Liver Fatty Acid-Binding Protein (L-Fabp) Modifies Intestinal Fatty Acid Composition and Adenoma Formation in Apc<sup>Min/+</sup> Mice

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Abstract
Evidence suggests a relationship between dietary fat intake, obesity, and colorectal cancer, implying a role for fatty acid metabolism in intestinal tumorigenesis that is incompletely understood. Liver fatty acid-binding protein (L-Fabp), a dominant intestinal fatty acid-binding protein, regulates intestinal fatty acid trafficking and metabolism, and L-Fabp deletion attenuates diet-induced obesity. Here, we examined whether changes in intestinal fatty acid metabolism following L-Fabp deletion modify adenoma development in Apc<sup>Min/+</sup> mice. Compound L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice were generated and fed a 10% fat diet balanced equally between saturated, monounsaturated, and polyunsaturated fat. L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice displayed significant reductions in adenoma number and total polyp area compared with Apc<sup>Min/+</sup> controls, reflecting a significant shift in distribution toward smaller polyps. Adenomas from L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice exhibited reductions in cellular proliferation, high-grade dysplasia, and nuclear β-catenin translocation. Intestinal fatty acid content was increased in L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice, and lipidomic profiling of intestinal mucosa revealed significant shifts to polyunsaturated fatty acid species with reduced saturated fatty acid species. L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice also showed corresponding changes in mRNA expression of enzymes involved in fatty acid elongation and desaturation. Furthermore, adenomas from L-Fabp<sup>−/-</sup>/Apc<sup>Min/+</sup> mice displayed significant reductions in mRNA abundance of nuclear hormone receptors involved in cellular proliferation and in enzymes involved in lipogenesis. These findings collectively implicate L-Fabp as an important genetic modifier of intestinal tumorigenesis, and identify fatty acid trafficking and metabolic compartmentalization as an important pathway linking dietary fat intake, obesity, and intestinal tumor formation. Cancer Prev Res; 6(10): 1026–37. ©2013 AACR.

Introduction
There is a strong foundation of epidemiologic and experimental work to support a role for environmental modifiers including high dietary fat intake and obesity in the pathogenesis of colorectal cancer, and increasing evidence suggests that the type of dietary fat is important (1–4). Several studies have found a strong association between saturated fat intake and colorectal cancer risk (5–7), whereas diets rich in ω-3 polyunsaturated fatty acids (PUFA) may suppress colorectal cancer development (8–10). These studies highlight the role of fatty acid metabolism in the pathogenesis of intestinal tumors and suggest that intestinal metabolism of specific dietary fats modify colorectal cancer risk. Despite the importance of these observations, however, little progress has been made toward a molecular genetic understanding of the pathways that link consumption of high saturated fat diets to intestinal tumorigenesis.

Fatty acid metabolism is a focal point for metabolic regulation, acting to modulate gene expression, growth and survival pathways in both physiologic and pathologic settings (11). Fatty acid-binding proteins (Fabps) include a large multigene family of highly abundant, cytosolic lipid-binding proteins that coordinate intracellular lipid trafficking and regulate metabolic and inflammatory pathways (11, 12). A dominant Fabp in mammalian intestine, liver fatty acid-binding protein (L-Fabp) plays a critical role in intestinal fatty acid trafficking and compartmentalization (13). Beyond a role in modulating lipid flux, however, the expression of L-Fabp within colorectal cancer tumor tissue has been linked to histologic (tumor) differentiation, the incidence of lymph node metastasis, and overall prognosis (14–16). Those studies imply that L-Fabp expression may...
modulate signaling pathways involved in colorectal cancer initiation and progression, although the mechanisms involved are currently obscure.

