Pathogenesis and reversal of liver fibrosis: Effects of genes and environment

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chapter 5
fasting reduces liver fibrosis in mouse model for chronic cholangyopathies
ABSTRACT

**Background:** Chronic cholangiopathies often lead to fibrosis, as a result of a perpetuated wound healing response, characterized by increased inflammation and excessive deposition of proteins of the extracellular matrix. Our previous studies have shown that food deprivation suppresses the immune response, which led us to postulate its beneficial effects on pathology when liver fibrosis driven by portal inflammation.

**Methods:** We investigated the consequences of fasting on liver fibrosis in Abcb4−/− mice that spontaneously develop it due to a lack of phospholipids in bile. The effect of up to 48 hours of food deprivation was studied by gene expression profiling, (immuno)histochemistry, and biochemical assessments of biliary output, and hepatic and plasma lipid composition.

**Results:** Bile composition in Abcb4−/− mice remained unchanged with fasting, in contrast to increased biliary output in the wild type counterparts. Markers of inflammation and cell proliferation dramatically decreased in livers of Abcb4−/− mice already after 12 hours of fasting. Reduced presence of activated hepatic stellate cells and actively increased tissue remodeling further propelled a decrease in parenchymal fibrosis in fasting.

**Conclusion:** This study is the first to show that food deprivation positively influences liver pathology in a fibrotic mouse model, opening a door for new strategies to improve liver regeneration in chronic disease.

*under revision*

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INTRODUCTION

Chronic cholangiopathies, like primary sclerosing cholangitis (PSC), are caused by progressive inflammation and scarring of the bile ducts in the liver (1). The inflammation impedes the flow of bile to the gut, which can lead to liver fibrosis (and cirrhosis as its ultimate stage), the final common phase of all chronic liver diseases. In general, liver injury is followed by a wound healing response, characterized by compensatory proliferation, inflammation and deposition of extracellular matrix (ECM) (2). Initially, fibrosis is reversible - normal architecture is restored by fibrolysis, and ECM producing cells are removed by apoptosis (3, 4). Recurrent chronic injury, however, results in an imbalance between fibrogenesis and fibrolysis, leading to scar formation, architectural distortion, cirrhosis and liver failure (5, 6). Liver transplantation is the only effective treatment, but surgical contraindications and the lack of donors urge for interventions that halt disease progression.

Starvation provokes a massive response of the body and an interorgan integration of the activities to prevent an irreversible loss of resources (7). When the reserves are low, there is necessity for a trade-off between the risk of starvation and disease (8, 9). It is not surprising therefore that the suppression of the immune response is also one of the major transcriptomics adaptations to food deprivation (10, 11). Given inflammation as a driving force in the pathogenesis of biliary fibrosis (12), we postulated that starvation-induced immune suppression could alleviate the pathology in sclerosing cholangitis. To assess the effect of fasting on liver fibrosis, we used the Abcb4<sup>−/−</sup> mice in which the lack of ABCB4 (ATP-binding cassette, subfamily b, member 4) leads to an absence of phosphatidylcholine in the bile, making it toxic. (13). Bile salts accumulate in the intrahepatic biliary system disrupting tight junctions and basement membranes of bile ducts, and lead to non-suppurative inflammatory cholangitis, periductal fibrosis shortly after birth, and ductular proliferation (14). Results of our study of fibrotic Abcb4<sup>−/−</sup> mouse livers, indeed, confirmed the hypothesis that food deprivation influenced pathology, and revealed an ameliorating effect on fibrosis of as short as 12 hours of fasting.

MATERIALS AND METHODS

Animals

We used 3 months old male Abcb4<sup>−/−</sup> (FVB background), and WT FVB mice as a control (Charles River, Maastricht, The Netherlands). The animals were kept separately in regular cages, and in grid bottom cages 24 h prior to and during fasting, to prevent coprophagia and intake of bedding. The study was approved by the AMC Animal Experiments Committee.

