Decreasing mitochondrial fission alleviates hepatic steatosis in a murine model of nonalcoholic fatty liver disease

Chad A. Galloway,1 Hakjoo Lee,2 Paul S. Brookes,1 and Yisang Yoon2
1Department of Anesthesiology, University of Rochester School of Medicine and Dentistry, Rochester, New York; 2Department of Physiology, Medical College of Georgia, Georgia Regents University, Augusta, Georgia

Submitted 19 May 2014; accepted in final form 24 July 2014

Galloway CA, Lee H, Brookes PS, Yoon Y. Decreasing mitochondrial fission alleviates hepatic steatosis in a murine model of nonalcoholic fatty liver disease. Am J Physiol Gastrointest Liver Physiol 307: G632–G641, 2014. First published July 31, 2014; doi:10.1152/ajpgi.00182.2014.—Mitochondria produce the majority of cellular ATP through oxidative phosphorylation, and their capacity to do so is influenced by many factors. Mitochondrial morphology is recently suggested as an important contributor in controlling mitochondrial bioenergetics. Mitochondria divide and fuse continuously, which is affected by environmental factors, including metabolic alterations. Underscoring its bioenergetic influence, altered mitochondrial morphology is reported in tissues of patients and in animal models of metabolic dysfunction. In this study, we found that mitochondrial fission plays a vital role in the progression of nonalcoholic fatty liver disease (NAFLD). The development of hepatic steatosis, oxidative/nitrative stress, and hepatic tissue damage, induced by a high-fat diet, were alleviated in genetically manipulated mice suppressing mitochondrial fission. The alleviation of steatosis was recapitulated in primary hepatocytes with the inhibition of mitochondrial fission. Mechanistically, our study indicates that fission inhibition enhances proton leak under conditions of free fatty acid incubation, implicating bioenergetic change through manipulating mitochondrial fission. Taken together, our results suggest a mechanistic role for mitochondrial fission in the etiology of NAFLD. The efficacy of decreasing mitochondrial fission in the suppression of NAFLD suggests that mitochondrial fission represents a novel target for therapeutic treatment of NAFLD.

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is one of the most prevalent metabolic diseases, with higher incidence in obese and diabetic individuals (9, 30). NAFLD is proposed to advance through a “multi-hit” mechanism (13, 18). Initially hepatic fat accumulation causes steatosis, which can progress to nonalcoholic steatohepatitis following a second hit, such as oxidative stress from increased levels of reactive oxygen species (ROS).

Mitochondria are central to cellular metabolism. Housed within mitochondria are the enzymes of the tricarboxylic acid cycle and β-oxidation, which produce reducing equivalents for the electron transport chain (ETC). Sequential oxidoreductase reactions occur in the complexes of the ETC and contribute to the generation of the proton-motive force that drives ATP synthesis. In converting metabolic inputs to usable cellular energy, the ETC also generates ROS as a byproduct (2, 40). Hyperlipidemic conditions enhance mitochondrial ROS production (6, 33, 36, 48), which contribute to the oxidative stress developed in NAFLD. Mitochondria are also morphologically dynamic, exhibiting shape change through fission and fusion (10, 45). Dynamin-related mechanoenzymes mediate mitochondrial fission and fusion (11, 12, 37). Dynamin-like-related protein 1 (DLP1/Drp1) constricts and severs mitochondrial tubules for fission. Mitofusins (Mfn1/2) and optic atrophy 1 (OPA1) fuse the outer and inner mitochondrial membranes, respectively. These processes are essential to normal cellular and mitochondrial physiology and bioenergetic homeostasis (22, 25, 41).

