Cystic Fibrosis and Nutrition: Linking Phospholipids and Essential Fatty Acids with Thiol Metabolism

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Annu. Rev. Nutr. 2008. 28:55–72

First published online as a Review in Advance on May 22, 2008

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

This article's doi: 10.1146/annurev.nutr.27.061406.093625

0199-9885/08/0821-0055$20.00

Key Words

choline, glutathione, redox balance, oxidative stress, docosahexaenoic acid, arachidonic acid, linoleic acid

Abstract

Cystic fibrosis (CF) is the most common lethal inherited disorder among Caucasians and results from mutation in the gene encoding the CF transmembrane conductance regulator. In addition to its multisystem clinical effects, the disease is characterized by increased proinflammatory mediators and oxidant stress, and systemic redox imbalance with reduced glutathione (GSH), together with alterations in circulating and tissue (n-6) and (n-3) fatty acids, particularly a decrease in docosahexaenoic acid. The metabolism of phospholipids and fatty acids is closely related to GSH through the methionine-homocysteine cycle, in which choline via betaine provides methyl groups to regenerate *S*-adenosylmethionine, important in generating phosphatidylcholine and amino acid precursors for GSH. Current research focuses both on fatty acid supplementations to normalize altered (n-6) to (n-3) fatty acid balance and decrease generation of (n-6) fatty acid-derived inflammatory mediators, and strategies to improve oxidant defenses and redox balance. However, further research is needed before such strategies can be included in clinical care of individuals with CF.

Contents

INTRODUCTION

Cystic fibrosis (CF) is the most common life-limiting inherited disorder among Caucasians, affecting about 1 in 3500 newborns, with an estimated 30,000 individuals affected in North America and 200,000 worldwide suffering from the disease (26). CF is caused by a mutation in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), a protein that functions primarily as a cyclic AMP-regulated anion channel on epithelial membranes with greatest selectivity for chloride (1, 2, 12, 88, 98, 100, 103). The major clinical features of CF include chronic progressive pulmonary disease, exocrine pancreatic insufficiency, chronic sinusitis, hepatobiliary disorders, and male infertility. Continual improvements in pancreatic enzyme formulations, nutrition, antibiotic therapies, and airway management over the past half century have led to marked improvements in life quality and life expectancy, to a median lifespan of 37 years in the United States (26). However, although pulmonary failure remains the leading cause of morbidity and mortality, CF is a complex disease that affects multiple organ systems, with a wide variability in severity and complications that are not readily explained by CFTR genotype or defective chloride transport (88, 137). Evidence is now accumulating to suggest that oxidative stress and altered redox balance, together with abnormalities in phospholipid and (n-6) and (n-3) fatty acid metabolism, play important roles in contributing to disease manifestations in individuals with CF. This review includes a brief overview of lipid and fatty acid digestion, absorption, and metabolism; addresses the link between phospholipid, choline, (n-6) and (n-3) fatty acids, redox balance, and thiol metabolism and their relevance to CF; and concludes with studies on the effects of (n-3) fatty acid supplementation in individuals with CF.

CF: cystic fibrosis

CFTR: cystic fibrosis transmembrane conductance regulator

CYSTIC FIBROSIS AND THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

CFTR is encoded by a 230 kb gene located at chromosome 7q31.3, spanning 250 kb with 27 exons, that is transcribed into a 6.5 kb mRNA that encodes the 1480 amino acid CFTR protein (100, 103). Over 1500 mutations in CFTR have been listed in the Cystic Fibrosis Mutation Database (**http://www.genet.sickkids.on.ca/cftr/**), and these are grouped into five classes based on the nature of the defect in CFTR. The most common mutation is a class II mutation (defective protein processing), in which a 3-base-pair deletion leads to deletion of phenylalanine from position 508 (ΔF508) (99). The absence of this single phenylalanine is believed to result in protein misfolding that leads to retention of CFTR in the endoplasmic reticulum and increased degradation rather than translocation to the membranes where CFTR is usually functional. The ΔF508 mutation is present in about 70% of patients

with CF, and over 50% of all individuals with CF are homozygous for ΔF508.

CFTR has a molecular weight between 140 and 170 kDA, depending upon the degree of posttranslational glycosylation, and functions as an integral membrane protein belonging to the ATP-binding cassette superfamily (1, 3, 6, 12, 98, 100, 103). When activated by cAMP/protein kinase, CFTR opens to form an ATP-gated channel to allow transport of chloride ions. Because the movement of water is linked osmotically to ion transport, defective epithelial CFTR functioning leads to increased sweat chloride content and to relatively thickened secretions that obstruct the airways and exocrine ducts, with effects most evident in the respiratory tract, exocrine pancreas, large intestine, and vas deferens. However, CFTR is also present in other organs and intracellular membranes and functions as a transporter involved in the regulation of other ion channels, including the epithelial Na^+ channel and voltage-gated K^+ channel, and other anions, notably bicarbonate, glutathione (GSH), and cytochrome P450 metabolites conjugated to GSH (3, 12, 36, 43, 50, 53, 79, 84, 85, 110).