We have previously shown that mice with germline deletion of L-Fabp (L-Fabp<sup>−/−</sup>) are protected against diet-induced obesity and hepatic steatosis when fed a high saturated fat diet, but not when fed a high PUFA diet (17–19). We have also shown significant alterations in enterohepatic fatty acid, cholesterol, and bile acid metabolism in L-Fabp<sup>−/−</sup> mice, findings which collectively show a role for L-Fabp as a metabolic sensor with a hierarchy of sensitivity to intraluminal and dietary fat species (20–22). These experimental observations, together with the findings linking dietary fat intake to colorectal cancer susceptibility, led us to ask whether alterations in intestinal fatty acid metabolism associated with L-Fabp deletion affect intestinal tumor initiation and progression. Here, we report that L-Fabp deletion significantly reduces intestinal adenoma formation in the Apc<sup>Min</sup> model of tumorigenesis and show that this protection is associated with alterations in the metabolic utilization of intestinal fatty acid species.

Materials and Methods

Animals and tissue processing

All studies were approved by the Animal Studies Committee at Washington University School of Medicine (St. Louis, MO) and conformed to criteria outlined in the NIH ‘Policy on Humane Care and Use of Laboratory Animals.’ L-Fabp<sup>−/−</sup>/Apc<sup>Min</sup> mice were generated and fed a 10% fat diet containing similar proportions of saturated, monounsaturated, and polyunsaturated fatty acids (#8626 Teklad Mouse Breeder Diet, Harlan Teklad). Mice were weighed and sacrificed at 104 ± 4 days of age. Intestines were formalin fixed and pinned for inspection with a dissecting microscope. Intestinal polyps were examined blinded to genotype and classified according to the location in the small intestine (SI). Each section was photographed using a Photometrics CoolSNAPpcf camera (Photometrics). Polyp size was quantitated using Metavue software (Molecular Devices). In separate groups of 104-day-old mice, normal intestine and polyp tissue were snap frozen for mRNA and protein expression studies. Additional groups of mice were sacrificed at 53 ± 2 days of age (before gross polyp development), and intestinal mucosa obtained for lipidomic profiling and gene expression studies. Where indicated, food consumption and fecal lipid studies were conducted on mice at approximately 10 weeks of age housed in metabolic cages (19).

Immunohistochemical studies, bromodeoxyuridine labeling, and dysplasia measurements

Four-micron sections were used to assess polyp histology following staining with hematoxylin and eosin. Dysplasia was assessed by an independent blinded gastrointestinal pathologist (I. Nalbantoglu). Proliferative activity within polyps was measured by bromodeoxyuridine (BrdUrd) incorporation as described previously (23). Immunohistochemical studies were conducted using rabbit anti-L-Fabp (a generous gift from Dr. Jeffrey Gordon, Washington University School of Medicine), rabbit anti-Fatty Acid Synthase (Bethyl Laboratories), or mouse anti-β-catenin (BD Biosciences). As shown previously (21), the anti-L-Fabp antiserum used in this study is specific for L-Fabp with no cross-reactivity to either ileal lipid-binding protein or intestinal fatty acid-binding protein (I-Fabp).

Western blotting

Tissue extracts were prepared as described (21) in denaturing sample buffer, separated by SDS-PAGE, and transferred onto a polyvinylidene difluoride membrane (Millipore). The membranes were probed sequentially with rabbit anti-L-Fabp, anti-PPAR-α, or anti-β actin (Sigma Aldrich). Proteins of interest were visualized with enhanced chemiluminescence reagents (GE Healthcare) and scanned using Image-Quant software (GE Healthcare Biosciences).

Analysis of tissue lipid content and fecal fat content

Frozen tissue (~100 mg) was homogenized and protein concentration determined (Bio-Rad; DC protein assay). Lipid extractions were conducted (21) and triglyceride, free fatty acid (FFA), and cholesterol content were determined using commercially available kits (Wako) and normalized to protein content of the homogenate. Fecal fat content was determined gravimetrically as previously described (17).

Lipidomic profiling

A modified Bligh–Dyer extraction method was used to extract lipids from scraped intestinal mucosa in the presence of internal standards. The FFAs were further derivatized into amides to improve the sensitivity of mass spectrometry (MS). Sample analysis was conducted with a Shimadzu 10A high-performance liquid chromatography (HPLC) system coupled to a TSQ Quantum Ultra triple quadrupole mass spectrometer. A pooled sample from each extracted study sample was used as the quality control sample. Data processing was conducted with Xcalibur software (Thermo). The concentration of lipids was calculated as concentration of internal standard multiplied by peak area ratio of analyte to internal standard, based on an assumption that the MS responses of analyte and internal standard are the same.