Metabolic diseases are associated with altered mitochondrial structure and bioenergetic deficiencies, which are accompanied by increased mitochondrial DNA damage and protein modification by ROS (5, 26, 36, 42). Increases in metabolic input, i.e., elevated glucose and/or free fatty acids, are associated with mitochondrial fragmentation in multiple tissues (21, 24, 31, 32, 46). Enhanced mitochondrial fission under conditions of greater metabolic flux coincides with elevated levels of ROS, causing oxidative damage and cell death (15, 33, 34, 39, 47, 49). Notably, suppression of mitochondrial fission in cells treated with high concentrations of glucose or fatty acids was sufficient to suppress increased ROS and cell death (32, 41, 46, 47). Mitochondrial fission is therefore proposed to be mechanistic precursor of enhanced ROS in metabolic excess and could be a target to relieve oxidative stress associated with metabolic disease. Indeed, we reported that decreasing mitochondrial fission in vivo suppressed oxidative stress and kidney dysfunction in a model of type I diabetes (19).

Given that mitochondrial fission is an effector for ROS increase in metabolic stimulation, we hypothesized that mitochondrial fission is integral to NAFLD. In the present study, we evaluated the role of mitochondrial fission in NAFLD using a high-fat (HF)-fed mouse model. Mice fed an HF diet presented with phenotypes consistent with fragmented mitochondria and metabolic dysfunction associated with NAFLD. Remarkably, transgenic inhibition of mitochondrial fission in HF-fed mice suppressed, not only hepatic oxidative stress, but also steatosis. In this manner, our findings demonstrate that the inhibition of mitochondrial fission is effective in preventing both the first and a secondary hit of NAFLD, highlighting a new therapeutic strategy for this disease.

MATERIALS AND METHODS

Transgenic mice, feeding regimen, and blood and tissue sampling. All experiments involving animals were performed according to procedures approved by the University Committee on Animal Resources at the University of Rochester Medical Center and IACUC of Georgia Regents University. Transgenic mice expressing the dominant-negative fission mutant DLPL-K38A in a doxycycline-inducible manner were created as previously described (19). DLPL-K38A ex-
pression was induced by diet (0.83 kcal/g protein; 0.78 kcal/g fat; 2.14 kcal/g carbohydrate) supplemented with 200 mg/kg doxycycline (Bioserv no. S3888). HF rodent diet containing 60% of daily caloric intake by fat (0.82 kcal/g protein; 3.24 kcal/g fat; 1.43 kcal/g carbohydrate) (Bioserv no. S3282) was supplied to mice beginning at 8 wk of age, ad libitum. A custom HF diet (Bioserv no. S3282) containing 200 mg/kg doxycycline was manufactured by Bioserv. Blood glucose levels were measured by tail vein sampling after an overnight (16 h) fast. For glucose tolerance tests, mice were fasted overnight prior to an intraperitoneal injection of a glucose bolus of 1 mg/g in 0.9% saline, and blood glucose values were then obtained. All mice were euthanized in the morning using CO2.

Histology and immunohistochemistry. Samples for electron microscopy (EM) were fixed by submersion, epoxy embedded, and imaged by transmission EM. Samples for histology were fixed in 4% paraformaldehyde. Hematoxylin and eosin-stained slides were prepared by a clinical pathology core. For nitro-tyrosine reactivity, rehydrated slices were incubated with anti-nitro-tyrosine, 1:250 overnight (Milipore no. AB5411), followed by a peroxidase-conjugated secondary antibody. Diaminobenzidine substrate was used to visualize immunoreactivity, with hematoxylin counterstaining.

Mitochondrial morphology assessment. Electron micrographs at ×8,000 were analyzed using ImageJ (NIH) to define the perimeter of mitochondria and calculate aspect ratio (AR = long axis/short axis) and form factor [FF = 1/circularity (4π × area/perimeter²)]. The relative levels of mitochondrial remodeling proteins were monitored in whole cell lysates by Western blot. Densitometry of Western blot signals (ImageJ) for mitochondrial fission factor (Mff), fission 1 (Fis1), DLP1, Mfn2, and OPA1 was corrected to β-actin and then normalized to values from mice fed control diet.