Defective CFTR functioning is associated with multiple disturbances, often described as constitutive or dysfunctional, which affect many organs and cells. These disturbances include increased proinflammatory and decreased anti-inflammatory cytokines, elevated markers of oxidative stress, decreased levels of GSH, dysregulation of pH, altered protein glycosylation and sialylation, high levels of disulfide-linked peptides, and abnormal apoptosis in epithelial cells and leukocytes (2, 11, 13, 30, 32, 41, 44, 46, 48, 68, 69, 71–74, 76, 88, 90, 97, 104, 106, 107, 112, 129, 137). The airway disease in CF is characterized by a persistent and excessive neutrophil-dominated inflammatory response, with elevated release of proinflammatory mediators, including leukotriene (LT) B4, the 5-lipoxygenase (LOX) product of arachidonic acid [ARA, 20:4(n-6)], and proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-8, and decreased levels of the anti-inflammatory IL-10 (2, 11, 30, 32, 48, 68, 69, 71–74, 90, 97, 107, 124) and ARA-derived lipoxins (65, 66). Notably, airway inflammation begins at an early age, also occurring in infants, with increased levels of IL-8 (a proinflammatory neutrophil chemoattractant) and activated NF-κβ, even without apparent infection (2, 32, 69, 71, 104, 107, 124). After pulmonary disease, liver disease including cholestasis, fatty infiltration of hepatocytes (steatosis), focal biliary fibrosis, and sometimes cirrhosis is the leading cause of morbidity and mortality among individuals with CF (24, 29, 75, 121). However, while impaired biliary epithelial secretory function due to defective CFTR activity is likely to contribute to reduced bile fluidity, decreased alkalinity, abnormal bile composition, and cholestasis, attempts to correlate CF liver disease to CF genotype have been unsuccessful, and many of the liver problems, including steatosis, may be the result of metabolic changes rather than simply bile duct obstruction (35, 78).

GSH (L-γ-glutamyl-L-cysteinyl-glycine) is a ubiquitous thiol that plays a central role in many processes sensitive to the redox state, including protection against oxidative stress, eicosanoid synthesis, generation of nitric oxide (NO), apoptosis, inflammatory responses and cytokine production, regulation of protein and DNA synthesis, gene expression, remodeling of extracellular matrix, surfactant phosphatidylcholine and mucolysis, and protein glutathionylation (132). GSH scavenges free radicals with oxidation to glutathione disulfide (GSSG), which is regenerated by the NADPH-dependent glutathione reductase. In individuals with CF, the normally high levels of GSH in lung epithelial lining fluid are reduced to about 5%–10% of normal, and GSH and the GSH/GSSG ratio is reduced in plasma, cells, and intracellular compartments (13, 44, 60, 76, 106, 122). The low systemic GSH together with the permeability of the CFTR anion channels to GSH has led to questions of whether defective CFTR functioning could contribute to impaired oxidant defense systems in CF (53, 70, 79). Decreased circulating and tissue (n-6) and (n-3)

GSH: glutathione

LT: leukotriene

LOX: lipoxygenase

ARA: arachidonic acid [20:4(n-6)]

IL: interleukin

NO: nitric oxide

GSSG: glutathione disulfide

DHA: docosahexaenoic acid [22:6(n-3)]

SAM: *S*-adenosylmethionine

PC: phosphatidylcholine

fatty acids, particularly the (n-3) docosahexaenoic acid (DHA), as well as decreased choline also occurs in individuals with CF (9, 22, 23, 25, 37, 45, 52, 59, 61, 77, 81, 82, 102, 105, 115, 116, 125). The (n-6) and (n-3) fatty acids are acyl moieties of membrane phospholipids that are released in response to agonist-stimulated membrane turnover, providing substrates for further metabolism to eicosanoids (8, 17). However, phospholipid metabolism is interrelated with thiol metabolism through the methionine-homocysteine cycle, in which choline serves to provide methyl groups for regeneration of methionine and *S*-adenosylmethionine (SAM), and amino acid precursors for the synthesis of GSH, and SAM provides methyl groups for the synthesis of phosphatidylcholine (PC) (4, 31, 42, 59, 60, 114, 134, 135). Important questions are whether alterations of lipid and thiol metabolism are functionally linked in individuals with defective CFTR function, and thus whether dietary interventions aimed at restoring normal lipid metabolism are likely to offer useful therapies to reduce the severity of disease in individuals with CF.

LIPIDS, ESSENTIAL FATTY ACIDS, AND THIOLS IN CYSTIC FIBROSIS

Overview of Lipid Digestion and Absorption

Typically, 90% of dietary fat is in the form of triacylglycerols, with smaller amounts of phospholipids, sphingolipids, partial glycerides, sterols, and fat-soluble vitamins. In addition to providing metabolic and storage energy, dietary fat provides the (n-6) and (n-3) polyunsaturated fatty acids, which owing to the absence of Δ-12 and Δ-15 desaturase enzymes are essential dietary nutrients for humans (55, 56). Choline is essential through its role in PC, sphingomyelin, and their metabolites, in acetylcholine, as a source of labile methyl groups, and as the precursor of betaine and phosphocholine, which also function as important osmolytes (114, 135).