Quantitative PCR

Normal intestinal tissue and tumor samples from individual animals were flash frozen and RNA extracted. Quantitative PCR was conducted in triplicate on an ABI Step One Plus Detection System (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems) and primer pairs (Supplementary Table S1) designed by Primer Express Software (Applied Biosystems). Relative gene expression was determined using the comparative threshold cycle method (Applied Biosystems User Bulletin I). Relative mRNA abundance in L-Fabp<sup>−/−</sup>/Apc<sup>Min</sup> mice was expressed as fold change compared with mRNA levels in normal
mucosa from Apc\textsuperscript{Min/+} mice, normalized to glyceraldehyde-3-phosphate dehydrogenase expression.

**PGE\textsubscript{2} analysis**

Normal tissue and tumor samples from individual (3 per genotype) animals were excised individually and flash frozen. Cleared homogenates were prepared (23) and analyzed using a PGE\textsubscript{2} monoclonal immunoassay kit (Cayman Chemical).

**Statistical analysis**

Statistical analysis was conducted using GraphPad Prism 4 software (GraphPad Software, Inc.). In most cases, Student t test was used to determine P values. For multiple group comparison, one-way ANOVA with Bonferroni post test was used to calculate the differences between the groups. Statistical significance was set at \( P \leq 0.05 \). All values are reported as mean ± SE.

**Results**

**L-Fabp deletion in the Apc\textsuperscript{Min/+} background attenuates small intestinal adenoma burden**

At 15 weeks, L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice manifested 35% fewer polyps (Fig. 1A) than Apc\textsuperscript{Min/+} mice, with a regional decrease in polyp multiplicity in all sections of small intestine (Fig. 1B), most evident in the proximal (Fig. 1C) and distal intestine. There was a 42% reduction in total small intestinal polyp area in L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice compared with Apc\textsuperscript{Min/+} mice (Fig. 1D). This reduction was most significant in the proximal and middle sections of small intestine with a trend toward reduced polyp area in the distal small intestine (Fig. 1E). Polyps were then divided into quartiles by size (area) and the distribution between genotypes was compared. L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice exhibited a significantly greater proportion of polyps in the smallest size quartile compared with Apc\textsuperscript{Min/+} mice (Fig. 1F). Our findings suggest that polyp number increases from proximal to distal small intestine in Apc\textsuperscript{Min/+} mice, an observation broadly consistent with our prior observations (23). We interpret the generally comparable reduction in polyp area (Fig. 1D) and polyp number (Fig. 1A) in L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice to imply that L-Fabp deletion reduces both polyp initiation and progression.

**L-Fabp deletion in the Apc\textsuperscript{Min/+} background reduces cellular proliferation, dysplasia, and Wnt signaling in small intestinal polyps**

We next examined histologic markers of adenoma progression and differentiation in polyps from both genotypes. L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice exhibited reduced proliferation in adenomas compared with Apc\textsuperscript{Min/+} mice in the proximal and middle intestine (Fig. 2A and B). There was a 26% reduction in the number of polyps containing high-grade dysplasia in L-Fabp\textsuperscript{−/−}Apc\textsuperscript{Min/+} mice compared with Apc\textsuperscript{Min/+} controls (Fig. 2C and D). Given the reduced proportion of tumors...
with high-grade dysplasia in \( L{-}\text{Fabp}^{-/} \) \( \text{Apc}^{\text{Min}}/+ \) mice, we evaluated nuclear translocation of \( \beta\)-catenin (Fig. 2E and F). We found a significant reduction in staining for nuclear \( \beta\)-catenin in proximal small intestinal polyps from \( L{-}\text{Fabp}^{-/} \) \( \text{Apc}^{\text{Min}}/+ \) mice compared with \( \text{Apc}^{\text{Min}}/+ \) mice. These findings suggest that \( L{-}\text{Fabp} \) deletion produces alterations in pathways related to cellular proliferation and differentiation in intestinal epithelium.

