for about 92 percent of dietary monounsaturated fatty acids. Monounsaturated fatty acids, including oleic acid and nervonic acid (24:1*n*-9), are important in membrane structural lipids, particularly nervous tissue myelin. Other monounsaturated fatty acids, such as palmitoleic acid, are present in minor amounts in the diet.

n-*6 Polyunsaturated Fatty Acids*

The primary *n*-6 polyunsaturated fatty acids are:

- 18:2 Linoleic acid
- 18:3 γ-Linolenic acid
- 20:3 Dihomo-γ-linolenic acid
- 20:4 Arachidonic acid
- 22:4 Adrenic acid
- 22:5 Docosapentaenoic acid

Linoleic acid cannot be synthesized by humans and a lack of it results in adverse clinical symptoms, including a scaly rash and reduced growth. Therefore, linoleic acid is essential in the diet. Linoleic acid is the precursor to arachidonic acid, which is the substrate for eicosanoid production in tissues, is a component of membrane structural lipids, and is also important in cell signaling pathways. Dihomo-γ-linolenic acid, also formed from linoleic acid, is also an eicosanoid precursor. *n*-6 Polyunsaturated fatty acids also play critical roles in normal epithelial cell function (Jones and

Kubow, 1999). Arachidonic acid and other unsaturated fatty acids are involved with regulation of gene expression resulting in decreased expression of proteins that regulate the enzymes involved with fatty acid synthesis (Ou et al., 2001). This may partly explain the ability of unsaturated fatty acids to influence the hepatic synthesis of fatty acids.

n-*3 Polyunsaturated Fatty Acids*

n-3 Polyunsaturated fatty acids tend to be highly unsaturated with one of the double bonds located at 3 carbon atoms from the methyl end. This group includes:

- 18:3 α-Linolenic acid
- 20:5 Eicosapentaenoic acid
- 22:5 Docosapentaenoic acid
- 22:6 Docosahexaenoic acid

α-Linolenic acid is not synthesized by humans and a lack of it results in adverse clinical symptoms, including neurological abnormalities and poor growth. Therefore, α-linolenic acid is essential in the diet. It is the precursor for synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are formed in varying amounts in animal tissues, especially fatty fish, but not in plant cells. EPA is the precursor of *n*-3 eicosanoids, which have been shown to have beneficial effects in preventing coronary heart disease, arrhythmias, and thrombosis (Kinsella et al., 1990).

Trans *Fatty Acids*

Trans fatty acids are unsaturated fatty acids that contain at least one double bond in the *trans* configuration. The *trans* double-bond configuration results in a larger bond angle than the *cis* configuration, which in turn results in a more extended fatty acid carbon chain more similar to that of saturated fatty acids rather than that of *cis* unsaturated, double-bond–containing fatty acids. The conformation of the double bond impacts on the physical properties of the fatty acid. Those fatty acids containing a *trans* double bond have the potential for closer packing or aligning of acyl chains, resulting in decreased mobility; hence fluidity is reduced when compared to fatty acids containing a *cis* double bond. Partial hydrogenation of polyunsaturated oils causes isomerization of some of the remaining double bonds and migration of others, resulting in an increase in the *trans* fatty acid content and the hardening of fat. Hydrogenation of oils, such as corn oil, can result in both *cis* and *trans* double bonds anywhere between carbon 4 and carbon 16. A major *trans* fatty acid is elaidic acid (9-*trans* 18:1).

During hydrogenation of polyunsaturated fatty acids, small amounts of several other *trans* fatty acids (9-*trans*,12-*cis* 18:2; 9-*cis*,12-*trans* 18:2) are produced. In addition to these isomers, dairy fat and meats contain 9-*trans* 16:1 and conjugated dienes (9-*cis*,11-*trans* 18:2). The *trans* fatty acid content in foods tends to be higher in foods containing hydrogenated oils (Emken, 1995).

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is a collective term for a group of geometric and positional isomers of linoleic acid in which the *trans/cis* double bonds are conjugated; that is, the double bonds occur without an intervening carbon atom not part of a double bond. At least nine different isomers of CLA have been reported as minor constituents of food (Ha et al., 1989), but only two of the isomers, *cis*-9,*trans*-11 and *trans*-10,*cis*-12, possess biological activity (Pariza et al., 2001). There is limited evidence to suggest that the *trans*-10,*cis*-12 isomer reduces the uptake of lipids by the adipocyte, and that the *cis*-9,*trans*-11 isomer is active in inhibiting carcinogenesis. Similarly, there are limited data to show that *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers inhibit atherogenesis (Kritchevsky et al., 2000).

CLA is naturally present in dairy products and ruminant meats as a consequence of biohydrogenation in the rumen. *Butyrivibrio fibrisolvens*, a ruminant microorganism, is responsible for the production of the *cis*-9,*trans*-11 CLA isomer that is synthesized as a result of the biohydrogenation of linoleic acid (Noble et al., 1974). The *cis*-9,*trans*-11 CLA isomer may be directly absorbed or further metabolized to *trans*-11 octadecenoic acid (vaccenic acid) (Pariza et al., 2001). After absorption, vaccenic acid can then be converted back to *cis*-9,*trans*-11 CLA within mammalian cells by Δ9 desaturase (Adlof et al., 2000; Chin et al., 1994; Griinari et al., 2000; Santora et al., 2000). Additionally, the biohydrogenation of several other polyunsaturated fatty acids has been shown to produce vaccenic acid as an intermediate (Griinari and Bauman, 1999), thus providing additional substrate for the endogenous production of *cis*-9,*trans*-11 CLA. Griinari and coworkers (2000) estimate that approximately 64 percent of the CLA in cow's milk is of endogenous origin.

Verhulst and coworkers (1987) isolated a microorganism, *Propionibacterium acnes*, that appears to have the ability to convert linoleic acid to *trans*-10,*cis*-12 CLA, an isomer of CLA that is found in rumen digesta (Fellner et al., 1999). *Trans*-10 octadecenoic acid is formed in the rumen via biohydrogenation of *trans*-10,*cis*-12 CLA, and both have been reported to be found in cow's milk (Griinari and Bauman, 1999). However, endogenous production of *trans*-10,*cis*-12 CLA from *trans*-10 octadecenoic acid does not occur because mammalian cells do not possess the Δ12 desaturase enzyme (Adlof et al., 2000; Pariza et al., 2001). Therefore, any *trans*-10,*cis*-12 CLA

isomer that is reported in mammalian tissue or sera would likely originate from gastrointestinal absorption.

Physiology of Absorption, Metabolism, and Excretion

Total Fat

Absorption. Dietary fat undergoes lipolysis by lipases in the gastrointestinal tract prior to absorption. Although there are lipases in the saliva and gastric secretion, most lipolysis occurs in the small intestine. The hydrolysis of triacylglycerol is achieved through the action of pancreatic lipase, which requires colipase, also secreted by the pancreas, for activity. In the intestine, fat is emulsified with bile salts and phospholipids secreted into the intestine in bile, hydrolyzed by pancreatic enzymes, and almost completely absorbed. Pancreatic lipase has high specificity for the *sn*-1 and *sn*-3 positions of dietary triacylglycerols, resulting in the release of free fatty acids from the *sn*-1 and *sn*-3 positions and 2-monoacylglycerol. These products of digestion are absorbed into the enterocyte, and the triacylglycerols are reassembled, largely via the 2-monoacylglycerol pathway. This pathway conserves the fatty acid at the *sn*-2 position. The triacylglycerols are then assembled together with cholesterol, phospholipid, and apoproteins into chylomicrons. Following absorption, fatty acids of carbon chain length 12 or less may be transported as unesterified fatty acids bound to albumin directly to the liver via the portal vein, rather than acylated into triacylglycerols.

Dietary phospholipids are hydrolyzed by pancreatic phospholipase A_2 and cholesterol esters by pancreatic cholesterol ester hydrolase. The lysophospholipids are re-esterified and packaged together with cholesterol and triacylglycerols in intestinal lipoproteins or transported as lysophospholipid via the portal system to the liver.

Chylomicrons enter the circulation through the thoracic duct. These particles enter the circulation and within the capillaries of muscle and adipose tissue. Chylomicrons come into contact with the enzyme lipoprotein lipase, which is located on the surface of capillaries. Activation of lipoprotein lipase apolipoprotein CII, an apoprotein present on chylomicrons, results in the hydrolysis of the chylomicron triacylglycerol fatty acids. Most of the fatty acids released in this process are taken up by adipose tissue and re-esterified into triacylglycerol for storage. Triacylglycerol fatty acids also are taken up by muscle and oxidized for energy or are released into the systemic circulation and returned to the liver.

Metabolism. Most newly absorbed fatty acids enter adipose tissue for storage as triacylglycerol. However, in the postabsorptive state or during exercise when fat is needed for fuel, adipose tissue triacylglycerol undergoes lipolysis and free fatty acids are released into the circulation. Hydrolysis occurs via the action of the adipose tissue enzyme hormone-sensitive lipase. The activity of this lipase is suppressed by insulin. When plasma insulin concentrations fall in the postabsorptive state, hormone-sensitive lipase is activated to release more free fatty acids into the circulation. Thus, in the postabsorptive state, free fatty acid concentrations in plasma are high; conversely, in the postprandial state, hormone-sensitive lipase activity is suppressed and free fatty acid concentrations in plasma are low.

Free fatty acids circulate in the blood bound to albumin. The major site of fatty acid oxidation is skeletal muscle. When free fatty acid concentrations are relatively high, muscle uptake of fatty acids is also high. As in liver, fatty acids in the muscle are transported via a carnitine-dependent pathway into mitochondria where they undergo β-oxidation, which involves removal of two carbon fragments. These two carbon units enter the citric acid cycle as acetyl coenzyme A (CoA), through which they are completely oxidized to carbon dioxide with the generation of large quantities of high-energy phosphate bonds, or they condense to form ketone bodies. Muscle can oxidize both fatty acids and glucose for energy. However, the uptake of fatty acids in excess of the needs for oxidation for energy by muscle does result in temporary storage as triacylglycerol (Bessesen et al., 1995). High uptake of fatty acids by skeletal muscle also reduces glucose uptake by muscle and glucose oxidation (Pan et al., 1997; Roden et al., 1996).

Fatty acids released from adipose tissue or to a lesser extent during hydrolysis of chylomicron and very low density lipoprotein (VLDL) triacylglycerols are also taken up and oxidized by the liver. Oxidation of fatty acids containing up to 18 carbon atoms occurs mainly in the mitochondria. Oxidation of excess fatty acids in the liver, which occurs in prolonged fasting and with high intakes of medium-chain fatty acids, results in formation of large amounts of acetyl CoA that exceed the capacity for entry to the citric acid cycle. These 2-carbon acetyl CoA units condense to form ketone bodies (e.g., acetoacetate and β-hydroxybutyrate) that are released into the circulation. During starvation or prolonged low carbohydrate intake, ketone bodies can become an important alternate energy substrate to glucose for the brain and muscle. High dietary intakes of medium-chain fatty acids also result in the generation of ketone bodies. This is explained by the carnitine-independent influx of medium-chain fatty acids into the mitochondria, thus by-passing this regulatory step of fatty acid entry into β-oxidation. Fatty acids of greater than 18 carbon atoms require chain shortening in peroxisomes prior to mitochondrial β-oxidation.

Fatty acids that do not enter into oxidative pathways can be re-esterified into triacylglycerols or other lipids. The major pathway for triacylglycerol synthesis in liver is the 3-glycerophosphate pathway, which shows a high degree of specificity for saturated fatty acids at the *sn*-1(3) position and for unsaturated fatty acids at the *sn*-2 position. In the liver, triacylglycerols can either be stored temporarily or incorporated into triacylglycerol-rich VLDL and released into the plasma. The triacylglycerol fatty acids of VLDL have the same fate as chylomicron triacylglycerol fatty acids. When VLDL triacylglycerols undergo lipolysis, the remaining triacylglycerol-depleted particle is called a VLDL remnant. These remnants are either removed directly by the liver or they are further metabolized in the vascular compartment to form low density lipoproteins (LDL).

Excretion. Fatty acids are generally catabolized entirely by oxidative processes from which the only excretion products are carbon dioxide and water. Small amounts of ketone bodies produced by fatty acid oxidation are excreted in urine. Fatty acids are present in the cells of the skin and intestine, thus small quantities are lost when these cells are sloughed.

Saturated Fatty Acids

Absorption. When saturated fatty acids are ingested along with fats containing appreciable amounts of unsaturated fatty acids, they are absorbed almost completely by the small intestine. In general, the longer the chain length of the fatty acid, the lower will be the efficiency of absorption. However, unsaturated fatty acids are well absorbed regardless of chain length. Studies with human infants have shown the absorption to be 75, 62, 92, and 94 percent of palmitic acid, stearic acid, oleic acid, and linoleic acid, respectively, from vegetable oils (Jensen et al., 1986). The absorption of palmitic acid and stearic acid from human milk is higher than from cow milk and vegetable oils (which are commonly used in infant formulas) because of the specific positioning of these long-chain saturated fatty acids at the *sn*-2 position of milk triacylglycerols (Carnielli et al., 1996a; Jensen, 1999). The intestinal absorption of palmitic acid and stearic acid from vegetable oils was 75 to 78 percent compared with 91 to 97 percent from fats with these fatty acids in the *sn*-2 position (Carnielli et al., 1996a). Still, absorption of stearic acid was over 90 percent complete in healthy adults when contained in triacylglycerols of mixed fatty acids (Bonanome and Grundy, 1989). Long-chain saturated fatty acids released into the lumen through the action of pancreatic lipase are less readily solubilized into mixed micelles than are unsaturated fatty acids; in the alkaline pH of the intestine they can form insoluble soaps with calcium and other divalent

cations and can be excreted (Carnielli et al., 1996a; Lucas et al., 1997; Tomarelli et al., 1968). Following absorption, long-chain saturated fatty acids are re-esterified along with other fatty acids into triacylglycerols and released in chylomicrons. Medium-chain saturated fatty acids (C8:0 and C10:0) are absorbed and transported bound to albumin as free fatty acids in the portal circulation and cleared by the liver. About two-thirds of lauric acid (C12:0) is transported with chylomicron triacylglycerols, whereas the remaining one-third enters the portal circulation as free fatty acids.

Metabolism. Pathways of oxidation of saturated fatty acids are similar to those for other types of fatty acids (see earlier section, "Total Fat"). Unoxidized stearic acid (9 to 14 percent) is rapidly desaturated and converted to the monounsaturated fatty acid, oleic acid (Emken, 1994; Rhee et al., 1997). For this reason, dietary stearic acid has metabolic effects that are closer to those of oleic acid rather than those of other long-chain saturated fatty acids. The saturated fatty acids, in contrast to *cis* mono- or polyunsaturated fatty acids, have a unique property in that they suppress the expression of LDL receptors (Spady et al., 1993). Through this action, dietary saturated fatty acids raise serum LDL cholesterol concentrations (Mustad et al., 1997).

Excretion. Saturated fatty acids, like other fatty acids, are generally completely oxidized to carbon dioxide and water.

cis-*Monounsaturated Fatty Acids*

Absorption. The absorption of *cis*-monounsaturated fatty acids (based on oleic acid data) is in excess of 90 percent in adults and infants (Jensen et al., 1986; Jones et al., 1985). The pathways of *cis*-monounsaturated fat digestion and absorption are similar to those of other fatty acids (see earlier section, "Total Fat").

Metabolism. Oleic acid, the major monounsaturated fatty acid in the body, is derived mainly from the diet. Small amounts also come from desaturation of stearic acid. Stable isotope tracer methods have shown that approximately 9 to 14 percent of dietary stearic acid is converted to oleic acid in vivo (Emken, 1994; Rhee et al., 1997). Based on the amount of stearic acid in the average diet (approximately 3 percent of energy), desaturation of dietary stearic acid is not a main source of oleic acid in the body. Oleic acid is oxidized, as are all other fatty acids, by β-oxidation. However, there is some evidence that oxidation of chylomicron-derived oleic acid is significantly greater than for palmitic acid (Schmidt et al.,

1999). The metabolic implications of the differential rates of oxidation of saturated, monounsaturated, and *cis* *n*-6 and *n*-3 fatty acids are not clear.

Excretion. Because oleic acid is highly absorbed, little is excreted. As for other fatty acids, the oxidation of monounsaturated fatty acids results in production of carbon dioxide and water.

n-*6 Polyunsaturated Fatty Acids*

Absorption. The digestion and absorption of *n*-6 fatty acids is efficient and occurs via the same pathways as that of other long-chain fatty acids (see earlier section, "Total Fat").

Metabolism. Both saturated and *n*-9 monounsaturated fatty acids can be synthesized from the carbon moieties of carbohydrate and protein. Mammalian cells do not have the enzymatic ability to insert a *cis* double bond at the *n*-6 position of a fatty acid chain, thus *n*-6 fatty acids are essential nutrients. The parent fatty acid of the *n*-6 series is linoleic acid. Studies using isotopically labeled linoleic acid have shown that adults and newborn infants can desaturate and elongate linoleic acid to form arachidonic acid (Emken et al., 1998, 1999; Salem et al., 1996; Sauerwald et al., 1997). The elongation of linoleic acid involves the sequential addition of two carbon units and desaturation involves insertion of a methylene-interrupted double bond towards the carboxyl terminus, thus preserving the position of the first *n*-6 double bond. These longer-chain, more polyunsaturated *n*-6 fatty acids are found primarily in membrane phospholipids, and since they can be formed only in animal cells, arachidonic acid is present in the diet only in animal tissue lipids.

Recent studies using stable isotopically labeled fatty acids to investigate the effect of gestational age and intrauterine growth on essential fatty acid desaturation and elongation have shown that the conversion of linoleic to arachidonic acid occurs as early as 26 weeks of gestation, and is in fact more active at earlier gestational ages (Uauy et al., 2000a). In addition to its role as a precursor to dihomo-γ-linolenic acid and arachidonic acid, linoleic acid has a specific role in acylceramides, which are important in maintaining the epidermal water barrier (Hansen and Jensen, 1985).

The 18 and 20 carbon *n*-9, *n*-6, and *n*-3 fatty acids compete for a common Δ6 and Δ5 desaturase. In vitro studies have shown the Δ6 desaturase enzymes preference occurs in the order 18:3*n*-3 > 18:2*n*-6 > 18:1*n*-9 (Brenner, 1974; Castuma et al., 1977). The formation of arachidonic acid and *n*-3 fatty acid metabolites also appears to be inhibited by the products of the reaction and by high amounts of substrate. Thus, high intakes of *n*-3 fatty acids or

arachidonic and linoleic acids will reduce the efficiency of conversion of linoleic acid to arachidonic acid and α-linolenic acid to its products (Emken et al., 1994, 1998, 1999). For example, Emken and coworkers (1994) reported that an intake of 30 g/d of linoleic acid resulted in a 40 to 54 percent lower conversion of stable isotopically labeled linoleic and α-linolenic acid to their metabolites compared to an intake of 15 g/d in healthy men. High dietary intakes of *n*-3 fatty acids result in reduced tissue arachidonic acid concentrations and synthesis of arachidonic acid-derived eicosanoids, with consequent effects on the balance of *n*-6 and *n*-3 fatty acid-derived eicosanoids that are produced. The reduction in arachidonic acid-derived eicosanoids due to high *n*-3 fatty acid intake involves effects on pathways of eicosanoid formation, in addition to reducing concentrations of precursor arachidonic acid availability.

Both the rate of oxidation to carbon dioxide and water and the acylation into different lipids differ among fatty acids of different chain length and unsaturation. Arachidonic acid is primarily found in tissue phospholipids, rather than in triacylglycerols or cholesterol esters. Retroconversion of adrenic acid to arachidonic acid occurs through cleavage of a 2-carbon unit from the carboxyl end of the fatty acid and may be important in maintaining adequate tissue concentrations of arachidonic acid. Besides being elongated to longer-chain fatty acids, arachidonic acid is the precursor to a number of eicosanoids (prostaglandins, thromboxanes, and leukotienes) that are involved in platelet aggregation, hemodynamics, and coronary vascular tone, which can have an effect on the onset of atherogenesis and coronary infarction (Kinsella et al., 1990).

Excretion. *n*-6 Fatty acids are almost completely absorbed and are either incorporated into tissue lipids, utilized in eicosanoid synthesis, or oxidized to carbon dioxide and water. Small amounts are lost during sloughing of cells from skin and other epithelial membranes.

n-*3 Polyunsaturated Fatty Acids*

Absorption. The digestion and absorption of *n*-3 fatty acids is similar to that of other long-chain fatty acids.

Metabolism. Humans are unable to insert a double bond at the *n*-3 position (*cis* 15) of a fatty acid of 18 carbons in length, and thus require a dietary source of *n*-3 fatty acids. The *n*-3 fatty acids cannot be formed from saturated, *n*-9 monounsaturated, or *n*-6 polyunsaturated fatty acids. The parent fatty acid of the *n*-3 series is α-linolenic acid, which can be further metabolized by elongation and desaturation to longer-chain, more highly

unsaturated metabolites using the same pathway and enzymes as those used for the *n*-6 fatty acids. α-Linolenic acid is desaturated by Δ6 desaturase, elongated, and then desaturated by Δ5 desaturase to form EPA, which is the precursor for series 3 eicosanoids and series 5 leukotrienes. The pathway leading from EPA to more highly unsaturated fatty acids involves the addition of two 2-carbon units, then a second Δ6 desaturation, after which the 24-carbon-chain fatty acid is transported to the peroxisomes and converted to DHA through one step of β-oxidation (Sprecher et al., 1995; Voss et al., 1991). DHA is a component of membrane structural lipids that are enriched in certain phospholipids, such as the ethanolamine phosphoglycerides and phosphatidylserine in nervous tissue, retina, and spermatozoa. α-Linolenic acid is not known to have any specific functions other than to serve as a precursor for synthesis of EPA and DHA.

High dietary intakes of EPA and DHA result in decreased tissue concentrations of arachidonic acid and increased concentrations of EPA and DHA, respectively. This results in changes in the balance of eicosanoids synthesized from the *n*-6 and *n*-3 fatty acids. The ability to convert α-linolenic acid to EPA and DHA differs among mammalian species. Studies using isotopically labeled α-linolenic acid, however, have shown that adults and newborn infants can desaturate and elongate α-linolenic acid to form DHA (Carnielli et al., 1996b; Salem et al., 1996; Sauerwald et al., 1996, 1997; Uauy et al., 2000a; Vermunt et al., 2000). Recent studies with infants have shown that the rates of conversion of α-linolenic acid to DHA appear to be higher in preterm infants and decrease with increasing gestational age (Uauy et al., 2000a). These types of studies have also shown that high intakes of α-linolenic acid result in reduced conversion to DHA (Vermunt et al., 2000).