Plasma and tissue collection and analytical procedures

The gallbladders in anesthetized mice (Hynorm and Diazepam, 1 ml/kg and 10 mg/kg respectively) were cannulated; bile was collected for 15 minutes and stored at -20°C. Blood sample was collected by cardiac puncture and plasma was stored at -20°C. The liver was quickly excised and parts were snap-frozen in liquid nitrogen and stored at -80°C, or fixed in 4% buffered formalin and embedded in paraffin. Total liver collagen was determined by measuring hydroxyproline content (15). Plasma concentrations of total cholesterol, triglycerides, FFA and choline-containing PL were determined using the equivalent colourimetric enzymatic kits (Wako Chemicals GmbH, Neuss, Germany and...
bioMérieux Benelux, Boxtel, The Netherlands). Biliary BS and PL content were analyzed as described (16). Biliary cholesterol was measured fluorescently (17). Tissue lipids were extracted using a chloroform–methanol-based (2:1 by volume) method (18); cholesterol and triglycerides were measured using the CHOD-PAP (Ecoline 25 cholesterol, Merek, Darmstadt, Germany) and GPO-PAP method (Ecoline S+, DiaSys Diagnostic Systems, Holzheim, Germany), and PL was measured with an enzymatic colourimetric assay (Biolabo, Maizy, France). To correct the obtained lipid values for the amount of tissue, the protein content of the liver was measured using the BCA method (Pierce, Perbio Science Nederland BV, Etten-Leur, The Netherlands).

**Histology and immunohistochemistry**

Four animals per time point and five sections per animal were analyzed. Paraffin sections were de-waxed and stained with hematoxylin and eosin (HE) for general histology, or with 0.2% picro-sirius red (PSR) to detect fibrillar collagen. Immunohistology was performed as described (19). Sections were incubated with rat IgG2b anti-mouse F4/80 monoclonal antibody (AbD Serotec, Oxford, UK) and Ki67 (Dako, Glostrup, Denmark). Primary antibodies were detected using appropriate secondary horseradish peroxidase-conjugated polyclonal goat IgG antibodies, directed against mouse IgG2a, or rat IgG2a (Southern Biotech, Birmingham, AL). Bound peroxidase activity was visualized using H₂O₂ and 3,3′-diaminobenzidine as chromogen. Sections were counterstained with hematoxylin.

**Reverse transcription, quantitative PCR and data analyses**

Total RNA was extracted from frozen livers with TRIzol reagent (Invitrogen, Breda, The Netherlands), and its quality assessed with RNA 6000 Nano LabChip® Kit in an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, USA). qPCR was performed as described (20). The average of three house-keeping genes (36b4, Hprt and cyclophilin b) was used to normalize the expression of the studied genes. mRNA concentration was calculated using the LinRegPCR program (21, 22).

**Western blot analysis**

was performed as described (20), using antibodies against MMP2 and MMP9 (goat anti-mouse; Bio-Rad Laboratories, Veenendaal, The Netherlands), with goat anti-rabbit IgG horseradish peroxidase conjugated (Santa Cruz Biotechnology) as secondary antibody. Chemiluminescence was quantified by Lumi-Imager F1 using CDP-Star (Roche, Mannheim, Germany), using β-actine as a reference.

**Statistical analysis**

ANOVA and two-tailed Student’s t-test were used to determine statistical significance. P<0.05 is denoted by asterisks in all figures, with error bars representing the standard deviation.

**RESULTS**

**Fasting reduces ECM deposition in the livers of Abcb4⁻/⁻ mice**

To investigate the effects of fasting on the fibrotic livers in Abcb4⁻/⁻ mice, we assessed the connective tissue by picro-Sirius red staining (Fig.1A). Parenchymal fibrosis was clearly reduced already after 12h of fasting, causing a decrease of inter-portal bridging.
Figure 1: Food deprivation ameliorates parenchymal fibrosis in the livers of Abcb4−/− mice. A) Representative Picro-sirius red immunostaining of liver sections of wild-type (WT) and Abcb4−/− mice, fed or starved for 12, 24 and 48h. Scale bar represents 100 µm. B) Hydroxyproline concentration (expressed in μg/mg wet tissue) in Abcb4−/− and WT livers, 0, 12, 24h and 48h after starvation started. C-I) Relative mRNA expression level of collagens 1, 3, 4 and fibronectin 1 (components of ECM), αSma and vimentin (markers of activated HSC), and Gfap (marker of quiescent HSC), in livers at 0, 12, 24 and 48h of fasting. mRNA expression is normalized by 3 house-keeping genes and shown as fold change compared with WT control at 0h. Asterisks denote significance compared to fed condition in the corresponding strain (P<0.05), and error bars represent SD.