Digestion and absorption is a multistep, sequential process in which dietary triacylglycerols, phospholipids, and sterol esters are solubilized and hydrolyzed for absorption (14, 96). The major steps involve triacylglycerol hydrolysis in the stomach by gastric lipase followed by continued hydrolysis by colipase-dependent pancreatic lipase in the small intestine, which releases unesterified fatty acids and *sn*-2 monoacylglycerols. These products of triacylglycerol hydrolysis are absorbed, reesterified into triacylglycerols, and secreted in chylomicrons (14, 96). In addition to the dietary intake, large amounts of PC are secreted into the intestine in bile, and this also requires digestion prior to absorption. The enterohepatic pool of PC is about 1 gm, with the bile pool circulating 5–10 times/d in healthy individuals (123). This results in a flux of endogenous PC into the intestine that can exceed the usual dietary intake of choline, which from all sources is about 0.5–1.0 g/d (61, 115). Digestion of dietary and biliary PC is considered to be accomplished largely by pancreatic phospholipase A_2, which hydrolyzes fatty acids from the *sn*-2 position of PC to release lysoPC and an unesterified fatty acid. LysoPC is absorbed and reesterified, contributing to intestinal lipoprotein synthesis, and is also transported to the liver bound to albumin (14, 96). The normal pancreas secretes several other lipid-hydrolyzing enzymes, including cholesterol ester hydrolase, phospholipase A_1, and pancreatic lipase-related proteins, although much less is known about the activity of these enzymes.

Defective CFTR function in the exocrine pancreas leads to decreased pancreatic fluid and bicarbonate secretion (131), which in about 85% of patients with CF results in pancreatic insuffiency with obstruction of pancreatic ducts by thick, sticky secretions (34). Replacement therapy with microencapsulated, enteric-coated pancreatic enzymes increases fat absorption from about 50%–60% in individuals with CF with untreated pancreatic insufficiency to about 85%, but residual fat malabsorption remains (16, 18, 19, 29, 34, 63). Decreased intestinal fluid and electrolyte secretions, which

result in decreased duodenual pH, thick viscoid secretions covering the brush border and microvilli, as well as abnormalities in intestinal permeability, gastrointestinal motility, and bile secretion and composition, however, are all problematic and likely to contribute to the inability of individuals with CF to achieve normal fat digestion despite pancreatic enzyme replacement (5, 24, 28, 29, 41, 51, 91, 101, 112, 129). Circulating levels of the (n-6) fatty acid linoleic acid [LA, 18:2(n-6)] and DHA are decreased, while ARA is either normal or decreased in individuals with CF (9, 22, 23, 25, 33, 37, 45, 49, 52, 77, 81–83, 94, 102, 105, 115, 116). Some evidence of impaired mucosal uptake of (n-6) fatty acids has been reported (63), although others have reported that (n-6) and (n-3) fatty acid absorption is not always diminished, with normal rates of LA absorption occurring when pancreatic enzyme supplements are provided and even when steatorrhea is present (87, 119). Moreover, studies to show that γ-linolenic acid [18:3(n-6)] and the (n-3) fatty acids eicosapentaenoic acid [EPA, 20:5(n-3)] and DHA given in triacylglycerol supplements are absorbed and incorporated into plasma, blood cell, and tissue phospholipids (21, 22, 33, 49, 52, 62, 68, 83, 94), which suggests that gastrointestinal pathways of triacylglycerol hydrolysis and (n-6) and (n-3) fatty acid uptake are functional in individuals with CF. Other recent studies have focused attention on phospholipid malabsorption in CF, possibly explained by impaired pancreatic HCO_3^- secretion, which leads to a low pH in the duodenum and jejunum (5, 101), inhibition of pancreatic phospholipase A_2 activity, and thus chronic malabsorption of choline-containing phosphoglycerides, with decreased plasma levels of choline and its metabolites betaine and dimethylglycine in individual with CF even when pancreatic enzyme supplements are provided (18, 19, 61). Together with evidence of increased choline turnover, increased ARA, release and increased generation of ARA-derived eicosanoids (10, 15, 48, 64, 68, 72, 89, 97, 117, 120, 133), the pattern of decreased (n-6) and (n-3) fatty acids and choline-containing lipids and metabolites (18, 19, 60, 61) suggests a vicious cycle whereby the turnover of membrane lipids is increased while the ability to regenerate normal membrane lipid compositions may be compromised by problems of altered choline and methyl metabolism and the characteristic low rates of DHA synthesis in humans (47, 57, 58, 95, 130).

Essential Fatty Acids and Their Metabolism

Although long known to be important sources of metabolic and storage energy, the (n-6) and (n-3) fatty acids are now appreciated for their roles as key metabolic regulators that enable tissues and cells to respond to and integrate physiological signals on many levels, including energy substrates, immunological, inflammatory and oxidative stressors, and neural activity. Following release from membrane phospholipids, (n-6) and (n-3) fatty acids serve as substrates for further metabolism to metabolites collectively termed eicosanoids, as ligands for membrane receptors and transcription factors that regulate gene expression; within membrane bilayers they regulate membrane properties, including the formation of lipid rafts, SNARE (soluble *N*-ethyl maleimide-sensitive factor attachment protein receptor) machinery for exocytosis and membrane trafficking, and membrane protein activities (8, 17, 57, 58).