Whereas the retroconversion of adrenic acid to maintain tissue arachidonic acid requires the removal of only a single 2-carbon unit, the retroconversion of DHA to EPA is more complex and involves the removal of the double bond at the Δ4 position, in addition to a 2-carbon unit. Supplementation with DHA is accompanied by an increase in EPA, which could be explained by retroconversion of DHA to EPA or by inhibition of further metabolism of EPA formed from α-linolenic acid (Brossard et al., 1996; Conquer and Holub, 1996; Nelson et al., 1997; Vidgren et al., 1997).

Excretion. *n*-3 Fatty acids are almost completely absorbed and either oxidized to carbon dioxide and water, incorporated into tissue lipids, or utilized in eicosanoid synthesis. Small amounts of *n*-3 fatty acids are lost during sloughing of skin and other epithelial cells.

Trans *Fatty Acids*

Absorption. As with other fatty acids, the coefficient of absorption of elaidic acid (18:1*t*) is about 95 percent (Emken, 1979). Studies in humans using pure triacylglycerols containing deuterated *cis* and *trans* octadecenoic acid isomers varying in melting point and double bond position suggest that the presence of *trans* double bonds in the fatty acyl chain has no measurable effect on efficiency of absorption (Emken, 1979, 1984).

Transport. *Trans* fatty acids are transported similarly to other dietary fatty acids and are distributed within the cholesteryl ester, triacylglycerol, and phospholipid fractions of lipoproteins (Vidgren et al., 1998). Platelet lipids also contain *trans* fatty acids and their composition reflects *trans* fatty acid intake, as do other tissues (except the brain) (Mensink and Hornstra, 1995).

Metabolism. The *trans* isomers of oleic acid and linoleic acid that are formed during partial hydrogenation of unsaturated vegetable oils have been suggested to have potential adverse effects on fetal and infant growth and development through inhibition of the desaturation of linoleic acid and α-linolenic acid to arachidonic acid and DHA, respectively (Koletzko, 1992; van Houwelingen and Hornstra, 1994). Many animal and in vitro studies, however, have involved much higher amounts of *trans* than all-*cis* polyunsaturated fatty acids (Hwang et al., 1982; Shimp et al., 1982). Other animal studies have suggested that the deleterious effects seen with high intakes of *trans* fatty acid do not occur with amounts comparable to those consumed in a normal human diet containing sufficient amounts of linoleic acid (Bruckner et al., 1982; Zevenbergen et al., 1988).

Available animal and human data indicate that adipose tissue *trans* fatty acid content reflects the content of the diet and that selective accumulation does not occur (Emken, 1984). More recent attention has been focused on validating the use of adipose *trans* fatty acid content as a measure of long-term dietary intake. In a study of Canadian individuals, Chen and colleagues (1995b) reported that adipose tissue *trans* fatty acid patterns, particularly those isomers found in partially hydrogenated vegetable fat, reflected dietary sources. Garland and coworkers (1998) also reported that adipose tissue *trans* fatty acid patterns correlated with intake and noted a stronger relationship with the isomers found in vegetable fat rather than animal fat. The authors cautioned that the later conclusion may have been due to the smaller between-person variability with animal versus vegetable *trans* fatty acid intake. In a letter to the editor regarding this study, Aro and Salminen (1998) suggested that the stronger correlation between adipose tissue *trans* fatty acid isomers found in hydrogenated vegetable fat

rather than animal fat may be attributable to different rates of metabolism of the *trans* isomers. Two groups have used adipose tissue *trans* fatty acid to corroborate dietary *trans* fatty acid intake derived from food frequency questionnaires and found a strong relationship (Lemaitre et al., 1998; London et al., 1991). Despite these observations, it should be noted that adipose tissue *trans* fatty acid profiles can be confounded by the retention of intermediate products of β-oxidation (Emken, 1995).

Excretion. *Trans* fatty acids are completely catabolized to carbon dioxide and water.

Clinical Effects of Inadequate Intakes

Total Fat

Impaired Growth. Dietary fat is a major source of body fuel. If intakes of fat, along with carbohydrate and protein, are inadequate to meet energy needs, the individual will be in negative energy balance. Depending on the severity and duration, this may lead to malnutrition or starvation. In an energy-sufficient diet, carbohydrate can replace fat as a source of energy. In some populations, fat intakes are very low and body weight and health are maintained by high intakes of carbohydrate (Bunker et al., 1996; Falase et al., 1973; Shintani et al., 2001). Clearly, humans have the ability to adapt metabolically to a wide spectrum of fat-to-carbohydrate intake ratios. In the short term, an isocaloric diet can be either very high or very low in fat with no obvious differences in health. The critical question therefore is, Are there optimal fat-to-carbohydrate ratios for long-term health, and if so, what are they? One potential concern over fat restriction is the potential for reduction in total energy intake, which is of particular relevance for infants and children, as well as during pregnancy when there is a relatively high energy requirement for both energy expenditure and for fetal development. Chapter 11 provides a detailed discussion on fat intake and growth.

Increased Risk of Chronic Diseases. Compared to higher fat intakes, low fat, high carbohydrate diets may modify the metabolic profile in ways that are considered to be unfavorable with respect to chronic diseases such as coronary heart disease (CHD) and diabetes (see Chapters 6 and 11). These changes include a reduction in high density lipoprotein cholesterol concentration, an increase in serum triacylglycerol concentration, and higher responses in postprandial glucose and insulin concentrations. This metabolic pattern has been associated with increased risk for CHD and type 2 diabetes

in intervention and prospective studies (see Chapter 11). Although changes in the metabolic profile do occur, strong evidence that low fat diets actually predispose to either CHD or diabetes does not exist. In fact, some populations that consume low fat diets and in which habitual energy intake is relatively high have a low prevalence of these chronic diseases (Falase et al., 1973; Shintani et al., 2001). Similarly, populations with high fat diets (i.e., ≥ 40 percent of energy) and a low prevalence of chronic diseases often include people who engage in heavy physical labor, are lean, and have a low family history of chronic diseases. Conversely, in sedentary populations, such as that of the United States where overweight and obesity are common, high carbohydrate, low fat diets induce changes in lipoprotein and glucose/insulin metabolism in ways that could raise risk for chronic diseases (see Chapter 11). Available prospective studies have not concluded whether low fat, high carbohydrate diets provide a health risk in the North American population.

Chronic nonspecific diarrhea in children has been suggested as a potential adverse effect of low fat diets. It is considered a disorder of intestinal motility that may improve with an increase in dietary fat intake in order to slow gastric emptying and alter intestinal motility (Cohen et al., 1979). Detailed discussion on fat intake and risk of chronic disease is provided in Chapter 11.

n-*6 Polyunsaturated Fatty Acids*

Certain polyunsaturated fatty acids were first identified as being essential in rats fed diets almost completely devoid of fat (Burr and Burr, 1929). Subsequently, studies in infants and children fed skimmed cow milk (Hansen et al., 1958, 1963) and patients receiving parenteral nutrition without an adequate source of essential fatty acids (Collins et al., 1971; Holman et al., 1982; Paulsrud et al., 1972) demonstrated clinical symptoms of a deficiency in humans. Because adipose tissue lipids in free-living, healthy adults contain about 10 percent of total fatty acids as linoleic acid, biochemical and clinical signs of essential fatty acid deficiency do not appear during dietary fat restriction or malabsorption when they are accompanied by an energy deficit. In this situation, release of linoleic acid and small amounts of arachidonic acid from adipose tissue reserves may prevent development of essential fatty acid deficiency. However, during parenteral nutrition with dextrose solutions, insulin concentrations are high and mobilization of adipose tissue is prevented, resulting in development of the characteristic signs of essential fatty acid deficiency. Studies on patients given fat-free parenteral feeding have provided great insight into defining levels at which essential fatty acid deficiency may occur. Without intervention, these patients develop clinical signs of a deficiency

in 2 to 4 weeks (Fleming et al., 1976; Goodgame et al., 1978; Jeppesen et al., 1998; Riella et al., 1975). In rapidly growing infants, feeding with milk containing very low amounts of *n*-6 fatty acids results in characteristic signs of an essential fatty acid deficiency and elevated plasma triene:tetraene ratios (see "*n*-6:*n*-3 Polyunsaturated Fatty Acid Ratio").

When dietary essential fatty acid intake is inadequate or absorption is impaired, tissue concentrations of arachidonic acid decrease, inhibition of the desaturation of oleic acid is reduced, and synthesis of eicosatrienoic acid from oleic acid increases. The characteristic signs of deficiency attributed to the *n*-6 fatty acids are scaly skin rash, increased transepidermal water loss, reduced growth, and elevation of the plasma ratio of eicosatrienoic acid:arachidonic acid (20:3*n*-9:20:4*n*-6) to values greater than 0.4 (Goodgame et al., 1978; Holman, 1960; Jeppesen et al., 2000; Mascioli et al., 1996; O'Neill et al., 1977). Other studies have utilized a ratio of 0.2 as indicative of an essential fatty acid deficiency (Holman et al., 1991; Jeppesen et al., 1998). In addition to the clinical signs mentioned above, essential fatty acid deficiency in special populations has been linked to hematologic disturbances and diminished immune response (Bistrian et al., 1981; Boissonneault and Johnston, 1983). Further discussion on this topic is included in "Findings by Life Stage and Gender Group—*n*-6 Polyunsaturated Fatty Acids."

n-*3 Polyunsaturated Fatty Acids*

Tissue levels of arachidonic acid, as well as the amounts of arachidonic acid and EPA- derived eicosanoids that are formed, have important effects on many physiological processes (e.g., platelet aggregation, vessel wall constriction, and immune cell function) via the biosynthesis of eicosanoids. Thus, the amount of *n*-3 fatty acids and their effects on arachidonic acid metabolism are relevant to many chronic diseases. EPA also appears to have specific effects on fatty acid metabolism, resulting in inhibition of hepatic triacylglycerol synthesis and VLDL secretion (Berge et al., 1999; Wong and Nestel, 1987). DHA, on the other hand, is highly enriched in specific phospholipids of the retina and nonmyelin membranes of the nervous system.

Studies in rodents and nonhuman primates have consistently demonstrated that prolonged feeding with diets containing very low amounts of α-linolenic acid result in reductions of visual acuity thresholds and electroretinogram A and B wave recordings, which were prevented when α-linolenic acid was included in the diet (Anderson et al., 1974; Benolken et al., 1973; Bourre et al., 1989; Neuringer et al., 1984, 1986; Wheeler et al., 1975). A variety of changes in learning behaviors in animals fed α-linolenic acid-deficient diets have also been reported (Innis, 1991). These studies have

involved feeding oils such as safflower oil, which contains less than 0.1 percent α-linolenic acid and is high in linoleic acid, as the sole source of fat for prolonged periods. The reduction in visual function is accompanied by decreased brain and retina DHA with an increase in docosapentaenoic acid (DPA, 22:5*n*-6). The compensatory increase in 22 carbon chain *n*-6 fatty acids results in maintenance of the total amount of *n*-6 and *n*-3 polyunsaturated fatty acids in neural tissue. DPA is formed from linoleic acid by similar desaturation and elongation steps used in the synthesis of DHA from α-linolenic acid. However, α-linolenic acid is clearly handled differently from linoleic acid. For example, rates of β-oxidation of α-linolenic acid are much higher than for linoleic acid (Clouet et al., 1989). This may suggest that immaturity or reduced enzyme activity is unlikely to explain lower DHA in the brain of young animals fed diets with low amounts of α-linolenic acid, and that DHA has specific metabolic functions that cannot be accomplished by DPA despite its structural similarity. Stable isotope studies have shown that infants can convert linoleic acid to arachidonic acid and α-linolenic acid to DHA (Carnielli et al., 1996b; Salem et al., 1996; Sauerwald et al., 1996, 1997; Uauy et al., 2000a), with the rate of conversion apparently higher in infants of younger gestational ages (Uauy et al., 2000a).

Unlike essential fatty acid deficiency (*n*-6 and *n*-3 fatty acids), plasma eicosatrienoic acid (20:3*n*-9) remains within normal ranges and skin atrophy and scaly dermatitis are absent when the diet is deficient in only *n*-3 fatty acids. Tissue concentrations of 22-carbon chain *n*-6 fatty acids increase, and DHA concentration decreases with a prolonged dietary deficiency of *n*-3 fatty acids accompanied by adequate *n*-6 fatty acids. Currently, there are no accepted plasma *n*-3 fatty acid or *n*-3 fatty acid-derived eicosanoid concentrations for indicating impaired neural function or impaired health endpoints. Further discussion on this topic is included in the next section.

EVIDENCE CONSIDERED FOR ESTIMATING THE REQUIREMENTS FOR TOTAL FAT AND FATTY ACIDS

Total Fat

Clinical endpoints of fat intake are trends, rather than defined endpoints, and therefore cannot be used to set an Estimated Average Requirement (EAR). The endpoints that strongly predict the relation of total fat intake to the development of chronic disease have been identified and are discussed in Chapter 11 for estimating Acceptable Macronutrient Distribution Ranges (AMDRs).

Growth

Because the amount of fat in the diet can have an impact on energy intake, a number of studies have been conducted to determine if diets containing less than 30 percent of energy from fat can impair growth of children (Boulton and Magarey, 1995; Foman et al., 1976; Lagström et al., 1999; Lapinleimu et al., 1995; Niinikoski et al., 1997a, 1997b; Obarzanek et al., 1997; Shea et al., 1993; Uauy et al., 2000b; Vobecky et al., 1995). These studies showed no effect of the level of dietary fat on growth when energy intake is adequate. Chapter 11 provides further discussion on this topic.

Fat Balance (Maintenance of Body Weight)

Because fat is an important source of energy, studies have been conducted to ascertain whether dietary fat influences energy expenditure and the amount of fat needed in the diet to achieve fat balance and therefore maintain body weight. These studies demonstrated that the amount of fat in the diet does not affect energy expenditure and thus the amount of energy required to maintain body weight (Hill et al., 1991; Leibel et al., 1992). Chapter 11 provides further discussion on this topic.

Saturated Fatty Acids

Saturated fatty acids are a potential fuel source for the body. In addition, they are important structural fatty acids for cell membranes and other functions and therefore are essential for body functions. These fatty acids, however, can be synthesized as needed for these functions from other fuel sources and have not been associated with any beneficial role in preventing chronic disease. Consequently, saturated fatty acids are not essential in the diet.

cis-*Monounsaturated Fatty Acids*

Monounsaturated fatty acids are a potential fuel source for the body and are a critical structural fatty acid for cell membranes and other functions. Monounsaturated fatty acids undoubtedly are required for many body functions. Nevertheless, monounsaturated fatty acids can be biosynthesized from other fuel sources and therefore are not essential in the diet.

n-*6 Polyunsaturated Fatty Acids*

Clinical signs of essential fatty acid deficiency are generally only found in patients with chronic fat malabsorption on parenteral nutrition and without an enteral or parenteral source of polyunsaturated fat. Early signs of essential fatty acid deficiency include rough and scaly skin, which if left untreated, develops into dermatitis (Jeppesen et al., 1998). In studies of patients with dermatitis who were receiving parenteral nutrition, the ratio of eicosatrienoic acid:arachidonic acid (20:3*n*-9:20:4*n*-6) in plasma was elevated. As described earlier, when present in adequate amounts, linoleic acid is converted to arachidonic acid through a multi-step process involving Δ6 and Δ5 desaturases (see Figure 8-1); however, in the absence of linoleic acid, Δ6 and Δ5 desaturases convert oleic acid to eicosatrienoic acid. The increase in eicosatrienoic acid concentration, which occurs in the absence of *n*-6 fatty acids or the combined absence of *n*-6 and *n*-3 fatty acids, led Holman (1960) to define a plasma triene:tetraene ratio of greater than 0.4 as evidence of essential fatty acid deficiency. More recently, a lower threshold of greater than 0.2 has been suggested (Holman et al., 1979; Jeppesen et al., 1998; Mascioli et al., 1996) because the average ratio was found to be 0.1 ± 0.08 (standard deviation) in populations of normal *n*-6 fatty acid status. Optimal plasma or tissue lipid concentrations of linoleic acid, arachidonic acid, and other *n*-6 fatty acids or the ratios of certain *n*-6:*n*-3 fatty acids have not been established.

Because the *n*-6 fatty acid intake is generally well above the levels needed to maintain a triene:tetraene ratio below 0.2 (even for very low fat diets), data on *n*-6 fatty acid requirements from traditional metabolic feeding studies are not available. Instead, studies with patients on total parenteral nutrition (TPN) solutions that contained very low amounts or were completely devoid of *n*-6 fatty acids have been used. In these studies, after developing an essential fatty acid deficiency, patients were treated with linoleic acid. Several case reports, small studies of two or three patients in which varying feeding designs were employed, or larger studies of patients with *n*-6 fatty acid deficiency caused by TPN have been documented (Barr et al., 1981; Collins et al., 1971; Goodgame et al., 1978; Jeppesen et al., 1998; Mascioli et al., 1979; Meng, 1983; Richardson and Sgoutas, 1975; Riella et al., 1975; Siguel et al., 1986; Wene et al., 1975; Wong and Deitel, 1981). These studies observed symptoms such as rash, scaly skin, and ectopic dermititis; reduced serum tetraene concentrations, increased serum triene concentration; and a triene:tetraene ratio greater than 0.4 after 2 to 4 weeks of TPN. Because of the lack of data on the *n*-6 fatty acid requirement in healthy individuals, an EAR cannot be set based on correction of a deficiency.

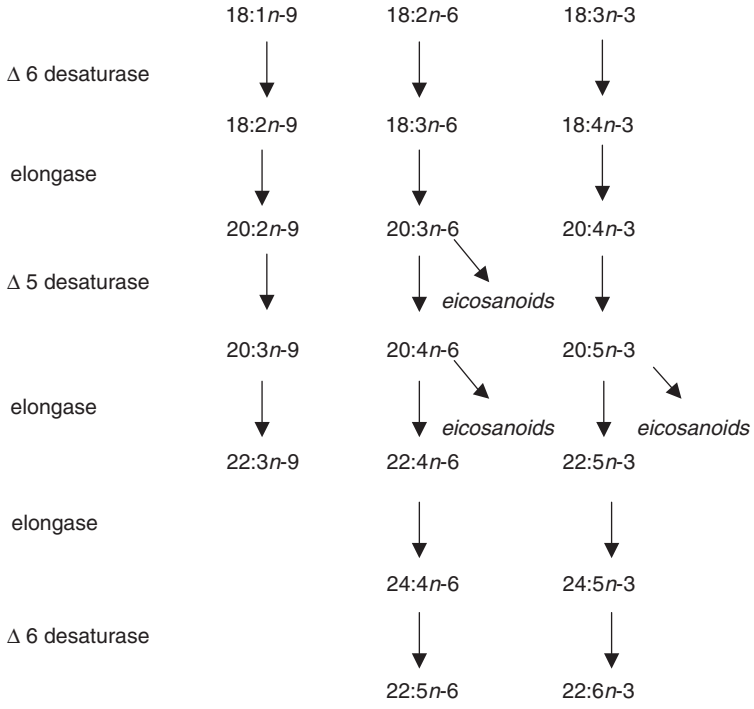

FIGURE 8-1 Biosynthesis of long-chain fatty acids.

n-*3 Polyunsaturated Fatty Acids*

n-*3 Polyunsaturated Fatty Acid Deficiency*

Some evidence for the essentiality of *n*-3 fatty acids in humans can be drawn from case reports of patients receiving parenteral nutrition with intravenous lipids containing an emulsion of safflower oil, which is very low in α-linolenic acid and high in linoleic acid. Biochemical changes of *n*-3 fatty acid deficiency include a decrease in plasma and tissue docosahexaenoic acid (DHA) concentrations. There is no accepted cut-off concentration of plasma or tissue DHA concentrations below which functions ascribed to *n*-3 fatty acids, such as visual or neural function, are impaired. Similarly, there are no accepted normal ranges for eicosapentaenoic acid (EPA) with respect to synthesis of EPA-derived eicosanoids or regulation of arachidonic acid metabolism and its eicosanoid metabolites, nor are there accepted clinical functional endpoints such as immune response.

Dietary or intravenous supplementation with oils containing α-linolenic acid, such as soybean oil, has been shown to increase red blood cell and plasma phospholipid DHA concentration in hospitalized patients with a long history of dietary *n*-3 fatty acid restriction (Bjerve et al., 1987a, 1987b; Holman et al., 1982). Sensory neuropathy and visual problems in a young girl given parenteral nutrition with an intravenous lipid emulsion containing only a small amount of α-linolenic acid were corrected when the emulsion was changed to one containing generous amounts of α-linolenic acid (Holman et al., 1982). Nine patients with an *n*-3 fatty acid deficiency had scaly and hemorrhagic dermatitis, hemorrhagic folliculitis of the scalp, impaired wound healing, and growth retardation (Bjerve, 1989). The possibility of other nutrient deficiencies, such as vitamin E and selenium, has been raised (Anderson and Connor, 1989; Meng, 1983). A series of papers have described low tissue *n*-3 fatty acid concentrations in nursing home patients fed by gastric tube for several years with a powdered diet formulation that provided about 0.5 to 0.6 percent of energy (0.65 to 0.86 g) as linoleic acid, and 0.02 percent of energy (30 to 50 mg) as α-linolenic acid (Bjerve et al., 1987a, 1987b). Skin lesions were resolved following supplementation with cod liver oil and soybean oil or ethyl linolenate (Bjerve et al., 1987a, 1987b). Concurrent deficiency of both *n*-6 and *n*-3 fatty acids in these patients, as in studies of patients supported by lipid-free parenteral nutrition, limits interpretation of the specific problems caused by inadequate intakes of *n*-3 fatty acids. Supplementation with cod liver oil and soybean oil, or feeding with a formula providing linoleic acid and α-linolenic acid or ethyl α-linolenic acid for 14 days, increased red blood cell arachidonic acid and DHA concentrations and gave some resolution of skin signs (Bjerve et al., 1987a, 1987b). Because of the lack of data on the *n*-3 fatty acid requirement in healthy individuals, an EAR cannot be set based on correction of a deficiency.

Growth and Neural Development

The membrane lipids of brain gray matter and the retina contain very high concentrations of DHA, particularly in the amino phospholipids phosphatidylethanolamine and phosphatidylserine. In these tissues, the concentration of DHA can exceed 50 percent of the fatty acids resulting in the presence of di-DHA phospholipid species. During *n*-3 fatty acid deficiency, DHA is tenaciously retained, thus most animal studies investigating the importance of *n*-3 fatty acids have used rats deprived of *n*-3 fatty acids for two or more generations. Small amounts of DHA are also present in cell membranes throughout the body. In these tissues, the phospholipid *sn*-1 chain is usually a saturated fatty acid (e.g., 16:0) and DHA is found on the *sn*-2 position. The developing brain accumulates large amounts of DHA

during pre- and postnatal development and this accumulation continues throughout the first two years after birth (Martinez, 1992). Evidence from autopsy analysis indicates that accumulation of DHA in the retina is complete by term birth (Martinez et al., 1988). Due to the accumulation of DHA during brain growth, the developing brain is more susceptible to *n*-3 fatty acid deficiency than the mature brain. However, the presence of DHA within the membrane hydrophobic interior can influence membrane order (fluidity), thickness, domain size, hydration, and permeability and activity of associated proteins and ion channels. Unesterified DHA also regulates the expression of a variety of genes and influences cell signaling mechanisms (Salem et al., 2001; Sinclair et al., 2000). Animal studies have shown that feeding a diet very low in α-linolenic acid results in reduced brain and retina DHA concentration, which is accompanied by reduced visual function and behavior in learning tasks (Benolken et al., 1973; Bourre et al., 1989; Neuringer et al., 1984; Wheeler et al., 1975). The decrease in DHA concentration in the brain and retina is compensated for by an increase in the *n*-6 fatty acid docosapentaenoic acid, and this leads to maintenance of the total polyunsaturated fatty acid content of the membrane. Reduced growth or changes in food intake have not been noted in the extensive number of studies in animals, including nonhuman primates fed for extended periods on otherwise adequate diets lacking *n*-3 fatty acids.