Total liver collagen, responsible for the structural integrity of the ECM and used as a marker for liver fibrogenesis (23), was determined by measuring hydroxyproline content. In Abcb4−/− mice it decreased 40, 70 and 80% at 12, 24 and 48h of fasting, respectively, and was not affected in WT animals (Fig.1B). mRNA expression of collagens 1 and 3 was lower in fasting in both Abcb4−/− and WT mice (Fig.1C-D). Expression of Col4 and fibronectin decreased at 12 and 24h (Fig.1E-F). These data strongly suggest a decrease in matrix deposition in response to fasting in Abcb4−/− mice.

Hepatic stellate cells (HSC) play a central role in ECM remodeling by production of MMPs and TIMPs (24, 25), and by deposition of fibrillar collagens type 1 and 3 (25). The impact of fasting was analyzed by measuring the markers for activated (α-Sma and vimentin) and quiescent HSC (Gfap). While quiescent were more abundant in the WT (Fig.1I), activated HSC were dominating in fibrotic Abcb4−/− livers (Fig.1G-H). A significant decrease in expression levels was observed for all three markers in Abcb4−/− mice, regardless of the duration of fasting (Fig.1F-I). In WT mice, these markers were downregulated after 12 and 24h of fasting. In support, the expression of fibronectin, which decreases starvation-induced apoptosis in rat HSC (26), was downregulated.
Clearly different response at 48h compared to previous time points does not come as a surprise (27), since such a long stretch in mice (comparable to several weeks in humans) provokes a complex survival response. Although we mainly focus on the effect of short and moderate fasting, we provide the later time point to complete the picture of the response in fibrotic livers.

**Fibrotic Abcb4−/− livers respond to fasting by matrix remodeling**

To assess the mechanism behind the reduced amount of ECM, we studied the components of the matrix remodeling machinery.

The expression levels of *Plau* and *Plat*, which activate MMPs (28), increased in prolonged fasting in Abcb4−/− mice (80% *Plat* and 30% *Plau*; Fig.2A-B), after an initial decrease in short fasting. In WT animals, food deprivation did not affect their gene expression. *Timp1*, which inhibits most MMP activity (29) and promotes the survival of HSC (30), was significantly downregulated by fasting in fibrotic livers at all time points (Fig.2C). *Tgfβ1*, which promotes hepatic fibrosis by prompting HSC differentiation into myofibroblasts, was 2-fold higher in fed fibrotic than in WT livers (Fig.2D). Compared to fed Abcb4−/−, *Tgfβ1* expression was 75, 70 and 50% lower at three fasting time points, respectively. Matrix remodeling in fasting, therefore, seems to be regulated by reduced expression of profibrotic cytokine *Tgfβ1* and thereby reduced number of activated HSC/MFs.

**Hepatic cell turnover is strongly reduced by fasting**

Abcb4−/− mice have an intrinsically high hepatocyte proliferation (31). We therefore analyzed the effect of fasting on proliferation markers *Pena* and *Ki67*. They decreased in both Abcb4−/− and WT mice (Fig.3B-C; *Pena* returned to control values in prolonged fasting). Immunostaining (Fig.3A) confirmed that the fibrotic livers of Abcb4−/− mice contained cycling hepatocytes and Ki67-positive cells in the portal tract. Ki67-positive cells were not found in fasted Abcb4−/− livers at any time point, or in any WT livers.
Figure 3: Fasting reduces originally high cell turnover in fibrotic livers of Abcb4−/− mice. A) Immunostaining for Ki67 in the liver sections of fed and 12h fasted WT and Abcb4−/− mice. Scale bar represents 100 µm. B-D) Gene expression of Ki67, Pena and p21 upon 0, 12, 24 and 48h of food deprivation. mRNA expression is normalized by 3 house-keeping genes and shown as fold change compared with WT control at 0h. Asterisks denote significance compared to fed condition in the corresponding strain (P<0.05), and error bars represent SD.

Fasting, therefore, was a strong enough stimulus to reduce proliferation in Abcb4−/− livers. This was apparently supported by the increased cell-cycle arrest, since the expression of p21, involved in repair of DNA damage, was strongly upregulated in Abcb4−/− mice (even 700% at 48h; Fig.3D).