Humans can synthesize saturated and monounsaturated fatty acids using carbons derived from degradation of carbohydrates, proteins or fats, with the major products being the saturated fatty acids palmitic acid (16:0), stearic acid (18:0) and their Δ-9 desaturase products palmitoleic acid (16:1n-7) and oleic acid (18:1n-9), **Figure 1**. However, due to the absence of Δ-12 and Δ-15 desaturase enzymes, mammals require a dietary source of (n-6) and (n-3) polyunsaturated fatty acids (55, 56). Once obtained from the diet, LA can be further metabolized by Δ-6 desaturation, elongation, and Δ-5 desaturation to ARA via dihomo-gamma-linolenic acid [DGLA, 20:3(n-6)], while α-linolenic acid [ALA, 18:3(n-3)] is metabolized to EPA [20:5(n-3)]. The pathway generally

LA: linoleic acid [18:2(n-6)]

EPA: eicosapentaenoic acid [20:5(n-3)]

DGLA: dihomo-γ-linolenic acid [20:3(n-6)]

ALA: alpha linolenic acid [18:3(n-3)]

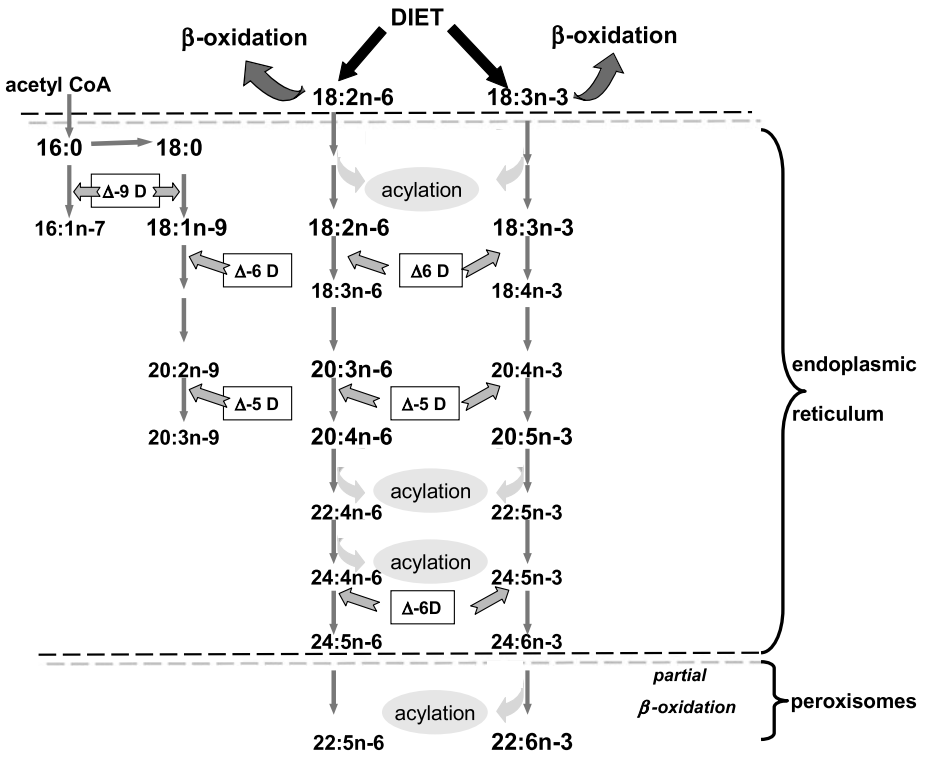

Figure 1

Schematic of major steps of fatty acid desaturation and elongation. Fatty acids are indicated using abbreviated nomenclature, which for major fatty acids discussed in the text are palmitic acid, 16:0, palmitoleic acid, 16:1(n-7); stearic acid, 18:0; oleic acid, 18:1(n-7); linoleic acid, 18:2(n-6); arachidonic acid, 20:4(n-6); linolenic acid, 18:3(n-3); eicosapentaenoic acid, 20:5(n-3); and docosahexaenoic acid, 22:6(n-3); Δ-9D, Δ-6D, Δ-5D, delta 9, 6 and 5 desaturases, respectively.

accepted for metabolism of EPA to DHA is that proposed by Sprecher and colleagues (113) and involves two sequential elongations of EPA to 24:6(n-3), followed by transport to the peroxisomes, then β-oxidation to yield DHA. Intermediate steps of translocation among cell compartments and their regulation remain unclear. Synthesis of the (n-6) docosapentaenoic acid [22:5(n-6)] is believed to occur through an analogous pathway and is increased in (n-3) fatty acid deficiency (55). However, Infante et al. (54) have suggested that synthesis of DHA and 22:5(n-6) occurs in the mitochondria, with separate enzymes for the (n-6) and (n-3) fatty acids. Regardless, there is now considerable evidence that the conversion of ALA to DHA is low in humans, particularly at the conversion of EPA to DHA, with the most important determinant of circulating levels of DHA in humans being the dietary intake of DHA itself (47, 57, 58, 80, 95, 130). Fatty acid desaturation, however, is subject to competition among the substrates and product inhibition (55). High dietary intakes of LA reduce circulating levels of EPA, likely due to inhibition of conversion of ALA to EPA, but do not increase ARA in humans (80). High dietary intakes of EPA and DHA, on the other hand, increase circulating and tissue levels of EPA and DHA and decrease ARA, and this also occurs in individuals with CF given supplemental doses of EPA plus DHA or DHA alone (83, 93, 94).