The essential role of α-linolenic acid appears to be its role as precursor for synthesis of EPA and DHA. Thus, the dietary *n*-3 fatty acid requirement involves the activity of the desaturase enzymes and factors that influence the desaturation of α-linolenic acid in addition to the amount of the *n*-3 fatty acid. The questions of whether term gestation infants can form DHA, or if DHA is required in the infant diet, has been studied extensively. Activity of Δ6 and Δ5 desaturases has been demonstrated in human fetal tissue from as early as 17 to 18 weeks of gestation (Chambaz et al., 1985; Rodriguez et al., 1998), and stable isotope studies have confirmed that preterm and term infants are able to convert α-linolenic acid to DHA (Carnielli et al., 1996b; Salem et al., 1996; Sauerwald et al., 1996, 1997; Uauy et al., 2000a). Furthermore, the ability to convert α-linolenic acid appears to be greater in premature infants than in older term infants (Uauy et al., 2000a), although variability among infants is large. Current information from stable isotope tracer studies does not provide quantitative whole body or organ data on the conversion of α-linolenic acid to DHA, whether the rate of conversion can meet the needs of the developing brain for DHA, or the effect of varying linoleic and α-linolenic acid intakes and ratios on conversion. Experimental studies suggest that the eye and certain brain cells, such as astrocytes, are able to synthesize DHA from α-linolenic acid (Moore et al., 1991; Wetzel et al., 1991). The contribution of synthesis of DHA in the brain and retina to the accumulation of

DHA in these organs is not known. In vivo studies, however, have shown that the brain does take up DHA from plasma (de la Presa Owens and Innis, 1999; Greiner et al., 1997).

A large number of clinical trials have been completed comparing growth, as well as measures of visual, motor, and mental development, in term infants fed formula with no DHA or with addition of DHA to approximate the amount in human milk. Some have included arachidonic acid or γ-linolenic acid (18:3*n*-6), the Δ6 desaturase product of linoleic acid. The results of these trials are summarized in Table 8-1. Several aspects of design are important in evaluating these studies. These include a prospective, double-blind design with a sufficient number of infants randomized to control for the multiple genetic, environmental, and dietary factors that influence infant development and to detect meaningful treatment effects (Gore, 1999; Morley, 1998); the amount and balance of linoleic and α-linolenic acid; the duration of supplementation; the age at testing and tests used; and the physiological significance of any statistical differences found. None of the studies in Table 8-1 reported differences in growth among infants fed formulas with DHA added.

Recent large, randomized trials did not find differences in visual evoked potential, visual acuity, or tests of mental and psychomotor development through at least the first 18 months in term infants fed formulas supplemented with DHA or DHA plus arachidonic acid (Auestad et al., 1997, 2001; Lucas et al., 1999; Scott et al., 1998). These studies used formulas with at least 1.1 percent α-linolenic acid and had linoleic:α-linolenic acid ratios close to 10:1. In the study by Scott and coworkers (1998), indices of early vocabulary development were lower in infants fed formula with DHA, but not in those fed formulas lacking DHA and arachidonic acid or containing both DHA and arachidonic acid. Birch and coworkers (1998, 2000) reported better visual evoked potential, but not visual acuity, and higher Bayley mental developmental indices scores in infants fed formulas with DHA or DHA plus arachidonic acid than in infants fed standard formula. Carlson and coworkers (1996a) on the other hand, found higher visual acuity at 2 months, but not at 4, 6, 9, or 12 months, in infants fed formula with DHA and arachidonic acid. Early studies by Makrides and colleagues (1995) reported better visual evoked potential acuity in infants fed formula with 0.36 percent DHA than infants given no dietary DHA. However, this group did not confirm this finding in subsequent studies with formulas containing 0.34 or 0.35 percent DHA (Makrides et al., 2000b). In addition, greater problem-solving ability has been reported among infants fed formula with DHA and arachidonic acid than in infants fed standard formula (Willatts et al., 1998).

The effect of low *n*-6:*n*-3 ratios (high *n*-3 fatty acids) on arachidonic acid metabolism is also of concern in growing infants. Several studies in

premature infants have reported an association between feeding *n*-3 long-chain fatty acids in the absence of arachidonic acid and reduced growth (Carlson et al., 1992, 1993, 1996b; Ryan et al., 1999). Scott and coworkers (1998) reported lower indices of language development in term infants fed formula with DHA, although not in infants fed formula with both DHA and arachidonic acid or with no DHA and arachidonic acid. Human milk from women in the United States and Canada following usual diets contains both arachidonic acid and DHA, usually in the range of 1:1 to 2:1. No evidence of reduced growth or outcome on developmental tests have been reported for infants fed formulas with both arachidonic acid and DHA in amounts similar to that contained in human milk. Infants fed formula with a ratio of linoleic:α-linolenic acid of 4.8:1 and no arachidonic acid had lower growth, as well as lower plasma arachidonic acid status, than infants fed a formula with a ratio of 44:1 (Jensen et al., 1997), and no differences in growth were found between infants fed formulas containing linoleic:α-linolenic acid ratios of 9.7:1 and 18.2:1. Additionally, no differences in growth were found among infants fed formulas with 1.7 or 3.3 percent α-linolenic acid with linoleic:α-linolenic acid ratios of 10:1 or 5:1, respectively (Makrides et al., 2000a).

In conclusion, randomized clinical studies on growth or neural development with term infants fed formulas currently yield conflicting results on the requirements for *n*-3 fatty acids in young infants, but do raise concern over supplementation with long-chain *n*-3 fatty acids without arachidonic acid. For these reasons, growth and neural development could not be used to set an EAR.

Trans *Fatty Acids and Conjugated Linoleic Acid*

Small amounts of *trans* fatty acids and conjugated linoleic acid are present in all diets. They can serve as a source of fuel energy for the body. However, there are no known requirements for *trans* fatty acids and conjugated linoleic acid for specific body functions.

FACTORS AFFECTING THE REQUIREMENTS

Fat Absorption and Aging

Aging in humans has been associated with a decrease in liver size and hepatic blood flow, slightly decreased serum albumin concentrations, and normal routine liver chemistries (Russell, 1992). Pancreatic secretion after initial stimulation with either secretin or pancreozymin is not diminished with age (Bartoš and Groh, 1969). Similarly, 72-hour fecal fat excretion in response to a dietary fat challenge in young (19 to 44 years of age) and old

TABLE 8-1 Randomized Studies of *n*-3 Fatty Acids and Neural and Visual Development in Full-Term, Formula-Fed Infants

Reference	Study Population[a]	Test/Age[b]	Fatty Acid[c]
Agostoni et al., 1995	*n* = 29 formula *n* = 29 formula + LC-PUFA	Brunet-Lézine psychomotor development test 4 mo	18:2*n*-6 18:3*n*-3 18:3*n*-6 (GLA) 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Makrides et al., 1995	*n* = 14 formula *n* = 12 formula + LC-PUFA	VEP acuity 16, 30 wk	18:2*n*-6 18:3*n*-3 18:3*n*-6 (GLA) 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Carlson et al., 1996a	*n* = 20 formula *n* = 19 formula + DHA + AA	Visual acuity 2, 4, 6, 9, 12 mo	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Agostoni et al., 1997	*n* = 30 formula *n* = 26 formula + LC-PUFA	DQ 24 mo	18:2*n*-6 18:3*n*-3 18:3*n*-6 (GLA) 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Auestad et al., 1997	*n* = 45 formula *n* = 43 formula + DHA *n* = 46 formula + DHA + AA	Sweep VEP 2, 4, 6, 9, 12 mo Visual acuity 2, 4, 6, 9, 12 mo	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Jensen et al., 1997	*n* = 20 each group	VER 4 mo	18:2*n*-6 18:3*n*-3 18:2*n*-6:18:3*n*-3 ratio
Birch et al., 1998	*n* = 21 formula *n* = 20 formula + DHA *n* = 19 formula + DHA + AA	Sweep VEP acuity 6, 17, 26, 52 wk Visual acuity 6, 17, 26, 52 wk	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)

Fatty Acid Content (% of fatty acids)				Results
Formula	Formula + LC-PUFA			Infants consuming formula supplemented with LC-PUFA scored significantly higher than standard formula group
11.1	10.8			
0.70	0.73			
—	0.30			
—	0.44			
—	0.30			
Formula	Formula + LC-PUFA			VEP acuity better in infants fed supplemented formula than in infants fed standard formula
16.79	17.44			
1.58	1.52			
0.05	0.27			
—	0.01			
	0.36			
Formula	Formula + DHA + AA			Infants fed formula supplemented with DHA + AA had higher visual acuity than infants fed standard formula at 2 mo, but not at 4, 6, 9, or 12 mo
21.9	21.8			
2.2	2.0			
—	0.43			
—	0.10			
Formula	Formula + LC-PUFA			No differences in DQ values
11.1	10.8			
0.70	0.73			
—	0.30			
—	0.44			
—	0.30			
Formula	Formula + DHA	Formula + DHA + AA		No differences in VEP or visual acuity
21.9	20.7	21.7		
2.2	1.9	1.9		
—	—	0.43		
—	0.23	0.12		
Formula #1	#2	#3	#4	No differences in VER
17.6	17.3	16.5	15.6	Infants fed formula with a ratio of 4.8 weighed less than infants fed formula with a ratio of 44
0.4	0.95	1.7	3.2	
44.0	18.2	9.7	4.8	
Formula	Formula + DHA	Formula + DHA + AA		Sweep VEP acuity better in infants fed supplemented formulas than in infants fed standard formula at 6, 17, and 52 wk, but not 26 wk
14.6	15.1	14.9		
1.49	1.54	1.53		
—	0.02	0.72		Visual acuity not different between groups
—	0.35	0.36		

continued

TABLE 8-1 Continued

Reference	Study Population[a]	Test/Age[b]	Fatty Acid[c]
Jørgensen et al., 1998	*n* = 11 formula *n* = 12 formula + DHA *n* = 14 formula + DHA + GLA	Sweep VEP acuity 4 mo	18:2*n*-6 18:3*n*-3 18:3*n*-6 (GLA) 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Scott et al., 1998	*n* = 42–45 formula *n* = 33–43 formula + DHA *n* = 38–46 formula + DHA + AA	Bayley scales of infant development 12 mo MacArthur communicative development 14 mo	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Lucas et al., 1999	*n* = 125 formula *n* = 125 formula + LC-PUFA	Bayley scales of infant development 18 mo	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)
Makrides et al., 2000a	*n* = 30 10:1 formula *n* = 28 5:1 formula	VEP acuity 16, 34 wk	18:2*n*-6 18:3*n*-3
Makrides et al., 2000b	*n* = 21 formula *n* = 23 formula + DHA *n* = 24 formula + DHA + AA	VEP acuity 16, 34 wk Bayley scales of infant development 12, 24 mo	18:2*n*-6 18:3*n*-3 20:4*n*-6 (AA) 22:6*n*-3 (DHA)

[a] LC-PUFA = long chain polyunsaturated fatty acids.
[b] VEP = visual evoked potential, DQ = developmental quotient, VER = visual evoked response.

(70 to 91 years of age) individuals suggests little change in the capacity to absorb fat (Arora et al., 1989). The ratio of mean surface area to volume of jejunal mucosa has been reported not to differ between young and old individuals (Corazza et al., 1986). Total gastrointestinal transit time appears to be similar between young and elderly individuals (Brauer et al.,

Fatty Acid Content (% of fatty acids)			Results
Formula	Formula + DHA	Formula + DHA + GLA	No differences in VEP acuity
12.01	11.95	12.67	
1.20	1.20	1.17	
—	—	0.54	
—	0.06	0.06	
—	0.32	0.32	
Formula	Formula + DHA	Formula + DHA + AA	No differences in mental and psychomotor development
21.9	20.7	21.7	Vocabulary production and comprehension lower in the formula + DHA group
2.2	1.9	1.9	
—	—	0.43	
—	0.23	0.12	
Formula	Formula + LC-PUFA		No differences in mental and psychomotor development
12.4	15.9		
1.1	1.4		
—	0.30		
—	0.32		
10:1 Formula	5:1 Formula		No differences in VEP acuity
16.9	16.6		
1.7	3.3		
Formula	Formula + DHA	Formula + DHA + AA	No differences in VEP acuity or Bayley scales of mental and psychomotor development
16.8	16.8	16.6	
1.5	1.2	1.0	
—	—	0.34	
—	0.35	0.34	

[c] GLA = γ-linolenic acid, AA = arachidonic acid, DHA = docosahexaenoic acid.

1981). Documented changes with age may be confounded by the inclusion of a subgroup with clinical disorders (e.g., atrophic gastritis). The presence of bile salt-splitting bacteria normally present in the small intestine of humans is of potential significance to fat absorption. No evidence of bacterial overgrowth has been reported in older individuals (Arora et

al., 1989). In addition, increases in fat malabsorption have not been demonstrated in normal elderly compared to younger individuals (Russell, 1992).

Exercise

Imposed physical activity decreased the magnitude of weight gain in nonobese volunteers given access to high fat diets (60 percent of energy) (Murgatroyd et al., 1999). In the exercise group, energy and fat balances (fat intake + fat synthesis – fat utilization) were not different from zero. Thus, high fat diets may cause positive fat balance, and therefore weight gain, only under sedentary conditions. These results are consistent with epidemiological evidence that show interactions between dietary fat, physical activity, and weight gain (Sherwood et al., 2000). Higher total fat diets can probably be consumed safely by active individuals while maintaining body weight. Although in longitudinal studies of weight gain, where dietary fat predicts weight gain independent of physical activity, it is important to note that physical activity may account for a greater percentage of the variance in weight gain than does dietary fat (Hill et al., 1989). Another endpoint that merits consideration is physical performance. High fat diets (69 percent of energy) do not appear to compromise endurance in trained athletes (Goedecke et al., 1999); however, athletes may not be able to train as effectively on short-term (less than 6 days) intakes of a high fat diet as on a high carbohydrate diet (Helge, 2000). This effect on training was not observed following long-term adaptation of high fat diets.

Genetic Factors

Studies of the general population may underestimate the importance of dietary fat in the development of obesity in subsets of individuals. Some data indicate that genetic predisposition may modify the relationship between diet and obesity (Heitmann et al., 1995). Additionally, some individuals with relatively high metabolic rates appear to be able to consume high fat diets (44 percent of energy) without obesity (Cooling and Blundell, 1998). Intervention studies have shown that those individuals susceptible to weight gain and obesity appear to have an impaired ability to increase fat oxidation when challenged with high fat meals and diets (Astrup et al., 1994; Raben et al., 1994). Animal studies show that there are important gene and dietary fat interactions that influence the tendency to gain excessive weight on a high fat diet (West and York, 1998). Once these genes are identified, further studies in humans will be feasible.

Alcohol

Alcohol is metabolized to acetylcoenzyme A in the liver and can enter all normal pathways for acetate metabolism, including the synthesis of fatty acids. The formation of nicotinamide adenine dinucleotide, resulting from ethanol oxidation, serves as a cofactor for fatty acid biosynthesis (Eisenstein, 1982). Similar to carbohydrate, alcohol consumption creates a shift in postprandial substrate utilization to reduce the oxidation of fatty acids (Schutz, 2000). Significant intake of alcohol (23 percent of energy) can depress fatty acid oxidation to a level equivalent to storing as much as 74 percent as fat (Murgatroyd et al., 1996). If the energy derived from alcohol is not utilized, the excess is stored as fat (Suter et al., 1992).

Interaction of n-*6 and* n-*3 Fatty Acid Metabolism*

The *n*-6 and *n*-3 unsaturated fatty acids are believed to be desaturated and elongated using the same series of desaturase and elongase enzymes (see Figure 8-1). The rate-limiting steps are the desaturases, rather than the elongase, enzymes. In vitro, the Δ6 desaturase shows clear substrate preference in the following order: α-linolenic acid > linoleic acid > oleic acid (Brenner, 1974). In addition, the formation of docosahexaenoic acid (DHA) from tetracosapentenoic acid (24:5*n*-3) involves a Δ6 desaturation to 24:6*n*-3 and then β-oxidation to yield 22:6*n*-3 (DHA) (Sprecher, 1992). It is not known if these are the Δ6 desaturases that are responsible for metabolism of linoleic acid and α-linolenic acid or a different enzyme (Cho et al., 1999). Many studies, primarily in laboratory animals, have provided evidence that the balance of linoleic and α-linolenic acid is important in determining the amounts of arachidonic acid, eicosapentaenoic acid (EPA), and DHA in tissue lipids. An inappropriate ratio may involve too high an intake of either linoleic acid or α-linolenic acid, too little of one fatty acid, or a combination leading to an imbalance between the two series. The provision of preformed carbon chain *n*-6 and *n*-3 fatty acids results in rapid incorporation into tissue lipids. Thus, the linoleic:α-linolenic acid ratio is likely to be of most importance for diets that are very low in or devoid of arachidonic acid, EPA, and DHA. The importance of the dietary linoleic:α-linolenic acid ratio for diets rich in arachidonic acid, EPA, and DHA is not known. Arachidonic acid is important for normal growth in rats (Mohrhauer and Holman, 1963). Later in life, risk of certain diseases may be altered by arachidonic acid and arachidonic acid-derived eicosanoids. Consequently, the desirable range of *n*-6:*n*-3 fatty acids may differ with life stage.

The regulation of *n*-6 and *n*-3 fatty acid metabolism is complex as the conversion of linoleic acid to arachidonic acid is inhibited by EPA and

DHA in humans, as well as arachidonic acid, α-linolenic acid, and linoleic acid itself (Chen and Nilsson, 1993; Emken et al., 1994, 1998, 1999; Sauerwald et al., 1996). Similarly, stable isotope studies have shown that increased intakes of α-linolenic acid result in decreased conversion of linoleic acid to its metabolites, and the amounts metabolized to longer-chain metabolites is inversely related to the amount oxidized (Vermunt et al., 2000). Unfortunately, very few studies are available on the rates of formation of arachidonic acid and DHA from their precursors in humans fed diets differing in linoleic acid and α-linolenic acid content, and with or without controlled amounts of arachidonic acid, EPA, and DHA.

Arachidonic acid is a precursor to a number of eicsanoids (e.g., thromboxane A_2, prostacylcin, and leukotriene B_4). These eicosanoids have been shown to have beneficial and adverse effects in the onset of platelet aggregation, hemodynamics, and coronary vascular tone. EPA has been shown to compete with the biosynthesis of *n*-6 eicosanoids and is the precursor of several *n*-3 eicosanoids (e.g., thromboxane A_3, prostaglandin I_3, and leukotriene B_5), resulting in a less thrombotic and atherogenic state (Kinsella et al., 1990).

n-*6*:n-*3 Polyunsaturated Fatty Acid Ratio*

Jensen and coworkers (1997) reported that infants fed formulas containing a linoleic acid:α-linolenic acid ratio of 4.8:1 had lower arachidonic acid concentrations and impaired growth compared to infants fed formulas containing ratios of 9.7:1 or higher. More recent, large clinical trials with infants fed formulas providing linoleic acid:α-linolenic acid ratios of 5:1 to 10:1 found no evidence of reduced growth or other problems that could be attributed to decreased arachidonic acid concentrations (Auestad et al., 1997, 2001; Makrides et al., 2000a). Clark and coworkers (1992) concluded that intake ratios less than 4:1 were likely to result in fatty acid profiles markedly different from those from infants fed human milk. Based on the limited studies, the linoleic acid:α-linolenic acid or total *n*-3:*n*-6 fatty acids ratios of 5:1 to 10:1, 5:1 to 15:1, and 6:1 to 16:1 have been recommended for infant formulas (Aggett et al., 1991; ISSFAL, 1994; LSRO, 1998).

In adult rats it has been determined that a linoleic acid:α-linolenic acid ratio of 8:1 was optimal in maintaining normal-tissue fatty acid concentrations (Bourre et al., 1996). Increasing the intake of linoleic acid from 15 to 30 g/d, with an increase in the linoleic:α-linolenic acid ratio from 8:1 to 30:1, resulted in a 40 to 54 percent decreased conversion of linoleic acid and α-linolenic acid to their metabolites in healthy men (Emken et al., 1994). Clinical studies with patients supported by total parenteral nutrition found resolution of signs of deficiency when a

parenteral lipid containing a linoleic acid:α-linolenic acid ratio of 6:1 was provided (Holman et al., 1982).

Clinical and epidemiological studies have addressed the *n*-6:*n*-3 fatty acid ratio, focusing on beneficial effects on risk of certain diseases associated with higher intakes of the *n*-3 fatty acids EPA and DHA, as reviewed in Chapter 11. The specific importance of the ratio in these studies cannot be assessed because the decreased ratio is secondary to an increased intake of fish or EPA and DHA from supplements. For example, low rates of heart disease in Japan, compared with the United States, have been attributed in part to a total *n*-6:*n*-3 fatty acid ratio of 4:1 (Lands et al., 1990), with about 5 percent energy as linoleic acid, 0.6 percent energy from α-linolenic acid, and 2 percent energy from EPA+DHA in Japan, compared with intakes of 6 percent energy from linoleic acid, 0.7 percent energy from α-linolenic acid, and less than 0.1 percent energy from EPA+DHA in the United States (Lands et al., 1992). Similarly, an inverse association between the dietary total *n*-6:*n*-3 fatty acid ratio and cardiovascular disease, cancer, and all-cause mortality (Dolecek and Grandits, 1991), as well as between fish intake and coronary heart disease mortality (Kromhout et al., 1985; Shekelle et al., 1985), have been reported. In other studies, however, no differences were found in coronary heart disease risk factors when a diet containing a total *n*-6:*n*-3 ratio of 4:1 compared to 1:1 was consumed (Ezaki et al., 1999), or in thrombotic conditions with a diet containing a total *n*-6:*n*-3 ratio of 3.3:1 compared with 10:1 (Nelson et al., 1991). Hu and coworkers (1999b) observed a weak relationship between the *n*-6:*n*-3 ratio and fatal ischemic heart disease since both α-linolenic acid and linoleic acid were inversely related to risk. Based on the limited studies in animals, children, and adults, a reasonable linoleic:α-linolenic acid ratio of 5:1 to 10:1 has been recommended for adults (FAO/WHO, 1994).