### Reduced \( L{-}\text{Fabp} \) expression in intestinal adenomas in \( \text{Apc}^{\text{Min}}/+ \) mice

Because germline deletion of \( L{-}\text{Fabp} \) results in reduced polyp burden, we examined the expression of \( L{-}\text{Fabp} \) in adenomas compared with normal intestinal mucosa in \( \text{Apc}^{\text{Min}}/+ \) mice (i.e., mice with a wild-type \( L{-}\text{Fabp} \) allele). A gradient of \( L{-}\text{Fabp} \) expression was observed in normal intestinal mucosa from proximal to distal small intestine.
L-Fabp deletion in the \( A^\text{Min}+/+ \) background increases intestinal free fatty acid, triglyceride, and cholesterol content and alters distribution of fatty acid species

We have previously shown protection against diet-induced obesity associated with alterations in the kinetics of intestinal fatty acid transport in female \( L^{-}\text{Fabp}^{-/-} \) mice (17, 19). We found that \( L^{-}\text{Fabp}^{-/-} A^\text{Min}+/+ \) mice weighed significantly less than their \( A^\text{Min}+/+ \) counterparts at 104 ± 4 days of age (Fig. 4A). \( L^{-}\text{Fabp}^{-/-} A^\text{Min}+/+ \) mice exhibited increased intestinal FFA, triglyceride, and cholesterol content compared with \( A^\text{Min}+/+ \) mice along with a concomitant decrease in serum FFAs and triglycerides (Fig. 4B and C). Analysis of fecal lipid content in mice from both genotypes revealed reduced fecal fat and a subtle yet significant increase in dietary fat absorption in \( L^{-}\text{Fabp}^{-/-} A^\text{Min}+/+ \) mice (Fig. 4D). These data suggest that L-Fabp deletion alters intestinal lipid trafficking in the \( A^\text{Min}+/+ \) background.

We next turned to a lipidomics approach to examine whether the changes in lipid abundance in mucosal
extracts from proximal small intestine of 53-day-old mice reflect a corresponding change in fatty acid species within lipid classes. As shown in Fig. 5A, there was a significant shift in relative abundance of intestinal FFA species between genotypes, with a decrease in total saturated fatty acid species and an increase in total PUFA species.

Figure 4. Reduced body weight and altered lipid trafficking and metabolism in L-Fabp−/−/ApcMin+ mice. A, reduced weight gain on 10% fat diet at 104 days. B, increased intestinal FFA (left), triglyceride (TG; center), and cholesterol (right) in 53-day-old L-Fabp−/−/ApcMin+ mice. C, reduced serum FFA (left) and TG (center), and unchanged serum cholesterol (right) in 53-day-old L-Fabp−/−/ApcMin+ mice. D, reduced fecal lipid content (left) and increased dietary fat absorption (right) in L-Fabp−/−/ApcMin+ mice. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.
**L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup>** mice. Apc<sup>Min/+</sup> mice also exhibited a significant increase in relative abundance of shorter chain saturated fatty acid species (14–18 carbons), whereas **L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup>** mice showed increased abundance of essential dietary PUFA (18:2, 18:3). There was also a significant shift in the relative abundance of fatty acid species in diacylglycerides between genotypes (Fig. 5B and Supplementary Table S2), with broadly similar patterns to those observed above for individual FFA species. Diacylglycerides containing 18:0 fatty acid were decreased in mucosal extracts from **L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup>** mice, and diacylglycerides containing arachidonic acid (20:4) were significantly increased in Apc<sup>Min/+</sup> mice. We then examined the relative composition of triglyceride fatty acid species in these extracts (Fig. 5C and Supplementary Table S3). The abundance of triglycerides that contain the saturated fatty acid species 18:0 was significantly decreased in L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup> mice, as was the abundance of triglycerides containing arachidonic acid (20:4). These data collectively suggest that L-Fabp functions as a critical component of intestinal fatty acid trafficking and compartmentalization, with a distinct hierarchy of fatty acid sensitivity. The findings further suggest that L-Fabp deletion results in a significant shift in metabolic channeling of diacylglycerides and triglycerides containing 20:4.