The metabolism of 18:1(n-9) by Δ-6 and Δ-5 desaturases to eicosatrienoic acid (20:3n-9) increases during deficiency of both LA and ALA, which together with the decrease in ARA leads to an increase in the ratio of 20:3(n-9)/ARA, known as the triene/tetraene ratio [20:3(n-9)/20:4(n-6)], to above 0.2 (55). Plasma triene/tetraene ratios are usually within the normal range in individuals with CF, except in patients with significant fat malabsorption (37, 105). It has long been known that individuals with CF have altered levels of fatty acids,

typically involving an increase in 16:1(n-7) and 18:1(n-9), decreased LA and DHA, and normal or decreased levels of ARA (9, 22, 23, 25, 37, 45, 52, 59, 77, 81, 82, 102, 105, 115, 116, 125). The monoenoic fatty acids 16:1(n-7) and 18:1(n-9) are products of Δ-9 desaturase (stearoyl CoA desaturase), an important enzyme in hepatic triacylglycerol metabolism (108). Whether the elevated levels of endogenously formed monoenoic fatty acids in individuals with CF is explained by increased hepatic de novo lipogenesis and the potential relevance to hepatosteatosis, also a common complication in CF (24, 29, 75), is not known.

Although early suggestions for the reduced levels of (n-6) and (n-3) fatty acids in individuals with CF focused on effects of low fat intakes, poor fatty acid absorption, and increased fatty acid oxidation to support the high energy requirements, it is now clear that mutations in the CFTR as well as chemical inhibition of Cl^- channels in vitro result in altered phospholipid and fatty acid turnover (10, 14, 15, 64, 89, 102, 105, 117, 120, 133). Many of the essential functions of (n-6) and (n-3) fatty acids are mediated through their roles as acyl moieties of membrane phospholipids from which they are released in response to agonist-stimulated phospholipase activity to serve as ligands for transcription factors, or substrates for further metabolism via cyclooxygenases (COXs), LOXs, epoxygenases, and cytochrome P450s (8, 17, 111). ARA is the major fatty acid in membrane phospholipids and is metabolized by COXs to prostaglandins, prostacyclins, and thromboxanes, by LOX to LTs and hydroperoxyeicosatetraenoates, and to the lipoxins that play key roles in inflammation control and resolution. DGLA and EPA are also substrates for COXs, LOXs, and epoxygenases, but in general their metabolites are less potent than those derived from ARA, such as the EPA-derived leukotriene B5 (LTB5), which is much less active as a neutrophil chemotractant than the ARA-derived LTB4 (17). COX also generates the E-series resolvins from EPA and D-series resolvins, docosatrienes and neuroprotectins from DHA that have anti-inflammatory actions, including decreased leukocyte infiltration and cytokine production (111).

COX: cyclooxygenase

The decrease in DHA with the normal or slight decrease in ARA in plasma and blood cell lipids in individuals with CF (9, 22, 23, 25, 37, 38, 45, 49, 52, 59, 77, 81, 82, 102, 105, 115, 116, 125) results in a relative increase in ARA compared with DHA. However, Freedman et al. (38) have reported lower LA but higher ARA in nasal biopsy samples from 7 individuals with CF and pancreatic insufficiency compared with 7 healthy controls, although ARA levels were not different in nasal mucosal scrapings from 21 individuals with CF and 16 controls or in rectal tissue from 7 individuals with CF and 9 controls. It is unclear whether infection or inflammation alters tissue ARA or if changes in ARA metabolism occur in some CFTR-regulated cells that are not evident from measures of circulating lipids. Regardless, lower DHA in membrane phospholipids could contribute to an increased release of ARA in response to agonist-stimulated phospholipase activity, leading to increased generation of ARA metabolites, such as prostaglandin E2 and LTB4, which have been found to be elevated in individuals with CF (48, 68, 72, 73, 97, 117, 133). On the other hand, other recent studies have focused on a decreased ability of individuals with CF to generate lipoxins, which are trihydroxytetraene-containing metabolites of ARA generated through interactions among epithelia, endothelia, monocytes, and platelets (65, 66). Lipoxins play important roles in neutrophilic inflammation by providing counter-regulatory signals to many proinflammatory mediators, such that early phases of inflammation are characterized by production of proinflammatory mediators such as LTB4, followed by synthesis of lipoxins to allow resolution and prevent progression to a chronic proinflammatory state (66). Recent studies have shown decreased levels of lipoxin A4 in broncheoaveolar fluid from individuals with CF (65), but a mechanism to explain an apparent defective switching from generation of proinflammatory LTs to anti-inflammatory lipoxins has not been identified. Whether altered circulating or tissue levels

of ARA are relevant to the ability to generate ARA-derived lipoxins is also unclear.

PE: phosphatidylethanolamine

Phospholipids, Essential Fatty Acids, and Their Interaction with Methyl Metabolism and Glutathione

CF is characterized by increased generation of oxidants and impaired antioxidant defenses, including low GSH (13, 32, 71, 106), low phospholipid DHA (55, 65, 67, 94–96, 119), and low choline and methyl transfer capacity (19, 59–61). Phospholipid and phospholipid fatty acid metabolism, generation of eicosanoids, and thiol metabolism are closely interrelated through the methionine-homocysteine cycle, which serves as a central axis connecting phospholipid and thiol metabolism, including the generation of GSH (4, 31, 60, 114, 132, 135) (**Figure 2**). In this cycle, PC is formed from phosphatidylethanolamine (PE), and choline provides methyl groups via betaine for regeneration of SAM via methionine. In turn, SAM is a key metabolite regulating entry of homocysteine into the transsulferation pathway leading to cysteine, a precursor of GSH (4, 132). In addition, choline is further metabolized to betaine, which donates a methyl group for remethylation of homocysteine to methionine, with dimethylglycine as the other product; further metabolism of dimethylglycine provides two methyl groups to the mitochondrial folate pool, with synthesis of glycine, a second amino acid constituent of GSH (31, 42).

