Abnormal n-6 fatty acid metabolism in cystic fibrosis is caused by activation of AMP-activated protein kinase

Obi C. Umunakwe and Adam C. Seegmiller
Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN

Abstract Cystic fibrosis (CF) patients and model systems exhibit consistent abnormalities in PUFA metabolism, including increased metabolism of linoleate to arachidonate. Recent studies have connected these abnormalities to increased expression and activity of the Δ6- and Δ5-desaturase enzymes. However, the mechanism connecting these changes to the CF transmembrane conductance regulator (CFTR) mutations responsible for CF is unknown. This study tests the hypothesis that increased activity of AMP-activated protein kinase (AMPK), previously described in CF bronchial epithelial cells, causes these changes in fatty acid metabolism by driving desaturase expression. Using CF bronchial epithelial cell culture models, we confirm elevated activity of AMPK in CF cells and show that it is due to increased phosphorylation of AMPK by Ca²⁺/calmodulin-dependent protein kinase kinase β (CaMKKβ). We also show that inhibition of AMPK or CaMKKβ reduces desaturase expression and reverses the metabolic alterations seen in CF cells. These results signify a novel AMPK-dependent mechanism linking the genetic defect in CF to alterations in PUFA metabolism. —Umunakwe, O. C., and A. C. Seegmiller. Abnormal n-6 fatty acid metabolism in cystic fibrosis is caused by activation of AMP-activated protein kinase. J. Lipid Res. 2014. 55: 1489–1497.

Supplementary key words arachidonic acid • fatty acid desaturase • linoleic acid • polyunsaturated fatty acid • adenosine 5′-monophosphate-activated protein kinase

Cystic fibrosis (CF) is a common inherited disease primarily affecting the pulmonary, gastrointestinal, endocrine, and reproductive systems, leading to significant morbidity and mortality (1). CF is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) (2), a cyclic AMP-activated anion channel located in the apical membrane of epithelial cells (3). Among the myriad manifestations of these mutations are consistent alterations in PUFA metabolism (4–6). Consequently, CF patients have characteristic alterations in PUFA composition, including decreased levels of linoleic acid (LA) and DHA in blood, which are accompanied by increased arachidonic acid (AA) in tissues (7, 8). The magnitude of these alterations correlates with disease severity, suggesting a link to pathophysiology (7, 9–12).

The PUFA alterations associated with CF have been recapitulated in models of CF. Both CFTR knockout (13, 14) and ΔF508 (15) mouse models exhibit changes similar to CF patients. A similar pattern is observed in cultured bronchial epithelial cells lacking CFTR (16, 17). These results suggest that PUFA alterations are intrinsically linked to loss of CFTR function. However, until recently, the mechanism of this linkage was largely unknown.

Recent studies have attributed alterations in PUFA levels in CF cells to changes in the activities of PUFA-metabolizing enzymes. This is particularly true for the n-6 PUFA metabolic pathway, which includes conversion of LA to AA through a series of desaturation and elongation reactions. These reactions are catalyzed by Δ6-desaturase (Δ6D), which is rate-limiting, elongase 5 (ELO5), and Δ5-desaturase (Δ5D) (18). Cultured bronchial epithelial cells lacking CFTR exhibit significantly greater expression and activity of both Δ5D and Δ6D, leading to reduced LA levels and increased AA levels, which is typical of CF (19). Furthermore, suppression of Δ5D and Δ6D overexpression by DHA supplementation reverses these PUFA alterations (20).

One potential candidate connecting CFTR mutations with PUFA metabolic enzymes is AMP-activated protein kinase.
kinase (AMPK). AMPK is a heterotrimeric protein, composed of a catalytic α subunit, and regulatory β and γ subunits, that is sensitive to changes in cellular metabolic status (21). When activated, it promotes net ATP synthesis by regulating a variety of cellular processes, including lipid metabolism. Through phosphorylation of downstream targets, AMPK induces cellular uptake and β-oxidation of fatty acids, and inhibits de novo synthesis of saturated and monounsaturated fatty acids (22, 23). While the effect of AMPK on PUFA desaturation and elongation is unknown, there is a clear connection between AMPK and CF, AMPK is part of a macromolecular complex that interacts with and regulates CFTR activity (24). This complex serves as a scaffold that connects CFTR and other ion channels to a number of signal transduction networks. Of note, CF bronchial epithelial cells exhibit greater AMPK activity than their WT counterparts (25).

Complete activation of AMPK requires phosphorylation of threonine-172 in the α-subunit by upstream kinases. In mammalian cells, the primary AMPK kinases are liver kinase B1 (LKB1) and CaMKγ/calmodulin-dependent protein kinase kinase β (CaMKKβ). While LKB1-mediated AMPK phosphorylation is dependent on intracellular AMP concentration, CaMKKβ-mediated AMPK phosphorylation is stimulated by increased intracellular Ca2+ concentration (26–28). AMPK activation in CF bronchial epithelial cells appears to be unrelated to intracellular AMP concentration (25). However, CF bronchial epithelial cells are known to exhibit aberrant calcium homeostasis and increased Ca2+ signaling (29–32), suggesting that CaMKKβ may mediate the observed increase in AMPK activity.

In the present study, we investigated the potential role of increased AMPK activity in altered PUFA metabolism in CF bronchial epithelial cells. Specifically, we tested the hypotheses that AMPK activity is enhanced in CF cells due to a Ca2+-dependent increase in phosphorylation of AMPK by CaMKKβ, and that increased AMPK activity leads to increased fatty acid desaturase expression and activity.

MATERIALS AND METHODS

Materials

STO-609 was obtained from EMD Millipore (Billerica, MA) and dissolved in 100 mM NaOH. BAPTA-AM (1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) was obtained from Abcam (Cambridge, MA) and dissolved in DMSO. EDTA was obtained from Mediatech (Manassas, VA). Dorosomphin dihydrochloride (compound C) and 5-aminomimidazole-4-carboxamide-1β-4-ribofuransoide (AICAR) were obtained from Tocris Bioscience (Minneapolis, MN) and dissolved in water. Rabbit monoclonal antibodies for detection of human AMPKα, phospho-AMPKα (T172), acetyl-CoA carboxylase (ACC), and phospho-ACC (S79) were obtained from Cell Signaling Technology (Beverly, MA). Polyclonal goat anti-rabbit secondary antibody was obtained from Abcam. Mouse monoclonal antibody for detection of human β-actin was obtained from Sigma-Aldrich (St. Louis, MO). Polyclonal sheep anti-mouse secondary antibody was obtained from GE Healthcare Life Sciences (Pittsburgh, PA). Radioactively labeled [1,14C]18:2n-6 (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Fatty acid methyl ester standards (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6) were purchased from NuChek Prep (Elysian, MN). HPLC grade solvents were purchased from Fisher Scientific (Pittsburgh, PA) and IN-Flow 2:1 liquid scintillation cocktail was purchased from IN/US Systems (Tampa, FL).

Cell culture

16HBE13 cell line and antisense cells were a gift from Dr. Pamela Davis (Case Western Reserve University School of Medicine, Cleveland, OH). IB3 and C38 cells were obtained from ATCC (Manassas, VA). Cells were grown in tissue culture flasks pre-coated with LHC basal media (Invitrogen, Carlsbad, CA) containing 0.1 mg/ml BSA (Sigma-Aldrich), 10 μg/ml human fibronectin (Sigma-Aldrich), and 3 μg/ml sodium phosphate buffer (Ortho Biotech Biomaterials, Palo Alto, CA). Complete culture medium consisted of minimum essential medium + glutamax (Invitrogen) supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% horse serum (Atlanta Biologicals, Lawrenceville, GA). Cells were grown at 37°C in a 5% CO2 humidified incubator. Medium was changed three times weekly. Experiments were performed after cells reached 100% confluence.

SDS-PAGE and immunoblotting

Total protein was isolated from cells using RIPA buffer (Sigma-Aldrich) and 2× Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, Waltham, MA). Protein concentrations were determined by BCA assay (Thermo Scientific). Protein samples were mixed 1:1 with 2× Laemmli sample buffer (Bio-Rad Laboratories, Hercules, CA) and boiled for 5 min. Then, volumes equivalent to 15–25 μg of protein were loaded into precast 4–20% gradient polyacrylamide gels (Bio-Rad). After electrophoresis, protein was transferred onto Immobilon-P polyvinylidene fluoride membranes (EMD Millipore). Membranes were blocked using 5% (w/v) blotting grade blocker (Bio-Rad) in TBS-Tween (Sigma-Aldrich). After antibody incubations, protein bands were detected by using SuperSignal West Pico chemiluminescent substrate (Thermo Scientific). Membranes were exposed to Amersham Hyperfilm ECL film (GE Healthcare). Films were scanned and densitometry was performed using Image J analysis software (National Institutes of Health). β-actin was used as a loading control. For repeat immunoblotting, membranes were stripped using Restore Western blot stripping buffer (Thermo Scientific).

Quantitative reverse transcription PCR

Specific primers for quantification of mRNA from FADS1 (ΔΔD), FADS2 (ΔD6), ELOVL5 (ELO5), and RPLP0 genes were described previously (19). Total RNA was isolated from cells using TRIzol (Invitrogen) according to the manufacturer’s instructions. Contaminating DNA was removed from the RNA samples using DNA-free (Ambion, Austin, TX) according to the manufacturer’s instructions. cDNA was synthesized from 1 μg of total RNA using iScript cDNA synthesis kit (Bio-Rad). Quantitative reverse transcription PCR (qRT-PCR) was performed in 10 μl reactions containing 50 ng cDNA, 156 nM forward and reverse primers, and 1× iTaq Universal SYBR Green (Bio-Rad) in 96-well plates. Each reaction was performed in duplicate. Ct values were determined using the CFX96 real-time PCR detection system with CFX Manager software (Bio-Rad). Relative mRNA levels were calculated using the comparative Ct method with RPLP0 as a reference gene.

Desaturase activity assay

Confluent cells were incubated in minimum essential medium containing 10% reduced-lipid fetal bovine serum (HyClone, Logan, UT) and 4.7 μM of [1,14C]18:2n-6 (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Fatty acid methyl ester standards (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6) were purchased from NuChek Prep (Elysian, MN). HPLC grade solvents were purchased from Fisher Scientific (Pittsburgh, PA) and IN-Flow 2:1 liquid scintillation cocktail was purchased from IN/US Systems (Tampa, FL).
Cells were then washed and incubated an additional 20 h in complete medium. Cells were scraped on ice and pelleted by centrifugation, then resuspended in 0.5 ml PBS. Lipids were extracted using a modified method of Folch, Lees, and Sloane Stanley (33). Briefly, lipids were extracted by addition of 3 ml chloroform-methanol (2:1, v/v). After centrifugation, the organic phase was separated and dried under nitrogen. Fatty acids were methylated by adding 0.5 ml of 0.5 N methanolic NaOH (AcrOs Organics, Geel, Belgium) and then heated at 100°C for 3 min, followed by addition of 0.5 ml BF₃ and heating at 100°C for 1 min. The resulting fatty acid methyl esters were extracted into 1 ml of hexane, followed by addition of 6.5 ml of water saturated with NaCl. After centrifugation, the hexane layer was retrieved and dried completely under nitrogen.

For HPLC analysis, fatty acid methyl esters were dissolved in 50 µl of acetonitrile, and 20 µl was injected into an HPLC instrument (Agilent 1200 series; Agilent Technologies, Santa Clara, CA) equipped with an Agilent Zorbax Eclipse XDB-C18 column, 4.6 × 250 mm, 5 µm. A guard column of 4.6 × 12.5 mm, 5 µm was used in conjunction with the analytical column. The fatty acids were separated using a binary solvent system. Solvent A consisted of HPLC grade water with 0.02% H₂PO₄, and solvent B was 100% HPLC grade acetonitrile. The solvent program started with 42% solvent A and 58% solvent B for 25 min, followed by a linear gradient from 58 to 61% solvent B over 2 min, a hold for 8 min, another linear gradient from 61 to 100% solvent B over 15 min, and a hold for 20 min, followed by reconstitution of the original conditions. The flow rate was 1 ml/min. Peaks were detected by UV absorbance at 205 nm and identified by comparison with retention times of unlabeled fatty acid methyl ester standards. Radioactivity from 14C-labeled fatty acid methyl esters was measured with a scintillation detector (β-RAM model 4, IN/US Systems) coupled to the HPLC. The counting efficiency of this detector is 90% for 14C with 5 cpm background.

Statistical analysis

Statistical differences between groups were evaluated by the Mann-Whitney test using STATA or by two-way ANOVA followed by Tukey’s honestly significant difference (HSD) post hoc test for multiple comparisons using R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

AMPK activity was studied in two cell culture models known to exhibit CF-related changes in PUFA composition (17, 19). The first was 16HBEo− bronchial epithelial cells stably transfected with plasmids expressing an oligonucleotide sequence complementary to CFTR in either the sense or antisense orientation (34). Cells transfected with the sense oligonucleotide (S cells) maintain normal CFTR expression, while CFTR expression is silenced in cells transfected with the antisense oligonucleotide (AS cells) (17, 34). The second model, IB3-1, was derived from the bronchial epithelium of a CF patient with a ΔF508/ W1282X CFTR genotype (35). The isogenic control cell line, C38, was generated by stable transfection of normal CFTR cDNA into IB3-1 cells.

Protein levels of phosphorylated AMPK (pAMPK)α and total AMPKα were measured by immunoblotting to determine relative AMPK activation in CF and control cells. In both cell models, pAMPK levels were significantly greater in CF (AS or IB3-1) cells than in the corresponding controls (S cells or C38 cells) (Fig. 1A). There was no significant difference in total AMPK protein levels between CF and control cells. Accordingly, the pAMPK/AMPK ratio was significantly greater in CF cells than in control cells. AMPK activity was assessed by measuring phosphorylation of ACC, which is phosphorylated by activated AMPK (21). In both cell models, phosphorylated ACC (pACC) levels and pACC/ACC ratios were significantly greater in CF than control cells (Fig. 1B), indicative of increased pAMPK activity.

To test the hypothesis that increased AMPK activity in CF cells results from increased CaMKKβ activity, cells were treated with STO-609, a specific inhibitor of CaMKKs (36). Treatment with STO-609 at two different concentrations caused a significant decline in pAMPK and pACC levels in CF cells only (Fig. 2A, B). There was no significant effect on total AMPK or ACC protein levels. Accordingly,

![Fig. 1. AMPK activity is increased in CF cells.](image-url)

Protein was isolated from CF (AS or IBe-1) and control (S or C38) cells 2 days post confluence as described in the Materials and Methods. pAMPK and total AMPK (A) and pACC and total ACC (B) were detected in all cell types by immunoblotting. Autoradiographs from representative immunoblots are shown. Autoradiographs were scanned and the relative intensity of each band was measured by densitometry. Bar graphs represent the mean ratio of pAMPK/AMPK or pACC/ACC as fold change relative to control cells. Data are presented as mean ± SEM (n = 3). *P < 0.05 by Mann-Whitney test. These results are representative of at least three independent experiments.
cells treated with compound C exhibited a significant dose-dependent decline in $\Delta 6D$ mRNA levels, such that they were equivalent to control cells at the highest dose tested (Fig. 3B). There was an even more dramatic decline in $\Delta 5D$ mRNA levels after compound C treatment that was seen in CF and control cells alike (Fig. 3C). Compound C treatment had no effect on ELO5 expression (not shown).

Previous studies indicated that increased $\Delta 6D$ and $\Delta 5D$ mRNA levels in CF cells correlate with increased desaturase activity (19, 20). Because $\Delta 6D$ is rate limiting, the conversion of $[^{14}C]$-labeled LA to $[^{14}C]$-labeled AA can be used as a measure of desaturase activity. As seen in previous studies, vehicle-treated CF cells displayed greater conversion of LA to AA when compared with control cells. This was indicated by increased detection of labeled AA and reduced detection of labeled LA resulting in an elevated AA/LA ratio in CF cells relative to control cells (Fig. 3D). Treatment with compound C resulted in increased LA and decreased AA levels, reducing the AA/LA ratio (Fig. 3D). Importantly, this treatment also eliminated the significant differences observed between vehicle-treated CF and control cells.

Modulators of AMPK activity were used to determine the role of AMPK activation of PUFA metabolism. Compound C (dorsomorphin dihydrochloride) is an inhibitor of AMPK that acts by binding directly to the kinase domain of the catalytic AMPKα subunit (38, 39). Accordingly, compound C treatment reduced pACC levels in CF and control cells, indicative of decreased AMPK activity (Fig. 3A).

As previously described (19, 20), vehicle-treated CF cells exhibited increased expression of both $\Delta 6D$ and $\Delta 5D$ compared with controls, as measured by qRT-PCR. However, CF cells treated with compound C exhibited a significant dose-dependent decline in $\Delta 6D$ mRNA levels, such that they were equivalent to control cells at the highest dose tested (Fig. 3B). There was an even more dramatic decline in $\Delta 5D$ mRNA levels after compound C treatment that was seen in CF and control cells alike (Fig. 3C). Compound C treatment had no effect on ELO5 expression (not shown).

For Fig. 2, postconfluent control (S) and CF (AS) cells were treated with STO-609 at the indicated concentrations (A, B) or EDTA (1 mM) and BAPTA-AM (100 μM) (C, D) for 24 h. Protein was isolated and immunoblotting performed using antibodies for pAMPK and total AMPK (A, C) and for pACC and total ACC (B, D). Autoradiographs from representative immunoblots are shown. Autoradiographs were scanned and the relative intensity of each band was measured by densitometry. Bar graphs represent the mean ratio of pAMPK/AMPK or pACC/ACC as fold change relative to control cells. Data are presented as mean ± SEM (n = 3). Statistical significance was determined by two-way ANOVA with Tukey’s HSD post hoc test for pairwise comparisons. Unlike letters denote significant differences ($P < 0.05$) in pairwise comparisons. These results are representative of at least three independent experiments.
AMPK and abnormal fatty acid metabolism in cystic fibrosis

would be expected to reduce levels of malonyl CoA, the product of ACC, and one of the substrates of ELO5, limiting LA → AA metabolism. The role of the AMPK pathway in PUFA metabolism was confirmed by inhibiting CaMKKβ. Treatment with STO-609, which reduced AMPK activity (Fig. 2), caused significant declines in both Δ6D and Δ5D mRNA levels in CF cells, which were more pronounced in CF cells (Fig. 5A, B). Ca²⁺ chelation with EDTA and BAPTA-AM caused similar effects, reducing Δ6D and Δ5D mRNA levels in CF cells to that of control cells (Fig. 5C, D). Accordingly, treatment with STO-609 reduced LA → AA metabolism to control cell levels.

The opposite effect was observed when cells were treated with the AMPK activator AICAR. When phosphorylated within cells, AICAR becomes ZMP, an AMP-analogue that increases AMPK phosphorylation and activity (40). Treatment with AICAR increased pAMPK and pACC levels in CF and control cells, indicative of AMPK activation (Fig. 4A). As expected, this treatment caused a significant increase in both Δ6D and Δ5D mRNA levels in CF and control cells (Fig. 4B, C). Similar to compound C, there was no effect of ELO5 expression (not shown). However, despite these changes, AICAR did not increase the rate of LA to AA conversion (Fig. 4D). This may be due to the inhibitory effect of ACC phosphorylation stimulated by AICAR. This suggests that AMPK activation may have a more complex role in PUFA metabolism, with both stimulatory and inhibitory effects depending on the specific conditions and substrates involved.
That both cell lines exhibit similar activation of AMPK implies that absence of functional CFTR protein at the cell surface is responsible for the AMPK activation. These results confirm those of a prior study indicating increased AMPK phosphorylation and activity in primary bronchial epithelial cells from CF patients (25). However, another study that transiently disrupted levels (Fig. 5E). Treatment with EDTA/BAPTA also reduced LA → AA metabolism, but in CF cells only (Fig. 5F).

**DISCUSSION**

Many studies have documented the consistent alterations in PUFA levels in the blood and tissues of CF patients and the potential role these alterations play in disease pathophysiology [reviewed in (4–6)]. However, the connection between mutations in the CFTR gene and changes in PUFA metabolism has remained elusive. This is the first study to elucidate a clear mechanistic pathway between these seemingly disparate observations. A schematic overview of these findings is presented in Fig. 6.

This study demonstrates increased phosphorylation and activity of AMPK in two different CF bronchial epithelial cell culture models (Fig. 1). A number of studies have confirmed alterations in PUFA metabolism in these cell lines (16, 17, 19, 20, 41, 42). These cell lines differ in their mechanism of CFTR silencing, one using antisense RNA to block CFTR translation (34), while the other carries the ΔF508 mutation that blocks transit of functional protein to the cell surface (35). That both cell lines exhibit similar activation of AMPK implies that absence of functional CFTR protein at the cell surface is responsible for the AMPK activation. These results confirm those of a prior study indicating increased AMPK phosphorylation and activity in primary bronchial epithelial cells from CF patients (25). However, another study that transiently disrupted levels (Fig. 5E). Treatment with EDTA/BAPTA also reduced LA → AA metabolism, but in CF cells only (Fig. 5F).