**L-Fabp deletion significantly alters mRNA expression of enzymes involved in fatty acid elongation and desaturation**

The demonstration of differences in fatty acid composition among intestinal FFA, diacylglyceride, and triglyceride species between genotypes prompted us to examine the expression of enzymes involved in fatty acid biosynthesis, elongation, and desaturation. We found that mRNA abundance of fatty acid elongases 5 and 6 (ELOVL5 and ELOVL6) and fatty acid desaturases 1 and 2 (FADS1 and FADS2) was reduced by 40% to 60%, whereas fatty acid synthase (FASN) mRNA was reduced 2-fold in small intestinal mucosa of 53-day-old L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup> mice (Fig. 5D). There was also a qualitative reduction in FASN protein expression as determined by immunostaining (Supplementary Fig. S1). Collectively, these findings imply that altered fatty acid trafficking as a result of L-Fabp deletion produces significant adaptive changes in long-chain fatty acid biosynthesis, elongation, and desaturation, which likely further contribute to the altered fatty acid profiles observed.

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**Figure 5.** Altered composition of intestinal FFA, diacylglycerides, and triglycerides in L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup> mice by lipidomics. A, altered relative abundance of FFA species with shift to PUFA species in L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup> mice. B, altered relative abundance of fatty acid species in diacylglyceride between genotypes. Diacylglyceride from Apc<sup>Min/+</sup> mice have an increased proportion of 18:0 fatty acids and arachidonic acid (20:4). C, altered relative abundance of fatty acid species in triglyceride. Triglyceride from Apc<sup>Min/+</sup> mice displayed increased relative abundance of saturated fatty acids and arachidonic acid (20:4). D, altered mRNA abundance of enzymes involved in fatty acid biosynthesis, elongation, and desaturation. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.

*P < 0.05; **P < 0.01; ***P < 0.001.
**L-Fabp deletion significantly alters mRNA expression of nuclear hormone receptors involved in fatty acid metabolism**

Given the pronounced differences in intestinal fatty acid composition in intestinal mucosa at 53 days of age, we investigated gene expression changes of transcription factors known to be involved in fatty acid metabolism and whose regulation has been implicated in intestinal tumorigenesis in normal mucosa and polyps from mice of both genotypes. The peroxisome proliferator-activated receptor family of transcription factors are activated by endogenous lipid ligands (24). We found that the abundance of both PPARα and PPARδ mRNAs was significantly reduced in adenomas compared with normal intestinal mucosa in ApcMin/C0 mice (Fig. 6A and B). There was a trend to reduced mRNA expression of PPARβ and PPARδ between polyps and normal intestine in L-Fabp−/−ApcMin+/+ mice, but PPARα protein expression was significantly reduced in polyps of L-Fabp−/−ApcMin+/+ mice (Figs. 6A and B and Supplementary Fig. S2). No significant differences were found between genotypes in the expression of PPARY (Fig. 6C). Sterol regulatory element-binding protein-1 (SREBP-1) is a master regulator of lipogenic pathways and has been shown to be upregulated in colorectal cancer (25). mRNA expression of SREBP-1c was significantly upregulated in polyps compared with normal mucosa in ApcMin+/+ mice, but significantly reduced in polyps from L-Fabp−/−ApcMin+/+ mice compared with expression in polyps in ApcMin+/+ mice (Fig. 6D).

Similarly, the NR4A orphan nuclear receptors are linked to the regulation of fatty acid metabolism, and expression of NR4A2, in particular, is increased in colorectal cancer (26, 27). mRNA abundance of NR4A2 was also upregulated in polyps from ApcMin+/+ mice and significantly reduced in polyps from L-Fabp−/−ApcMin+/+ mice (Fig. 6E). Given the changes in NR4A2 and the known modulation of COX-mediated prostaglandin signaling by NR4A2, we then measured mRNA abundance of COX-2 and levels of prostaglandin E2 (PGE2) in normal and polyph tissue from mice of both genotypes. There was a trend toward reduced COX-2 mRNA abundance (37% reduction in relative mRNA abundance, P = 0.13) and reduced PGE2 levels (62 μg PGE2/mg protein vs. 83 μg PGE2/mg protein, P = 0.3) in polyps from L-Fabp−/−ApcMin+/+ mice compared with ApcMin+/+ mice, despite similar expression levels in normal intestine (data not shown). These results collectively suggest that intestinal tumorigenesis is accompanied by altered expression of key transcription factors that govern fatty acid metabolic pathways important in sustaining cell growth and proliferation, and that L-Fabp deletion seems to prevent these metabolic changes at the transcriptional level.