PC is also formed by the cytidine diphosphocholine pathway, which requires preformed

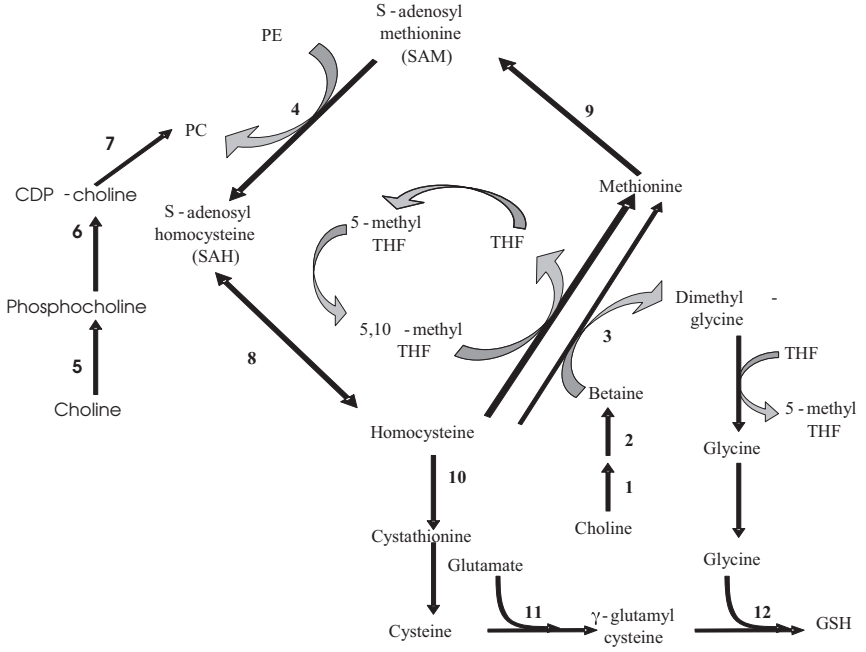

Figure 2

Schematic of the interaction of phospholipid metabolism with the methionine-homocysteine-choline cycle and glutathione synthesis. Enzymes shown are (*1*) choline oxidase; (*2*) betaine aldehyde dehydrogenase; (*3*) betaine homocysteine methyltransferase (BHMT); (*4*) phosphatidylethanolamine-*N*-methyltransferase; (*5*) choline kinase; (*6*) CTP: phosphocholine; (7) CDP-choline: 1,2-diacylglycerol choline phosphotransferase; (*8*) *S*-adenosyl-SAH hydrolase; (*9*) methionine adenosyltransferase; (*10*) cystathione β synthase; (*11*) γ glutamyl cysteine synthetase; (*12*) glutathione (GSH) synthetase. THF, tetrahydrofolate.

choline derived from the diet or by synthesis in the phosphatidylethanolamine-*N*-methyltransferase (PEMT) pathway using PE and SAM as the substrates (**Figure 2**). In the PEMT pathway, transfer of methyl groups from SAM to PE by PEMT generates PC with *S*-adenosylhomocysteine (SAH) as the other product (114, 134, 135). Although the PEMT pathway leads to the de novo synthesis of choline, it does not fulfill the needs for choline in the absence of an adequate choline intake (134, 135). Choline deficiency is associated with many abnormalities relevant to CF, including hepatic steatosis, elevated liver enzymes, decreased SAM and betaine, increased plasma homocysteine, and increased lymphocyte apoptosis (4, 27, 135). SAH is hydrolyzed to adenosine and homocysteine, which can be methylated to regenerate methionine via the 5,10-methylenetetrahydrofolate reductase pathway or betaine-homocysteine *S*-methyltransferase pathway, or it can irreversibly enter the transsulfuration pathway leading to cysteine, and thus GSH (**Figure 2**). A decrease in SAM and an increase in the SAM/SAH ratio signal a deficit in methyl transfer, with counterregulation to conserve methyl groups. SAM is also a positive modulator of cystathionine β-synthase (CβS), a redox-sensitive enzyme that regulates entry of homocysteine to the transsulfuration pathway (31, 132). In individuals with CF, systemic, epithelial lining fluid, lymphocyte, tissue, and mitochondrial levels of GSH are decreased (13, 44, 60, 76, 106, 122), plasma homocysteine and SAH are increased, and the SAM/SAH ratio, and choline, betaine, and dimethylglycine are decreased (19, 59–61). GSH is pivotal in protection against oxidative stress through its role in scavenging free radicals as well as in eicosanoid synthesis, generation of NO, activation of T-lymphocytes, and polymorphonuclear lymphocytes, and in cytokine production (132). Recent studies have also focused on the ability of *S*-nitroglutathione to activate CFTR via posttranscriptional protein modification, potentially offering a mechanism whereby defective CFTR can be rescued from degradative pathways (20, 136). In addition, because a decrease in GSH/GSSG activates signaling pathways such as protein kinases, nuclear factor κB, mitogen-activated protein kinase, and apoptosis, the ability to maintain and regenerate GSH is critically linked to inflammatory responses (132). Notably, NO levels are also decreased in individuals with CF (67, 86), with the decrease in NO being greater in individuals with the lowest plasma ARA/DHA levels (67). Choline and betaine are one of the few sources of methyl groups, both for the betaine-homocysteine *S*-methyltransferase pathway and folate pool, thus serving important roles in the regeneration of SAM, and thus PC biosynthesis, and in generating amino acid precursors for GSH synthesis (**Figure 2**). The reduced plasma PC/PE and SAM/SAH ratios, and decreased choline, betaine, and dimethylglycine in CF (19, 59, 61), raise the possibility that deficiency of methyl groups contributes to impaired redox balance in addition to an altered membrane lipid environment. In one recent short-term study, supplementation with water-soluble forms of choline increased systemic methyl transfer capacity and redox balance, as measured by the plasma SAM/SAH and GSH/GSSG ratios, although the 14-day supplementation study included no measures of functional outcome (60).

