Omega-3 Fatty Acids Protect Fatty and Lean Mouse Livers After Major Hepatectomy

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Objective: The aim of this study was to assess the effect of Ω3 fatty acids (Ω3FA) on fatty and lean liver in hepatic surgery.

Background: The global spread of energy-dense diets has led to an endemic rise in fatty liver disease and obesity. Besides metabolic pathologies, steatosis enhances hepatic sensitivity to ischemia reperfusion (I/R) and impedes liver regeneration (LR). Steatosis limits the application of liver surgery, still the main curative option for liver cancer. Ω3FA are known to reverse steatosis, but how these lipids affect key factors defining surgical outcomes—that is, I/R, LR, and liver malignancy—is less clear.

Methods: We established a standardized mouse model of high fat diet (HFD)-induced steatosis followed by Ω3FA treatment and the subsequent assessment of Ω3FA effects on I/R, LR, and liver malignancy (n = 5/group), the latter through a syngeneic metastasis approach. Fatty liver outcomes were compared with lean liver to assess steatosis-independent effects. Nonparametric statistics were applied.

Results: Ω3FA reversed HFD-induced steatosis and markedly protected against I/R, improved LR, and prolonged survival of tumor-laden mice. Remarkably, these beneficial effects were also observed in lean liver, albeit at a smaller scale. Notably, mice with metastases in fatty versus lean livers were associated with improved survival.

Conclusions: Ω3FA revealed multiple beneficial effects in fatty and lean livers in mice. The improvements in I/R injury, regenerative capacity, and oncological outcomes await confirmatory studies in humans.

Keywords: Ω-3 polyunsaturated fatty acids, ischemia-reperfusion injury, liver regeneration, liver surgery

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ML, BH, MF, JFD did the experimental design. ML, PL, PKa, PKr, CT, NC did the data acquisition and analyses. BH, ML, MF, JFD, RG did the data interpretation. BH, ML did the study concept and design. BH, ML, PAC did the manuscript writing and critical revision. BH, PAC provided funding for this study. All authors approved the final version.

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The increasing consumption of fat-laden diets is putting a strain on the liver. Many societies experience an endemic rise in obesity and the metabolic syndrome (MS), often already apparent in children and young adults.[1] Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the MS and is thought to underlie its hallmark insulin resistance and its associated cardiovascular risks.[2]

Surgical removal remains the only curative option for liver tumors with an increased risk of liver failure in the presence of hepatic steatosis.[3] To prevent blood loss during liver resection, portal inflow is temporarily occluded, leading to an ischemic period that may cause significant liver injury upon reperfusion. Steatosis not only enhances the liver's sensitivity toward ischemic insults but also impedes its regenerative capacity in terms of both repairing ischemic injury and compensating for lost volume following resection.[4,5] Together, these features lead to an elevated rate of postoperative complications that restrict surgical options in patients with fatty liver.[5]

Simple and well-tolerated means to negate the impact of liver fat on surgical outcomes are highly desired. Omega-3 polyunsaturated fatty acids (Ω3FA), most known for their potential benefits to cardiovascular health,[6] may combine many properties sought for liver surgery. Although mechanisms underlying Ω3FA action are incompletely understood, observations such as the coupling of enhanced energy turnover with anti-inflammatory mechanisms are likely related to the potent anti-steatotic effects described for Ω3FA in liver.[7–9] Of note, Ω3FA have been reported to suppress steatosis also in a mouse model of diet-induced obesity[10] and in NAFLD patients.[11] The anti-inflammatory properties of Ω3FA further translate into an efficient protection of animal liver from ischemia-reperfusion (I/R) injury. We have shown that Ω3FA reduce I/R injury in lean mouse liver via engagement of Gpr120, which imprints an anti-inflammatory M2 polarization phenotype onto Kupffer cells.[12] Likewise, Ω3FA were also effective against I/R in the *ob/ob* model of NAFLD.[13] In addition, Ω3FA seem to improve recovery of lean liver from resection-induced liver failure, or of fatty liver after standard resection.[14,15] Whether such effects are indirect (eg, by protecting from injury) or direct (eg, by promoting hepatocyte proliferation) remains unclear, but the resulting benefits can also be observed in the clinic.[16] Finally, Ω3FA have been associated with anti-cancerous properties in a variety of neoplasms.[17] The effects of Ω3FA on colorectal liver metastasis (CRLM)—the most common indication for liver surgery in the Western world—are unclear. In a syngeneic rat model, Ω3FA promoted CRLM development, while in another study—based on the same rats and cancer cells—Ω3FA revealed beneficial effects.[18,19]

The effects on steatosis, hepatic I/R, liver recovery after resection, and liver tumors together suggest a fallow potential of Ω3FA to improve outcomes in liver surgery. What remains unknown is (i) whether Ω3FA's beneficial effects are simply due to fat reduction, (ii) whether Ω3FA can directly promote parenchymal regeneration after resection, and (iii) whether Ω3FA do counteract the formation or growth of liver tumors, particularly in a fatty background. Moreover, it is not established whether the single effects

are bound to a given model or rather are a general feature of Ω3FA. To address these questions, we designed a standardized mouse model of fatty liver surgery based on a high fat diet (HFD). This model enabled us to study the effects of dietary Ω3FA on steatosis, I/R, liver regeneration, and tumor risk in the settings of pre-existing NAFLD compared with a lean background. Our findings strongly encourage the pre-operative use of Ω3FA to expand the application of surgery for fatty liver.