**Discussion**

The relationship between dietary fat intake, obesity, and colorectal cancer has focused attention on the pathways that integrate elements of intestinal lipid metabolism and pathways involved in tumorigenesis. We have previously shown that L-Fabp plays an important role in the metabolic response to dietary fat and is a modifier of the obesity trait associated with high saturated fat feeding (18, 19). The key finding of this study is that L-Fabp deletion in ApcMin+/+ mice not only protects against weight gain, but also significantly attenuates intestinal tumor initiation and progression. This protection from adenoma development is associated with alterations in intestinal fatty acid and triglyceride content and composition and in the expression of transcription factors and enzymes involved in lipogenesis and energy usage. Taken together, these findings suggest that L-Fabp is an important genetic modifier of intestinal tumorigenesis and identify fatty acid trafficking as a novel pathway linking dietary fat intake, obesity, and intestinal adenoma formation.

The requirement of fatty acid and lipid substrates for membrane synthesis, energy homeostasis, and cell signaling is an increasingly recognized hallmark of neoplastic transformation (28, 33, 34). These fatty acids are either imported from the extracellular milieu, synthesized de novo, or mobilized from intracellular stores through specific adaptations in lipid metabolism critical for tumor cell growth and proliferation. Consistent with the need for robust lipid synthesis, neoplastic cells show increased inhibition (28–31). Here, we show that mRNA expression of three key lipogenic enzymes, acetyl coA carboxylase (ACC), FASN, and stearoyl-coA desaturase (SCD-1), were all significantly upregulated in the transformation from normal intestinal mucosa to adenoma in ApcMin+/+ mice (Fig. 6F–H). However, there was no corresponding change in mRNA expression for these genes in L-Fabp−/−ApcMin+/+ mice (Fig. 6F–H and Supplementary Fig. S2). Alterations in fatty acid lipolysis pathways have also been shown to be part of the adaptive changes in lipid metabolism in colorectal cancer (26). The expression of hormone-sensitive lipase (HSL), an enzyme involved in lipolysis, was significantly reduced in polyps from L-Fabp−/−ApcMin+/+ mice compared with normal mucosa, whereas expression remained unchanged in ApcMin+/+ mice (Fig. 6I). Finally, the expression of cannabinoid receptor 1 (CB1), an endocannabinoid receptor implicated in the control of energy and metabolism through alterations in fatty acid balance and known to be altered in colorectal cancer (32), was significantly upregulated in the polyps of ApcMin+/+ mice compared with normal mucosa, whereas its expression remained unchanged in L-Fabp−/−ApcMin+/+ mice (Fig. 6J). Taken together, these results suggest that L-Fabp deletion modulates pathways of de novo fatty acid lipogenesis and influences the metabolic compartmentalization of fatty acid. These metabolic shifts, in turn, are associated with decreased intestinal adenoma burden in L-Fabp−/−ApcMin+/+ mice.
Figure 6. Alterations of tumor lipid metabolism in polyps from L-Fabp<sup>−/−</sup>/Apc<sup>Min/+</sup> mice compared with Apc<sup>Min/+</sup> mice. Altered mRNA expression in proximal small intestine polyps (P) compared with normal proximal small intestine (N) of the PPAR family (A–C), SREBP1C (D), NR4A2 (E), enzymes involved in de novo lipogenesis (F–H), lipolysis (I) and energy usage (J). Gene expression is normalized to expression in normal proximal small intestinal mucosa of Apc<sup>Min/+</sup> mice. *, P ≤ 0.05.
expression of critical enzymes involved in lipogenesis, including ACC, FASN, and SCD1 (33). Studies have shown that inhibiting each of these enzymes in vitro and/or in vivo results in diminished tumor cell proliferation, decreased cell viability, or decreased tumor size, suggesting that interruption of fatty acid synthesis may be a potential chemo- therapeutic strategy in the treatment of cancer (35–41). Here, we show upregulated expression of lipogenic enzymes ACC1, FASN, and SCD1 in intestinal adenomas in ApcMin/+ mice, yet find no corresponding increase in these lipogenic genes in L-Fabp–/– ApcMin/+ mice (Fig. 6). This suggests that alterations in fatty acid metabolic trafficking caused by L-Fabp deletion may reduce the ability of neoplastic cells to upregulate lipogenesis necessary to support the initiation and/or proliferation of intestinal tumors. These findings suggest that the adaptations in fatty acid trafficking and lipogenesis observed during tumorigenesis in ApcMin/+ mice are reduced or delayed in L-Fabp–/– ApcMin/+ mice, resulting in reduced intestinal adenoma formation and proliferation. Further experimental confirmation using more direct measures of lipid substrate usage and turnover will, of course, be required to be able to make firm conclusions about the relative importance of these pathways implicated in intestinal tumorigenesis.