The frequency of apoptotic changes (Councilman bodies) did not change dramatically in Abcb4−/− mice upon fasting, especially in acinar zones 2 (Fig.4A). Similarly, mRNA concentrations of proapoptotic markers Bax and Apaf barely changed in Abcb4−/− mice (Fig.4B-C), while in the WT they significantly decreased with fasting (40-60%). Expression of antiapoptotic Bcl-xl was unaffected by fasting in fibrotic livers, though it has strongly increased in prolonged fasting in WT mice (Fig.4D). This suggests a possible different mechanism behind apoptosis between the highly proliferative livers of fed Abcb4−/− mice, and those affected by fasting.

**Biliary output and hepatic and plasma lipid composition in fasted Abcb4−/− mice**

In contrast to the earlier reported increased biliary output in fasted WT mice (32), bile flow in Abcb4−/− mice tended to decrease after 12h (40%, P<0.06; Table 1). As expected, PL were barely measurable in Abcb4−/− animals. Cholesterol concentration was 2.7-6.5 fold lower during fasting compared to WT, while none of the measured biliary secretion rates – BS, cholesterol or PL- was affected by fasting, indicating that the Abcb4−/− mice had lost the adaptive biliary response to fasting seen in WT animals.
Figure 4: Apoptosis in fasted fibrotic livers. A) Hematoxylin-eosin staining of liver sections of fed and fasted WT and Abcb4/− mice. Scale bar represents 100 µm. B-D) Relative mRNA expression level of Bax, Apaf, and Bcl-xl, after 0, 12, 24h and 48h of fasting. mRNA expression is normalized by 3 house-keeping genes and shown as fold change compared with WT control at 0h. Asterisks denote significance compared to fed condition in the corresponding strain (P<0.05), and error bars represent SD.

Cholesterol concentration in the liver of Abcb4/− mice increased during fasting (Table 1), hepatic triglyceride concentration was strongly increased, while that of PL did not change. Hepatic TG and cholesterol concentrations were somewhat lower in fed Abcb4/− mice than in their WT counterparts, but fasting altered them so that both lipids were present in higher concentration in Abcb4/− mice.

To assess the lipid status in the systemic circulation of fasted Abcb4/− mice, we measured the plasma concentrations of cholesterol, TG, FFA, and PL (Table 1). Similarly to WT, plasma cholesterol concentration increased after 12 and 24h of fasting, TG concentration strongly decreased in longer fasting, PL concentrations remained stable, while FFA concentration increased (50 and 25% after 12 and 24h). Initially, plasma cholesterol level in fed Abcb4/− mice was two-fold lower than in the WT, and remained such also in fasting.
Fasting reduces hepatic inflammation in Abcb4−/− mice

Bile duct proliferation and portal inflammation (13) are the most prominent histopathological features of Abcb4−/− mice and are caused by secretion of toxic bile (33, 34). To assess the effect of fasting on portal inflammation, we analyzed the presence of inflammatory cells in livers of Abcb4−/− and WT mice.

Markers for activated monocytes and macrophages (Cd11b; Cd11c), neutrophils (Mpo), circulating monocytes (Mpd1), and liver-resident Kupffer cells (F4/80), as well as inflammatory marker Tnfα, were clearly (and expectedly) higher in fibrotic than in control livers (Fig. 5A-E; Mpo and Mpd1 not shown). Inflammation seemed attenuated in fasted fibrotic livers by significantly lowered expression of general markers F4/80, Cd11b and Cd11c. In fibrotic livers of fed Abcb4−/− mice, immunostaining for F4/80 showed areas with abundant periportal inflammation, with large numbers of F4/80 positive macrophages and relatively few F4/80 positive Kupffer cells in the adjacent lobules (Fig. 5A, top right panel). More Kupffer cells were present in the areas where periportal inflammation was less abundant. A striking shift in localization of F4/80 macrophages was observed at 12 and 24h of fasting (Fig. 5A, two mid-right panels). The periportal tracts contained far less F4/80 macrophages, whereas their number was increased in acinar zone 2. Upon 24 and 48h of fasting the number of macrophages was further reduced, with adherent F4/80 macrophages now often present in central veins (Fig. 5A, two bottom-right panels). In WT mice, the relative number of F4/80 positive Kupffer cells decreased only after 48h of starvation (Fig. 5A, left panels). A decrease in expression of proinflammatory marker Irf5 that promotes inflammatory macrophage polarization