Primary hepatocyte isolation and treatments. Primary hepatocytes were isolated from 8–12-wk-old mice as previously reported (19) and plated in low-glucose DMEM containing 10% FBS. DLP1-K38A was expressed by adenoviral infection (Ad-DLP1-K38A), and green fluorescent protein (GFP)-expressing virus (Ad-GFP) was used to control for infection (19). In fat-loading experiments, serum-free DMEM was supplemented with 200 μM palmitate-BSA complex or 0.2% fatty acid-free BSA alone as a control, for 24 h before Oil
Red O staining. Palmitate was conjugated with BSA as previously described (43).

Oxygen consumption analyses. Whole cell oxygen consumption rate (OCR) was measured in intact primary hepatocytes using a Clark-type oxygen electrode in a sealed chamber (Mitocell 200 system, Strathkelvin Instruments). Oligomycin was added to induce ATP synthase inhibition, and maximal OCR was induced by addition of the protonophore trifluorocarbonyl cyanide phenylhydrazone (FCCP). OCR in the presence of 200 μM palmitate-BSA or 0.2% BSA control was measured simultaneously using a dual-oxygen chamber in serum-free low-glucose DMEM. Once a steady basal OCR was reached, palmitate or BSA, oligomycin, FCCP, and antimycin A (inhibitor of complex III) were sequentially injected to the chambers. OCRs were obtained by subtracting OCRantimycin A.

Statistical analyses. To estimate the effect of HF feeding, separate mixed-effect models were fit to the repeated measures of percentage weight gain and blood glucose level on each mouse with these treatments for 24 wk. To compare the effects of the HF diet, doxycycline (Dox), and genotypes in combination, separate mixed-effect models were fit to the repeated measures of weight and percentage weight gain on each mouse. These models were each fit by restricted-maximum-likelihood estimation using the mixed procedure in SAS version 9.3. Least-squares mean differences and contrasts between treatments were estimated at prespecified time points. All other experimental end points were evaluated by Student’s t-test.

RESULTS

HF feeding in B6SJL/129 mice results in pathophysiology consistent with NAFLD. To investigate a role of mitochondrial fission in NAFLD in vivo, we established an HF feeding regimen in the background strain of our transgenic mice expressing a mutated DLP1, DLP1-K38A. These mice were generated by crossing ROSA26-rT A mice in the 129 genetic background with mice carrying TRE-DLP1-K38A in a B6SJL background (19). One group of mice was maintained on an HF-feeding regimen and another on control diet from 8 wk of age. HF-fed mice were significantly heavier beginning 2 wk into the regimen (Fig. 1A). At the conclusion (24 wk), HF-fed mice had gained 70% of their original body weight vs. 19% in control diet-fed mice (P < 0.0001) (Fig. 1B). At week 17, HF-fed mice had an average blood glucose level of 190 mg/dl, significantly higher (P < 0.0001) than the blood glucose level of 142 mg/dl in control diet-fed mice. Increased blood glucose levels were maintained through 24 wk with final fasted blood glucose levels nearly double in HF-fed mice vs. control diet-fed mice (Fig. 1C). Consistent with metabolic dysfunction, HF-fed mice had delayed clearance of glucose in a glucose tolerance test at week 20, with elevations in both fasted and final glucose values (Fig. 1D). Elevated blood glucose levels
were maintained despite an approximately threefold increase in fasted insulin levels in the HF-fed mice (Fig. 1E), indicative of insulin resistance.

A hallmark of NAFLD is the development of hepatic steatosis. Livers from 24-wk HF-fed mice displayed histology consistent with pathological hepatic steatosis, with large intracellular voids where fatty infiltrates resided (Fig. 1F). Additionally, macrovesicular steatosis along with hepatocyte ballooning were observed in HF-fed mice (Fig. 1F, insets). Collectively, these results demonstrate that B6SJL/129 mice on an HF diet recapitulate whole body and hepatic pathologies commonly associated with obese and type II diabetic phenotypes in patients with NAFLD.