The aforementioned adaptive changes in tumor lipid metabolism are proposed to reflect activation of oncogenic pathways that in turn alter the activity of key transcription factors (42). For example, the transcriptional regulation of lipogenic genes in colorectal neoplasia has been shown to be regulated by SREBP-1 (25). Here, we show that L-Fabp deletion significantly reduces the expression of SREBP-1 in intestinal polyps. Our findings suggest that this decrease is associated with reduced lipogenesis and fewer and smaller intestinal polyps. We also showed reduced expression of the nuclear orphan receptor NR1A2 in L-Fabp–/– ApcMin/+ mice, which is of interest in relation to the recent studies showing that this gene plays a key role integrating eicosanoid and fatty acid metabolic pathways (26, 27). We showed increased NR1A2 expression in polyps from ApcMin/+ mice but not in polyps from L-Fabp–/– ApcMin/+ mice (Fig. 6). These data, together with the lipidomic data showing decreased abundance of diacylglyceride and triglyceride species containing 20:4 fatty acid in mucosal extracts from L-Fabp–/– ApcMin/+ mice and the trend toward reduced COX-2 mRNA and PGE2 in polyps from L-Fabp–/– ApcMin/+ mice, suggest that L-Fabp expression may modulate eicosanoid and prostanoid signaling, pathways that are important in intestinal tumorigenesis.

An unresolved question is whether the changes in proliferation and gene expression in polyps from L-Fabp–/– ApcMin/+ mice arise as a direct result of altered intracellular fatty acid trafficking and compartmentalization within neoplastic cells, or whether these changes reflect altered lipid substrate availability and usage in the extracellular milieu that restrict polyp growth. These questions assume additional significance because L-Fabp expression was consistently reduced in intestinal polyps compared with normal intestinal mucosa in ApcMin/+ mice and apparent even in 8-week-old mice, suggesting that loss of L-Fabp is an early event in intestinal tumorigenesis. Consistent with this, studies in human colorectal cancer have shown changes in L-Fabp expression in early-stage intestinal adenomas with progressive loss of L-Fabp expression during colorectal cancer development and progression, with the lowest levels of expression occurring in poorly differentiated tumors (14, 43). These findings suggest that L-Fabp expression is important in the promotion of normal intestinal differentiation, and that pathways involved in regulating L-Fabp expression may be disrupted in transformed and dedifferentiated enterocytes. Another possibility is that the transformation of normal intestinal epithelium into dysplastic adenoma and adenoma progression is dependent on the transfer of lipid substrates from surrounding normal intestinal epithelium. Recently, Nieman and colleagues reported reduced tumor burden and metastases in FABP4–/– mice compared with wild-type mice (44), along with reduced transfer of lipid substrates from adipocytes to ovarian cancer cells. Those findings raise the question of whether similar adaptations may in part explain our current observations. In this scenario, deletion of L-Fabp may result in altered trafficking of substrates along with alterations in key pathways of lipogenesis, reducing the availability of substrates necessary for tumorigenesis. Thus, it is possible that the attenuation in polyph burden in L-Fabp–/– ApcMin/+ mice reflects altered lipid trafficking required for the earliest stages of adenoma progression. Our findings bear further consideration in the context of other work showing that low-density lipoprotein receptor-related protein 1 modulates intracellular cholesterol storage and fatty acid synthesis through Wnt signaling pathways (45). These findings have added significance because L-Fabp regulates hepatobiliary cholesterol metabolism in vivo (17, 22). Here, we find increased intestinal cholesterol content associated with reduced lipogenesis and nuclear β-catenin translocation in L-Fabp–/– ApcMin/+ mice, raising the possibility that L-Fabp may also play a role in the crosstalk between sterol and oxysterol metabolism and Wnt signaling pathways.