Impact of Trans *Fatty Acids on* n-*6 and* n-*3 Metabolism*

The *trans* isomers of oleic acid and linoleic acid, which are present in hydrogenated vegetable oils and meats, have been suggested to have adverse effects on growth and development through inhibition of the desaturation of linoleic acid and α-linolenic acid to arachidonic acid and DHA, respectively (Sugano and Ikeda, 1996). Desaturation and elongation of *trans* linoleic and α-linolenic acid isomers containing a double bond at the *cis*-12 and *cis*-15 position, respectively, with formation of 20 and 22 carbon chain metabolites that could be incorporated into mem-brane lipids, have also been suggested. In vitro studies and studies with animals fed diets high in *trans* fatty acids have found evidence of reduced essential *n*-6 and *n*-3 fatty acid desaturation (Cook, 1981; Rosenthal and Doloresco, 1984). An inverse association between total *trans* fatty acids and arachidonic

acid and DHA concentrations in plasma cholesteryl esters, and between plasma cholesteryl esters, elaidic acid (18:1*trans*), and birth weight of premature infants has been reported (Koletzko, 1992). Studies in term infants found no relation between *trans* fatty acids and length of gestation, birth weight, or birth length (Elias and Innis, 2001). Similarly, an inverse association between plasma phospholipid *trans* fatty acids and arachidonic acid has been found for children aged 1 to 15 years (Decsi and Koletzko, 1995). The industrial hydrogenation of vegetable oils results in destruction of *cis* essential *n*-6 and *n*-3 fatty acids and the formation of *trans* fatty acids (Valenzuela and Morgado, 1999). It is not clear if differences in dietary intakes of *n*-6 and *n*-3 fatty acids, rather than inhibition of linoleic acid and α-linolenic acid desaturation by *trans* fatty acids, explains the statistical inverse associations between *trans* and *n*-6 and *n*-3 fatty acids reported in some studies (Craig-Schmidt, 2001). Based on the much greater affinity of the Δ6 desaturase for *cis n*-6 and *n*-3 fatty acids than monounsaturated fatty acids (Brenner, 1974; Castuma et al., 1977), and on experimental work that shows that inhibition of the Δ6 desaturation of linoleic acid is not of concern with linoleic acid intakes above about 2 percent of energy (Zevenbergen et al., 1988), it seems unlikely that inhibition of essential fatty acid metabolism by *trans* fatty acids is of concern for practical human diets.

FINDINGS BY LIFE STAGE AND GENDER GROUP

Total Fat
Infants Ages 0 Through 12 Months

Method Used to Set the Adequate Intake

No functional criteria of fat have been demonstrated that reflects a response to dietary intake in infants. Thus, the recommended intakes of total fat are based on an Adequate Intake (AI) that reflects the observed mean fat intake of infants principally fed human milk.

Ages 0 Through 6 Months. Fat is the major single source of energy in the diet of infants exclusively fed human milk. The high intake of fat and the energy density that it provides to the diet are important in providing the energy needed for rapid growth during early infancy. Thus, the recommended intake of total fat for infants 0 through 6 months of age is based on an AI that reflects the observed mean fat intake of infants fed human milk. Table 8-2 shows the concentration and proportion of energy from fat provided by mature human milk from women delivering at term gestation. Assuming an intake of 0.78 L/d of human milk by infants exclusively fed

human milk (Chapter 1) and a mean milk fat content of 40 g/L, the AI for fat is 31 g/d. This AI assumes that the energy requirements of the young infant are being met. The mean energy content of mature human milk is 650 kcal/L (Chapter 5), thus dietary fat represents 55 percent of total energy intake for infants 0 through 6 months of age. Fomon and coworkers (1976) reported that the length and weight of infants were not different when fed formula and strained food providing 29 or 57 percent of energy from fat. Thus, an intake of 55 percent energy most likely exceeds the minimum percent needed for optimal growth of healthy infants.

Ages 7 Through 12 Months. The proportion of energy from dietary fat decreases during the second 6 months of age when complementary foods, specifically infant cereals, vegetables, and fruits, are added to the diet of the infant. The average concentration of fat in milk is approximately 40 g/L during the second 6 months of lactation (Table 8-2). The infant consumes about 0.6 L/d of human milk during the second 6 months (Chapter 1), with additional energy and nutrients provided by complementary foods, thus achieving total energy and essential nutrient needs of the infant 7 through 12 months of age.

The AI for the older infants is set based on the average intake of fat ingested from human milk and complementary foods (Chapter 1). Data from the Continuing Survey of Food Intakes by Individuals (CFSII) indicate that the average intake of fat from complementary foods by older infants is approximately 5.7 g/d. Therefore, the average fat intake from human milk and complementary foods would be 30 g/d ([0.6 L/d × 40 g/L] + 5.7) after rounding. The average energy intake from human milk is 390 kcal/d (0.6 L/d × 650 kcal/L) and from complementary foods is 281 kcal/d (CFSII), or a total energy intake of 671 kcal/d. Therefore, for infants 7 though 12 months of age, 40 percent of energy from fat is consumed from human milk and complementary foods.

Total Fat AI Summary, Ages 0 Through 12 Months

AI for Infants

0–6 months	**31 g/d of fat**
7–12 months	**30 g/d of fat**

Special Considerations

Conventional milk-based infant formulas contain approximately 48 percent of energy intake as fat (LSRO, 1998). The most common sources of fat in infant formulas are soybean oil, safflower oil, sunflower oil, coconut oil, and palm oil.

TABLE 8-2 Total Fat Content in Term Human Milk of Women in the United States and Canada

Reference	Study Population/ Stage of Lactation[a]	Total Fat Content (g/L)	Total Fat Content (% of total energy)	Total Energy[b] (kcal/L)
Anderson et al., 1983	9 women			
	3 d pp	18 ± 6	31.3	510 ± 90
	7 d pp	31 ± 10	43.6	630 ± 98
	14 d pp	37 ± 10	49.0	670 ± 100
Bitman et al., 1983	8–41 women			
	3 d pp	20.4 ± 3.2		
	7 d pp	28.9 ± 3.1		
	21 d pp	34.5 ± 3.7		
	42 d pp	31.9 ± 4.3		
	84 d pp	48.7 ± 6.2		
Dewey and Lönnerdal, 1983	13–18 women			
	1 mo pp	49.2 ± 10.5	55.9	781 ± 100
	2 mo pp	45.8 ± 9.7	54.0	753 ± 92
	3 mo pp	45.8 ± 16.5	55.2	736 ± 148
	4 mo pp	46.2 ± 18.6	52.1	787 ± 173
	5 mo pp	43.6 ± 16.7	51.8	747 ± 148
	6 mo pp	43.0 ± 19.6	51.0	748 ± 183
Butte et al., 1984	45 women			
	1 mo pp		47.8	
	2 mo pp		47.8	
	3 mo pp		45.7	
	4 mo pp		47.6	
Dewey et al., 1984	119 samples			
	4–6 mo pp	44.1 ± 18.5	60.2[c]	
	7–11 mo pp	34.5 ± 15.3	47.1[c]	
	12–20 mo pp	48.4 ± 1.19	66.0[c]	
Ferris et al., 1988	12 women			
	2 wk pp	39.8 ± 9.9	45.2	781 ± 125
	6 wk pp	44.1 ± 11.7	51.9	753 ± 77
	12 wk pp	48.7 ± 11.9	54.5	792 ± 93
	16 wk pp	55.0 ± 10.9	58.8	829 ± 122
Innis and Kuhnlein, 1988	12 Vancouver women	31 ± 3		

TABLE 8-2 Continued

Reference	Study Population/ Stage of Lactation[a]	Total Fat Content (g/L)	Total Fat Content (% of total energy)	Total Energy[b] (kcal/L)
Nommsen et al., 1991	46–70 women			
	3 mo pp	36.2 ± 7.0	46.1	697 ± 67
	6 mo pp	37.7 ± 9.6	47.3	707 ± 92
	9 mo pp	38.1 ± 8.0	47.7	709 ± 74
	12 mo pp	37.0 ± 11.3	46.7	706 ± 110
Chen et al., 1995a	198 samples			
	3–4 wk pp	31.58 ± 9.37		

[a] pp = postpartum.
[b] Calculated using 8.87 kcal/g of fat.
[c] Percent of energy determined from mean energy content of all milk samples during 7–20 mo pp (650 kcal/L).

Children and Adolescents Ages 1 Through 18 Years

A number of studies have been conducted to ascertain whether a certain amount of fat is needed in the diet to provide normal growth in children. These data generally conclude that there is no effect of fat intake on growth when consumed at levels as low as 21 percent of energy and provided that the energy intake is adequate (Boulton and Magarey, 1995; Fomon et al., 1976; Lagström et al., 1999; Lapinleimu et al., 1995; Niinikoski et al., 1997a, 1997b; Obarzanek et al., 1997; Shea et al., 1993) (see Chapter 11). There is insufficient evidence to identify a defined intake level of fat to prevent obesity or chronic diseases. Based on this lack of evidence and the lack of an effect of fat intake on growth, neither an AI nor an Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) are set for children and adolescents.

Adults Ages 19 Years and Older

The amount of total energy as fat in the diet can vary from 10 to 50 percent without differing effects on short-term health (Jéquier, 1999). When men and women were fed isocaloric diets containing 20, 40, or 60 percent fat, there was no difference in total daily energy expenditure (Hill et al., 1991). Similar observations were reported for individuals who consumed diets containing 10, 40, or 70 percent fat (Leibel et al., 1992) and men fed 9 to 79 percent fat (Shetty et al., 1994). In addition, a number

of studies have reported on the impact of or the relationship between low and high fat diets and the indicators for and risk of chronic diseases (e.g., coronary heart disease, diabetes, and obesity) (see Chapter 11). There are insufficient data, however, to identify a defined intake level for fat based on maintaining fat balance or on the prevention of chronic diseases. Therefore, neither an AI nor an EAR and RDA are set.

Saturated Fatty Acids

There is no evidence to indicate that saturated fatty acids are essential in the diet or have a beneficial role in the prevention of chronic diseases. Therefore, neither an AI nor an EAR and RDA are set.

cis n-*9 Monounsaturated Fatty Acids*

There is no evidence to indicate that monounsaturated fatty acids are essential in the diet, and monounsaturated fatty acids have no known independent role in preventing chronic diseases. Therefore, neither an AI nor an EAR and RDA are set.

n-*6 Polyunsaturated Fatty Acids*
Infants Ages 0 Through 12 Months

Method Used to Set the AI

A series of papers reported skin lesions and poor growth in infants fed skimmed cow milk, which is very low in *n*-6 fatty acids (Hansen et al., 1958, 1963). Cuthbertson (1976) concluded that less than 50 mg/100 kcal of linoleic acid (0.45 percent energy) can provide normal health and well-being during infancy. Studies on the essential fatty acid status of older individuals have established that about 2 percent energy from *n*-6 polyunsaturated fatty acids (linoleic acid) will prevent abnormal elevation of the triene:tetraene ratio (20:3*n*-9:20:4*n*-6) and clinical signs of essential fatty acid deficiency during parenteral nutrition (Barr et al., 1981). Interpretation, however, is complicated because linoleic acid in the soybean oil emulsion used to provide *n*-6 fatty acids can also be expected to inhibit synthesis of eicosatrienoic acid (20:3*n*-9) (Brenner, 1974), and thus reduce the triene:tetraene ratio. Furthermore, children are expected to require higher amounts of *n*-6 fatty acids than adults in order to support deposition of *n*-6 fatty acids in cell membranes of growing tissues. This suggests that a margin of safety is prudent.

Ages 0 Through 6 Months. An AI can be set based on the average amount of *n*-6 polyunsaturated fatty acids provided by human milk. Table 8-2 provides the fat and energy content of human milk. Human milk contains 5.6 g/L (14 percent *n*-6 fatty acid in milk × 40 g/L) of *n*-6 polyunsaturated fatty acids (Table 8-3).

Based on an average intake of 0.78 L/d of human milk (Chapter 1), the AI is 4.4 g/d (0.78 L/d × 5.6 g/L). The energy content of human milk is approximately 650 kcal/L (Chapter 5) and therefore provides 507 kcal/d (650 kcal/L × 0.78 L/d). Thus, *n*-6 polyunsaturated fatty acids contribute approximately 8 percent of daily energy intake. The various *n*-6 fatty acids that are naturally present in human milk can contribute to this AI.

Ages 7 Through 12 Months. The period from 7 through 12 months of age is a time of major transition in the diet, from infants exclusively fed human milk or infant formulas that provide large amounts of dietary fat to a diet containing a variety of foods in addition to milk or formula. The infant consumes about 0.6 L/d of human milk during the second 6 months of life (Chapter 1), with additional energy and nutrients provided by complementary foods, thus achieving total energy and essential nutrient needs. The AI for older infants is set based on the average intake of *n*-6 polyunsaturated fatty acids ingested from human milk and complementary foods (Chapter 1). Data from CFSII indicates that the average intake of *n*-6 polyunsaturated fatty acids from complementary foods by older infants is approximately 1.2 g/d. Therefore, the AI for *n*-6 polyunsaturated fatty acids is 4.6 g/d ([0.6 L/d × 5.6 g/L] + 1.2) after rounding. The average fat energy coming from human milk is 390 kcal/d (0.6 L/d × 650 kcal/L), and from complementary foods is 281 kcal/d (CFSII), for a total energy intake of 671 kcal/d. Therefore, 6 percent of energy from *n*-6 polyunsaturated fat is consumed via human milk and complementary foods.

n-*6 Polyunsaturated Fatty Acids AI Summary, Ages 0 Through 12 Months*

AI for Infants

0–6 months	**4.4 g/d of *n*-6 polyunsaturated fatty acids**
7–12 months	**4.6 g/d of *n*-6 polyunsaturated fatty acids**

Special Considerations

The polyunsaturated vegetables oils (e.g., safflower oil and soybean oil) used in the manufacture of infant formulas contain abundant amounts (45 to 70 percent of total fatty acids) of linoleic acid. The minimum permissible amount of linoleic acid found in infant formulas is 2.7 percent of

TABLE 8-3 *n*-6 Polyunsaturated Fatty Acid Content in Term Human Milk of Women in the United States and Canada

			Content in Human Milk	
Reference	*n*	*n*-6 Fatty Acid	% of Total Fatty Acids	% of Total Energy[a]
Putnam et al., 1982	9	18:2	15.8 ± 0.61	8.62
		20:2	0.4 ± 0.03	0.22
		20:3	0.4 ± 0.03	0.22
		20:4	0.6 ± 0.03	0.33
		22:4	0.2 ± 0.02	0.11
		22:5	0.1 ± 0.02	0.05
		Total	17.50	9.55
Bitman et al., 1983	6	18:2	15.58 ± 1.99	8.50
		20:2	0.18 ± 0.20	0.10
		20:3	0.53 ± 0.15	0.29
		20:4	0.60 ± 0.29	0.33
		22:4	0.07 ± 0.16	0.04
		22:5	0.03 ± 0.08	0.02
		Total	16.99	9.28
Harris et al., 1984	8	18:2	15.3 ± 3.3	8.35
		20:3	0.3 ± 0.1	0.16
		20:4	0.4 ± 0.1	0.22
		Total	16.0	8.73
Finley et al., 1985	172	18:2	16.49 ± 4.80	9.00
		20:2	0.38 ± 0.15	0.21
		20:3	0.28 ± 0.09	0.15
		20:4	0.29 ± 0.08	0.16
		Total	17.44	9.52
Innis and Kuhnlein, 1988	12	18:2	12.7 ± 1.8	6.93
		20:2	0.4 ± 0.1	0.22
		20:4	0.7 ± 0.0	0.38
		22:5	0.2 ± 0.1	0.11
		Total	14.0	7.64
Chen et al., 1995a	198	18:2	10.47 ± 2.62	5.72
		18:3	0.08 ± 0.06	0.04
		20:2	0.17 ± 0.37	0.09
		20:3	0.26 ± 0.09	0.14
		20:4	0.35 ± 0.11	0.19
		22:4	0.04 ± 0.05	0.02
		22:5	0.01 ± 0.02	0.01
		Total	11.38	6.21

TABLE 8-3 Continued

Reference	*n*	*n*-6 Fatty Acid	Content in Human Milk	
			% of Total Fatty Acids	% of Total Energy[a]
Innis and King, 1999	103	18:2	12.1 ± 0.35	6.60
		18:3	0.1 ± 0.00	0.05
		20:2	0.3 ± 0.01	0.16
		20:3	0.3 ± 0.01	0.16
		20:4	0.4 ± 0.01	0.22
		22:4	0.1 ± 0.00	0.05
		Total	13.3	7.24

[a] Calculated using the following values: 40 g of fat/L of milk, 8.87 kcal/g of fat, 650 kcal/L of milk.

energy (Infant Formula. Nutrient Specifications. 21 C.F.R. §107.100, 1985); however, formulas provide higher amounts than this level.

Children and Adolescents Ages 1 Through 18 Years

Method Used to Set the AI

No specific information is available on the amount of linoleic acid required to correct the symptoms of an *n*-6 polyunsaturated fatty acid deficiency. In the absence of this information, an AI is set based on the median intake of linoleic acid consumed in the United States where the presence of an *n*-6 fatty acid deficiency is basically nonexistent in the free-living population (Appendix Table E-9), and rounding.

Linoleic Acid AI Summary, Ages 1 Through 18 Years

AI for Children

1–3 years	**7 g/d of linoleic acid**
4–8 years	**10 g/d of linoleic acid**

AI for Boys

9–13 years	**12 g/d of linoleic acid**
14–18 years	**16 g/d of linoleic acid**

AI for Girls

9–13 years	**10 g/d of linoleic acid**
14–18 years	**11 g/d of linoleic acid**

Adults Ages 19 Years and Older

Method Used to Set the AI

Various studies on adult patients receiving total parenteral nutrition have shown that linoleic acid intakes of as little as 7.4 to 8 g/d reverses the symptoms of deficiency (Barr et al., 1981; Collins et al., 1971; Goodgame et al., 1978; Jeppesen et al., 1998; Wong and Deitel, 1981). There is inadequate information, however, to set an EAR for healthy individuals. In the absence of this information, an AI is set based on the median intake of linoleic acid in the United States where the presence of an *n*-6 fatty acid deficiency is basically nonexistent in the free-living population (Appendix Table E-9). The highest median intakes have been used, each for men and women 19 to 50 years of age. Energy expenditure increases fat oxidation (Calles-Escandon et al., 1996) and linoleic acid is readily used for energy (Cunnane et al., 2001). Therefore, the AI for older men and women (greater than 50 years of age), whose energy expenditure is less than younger adults, is based on the highest median intake within this age range and rounding.

Linoleic Acid AI Summary, Ages 19 Years and Older

AI for Men

19–30 years	**17 g/d of linoleic acid**
31–50 years	**17 g/d of linoleic acid**
51–70 years	**14 g/d of linoleic acid**
> 70 years	**14 g/d of linoleic acid**

AI for Women

19–30 years	**12 g/d of linoleic acid**
31–50 years	**12 g/d of linoleic acid**
51–70 years	**11 g/d of linoleic acid**
> 70 years	**11 g/d of linoleic acid**

Pregnancy

Method Used to Set the AI

The demand for *n*-6 fatty acids for incorporation into placental tissue and the developing fetus during gestation must be met by *n*-6 fatty acids from maternal tissues or through dietary intake. Longitudinal studies have reported a decrease in plasma arachidonic acid concentration in pregnant women (Ghebremeskel et al., 2000; Sanjurjo et al., 1993). Lower arachidonic acid concentrations have also been reported for red blood cell phospholipids of pregnant women compared with nonpregnant women (Ghebremeskel et al., 2000). It is not clear that this reflects an increased need for *n*-6 fatty acids that was not met in the women in these studies, or whether changes in maternal *n*-6 fatty acid concentrations are normal physiological responses explained by the changes in endocrine status, lipoprotein and lipid metabolism, or nutrient transfer to the fetus. There is no evidence that maternal dietary intervention with *n*-6 fatty acids has any effect on fetal or infant growth and development in women meeting the requirements for *n*-6 fatty acids.

Because of a lack of evidence for determining the requirement during pregnancy, the AI is set based on the median linoleic acid intake of pregnant women in the United States where a deficiency is basically nonexistent in noninstitutionalized populations (Appendix Table E-9), and rounding.

Linoleic Acid AI Summary, Pregnancy

AI for Pregnant Women

14–18 years	**13 g/d of linoleic acid**
19–30 years	**13 g/d of linoleic acid**
31–50 years	**13 g/d of linoleic acid**

Lactation

Method Used to Set the AI

As stated above, there is no evidence that maternal dietary intervention with *n*-6 fatty acids has any effect on infant growth and development in women meeting the requirements for *n*-6 fatty acids. Because of a lack of evidence for determining the requirement during lactation, the AI is set based on the median linoleic acid intake of lactating women in the United States where a deficiency is basically nonexistent in noninstitutionalized populations (Appendix Table E-9), and rounding.

Linoleic Acid AI Summary, Lactation

AI for Lactation	
14–18 years	**13 g/d of linoleic acid**
19–30 years	**13 g/d of linoleic acid**
31–50 years	**13 g/d of linoleic acid**

n-*3 Polyunsaturated Fatty Acids Infants Ages 0 Through 12 Months*

Method Used to Set the AI

Human milk contains α-linolenic acid (18:3), eicosapentaenoic acid (EPA, 20:5), and docosahexaenoic acid (DHA, 22:6) (Table 8-4), but the amounts present are highly variable and depend on the amounts present in the mother's diet. Concentrations of about 0.7 to 1.4 percent DHA have been reported for women who eat large amounts of fish and other marine foods (Innis and Kuhnlein, 1988; Kneebone et al., 1985). Blood concentrations of DHA appear to show little metabolic regulation and increase with increasing DHA intake in breast-fed infants (Gibson et al., 1997; Innis and King, 1999; Sanders and Reddy, 1992) or formula-fed infants (Auestad et al., 1997; Carlson et al., 1996a; Innis et al., 1996; Makrides et al., 1995), as they do in adults. Numerous studies have shown that infants fed formulas with no DHA have lower plasma and red blood cell DHA concentrations than infants fed human milk or formulas with DHA (Auestad et al., 1997; Carlson et al., 1986, 1996a; Innis et al., 1996; Makrides et al., 1995; Ponder et al., 1992; Putnam et al., 1982). Similarly, the plasma and red blood cell DHA concentrations are lower in infants breast-fed by mothers with vegetarian rather than omnivorous diets (Sanders and Reddy, 1992). Evidence of DHA depletion based on functional endpoints has not been reported for populations or subgroups that have diets containing no DHA but with adequate α-linolenic acid.