PEMT: phosphatidylethanolamine-*N*-methyltransferase

SAH: *S*-adenosylhomocysteine

Disturbances in methyl metabolism also lead to alterations in the fatty acid composition of circulating and tissue phospholipids explained at least in part by the intersection of pathways of PC biosynthesis with the methionine-homocysteine cycle (**Figure 2**). Because PE has higher levels of DHA than PC, PC formed by methylation of PE using methyl groups from SAM has higher levels of DHA than PC derived from the cytidine diphosphocholine pathway, which uses diglyceride as a substrate (86). Decreased PEMT activity results in a marked decrease in plasma and liver PC DHA in animals (127). The ratio of PE/PC is increased, with an inverse relationship between methionine, but positive relationship between SAH and homocysteine and plasma PE in individuals with CF (59), consistent with reduced biosynthesis of PC via PEMT, and defective regeneration of SAM

(59, 60). However, the extent to which altered pathways of PC synthesis explain the low circulating and tissue DHA consistently found in individuals with CF is not known, although a recent case report described increased plasma and red blood cell DHA in a child with CF following supplementation with methyl groups from 5-methyltetrahydrofolate (109). These observations raise the question of whether the low DHA reported in individuals with CF are the consequence of altered methyl and phospholipid metabolism rather than defective fatty acid desaturation.

Interventions with Essential Fatty Acids in Cystic Fibrosis

The heightened inflammatory response together with the increased production of ARA-derived eicosanoids and decreased circulating and tissue levels of DHA in individuals with CF has led to interest in supplementation with EPA and DHA with the aim of decreasing ARA-derived eicosanoids, favoring synthesis of the less inflammatory EPA-derived eicosanoids and increasing tissue levels of DHA. Additional impetus for clinical studies with DHA arose from some studies reporting protection from abnormal manifestation in several organs of $cftr^{-/-}$ mice given high doses of DHA (7, 39, 40, 92). Studies by Freedman et al. (39) with $cftr^{-/-}$ mice reported a dose-dependent decrease in ARA and increase in DHA in tissue lipids with increasing doses of 0.5–40 mg/d DHA; 40 mg/d, but not 10 mg/d, DHA or EPA protected from abnormal manifestations in pancreas acinar cells, ileum, and lung, and blocked *Pseudomonas aeroginosa* endotoxin-induced inflammation in the lung. Total lipids from the ileum, lung, and pancreas of $cftr^{-/-}$ mice had increased ARA but had decreased DHA when compared with wild-type mice (39). In a subsequent report, 40 mg/d DHA decreased neutrophil infiltration into the lung aveolar spaces following exposure to aerosolized *Pseudomonas aeroginosa*, and decreased the elevated 6-keto PGF1α, PGF2α, PGE2, and TxB2 in bronchoaveolar lavage fluid of $cftr^{-/-}$ compared with wild-type mice, although LTB4 levels, which are increased in humans with CF (32, 48, 68, 71, 72, 74, 94) were not different in the $cftr^{-/-}$ and wild-type mice and not altered by DHA treatment (40). In the same mouse model, 40 mg/d DHA also decreased the severity of liver disease, primarily due to a decrease in periportal inflammation (130). Studies of pancreatic lipid fatty acids revealed higher 20:3(n-6) but lower 18:2(n-6) in PC plus phosphatidylinositol and phosphatidylserine, and lower 18:2(n-6) but higher 20:3(n-6), 22:4(n-6), 22:5(n-6), and 22:5(n-3) in PE, with no differences in ARA or DHA in any lipid between the $cftr^{-/-}$ and wild-type mice; it was concluded that there was increased flux of (n-6) fatty acids beyond ARA (92). In contrast, Werner et al. (128) found no difference in pancreatic, lung, or jejunum ARA or DHA in $cftr^{-/-}$ mice or homozygous ΔF508 mice and wild-type mice, and using uniformally ^{13}C-labeled LA and ALA, also found no evidence of altered conversion of LA and ALA to ARA or DHA, respectively, in $cftr^{-/-}$ mice compared with wild-type littermates. Regardless, histological evidence has been published to show marked reduction in abnormal manifestations in several organs owing to high-dose DHA, not EPA supplementation, equivalent to about 1 g/kg in $cftr^{-/-}$ mice (7, 39, 40), although it should be noted that usual dietary intakes of DHA in humans are in the range of 100–400 mg/person/d.