The development of hepatic steatosis is coincident with altered mitochondrial morphology. We next analyzed hepatic mitochondrial morphology in 24-wk HF-fed mice. Electron micrographs indicated that mitochondria in livers from HF-fed mice tended to be shorter and more round than those in control diet-fed mice (Fig. 2, A and B). Cristae morphology and matrix density were similar between the two groups (Fig. 2, A′ and B′). Mitochondrial shape was assessed by AR and FF (19, 46). Perfectly round mitochondria exhibit a value of 1 for both AR and FF, with values increasing as mitochondria become longer and branched (28). The average AR and FF were significantly lower in HF-fed mice (Fig. 2C), demonstrating that HF diet induces small and round mitochondria, indicative of mitochondrial fragmentation. Western blot analyses of liver lysates from control diet and HF-fed mice revealed increases of the fission proteins Mff and DLP1 (Fig. 2D). Consistent with prior observations in skeletal muscle (1), there was also a significant decrease in the fusion protein Mfn2 in the HF-fed group. These alterations in the fusion/fission machinery are consistent with the small round morphology observed by EM.

The decrease of mitochondrial fission during HF feeding suppresses hepatic steatosis and oxidative stress. To investigate a role of mitochondrial fission in NAFLD, we utilized transgenic mice inducibly expressing the dominant-negative fission mutant DLP1-K38A (19). These mice are double transgenic (dTg), carrying both rtTA and DLP1-K38A, and the expression of DLP1-K38A in these mice requires Dox. We previously showed Dox-induced expression of DLP1-K38A in the liver of these mice (19). We found that Dox feeding alone significantly increased body weight in both control (rtTA) and dTg mice (P < 0.0001) (Fig. 3A). Weight gain at the end of the 24-wk Dox treatment was ~50% of the original body weight for both control and dTg (Fig. 3B), with no significant difference between control and dTg. The effect of adding HF diet to Dox significantly increased the average weight of mice (P = 0.0047) (Fig. 3A). Control mice fed HF with Dox showed an approximate 65% increase of body weight (Fig. 3B), signifi-

![Fig. 3. Enhanced weight gain with expression of the dominant negative DLP1-K38A in mice fed HFD. A and B: body weights of rtTA mice (cont) or rtTA & DLP1-K38A double transgenic (dTg) fed control diet or HFD supplemented with doxycycline (Dox). Dox diet alone increased body weight, and HFD and Dox further increased it. DLP1-K38A-expressing mice showed enhanced weight gain compared with control mice fed the same diet at weeks 18 and 24. ****P ≤ 0.0001. C: final fasted blood glucose (BG) values showing HFD-induced hyperglycemia in both control and dTg mice. Error bars represent SE, (n = 4–7).](http://ajpgi.physiology.org/).
significantly more than those receiving Dox alone ($P < 0.0001$). dTg mice fed HF and Dox gained a remarkable 102% at the end of 24-wk feeding (Fig. 3, A and B), 37% more than control mice fed HF and Dox ($P < 0.0001$). Weight gain was similar in control and dTg up to the weeks 12–14. As observed previously (44), the gain slowed in control mice beginning around week 14. However, dTg mice continued weight gain throughout (Fig. 3A). This difference is highlighted in the calculated weight gain percentage at 6-wk intervals, showing significant differences at weeks 18 and 24 (Fig. 3B). The transition from rapid to slow gain in HF diet could be attributed to changes in energy intake and metabolic efficiency (44). Although it is currently unclear, the lack of transition in weight gain rate in dTg suggests that a decrease in mitochondrial fission may alter energy metabolism. We found that Dox alone did not increase the fasted blood glucose level despite a significant weight gain (Fig. 3C). However, HF plus Dox significantly increased blood glucose (Fig. 3C), consistent with the notion that the observed hyperglycemia is due to HF diet.

We next examined liver histology after the 24-wk feeding period. Prolonged treatment with tetracycline derivatives has been shown to cause liver steatosis (17). However, we observed no change in liver histology after 24-wk Dox feeding (Fig. 4, A, B, and E), indicating that a prolonged Dox supplementation has little effect on hepatic steatosis despite significant weight gain. The addition of HF diet to Dox resulted in a substantial increase in steatosis (Fig. 4C). Identical to our observations with HF feeding alone (Fig. 1), the histology depicted macrosteatosis after HF/Dox feeding. Remarkably, however, we found that the same diet (HF/Dox) yielded markedly reduced hepatic steatosis in dTg mice that expressed DLP1-K38A (Fig. 4D). Quantification revealed a threefold reduction of steatosis by DLP1-K38A expression (Fig. 4F), indicating that inhibition of mitochondrial fission was protective against development of steatosis. Because body weight and blood glucose levels remained elevated in dTg mice, suppression of the steatotic phenotype by DLP1-K38A is not likely due to a global recovery of the metabolic phenotype, but it is likely through a hepatocyte autonomous mechanism.

