Altered de novo lipogenesis contributes to low adipose stores in cystic fibrosis mice

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Bederman I, Perez A, Henderson L, Freedman JA, Poleman J, Guentert D, Ruhrkraut N, Drumm ML. Altered de novo lipogenesis contributes to low adipose stores in cystic fibrosis mice. Am J Physiol Gastrointest Liver Physiol 303: G507–G518, 2012. First published June 7, 2012; doi:10.1152/ajpgi.00451.2011.—Cystic fibrosis (CF) mouse models exhibit exocrine pancreatic function, yet they do not develop adipose stores to the levels of non-CF mice. CF mice homozygous for the Cftr mutation (F508del) at 3 wk (postweaning) and 6 wk (young adult) of age had markedly less adipose tissue than non-CF mice. Food intake was markedly lower in 3-wk-old CF mice but normalized by 6 wk of age. Both 3- and 6-wk-old mice had dietary lipid absorption and fecal lipid excretion comparable to non-CF mice. Hepatic de novo lipogenesis (DNL), determined by 2H incorporation, was reduced in CF mice. At 3 wk, F508del mice had significantly decreased DNL of palmitate and stearate, by 83% and 80%, respectively. By 6 wk, DNL rates in non-CF mice remained unchanged compared with 3-wk-old mice, while DNL rates of F508del mice were still reduced, by 33% and 40%, respectively. Adipose tissue fatty acid (FA) profiles were comparable in CF and non-CF mice, indicating that adipose differences are quantitative, not qualitative. A correspondingly lower content of 2H-labeled FA was found in CF adipose tissue, consistent with reduced deposition of newly made hepatic triglycerides and/or decreased adipose tissue lipogenesis. Hepatic transcriptome analysis revealed lower mRNA expression from several genes involved in FA biosynthesis, suggesting downregulation of this pathway as a mechanism for the reduced lipogenesis. These novel data provide a model for altered lipid metabolism in CF, independent of malabsorption, and may partly explain the inability of pancreatic enzyme replacement therapy to completely restore normal body mass to CF patients.

dipose tissue; F508del

LOW BODY WEIGHT FOR AGE is an independent risk factor for decline in pulmonary function in cystic fibrosis (CF) patients (22, 35). Additionally, body mass index (BMI, used as a proxy for nutritional status) was shown to positively correlate with lung function (43). Consequently, increasing body weight and maintaining healthy BMI are important goals of CF clinical care (42), with the greatest emphasis on increasing calories from fat (42). Despite recommended nutritional intakes of high-fat, high-calorie diets (~40–50% fat as kilocalories), CF patients, on average, do not reach values of the non-CF population with respect to morphometric indexes (31, 44, 45, 52).

The mechanisms underlying impaired growth of CF patients have been attributed predominantly to malnutrition from loss or absence of exocrine pancreatic activity and cachexia from chronic lung infections (4, 26). Significant advancements in pancreatic enzyme supplementation, aggressive nutritional interventions, and improvements in the treatment of infection and inflammation of the lungs have led to increased weight in CF patients (42). Enzyme replacement therapy and dietary maneuvers are usually initiated at the time of diagnosis, typically well within the 1st yr of life. A recent analysis of prospective and retrospective cohort studies indicated that higher energy intake by CF patients resulted in improved weight gain, but conclusive evidence regarding stature was lacking (42). Accordingly, CF patients are able to gain weight, if more substrate is provided. In contrast, malnutrition-induced stunting in non-CF children is largely reversible (47), particularly when initiated at an early age, suggesting that our understanding of the mechanisms involved in CF-related growth retardation is incomplete.

CF mice provide a model to study these processes, as they display suppressed growth hormone signaling and growth retardation (37, 41), similar to humans. However, existing data indicate readily detectable exocrine pancreatic function (6, 19) and, thus, bring into question the mechanisms behind growth suppression via malnutrition. These animals do exhibit impaired gastrointestinal physiology, experiencing a high incidence of intestinal obstruction (12, 17, 34), inflammation (33), and bacterial overgrowth (32), and because of these characteristics, it has been suggested that the absence of Cftr from the intestines results in malabsorption. Bijlevelds and colleagues (5) recently investigated various aspects of fat absorption and metabolism in Cftr-null and F508del mice. They reported that fecal lipid excretion and fat absorption in F508del mice were normal but found some evidence of increased fecal lipids and impaired lipolysis of dietary triglycerides (TGs) in Cftr-null mice. In other work, they demonstrated no impairment of the metabolism of essential fatty acids in either mouse model (49). Using conditional knockout mouse models, Hodges et al. (18) recently demonstrated the causal relationship between the absence of Cftr in the intestinal epithelium and gut obstruction, but they also demonstrated that this did not impair growth and that when Cftr function was restored in the intestinal epithelium, obstruction was prevented but growth remained low and indistinguishable from CF mice. In summary, the causality for reduced growth and low adiposity in CF mice is multifaceted and has not been fully elucidated, thus providing a framework for the studies reported here.

De novo lipogenesis (DNL) is a metabolic pathway in which carbohydrates are converted to fatty acids and, subsequently, VLDL particles that transport lipids to adipose tissue stores (48). DNL is thought to occur to a greater extent in the liver and to some extent in the adipose tissue. Since typical mouse chow is high-carbohydrate and low-fat, mice rely on the
high-carbohydrate content as substrates to synthesize lipids de novo for fat accretion during growth (15). We and others have shown that rodents (mice and rats) indeed have high rates of DNL (1, 3, 7). In this work, we first examined morphometric indexes and body composition as reflected by the inguinal adipose tissue in postweaning (3-wk-old) and young adult (6-wk-old) F508del (CF) mice and compared them with non-CF mice. Next, we analyzed lipid balance by measuring food intake and fecal excretion. We found that growing CF mice have markedly decreased adipose tissue storage without lipid imbalance. Thus we hypothesize that CF mice have reduced DNL and that this contributes to the adipose tissue deficit of these animals. We tested this hypothesis in the work presented here. Namely, DNL was measured using incorporation of 2H from 2H2O into C-H bonds of TG-bound palmitate isolated from the liver and adipose tissue. As expected, hepatic and adipose lipogenesis rates were high in non-CF mice. Weaning and young adult CF mice had markedly decreased rates of hepatic and adipose lipogenesis, consistent with our hypothesis. In weaning mice, the decrease in lipogenesis positively correlated with decreased food intake. In young adult mice, food intake was normal, but lipogenesis was still significantly decreased. These novel data support our hypothesis that decreased hepatic and adipose lipogenesis significantly contributes to the adipose deficit in CF mice. To further understand mechanisms for decreased lipogenesis, we performed hepatic transcriptomics in weaning mice, which revealed many significant changes in genes involved in this pathway.