Several studies have been published that involve small numbers of subjects with CF who were supplemented with (n-3) fatty acids from fish oils rich in EPA and DHA, mixtures of fish and vegetable oils, and algal oils rich in DHA, although with varying doses and for varying durations. As expected, supplementation with EPA and DHA increases EPA and DHA in plasma, erythrocytes, platelets, neutrophils, and tissue lipids, usually with a decrease in ARA in individuals with CF (21, 25, 33, 52, 62, 74, 83, 93, 94). In a recent study, 50 mg/kg DHA from algal oil given daily for six months decreased the plasma phospholipid 20:3(n-6) from 4.4% to 2.9% and ARA from 12.2% to 7.6%, whereas DHA increased from

2.3% to 11.5% of total fatty acids, with similar changes in the erythrocyte lipids, a greater than four-fold increase in rectal tissue DHA, although no changes in pulmonary function or liver enzymes were found (83). Similarly, supplementation with 200–400 mg EPA + 100–200 mg DHA/d for eight months decreased ARA and increased DHA in red cell lipids of individuals with CF, with a small but significantly decreased forced expiratory volume in one second (FEV-1) of 57% after intervention compared with 61% at the start of the study, although the total number of days of antibiotic use during the study was 391 compared with 721 for the eight months preceding the study (33). In a small, uncontrolled pilot study, two children under 25 kg were given 1800 mg EPA + 1200 mg DHA and three children over 25 kg were given 2700 mg EPA + 1800 mg DHA/d in six-week cycles of supplementation or no supplementation for one year. One child died (whether supplementation with EPA and DHA at a time when infection occurred had an adverse effect could not be answered); the remaining four children showed no apparent change in lung function or hospitalizations (118). In an early study, Henderson et al. (52) provided 3.2 g EPA + 2.2 g DHA/d as fish oil for six weeks: Two of the seven subjects withdrew because of eructation or diarrhea; the plasma EPA/ARA increased nine-fold in the five individuals with CF who completed the study. More recently, an increase in DHA, with no change in ARA in duodenal tissue obtained by biopsy, was reported for four adults with CF assigned to take 70 mg/kg DHA/d for six weeks, but there were no changes in tests of liver or lung function (49). In contrast, Kurlandsky et al. (74) gave 100–132 mg/kg/d (n-3) fatty acids from fish oil as ethyl esters to children with CF and after six weeks found increased platelet phospholipid EPA and DHA, no significant effects on pulmonary function, and a decrease in serum LTB_4. Consistent with the latter finding, 200–600 mL of a mixture of 90 mg 18:4(n-3), 180 mg 18:3(n-6), 200 mg EPA + 100 mg DHA/200 mL for six months increased DGLA and EPA, decreased ARA in neutrophil phospholipids, and decreased the ratio of LTB4/LTB5 released by neutrophils ex vivo in response to calcium ionophore stimulation (94). In individuals colonized with *Pseudomonas aeroginosa*, 2.7 g EPA for six weeks decreased sputum volume, improved FEV-1 and Shwachman score, and increased the sensitivity of neutrophils ex vivo to LTB4-induced chemotaxis, used as a measure of chronic in vivo LTB4 exposure (77). These latter studies provide some evidence that high supplementation with EPA and DHA may decrease the generation of ARA-derived eicosanoids, such as LTB4, although the effects on generation of the anti-inflammatory ARA-derived lipoxins or metabolites derived from DHA are not yet known (65, 66, 111). Studies using murine models of CF also point to a specific effect of high-dose DHA in alleviating abnormal manifestations in several organs as well as in reducing the excessive response to infection in the lungs (7, 39, 40). Further studies are needed to address whether oils enriched in DHA are a safe and efficacious treatment to reduce the severity of complications in individuals with CF.

FEV-1: forced expiratory volume in one second

SUMMARY

CF is a complex disease with a wide range of clinical problems, affecting many organs and with differing manifestations and severity not readily explained by genotype alone, but for which a heightened inflammatory response and impaired oxidant defenses are likely to play a central role (32, 88, 137). Membrane phospholipid (n-6) and (n-3) fatty acids are released following agonist-stimulated phospholipid turnover and are further metabolized to pro- and anti-inflammatory mediators. Circulating and tissue lipids of individuals with CF are characterized by alterations in (n-6) and (n-3) fatty acids, specifically including decreased LA and DHA (17). GSH plays a central role in responding to and protecting against oxidative stress, and through transport of NO, may also activate and rescue CFTR from degradation (20, 132, 136). Recent advances have underscored the metabolic interconnection between

phospholipids and thiol metabolism through the methionine-homocysteine cycle that serves to generate amino acid precursors for GSH and PC enriched in DHA (4, 31, 60, 114, 135). However, it is not yet known whether dietary-based interventions aimed at correcting the reduced methyl transfer capacity and redox balance in individuals with CF will improve the ability to respond to oxidative stressors, reduce the severity of complications arising from the disease, or influence the abnormal fatty acid patterns. In murine models of CF, very high doses of DHA protect against the severe organ manifestations that result from the absence of CFTR function (7, 39, 40). Most studies in humans with CF have focused on supplementation with EPA and DHA to both reduce ARA-derived inflammatory mediators and to increase circulating and tissue DHA, but with mixed clinical results (17). On the other hand, recent studies focusing on the role of GSH in protein S-nitrosylation have raised the possibility of rescue of class II mutations such as the ΔF508 from entering degradative pathways. Whether the nutritional environment, including provision of essential (n-6) and (n-3) fatty acids, and methyl donors such as choline, influence redox balance and the functional roles of GSH in human disease and health is a new area of nutrition research awaiting exploration.

DISCLOSURES

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Annual Review of Nutrition
Volume 28, 2008

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Errata

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