To further evaluate mechanisms of the protective effect exerted by DLP1-K38A expression, oxidative stress was examined histologically by nitro-tyrosine reactivity, which is increased when superoxide is overproduced. In HF/Dox-fed control mice, most nitro-tyrosine reactivity was associated with
fatty infiltrates (Fig. 5B), and quantitation showed an approximately sixfold increase in HF/Dox over Dox alone in control mice (Fig. 5D). DLP1-K38A expression reduced the nitrotyrosine signal by fivefold in HF/Dox (Fig. 5, C and D), demonstrating that decreased mitochondrial fission ameliorates HF-induced oxidative stress.

Elevated serum levels of alanine transaminase (ALT) are a marker of liver damage. Serum ALT levels were increased nearly fourfold in control mice fed HF/Dox compared with Dox alone. DLP1-K38A expression in HF-fed mice substantially decreased serum ALT levels (Fig. 5E), indicating decreased hepatic damage. However, no significant changes were found for aspartate amino transferase (AST) and alkaline phosphatase (ALP) levels (Fig. 5E). AST levels are known to be less specific to steatotic liver damage in murine models (23). The lack of change in ALP levels suggests that the damage does not involve the bile duct.

These data demonstrate that inhibiting mitochondrial fission suppresses lipid accumulation in HF-fed mice.

Inhibition of mitochondrial fission through DLP1-K38A expression suppresses lipid accumulation in primary hepatocytes. To investigate hepatocyte-autonomous effects of mitochondrial fission on steatosis and oxidative stress, we utilized a primary hepatocyte culture system. Primary mouse hepatocytes were infected with either adenovirus expressing DLP1-K38A (Ad-DLP1-K38A) to inhibit mitochondrial fission or Ad-GFP as control. To monitor lipid accumulation, hepatocytes were incubated with 200 μM palmitate-BSA or BSA alone, and lipid accumulation was assessed by Oil Red O staining after 24 h. In control cells, palmitate loading increased the Oil Red O-stained area by 1.6-fold vs. BSA alone (Fig. 6, A, B, and E). However, palmitate loading did not increase lipid accumulation in hepatocytes expressing DLP1-K38A (Fig. 6, C, D, and E). These data demonstrate that inhibition of mitochondrial fission in hepatocytes suppresses lipid accumulation.

Expressing DLP1-K38A in hepatocytes utilizing palmitate increases proton leak. Our observations that DLP1-K38A expression suppresses steatosis without preventing an obese phenotype suggest that decreased mitochondrial fission may...
alter energy metabolism in a hepatocyte autonomous manner, supported by in vitro fat-loading experiments in primary hepatocytes (Fig. 6). Thus we analyzed the respiratory capacity of hepatocytes subjected to palmitate treatment. OCR was measured with consecutive additions of 200 μM palmitate-BSA complex or BSA alone, followed by oligomycin, and FCCP (Fig. 7). Antimycin A was added at the end to measure and correct for nonmitochondrial OCR. Basal OCRs were not significantly different between GFP- and DLP1-K38A-expressing cells (Fig. 7, A–D). Addition of BSA did not increase respiration (OCR_{BSA}/OCR_{basal} = 1.05 for Ad-GFP and 1.03 for Ad-DLP1-K38A) (Fig. 7, A, C, and E). In contrast, addition of palmitate-BSA complex (Pal) significantly increased OCR by >30% in both control and DLP1-K38A cells. The OCR_{Pal}/OCR_{basal} ratios were 1.32 for Ad-GFP and 1.31 for Ad-DLP1-K38A, showing no difference in the OCR increase between control and DLP1-K38A cells (Fig. 7, B, D, and E). These results indicate that hepatocytes readily utilize palmitate as a respiratory substrate and that DLP1-K38A expression does not alter fatty acid oxidation.