METHODS

Animals and Diets

All animal experiments were approved by the Veterinary Office of Zurich, Switzerland. Male mice (C57BL6, Harlan) were purchased at the age of 8 to 10 weeks and kept on a 12-hour day/night cycle. After accommodation for 1 week, animals were either exposed to HFD (total energy 22 MJ/kg, 60 kJ% from fat and 8.4 kJ% sucrose) or a control diet (CD, total energy 15 MJ/kg, 11 kJ% from fat without sucrose) for 6 weeks (ssniff, Soest, Germany). During treatment, overall calories in HFD were reduced by 3 MJ/kg to mimic mild pre-operative calorie restriction, and for Ω3FA treatment, fish oil (mainly eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) replaced 45% of total fat while maintaining 8.4 kJ% sucrose (Supplementary Figure 1A, http://links.lww.com/SLA/B84). Each treatment group included 5 animals, with a total of 180 animals used for the experimental setup.

Lipid Quantification Using Small Animal MRI and Chemical Analysis

To assess liver fat content longitudinally in vivo, a small-animal 4.7-Tesla magnetic resonance imaging (MRI) scanner (Bruker BioSpin MRI, Inc., Massachusetts) was used (Supplementary Figure 1B, http://links.lww.com/SLA/B84).[20] Liver fat was chemically quantified by the Vanillin method.[21]

Surgical Procedures

Isoflurane inhalation (2% to 4%) anesthesia (Pittman-Moore, Chicago, IL) including subcutaneous injection of buprenorphine (0.1 mg/kg) was used for all surgical interventions. For ischemia/reperfusion (I/R) experiments, 68% hepatic ischemia was induced as described.[22] Animals were sacrificed 6 hours after reperfusion, a time point that well reflects injury levels at later reperfusion times.[22] Hepatectomies (68% for normal liver regeneration, 86% for inducing liver failure) were performed as reported.[23] Omegaven (parenteral fish oil emulsion; Fresenius Kabi AG, Oberdorf, Switzerland), was intravenously (i.v.) injected 1 hour before hepatectomy at 0.2 g/kg. Cell solutions (10^5/100 μl phosphate-buffered saline [PBS]) of exponentially growing MC-38 colorectal cancer cells were injected into the portal vein under selective clamping as described,[24] inducing tumors limited to the right and caudate lobes.

Cell Culture

MC-38 cells were cultured as reported.[24] Cultures were tested negative for mycoplasma at their onset (PCR Mycoplasma Test Kit; PromoCell Gmbh, Heidelberg, Germany).

Histological Analyses

Immunohistochemistry on archived liver sections was performed for Ki67 (Abcam ab16667) and pH3 (Abcam ab92628) as described.[23] Quantification was done by blindly counting nuclear positivity in 10 random visual fields.

Serum and Tissue Analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using a multiple biochemical analyzer (Dri-Chem 4000i; Fujifilm, Tokyo, Japan). Serum Hmgb1 levels were assessed by ELISA (Shino-Test, Japan, via IBL International). The malondialdehyde assay (MDA) from Oxis (Bioxytech #21012) was used to estimate lipid peroxidation.

Quantitative Real-Time PCR (qPCR)

Quantitative real-time polymerase chain reactions were run on a ABI Prism 7000 Sequence Detector System (PE Applied Biosystems, Rotkreuz, Switzerland) using following TaqMan gene expression assays (PE Applied Biosystems, Rotkreuz, Switzerland): *Il1b* (Mm00434228_m1), *Emr1* (Mm00802530_m1), *Tnfa* (Mm00443258_m1), and normalization control *18S rRNA*.[23]

Statistical Analysis

Each group consisted of n = 5 unless otherwise stated. Data are presented as mean ± SD. Group differences were assessed by Mann-Whitney testing throughout the manuscript. Survival was assessed by Kaplan-Meier analysis. Significance levels are categorized by stars ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Equicaloric Ω3FA Substitution for the Reduction of High Fat Diet Induced Steatosis

The experimental set up is shown in Figure 1A. Six weeks of HFD feeding led to a gain in mean bodyweight from 21.5 g (±1.3) to 34.3 g (±2.9), rising to 40.6 g (±3.9) after 10 weeks in the HFD control group (Figure 1B). In the HFD-Ω3FA group, bodyweight increased to 39.2 g (±4.9) indicating that Ω3FA treatment has little effect on diet-induced obesity ($P = 0.686$). Liver weight grew from 0.9 g (±0.1) to 1.8 g (±0.2) after 6 weeks and 1.9 g (±0.3) after 10 weeks in the HFD group, but was decreased by Ω3FA from 1.8 g (±0.2 g) to 1.5 g (±0.1, $P = 0.016$). Both groups had a comparable food intake during treatment (HFD 360.4 g/cage vs HFD-Ω3FA 356.5 g/cage). On histology, lipid accumulation was seen in hepatocytes after 6 weeks of HFD. After 10 weeks, severe microsteatosis with rare signs of macrosteatosis (lipid droplets, displaced hepatocyte nuclei) was present in the HFD group, while steatosis was markedly diminished in the HFD-Ω3FA group and marginal in the CD group (Figure 1C). Histology revealed no signs of fibrosis or significant immune infiltration. No significant elevations in hepatic lipid peroxidation, serum Hmgb1 levels, and inflammatory markers were noted in the HFD group (Supplementary Figure 2, http://links.lww.com/SLA/B84). Therefore, 10 weeks of HFD induce simple steatosis in mouse liver.

Chemical analysis confirmed steatosis, with lipid contents after 6 weeks being 41.1 (±12.2) and 14.2 μg/mg (±2.4, $P = 0.002$) in liver tissue from HFD and CD animals, respectively (Figure 1D). Similarly, liver fat assessed by MRI was significantly elevated after 6 weeks HFD compared with CD, further increased after 10 weeks of HFD, but was drastically reduced in the HFD-Ω3FA group (Figure 1D). Therefore, MRI was used to longitudinally monitor steatotic development and treatment effects in vivo.