**Table 1: Biliary output and hepatic and plasma lipid composition in up to 48 hours fasted Abcb4−/− and wild type mice.** The values are given as mean ± S.D. Asterisks denote significance (P<0.05)

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<td><strong>biliary lipids</strong></td>
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<td>bile flow (µl/min.100g)</td>
<td>9.5 ± 1.64</td>
<td>3.7 ± 0.9</td>
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<td>bile salts (nmol/min.100g)</td>
<td>5.6 ± 0.29</td>
<td>6.5 ± 0.4*</td>
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<tr>
<td>cholesterol (nmol/min.100g)</td>
<td>1.06</td>
<td>6.2 ± 0.8*</td>
</tr>
<tr>
<td>phospholipids (nmol/min.100g)</td>
<td>6.6 ± 1.00</td>
<td>24 h</td>
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<tr>
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<th>Abcb4−/−</th>
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<td><strong>hepatic lipids</strong></td>
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<tr>
<td>triglycerides (nmol/mg)</td>
<td>5.7 ± 0.8</td>
<td>7.9 ± 1.1</td>
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<tr>
<td>cholesterol (nmol/mg)</td>
<td>15.2 ± 0.8*</td>
<td>11.7 ± 1.6*</td>
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<tr>
<td>phospholipids (nmol/mg)</td>
<td>17.6 ± 1.2*</td>
<td>12.8 ± 2.4*</td>
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<td>triglycerides (mmol/L)</td>
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<td>4.3 ± 0.1*</td>
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<tr>
<td>free fatty acids (mmol/L)</td>
<td>2.5 ± 0.1*</td>
<td>4.5 ± 0.1*</td>
</tr>
<tr>
<td>phospholipids (mmol/L)</td>
<td>2.2 ± 0.2</td>
<td>4.9 ± 0.2*</td>
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Fasting reduces hepatic inflammation in Abcb4−/− mice
(35), and an increase of Ym1, a marker for alternatively activated macrophages, indicate a shift towards alternatively activated macrophages in fasted fibrotic livers.

![Figure 5: Fasting affects inflammatory status and macrophage localization in fibrotic livers. A) Representative Immunostainings for F4/80 in the livers of fed and fasted WT and Abcb4^{−/−} mice. B-G) Relative mRNA level of F4/80, Tnfα, Cd11b, Cd11c, Irf5 and Ym1 upon 0, 12, 24 and 48h of fasting. mRNA expression is normalized by 3 house-keeping genes and shown as fold change compared with WT control at 0h. Asterisks denote significance compared to fed condition in the corresponding strain (P<0.05), and error bars represent SD.]

**DISCUSSION**

This study reveals that fasting causes a reduction of liver fibrosis in a mouse model for biliary cholangiopathies. Rapid adaptive response to food deprivation in Abcb4^{−/−} mice (already after 12h) lowered inflammation, decreased hepatocyte proliferation, lowered the number of activated HSC/MFs, decreased production of ECM components, and increased expression of genes involved in tissue remodeling, leading ultimately to amelioration of liver fibrosis.

Collagen, responsible for the structural integrity of the ECM, was strongly affected by fasting. Within 48 hours, total collagen synthesis in livers of Abcb4^{−/−} mice (measured by hydroxyproline) decreased to 20%, similarly to the response found in the articular cartilage of fasted guinea pigs (36). This corresponds with a notion that collagen production is sensitive to changes in food intake, and that malnutrition may have profound effects on its production (37). Amount and the types of produced collagen are regulated by the nutritional state of the animal or by the disease processes - either directly as in wound healing, inflammation, and fibrosis, or indirectly, as in starvation and diabetes. In advanced starvation, collagen is one of the two major sources of energy.
fibril-forming collagens 1 and 3 and vimentin, predominantly synthesized by activated HSC (39), suggesting that the process of liver remodeling was specifically induced by food deprivation.