**METHODS**

**Husbandry and Genotyping**

CF mice used in this study were homozygous for the F508del mutation in Cftr (Cftrtm1Kth) (51). Mice were bred to congenicity by mating homozygous mice. We tested this hypothesis in the work presented here. Namely, DNL was measured using incorporation of 2H from 2H2O into C-H bonds of TG-bound palmitate isolated from the liver and adipose tissue. As expected, hepatic and adipose lipogenesis rates were high in non-CF mice. Weaning and young adult CF mice had markedly decreased rates of hepatic and adipose lipogenesis, consistent with our hypothesis. In weaning mice, the decrease in lipogenesis positively correlated with decreased food intake. In young adult mice, food intake was normal, but lipogenesis was still significantly decreased. These novel data support our hypothesis that decreased hepatic and adipose lipogenesis significantly contributes to the adipose deficit in CF mice. To further understand mechanisms for decreased lipogenesis, we performed hepatic transcriptomics in weaning mice, which revealed many significant changes in genes involved in this pathway.

**Fecal Sample Collection**

CF and non-CF mice were allowed to defecate naturally, and feces were collected and immediately frozen in liquid nitrogen. Before the samples were processed, they were lyophilized to remove moisture and weighed. TGs were extracted using the procedure of Folch et al. (13).

**De Novo Lipogenesis (Stable Isotope Protocol)**

We determined the rates of hepatic and adipose tissue lipogenesis following the incorporation of 2H from 2H2O into newly made TG-bound fatty acids, as published previously (2). At 3 PM, CF and non-CF mice were given an intraperitoneal injection of 2H-labeled saline (28 ml/kg body wt of 9 g of NaCl in 1,000 ml of 99.9% 2H2O). Then the mice were returned to their cages and maintained on 6% 2H-labeled drinking water overnight. This procedure maintains a steady-state labeling of body water at ~3.0–3.5% 2H molar percent enrichment. After ~19 h of exposure to 2H2O, mice were weighed, anesthetized with carbon dioxide, and exsanguinated. Blood samples were collected, livers were freeze-clamped using Wollenberger tongs and weighed, and epididymal fat pads were carefully excised, weighed, and frozen. All samples and tissues were kept at ~86°C until they were processed.

**10-Day-Old Pups**

An additional group of 10-day-old non-CF (n = 27, male-to-female ratio 12:12) and CF (n = 23, male-to-female ratio 15:11) mice was used to rule out the confounding effects of weaning period on DNL and gene expression. Pups were taken from mothers at precisely day 10, weighed, and given an intraperitoneal injection of 2H2O, as described above. As pups were fed milk exclusively, no 2H in drinking water was given. After 3 h of isotopic equilibration, pups were euthanized, and liver and blood samples were taken and frozen. All samples and tissues were kept at ~86°C until they were processed.

**Analytical Procedures**

Isotopic enrichments of body water and TG-bound fatty acids and concentration profiles of tissue fatty acids were determined using a mass selective detector (model 5973N, Agilent) equipped with a gas chromatography system [gas chromatography/mass spectrometry (GC/MS); model 6890, Agilent]. A DB-17MS capillary column (30 m × 0.25 mm × 0.25 μm) with a helium flow of 1 ml/min was used in all assays. Samples were analyzed in selected ion monitoring mode using electron impact ionization. Ion dwell time was set to 10 ms.

**2H labeling of body water.** The 2H labeling of body water was determined by exchange with acetone, as previously described by Katanik et al. (20). Briefly, 20 μl of whole blood or standard were reacted overnight at room temperature with 4 μl of 10 N KOH and 4 μl of 5% acetone in acetonitrile. 2H-labeled acetone was then extracted by addition of 500 μl of chloroform and dried with sodium sulfate, 100 μl were transferred to the GC/MS vial insert, and a 1-μl sample was injected into the GC/MS in split mode (1:40). Acetone mass-to-charge ratios (m/z) 58 and 59 were monitored. Isotopic enrichment was determined as m/z 59/(58 + 59) and corrected using a standard curve.

**2H labeling of TG-bound fatty acids.** The 2H labeling of TG-bound palmitate and stearate was determined from organic extracts of liver and adipose tissue. Briefly, liver and adipose tissue TGs were extracted using the Folch extraction method, as described elsewhere (13). Isolated TGs were saponified by reaction with 1 ml of 1 N KOH [70% ethanol (vol/vol)] at 90°C for 2 h. After acidification, fatty acids were extracted three times with hexane. Combined hexane extracts were evaporated to dryness, and fatty acids were converted to their trimethylsilyl derivatives by the following derivatization procedure: 80 μl of bis(trimethylsilyl) trifluoroacetamide + 10% trimethylchlo- rosilane (Regis, Morton Grove, IL) were added to the dried samples and reacted for 30 min at 75°C. Derivatized sample was transferred to the GC/MS vial insert, and 1 μl was injected into the GC/MS in split mode (1:40). The following ions (313–317 and 341–345) were monitored for palmitate and stearate, respectively. Isotopic enrichment was determined for each correct mass isotopomer as M2/M0 (M0 = M1).