Altogether, our HFD mouse feeding protocol designed to mimic western diet induces obesity and simple steatosis, while CD feeding is associated with little gain in body or liver weight [6 weeks: body 26.9 g (±2.0)/liver 1.3 g (±0.2); 10 weeks: 28.6 (±2.6)/1.5 g (±0.3); CD-Ω3FA 10 weeks: 30.9 (±3.4)/1.2 g (±0.1); $P = 0.530$ for bodyweight, $P = 0.111$ for liverweight]. Four weeks of Ω3FA treatment via dietary fat substitution are associated with a reduction in steatosis to levels similar to those in CD-fed animals

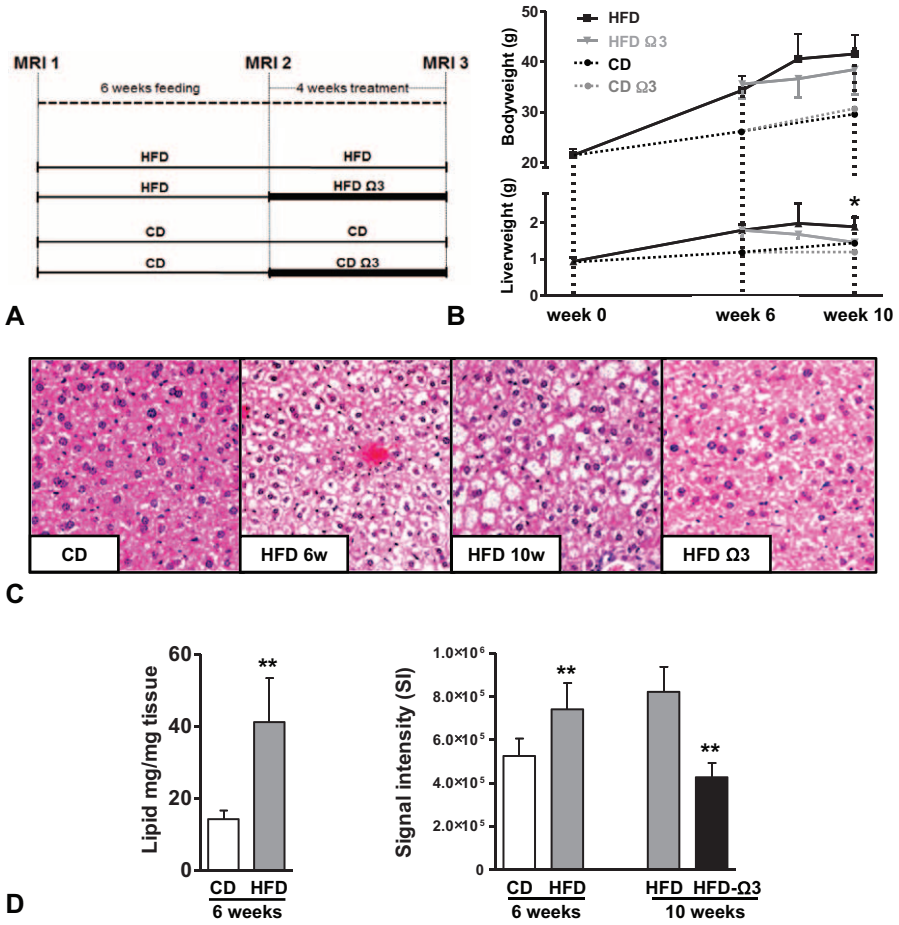

FIGURE 1. Antisteatotic effects of Ω3FA in a model of HFD-induced fatty liver disease. A, Experimental design showing schedules of HFD/CD feeding and Ω3FA treatment. Effects were assessed subsequent to the treatment unless otherwise stated. B, Body and liver weight during the feeding and treatment periods. C, Liver histology of mice exposed to CD or HFD for 6 weeks, and to another 4 weeks of HFD or HFD along with Ω3FA substitution. Pale cytoplasmic spots represent lipid vesicles. D, Chemical analysis of hepatic fat content after differential feeding, and MRI assessment of liver fat content after the feeding/treatment period. For all figures: n = 5/group unless otherwise stated; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[SI 427813 (±80,847) vs SI 526322 (±65,886), $P = 0.056$), Figure 1D], consistent with the reported Ω3FA-defattening effects.[7–11] Therefore, our HFD protocol yields a clinically relevant model to study how pre-operative Ω3FA treatment impacts surgical outcomes (I/R injury, liver regeneration, and malignant risk) complicated through fatty liver.

Ω3FA Protect Fatty Liver Against Ischemia Reperfusion

Steatosis increases the sensitivity of liver toward I/R.[13] Ω3FA can protect against I/R in genetic models of NAFLD; however, whether protection can be also achieved in diet-induced steatosis is unknown.

Following treatment, HFD and HFD-Ω3FA mice were exposed to partial hepatic ischemia for 60 minutes. Six hours after reperfusion,[22] liver injury was assessed via multiple parameters. Necrosis on histology was visibly reduced in the HFD-Ω3FA versus the HFD group (Figure 2A), as was sinusoidal plugging indicated by stuck erythrocytes. Likewise, marked reductions through Ω3FA treatment were noted for the injury markers ALT/AST, and for Hmgb1, released by necrotic cells and propagating parenchymal injury following reperfusion (Figure 2B).[25] Therefore, Ω3FA treatment of mouse liver with diet-induced steatosis offers effective protection against hepatic I/R.