Chronic liver diseases are characterized by aberrant matrix deposition, calling for our attention to the role of ECM in resolution of liver fibrosis. Tissue remodeling is regulated by MMPs, involved in the ECM degradation, and TIMPs, their endogenous inhibitors. Their subtle balance maintains liver fibrogenesis. Tissue homeostasis is further regulated by proteolytic activity of the PLAU/PLAT/plasmin, responsible for the maintenance of the physiologic levels of ECM (40). PLAU promotes ECM degradation through activation of MMPs (MMP-2, -3 and -9; (41, 42), increases the differentiation of hepatic stem cells, and HGF-dependent regeneration of hepatocytes (43). PLAT protects ECM proteins from proteolytic degradation and helps expedite wound healing (44). In fed Abcb4⁺/⁻ mice, hepatic Plan and Plat expression levels were much higher than in their WT counterparts (3- and 8-fold, respectively). Short and moderate fasting initially reduced the expression of both, but prolonged fasting posed a demand for higher mRNA concentrations, indicating an ongoing balancing action between fibrolysis and fibrogenesis. Together with strongly (6-fold) reduced Timp1 expression, our data indicate that fibrotic livers responded to food deprivation by an active ECM remodeling. Matrix remodeling and in fasting was further supported by reduced expression Mmp13. Expression of this collagenase mainly produced by Kupffer cells is high in collagen 1α1 r/r mouse model that fails to recover from fibrotic liver injury, due to resistance to MMP degradation of ECM (45). However, given the contradictory findings by other groups (46), the role of Mmp13 in (resolution of) liver fibrosis remains controversial.

Apart from ECM synthesis and degradation, tissue remodeling comprises cell proliferation, death, and migration. In general, hepatocyte proliferation is low under normal conditions, except to compensate for a loss of cells (47) and it gets further reduced when the energy supply is low (48). Caloric restriction induces apoptosis and decreases hepatocyte proliferation in a murine strain with a high incidence of spontaneous liver tumors (49). We now show that, as a part of tissue remodeling, fibrotic livers of Abcb4⁻/⁻ mice respond to fasting mainly by a decrease in their pathologically high proliferation. Furthermore, consistently with previously shown increased apoptosis in HSCs in response to nutrient deprivation (26), this study shows a decrease in number of activated and quiescent stellate cells, most likely in response to decreased production of fibronectin and TIMP1, which both prevent HSCs apoptosis (26, 30).

The impressive decrease in inflammation in Abcb4⁻/⁻ mice, the macrophage migration from the periportal tract towards the central vein, and the shift from classically to alternatively activated macrophages, seem a likely driving force for improved pathology (50). Overall, altered concentrations of cytokines, metabolic and hormonal trophic factors induced by fasting could decrease inflammation in fibrotic livers. Notably, profibrotic cytokine leptin that increases TIMP1 expression (51) is strongly reduced by fasting (52). Fasting increases circulating corticosterone (53, 54), decreasing thereby the production of cytokines and interleukins (55, 56), with an immunosuppressive effects in humans (57, 58) and mice (59, 60). In Abcb4⁻/⁻ mice, persistent hepatic
inflammation triggers profibrotic signaling via activation of cytokines, promoting the formation of MFs, which in turn synthesize elevated amounts of ECM proteins (61, 62). TGF-β1, the most potent fibrogenic cytokine, prompts HSC differentiation into myofibroblasts, by enhancing expression of TIMPs (that block ECM degradation), and by directly stimulating synthesis of interstitial fibrillar collagens (12). Liver fibrosis in Abcb4−/− mice was attenuated in fasting, most likely driven by downregulation of Tgfβ1, which reduced number of activated HSC. Downregulation of a number of proinflammatory markers, a.o. Tnfa and Irf5 (a transcriptional activator of pro-inflammatory cytokines and chemokines (35)), clearly points to fasting-induced suppression of immune response. The change in number, but also localization of resident and infiltrated macrophages, may be causal in resolution of the parenchymal fibrosis (e.g. by altered production of MMPs (46, 63), especially given the pivotal but divergent roles of macrophages in matrix remodelling - favoring ECM accumulation during ongoing injury, and enhancing matrix degradation during recovery (64). In the starved animals, the high energy demand of the immune response (e.g. for production of acute phase response proteins) leads to a massive change in hepatic transcriptome directed towards its suppression (11, 50). The decrease in inflammation in Abcb4−/− mice, is therefore compatible with our previous notion that the suppression of immune response (subserving energy preservation) is one of the highlights of the overall body’s fasting response (50).

In conclusion, this study demonstrates that fasting leads to alleviation of biliary fibrosis by decreased inflammation and by actively increased matrix remodeling. Fasting in the fibrotic Abcb4−/− model may help improve understanding of the mechanisms of resolution, and inform strategies to improve liver regeneration in chronic disease.

References