Several autopsy studies have reported lower DHA concentrations in the brains of infants fed formulas that contain no DHA compared with infants fed human milk (Byard et al., 1995; Farquharson, 1994; Farquharson et al., 1992, 1995; Jamieson et al., 1994, 1999; Makrides et al., 1994). In addition, brain DHA accumulation continues in both breast-fed and formula-fed infants for at least 40 weeks of life, but the accumulation is at a greatly reduced rate in formula-fed infants (Makrides et al., 1996). Although many infant formulas contain similar amounts of α-linolenic acid as human milk, the dietary supply of only α-linolenic acid and no DHA in formulas may be inadequate to supply the infant brain with DHA (Farquharson,

TABLE 8-4 *n*-3 Polyunsaturated Fatty Acid Content in Term Human Milk of Women in the United States and Canada

			Content in Human Milk	
Reference	*n*	*n*-3 Fatty Acid	% of Total Fatty Acids	% of Total Energy[a]
Putnam et al., 1982	9	18:3	0.8 ± 0.09	0.44
		20:5	0.1 ± 0.03	0.05
		22:5	0.1 ± 0.01	0.05
		22:6	0.1 ± 0.01	0.05
		Total	1.1	0.59
Bitman et al., 1983	6	18:3	1.03 ± 0.21	0.56
		20:5	trace	trace
		22:5	0.11 ± 0.15	0.06
		22:6	0.23 ± 0.14	0.13
		Total	1.37	0.75
Harris et al., 1984	8	18:3	0.8 ± 0.5	0.44
		20:5	trace	trace
		22:5	trace	trace
		22:6	0.1 ± 0.1	0.05
		Total	0.9	0.49
Finley et al., 1985	172	18:3	1.56 ± 0.43	0.85
		22:6	0.06 ± 0.004	0.03
		Total	1.62	0.88
Innis and Kuhnlein, 1988	12	18:3	0.6 ± 0.2	0.33
		20:5	0.2 ± 0.2	0.11
		22:5	0.4 ± 0.1	0.22
		22:6	0.4 ± 0.1	0.22
		Total	1.6	0.88
Chen et al., 1995a	198	18:3	1.16 ± 0.37	0.63
		20:4	0.06 ± 0.06	0.03
		20:5	0.05 ± 0.05	0.03
		22:5	0.08 ± 0.06	0.04
		22:6	0.14 ± 0.10	0.08
		Total	1.49	0.81
Innis and King, 1999	103	18:3	1.4 ± 0.07	0.76
		20:5	0.1 ± 0.01	0.05
		22:5	0.2 ± 0.02	0.11
		22:6	0.2 ± 0.03	0.11
		Total	1.9	1.03

[a] Calculated using the following values: 40 g of fat/L of milk, 8.87 kcal/g of fat, 650 kcal/L of milk.

1994). Animal studies have shown that dietary DHA is incorporated into brain tissue to a greater extent than is DHA that is biosynthesized from α-linolenic acid (Abedin et al., 1999; Sinclair, 1975). Furthermore, administration of dietary α-linolenic acid was not effective in restoring brain DHA concentrations in chicks deficient in *n*-3 fatty acids (Anderson et al., 1990). Therefore, the DHA content of the brain may depend more heavily upon the dietary supply of DHA rather than its precursor, α-linolenic acid. Randomized clinical studies on growth or neural development with term infants fed formulas currently yield conflicting results on the requirement for *n*-3 fatty acids in young infants (see "Evidence Considered for Estimating the Requirement for Total Fat and Fatty Acids").

Ages 0 Through 6 Months. *n*-3 Polyunsaturated fatty acids provide DHA that is important for the developing brain and retina. Human milk is assumed to meet the *n*-3 fatty acid requirements of the infants fed human milk. Therefore, an AI for *n*-3 fatty acids is based on the amount of *n*-3 fatty acids, total fat, and energy provided by human milk. Table 8-2 shows the fat and energy content of human milk. Human milk contains approximately 0.63 g/L (1.58 percent *n*-3 fatty acids × 40 g/L total fat) of *n*-3 polyunsaturated fatty acids (Table 8-4). The AI is based on the average amount of milk consumed by the infant (0.78 L/d) and the *n*-3 fatty acid concentration in human milk. Therefore, the AI is set at 0.5 g/d (0.78 L/d × 0.63 g/L), after rounding, which provides approximately 4.5 kcal/d. Because human milk provides 650 kcal/L (Chapter 5) or 507 kcal/d (650 kcal/L × 0.78 L/d), an AI of 0.5 g/d of *n*-3 polyunsaturated fatty acids represents approximately 1 percent (4.5 ÷ 507) energy intake, after rounding. The various *n*-3 fatty acids that are naturally present in human milk can contribute to this AI.

Ages 7 Through 12 Months. While the energy requirement relative to body weight decreases in the second 6 months of life (see Chapter 5), autopsy analyses suggest that brain DHA accretion continues at a similar rate from 0 through 24 months of age (Martinez, 1992). The AI for older infants is set based on the average intake of *n*-3 fatty acids ingested from human milk and complementary foods (Chapter 1). Data from CFSII indicate that the average intake of *n*-3 fatty acids from complementary foods by older infants is approximately 0.11 g/d. Therefore, the AI is 0.5 g/d [0.6 L/d × 0.63 g/L] + 0.11), after rounding, which represents approximately 4.5 kcal/d. The average energy intake from human milk is 390 kcal/d (0.6 L/d × 650 kcal/L), and from complementary foods is 281 kcal/d (CFSII), for a total energy intake of 671 kcal/d. Therefore, approximately 0.67 percent (4.5 kcal/d ÷ 671 kcal/d) of energy is consumed as *n*-3 polyunsaturated fatty acids from human milk and complementary foods.

n-*3 Polyunsaturated Fatty Acid AI Summary, Ages 0 Through 12 Months*

AI for Infants	
0–6 months	**0.50 g/d of *n*-3 polyunsaturated fatty acids**
7–12 months	**0.50 g/d of *n*-3 polyunsaturated fatty acids**

Special Considerations

Vegetable oils that provide α-linolenic acid are used in the manufacture of infant formulas. The U.S. Code of Federal Regulations does not currently specify minimum or maximum levels of α-linolenic acid for infant formulas. At the present time, DHA is not directly added to infant formulas. Information from clinical trials with term infants fed formulas with DHA are inconsistent, and associations between lower growth and delays on some developmental tests have been noted in preterm and term infants fed formulas containing DHA, but not arachidonic acid. Definitive evidence that this is due to the absence of arachidonic acid or explained by antagonism between DHA and *n*-6 fatty acids is not available. DHA is added to infant formula ingredients in the form of oils from fish oils, egg total lipids, egg phospholipids, and oil from single cell microorganisms.

Children and Adolescents Ages 1 Through 18 Years

Method Used to Set the AI

One case study of a 6-year-old girl on total parenteral nutrition (TPN) reported that the TPN solution, which was low in α-linolenic acid and provided approximately 0.08 g/d, resulted in episodes of numbness, weakness, blurred vision, and the inability to walk (Holman et al., 1982). Analysis of the girl's plasma fatty acids confirmed a low *n*-3 fatty acid concentration. It was determined that 1.625 g/d of α-linolenic acid reversed the abnormal neurological symptoms. Bjerve and coworkers (1988) reported low plasma *n*-3 fatty acid concentrations and poor growth in a child fed approximately 0.54 g/d of α-linolenic acid via a gastric tube. Growth was somewhat improved by the addition of 0.56 g/d of α-linolenic acid.

Because of a lack of evidence for determining the requirement for *n*-3 fatty acids during childhood, an AI is set based on the median intake of α-linolenic acid in the United States where a deficiency is basically nonexistent in noninstitutionalized populations (Appendix Table E-11), and rounding. Small amounts of EPA and DHA can contribute toward reversing an *n*-3 fatty acid deficiency (Bjerve, 1989; Bjerve et al., 1987a, 1987b, 1989) and can therefore contribute toward the AI for α-linolenic acid.

EPA and DHA contribute approximately 10 percent of the total *n*-3 fatty acid intake and therefore this percent contributes toward the AI for α-linolenic acid (Appendix Tables E-10, E-12, and E-14).

α-*Linolenic Acid AI Summary, Ages 1 Through 18 Years*

AI for Children

1–3 years	**0.7 g/d of α-linolenic acid**
4–8 years	**0.9 g/d of α-linolenic acid**

AI for Boys

9–13 years	**1.2 g/d of α-linolenic acid**
14–18 years	**1.6 g/d of α-linolenic acid**

AI for Girls

9–13 years	**1.0 g/d of α-linolenic acid**
14–18 years	**1.1 g/d of α-linolenic acid**

Adults Ages 19 Years and Older

Method Used to Set the AI

Several studies involving adult patients who were fed by gastric tube showed that an *n*-3 fatty acid (α-linolenic acid) deficiency could occur with intakes ranging from 0.015 to 0.095 g/d of α-linolenic acid (Bjerve, 1989; Bjerve et al., 1987a, 1987b, 1989), whereas intakes of as low as 0.3 g/d prevented the symptoms of a deficiency (Bjerve et al., 1987a). There were insufficient data, however, to set an EAR for free-living healthy adults.

Because of a lack of evidence for determining the requirement for *n*-3 fatty acids, an AI is set based on the highest median intake of α-linolenic acid by adults in the United States where a deficiency is basically nonexistent in noninstitutionalized populations (Appendix Table E-11), and rounding. Small amounts of EPA and DHA can contribute toward reversing an *n*-3 fatty acid deficiency (Bjerve, 1989; Bjerve et al., 1987a, 1987b, 1989). EPA and DHA contribute approximately 10 percent of the total *n*-3 fatty acid intake and therefore this percent contributes toward the AI for α-linolenic acid (Appendix Tables E-10, E-12, and E-14).

α-*Linolenic Acid AI Summary, Ages 19 Years and Older*

AI for Men	
19–30 years	**1.6 g/d of α-linolenic acid**
31–50 years	**1.6 g/d of α-linolenic acid**
51–70 years	**1.6 g/d of α-linolenic acid**
> 70 years	**1.6 g/d of α-linolenic acid**
AI for Women	
19–30 years	**1.1 g/d of α-linolenic acid**
31–50 years	**1.1 g/d of α-linolenic acid**
51–70 years	**1.1 g/d of α-linolenic acid**
> 70 years	**1.1 g/d of α-linolenic acid**

Pregnancy and Lactation

Method Used to Set the AI

The demand for *n*-3 polyunsaturated fatty acids for incorporation into placental tissue and for the developing fetus during gestation, as well as for secretion of *n*-3 polyunsaturated fatty acids in milk during lactation, must be met by *n*-3 fatty acids from maternal tissues or through dietary intake. Several studies have reported lower plasma and red blood cell lipid DHA concentrations in pregnant and lactating women compared with nonpregnant, nonlactating women (Ghebremeskel et al., 2000; Holman et al., 1991). It is not clear that this reflects declining DHA status due to inadequate *n*-3 fatty acid intakes in the women in these studies. An alternative explanation is that changes in maternal DHA concentrations are normal physiological responses to the changes in endocrine status, lipoprotein and lipid metabolism, or nutrient transfer that accompany pregnancy and lactation. However, supplementation with fish oil during pregnancy does increase DHA in both the mother and the newborn infant, and supplementation with fish oil during lactation increases the concentration of DHA in the mother's milk and in the infant's blood (Connor et al., 1996; Henderson et al., 1992; van Houwelingen et al., 1995). Dietary fatty acids are almost completely absorbed, and an increase in blood DHA concentration following the increase in intake with fish oil supplementation is to be expected. Evidence is not available to show that increasing intakes of DHA in pregnant and lactating women consuming diets that meet requirements for *n*-6 and *n*-3 fatty acids have any physiologically significant benefit to the infant. Population comparative studies have found higher birthweights and longer gestation for women in the Faroe Islands than in Denmark (Olsen et al., 1989). This has been attributed to a higher intake of EPA from fish

and other marine foods, leading to *n*-3 fatty acid-induced inhibition of the *n*-6 fatty acid-derived eicosanoids that are important in cervical ripening and initiation of parturition. Subsequent intervention studies indicate that 10.8 g of supplemental *n*-3 fatty acids from fish oil is associated with an increase in gestation of about 4 days (Olsen et al., 1992).

Because of a lack of evidence for determining the requirement for *n*-3 fatty acids during pregnancy and lactation, an AI is set based on the median intake of α-linolenic acid in the United States where a deficiency is basically nonexistent in noninstitutionalized populations (Appendix Table E-11), and rounding. Small amounts of EPA and DHA can contribute toward reversing an *n*-3 fatty acid deficiency (Bjerve, 1989; Bjerve et al., 1987a, 1987b, 1989), and can therefore contribute toward the AI for α-linolenic acid.

α-*Linolenic Acid AI Summary, Pregnancy and Lactation*

AI for Pregnancy

14–18 years	**1.4 g/d of α-linolenic acid**
19–30 years	**1.4 g/d of α-linolenic acid**
31–50 years	**1.4 g/d of α-linolenic acid**

AI for Lactation

14–18 years	**1.3 g/d of α-linolenic acid**
19–30 years	**1.3 g/d of α-linolenic acid**
31–50 years	**1.3 g/d of α-linolenic acid**

Special Considerations

The ratio of linoleic acid:α-linolenic acid in the diet is important because linoleic acid and α-linolenic acid compete for the same desaturase enzymes. Thus, a high ratio of linoleic acid:α-linolenic acid can inhibit the conversion of α-linolenic acid to DHA, while a low ratio will inhibit the desaturation of linoleic acid to arachidonic acid. The linoleic acid:α-linolenic acid ratio, however, is likely to be of greatest importance in diets that are very low or devoid of arachidonic acid, EPA, and DHA.

The available data, although limited, suggest that linoleic:α-linolenic acid ratios below 5:1 may be associated with impaired growth in infants (Jensen et al., 1997). Although a ratio of 30:1 has been shown to reduce further metabolism of α-linolenic acid, sufficient dose–response data are not available to set an upper range for this ratio with confidence. Assuming an intake of *n*-6 fatty acids of 5 percent energy, with this being mostly linoleic acid, the α-linolenic acid intake at a 5:1 ratio would be 1 percent of energy.

Trans *Fatty Acids*

There are no data available to indicate a health benefit from consuming *trans* fatty acids. Therefore, neither an AI nor an EAR and RDA are established for *trans* fatty acids.

INTAKES OF TOTAL FAT AND FATTY ACIDS

Total Fat

Food Sources

Both animal- and plant-derived food products contain fat. The principal foods that contribute to fat intake are butter, margarine, vegetable oils, visible fat on meat and poultry products, whole milk, egg yolks, nuts, and baked goods (e.g., cookies, doughnuts, and cakes). Over 95 percent of total fat intake is in the form of triacylglycerols. As discussed below, the type of fat present in these food products varies.

Dietary Intake

Intake data from the Continuing Survey of Food Intakes of Individuals (CFSII) (1994–1996, 1998) showed that the median total fat intake ranged from 65 to 100 g/d for men and 48 to 63 g/d for women (Appendix Table E-5). These intake ranges represent approximately 32 to 34 percent of total energy (Appendix Table E-6). During 1990 to 1997, median intakes of fat ranged from 32 to 34 percent and 30 to 33 percent of energy in Canadian men and women, respectively (Appendix Table F-3).

A longitudinal study in the United States found that dietary fat represented 48, 41, 35, and 30 percent of total energy intakes at 3, 6, 12, and 24 months of age, respectively (Butte, 2000). The Third National Health and Nutrition Examination Survey (NHANES) estimated that children 2 to 19 years of age consumed an average of 34 percent of total energy as fat, with little difference across the individual age groups (Troiano et al., 2000). Comparison of data collected across the three NHANES studies conducted since the early 1970s shows that children and adolescents across all race, gender, and age groups have decreased their total fat intake. Mean age-adjusted fat intakes have declined from 36 to 37 percent to 33 to 34 percent of total energy (Troiano et al., 2000). About 23 percent of children 2 to 5 years old, 16 percent of children 6 to 11 years old, and 15 percent of adolescents 12 to 19 years old had dietary fat intakes equal to or less than 30 percent of total energy intakes.

Saturated Fatty Acids

Food Sources

Sources of saturated fatty acids tend to be foods of animal sources, including whole milk, cream, butter, cheese, and fatty meats such as pork and beef (USDA/HHS, 2000). Certain oils, however, such as coconut, palm, and palm kernel oil, also contain relatively high amounts of saturated fatty acids. Saturated fatty acids provide approximately 20 to 25 percent of energy in human milk (Table 8-5).

Dietary Intake

Based on intake data from CFSII (1994–1996, 1998), median saturated fatty acid intake ranged from approximately 21 to 34 g/d for men and 15 to 21 g/d for women (Appendix Table E-7). Data from NHANES III indicated that saturated fatty acids provided 11 to 12 percent of energy in adult diets and ranged from 12.2 to 13.9 percent of energy for children and adolescents (CDC, 1994). NHANES III reported that 9 percent of children 2 to 11 years old and 7 percent of those 12 to 19 years old had saturated fatty acid intakes of less than 10 percent of total energy (Troiano et al., 2000). During 1990 to 1997, median intakes of saturated fatty acids ranged from approximately 10 to 12 percent of energy for Canadian men and women (Appendix Table F-4).

Cis-*Monounsaturated Fatty Acids*

Food Sources

About 50 percent of monounsaturated fatty acids are provided by animal products, primarily meat fat (Jonnalagadda et al., 1995). Oils that contain monounsaturated fatty acids include canola and olive oils. Monounsaturated fatty acids provide approximately 20 percent of energy in human milk (Table 8-6).

Dietary Intake

Based on intake data from CFSII (1994–1996, 1998), median monounsaturated fatty acid intake ranged from approximately 25 to 39 g/d for men and 18 to 24 g/d for women (Appendix Table E-8). Data from the 1987–1988 Nationwide Food Consumption Survey indicated that mean intakes of monounsaturated fatty acids were 13.6 to 14.3 percent of energy (Ganji and Betts, 1995).

TABLE 8-5 Saturated Fatty Acid Content in Term Human Milk of Women in the United States and Canada

			Content in Human Milk	
Reference	*n*	Saturated Fatty Acid	% of Total Fatty Acids	% of Total Energy[a]
Putnam et al., 1982	9	8:0	0.3	0.16
		10:0	1.4	0.76
		12:0	6.2	3.38
		14:0	7.6	4.15
		16:0	20.5 ± 0.70	11.19
		18:0	9.0 ± 0.46	4.91
		20:0	0.3 ± 0.02	0.16
		21:0	0.1 ± 0.02	0.05
		24:0	0.5 ± 0.01	0.27
		Total	45.9	25.03
Bitman et al., 1983	6	10:0	0.97 ± 0.28	0.53
		12:0	4.46 ± 1.17	2.43
		14:0	5.68 ± 1.36	3.10
		15:0	0.31 ± 0.07	0.17
		16:0	22.20 ± 2.28	12.12
		17:0	0.49 ± 0.36	0.27
		18:0	7.68 ± 1.85	4.19
		20:0	0.32 ± 0.11	0.17
		21:0	0.17 ± 0.12	0.09
		Total	42.28	23.07
Harris et al., 1984	8	10:0	trace	trace
		12:0	4.2 ± 1.3	2.29
		14:0	5.9 ± 0.7	3.22
		16:0	22.8 ± 1.6	12.45
		18:0	8.2 ± 1.2	4.48
		Total	41.1	22.44
Finley et al., 1985	172	8:0	0.16 ± 0.11	0.09
		10:0	1.10 ± 0.30	0.60
		12:0	5.56 ± 1.68	3.03
		14:0	8.01 ± 2.46	4.37
		16:0	23.28 ± 3.35	12.71
		18:0	8.06 ± 1.58	4.40
		Total	46.17	25.20
Innis and Kuhnlein, 1988	12	10:0	1.2 ± 0.2	0.66
		12:0	5.2 ± 0.7	2.84
		14:0	6.7 ± 0.5	3.66
		16:0	22.1 ± 2.7	12.06
		18:0	8.2 ± 0.8	4.48
		Total	43.4	23.70

continued

TABLE 8-5 Continued

Reference	*n*	Saturated Fatty Acid	Content in Human Milk: % of Total Fatty Acids	Content in Human Milk: % of Total Energy[a]
Chen et al., 1995a	198	10:0	1.39 ± 0.59	0.76
		12:0	5.68 ± 2.01	3.10
		14:0	6.10 ± 1.73	3.33
		15:0	0.37 ± 0.12	0.20
		16:0	18.30 ± 2.25	9.99
		17:0	0.32 ± 0.08	0.17
		18:0	6.15 ± 0.97	3.36
		20:0	0.15 ± 0.09	0.08
		Total	38.46	20.99
Innis and King, 1999	103	10:0	0.6 ± 0.03	0.33
		12:0	4.1 ± 0.15	2.24
		14:0	6.1 ± 0.21	3.33
		16:0	19.4 ± 0.28	10.59
		18:0	7.2 ± 0.15	3.93
		20:0	0.2 ± 0.00	0.11
		22:0	0.1 ± 0.00	0.05
		24:0	0.1 ± 0.00	0.05
		Total	37.8	20.63

[a] Calculated using the following values: 40 g of fat/L of milk, 8.87 kcal/g of fat, 650 kcal/L of milk.

n-*6 Polyunsaturated Fatty Acids*

Food Sources

Sources of *n*-6 polyunsaturated fatty acids include nuts, seeds, certain vegetables, and vegetable oils such as soybean oil, safflower oil, and corn oil. Certain oils, such as blackcurrant seed oil and evening primrose oil, are high in γ-linolenic acid (18:3*n*-6), which is an intermediate in the conversion of linoleic acid to arachidonic acid. Arachidonic acid is formed from linoleic acid in animal cells, but not plant cells, and is present in the diet in small amounts in meat, poultry, and eggs. Arachidonic acid is not present in plant-derived fats and oils.

TABLE 8-6 Monounsaturated Fatty Acid Content in Term Human Milk of Women in the United States and Canada

			Content in Human Milk	
Reference	*n*	Monounsaturated Fatty Acid	% of Total Fatty Acids	% of Total Energy[a]
Putnam et al., 1982	9	18:1	37.6 ± 0.75	20.52
		20:1	0.9 ± 0.07	0.49
		22:1	0.1 ± 0.02	0.05
		Total	38.6	21.06
Bitman et al., 1983	6	16:1	3.83 ± 0.39	2.09
		18:1	35.51 ± 2.73	19.38
		Total	39.34	21.47
Harris et al., 1984	8	16:1	2.5 ± 0.6	1.36
		18:1	32.6 ± 3.3	17.79
		20:1	0.5 ± 0.1	0.27
		Total	35.6	19.42
Finley et al., 1985	172	16:1	3.02 ± 0.77	1.65
		18:1	31.72 ± 3.81	17.31
		Total	34.74	18.96
Innis and Kuhnlein, 1988	12	16:1	3.3 ± 0.6	1.80
		18:1	36.3 ± 2.7	19.81
		20:1	0.7 ± 0.3	0.38
		22:1	0.2 ± 0.1	0.11
		Total	40.5	22.10
Chen et al., 1995a	198	14:1	0.28 ± 0.08	0.15
		16:1	2.68 ± 0.69	1.46
		17:1	0.21 ± 0.06	0.11
		18:1	36.09 ± 3.51	19.70
		20:1	0.53 ± 0.22	0.29
		22:1	0.02 ± 0.03	0.01
		Total	39.81	21.72
Innis and King, 1999	103	14:1	0.2 ± 0.01	0.11
		16:1	2.5 ± 0.08	1.36
		18:1	35.7 ± 0.41	19.49
		20:1	0.6 ± 0.05	0.33
		22:1	0.2 ± 0.02	0.11
		24:1	0.1 ± 0.01	0.05
		Total	39.3	21.45

[a] Calculated using the following values: 40 g of fat/L of milk, 8.87 kcal/g of fat, 650 kcal/L of milk.