Ω3FA Accelerate the Regeneration of Fatty Liver After Resection

Lipid accumulation in hepatocytes diminishes hepatoregenerative capacities, thereby increasing the likelihood of postoperative complications including liver failure.[4,5] After 68% hepatectomy (68%HX) in lean liver, the original liver weight is gradually regained (by 50% after 48 hours, 70% after 96 hours) and completely restored after 1 week.[23] HFD-induced steatosis was associated with a delayed liver weight regain after 68%HX (45% after 48 hours, 50% after 96 hours). Ω3FA treatment improved regeneration patterns to those seen in lean mice (50% after 48 hours, 70% after 96 hours), suggesting that Ω3FA can normalize regenerative capacity (Figure 3A). Ω3FA treatment also improved the liver-to-body weight ratio (LW/BW) after resection (Figure 3A), however less pronounced than for percentage increase, because Ω3FA differentially affect liver versus body weight (Figure 1B).

Counts for Ki67, marking all cycling cells, were elevated in the HFD-Ω3FA versus HFD group at most times examined after 68%HX (Figure 3B). To assess cell cycle progression, we examined pH3, a differential marker for cells in the G_2 (weak nuclear positivity) and M (bold nuclear positivity) cell cycle phases.[23] The ratio of bold versus total pH3 counts thus reflects the proportion of mitotic cells among cells past the S-phase. Compared with the HFD group, Ω3FA-treated liver displayed a significantly increased pH3 ratio at 72 and 96 hours postresection (Figure 3C). Therefore, Ω3FA treatment of

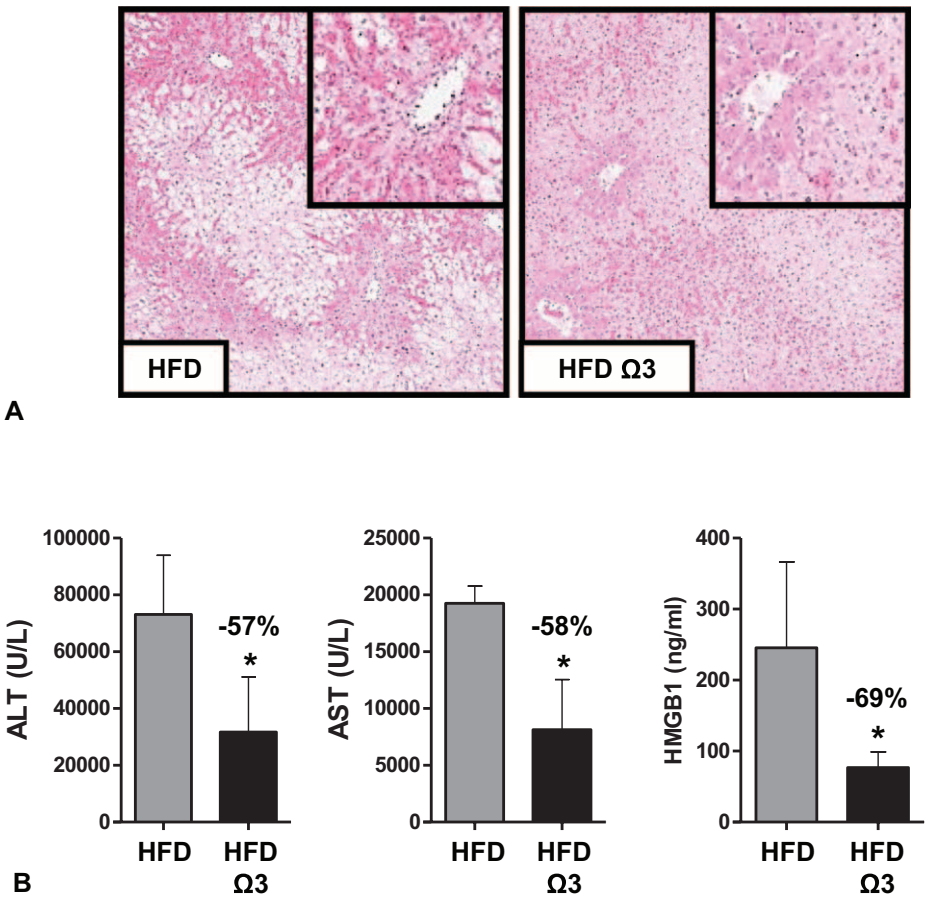

FIGURE 2. Ω3FA effects on I/R injury in fatty liver 6 hours after reperfusion. A, Histological necrosis. B, Serum ALT, AST, and Hmgb1 levels.

fatty liver promotes entry of hepatocytes into cell cycle and their mitotic progression at times when hepatocellular division usually is completed.[23]

Ω3FA Contribute to Hepatoprotection Also Independent of Fat Reduction

To investigate whether the protection of fatty liver against I/R through Ω3FA is solely due to steatosis reduction,[26] experiments were repeated in mice fed on CD for 6 weeks, and then exposed or not to CD diet enriched in Ω3FA.

After 10 weeks feeding, CD mice present with little steatosis in their liver (Figure 1C, D). Accordingly, a 4-week Ω3FA led to an insignificant reduction in lipid content (Figure 4A). When Ω3FA-CD animals were subjected to hepatic ischemia, injury levels were reduced by about a half compared with the CD group (Figure 4B, C).

We conclude that in addition to the reduced sensitivity toward I/R following fat reduction, Ω3FA also exert steatosis-independent protection against hepatic ischemia.