Lipidomic profiling revealed altered intestinal FFA, diacylglyceride, and triglyceride fatty acid species between genotypes, with a shift from saturated fatty acids in ApcMin/+ mice to PUFAs in L-Fabp–/– ApcMin/+ mice. These findings, in conjunction with our previous studies showing reduced diet-induced obesity and hepatic steatosis in L-Fabp–/– mice fed a high saturated but not PUFA diet, suggest that L-Fabp deletion alters the metabolism of specific fatty acid species. Although in vitro data show that L-Fabp has higher affinity for long-chain PUFA than saturated fatty acid species, the adaptations that take place in vivo are more complicated to interpret because both L-Fabp and L-Fabp are abundantly expressed within enterocytes. Studies have shown different functions for murine L-Fabp and L-Fabp in intestinal fatty acid trafficking and energy homeostasis that may explain some of the subtle differences in fatty acid composition in different lipid classes noted in our study (46). For example, it is possible that L-Fabp plays a
larger role in saturated fatty acid metabolism and L-Fabp assumes a larger role in PUFA metabolism. Alternatively, our findings are also consistent with a role for L-Fabp in the metabolism of essential dietary PUFA. In this scenario, the shift in relative abundance from increased saturated fatty acids in ApC<sup>-/-</sup> mice to increased PUFAs in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice may reflect reduced usage and metabolism of essential dietary PUFA precursors and increased reliance on saturated fatty acids in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice. This differential processing of dietary fatty acids in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice might then lead to reduced production of longer chain ω-3 and ω-6 fatty acids involved in pathways of tumor initiation and progression, the result of defective trafficking caused by L-Fabp deletion. Although fatty acid usage was not specifically examined in the current study, the observation that diacylglyceride and triglyceride species containing arachidonic acid were more abundant in ApC<sup>-/-</sup> mice compared with L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice, further substantiates the possibility that there might be reduced production of important eicosanoid and prostanoid precursors in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice. In support of this possibility, we found trends toward reduced COX-2 and PGE<sub>2</sub> expression in polyps from L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice compared with ApC<sup>-/-</sup> mice. In addition, we found significant reductions in mRNA expression of enzymes involved in fatty acid elongation and desaturation, which also suggest that alterations in fatty acid trafficking in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice may reduce generation of long-chain fatty acid substrates necessary for tumor initiation and progression. Confirmation of this hypothesis will require further studies in these lines of mice to examine whether changes in eicosanoid precursor and PGE production play a role in reduced polypr growth in L-Fabp<sup>+/+</sup>/-Apc<sup>-/-</sup> mice.

In conclusion, the present findings suggest that an array of adaptive changes in fatty acid metabolism, focused on maximizing lipogenesis and suppressing fatty acid oxidation, occurs in intestinal tumors to support the lipid substrate requirements of rapidly proliferating dysplastic cells. We propose that L-Fabp modulates intestinal tumorigenesis by altering cellular fatty acid and lipid trafficking and compartmentalization and by altering lipid substrate availability. The net results of these alterations are reduced lipid synthesis and usage for energy homeostasis, membrane synthesis, and lipid signaling required for tumor initiation and progression. Our results suggest that lipid trafficking may represent a novel pathway in understanding mechanisms involved in intestinal tumorigenesis and raise the possibility that strategies to target fatty acid flux may eventually become a therapeutic target in the prevention and treatment of intestinal tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
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References


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