Dietary Intake

Based on intake data from CFSII (1994–1996, 1998), median *n*-6 polyunsaturated fatty acid (linoleic acid) intake ranged from approximately 12 to 17 g/d for men and 9 to 11 g/d for women (Appendix Table E-9).

Polyunsaturated fatty acids have been reported to contribute approximately 5 to 7 percent of total energy intake in diets of adults (Allison et al., 1999; Fischer et al., 1985). Most (approximately 85 to 90 percent) *n*-6 polyunsaturated fatty acids are consumed in the form of linoleic acid. Other *n*-6 polyunsaturated fatty acids, such as arachidonic acid and γ-linolenic acid, are present in small amounts in the diet.

n-*3 Polyunsaturated Fatty Acids*

Food Sources

The major sources of *n*-3 fatty acids include certain vegetable oils and fish (Kris-Etherton et al., 2000). Vegetable oils such as soybean and flaxseed oils contain high amounts of α-linolenic acid. Fish oils provide a mixture of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and fatty fish are the major dietary sources of EPA and DHA. Smaller amounts are also present in meat and eggs.

Dietary Intake

Based on intake data from CFSII (1994–1996, 1998), the total *n*-3 fatty intake for men and women ranged from approximately 1.3 to 1.8 g/d and 1.0 to 1.2 g/d, respectively (Appendix Table E-10). These findings are similar to that reported by Kris-Etherton and coworkers (2000), who also reported that the average intake of *n*-3 polyunsaturated fatty acids was approximately 0.7 percent of energy. The median intake of α-linolenic acid ranged from approximately 1.2 to 1.6 g/d for men and 0.9 to 1.1 g/d for women (Appendix Table E-11). For all adults, the median intakes of EPA and DHA ranged from 0.004 to 0.007 and 0.052 to 0.093 g/d, respectively (Appendix Tables E-12 and E-14). The median intake of DHA ranged from 0.066 to 0.093 g/d for men and 0.052 to 0.069 g/d for women (Appendix Table E-14). Docosapentaenoic acid provided only 0.001 to 0.005 g/d (Appendix Table E-13).

Trans *Fatty Acids*

Food Sources

Reports listing the *trans* fatty acid level in selected food items are available from the United States (Enig et al., 1990; Litin and Sacks, 1993; Michels and Sacks, 1995), Canada (Ratnayake et al., 1993), and Europe (Aro et al., 1998a, 1998b, 1998c; Michels and Sacks, 1995; van Erp-baart et al., 1998; van Poppel et al., 1998). More recently, a comprehensive U.S. database was compiled by the U.S. Department of Agriculture (ARS, 2001) that included a description of the methodology used to formulate the nutrient values (Schakel et al., 1997). *Trans* fatty acids are present in foods containing traditional stick margarine (3.04 g *trans* fatty acids/serving) and vegetable shortenings (2.54 g/serving) that have been subjected to hydrogenation, as well as in milk (0.22 g/serving), butter (0.40 g/serving) and meats (0.01 to 0.21 g/serving) (Emken, 1995). Therefore, foods that are contributors of *trans* fatty acids include pastries, fried foods (e.g., doughnuts and french fries), dairy products, and meats. Human milk contains approximately 1 to 5 percent of total energy as *trans* fatty acids (Table 8-7) and similarly, infant formulas contain approximately 1 to 3 percent (Ratnayake et al., 1997).

Dietary Intake

Estimating the amount of *trans* fatty acids in the food supply has been hampered by the lack of an accurate and comprehensive database from which to derive the data and the trend towards the reformulation of products over the past decade to reduce levels. This latter issue complicates analysis of historical food intake data. Additionally, the variability in the *trans* fatty acid content of foods within a food category is extensive and can introduce substantial error when the calculations are based on food frequency questionnaires that heavily rely on the grouping of similar foods (Innis et al., 1999). *trans* Fatty acid intake is not currently collected in U.S. national surveys.

Early reports suggested a wide range of *trans* fatty acid intakes, from 2.6 to 12.8 g/d (Emken, 1995). The lower estimated intakes tended to be derived from food frequency data, whereas the higher estimated intakes tended to be derived from food availability data. More recent data from food frequency questionnaires collected in the United States suggest average *trans* fatty acid intakes of 1.5 to 2.2 percent of energy (Ascherio et al., 1994; Hu et al., 1997), or 5.2 percent of total dietary fat (Lemaitre et al., 1998). Intakes of about 1 to 2 percent of energy have been reported for women in Canada, although the range of intakes was wide (Elias and Innis,

TABLE 8-7 *Trans* Fatty Acid Content in Term Human Milk of Women in the United States and Canada

Reference	Study Population/Stage of Lactation[a]	*Trans* Fatty Acid	Content in Human Milk	
			% of Total Fatty Acids	% of Total Energy[b]
Gibson and Kneebone, 1981	120 women, 40–45 d pp	16:1 18:1	trace ~ 10	trace ~ 5.46
Chappell et al., 1985	7 women, 1–37 d pp	18:1(9) 18:1(7) 18:1(5) 18:2(6) c,t+t,c[c] Total	2.6 ± 0.4 0.1 ± 0.03 0.1 ± 0.04 0.1 ± 0.4 2.9	1.42 0.05 0.05 0.05 1.57
Chen et al., 1995a	198 samples, 3–4 wk pp	Total *trans*	7.19 ± 3.03	3.92
Innis and King, 1999	103 women, 2 mo pp	Total *trans*	7.1 ± 0.32	3.88

[a] pp = postpartum.
[b] Calculated using the following values: 40 g of fat/L of milk, 8.87 kcal/g of fat, 650 kcal/L of milk.
[c] c,t+t,c = *cis, trans* and *trans, cis*.

2001, 2002). Most recently, *trans* fatty acid intake was estimated from existing CFSII data (Allison et al., 1999). The mean *trans* fatty acid intake for the U.S. population aged 3 years and older was 2.6 percent of total energy intake.

Conjugated Linoleic Acid

Food Sources

The average concentration of conjugated linoleic acid (CLA) in dairy products and ruminant meats is approximately 5 mg of CLA/g of fat (Chin et al., 1992). Although numerous CLA isomers have been reported to be found in meat, milk, and dairy products (Ha et al., 1989), the *cis*-9,*trans*-11 isomer is the predominant form of CLA present in these foods (Ma et al., 1999). The conjugated linoleic acid content of milk can vary depending on a number of factors, such as animal feed diet, pasture grazing, supple-

ment use, and number of lactations (MacDonald, 2000). Ma and coworkers (1999) reported values of 1.8 mg of CLA/g of fat for skim milk, 3.4 mg/g for whole milk, 4.3 mg/g for 1 percent milk, 5.0 mg/g for 2 percent milk, and 5.5 mg/g for half-and-half cream. In addition, values ranged from 2.7 to 6.2 mg of CLA/g of fat for various cheeses and 1.2 to 3.2 mg of CLA/g of fat for different types of raw and cooked beef products.

Dietary Intake

Recent analysis of duplicate food portions indicates CLA intake in the United States is in the range of 151 to 212 mg/d (Ritzenthaler et al., 2001). The average intake of *cis*-9,*trans*-11 octadecadienoic acid in a small group of Canadians was recently estimated to be about 95 mg/d (Ens et al., 2001). Based on the CLA content in the Health Canada National Nutritious Food Basket 1998 for purchased quantities, *cis*-9,*trans*-11 CLA intake for men and women was 332 and 295 mg/d, respectively. These values assume that all food purchased is actually eaten. From food records it is clear that the pattern of CLA intake is highly variable among individuals and from day-to-day for individuals themselves. Estimates from information on foods purchased, however, are higher than estimates from reported food intake data; therefore, the two data sets are not comparable.

ADVERSE EFFECTS OF OVERCONSUMPTION

Total Fat

A Tolerable Upper Intake Level (UL) was not set for total fat because of the lack of a defined intake level at which an adverse effect, such as obesity, can occur (see Chapter 11). An Acceptable Macronutrient Distribution Range (AMDR) for fat intake, however, has been estimated based on adverse effects from consuming low fat and high fat diets (Chapter 11).

Saturated Fatty Acids

Hazard Identification

Elevated LDL Cholesterol Concentration and Risk of CHD. Several hundred studies have been conducted to assess the effect of saturated fatty acids on serum cholesterol concentration. In general, the higher the intake of saturated fatty acids, the higher the serum total (Figure 8-2) and low density lipoprotein (LDL) cholesterol concentrations (Figure 8-3). Regression analyses of such studies have suggested that for each 1 percent increase

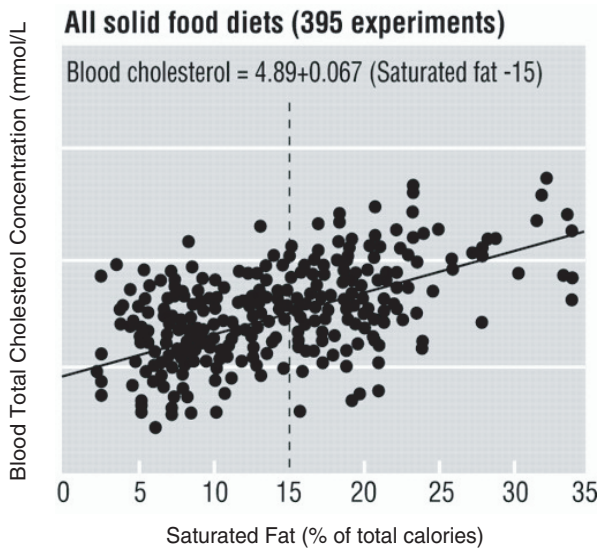

FIGURE 8-2 Relationship between blood total cholesterol concentrations and saturated fatty acid intake. Reprinted, with permission, from Clarke et al. (1997). Copyright 1997 by the *British Medical Journal*.

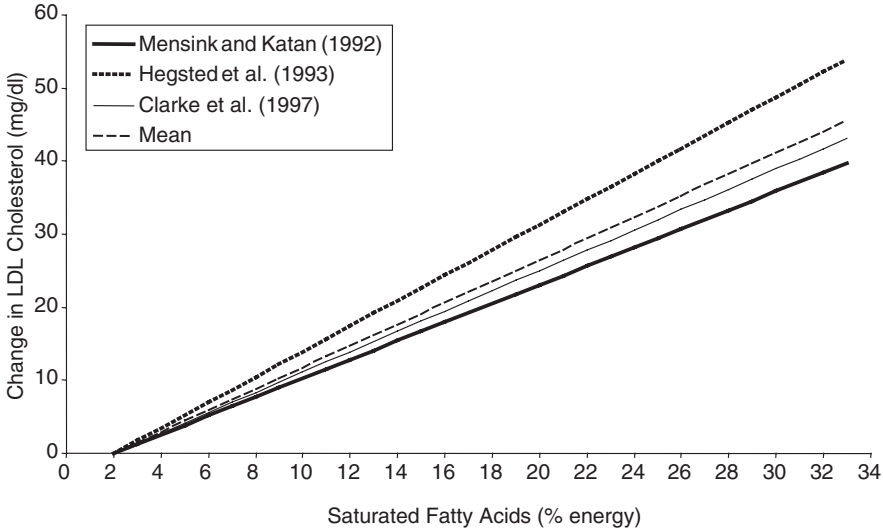

FIGURE 8-3 Calculated changes in serum low density lipoprotein cholesterol concentration in response to percent change in dietary saturated fatty acids. Three regression equations were used to establish the response curves. The range in saturated fatty acid intake was 2.2 to 33 percent of energy.

in energy from saturated fatty acids, serum LDL cholesterol concentration increases by 0.033 mmol/L (Mensink and Katan, 1992), 0.036 mmol/L (Clarke et al., 1997), or 0.045 mmol/L (Hegsted et al., 1993). Although all fats will increase serum high density lipoprotein (HDL) cholesterol concentration relative to carbohydrate, the increase attributable to saturated fats is greater than that observed for monounsaturated and polyunsaturated fatty acids. Serum HDL cholesterol concentration increases by 0.011 to 0.013 mmol/L for each 1 percent increase in saturated fat (Clarke et al., 1997; Hegsted et al., 1993; Mensink and Katan, 1992).

Similar to that observed for saturated fatty acid intake and LDL cholesterol concentration, there is a positive linear relationship between serum total and LDL cholesterol concentrations and risk of coronary heart disease (CHD) or mortality from CHD (Jousilahti et al., 1998; Neaton and Wentworth, 1992; Sorkin et al., 1992; Stamler et al., 1986; Weijenberg et al., 1996). Results from the Zutphen Elderly Study estimated that the relative risk of CHD mortality was 1.4 with a corresponding increase of 1 mmol/L of total serum cholesterol concentration (Weijenberg et al., 1996). It has been estimated that a 10 percent reduction in serum cholesterol concentration would reduce CHD mortality by 20 percent (Jousilahti et al., 1998).

A number of epidemiological studies have reported an association between saturated fatty acid intake and risk of CHD. The majority of these studies have reported a positive relationship between saturated fatty acid intake and risk of CHD and CHD mortality (Goldbourt et al., 1993; Hu et al., 1997, 1999a, 1999c; Keys et al., 1980; McGee et al., 1984). Ascherio and coworkers (1996) concluded that the association between saturated fatty acid intake and risk of CHD was not strong; however, saturated fat and the predicted effects on blood cholesterol concentrations did affect risk. No association between saturated fatty acid intake and coronary deaths was observed in the Zutphen Study or the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Kromhout and de Lezenne Coulander, 1984; Pietinen et al., 1997).

Although all saturated fatty acids were originally considered to be associated with increased adverse health outcomes, including increased blood cholesterol concentrations, it later became apparent that saturated fatty acids differ in their metabolic effects (e.g., potency in raising blood cholesterol concentrations). In general, stearic acid has been shown to have a neutral effect on total and LDL cholesterol concentrations (Bonanome and Grundy, 1988; Denke, 1994; Hegsted et al., 1965; Keys et al., 1965; Yu et al., 1995; Zock and Katan, 1992). While palmitic, lauric, and myristic acids increase cholesterol concentrations (Mensink et al., 1994), stearic acid is more similar to oleic acid in its neutral effect (Kris-Etherton et al., 1993). Furthermore, a stearic acid-rich diet has been shown to improve

thrombogenic and atherogenic risk factor profiles (Kelly et al., 2001). However, it is impractical at the current time to make recommendations for saturated fatty acids on the basis of individual fatty acids.

Mortality. A number of studies have demonstrated a positive association between serum cholesterol concentration and the incidence of mortality (Conti et al., 1983; Corti et al., 1997; Haheim et al., 1993; Klag et al., 1993; Martin et al., 1986). Some studies, however, have reported an increased risk of non-CHD mortality, especially cancer, with low serum cholesterol concentration, suggesting a "U" or "J" shaped curve (Agner and Hansen, 1983; Frank et al., 1992; Kagan et al., 1981). The Poland and United States Collaborative Study on Cardiovascular Epidemiology showed an increased risk for cancer with low serum cholesterol concentrations in Poland, but not in the United States (Rywik et al., 1999). It was concluded that various nutritional and non-nutritional factors (obesity, smoking, alcohol use) were confounding factors, resulting in the differences observed between the two countries. As a specific example, body fat was shown to have a "U" shaped relation to mortality (Yao et al., 1991).

Obesity. A number of studies have attempted to ascertain the relationship between saturated fatty acid intake and body mass index, and these results are mixed. Saturated fatty acid intake was shown to be positively associated with body mass index or percent of body fat (Doucet et al., 1998; Gazzaniga and Burns, 1993; Larson et al., 1996; Ward et al., 1994). In contrast, no relationship was observed for saturated fatty acid intake and body weight (González et al., 2000; Ludwig et al., 1999; Miller et al., 1994).

Impaired Glucose Tolerance and Risk of Diabetes. Epidemiological studies have been conducted to ascertain the association between the intake of saturated fatty acids and the risk of diabetes. A number of these studies found no relationship (Colditz et al., 1992; Costa et al., 2000; Salmerón et al., 2001; Sevak et al., 1994; Virtanen et al., 2000). Several large epidemiological studies, however, showed increased risk of diabetes with increased intake of saturated fatty acids (Feskens et al., 1995; Hu et al., 2001; Marshall et al., 1997; Parker et al., 1993). The Normative Aging Study found that a diet high in saturated fatty acids was an independent predictor for both fasting and postprandial insulin concentration (Parker et al., 1993). A reduction in saturated fatty acid intake from 13.9 to 7.8 percent of energy was associated with an 18 percent decrease in fasting insulin and a 25 percent decrease in postprandial insulin concentrations.

Findings from short-term intervention studies tend to suggest a lack of adverse effect of saturated fatty acids on risk indicators for diabetes in

healthy individuals. Postprandial glucose and insulin concentrations were not significantly different in men who ingested three different levels of saturated fatty acids (Roche et al., 1998). Fasching and coworkers (1996) reported no difference in insulin secretion or sensitivity in men who consumed a 33 percent saturated, monounsaturated, or polyunsaturated fatty acid diet. There was no difference in postprandial glucose or insulin concentration when healthy adults were fed butter or olive oil (Thomsen et al., 1999). Louheranta and colleagues (1998) found no difference in glucose tolerance and insulin sensitivity in healthy women fed either a high oleic or stearic acid diet. In contrast, results of the KANWU study indicate that consumption of high levels (18 percent of energy) of saturated fats can significantly impair insulin sensitivity (Vessby et al., 2001).

Summary

Intakes above an identified UL indicate a potential risk of an adverse health effects. There is a positive linear trend between total saturated fatty acid intake and total and LDL cholesterol concentration and increased risk of CHD. A UL is not set for saturated fatty acids because any incremental increase in saturated fatty acid intake increases CHD risk. It is neither possible nor advisable to achieve 0 percent of energy from saturated fatty acids in typical whole-food diets. This is because all fat and oil sources are mixtures of fatty acids, and consuming 0 percent of energy would require extraordinary changes in patterns of dietary intake, such as the inclusion of fats and oils devoid of saturated fatty acids, which are presently unavailable. Such extraordinary adjustments may introduce undesirable effects (e.g., inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks. It is possible to consume a diet low in saturated fatty acids by following the dietary guidance provided in Chapter 11.

Cis-*Monounsaturated Fatty Acids*

Hazard Identification

Cardiovascular Disease. Within the range of usual intake, there are no clearly established adverse effects of *n*-9 monounsaturated fatty acids in humans. There is some preliminary evidence that a meal providing 50 g of fat from olive oil reduced brachial artery flow-mediated vasodilation by 31 percent in 10 healthy, normolipidemic individuals versus canola oil or salmon (Vogel et al., 2000). In addition, there is evidence from nonhuman primates that a diet rich in *n*-9 monounsaturated fatty acids promotes

atherosclerosis just as much as a diet containing isocaloric amounts of saturated or polyunsaturated fatty acids (Rudel et al., 1997). Dietary monounsaturated fatty acids induce atherogenesis due to greater hepatic lipid concentrations (i.e., triacylglycerol, free cholesterol, and cholesteryl ester), as well as the high degree of cholesteryl oleate enrichment in plasma cholesteryl esters. Overconsumption of energy related to a high *n*-9 monounsaturated fatty acid and high fat diet is another potential risk associated with excess consumption of monounsaturated fatty acids. *n*-9 Monounsaturated fatty acid intake may result in an increase in energy intake from saturated fatty acids due to the simultaneous occurrence of saturated and *n*-9 monounsaturated fatty acids in animal fats.

The *n*-7 monounsaturated fatty acid, palmitoleic acid, behaves like saturated fatty acids in raising LDL cholesterol concentration (Nestel et al., 1994). Watts and coworkers (1996) reported a positive correlation between palmitoleic acid and progression of CHD.

Cancer. While most epidemiological studies indicate that monounsaturated fatty acid intake is not associated with increased risk of most cancers (Holmes et al., 1999; Hursting et al., 1990; van Dam et al., 2000; van den Brandt et al., 1993), a few studies have observed a positive association. There is some epidemiological evidence for a positive association between oleic acid intake and breast cancer risk in women with no history of benign breast disease (Velie et al., 2000). In addition, one study reported that women with a family history of colorectal cancer who consumed a diet high in mono- and polyunsaturated fatty acids were at greater risk of colon cancer than women without a family history (Slattery et al., 1997). Giovannucci and coworkers (1993) reported a positive association between monounsaturated fatty acid intake and risk of advanced prostate cancer, while two studies observed increased risk of lung cancer (De Stefani et al., 1997; Veierød et al., 1997).

Summary

Based on the lack of adequate data on adverse effects of monounsaturated fatty acids, a UL is not set.

n-*6 Polyunsaturated Fatty Acids*

A UL is not set for *n*-6 polyunsaturated fatty acids because of the lack of a defined intake level at which an adverse effect can occur (see Chapter 11). An AMDR for *n*-6 polyunsaturated fatty acids, however, is estimated based on adverse effects from consuming a diet low or high in *n*-6 polyunsaturated fatty acids (Chapter 11).

n-*3 Polyunsaturated Fatty Acids*

Because the longer-chain *n*-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are biologically more potent than their precursor, α-linolenic acid, much of the work on the adverse effects of this group of fatty acids has been on DHA and EPA.

Hazard Identification

Immune Function. Numerous studies have shown suppression of various aspects of human immune function in vitro or ex vivo in peripheral blood mononuclear cells, or in isolated neutrophils or monocytes in individuals provided *n*-3 polyunsaturated fatty acids as a supplement or as an experimental diet compared with baseline values before the intervention (Table 8-8). The minimum dose observed for such an effect was 0.9 g/d of EPA and 0.6 g/d of DHA given as fish oil for 6 to 8 weeks to healthy adults (Cooper et al., 1993). The level of EPA that caused some type of immunosuppression ranged from 0.9 to 9.4 g/d when fed for 3 to 24 weeks. The level of DHA that caused immunosuppression ranged from 0.6 to 6.0 g/d (Table 8-8).