Ω3FA Promote Liver Regeneration in a Direct Way

To determine whether Ω3FA can improve liver regeneration also independent of fat reduction,[26] CD-fed mice treated or not with Ω3FA were subjected to 68%HX.

Regenerative improvements through Ω3FA were less pronounced in lean versus fatty liver. The percentage gain in liver weight was significantly raised by Ω3FA at 96 hours postresection time, while LW/BW tended to be increased (Figure 5A). Ki67 and pH3 counts confirmed the improvements following Ω3FA treatment, with more hepatocytes in cycle and with accelerated mitotic progression at 96 hours (Figure 5B, C). To provide additional evidence for a pro-regenerative effect of Ω3FA in lean liver, we tested an 86%HX model of regenerative deficiency due to defective cell cycle progression.[23] Ω3FA (or vehicle[12]) were i.v. injected as an emulsion (Omegaven) 1 hour before surgery to reveal their direct effects. Examining 96 hours post 86%HX, when regeneration is most delayed,[23] Omegaven increased liver weight (Figure 5D), consistent with a direct pro-regenerative effect on liver.

Ω3FA Counteract Colorectal Liver Metastases in a Syngeneic Model

Given the pro-regenerative effects of Ω3FA, their impact on liver malignancy was unclear.

To estimate potential effects of Ω3FA on hepatic malignancy, we used a syngeneic, orthotopic mouse model of CRLM, wherein MC-38 cancer cells are injected in the portal vein under selective clamping, leading to lobe-restricted tumor growth that is the major determinant of survival.[24] We tested 2 settings (Figure 6A): the direct impact of Ω3FA treatment on the establishment and growth of CRLM (cancer cell injection before treatment), and the indirect impact, that is, how CLRM development is affected after liver has been treated with Ω3FA (injection after treatment, similar to surgery-induced disease recurrence). Tumor inoculation before treatment led to a median survival of 36 days (from injection) in HFD animals (Figure 6B), with median liver weight at 4.3 g (±1.4). In contrast,

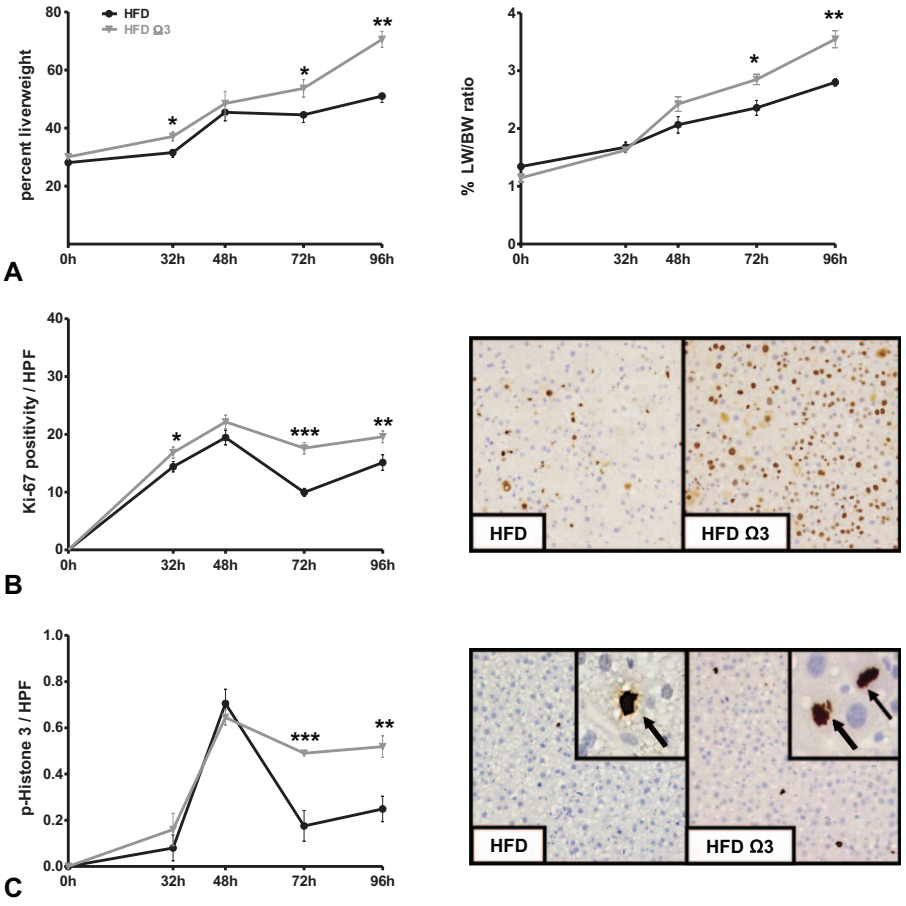

FIGURE 3. Ω3FA effects on regeneration of fatty liver after 68% hepatectomy. A, Percent liver weight after hepatectomy in HFD and HFD-Ω3FA mice. B, LW/BW after hepatectomy. C, Percentage of Ki67-positive hepatocytes after hepatectomy. Photographs show representative examples of Ki67 staining. D, Differential pH3 counts after hepatectomy. The ratio displays the number of hepatocytes with bold pH3 staining (marking M cells, see examples marked by arrows) divided by the number of all pH3-positive cells (marking G_2 and M cells).

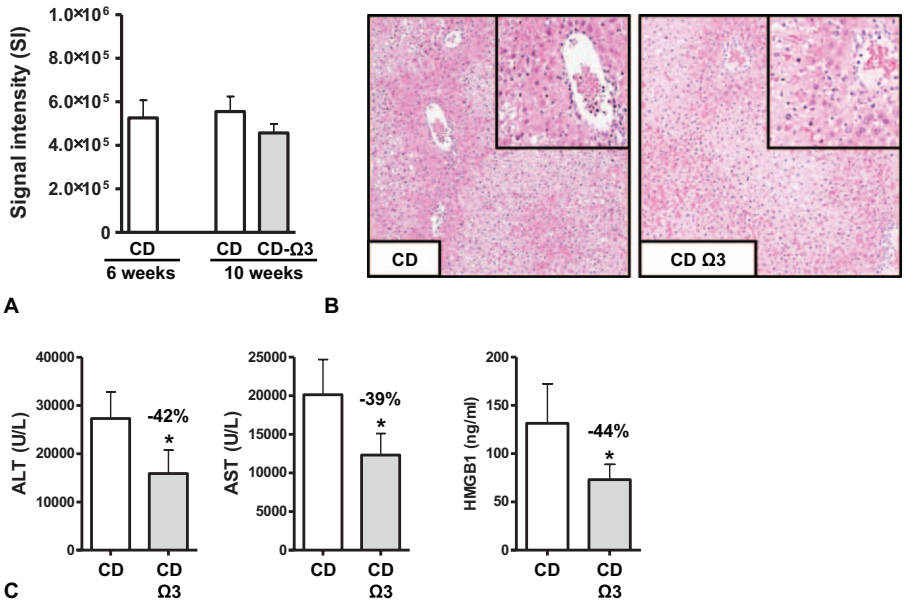

FIGURE 4. Ω3FA effects on I/R injury in CD liver. A, MRI signal intensity representing liver fat in mice on a CD and treated or not with Ω3FA (see Fig. 1D for HFD liver). Note the small effect of Ω3FA treatment on the MRI signal. B, Histological necrosis and C, Serum ALT, AST, and Hmgb1 levels at 6 hours after reperfusion.

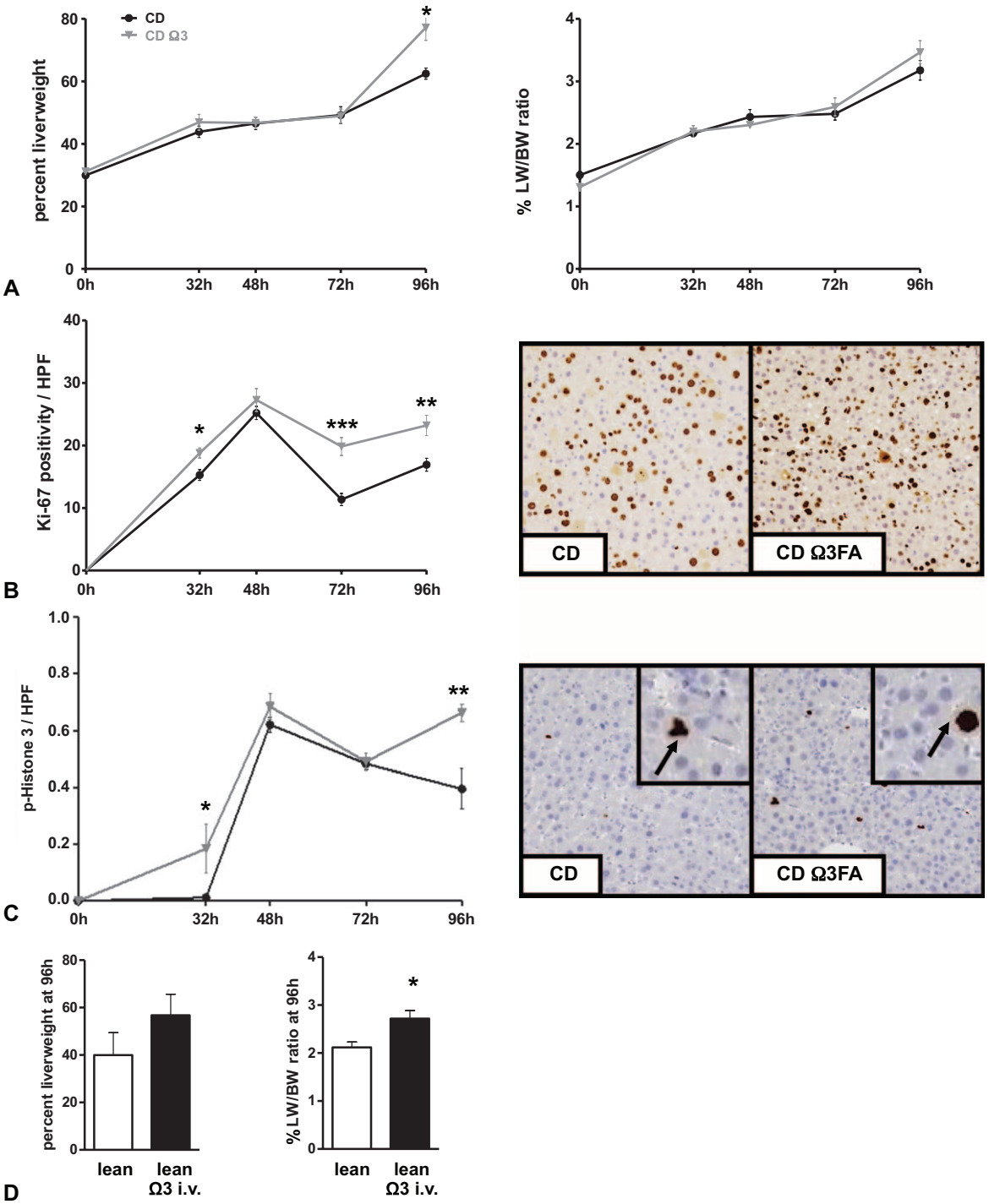

FIGURE 5. Ω3FA effects on regeneration of CD liver after hepatectomy. A, Percent liver weight and LW/BW after hepatectomy in CD or CD-Ω3FA mice. B, Percentage of Ki67-positive hepatocytes after hepatectomy. C, Differential pH3 counts after hepatectomy. Arrows mark pH3-positive mitotic cells. D, Percent liver weight and LW/BW at 96 hours after extended (86%) hepatectomy with/without concomitant Ω3FA injection.

survival in HFD-Ω3FA animals was 59 days ($P = 0.07$) and liver weight 3.8 g (±0.4), suggesting Ω3FA inhibit CRLM in pre-existing steatosis. For tumor inoculation after treatment, survival after injection was 40 days [liver weight 5.2 g (±1.7) in the HFD group and was extended to 47 days ($P = 0.156$; liver weight 5.6 g (±1.1)] in the HFD-Ω3FA group (Figure 6C). To better assess the latter differences, liver weight (n = 3/group) was measured at day 30 postinjection and was 2.9 g (±0.5) for HFD and 1.5 g (±0.3), $P = 0.100$) for

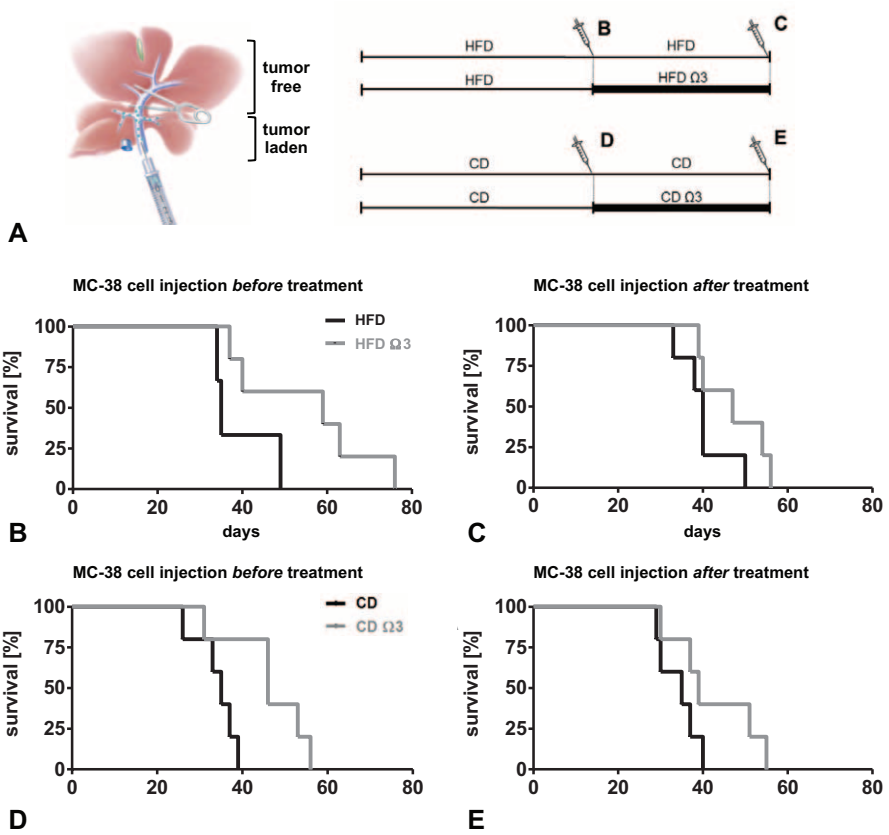

FIGURE 6. Ω3FA effects on CRLM in HFD and CD liver. A, Selective portal vein injection of cancer cells and experimental setup to estimate Ω3FA effects on CRLM. B, Kaplan-Meier survival curves for HFD/HFD-Ω3FA mice injected with cancer cells before treatment. C, Survival for HFD/HFD-Ω3FA mice injected with cancer cells after treatment. D, Survival for CD/CD-Ω3FA mice injected with cancer cells before treatment. E, Survival for CD/CD-Ω3FA mice injected with cancer cells after treatment.

HFD-Ω3FA animals, indicating Ω3FA delay CRLM development as confirmed on MRI (Supplementary Figure 3, http://links.lww.com/SLA/B84). Therefore, Ω3FA maintain some anticancer effects in liver also after treatment cessation.

Above experiments were repeated in mice on a CD. For inoculation before treatment, survival was 35 days in CD and 46 days in CD-Ω3FA mice [$P = 0.027$; liver weights 3.6 g (±0.9) and 4.5 g (±1.5), respectively]. For inoculation after treatment, survival was 35 days in CD and 39 days in CD-Ω3FA mice [$P = 0.137$; liver weights 4.8 g (±1.9) and 4.3 g (±1.3), respectively]. Therefore, Ω3FA may also inhibit CRLM in lean liver, albeit modestly.

We conclude that Ω3FA may exert anticancer effects on both fatty and lean liver even beyond their administration. Ω3FA are hence unlikely to compromise oncological outcomes in liver surgery.

DISCUSSION

In this study, we showed in a mouse model of HFD-induced NAFLD that preoperative Ω3FA treatment confers several benefits influencing the outcome of major surgery. We observed that steatotic reduction associated with the use of Ω3FA has relevant positive consequences on (i) the sensitivity of liver to I/R, (ii) its regenerative capacity following tissue loss, and (iii) seemingly also on CRLM, a prime indication for liver surgery. Furthermore, we found that Ω3FA maintain—at a smaller scale—these beneficial effects in the absence of steatosis, indicating the mode of action of Ω3FA extends beyond the sole reduction of fat. Therefore, Ω3FA may suit to expand the application of liver surgery in situations of both fatty and nonfatty liver surgery.

The health benefits of Ω3FA are manifold and cannot be attributed to a single mechanism. Dietary Ω3FA comprise a number of fatty acids, of which EPA and DHA are the major constituents. Apart from modulating the composition and function of cell membranes, Ω3FA can activate specific receptors (eg, GPR120[12]) and are further metabolized into a series of bioactive lipids (eg, resolvins, protectins), which participate in cellular signaling and are intermingled with other lipid signaling systems, such as by acting as ligands of cannabinoid-type receptors.[27,28] This underlying complexity suggests that detailed action mechanisms of Ω3FA will vary to provide dynamic responses toward specific situations. More broadly, Ω3FA however do have phenotypic effects that may be considered as general attributes of these lipids. The anti-inflammatory action (eg, nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) inhibition), the promotion of a healthy endothelial phenotype (eg, NO production), and the changes in energy turnover (eg, promotion of β-oxidation, inhibition of mammalian target of rapamycin [mTOR] signaling) reminiscent of a fasting response (eg, activation of silent mating type information regulation 2 homolog 1 [SIRT1], 5′ adenosine monophosphate-activated protein kinase [AMPK]) likely are properties of Ω3FA that contribute to their beneficial effects on steatosis, hepatic I/R, liver regeneration, and cancer.[9,29–32]

Inflammation, endothelial dysfunction, and alterations in oxidative metabolism have all been causally linked to steatosis.[10,33,34] We have previously shown that relieving steatosis-associated vasoconstriction via Ω3FA normalizes the oversensitivity toward I/R,[13] while Ω3FA-GPR120 mediated inhibition of inflammatory Kupffer cells protects lean liver akin to fasting.[12,25] Endothelial effects clearly will add, because sinusoids are the first to experience the consequences of ischemia. Proper endothelial function likewise is a prerequisite for liver regeneration after resection,[35] and the improvement of the defective regeneration seen in aged liver is associated with the re-installation of vascular integrity.[36] How Ω3FA's anti-inflammatory

action affects liver regeneration is unclear; for example, secretion of tumor necrosis factor (TNF) from Kupffer cells is needed for regeneration,[37] but excess TNF, often elevated in fatty liver,[38] causes liver failure after hepatectomy.[39] Furthermore, targeting inflammatory Kupffer cells has a little effect on regeneration,[40] suggesting that inflammatory contributions to regeneration are more complex than thought.

Inflammation and pathological angiogenesis are hallmarks of cancer; the trend seen for Ω3FA in inhibiting CRLM is hence not surprising.[32,41] A tendency to prolonged survival through Ω3FA was observed in all 4 settings (HFD vs CD, injection before vs after treatment) we tested, indicating that Ω3FA may be beneficial, but certainly not harmful, with regard to CRLM. The largest effect on survival was seen when cancer cells were injected before treatment, thus when the early CRLM development was fully exposed to Ω3FA. When injected after treatment, Ω3FA levels are expected to decline over time, explaining their diminished ''anticancer'' effects. Of note, survival in general was shortened in lean versus fatty liver, perhaps suggesting an anti-malignant effect for steatosis. We speculate this was due to better perfusion in lean liver,[13] facilitating migration/transport of cancer cells from the portal vein into the sinusoids and thereby increasing the initial malignant load. Although controversial, some clinical observations have reported a reduced incidence of CRLM in steatotic patients and an improved survival in steatotic/obese CRLM patients,[42–45] consistent with steatosis creating a microenvironment unfavorable to invasion and colonization of liver by malignant intestinal cells.

Altogether, Ω3FA treatment of fatty and lean liver was beneficial across the key aspects related to major liver surgery. To foster a potential clinical application, we have initiated a clinical trial (NCT01884948) to assess the impact of Ω3FA on outcomes after major liver surgery. This study should also inform on the Ω3FA activities in I/R, regeneration, and steatosis in patients.[46]

The use of Ω3FA as a measure to expand the application of surgery to fatty liver, or lean liver requiring extended resection, comes with several advantages; the pre-operative administration of Ω3FA is simple, either as a food supplement or via intravenous injection (Omegaven). Therefore, little issues with compliance are expected, unlike for the currently recommended NAFLD treatment, that is, exercise combined with caloric restriction.[31] Given that Ω3FA are being ingested as part of our food and have been assessed in various trials, potential side effects will not hamper their clinical use.[47] Indeed, prolonged application such as needed for ''defattening'' may exert additional benefits, including the mitigation of the MS, cardiovascular disease, or other comorbidities associated with NAFLD.[31] More research is needed to identify which of the Ω3FA lipids and at what dosages may induce specific benefits in a given setting.

In conclusion, our findings suggest a potential of Ω3FA in extending surgical options for both fatty and lean livers. Apart from the steatotic changes, the benefits of Ω3FA include an increased tolerance toward ischemic reperfusion injury, an improved regenerative capacity, and a trend toward lower mortality due to liver tumors. These effects partially are not only a consequence of the Ω3FA-associated defattening but also occur in the absence of significant steatosis, implying that the anti-inflammatory, vascular, and metabolic effects of these lipids are likewise beneficial to physiological liver function. Their ease of application, their safety profile, their affordability, and their many potential health benefits with regard to comorbidities or oncological indications advocate their use in the clinic. Considering the endemic rise in fatty liver disease, Ω3FA-based strategies hold a strong position in improving outcomes and should be tested in convincing clinical studies.

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