Total isotopic enrichment of fatty acids was determined as the sum of all isotopic enrichments, such as M1 + (M2 × 2) + ... (Mn × n). Total
enrichment was divided by the factors 22 and 24, the numbers of \(^2\)H atoms per carbon incorporated for palmitate and stearate, respectively, resulting in fractional enrichment (FA_{fraction}) (24). Finally, fractional lipogenesis was determined using a precursor-product relationship of \(\text{FA}_{\text{fraction}}/\text{H}-\text{labeled body water } \times \text{time (days)}\).

**Tissue concentration profiles of TG-bound fatty acids.** We determined concentration profiles of myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic [18:2(n-6)], and linolenic [18:3(n-3)] fatty acids extracted from feces, liver, and adipose tissue. Fatty acids were extracted as described above. Concentrations of fatty acids were determined by comparing the abundance of \(m/z\) 285, 313, 341, 339, 337, and 335, respectively, with that of heptadecanoic acid (17:0), \(m/z\) 327, added as internal standard. Since ionization efficiency of various fatty acids is different, we premixed equal quantities of each fatty acid to determine the correction factors (1.0 for myristate, 0.95 for palmitate, 0.9 for oleate, 0.5 for linoleate and 0.3 for linolenate). Each sample was run in duplicate.

**Transcriptional profiling.** RNA was extracted from the livers of 3-wk-old F508del and non-CF mice with the RNeasy Midi Kit (Qiagen, Valencia, CA), and RNA integrity was determined by formaldehyde-agarose gel. cDNA was prepared with the Illumina TotalPrep RNA Amplification Kit (Applied Biosystems/Ambion, Austin, TX), with 500 ng as the starting point. Similar amounts of total amplified labeled cRNA were obtained for all samples (27.1 \pm 2.7 (SE) \mu g, \(n = 8\)), as determined by the Nanodrop 1000 (Nanodrop, Wilmington, DE). Biotin-Cy3-labeled cRNA (750 ng per sample) was loaded onto a MouseRef-8 v2.0 Expression BeadChip (Illumina, San Diego, CA) and processed according to the manufacturer’s instructions. The BeadChip was scanned using a BeadArray reader (Illumina), and data were transferred to GenomeStudio software for initial assessment of quality control.

Internal controls were included in each Illumina Gene Expression Bead to evaluate the quality of the data. Sample-independent metrics, assessed by oligonucleotides spiked into the hybridization solution, indicated no problem with hybridization, washing, or staining. Sample-dependent metrics, assessed by looking at the number of genes detected as present, the 95th intensity profile, and signal-to-noise ratios, also indicated no problem with any of the samples.

After the quality of the data was established, the whole data set was exported from GenomeStudio software into Integromics Biomarker Discovery (IBD) for TIBCO Spotfire software (IBD, Madrid, Spain) for subsequent analysis. In IBD, variance-stabilizing transformation (critical for subsequent inference to identify differential genes) and quantile normalization (to remove from the expression measures any systematic trends that arise from the microarray technology, rather than from differences between probes or between target RNA samples hybridized to the array) were applied to the data.

After data were normalized as described above, differentially expressed genes were determined using significance analysis of microarrays (SAM), a nonparametric method developed at Stanford University (http://www-stat.stanford.edu/~tibs/SAM/sam.pdf) and included in the IBD software. SAM assigns a score to each gene and uses permutations to estimate the percentage of genes identified by chance and if the expression of any gene is significantly related to the response. The cutoff for significance was determined by an adjusted \(P\) value/Q value <0.05. The data discussed in this publication have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number GSE33319 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33319).

**Functional analysis.** Pathway Studio 8 (Ariadne Genomics, Rockville, MD) was used to identify functional pathways enriched with the significantly expressed genes. Gene set enrichment analysis is based on the work of Subramanian et al. (45a, 45b). Since, in most cases, the expression of genes occurs in a coordinated way, where transcription factors induce or repress the expression of a number of target genes at the same time, an approach more powerful than one that compares the expression of each gene separately between groups involves examination of the expression of sets of genes that are known to be coregulated or coexpressed. This analysis first calculates an enrichment score that indicates which gene sets are overrepresented within a ranked list; then a statistical significance (nominal \(P\) value) of that enrichment score is calculated, and finally the \(P\) value is adjusted for multiple hypothesis testing. Gene set categories included in the analysis included Gene Ontology, Ariadne Ontology, and Ariadne curated metabolic and signaling pathways.

**Real-time quantitative PCR.** RNA was extracted as described above, and 1 \mu g of total RNA from each sample was reverse-transcribed into cdNA using a Transcriptor First Strand cdNA Synthesis Kit (Roche, Indianapolis, IN) in a MJ Mini Personal Thermal Cycler (Bio-Rad, Hercules, CA). The cdNA product was diluted 1:10, and 4 \mu l of this reaction were used for the PCR according to instructions provided with the TaqMan Gene Expression System (Applied Biosystems, Carlsbad, CA). Assays-on-Demand Gene Expression products used were as follows: Mm00777290_m1 for stearoyl-CoA desaturase 1 (SCD1) and 4353241E for mouse \(\beta\)-actin. Reactions were run in duplicate on a Template III 96-well optical reaction plate (USA Scientific, Orlando, FL) in a StepOnePlus Real-Time PCR System (Applied Biosystems) using the following protocol: 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. Values (means \pm SE) were normalized to \(\beta\)-actin and expressed as 2^\text{ΔΔCT} [2^{\text{ΔCT}(\beta\text{-actin}) - \text{C}_{\text{ΔT}(SCD1)}] \). Measurements of intestinal bacterial load. Intestinal bacterial load was determined as previously described (32, 50) with some modifications. Mice (5 non-CF and 3 CF) were weaned at 21 days to solid chow plus CoLyte (half strength) and euthanized at 6 wk of age. Immediately after the animals were euthanized, the small intestine was removed in its entirety from stomach to cecum and flushed with cold PBS containing 10 mM dithiothreitol. This material was centrifuged at 7,000 rpm for 45 min at 4°C, and the pellet was kept at −20°C until it was further processed. DNA was extracted from the pellet using the QIAamp DNA Stool Mini Kit (Qiagen). Microbial load was measured by RT-PCR using microbial 16S (small ribosomal subunit gene) broad-range (universal) primers: 5'-GTG STG CAY GGY TGT CGT CA-3' (forward) and 5'-AGC TCR TCC MCA CCT TCC TC-3' (reverse). A standard curve was generated using the 16S PCR product from Escherichia coli cloned into the pDRIVE plasmid (a generous gift from Robert C. De Lisle), as well as the sequence of the more universal primers used in this study. PCRs were run in duplicate on a Template III 96-well optical reaction plate (USA Scientific) in a StepOnePlus Real Time PCR System (Applied Biosystems) using Power SYBR Green (Applied Biosystems). For each reaction, 1 \mu l of extracted DNA was used as the template with the following protocol: 10 min at 95°C, 40 cycles of 15 s at 95°C, 1 min at 60°C, and 30 s at 70°C; a melting curve was performed at the end in the range of 55–90°C. Values (means \pm SE) are expressed as log(copies 16S) per the entire intestine.