**Fig. 5.** Inhibiting CaMKKβ diminishes desaturase expression and activity. Postconfluent control (S) and CF (AS) cells were treated with STO-609 at the indicated concentrations (A, B, E) or EDTA (1 mM) and BAPTA-AM (100 μM) (C, D, F) for 24 h prior to analysis. RNA was isolated and relative Δ6D (A, C) and Δ5D (B, D) mRNA levels were determined by qRT-PCR using the RPLP0 gene as an invariant control as described in the Materials and Methods. Data are shown as mean ± SEM (n = 3). Findings are representative of at least two independent experiments. Statistical differences were determined by two-way ANOVA with Tukey’s HSD post hoc test for pairwise comparisons. Unlike letters denote significant differences (P < 0.05) in pairwise comparisons. E, F: After treatment, cells were incubated in medium containing [14C]LA for 4 h. Cells were washed and then incubated in medium without [14C]LA for 20 additional hours. Cells were harvested, fatty acids isolated, and conversion of [14C]LA to [14C]AA was measured by HPLC coupled with scintillation counting as described in the Materials and Methods. Bar graphs indicate the percent of total counts detected in LA or AA peaks and as the ratio of AA/LA. Data are shown as mean ± SEM (n = 3). Statistical significance was determined by two-way ANOVA with Tukey’s HSD post hoc test for pairwise comparisons. Unlike letters denote significant differences (P < 0.05) in pairwise comparisons. These results are representative of at least two independent experiments.

**Fig. 6.** Schematic of mechanism linking loss of CFTR to elevated desaturase activity. Loss of CFTR function due to mutation causes disordered Ca2+ metabolism leading to increased intracellular Ca2+ concentrations. This activates CaMKKβ, which phosphorylates and activates AMPK. Increased AMPK activity indirectly stimulates expression and consequently, activity of Δ6D and Δ5D. This increases conversion of LA → AA, which results in the characteristic PUFA abnormalities observed in CF. Direct effects are indicated by solid lines, while indirect effects are indicated by dashed lines.
expression and activity is not known, but there are a number of potential mechanisms. AMPK has been shown to phosphorylate and activate PPARγ coactivator 1α (PGC-1α), a coactivator of PPARα (50–52). Activation of PPARα has been shown to stimulate Δ6D expression and activity by binding to a PPAR response element in its promoter (53). AMPK can also alter gene expression by histone modification. AMPK can phosphorylate and inhibit a subset of histone deacetylases (HDACs) (54, 55), as well as directly phosphorylating histone H2B (56), both of which stimulate transcription. Interestingly, altered HDAC activity has been observed in CF cells (57, 58). Whether AMPK induces Δ6D and Δ5D expression and activity through one of these mechanisms will need to be examined experimentally.

The present study focuses on bronchial epithelial cells. However CF-related PUFA alterations have been observed in multiple CFTR-expressing tissues and in plasma of both model organisms and patients (7, 13). This has been connected to increased Δ6D and Δ5D mRNA expression in the lung and intestinal epithelium of CF mice (S. Njoroge, M. Laposata, and A. C. Seegmiller, unpublished observations). Although AMPK activity has not been measured in other CF tissues, it is possible that AMPK activation is responsible for PUFA alterations in other tissues. Alternatively, it is possible that pulmonary epithelium is a major contributor to PUFA alterations in blood and other tissues. For example, Witters et al. (59) recently reported that lung transplantation appeared to correct plasma PUFA alteration in CF patients.

Finally, the findings in the present study raise the possibility that the AMPK pathway could be a therapeutic target in CF. Studies in a CF mouse model indicate that correction of the PUFA alterations by dietary supplementation with large doses of DHA can ameliorate CF-related patholgy (15). However, replicating this result in human studies has been challenging (4). With demonstration that AMPK plays a role in altered PUFA metabolism in CF, it is conceivable that interventions targeting the AMPK signaling pathway either alone or as an adjuvant to PUFA supplementation may have therapeutic benefit in CF patients.

The authors express deep gratitude to Dr. Michael Laposata for many helpful discussions and Eva Henderson and the Vanderbilt University Medical Center Molecular Cell Biology Resource Core for the design and synthesis of primers.

REFERENCES