The data in single treatment studies comparing baseline versus post-supplementation immune function indicate that *n*-3 polyunsaturated fatty acids, especially EPA and DHA at levels 7 to 15 times greater than typical current U.S. intakes, diminish the potential of the immune system to attack pathogens (Kelley et al., 1998, 1999; Lee et al., 1985; Schmidt et al., 1989). This diminished ability, however, is also associated with suppression of inflammatory responses, suggesting benefits for individuals suffering from autoimmune diseases such as rheumatoid arthritis. It seems that the same doses of *n*-3 fatty acids that may be beneficial in chronic disease prevention are doses that are also immunosuppressive.

Several studies using a design of comparison across treatment groups (Blok et al., 1997; Kelley et al., 1998; Mølvig et al., 1991; Yaqoob et al., 2000), rather than comparison within individuals with a baseline, have shown a lack of several potential adverse effects of EPA and DHA supplementation on human immune cell functions. In one key study, 58 healthy men were given daily supplements of 0, 3, 6, or 9 g/d of a fish-oil supplement (EPA intake of 0, 0.81, 1.62, or 2.43 g/d and DHA intake of 0, 0.16, 0.33, or 0.49 g/d) for 1 year (Blok et al., 1997). Ex vivo endotoxin-stimulated production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, or IL-1Ra (IL-1 receptor antagonist) did not differ among treatments up to 6 months after the fish-oil supplementation was stopped. These data support a lack of long-term adverse effect of fish-oil supplementation on cytokine activity.

TABLE 8-8 Effects of *n*-3 Fatty Acid Intake on Immune Function

Reference	Study Design	*n*-3 Fatty Acid Dose (Daily)[a]
Lee et al., 1985	7 men 6 wk	MaxEPA (3.2 g EPA, 2.2 g DHA)
Endres et al., 1989	9 men 6 wk	MaxEPA (2.75 g EPA, 1.85 g DHA)
Schmidt et al., 1989	12 men 6 wk	Cod liver oil (2.5 g EPA)
Kelley et al., 1991	10 men 56-d crossover	Basal diet Flaxseed oil-supplemented diet (20 g 18:3*n*-3)
Meydani et al., 1991	6 young women, 6 older women 12 wk	ProMega (1.68 g EPA, 0.72 g DHA)
Mølvig et al., 1991	8 men 9 men 8 men 7 wk	Placebo oil Fish oil (1 g EPA, 0.5 g DHA) Fish oil (2 g EPA, 1 g DHA)
Thompson et al., 1991	6 men, 6 women 4-wk crossover	MaxEPA (2.16 g EPA) 12 g olive oil
Virella et al., 1991	4 men fed fish oil, 2 men fed olive oil 6 wk	Fish oil (2.4 g EPA)
Yamashita et al., 1991	3 adults 1 d	3 g EPA, infused
Cooper et al., 1993	8 men and women 6–8 wk	Fish oil (0.9 g EPA, 0.6 g DHA)
Endres et al., 1993	9 men 6 wk	MaxEPA (2.75 g EPA, 1.85 g DHA)
Meydani et al., 1993	7 women, 3 men 24 wk after 6 wk on typical U.S. diet (baseline)	Low fat, high fish diet (1.23 g EPA + DHA)
Sperling et al., 1993	5 women and 3 men with rheumatoid arthritis 10 wk	SuperEPA (9.4 g EPA, 5.0 g DHA)

Results[b]
Depressed neutrophil LTB_4, 6-*trans*-LTB_4, 5-HETE, and endothelial adherence, monocyte LTB_4 and 5-HETE, neutrophil chemotaxis
Depressed PBMC IL-1β, IL-1α, TNF, PGE_2, and neutrophil chemotaxis
Depressed neutrophil migration, monocyte cell density (marker of monocyte migration)
Depressed PBMC proliferation in response to T-cell mitogen but not to B-cell mitogen with flaxseed oil-supplemented diet
Depressed PBMC IL-1β and IL-6 (greater in older women), TNF and IL-2 (older women only)
Depressed PBMC proliferation, IL-1β in PBMCs and monocytes with *n*-3 fatty acids PBMC secretion of IL-1β, TNF-α, PGE_2 or LTB_4 not affected by *n*-3 fatty acids
Depressed neutrophil chemiluminescence (marker of neutrophil function) with MaxEPA diet
Depressed PBMC IL-2
Depressed NK cell activity of PBMCs
Typhoid vaccine injection site less inflamed, postvaccination tachycardia inhibited, depressed blood IL-1 and IL-6 concentrations
Depressed PBMC IL-2 and proliferation
Depressed PBMC IL-1β, TNF, IL-6, PGE_2, CD_{4+} lymphocytes, and lymphocyte proliferation, delayed-type hypersensitivity
Depressed neutrophil chemotaxis, inositol tris-phosphate formation, and LTB_4, monocyte LTB_4

continued

TABLE 8-8 Continued

Reference	Study Design	*n*-3 Fatty Acid Dose (Daily)[a]
Gallai et al., 1995	20 patients with relapsing/remitting multiple sclerosis and 15 controls 6 mo	Fish oil (3.06 g EPA, 1.86 g DHA)
Caughey et al., 1996	30 men 4-wk diet + 4-wk diet with fish oil	Flaxseed oil-enriched diet and fish oil (EPA 1.62 g, DHA 1.08 g) Sunflower oil diet and fish oil (EPA 1.62 g, DHA 1.08 g)
Hughes et al., 1996	3 men, 3 women 3 wk	EPA Forte (0.93 g EPA, 0.63 g DHA)
Blok et al., 1997	58 men 1 y	0, 3, 6, or 9 g fish oil (0, 0.81, 1.62, or 2.43 g EPA; 0, 0.16, 0.33, or 0.49 g DHA)
Kelley et al., 1998	4 men 7 men 120 d	Basal diet DHA-enriched oil (6 g DHA)
Kelley et al., 1999	4 men 7 men 120 d	Basal diet DHA-enriched oil (6 g DHA)
Yaqoob et al., 2000	5 men, 3 women 7 men, 1 woman 3 other groups of 8 fed other oils, but all comparable to placebo 12-wk parallel	Placebo oil (3:1 coconut and soybean oils) Fish oil (2.1 g EPA, 1.1 g DHA)

[a] EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.
[b] LTB_4 = leukotriene B_4, 5-HETE = 5-hydroxyeicosatetraenoic acid, PBMC = peripheral blood mononuclear cell, IL = interleukin, TNF = tumor necrosis factor, PGE_2 = prosta-

In studies using multitreatment parallel designs, potential adverse effects of *n*-3 fatty acids on immune function that were observed include decreased expression of monocyte major histocompatibility complex antigens and cell surface adhesion proteins (Hughes et al., 1996), decreased peripheral blood mononuclear cell (PBMC) proliferation and IL-1β in

Results[b]
Depressed PBMC IL-1β, TNF-α, IL-2 and IFN-γ, PGE_2, and LTB_4, serum-soluble IL-2 receptors
Depressed PBMC TNF-α, IL-1β, TxB_2, and PGE_2 with flaxseed oil-enriched diet Greater decreases in PBMC TNF-α, IL-1β, and TxB_2 in both groups after fish-oil supplementation
Depressed monocyte surface proteins: HLA-DR, HLA-DP, HLA-DQ, ICAM-1, LFA-1
No effect on whole blood IL-1β, TNF-α, or IL-1 receptor antagonist
Decreased white blood cells PBMC proliferation and delayed-type hypersensitivity not different between groups
Depressed PBMC IL-1β and TNF-α production, in vitro PBMC PGE_2 and LTB_4 secretion
No effect of fish oil on PBMC NK cell activity, proliferation, types of blood lymphocytes, IL-1α, IL-1β, TNF-α, IL-2, IL-10, and IFN-γ

glandin E_2, NK cell = natural killer cell, IFN-γ = interferon-γ, TxB_2 = thromboxane B_2, HLA = human leukocytes antigen, ICAM = intercellular adhesion molecule, LFA = leukocyte function-associated antigen.

PBMCs and monocytes (Mølvig et al., 1991), decreased PBMC IL-2 (Virella et al., 1991), decreased but still clinically normal neutrophils (Kelley et al., 1998), and decreased tachycardia and inflammation after typhoid vaccine (Cooper et al., 1993).

All of the single treatment studies comparing individuals fed *n*-3 polyunsaturated fatty acids before and after supplementation showed immunosuppressive effects. Differences in study design (single treatment versus multitreatment parallel designs) seem to be quite significant in determining whether *n*-3 fatty acid supplementation exerts immunosuppression or not. There is no clear basis to prefer one type of study design to the other. For example, the difference in results between Caughey and colleagues (1996) (a baseline comparison study) and Blok and colleagues (1997) (a group comparison study) is not accounted for by greater variability in measurements by the latter group. The standard deviation for whole blood TNF-α was no more than 5 percent of the mean in the study by Blok and coworkers (1997), and the standard deviation for mononuclear cell TNF-α was 25 to 45 percent of the mean in the study by Caughey and coworkers (1996). In another study using intertreatment comparisons of control versus men given fish oil for 7 weeks, secretions of IL-1β and TNF-α were not suppressed by fish-oil feeding, but lysates of peripheral blood mononuclear cells from people given fish oil contained less IL-1β and TNF-α than did cells from controls (Mølvig et al., 1991). Therefore, the study by Mølvig and colleagues (1991) showed some concurrence with that of Blok and colleagues (1997) and Caughey and colleagues (1996).

Another alternative is to extrapolate from animal studies using model species that are known to have similar immune system components and responsiveness compared to humans. Detailed characterization of appropriateness of animal models for extrapolation to humans with respect to immunosuppression has not been done. A few animal studies have shown the effects of dietary *n*-3 fatty acids on response to infection (Chang et al., 1992; Fritsche et al., 1997). At this time, there are not sufficient data to support establishing an UL for EPA and DHA based on infection responsiveness.

Bleeding and Increased Risk of Hemorrhagic Stroke. One of a number of factors that has been suggested to link *n*-3 polyunsaturated fatty acid intake with reduced risk of CHD is reduced platelet aggregation, and therefore prolonged bleeding time. The platelet count can decline by as much as 35 percent; however, the count does not usually fall below the lower limit of normal (Goodnight et al., 1981). Although prolonged bleeding times have been shown to be beneficial in preventing heart disease, bleeding times can become prolonged enough to result in excessive bleeding and bruising. Intervention studies that have examined the effects of *n*-3 fatty acids on bleeding time are mixed. A number of short-term studies (4 to 11 weeks) have shown significant increased bleeding time with taking EPA/DHA supplements ranging from 2 to 15 g/d (Cobiac et al., 1991; De

Caterina et al., 1990; Levinson et al., 1990; Lorenz et al., 1983; Mortensen et al., 1983; Sanders et al., 1981; Schmidt et al., 1990, 1992; Smith et al., 1989; Thorngren and Gustafson, 1981; Wojenski et al., 1991; Zucker et al., 1988), whereas other studies using similar intake levels resulted in no difference (Blonk et al., 1990; Freese and Mutanen, 1997; Rogers et al., 1987). Analysis of these studies collectively indicated no dose–response for EPA and DHA intake and the percent increase in bleeding time. Schmidt and coworkers (1992) reported increased bleeding times when 3.1 g/d of EPA and DHA were given for 6 weeks and 9 months. None of the above studies reported excessive bleeding times, bleeding episodes, or bruising.

Dietary feeding studies that provided approximately 2 percent of energy as EPA and DHA from salmon did not result in increased bleeding time compared to a stabilization diet that contained only 0.3 percent of energy as EPA and DHA (Nelson et al., 1991). Excessive cutaneous bleeding time and reduced in vitro platelet aggregability have been reported in Greenland Eskimos (Dyerberg and Bang, 1979; Dyerberg et al., 1978) who ingest on average 6.5 g/d (3.8 percent of energy) of EPA and DHA derived mainly from seal (Bang et al., 1980). A tendency to bleed from the nose and urinary tract was observed among the Greenland Eskimos (Bang and Dyerberg, 1980). One study comparing perirenal adipose tissue fatty acid profiles with incidence of hemorrhagic stroke in human autopsy cases from Greenland showed that the amounts of EPA and DHA in the adipose tissue of 4 hemorrhagic stroke victims was greater than in 26 control cases with no cerebral pathology (Pedersen et al., 1999). Furthermore, ecological studies have suggested an increased risk of hemorrhagic stroke among Greenland Eskimos (Kristensen, 1983; Kromann and Green, 1980). A recent prospective study in the United States showed no association between intake of *n*-3 fatty acids and risk of hemorrhagic stroke (Iso et al., 2001). The median intake levels for the quintiles of *n*-3 polyunsaturated fat intake, however, ranged from only 0.077 to 0.481 g/d, which reflects the relatively low intake level of *n*-3 fatty acids in the Unites States.

Oxidative Damage. Long-chain polyunsaturated fatty acids, particularly DHA and EPA, are vulnerable to lipid peroxidation, resulting in oxidative damage of various tissues. Numerous feeding studies using laboratory animals have demonstrated increased lipid peroxidation and oxidative damage of erythrocytes, liver, and kidney membranes and bone marrow DNA with consumption of DHA (Ando et al., 1998; Song and Miyazawa, 2001; Umegaki et al., 2001; Yasuda et al., 1999). The oxidative damage was shown to be reduced or prevented with the coconsumption of vitamin E (Ando et al., 1998; Leibovitz et al., 1990; Yasuda et al., 1999).

Summary

While there is evidence to suggest that high intakes of *n*-3 polyunsaturated fatty acids, particularly EPA and DHA, may impair immune response and result in excessively prolonged bleeding times, it is not possible to establish a UL. Studies on immune function were done in vitro and it is difficult, if not impossible, to know how well these artificial conditions simulate human immune cell response in vivo. Data on EPA and DHA intakes and bleeding times are mixed and a dose–response effect was not observed. Although excessively prolonged bleeding times and increased incidence of bleeding have been observed in Eskimos, whose diets are rich in EPA and DHA, information is lacking to conclude that EPA and DHA were the sole basis for these observations. At the 99th percentile of intake, the highest intakes of dietary EPA and DHA were 0.662 and 0.651 g/d, respectively, in men 71 years of age and older (Appendix Tables E-12 and E-14). This EPA + DHA intake (1.31 g/d) is much lower than that for Greenland Eskimos (6.5 g/d). EPA and DHA are available as dietary supplements, and until more information is available on the adverse effects of EPA and DHA, these supplements should be taken with caution.

Special Considerations

A few special populations have been reported to exhibit adverse effects from consuming *n*-3 polyunsaturated fatty acids. Despite the favorable effects of *n*-3 fatty acids on glucose homeostasis, caution has been suggested for the use of n-3 fatty acids in those individuals who already exhibit glucose intolerance or diabetic conditions (Glauber et al., 1988; Kasim et al., 1988) that require increased doses of hypoglycemic agents (Friday et al., 1989; Stacpoole et al., 1989; Zambon et al., 1992). Increased episodes of nose bleeds have been observed in individuals with familial hypercholesterolemia during fish-oil supplementation (Clarke et al., 1990). Anticoagulants, such as aspirin, warfarin, and coumadin, will prolong bleeding times and the simultaneous ingestion of n-3 fatty acids by individuals may excessively prolong bleeding times (Thorngren and Gustafson, 1981). Therefore, the subpopulations described above should take supplements containing EPA and DHA with caution.

Trans *Fatty Acids*

Hazard Identification

Total and LDL Cholesterol Concentrations. Prior to 1980 there was generally little concern about the trend toward increased consumption of

hydrogenated fat in the U.S. diet, especially when the hydrogenated fats displaced fats relatively high in saturated fatty acids (Denke, 1995). During the early 1980s studies showed a hypercholesterolemic effect of *trans* fatty acids in rabbits (Kritchevsky, 1982; Ruttenberg et al., 1983). Renewed interest in the topic of hydrogenated fat in human diets, or more precisely *trans* fatty acid intake, started in the early 1990s. The availability of a methodology to distinguish the responses of individual lipoprotein classes to dietary modification expanded the depth to which the topic could be readdressed.

A report from the Netherlands suggested that a diet enriched with elaidic acid (a subfraction of 18:1 *trans*) compared to one enriched with oleic acid (18:1 *cis*) increased total and LDL cholesterol concentrations and decreased HDL cholesterol concentrations, hence resulting in a less favorable total cholesterol:HDL cholesterol ratio (Mensink and Katan, 1990). Consumption of a diet enriched with saturated fatty acids resulted in LDL cholesterol concentrations similar to those observed after individuals consumed the diet high in elaidic acid, but HDL cholesterol concentrations were similar to those observed after individuals consumed the diet high in oleic acid. A number of similar studies have been published since then and have reported that hydrogenated fat/*trans* fatty acid consumption increases LDL cholesterol concentrations (Aro et al., 1997; Judd et al., 1994, 1998; Louheranta et al., 1999; Müller et al., 1998; Sundram et al., 1997) (Tables 8-9, 8-10, and 8-11). Recent data have demonstrated a dose-dependent relationship between *trans* fatty acid intake and the LDL:HDL ratio and when combining a number of studies, the magnitude of this effect is greater for *trans* fatty acids compared with saturated fatty acids (Figure 8-4) (Ascherio et al., 1999).

Similar to the metabolic clinical trial data, studies in free-living individuals asked to substitute hydrogenated fat for other fat in their habitual diet resulted in higher concentrations of total and LDL cholesterol (Table 8-11) (Nestel et al., 1992b; Noakes and Clifton, 1998; Seppänen-Laakso et al., 1993).

No studies have been conducted to evaluate the effect of *trans* fatty acids that are present in meats and dairy products on LDL concentrations. The relative effect of *trans* fatty acids in meat and dairy products on LDL cholesterol concentration would be small compared to hydrogenated oils because of the lower levels that are present, and because any rise in concentration would most likely be due to the abundance of saturated fatty acids.

HDL Cholesterol Concentrations. The data related to the impact of hydrogenated fat/*trans* fatty acids compared with unhydrogenated oil/*cis* fatty acids on HDL cholesterol concentrations are less consistent than for LDL cholesterol concentrations (Tables 8-9, 8-10, and 8-11). As reported

TABLE 8-9 Dietary *Trans* Fatty Acids (TFA) and Blood Lipid Concentration: Controlled Feeding Trials

Reference	Study Population	Diet[a]
Mensink and Katan, 1990; Mensink et al., 1992	79 men and women, avg 25–26 y	3-wk crossover, 40% fat 10% 18:1 10% SF 10% TFA
Zock and Katan, 1992	56 healthy men and women	3 wk crossover, 41% fat 18:2 18:0 TFA
Judd et al., 1994	58 men and women	6-wk crossover, 40% fat 18:1 SFA moderate TFA high TFA
Aro et al., 1997	80 healthy men and women, 20–52 y	5-wk intervention, 33% fat 18:0 TFA
Sundram et al., 1997	27 men and women, 19–39 y	4-wk crossover, 31% fat 18:1 16:0 12:0 + 14:0 TFA
Louheranta et al., 1999	14 healthy women, avg 23 y	4-wk crossover, 37% fat 18:1 TFA
Judd et al., 2002	50 men	5-wk crossover, 39% fat 18:1 18:0 TFA/18:0 TFA

[a] SF = saturated fat, SFA = saturated fatty acids.
[b] LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, Lp(a) = lipoprotein(a).

	Blood Lipid Concentrations[b]		
TFA (% of energy)	LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (mg/L)
0	2.67[c]	1.42[c]	32[c]
1.8	3.14[d]	1.42[c]	26[d]
10.9	3.04[e]	1.25[d]	45[e]
0.1	2.83[c]	1.47[c]	
0.3	3.00[d]	1.41[d]	
7.7	3.07[d]	1.37[d]	
0.7	3.34[c]	1.42[c]	
0.7	3.64[d]	1.40[c,d]	
3.8	3.54[e]	1.47[e]	
6.6	3.60[d,e]	1.38[d]	
0.4	2.89[c]	1.42[c]	270[c]
8.7	3.13[d]	1.22[d]	308[d]
0	3.17	1.25	128.3
0	3.15	1.26	122.0
0	3.57	1.18	134.3
6.9	3.81	1.05	153.3
0	2.53	1.37	225 (units/L)
5.1	2.64	1.31	220 (units/L)
0	2.95[c]		
0	3.10[d]		
4	3.32[e]		
8	3.36[e]		

[c,d,e] Within each study, LDL-C, HDL-C, or Lp(a) concentrations that are significantly different between treatment groups have a different superscript.

TABLE 8-10 Hydrogenated Fat Intake and Blood Lipid Concentrations: Controlled Feeding Trials

Reference	Study Population	Diet[a]
Lichtenstein et al., 1993	14 men and women, 44–78 y	32-d crossover, 30% fat Baseline Corn oil Corn oil margarine
Almendingen et al., 1995	31 men, 21–46 y	3-wk crossover, 33–36% fat Butter PHFO PHSO
Judd et al., 1998b	46 men and women, 28–65 y	5-wk crossover, 34% fat PUFA-M Butter TFA-M
Müller et al., 1998	16 healthy females, 19–30 y	14-d crossover, 31–32% fat Vegetable oil PHFO
Lichtenstein et al., 1999	36 men and women, > 50 y	35-d crossover, 30% fat Soybean oil Semiliquid margarine Butter Soft margarine Shortening Stick margarine

[a] PHFO = partially hydrogenated fish oil, PHSO = partially hydrogenated soybean oil, PUFA-M = margarine containing polyunsaturated fatty acids, TFA-M = margarine containing *trans* fatty acids.
[b] TFA = *trans* fatty acids.

for LDL cholesterol concentrations, the effect of hydrogenated fat/*trans* fatty acids on HDL cholesterol concentrations, if present, is likely to be dose-dependent (Judd et al., 1994). The preponderance of the data suggests that hydrogenated fat/*trans* fatty acids, relative to saturated fatty acids, result in lower HDL cholesterol concentrations (Ascherio et al., 1999; Zock and Mensink, 1996; Zock et al., 1995). Because of the potentially

	Blood Lipid Concentrations[c]		
TFA[b] (% of energy)	LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (mg/L)
0.77	3.96[d]	1.24[d]	140[d]
0.44	3.23[e]	1.14[e]	160[d]
4.16	3.49[e]	1.11[e]	130[d]
0.9	3.81[d]	1.05[d]	194[d]
8.0	3.94[d,f]	0.98[e]	234[e]
8.5	3.58[e]	1.05[d]	238[e]
2.4	3.21[d]	1.24[d]	197[d]
2.7	3.44[e]	1.27[d]	186[e]
3.9	3.27[f]	1.24[d]	202[d]
1.1	2.63[d]	1.32[d]	212[d]
1.7	2.87[e]	1.28[d]	225[d]
0.55	3.98[d]	1.11[d,e]	230
0.91	4.01[d,e]	1.11[d,e]	230
1.25	4.58[f]	1.16[e]	220
3.30	4.11[d,e]	1.11[d,e]	240
4.15	4.24[e]	1.11[d,e]	240
6.72	4.34[e]	1.01[d]	240

[c] LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, Lp(a) = lipoprotein(a).
[d,e,f] Within each study, LDL-C, HDL-C, or Lp(a) concentrations that are significantly different between treatment groups have a different superscript.

differential effects of hydrogenated fat/*trans* fatty acids on LDL and HDL cholesterol concentrations, concern has been raised regarding their effect on the total cholesterol or LDL cholesterol:HDL cholesterol ratio (Ascherio et al., 1999). However, with respect to dietary fat recommendations, the strategy to improve the total cholesterol or LDL cholesterol:HDL

TABLE 8-11 Dietary *Trans* Fatty Acids (TFA), Hydrogenated Fat, and Blood Lipid Concentrations: Free-Living Trials

Reference	Study Population	Diet[a]
Nestel et al., 1992a	26 mildly hypercholesterolemic men, 27–57 y	4-wk crossover, 42% fat Control 1 Control 2 Blend 1 Blend 2
Nestel et al., 1992b	27 mildly hypercholesterolemic men, 30–63 y	3-wk crossover, 36–37% fat Control 18:1 TFA 16:0
Seppänen-Laakso et al., 1993	57 men and women, middle-aged	12-wk crossover to 1 of 2 diets, 39–43% fat Margarine Rapeseed Olive oil
Wood et al., 1993a	38 healthy men, 30–60 y	6-wk crossover, 38% fat Butter Butter-sunflower Butter-olive Hard margarine Soft margarine
Wood et al., 1993b	29 healthy men, 30–60 y	6-wk crossover, 37% fat Butter Crude palm Margarine Refined palm Refined palm+sunflower Sunflower oil
Chisholm et al., 1996	49 hypercholesterolemic men and women, avg 47 y	6-wk crossover, 26–27% fat Butter Margarine

	Blood Lipid Concentrations[c]		
TFA (% of energy)	LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (units/L)
3.8	4.13[c]	1.11[c]	
3.7	4.03[c,d]	1.15[c]	
6.7	3.92[d,e]	1.10[c]	
6.6	3.83[e]	1.11[c]	
< 1	4.22[c]	0.98[c]	235[c]
1.4	3.90[d]	0.98[c]	236[c]
5.7	4.27[c]	0.98[c]	296[d]
< 1	4.16[c]	1.09[d]	249[e]
	Change from baseline	Change from baseline	
2.9	–0.20	+0.05	
0	–0.30	–0.01	
0	–0.32	0.00	
2.1	3.78[c]	1.22[c]	
1.0	3.49[d]	1.19[c]	
1.0	3.59[d]	1.22[c]	
11.1	3.47[d]	1.16[c]	
0	3.26[e]	1.16[c]	
0.2	3.52[c]	1.03[c]	
0	3.36[c]	1.03[c]	
3.0	3.36[c]	1.00[c]	
0	3.41[c]	1.06[d]	
0	3.41[c]	1.03[c]	
0	3.23[d]	1.00[c]	
1.4	4.21[c]	1.26[c]	223[c]
3.6	3.82[d]	1.24[c]	249[c]

continued

TABLE 8-11 Continued

Reference	Study Population	Diet[a]
Noakes and Clifton, 1998	38 mildly hyperlipidemic men and women	3-wk crossover, 2 groups, 31–35% fat Canola + TFA TFA-free canola Butter PUFA + TFA TFA-free PUFA Butter

[a] PUFA = polyunsaturated fatty acids.
[b] LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, Lp(a) = lipoprotein(a).

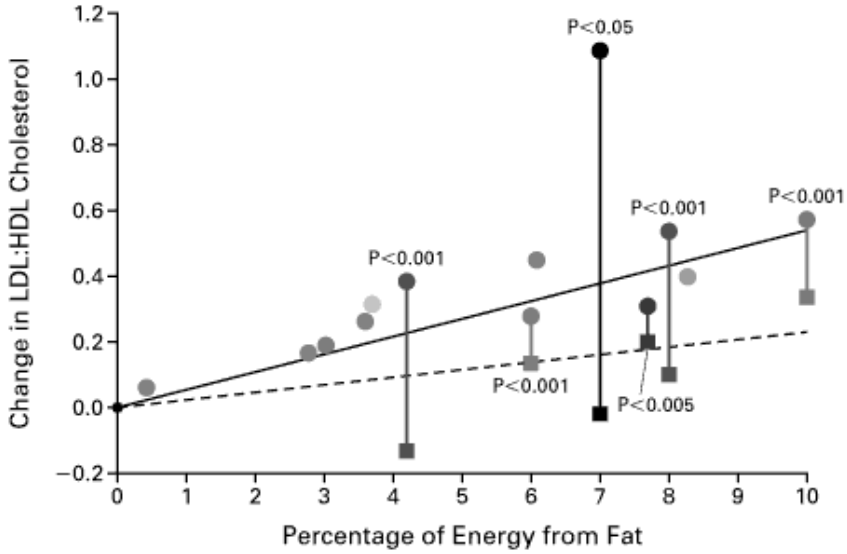

FIGURE 8-4 Change in the low density lipoprotein (LDL):high density lipoprotein (HDL) cholesterol concentration with increasing energy intake from saturated and *trans* fatty acids. Solid line represents the best-fit regression for *trans* fatty acids. Dotted line represents the best-fit regression for saturated fatty acids. Reprinted, with permission, from Ascherio et al. (1999). Copyright 1999 by the Massachusetts Medical Society.

	Blood Lipid Concentrations[c]		
TFA (% of energy)	LDL-C (mmol/L)	HDL-C (mmol/L)	Lp(a) (units/L)
3.3	3.64[c]	1.19[c]	
0	3.61[c]	1.28[c]	
1.1	4.14[d]	1.20[c]	
3.6	4.23[c]	1.17[c]	
0	3.98[d]	1.23[c]	
1.2	4.70[e]	1.27[c]	

[c,d,e] Within each study, LDL-C, HDL-C, or Lp(a) concentrations that are significantly different between treatment groups have a different superscript.

cholesterol ratio would not be different from that to decrease LDL cholesterol concentrations.

Lp(a) Concentrations. Lipoprotein(a) (Lp(a)) concentrations in plasma have been associated with increased risk for developing cardiovascular and cerebrovascular disease, possibly via inhibition of plasminogen activity (Lippi and Guidi, 1999; Nielsen, 1999; Wild et al., 1997). Lp(a) is a lipoprotein particle similar to LDL with respect to its cholesterol and apolipoprotein B100 content, but it also contains an additional apolipoprotein termed apo(a) (Lippi and Guidi, 1999; Nielsen, 1999). Lp(a) concentrations have been reported by some investigators to be increased after the consumption of diets enriched in hydrogenated fat/*trans* fatty acids (Tables 8-9, 8-10, and 8-11) (Almendingen et al., 1995; Aro et al., 1997; Lichtenstein et al., 1999; Mensink et al., 1992; Nestel et al., 1992b; Sundram et al., 1997), but not by all (Chisholm et al., 1996; Judd et al., 1998; Lichtenstein et al., 1993; Louheranta et al., 1999; Müller et al., 1998). The magnitude of the mean increases in Lp(a) concentrations reported to date that is associated with *trans* fatty acid intake for the most part would not be predicted to have a physiologically significant effect on cardiovascular disease risk. However, an unresolved issue at this time is the potential effect of relatively high levels of *trans* fatty acids in individuals with initially high concentrations of Lp(a).

Hemostatic Factors. The effect of *trans* fatty acids on hemostatic factors has been assessed by a number of investigators (Almendingen et al., 1996; Mutanen and Aro, 1997; Sanders et al., 2000; Turpeinen et al., 1998; Wood et al., 1993b) (Table 8-12). In general, these researchers have concluded that hydrogenated fat/*trans* fatty acids had little effect on a variety of hemostatic variables. Similarly, Müller and colleagues (1998) reported that hemostatic variables were unaffected by the substitution of a vegetable oil-based margarine relatively high in saturated fatty acids when compared with a hydrogenated fish oil-based margarine.

Susceptibility of LDL to Oxidation. Hydrogenated fat/*trans* fatty acids have consistently been reported to have little effect on the susceptibility of LDL to oxidation (Cuchel et al., 1996; Halvorsen et al., 1996; Nestel et al., 1992b; Sørensen et al., 1998) (Table 8-12).

Blood Pressure. A few reports addressed the issue of *trans* fatty acid intake and blood pressure (Mensink et al., 1991; Zock et al., 1993) (Table 8-12). The authors concluded that consumption of diets high in saturated, mono-unsaturated, or *trans* fatty acids resulted in similar diastolic and systolic blood pressures.

CHD. Similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentrations (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). Some evidence also suggests that *trans* fatty acids result in lower HDL cholesterol concentrations (Table 8-13). Hence, the net result is a higher total cholesterol or LDL cholesterol:HDL cholesterol ratio (Judd et al., 1994; Lichtenstein et al., 1999; Zock and Katan, 1992). This finding, combined with data from prospective cohort studies (Ascherio et al., 1996; Gillman et al., 1997; Hu et al., 1997; Pietinen et al., 1997; Willett et al., 1993) (Table 8-13), has lead to the concern that dietary *trans* fatty acids are more deleterious with respect to CHD than saturated fatty acids (Ascherio et al., 1999).

Summary

Similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentration, and therefore increased risk of CHD. A UL is not set for *trans* fatty acids because any incremental increase in *trans* fatty acid intake increases CHD risk. Because *trans* fatty acids are unavoidable in ordinary, nonvegan diets, consuming 0 percent of energy would require significant changes in patterns of dietary intake. Such adjustments may introduce undesirable effects (e.g., elimination of commercially prepared foods and dairy products and meats that

contain *trans* fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks. It is possible to consume a diet low in *trans* fatty acids by following the dietary guidance provided in Chapter 11.

RESEARCH RECOMMENDATIONS

Total Fat

- Studies are needed that examine the effects of alterations in the level of total fat in the context of a low saturated fatty acid diet on blood lipid concentrations and glucose-insulin homeostasis in individuals with defined metabolic syndromes, such as type 1 and type 2 diabetes.
- Randomized and blinded long-term (greater than 1 year) studies are needed on the effect of dietary fat versus carbohydrate on body fatness.

Saturated Fatty Acids

- Further examination of intakes at which significant risk of chronic diseases can occur is needed.
- Data that examine the indicators for and risk of chronic disease at low levels of saturated fatty acid intake are necessary.

Cis-*Monounsaturated Fatty Acids*

- Information is needed to assess energy balance in free-living individuals who have implemented a diet high in monounsaturated fatty acids versus a diet lower in monounsaturated fatty acids (and higher in carbohydrate).
- Additional information is needed on the effects of alterations in the level of monounsaturated fatty acid in the context of a low saturated fatty acid diet on blood lipid concentrations and glucose–insulin homeostasis in individuals with defined metabolic syndromes, such as type 1 and type 2 diabetes.
- Studies are needed to evaluate cardiovascular disease risk status and risk of other chronic diseases in individuals consuming a high monounsaturated fatty acid diet versus a diet lower in monounsaturated fatty acids (and higher in carbohydrate).
- An evaluation of the nutritional adequacy and nutrient profile of free-living individuals following a self-selected high monounsaturated fatty acid diet is necessary.
- Studies that assess the effects of a high monounsaturated fatty acid diet on endothelial function and atherogenesis are needed.

TABLE 8-12 *Trans* Fatty Acid (TFA) Intake and Blood Clotting, Low Density Lipoprotein (LDL) Oxidation, and Blood Pressure

Reference	Study Population	Diet[a]	TFA (% of energy)
Clotting			
Wood et al., 1993b	29 men, 30–60 y	6-wk crossover, 37% fat	
		Butter	0.2
		Crude palm oil	0
		Margarine	3.0
		Refined palm oil	0
		Refined palm+sunflower	0
		Sunflower oil	0
Almendingen et al., 1996	31 men, avg 27 y	3-wk crossover, 33–36% fat	
		PHSO	8.5
		PHFO	8.0
		Butter	0.9
Mutanen and Aro, 1997	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 33–34% fat	
		High 18:0	0.4
		High TFA	8.7
Turpeinen et al., 1998	80 men and women, 20–52 y	5-wk crossover to 1 of 2 diets, 32–34% fat	
		18:0	0.4
		TFA	8.7
Sanders et al., 2000	16 men and women, 18–32 y	1 test-meal crossover, 7% or 65% fat	
		18:1	0.1
		18:1 trans	24.7
		18:0	0
		16:0	0.2
		MCT	0
		Low fat	0
Oxidation			
Cuchel et al., 1996	14 men and women, 44–78 y	32-d crossover, 30% fat	
		Corn oil	0.44
		Corn oil+margarine	4.16

Results[b]		Comments
TxB_2 (pg/mL)	6-keto-$PGF_{1\alpha}$ (pg/mL)	
35	89	
41	94	
40	86	
40	87	
36	100	
62	95	
Fibrinogen (g/L)	PAI-1 activity (units/mL)	
3.0	13.5	For PHSO, greater PAI-1 activity than PHFO or butter
2.9	10.7	Increased fibrinogen with butter diet
3.1	8.8	No significant difference in factor VII, fibrinogen peptide A, β-thromboglobulin, or tissue plasminogen activator
Fibrinogen (g/L)		No marked difference in factor VII coagulation activity, tissue type plasminogen activity, or PAI-1 activity
3.62		
3.61		
		No difference in TxB_2 production or ADP-induced platelet aggregation in vitro
		Significant increase in collagen-induced aggregation with 18:0 diet
$FVII_c$ (% standard)	$FVII_a$ (ng/mL)	No significant differences in factor VII coagulation activity; factor VII-activated concentrations were significantly higher with 18:1, 18:1 trans, 18:0, and 16:0 diets
124	2.7	
122	1.9	
114	1.9	
112	2.1	
112	1.5	
99	1.4	
		No difference in susceptibility to LDL oxidation

continued

TABLE 8-12 Continued

Reference	Study Population	Diet[a]	TFA (% of energy)
Halvorsen et al., 1996	29 men, 21–46 y	19-d crossover, 33–36% fat	
		Butter	0.9
		PHSO	8.5
		PHFO	8.0
Sørensen et al., 1998	47 men, 29–60 y	4 wk, consumed 30 g/d of 1 of 2 margarines	mol % of fat
		Sunflower oil	0.79
		Fish oil, enriched	0.98
Blood pressure			
Mensink et al., 1991	59 men and women, 19–57 y, normo-tensive	3-wk crossover, 39–40% fat	
		18:1	0
		TFA	10.9
		SFA	1.8
Zock et al., 1993	55 men and women, 19–49 y	3-wk crossover, 40–43% fat	
		18:2	0.1
		18:0	0.3
		TFA	7.7

[a] PHSO = partially hydrogenated soybean oil, PHFO = partially hydrogenated fish oil, MCT = medium-chain triacylglycerol, SFA = saturated fatty acid.

n-*6 Polyunsaturated Fatty Acids*

- In metabolic and large observational studies, comparison should be made of the benefits of α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) across a range of *n*-6 polyunsaturated fatty acid intakes.
- Using good biomarkers for low density lipoprotein oxidation and cancer susceptibility, assessments are needed of the potential adverse effects of diets at levels of *n*-6 polyunsaturated fatty acids greater than 10 percent of energy.
- Studies that assess the effects of a high *n*-6 polyunsaturated fatty acid diet on markers of endothelial function and inflammation are needed.

Results[b]		Comments
Dienes (nmol/mg LDL)	Formation rate (nmol/mg LDL × min)	No significant differences in conjugated dienes, lipid peroxides, uptake by macrophages, or electrophoretic mobility of LDL
1,020	10	
1,034	10	TFA does not alter susceptibility to LDL oxidation
1,107	10	
Dienes (nmol/g)	Oxidation rate (nmol/mg × min)	Fish oil consumption compared with sunflower oil margarine had no effect on LDL size and led to minor changes in LDL oxidation resistance
445	10.4	
468	10.2	
SBP (mmHg)	DBP (mmHg)	No effect of TFA intake on blood pressure
113	66	
112	67	
112	67	
SBP (mmHg)	SBP (mmHg)	No effect of TFA intake on blood pressure
114	68	
113	70	
113	69	

[b] TxB_2 = thromboxane B_2, 6-keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$, PAI-1 = plasminogen activator inhibitor type 1, $FVII_c$ = factor VII coagulant activity, $FVII_a$ = factor VII activated, SBP = systolic blood pressure, DBP = diastolic blood pressure.

- Further research is needed to address the potentially important relationships between the amount of *n*-3 and *n*-6 fatty acids and glucose tolerance suggested by studies of fatty acid composition in affected individuals.

n-*3 Polyunsaturated Fatty Acids*

- Randomized clinical trials are needed of EPA+DHA, EPA, and DHA to evaluate their impact on cancer (i.e., colon, breast, prostate). The use of biomarkers for cancer susceptibility may expedite such studies.

TABLE 8-13 Dietary *Trans* Fatty Acids (TFA): Epidemiological Studies

Reference	Study Design[a]	Dietary and Other Information
Lipoprotein concentration		
Siguel and Lerman, 1993	47 CAD cases 56 controls Case-control	No dietary intake information
Coronary heart disease (CHD)		
Hudgins et al., 1991	76 men, 23–78 y Cross-sectional	No dietary intake information
Troisi et al., 1992	748 men, 43–85 y Cross-sectional	Food frequency questionnaire, multivariate analysis
Willett et al., 1993	Women, 431 CHD cases Cohort, 8-y follow-up	Food frequency questionnaire, multivariate analysis
Ascherio et al., 1994	239 MI cases 282 controls Case-control	Food frequency questionnaire, multivariate analysis
Kromhout et al., 1995	12,763 men, 40–59 y Cohort, 25-y follow-up	Weighed food record
Ascherio et al., 1996	43,757 men, 40–75 y Cohort, 6-y follow-up	Food frequency questionnaire, multivariate analysis

Results[b]			Comments[c]
Plasma	Case	Control	TFA negatively associated with HDL
TFA (%)	1.38	1.11	TFA positively associated with LDL and TAG
HDL (mmol/L)	0.88	1.34	
LDL (mmol/L)	3.78	2.97	
TAG (mmol/L)	1.78	0.97	
Total TFA in adipose tissue was 4.4% of total fatty acids			Total TFA content in adipose tissue was not significantly related to risk factors of CHD (e.g., age, BMI, LDL, cholesterol, blood pressure)
TFA intake was directly related to total ($r = 0.07$, $P = 0.04$) and LDL ($r = 0.09$, $P = 0.01$) cholesterol			An increased TFA intake from 2.1 to 4.9 g/d increased the risk of MI by 27%
TFA intake (% energy)	RR of CHD		Positive association with TFA intake and risk of CHD
1.3	1.0		
1.8	1.4		
2.2	1.25		
2.6	1.55		
3.2	1.8		
TFA intake (g/d)	RR of MI		Positive association of TFA intake and risk of myocardial infarction
1.69	1.0		
2.48	0.73		
3.35	1.24		
4.52	1.63		
6.51	2.28		
Correlation between 18:1*trans* intake and CHD mortality is 0.78 ($p < 0.001$)			
TFA intake (g/d)	RR of MI		TFA intake directly associated with risk of MI
1.5	1.0		
2.2	1.20		
2.7	1.24		
3.3	1.27		
4.3	1.40		

continued

TABLE 8-13 Continued

Reference	Study Design[a]	Dietary and Other Information
Gillman et al., 1997	Men, 45–64 y 267 CHD cases Cohort, 21-y follow-up	24-h recall, multivariate analysis
Hu et al., 1997	Women, 34–59 y 939 MI cases Cohort, 14-y follow-up	Food frequency questionnaire, multivariate analysis
Pietinen et al., 1997	Smoking men, 50–69 y 1,399 coronary events 635 coronary deaths Cohort, 6.1-y follow-up	Food frequency questionnaire, multivariate analysis
Tavani et al., 1997	Women, 18–74 y 429 MI cases 866 controls Case-control	Questionnaire on selected indicator foods, multivariate analysis
Cancer		
Kohlmeier et al., 1997	Women, 50–74 y 291 breast cancer cases 407 controls Case-control	No diet information

Results[b]			Comments[c]
Margarine intake (tsp/d)	No. of events (/1,000) Period 1	Period 2	RR for CHD for each increment of 1 tsp/d was 0.99 for follow-up period 1 and 1.12 for period 2
0	77	65	Modest risk of CHD with increasing margarine intake
1–4	42	35	
≥ 5	18	30	
TFA intake (% energy)	RR of MI		RR for 2% increment in energy from TFA intake was 1.93
1.3	1.0		
1.7	1.07		
2.0	1.10		
2.4	1.13		
2.9	1.27		
TFA intake (g)	RR of major coronary event		Positive association between TFA intake and risk of coronary death
1.0	1.00		
1.7	1.10		
2.0	0.97		
2.7	1.07		
6.2	1.14		
TFA intake (g)	RR of coronary death		
1.0	1.00		
1.7	1.05		
2.0	1.12		
2.7	0.90		
6.2	1.39		
Margarine intakes	RR of MI		The association with margarine could explain about 6% of MI in this population
No or low	1.0		
Medium or high	1.5		
Adipose TFA concentration	OR of breast cancer		Risk for breast cancer is based on the relative concentration of TFA and PUFA
TFA	1.46		
TFA within lowest PUFA tertile	3.65		
TFA within highest PUFA tertile	0.97		

continued

TABLE 8-13 Continued

Reference	Study Design[a]	Dietary and Other Information
Tuyns et al., 1988	35–75 y 453 colon cancer cases 365 rectal cancer cases 2,851 controls Case-control	Dietary history

[a] CAD = coronary artery disease, CHD = coronary heart disease, MI = myocardial infarction.
[b] HDL = high density lipoprotein cholesterol, LDL = low density lipoprotein cholesterol, TAG = triacylglycerol, RR = relative risk, OR = odds ratio, PUFA = polyunsaturated fatty acid.

- Randomized clinical trials on the use of EPA+DHA, EPA, and DHA in treatment of inflammatory disorders (e.g., Crohn's disease, arthritis, psoriasis, asthma) and infections are needed.
- Studies of EPA+DHA, EPA, and DHA supplementation in the elderly to prevent degenerative diseases of the central nervous system and retina, such as dementia, age-related macular degeneration, and night blindness are needed.

Trans *Fatty Acids*

- A comprehensive database needs to be developed for the *trans* fatty acid content of the United States food supply; this database could then be used to determine the *trans* fatty acid intakes in different age and socioeconomic groups.
- An assessment of major sources of *trans* fatty acids currently in the marketplace is needed, along with development of alternatives similar to that done for foods high in saturated fatty acids.
- Studies that distinguish *trans* fatty acid isomers from plants and animals with respect to the relative impact on blood lipid and lipoprotein concentrations are needed.
- In light of the wide variability of *trans* fatty acid intakes within food categories, the development of a biochemical marker for *trans* fatty acid intake, independent of self-reported intake data, is needed.

Results[b]	Comments[c]
	There was no increased risk of either cancers with increased consumption of margarine

[c] BMI = body mass index.

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