**RESULTS**

**Morphometric Indexes**

CF mice and humans are characterized by delayed and reduced growth. In humans with CF, not only are height and weight values lower than in non-CF individuals, but also BMI is lower, indicating that delayed growth also impacts body composition. The marked decrease in body weight was evident in 10-day-old mice \([5.1 \pm 0.2 \text{ and } 3.3 \pm 0.2 \text{ g for non-CF (n = 27) and CF (n = 23), respectively, } P < 0.0001]\). Figure 1 shows morphometric indexes of growth of 3- and 6-wk-old mice. Body weight was significantly lower in CF than non-CF mice \([29\% (P < 0.01) \text{ and } 27\% (P < 0.01) \text{ for } 3- \text{ and } 6-wk-old \text{ mice.} \)

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mice, respectively; Fig. 1A]. Body length was significantly lower in CF than non-CF mice at 3 and 6 wk [9.1% (P < 0.01) and 10% (P < 0.0001), respectively; Fig. 1B]. CF mice had significantly lower and similar BMI values at 3 and 6 wk (~14%, P < 0.05; Fig. 1C). This is not surprising, since weight was decreased much more than length in CF mice. Data in Fig. 1 are comparable to those previously reported by us and others (37, 41, 51). In CF patients, the low BMI values predominantly reflect reduced fat mass. To determine whether the decrease in body weight was largely attributed to reduced adipose tissue, we measured body composition (fat mass).

Body Composition

To assess body composition differences in CF mice, we weighed inguinal fat pads representing visceral fat (epididymal for males and omental for females). Figure 2 shows absolute and relative (expressed as percentage of body weight) weights of inguinal fat pads. At 3 and 6 wk, the absolute weights of fat pads were significantly smaller in CF mice [by 60% (P < 0.01) and 40% (P < 0.001), respectively]. When fat pad weights were normalized to body weight, CF mice still had significantly less fat storage (by 30% and 20%, P < 0.05, at 3 and 6 wk, respectively). These data indicate that body weight reduction in CF mice is largely due to decreased fat mass.

Food Intake and Feed Efficiency

We measured food consumption, because reduced caloric intake could explain the observed reductions in body weight/fat mass. Figure 3 shows daily food intake expressed per mouse and per gram mouse weight and feed efficiency. As shown in Fig. 3A, 3-wk-old CF mice consumed significantly less chow (~55%, P < 0.001) per day; however, food intake was not different between CF and non-CF mice by 6 wk of age. When food intake was normalized per body weight (Fig. 3B), CF mice still consumed less food at 3 wk but not at 6 wk. At 3 wk of age, non-CF and CF mice consumed 243 and 108 mg of lipid per day, respectively, while at 6 wk of age, non-CF and CF mice consumed comparable amounts, 288 and 261 mg of lipid per day, respectively. At 3 wk of age, feed efficiency was significantly higher in CF mice, indicating that consumed calories were utilized, consistent with fecal fat analyses (see below). These results indicate that caloric intake was inadequate to support growth in 3-wk-old mice.

Fecal Lipid Analysis

As an indicator of intestinal lipid absorption, we determined fecal lipid content. Figure 4 shows fecal lipid profiles of essential [oleic (18:1), linoleic (18:2), and linolenic (18:3)] and nonessential [myristic (14:0), palmitic (16:0), and stearic (18:0)] fatty acids for 3- and 6-wk-old mice. Fatty acid profiles were compared with those in the consumed chow. In all profiles, concentrations of fecal fatty acids were substantially lower than those in the rodent chow, suggesting that the majority of the dietary fat was absorbed. We did not find any significant differences in the excretion of fecal fatty acids between CF and non-CF mice. For example, 3- and 6-wk-old non-CF mice excreted 9.5 ± 0.5 and 9.6 ± 1.2 mg of total fecal TG per gram solid, respectively, while 3- and 6-wk-old CF mice excreted 9.4 ± 0.8 and 12.3 ± 1.2 mg of total fecal TG per gram solid, respectively. Thus, from a comparison of lipid intake with lipid excretion, it is apparent that CF mice do not have steatorrhea when fed this diet. These measurements agree...
well with observations by Bijvelds et al. (5), who also concluded that the dietary fatty acids were absorbed equally well in F508del mice. To further elucidate the causes for reduced growth/adiposity, we measured de novo lipid synthesis in the lipogenic tissues, i.e., liver and adipose tissue.