The OCRs in the presence of oligomycin and FCCP represent leak respiration and maximum respiration, respectively (3). A substantial increase of maximum respiration was observed in the presence of palmitate in control cells, compared with BSA (Fig. 7, A and B), presumably attributable to increased electron input to the respiratory chain through palmitate oxidation. This observation supports a notion that substrate oxidation has a significant control over uncoupled maximum respiration (3). The capacity of the palmitate-induced increase in uncoupled respiration was maintained in DLP1-K38A-expressing cells (Fig. 7, C and D), indicating that fission inhibition does not affect the respiratory complex activities. In contrast, further respiration analyses found that DLP1-K38A expression significantly increased proton leak in the presence of palmitate. The leak respiration (OCR_{Olm}) was generally higher in DLP1-K38A cells compared with control in the presence of BSA (13.2 vs. 11.8 nmol/10^6 cells per minute, respectively), and this difference became larger with palmitate addition (15.2 for control and 18.8 nmol/10^6 cells per minute for DLP1-K38A). Substantiating this observation, OCR_{Olm}/OCR_{basal} was significantly higher in DLP1-K38A cells in the presence of palmitate (Fig. 7F). These results indicate that expression of DLP1-K38A increases the inner membrane proton leak with the palmitate treatment.

An increase of proton leak accelerates electron transport and thus promotes substrate oxidation. In this manner, hepatocytes expressing DLP1-K38A and subjected to palmitate or HF diet could catabolize the fat more rapidly, resulting in reduced lipid accumulation. Additionally, because increased proton leak decreases mitochondrial ROS production, DLP1-K38A expression would also suppress ROS levels and oxidative stress in HF diet. In summary, our study indicates that inhibiting mitochondrial fission lessens NAFLD by limiting steatosis and oxidative stress, presumably through an increase of proton leak.

DISCUSSION

Circumstantial observations have associated mitochondrial morphology changes with alterations in metabolic flux. Metabolic stimuli (e.g., glucose and free fatty acids) were shown to evoke changes in mitochondrial morphology, mostly a profission phenotype (20, 45), which is consistent with our in vivo observations reported here (Figs. 1 and 2). This relationship between mitochondrial morphology and bioenergetic function, coupled with the observed mitochondrial dysfunction in patients with NAFLD, prompted us to hypothesize that changes in mitochondrial morphology are critical to the progression of the disease.

We previously reported that DLP1-K38A expression decreased ROS levels and oxidative stress in hyperglycemia both in vitro and in vivo (19, 46, 47), foreshadowing the suppression of oxidative stress/tissue damage by DLP1-K38A in the hepatic tissue of NAFLD model (Fig. 5). Rather unexpectedly, we found that the expression of DLP1-K38A significantly suppressed hepatic steatosis. Our in vitro studies showed that inhibition of mitochondrial fission increased proton leak with palmitate treatment, consistent with our prior report (19) and possibly accounting for the decreased lipid accumulation. Pro-
ton leak is nonproductive in oxidative phosphorylation, promoting futile use of substrate. Furthermore, it has been shown that treatment of hepatic cells with palmitate enhances ROS production from ETC (33), linked to mitochondrial hyperpolarization. Under these conditions, increased proton leak decreases ROS production (4). An HF feeding regimen in mice has been reported to increase the uncoupling protein-2 (UCP2) level, decreasing the membrane potential through proton leak and effectively reducing ROS production (16). Although we did not observe an increase in UCP2 after 24-wk HF diet in our current study (results not shown), the study supports a similar proton leak-mediated mechanism in suppression of ROS generation. We reason that proton leak induced by DLP1-K38A expression, not only decreased steatosis, but also lowered oxidative stress in the livers of HF-fed mice. Further studies investigating the fate of the palmitate and alternative manipulations of mitochondrial fission are necessary to elaborate on the mechanistic underpinnings and generality of the proton leak effect. In addition, the mechanisms linking inhibited mitochondrial fission to greater proton leak remain to be elucidated.