**De Novo Hepatic Lipogenesis**

To measure DNL, we utilized the $^2$H incorporation method (see MATERIALS AND METHODS) (2). Briefly, mice were given a bolus of $^2$H-labeled water and kept on $^2$H-enriched drinking water to maintain $^2$H concentration at a steady state. On the basis of the body weight of each of the animals and the assumption that body water accounted for 75% of body weight, we calculated that the bolus injection should have enriched body water $^2$H to the expected range of 3–3.5%. We validated this assumption by comparing body water $^2$H molar percent enrichment in all groups (non-CF vs. CF: 3.0 $\pm$ 0.1% vs. 2.8 $\pm$ 0.2% at 3 wk, and 3.4 $\pm$ 0.1% vs. 3.5 $\pm$ 0.1% at 6 wk). These results were used to compute fractional DNL rates (see MATERIALS AND METHODS). In 10-day-old mice, the DNL rate was low and not different among groups: 13.7 $\pm$ 0.5 and 14.9 $\pm$ 0.6% for non-CF ($n = 27$) and CF ($n = 23$), respectively. A low DNL rate was expected in suckling animals, i.e., those fed a high-fat low-carbohydrate diet. As the mice were weaned onto low-fat high-carbohydrate chow, the DNL rate increased markedly. Figure 5 shows hepatic fractional synthesis rates of palmitic and stearic fatty acids at 3 and 6 wk of age. As expected in growing animals, DNL was high in control mice (palmitate and stearate accounted for $63\%$ and $48\%$, respectively, of the hepatic fatty acid pool synthesized per day). De novo synthesis of palmitate and stearate was $\sim 75\%$ lower in the livers of CF mice ($P < 0.0001$) than controls. At 6 wk of age, the DNL rate of non-CF mice remained the same, while DNL in CF mice significantly increased from 3 wk ($P < 0.001$), although it was still lower (by $\sim 35\%$ for palmitate and stearate) than in non-CF mice. On the basis of the amount of $^2$H labeling, the majority of stearate appears to be derived from newly made palmitate, rather than elongation of existing palmitate, in agreement with previously published findings by Lee et al. (24). In the livers of 3-wk-old animals, the fraction of stearate derived from palmitate was slightly, but significantly, higher ($P < 0.02$) in CF livers: 77 $\pm$ 0.8% vs. 82 $\pm$ 2.3% for non-CF and CF, respectively. In the livers of 6-wk-old animals, the fraction of stearate was not significantly different (78 $\pm$ 3.0% and 79 $\pm$ 6.0% for non-CF and CF, respectively). These data indicate that conversion of carbohydrates to new lipids was markedly decreased in growing mice.

**Absolute Hepatic Lipogenesis**

Using fractional lipogenesis rates, total liver palmitate concentrations, and liver weights, we estimated absolute lipogenesis rates for palmitic acid. At 3 wk, the hepatic palmitate pool was slightly higher in CF than non-CF mice (10.3 $\pm$ 0.3 vs. 8.8 $\pm$ 0.3 mg/g tissue), but since the fractional synthesis rate was reduced to a greater extent in CF (see above), the absolute palmitate synthesis rate was significantly lower in CF than non-CF mice (1.2 $\pm$ 0.1 vs. 4.5 $\pm$ 0.4 mg/day, $P < 0.00001$). At 6 wk, hepatic palmitate pools were not different between non-CF and CF mice (14.2 $\pm$ 1.5 and 11.9 $\pm$ 1.2 mg/g tissue, 12%).

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**Fig. 3.** Dietary intake and feed efficiency (FE). A: daily food intake. B: relative food intake. C: feed efficiency. Values are means $\pm$ SE of 15 3-wk-old non-CF and 9 F508del mice and 20 6-wk-old non-CF and 16 F508del mice. Student’s t-test was used to determine significance.

**Fig. 4.** Concentration profile of fecal fatty acids [myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2,n-6), and linolenic (18:3,n-3)] in 3-wk-old (A) and 6-wk-old (B) mice. Values are means $\pm$ SE of 15 3-wk-old non-CF and 9 F508del mice and 20 6-wk-old non-CF and 16 F508del mice. Data were normalized per gram of dry tissue weight.
respectively, \( P > 0.05 \), but since the fractional rate was still significantly lower in CF mice, the absolute rate was significantly lower in CF than non-CF mice (4.7 ± 0.4 vs. 6.8 ± 0.6 mg/day, \( P < 0.01 \)).

**Hepatic Profile of TG-Bound Fatty Acids**

To determine whether alterations in hepatic DNL caused changes in hepatic lipids, we measured the concentration profile of hepatic TG-bound fatty acids (Fig. 5, C and D). Long-chain saturated fatty acids were significantly elevated in the F508del livers of 3-wk-old mice [14:0, 58% (\( P < 0.05 \)); 16:0, 20% (\( P < 0.001 \)); and 18:0, 16% (\( P < 0.001 \))], but not 6-wk-old mice. Mono- and polyunsaturated fatty acids were not different in either of the age groups. Total liver lipids were not different between CF and non-CF mice at 3 wk (106.6 ± 5.4 and 118.4 ± 11.9 \( \mu \)mol/g tissue) or 6 wk (148.6 ± 10.1 and 132.7 ± 10.4 \( \mu \)mol/g tissue). These results indicate that elongation of myristate (14:0) and further processing of palmitate (16:0) and stearate (18:0) (desaturation and elongation) were affected in 3-wk-old CF mice. Together, these data suggest that, at 3 and 6 wk, absolute production of palmitate was significantly diminished in CF mice. The lower hepatic output of new lipids could influence growth of adipose tissue stores.

**Age-Dependent Changes in Lipogenesis**

Age-dependent changes in lipid metabolism are shown in Fig. 6. Changes in hepatic DNL of palmitate (previously shown in Fig. 5, A and B) are shown in Fig. 6A, while changes in hepatic SCD1 gene expression, measured by RT-PCR, are shown in Fig. 6B. From the profiles, it is apparent that both lipogenic processes are decreased in suckling (10-day-old) non-CF and CF mice; however, once the dietary switch to...
high-carbohydrate chow occurs at weaning, non-CF mice respond by increasing lipogenic activity, while CF mice show no change, consistent with lower food intake at this age. The greatest activation in both lipogenic processes in CF mice is observed at 6 wk of age. These age-dependent changes are consistent with delayed accretion of adipose tissue in CF mice (Fig. 2).

Adipose Tissue Lipogenesis

In the fed state, adipose tissue TGs derive their fatty acids from 1) chylomicrons carrying dietary lipids, 2) hepatic VLDLs carrying newly synthesized lipids, and, to a much lesser extent, 3) de novo synthesis in adipocytes. $^2$H content of TG-bound fatty acids extracted from the fat pads was measured to assess the overall rate at which newly synthesized fatty acids become adipose TGs. Figure 7 shows fractional DNL of palmitate and stearate isolated from adipose tissue TGs of 3- and 6-wk-old mice. At 3 wk, CF mice had significantly less $^2$H in adipose tissue fatty acids [%= 3-fold less palmitate ($P < 0.01$) and 5-fold less stearate ($P < 0.05$)]. At 6 wk, $^2$H content of adipose tissue in non-CF mice decreased compared with 3-wk-old mice. That is, the proportion of $^2$H-labeled fat decreased as the fat pool increased with age. In CF mice, the proportion of $^2$H-labeled palmitate and stearate did not change with age, consistent with the increase in fatty acid synthesis from 3 to 6 wk and the increase in pool size. When adipose tissue size is taken into account, net labeling from lipogenesis and deposition can be calculated by multiplying fractional labeling (reflecting synthesis and deposition) by the size of the lipid pool. Absolute lipogenesis of non-CF mice was 4.5- and 1.6-fold greater than that of CF mice at 3 and 6 wk of age, respectively. These data suggest that fatty acid delivery to the adipose tissue and adipose tissue turnover were decreased in growing CF mice.

Adipose Tissue Profile of TG-Bound Fatty Acids

We and others previously demonstrated that, regardless of the dietary fatty acid composition, C57BL/6J mice maintain the same composition of TG-bound fatty acids in adipose tissue lipids. To assess whether there was a differential remodeling of adipose tissue TG-bound fatty acids, we analyzed profiles of adipose tissue lipids (Fig. 7). Profiles of TG-bound fatty acids at 3 and 6 wk of age are shown in Fig. 7, C and D. We found that the profile of essential fatty acids in CF mice was significantly different from that in non-CF mice only at 3 wk of age [18:2, $\sim$ 25% ($P < 0.05$) and 18:3, $\sim$ 48% ($P < 0.005$)]. Fatty acid profiles of 6-wk-old mice were not different among CF and non-CF mice. These data indicate that lower dietary input caused the lower concentration of essential fatty acids in the adipose tissue.

Hepatic Transcriptome Analyses

Data from liver and adipose tissue lipid metabolism indicate significant alterations in de novo synthesis and elongation and desaturation metabolic pathways. Thus we studied modulation of gene expression of lipid metabolic pathways by examining the hepatic transcriptome. Hepatic RNA was extracted and...
probed using the Illumina MouseRef-8 v2.0 Expression BeadChip, which surveys >25,000 well-annotated RefSeq transcripts, corresponding to >19,100 unique genes. To determine which genes were differentially expressed in the livers of CF vs. non-CF mice, only genes (probes) considered present in all the samples (non-CF and CF) were entered in the analysis, as well as genes considered present in all the non-CF samples independently of their status in the CF samples, and vice versa. These allowed us to take into account genes whose expression may be suppressed or activated in CF or non-CF mice.

Figure 8A lists differentially expressed genes, together with gene ID, symbol, and fold change from non-CF mice. Figure 8B shows a schematic representation of the fatty acid biosynthesis pathway annotated with the fold changes (CF vs. non-CF) for 3-wk-old mice [adapted from GenMAPP (38)]. Overall, mRNA levels for genes involved in the fatty acid pathway are decreased in 3-wk-old CF compared with non-CF mice, but the major downregulation of gene expression is seen at three key points of the pathway: 1) ATP citrate lyase (Acly), which catalyzes generation of lipogenic acetyl-CoA in the cytosol from mitochondrial citrate, a critical reaction step providing a carbon source for DNL; 2) scd1, which catalyzes conversion of palmitate (16:0) and stearate (18:0) to palmitoleate [16:1(n-7)] and oleate (18:1), respectively; and 3) the family of short-chain (Acas2) and long-chain (Acsl3) acyl-CoA synthetases, which are responsible for converting free fatty acids to their acyl-CoA analogs. Acyl-CoA synthetase 3 (Acsl3), besides the mentioned role, has recently been shown to regulate the activity of several lipogenic transcription factors, their target gene expression, and the rates of DNL (8). These data indicate that gene expression along the lipogenesis pathway is significantly altered in 3-wk-old mice. This is in agreement with decreased hepatic lipogenesis, desaturation, and elongation of fatty acids.

Desaturation Indexes

After the significant decrease of SCD1 gene expression, we measured desaturation ratios of palmitoleate to palmitate and oleate to stearate in the liver and adipose tissue (Table 1). In agreement with transcriptome results, both desaturation ratios were significantly decreased in 3-wk-old animals. CF-non-CF differences were more pronounced in the liver than adipose tissue. In the case of 6-wk-old mice, only oleate-to-stearate ratios were significantly decreased.

Bacterial Load

To rule out small intestinal bacteria overgrowth as a factor in the reduced growth in CF mice, we measured bacterial load in the small intestine of mice fed solid chow and CoLyte (half strength) from weaning at 21 days until 6 wk of age. We found similar bacterial load in the small intestine of non-CF (n = 5) and CF (n = 3) mice (7.7 ± 0.1 vs. 7.4 ± 0.2 log copy number of 16S, respectively).

DISCUSSION

Low body fat is a well-documented trait for pediatric and adult CF populations (31, 44); however, no data exist on body composition of murine models of CF. Here we present data that show markedly reduced adipose tissue stores in F508del mice. We investigated mechanisms responsible for the reduced adipose stores in F508del mice. Two separate age groups were
DECREASED LIPOGENESIS IN CF MICE

Table 1. Desaturation ratios of triglyceride-bound long-chain fatty acids

<table>
<thead>
<tr>
<th></th>
<th>3-Week-Old</th>
<th>6-Week-Old</th>
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<tbody>
<tr>
<td></td>
<td>Non-CF (n = 15)</td>
<td>F508del (n = 9)</td>
</tr>
<tr>
<td>Palmitoleic-to-palmitic ratio (16:1/16:0)</td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.0039 ± 0.0004</td>
<td>0.0020 ± 0.0004*</td>
</tr>
<tr>
<td>WAT</td>
<td>0.0731 ± 0.0029</td>
<td>0.0524 ± 0.0054*</td>
</tr>
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| Oleic-to-stearic ratio (18:1/18:0) |                     |                           |                       |                     |                           |                       |
| Liver          | 0.6467 ± 0.0396           | 0.3611 ± 0.0240*          | 44.2                  | 1.1922 ± 0.1057      | 0.8500 ± 0.0719*           | 28.7                  |
| WAT            | 9.0901 ± 1.0350           | 6.9120 ± 0.7031†          | 23.9                  | 7.8931 ± 0.3807      | 6.8988 ± 0.3245*           | 12.6                  |

Values are means ± SE; n, number of mice. CF, cystic fibrosis; WAT, white adipose tissue. Student’s t-test was used to determine significance: *P < 0.05; †P = 0.06.

studied: 1) immediately postweaning (3 wk old) and 2) young adult (6 wk old). Both groups represent some of the most active periods of the mouse’s growth; thus we reasoned that we should detect the most significant changes by studying these two age groups. Indeed, similar to the null mice that we previously studied (37), we now demonstrate significant growth reduction in height and weight and, consequently, lower BMI in F508del mice. Similar reduction in morphometric indexes is typically observed in CF patients (44, 45, 52). Furthermore, we analyzed body composition of the mice by weighing the inguinal fat pads, a large fat storage depot in mice, which in non-CF mice constitutes about one-third of the total fat. Our analyses show a significant decrease in the inguinal fat pad weight, in absolute weight and as a proportion of total body weight, suggesting that CF mice remained significantly leaner for their length than non-CF mice; i.e., they were disproportionally lean. We found that food intake of CF mice, which in non-CF mice constitutes about one-third of the total daily intake, was significantly lower in the 3-wk-old group and not in the 6-wk-old group. Feed efficiency of CF mice was higher at 3 wk of age, suggesting that their intake was insufficient for their growth needs, but by 6 wk of age, intake and feed efficiency were similar between the two groups.

Norkina et al. (32) found small intestinal bacterial overgrowth (SIBO) in Cftr−/− mice and demonstrated that SIBO contributes to poor growth and low body weight. They showed that treatment of CF and wild-type mice with antibiotics decreased SIBO load and improved body weight of CF mice but did not adversely affect wild-type mice. The mechanisms of body weight improvement are not clear but were further investigated by Wouthuyzen-Bakker et al. (50), who showed that antibiotic treatment improved uptake of long-chain fatty acids in CF mice but had no effect on total fat absorption. They report that F508del mice had no SIBO, compromised nutritional status, and/or augmented body weight (50). The differences between these studies are not clear but suggest that strain differences, diet, and other conditions may play a role in the degree of SIBO in CF mice. For example, Canale-Zambrano et al. (9) demonstrated substantial SIBO in 4-day old Cftr−/− (BALBc) pups, independent of nutrition. Given these recent data, we tested whether reduced growth in our F508del mice can be attributed to SIBO. We found that bacterial load in our F508del mice was similar to that in their wild-type littermates, thus providing no explanation for reduced growth.

Analysis of excreted fecal lipids showed that CF and non-CF mice in both age groups had apparently normal lipid excretion, which is in agreement with previously reported findings in F508del mice (5, 49). After confirming that absorption of dietary lipids was normal, we tested whether the contribution of newly synthesized lipids to adipose tissue stores was altered. Indeed, DNL rate of palmitate was significantly reduced ~6 and ~1.5-fold in 3- and 6-wk-old mice, respectively. The synthesis rate of stearate, from the elongation of palmitate, mirrored the latter results. In addition, we analyzed TGs extracted from adipose tissue and determined, based on 3H labeling, that at 3 wk of age, CF mice made/deposited significantly less lipid, while at 6 wk the fractional lipogenesis rate slightly increased in CF mice, but relative to the total pool size (i.e., size of the fat pad) it was still nearly one-half that of non-CF mice. Overall, absolute synthesis/deposition of the adipose tissue lipids in CF mice was one-fifth to one-half of that in non-CF mice at 3 and 6 wk of age, respectively. A mechanistic explanation of these findings is lacking, but examination of the hepatic transcriptome from 3-wk-old mice revealed a significant decrease in gene expression of lipogenic enzymes, including enzymes for three different rate-limiting steps of lipogenesis, implying a somewhat general suppression of lipogenesis.

We found significant alterations in lipid metabolism in 3-wk-old, postweaning, F508del mice (reduced lipid intake and DNL). At this age, non-CF mice develop substantial adipose tissue, but the reduced lipid flux in CF mice appears to result in reduced adipogenesis. Between 3 and 6 wk of age, CF and non-CF mice increased their adipose stores seven- to eightfold, but because of the smaller initial compartment of the adipose tissue, the CF mice are not able to attain non-CF stores, thus leaving adult mice with reduced potential for “buffering” lipid fluxes (14). A primary function of adipose tissue is to “buffer daily lipid flux,” a phrase coined by Frayn (14). This means that, during postprandial periods, the adipose tissue maintains a relatively low circulating pool of TGs (~1–2 mg in a mouse) compared with the dietary TG intake (between 100 and 250 mg) by having adipose tissue to take up the bulk of dietary TGs. In this way, adipose tissue functions as a buffer to “spare” nonadipose tissues from excessive lipid flux, which could potentially lead to such effects as insulin resistance, a trait that affects many CF patients. Decreased adipose tissue in 6-wk-old F508del mice would potentially limit deposition of dietary lipid, thus forcing it to be “routed” elsewhere, i.e., other tissues causing lipotoxicity and/or oxidation. However, we did not detect increased liver TGs, suggesting that excess TGs may be oxidized (see below for discussion of SCD1 deficiency). The situation would be worse in mice fed a high-fat diet, where...
dietary lipid flux is greatly increased, thus requiring greater “buffering” capacity from adipose tissue. Indeed, our data show that when CF mice were fed a high-fat diet, they remained lean, while their non-CF counterparts became obese (Bederman et al., unpublished observations). Additionally, in CF mice fed a high-fat diet, hepatic TGs were elevated, indicating impaired clearance. Buffering capacity of adipose tissue is especially relevant for CF patients who consume diets high in saturated fat. Potentially because of reduced buffering capacity, CF patients have only marginal weight gain and have elevated oxidative stress. Further studies are needed to investigate the ramifications of decreased adipose tissue for lipid homeostasis in CF patients and animal models, especially under the conditions of high-fat diet feeding. The ramifications of the apparent lipodystrophy presented here remain to be determined but would likely affect exercise capacity and other metabolic functions influenced by adipose stores.

Dietary composition is an established modulator of DNL; i.e., low DNL rates are found when mice are nursing, as the high-fat content of the milk causes DNL suppression, as reviewed in detail by Girard et al. (15). Studies in rodents showed that hepatic DNL is rapidly (6–24 h) induced by weaning from high-fat milk to the low-fat high-carbohydrate chow (21, 39, 40). The increase in carbohydrate/insulin levels rapidly induces gene expression of lipogenic enzymes and causes increased lipogenic flux through the DNL pathway, as reviewed in detail by Towle et al. (46). To demonstrate low lipogenesis during the suckling period, we additionally measured DNL in the livers of F508del mice. This, in turn, resulted in low uptake of carbohydrates into adipose tissue. These results provide a potential mechanism by which nutrient and lipid homeostasis is influenced by adipose tissue.

The causes of reduced food intake in F508del mice at weaning remain unknown. Consistent with our findings in rodent chow, Norkina et al. (32) reported reduced food intake (20–25%) when weaning null mice were fed Peptamen liquid diet. Also consistent with our observations in 6-wk-old mice, Wouthuyzen-Bakker et al. (50) recently reported no difference in chow intake: 19.5 ± 4.2 and 18.8 ± 1.4 kcal/day in non-CF and F508del mice, respectively (Table 1 in Ref. 50). Our findings of reduced food intake in CF mice are clinically relevant, in that studies report reduced appetite and food intake in children with CF (36). Appetite stimulants are commonly prescribed for CF patients diagnosed with nutritional failure (30). Since our CF mice do not have spontaneous lung infections and have sufficient exocrine pancreatic function, they represent a relevant model to study mechanisms of reduced food intake and its relationship to nutrient absorption and lipid homeostasis. This is demonstrated by our findings of low levels of adipose tissue-bound essential fatty acids in 3-wk-old mice, likely resulting from reduced food intake in the absence of fecal malabsorption. This is consistent with data in CF patients showing deficiency of essential fatty acids associated with decreased dietary intake and impaired absorption (23). Furthermore, the relationship between decreased food intake and delayed upregulation of lipogenesis is not clear. Did decreased food intake cause decreased hepatic DNL directly, or did low DNL elsewhere cause decreased food intake and, subsequently, decreased hepatic DNL? We pose this question based on the work of Loftus et al. (28) and others (11) showing that decreased fatty acid synthase expression/lipogenesis in the hypothalamus controls food intake. Future experiments are needed to further elucidate the relationship between food intake control and lipid synthesis in CF. We are currently using conditional tissue-specific knockout CF mice to elucidate the intertissue cross talk with respect to lipid metabolism.

We and others have shown that the DNL rate is high in growing non-CF mice (3, 7, 24, 25). Humans typically consuming high-fat diets, compared with rodents, may not require the same degree of DNL for adipose tissue growth. However, a recent review by Lodhi et al. (27) sheds a new light on DNL in humans in the name of “lipoexpediency,” i.e., “the notion that lipids can be directed toward benefit, even in the setting of lipid abundance.” Namely, they provide experimental evidence that DNL, besides the traditionally accepted role of disposal of carbohydrates into storage lipids, may also make ligands/signaling molecules for modulation of peroxisome proliferator-activated receptors, insulin sensitivity, protein modification, and food intake regulation. We showed that SCD1 gene expression was significantly downregulated in young CF mice. This may be linked with reduced DNL, since it provides substrates [palmitate (16:0) and stearate (18:0)] for SCD1. Consequently, synthesis of the SCD1 products palmitoleate [16:1(n-7)] and olate (18:1) would be significantly decreased. Indeed, this was the case in adipose tissue desaturation ratios in 3-wk-old CF mice. This would be important for CF patients in light of the recent discovery of a new role for adipose tissue-derived palmitoleate as a “lipokine” regulating peripheral insulin sensitivity and hepatic TG clearance and, possibly, reducing inflammation (10). Our findings of reduced DNL in mice are especially relevant for CF patients who consume high-fat diets, since DNL is also reduced in young CF patients, and a high-fat diet would further suppress DNL, potentially limiting synthesis of important signaling molecules. Studies in CF patients are needed to elucidate whether hepatic and whole body lipogenesis are affected.

In summary, we found decreased hepatic lipogenesis and correspondingly low expression of lipogenic enzymes in the livers of F508del mice. This, in turn, resulted in low uptake of TGs into adipose tissue. These results provide a potential mechanism contributing to adipose tissue deficiency in F508del mice with implications for CF patients.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
AUTHOR CONTRIBUTIONS

I.B., A.P., and M.L.D. approved the final version of the manuscript. I.B., A.P., and M.L.D. approved the final version of the manuscript. I.B., L.H., and M.L.D. approved the final version of the manuscript. A.P., L.H., D.G., and N.R. prepared the figures. I.B., A.P., and M.L.D. drafted the manuscript. I.B., A.P., and M.L.D. edited and revised the manuscript. I.B., A.P., and M.L.D. approved the final version of the manuscript.

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