It is counterintuitive that DLP1-K38A expression enhances weight gain in HF-fed mice because proton leak would be predicted to promote weight loss via futile burning of substrates. However, the control of body weight involves the integrated physiology of whole body energy metabolism, in which the hypothalamus regulates energy homeostasis through coordinating food intake and energy expenditure. In our transgenic mice, DLP1-K38A expression is driven by ubiquitous promoters (CMV and ROSA26), and, although expression of DLP1-K38A in the brain was limited in dTg mice (19), it is possible that a limited expression still affects neuronal function in hypothalamus and contributes to the weight gain. Concordant with this, a recent report indicated that preventing mitochondrial fusion in Agrp neurons of hypothalamus decreased weight gain on an HF diet (14). More directly related to our observation of proton leak, silencing DLP1 in the glucose-sensing hypothalamic region was found to decrease ROS production, whereas increasing uncoupled respiration (7). The blunted ROS signaling resulted in decreased satiation, inducing increased food intake (7). Further studies utilizing brain- or liver-specific expression of DLP1-K38A will further delineate...
the role of mitochondrial fission in regulating body weight and energy homeostasis.

We observed that additional weight gain in HF-fed DLP1-K38A mice was coincident with increased visceral adipose tissue. Apolipoprotein B100 (ApoB100) is an essential scaffold protein secreted with hepatic lipid particles. Palmitate incubation and endoplasmic reticulum (ER) stress, conditions associated with enhanced ROS, are reported to inhibit ApoB100 secretion from liver (8, 35), a potential cause of hepatic steatosis. We observed a decrease in hepatic oxidative stress by DLP1-K38A expression. Possibly, a reduced sensing of ROS under DLP1-K38A expression could allow more hepatic triglycerides to be secreted with ApoB100, redistributing hepatic fat to adipose tissue. It has been shown that NAFLD is accompanied by the ER stress (29, 38). Given that the ER-mitochondria contact plays a role in ER stress and that the ER is integral to fatty acid homeostasis (18), it is possible that inhibition of mitochondrial fission may provide a protective effect through decreasing ER stress in HF conditions. Interestingly, one report suggests that DLP1 may be involved in the ER remodeling (37) although this process has not been extensively studied. A direct role for remodeling the ER architecture by DLP1 and its possible role in lipid synthesis and subsequent secretion would be a potential line of investigation.

The progression of NAFLD requires, not only the development of steatosis, but also an additional secondary hit that has been proposed to be oxidative stress, the result of enhanced mitochondrial ROS production. Targeting mitochondrial ROS in NAFLD treatment has precedence in clinical trials with MitoQ (27), a mitochondrially directed antioxidant, although it was prematurely ended because of poor enrollment (clinical-trials.gov ID: NCT01167088). Currently, a clinical trial assessing the efficacy of RGMX001, a mixture of antioxidants (vitamin E and silymarin) combined with carnitine to treat NAFLD, lends credence to this approach (clinicaltrials.gov ID: NCT01511523). Our study found that decreasing mitochondrial fission significantly decreased both steatosis and oxidative stress in HF diet, suggesting that it has a role in regulating hepatic lipid handling and oxidative stress associated with NAFLD. Controlling both critical events in NAFLD progression by mitochondrial fission may provide an effective therapeutic avenue for preventing disease progression.

ACKNOWLEDGMENTS

The authors thank Dr. Shannon Bailey of UAB for her critical reading of the manuscript.

GRANTS

This work was supported by National Institutes of Health Grants DK078618 and DK061991 to Y. Yoon, the American Heart Association fellowship 12POST9430003 and Department of Anesthesiology discretionary funds to C. Galloway, and R01-HL71158 to P. Brooks. Some statistical analyses in this publication were supported by the University of Rochester CTSA award number UL1 TR000042. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES