METABOLIC, ENDOCRINE, AND GENITOURINARY PATHOBIOLOGY

Impaired Fasting-Induced Adaptive Lipid Droplet Biogenesis in Liver-Specific *Atg5*-Deficient Mouse Liver Is Mediated by Persistent Nuclear Factor-Like 2 Activation

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Lipid droplets (LDs) are intracellular organelles that store neutral lipids as energy reservoir. Recent studies suggest that autophagy is important in maintaining the homeostasis of intracellular LDs by either regulating the biogenesis of LDs, mobilization of fatty acids, or degradation of LDs in cultured cells. Increasing evidence also supports a role of autophagy in regulating glucose and lipid metabolism *in vivo* in mammals. In response to fasting/starvation, lipids are mobilized from the adipose tissue to the liver, which increases the number of intracellular LDs and stimulates fatty acid oxidation and ketogenesis. However, it is still controversial and unclear how impaired autophagy in hepatocytes affects the biogenesis of LDs in mouse livers. In the present study, it was demonstrated that hepatic autophagy-deficient (L-*Atg5*) knockout mice had impaired adaptation to fasting-induced hepatic biogenesis of LDs. The maladaptation to fasting-induced hepatic biogenesis of LDs in L-*Atg5* knockout mouse livers was not due to hepatic changes of *de novo* lipogenesis, secretion of very-low-density lipoprotein or fatty acid β -oxidation, but it was due to persistent nuclear factor-like 2 activation because biogenesis of LDs restored in L-*Atg5*/nuclear factor-like 2 double-knockout mice. (*Am J Pathol* 2018, 188: 1833–1846; <https://doi.org/10.1016/j.ajpath.2018.04.015>)

Lipid droplets (LDs) are intracellular organelles that primarily store neutral lipids such as triglycerides (TGs) and sterol esters within cells. It is generally thought that LDs are originated from the endoplasmic reticulum, which bud off into the cytoplasm to form the LDs with the limiting phospholipid monolayer.^{1,2} TGs are synthesized from free fatty acids (FFAs) and glycerol-3-phosphate by several key enzymes in the endoplasmic reticulum. First, glycerol-3-phosphate is acylated by glycerol-3-phosphate acyltransferases to generate lysophosphatidic acid. Second, lysophosphatidic acid is then converted to phosphatidic acid by adding an additional acyl chain via family members of 1-acylglycerol-3-phosphate acyltransferases. Third, diacylglycerol is synthesized from phosphatidic acid by the lipid proteins that have phosphatidate phosphatase activity in mammals. Finally, diacylglycerol O-acyltransferase 1 and 2 mediate the final step of

esterifying diacylglycerol to produce TGs, which is then packed into LDs.^{1,2} The formation of LDs serves as a reservoir of energy for the excess FFAs because TGs can be released when nutrient is scarce. Moreover, the formation of LDs can also help to protect cells against the cytotoxicity of FFAs, which is termed lipotoxicity.^{3,4}

Humans and animals have developed sophisticated adaptive mechanisms in response to the fluctuation of nutrient availability through evolution. In response to

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decreased energy and nutrient intake, the concentrations of circulating glucose and insulin are low, which stimulates the lipolysis of LDs at the adipose tissue.⁵ In the fasted state, the levels of catecholamines and cellular cAMP increase in adipose tissue that activate protein kinase A. Activated protein kinase A then phosphorylates a number of LD-associated proteins, including perilipin 1, hormone sensitive lipase, and comparative gene identification-58. Comparative gene identification-58 binds with patatin-like phospholipase domain containing 2/adipose TG lipase, which increases the catalytic activity of patatin-like phospholipase domain containing 2/adipose TG lipase. The stored TGs in LDs are sequentially hydrolyzed into diacylglycerol and monoacylglycerol and eventually into nonesterified FFAs and glycerol by patatin-like phospholipase domain containing 2/adipose TG lipase, hormone sensitive lipase, and monoacylglycerol lipase.³ Increased adipose tissue lipolysis then mobilizes FFAs to the liver, resulting in the increased hepatic accumulation of lipid, whereby they are oxidized to generate acetyl-CoA and ketone bodies to produce energy.^{6,7}

In addition to the neutral lipase-mediated hydrolysis of TGs in LDs, macroautophagy (hereafter referred to as autophagy) mediates lipid transport from LDs to the lysosomes for lipolysis.^{3,8,9} Autophagy is an intracellular degradation system via the formation of autophagosome and the later autolysosomes. Autophagy degrades long-lived proteins, organelles, and other cellular contents to provide nutrients for cell survival under starvation conditions.^{10,11} Recent evidence suggests that autophagy-related (Atg) proteins are associated with LDs and autophagy may also degrade LDs or at least a portion of LDs, a process that is termed as lipophagy.¹² Impaired hepatic autophagy has been reported in nonalcoholic fatty liver disease and type 2 diabetes.¹³ In addition, pharmacologic modulation of autophagy pathway improves both nonalcoholic and alcoholic steatosis in mice.^{14–16} These observations support an important role of autophagy/lipophagy in regulating lipid metabolism during the pathogenesis of nonalcoholic and alcoholic fatty liver diseases. Of interest, the numbers of LDs increased rather than decreased when autophagic flux increased under starvation conditions in several cell culture models.^{17–19} Moreover, pharmacologic inhibition of autophagy or genetic knockout (KO) of Atg5 in mouse embryonic fibroblasts (MEFs) resulted in decreased numbers of LDs after starvation.¹⁹ Increased formation of LDs has been suggested to counteract the potential lipotoxicity due to the release of FFAs by autophagy/lipophagy-mediated TG hydrolysis in these cultured cells.¹⁹ In line with these *in vitro* findings, liver-specific Atg7 or FIP200 KO mice also had decreased numbers of LDs in the liver after overnight fasting.^{20,21} The mechanisms of how impaired hepatic autophagies lead to decreased formation of LDs in mouse livers after fasting are not clear. Under starvation conditions, the physiological adaptive mechanisms in mice could be

different from cultured cells. In response to starvation, there are active cross talks among different organs/tissues and changes of circulating endocrine factors in mice, which are all lacking in cultured cells. To maintain systemic energy balance during starvation, liver increases the uptake of FFAs derived from the increased lipolysis in white adipose tissues, resulting in rapid body fat mobilization and accumulation of LDs in the liver.^{3,5} FFAs activate peroxisome proliferator-activated receptor α (PPAR α), which increases hepatic FA oxidation and ketogenesis. PPAR α may also increase the production of hormone fibroblast growth factor (FGF) 21 from the liver during fasting to further stimulate hepatic FA oxidation and ketogenesis.⁷

Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) is a transcription factor, which is activated to up-regulate the expression of cellular antioxidant genes in response to oxidative stress.^{22,23} Under normal conditions, Nrf2 binds with Kelch-like ECH-associated protein 1 (Keap1), which recruits Cullin 3-Ring Box Protein 1 E3 ubiquitin ligase complex and targets Nrf2 for degradation by the 26S proteasome. Oxidative/electrophilic stress modifies a key cysteine residue of Keap1, resulting in the disruption of Keap1-dependent degradation of Nrf2 that allows the nuclear translocation of Nrf2. Once it enters the nuclei, Nrf2 then forms a heterodimer with small musculoaponeurotic fibrosarcoma protein, which activates the target gene transcription by binding to the antioxidant response element. This above-mentioned Nrf2 regulation is termed canonical Nrf2 activation.²⁴ In addition to canonical Nrf2 activation, Nrf2 can also be activated by the noncanonical pathway through SQSTM1/p62 (hereafter referred to as p62), a protein that generally accumulates in cells with impaired autophagy.^{25–27} p62 directly interacts with Keap1, resulting in Nrf2 accumulation and activation.^{28,29} We and others previously demonstrated that persistent activation of Nrf2 is responsible for the profound pathologic changes, including hepatocyte damage, liver inflammation, fibrosis, compensatory proliferation, and benign tumor formation, in liver-specific Atg5 (L-Atg5) and L-Atg7 KO mice.^{27,30,31} Interestingly, accumulating evidence suggests that Nrf2 also regulates lipid metabolism and plays a role in fatty liver diseases although conflicting observations have been reported, likely due to different diets and mouse models that were used.^{32–35} However, the role of Nrf2 activation in regulating hepatic lipid metabolism in autophagy-deficient mouse livers is unclear.

In the present study, we demonstrated that hepatic autophagy-deficient mice (L-Atg5 KO mice) had impaired adaptation to fasting-induced hepatic accumulation of LDs. The maladaptation to fasting-induced hepatic accumulation of LDs was not due to changes of *de novo* lipogenesis, secretion of hepatic very-low-density lipoprotein (VLDL) or FA β -oxidation but was associated with persistent Nrf2 activation in L-Atg5 KO mouse livers.

Materials and Methods

Cell Culture and Primary Hepatocyte Isolation

Atg5^{-/-} (*Atg5* KO) and *Atg7*^{-/-} (*Atg7* KO) MEFs and their matched wild-type (WT) MEFs were generously donated by Dr. Noboru Mizushima (University of Tokyo, Tokyo, Japan) and Dr. Masaaki Komatsu (Niigata University School of Medicine, Niigata, Japan), respectively. MEFs were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Gibco, Gaithersburg, MD), supplemented with L-glutamine and penicillin/streptomycin. All cell culture materials were obtained from Invitrogen (Carlsbad, CA), and all chemicals were obtained from Sigma-Aldrich (St. Louis, MO) if not specifically stated. Mouse hepatocytes were isolated by a retrograde, nonrecirculating perfusion of livers with 0.05% Collagenase Type IV (Sigma-Aldrich) as described previously.³⁶ Cells were cultured in William's medium E with 10% fetal bovine serum but no other supplements for 2 hours for attachment. Cells were then cultured in the same medium without serum overnight before they were loaded with oleic acid (OA; 250 μ mol/L). Briefly, a 20 mmol/L solution of OA in 0.01 N NaOH was incubated at 70°C for 30 minutes, and FA soaps were then complexed with 5% bovine serum albumin (BSA) in phosphate-buffered saline at a 7:1 molar ratio of FAs to BSA as we described previously.³⁷ The OA/BSA conjugates were administered to the cultured cells. Five percent BSA was used as a vehicle control. All cells were maintained in a 37°C incubator with 5% CO₂.

Antibodies and Reagents

Antibodies used in the study were CD36 (ab64014; Abcam, Cambridge, UK), rabbit anti-*Atg5* antibody (PM050; MBL, Woburn, MA), p62 (H00008878-M01; Abnova, Taiwan), β -Actin (a5541; Sigma-Aldrich), and glyceraldehyde-3-phosphate dehydrogenase (2118; Cell Signaling Technology, Danvers MA). The rabbit polyclonal anti-LC3B antibody was made by using a peptide that represented the NH₂-terminal 14 amino acids of human LC3B and an additional cysteine (PSEKTFKQRRTFEQC) as described before.³⁸ The secondary antibodies used in this study were horseradish peroxidase-conjugated goat anti-mouse (115-035-062; Jackson ImmunoResearch, West Grove, PA) or rabbit antibodies (111-035-045; Jackson ImmunoResearch).

Animal Experiments

Atg5^{flox/flox} mice (C57BL/6J129) were generated by Dr. Noboru Mizushima as described previously³⁹ and were crossed with Albumin-Cre mice (Alb-Cre, C57BL/6J) (The Jackson Laboratory, Bar Harbor, ME) for at least eight generations to generate *L-Atg5* KO mice. *Nrf2*^{-/-} (C57BL/6J) mice were obtained from The Jackson Laboratory and were further crossed with *Atg5* F/F, Alb Cre+ mice to generate *Atg5* F/F, Alb Cre+/*Nrf2*^{-/-} double-knockout (DKO) mice.

Alb Cre-matched littermates were used as control WT mice in this study. All animals received humane care. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center. Two- to 3-month-old male *L-Atg5* KO, *L-Atg5* KO/*Nrf2*^{-/-} (*L-Atg5*/*Nrf2* DKO), *Nrf2*^{-/-} (*Nrf2* KO), and matched WT littermates were either on regular chow diet or fasted for overnight. After overnight fasting, some mice were refed with chow diet for another 6 hours before they were euthanized. Liver tissues were harvested, and hematoxylin and eosin staining as well as Oil Red O staining of liver sections were performed as described previously.⁴⁰ Liver TG and cholesterol were extracted and quantified as described previously.⁴⁰ Total liver lysates were prepared by using radio-immunoprecipitation assay buffer [1% NP40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl (lauryl) sulfate] with protease inhibitor.

Immunoblot Assay

Total liver lysates (20 μ g per sample) were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with primary antibodies, followed by secondary horseradish peroxidase-conjugated antibodies. SuperSignal West Pico chemiluminescent substrate (Pierce, Waltham, MA) was used for the membrane development.

Serum Lipid and Other Biochemical Analysis

Serum cholesterol (Pointe Scientific, Canton, MI), TG (Pointe Scientific), FFAs (BioVision, San Francisco, CA), and free glycerol (Sigma-Aldrich) as well as β -hydroxybutyrate (β -HB) (BioVision) were measured with colorimetric assay kits according to the manufactures' instructions. Pooled plasma samples (five to six mice) were used for fast protein liquid chromatographic analysis of lipoprotein profile, which was conducted by the Mouse Metabolic Phenotyping Center at the University of Cincinnati (Cincinnati, OH). Serum FGF21 levels were measured by using a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN). All other reagents were either from Sigma-Aldrich or Invitrogen.

Fluorescence Microscopy

Cultured primary hepatocytes and MEFs were seeded in 12-well plates with microscope cover glasses (Thermo Fisher Scientific, Waltham, MA). Cells were loaded with 250 μ mol/L OA or BSA control for 6 hours. After treatments, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline. Cells were further stained with 1 μ mol/L Bodipy 493/503 and 1 μ mol/L Hoechst 33342. An inverted confocal microscope (Nikon, Melville, NY) or a Nikon Eclipse 200 fluorescence microscope was used for all imaging studies.

Electron Microscopy

Liver tissues were cut into small pieces and fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4), followed by 1% OsO₄. Thin sections were stained with uranyl acetate and lead citrate after dehydration. Images were acquired digitally by using a JEM 1011CX electron microscope (JEOL, Peabody, MA). Randomly selected 15 to 20 cells under each condition were used to quantify the number of LDs and autophagosomes.

Real-Time PCR

Trizol (Invitrogen) was used to isolate liver RNA, and RNA was reverse transcribed into cDNA by RevertAid Reverse Transcriptase (Fermentas, Burlington, ON, Canada). Real-time quantitative PCR was used to quantify the genes for lipid metabolism and was performed on an Applied Biosystems Prism 7900HT real-time PCR instrument (ABI, Foster City, CA), using Maxima SYBR green/roX quantitative PCR reagents (Fermentas). The primer sequences are described in [Table 1](#). *Actb* was used for normalization.

Results

Loss of Nrf2 Suppresses the Impaired LD Accumulation in L-Atg5 KO Mouse Livers after Fasting

To study the role of autophagy and Nrf2 activation in fasting-induced adaptive accumulation of LD in the liver, WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were fasted overnight. Consistent with previous reports,^{7,20,32,41} Oil Red O staining for neutral lipids showed increased hepatic steatosis in fasted WT mice as expected. In contrast, fasting-induced steatosis was almost absent in the L-Atg5 KO mouse livers ([Figure 1A](#)). Electron microscopic studies also showed increased accumulation of LDs in WT mouse livers after overnight fasting ([Figure 1, B and C](#)). L-Atg5 KO mice have slightly increased number of LDs, but most of these LDs were small and enwrapped in the aberrant multimembrane structures after fasting ([Figure 1, B and C](#)). L-Atg5 KO mice have persistent Nrf2 activation, which contributed to the liver pathogenesis, including cell death, inflammation, fibrosis, and tumorigenesis in L-Atg5 KO mice.^{26,27} Interestingly, L-Atg5/Nrf2 DKO and Nrf2 KO mice had the normal hepatic fasting response as demonstrated by the increased hepatic accumulation of LDs after fasting ([Figure 1, A–C](#)). The levels of hepatic TGs and cholesterol levels in WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were further determined under the fed, fasting, and refed conditions. Consistent with the Oil Red O staining results, the levels of fasting hepatic TGs markedly increased (approximately sixfold) compared with the fed state, which declined almost to the levels of the fed state after refed in WT mice ([Figure 2A](#)). The levels of hepatic cholesterol, serum TGs, and cholesterol did not change significantly under the fed, fasting, and refed

conditions in WT mice ([Figure 2, B and C](#)). Strikingly, the levels of hepatic TGs remained almost unchanged regardless of the fed, fasting, or refed condition in L-Atg5 KO mice. Similar to WT mice, the levels of hepatic cholesterol and serum TGs did not change in L-Atg5 KO mice under the fed, fasting, and refed conditions except that the serum TG levels were slightly higher in L-Atg5 KO mice than that of WT mice at the basal (fed) state. In addition, the serum TG levels in Nrf2 single-KO mice were also relatively higher than the other genotypes of mice under the fed, fasting, and refed conditions ([Figure 2C](#)). However, the serum cholesterol levels were significantly higher in L-Atg5 KO mice than their matched WT mice regardless of the fed, fasting, and refed conditions ([Figure 2D](#)), suggesting that L-Atg5 KO mice have hypercholesterolemia. Fast protein liquid chromatographic analysis confirmed that serum VLDL-TG levels were relatively higher in fasting Nrf2 KO mice ([Figure 2E](#)). Consistent with the increased total cholesterol levels in L-Atg5 KO mice, fast protein liquid chromatographic analysis also showed that fasting serum levels of LDL cholesterol and high-density lipoprotein cholesterol were much higher in L-Atg5 KO mice. However, L-Atg5/Nrf2 DKO and Nrf2 KO mice had almost the same levels of fasting serum cholesterol profiles with the WT mice ([Figure 2, F and G](#)). The body weight of age-matched WT and L-Atg5 KO mice was comparable from 2 to 9 months old ([Supplemental Figure S1A](#)). As reported previously,²⁶ the liver-to-body weight ratio was much higher in L-Atg5 KO mice than their matched WT mice, suggesting that L-Atg5 KO mice have hepatomegaly. In response to the fasting and refed conditions, the ratio of liver-to-body weight slightly decreased in WT and L-Atg5 KO mice. L-Atg5/Nrf2 DKO and Nrf2 KO mice had almost the same ratio of liver-to-body weight with the WT mice and did not change in response to the fasting and refed conditions ([Supplemental Figure S1B](#)). Taken together, these results indicated that fasting-induced hepatic LD accumulation was impaired in L-Atg5 KO mice, which was corrected by further deletion of Nrf2 in L-Atg5 KO mice.

Autophagy and Nrf2 Signaling Changes in WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO Mice in Response to Nutrient Changes

Increased hepatic protein levels of p62 and microtubule-associated protein 1 light chain 3 (LC3)-I form were found in L-Atg5 KO mice, indicating the defect of autophagy in these mouse livers ([Supplemental Figure S2](#)). The levels of p62 and LC3-I decreased in L-Atg5/Nrf2 DKO mice compared with L-Atg5 KO mice, suggesting Nrf2 may regulate p62 and LC3 at the transcription level as previously reported.^{42,43} In addition to increasing LD accumulation, fasting also increased the number of autophagosomes in WT mouse livers ([Supplemental Figure S3, A and B](#)). Some of the double membrane structures had enveloped mitochondrion ([Supplemental Figure S3A](#)). Close association of LDs was also found with intracellular vacuoles and some LDs

Table 1 List of Primers Used for Real-Time Quantitative PCR

Gene	Forward primer	Reverse primer
<i>Abca1</i>	5'-GCTTGTTGGCCTCAGTTAAGG-3'	5'-GTAGCTCAGGCGTACAGAGAT-3'
<i>Abcg5</i>	5'-AGGGCCTCACATCAACAGAG-3'	5'-GCTGACGCTGTAGGACACAT-3'
<i>Abcg8</i>	5'-CTGTGGAATGGGACTGTACTTC-3'	5'-GTTGGACTGACCACTGTAGGT-3'
<i>Acaca</i>	5'-CTCCAGGACAGCACAGATCA-3'	5'-TGACTGCCGAAACATCTCTG-3'
<i>Acox</i>	5'-CAGGAAGAGCAAGGAAGTGG-3'	5'-CCTTCTGGCTGATCCCATA-3'
<i>Actb</i>	5'-TGTTACCAACTGGGACGACA-3'	5'-GGGGTGTGAAGGTCTCAA-3'
<i>Cd36</i>	5'-ATGGGCTGTGATCGGAACG-3'	5'-AGCCAGGACTGCACCAATAAC-3'
<i>Cpt1a</i>	5'-CCAGGCTACAGTGGGACATT-3'	5'-GAACTTGCCCATGTCCTTGT-3'
<i>Crot</i>	5'-TACTTTTACCACGGCCGAAC-3'	5'-GACGGTCAAATCCTTTTCCA-3'
<i>Dgat1</i>	5'-TCCGTCCAGGGTGGTAGTG-3'	5'-TGAACAAAGAATCTGCAGACGA-3'
<i>Dgat2</i>	5'-GCGCTACTTCCGAGACTACTT-3'	5'-GGGCCTTATGCCAGGAAACT-3'
<i>Fasn</i>	5'-TGGGTCTAGCCAGCAGAGT-3'	5'-ACCACCAGAGACCGTTATGC-3'
<i>Fgf21</i>	5'-CTGGGGGTCTACCAAGCATA-3'	5'-CACCCAGGATTTGAATGACC-3'
<i>Gyk</i>	5'-ATCCGCTGGCTAAGAGACAACC-3'	5'-TGCACTGGGCTCCCAATAAGG-3'
<i>Hmgcr</i>	5'-TCTTGTTGGAATGCCTTGTGA-3'	5'-GCGACTATGAGCGTGAACAA-3'
<i>Insig1</i>	5'-CACGACCACGTCTGGAATAT-3'	5'-TGAGAAGAGCACTAGGCTCCG-3'
<i>Ldlr</i>	5'-TGACTCAGACGAACAAGGCTG-3'	5'-ATCTAGGCAATCTCGGTCTCC-3'
<i>Gapdhm</i>	5'-AGGTTTCAAGATCGCAATGG-3'	5'-CTCCTTGGTGCTCCACTAGC-3'
<i>Acadm</i>	5'-CTCGCCATCGCCCTCACTG-3'	5'-ACCGCTCACTCGCTCTTTGC-3'
<i>Mttp</i>	5'-CTCTTGGCAGTGCTTTTCTCT-3'	5'-GAGCTTGTATAGCCGCTCATT-3'
<i>Pgc1a</i>	5'-ATGTGTGCGCTTCTTGCTCT-3'	5'-ATCTACTGCCTGGGGACCTT-3'
<i>Ppara</i>	5'-ATGCCAGTACTGCCGTTTTC-3'	5'-GGCCTTGACCTTGTTTCATGT-3'
<i>Pparg</i>	5'-TTTTCAGGGTGCCAGTTTC-3'	5'-AATCCTTGCCCTCTGAGAT-3'
<i>Scarb1</i>	5'-TTTGAGTGGTAGTAAAAAGGGC-3'	5'-TGACATCAGGGACTCAGAGTAG-3'
<i>Scd1</i>	5'-TGCGATACACTCTGGTGCTC-3'	5'-TAGTCGAAGGGGAAGGTGTG-3'
<i>Srebp1c</i>	5'-GATCAAAGAGGAGCCAGTGC-3'	5'-TAGATGGTGGCTGCTGAGTG-3'
<i>Srebp2</i>	5'-GCGTTCTGGAGACCATGGA-3'	5'-ACAAAGTTGCTCTGAAAACAAATCA-3'

intruded into the vacuoles (Supplemental Figure S3A), mimicking the lipophagy-like structures as reported previously,¹² although these vacuoles appeared to be single-membrane structures. The nature of these single-membrane vacuoles remained to be determined in the future, which was beyond the scope of the present study. The hepatic protein levels of glutamate-cysteine ligase catalytic unit (GCLC), glutamate-cysteine ligase modifier unit (GCLM), and NAD(P)H quinone dehydrogenase 1 (NQO1), three well-known Nrf2 target genes, markedly increased in L-Atg5 KO mouse livers compared with WT mice, although the levels of GCLC, GCLM, and NQO1 seemed less affected by the fed, fasting, and refed conditions. The increased hepatic levels of GCLC, GCLM, and NQO1 in L-Atg5 KO mice was largely blunted in L-Atg5/Nrf2 DKO mice (Supplemental Figure S2). Taken together, these data confirmed that L-Atg5 KO mice have impaired hepatic autophagy and persistent hepatic Nrf2 activation.

Decreased Hepatic LD Accumulation in L-Atg5 KO Mice Is Not Due to Decreased Expression of *de Novo* Lipogenic Genes or Increased Expression of FA β -Oxidation Genes

At least four factors can contribute to liver steatosis, including i) increased *de novo* FA synthesis, ii) decreased

VLDL-TG secretion, iii) decreased FA β -oxidation, and iv) increased hepatic FA uptake. Quantitative PCR analysis was next performed to determine the mRNA expression of genes involved in lipid metabolism. The expression of many genes involved in *de novo* lipid synthesis, including *Scd1* and *Acaca*, did not change significantly in response to fasting in both WT and L-Atg5 KO mice. The expression of hepatic *Pparg* did not change significantly in WT mice after fasting, although it increased in L-Atg5 KO mice after fasting and remained higher after the refed condition. The expression of hepatic FA synthase (*Fasn*) and sterol regulatory element-binding transcription factor 1c (*Srebp1c*) decreased after fasting in both WT and L-Atg5 KO mice. Interestingly, the expression levels of *Fasn*, *Scd1*, *Acaca*, and *Srebp1c* were significantly higher in L-Atg5/Nrf2 DKO and Nrf2 KO mice than WT and L-Atg5 KO mice under the fed condition but decreased significantly under the fasted and refed conditions (Figure 3A). Western blot analysis was then performed to determine the changes of some of the key *de novo* lipid synthesis proteins. The protein levels of FASN, acetyl-CoA carboxylase, and matured SREBP1 (mSREBP1) either decreased or remained unchanged under the fasted conditions but increased under the refed conditions in WT mice (Supplemental Figure S4). These results generally supported the notion that *de novo* lipid synthesis was not a main factor for fasting-induced hepatic steatosis. Compared with WT mice, L-Atg5 KO mice had relatively higher basal levels of

Figure 1 Fasting-induced hepatic accumulation of lipid droplets (LDs) is impaired in hepatic autophagy-deficient (L-Atg5) knockout (KO) mice, which can be corrected by further deletion of nuclear factor-like (Nrf2). **A:** WT, L-Atg5 KO, L-Atg5/Nrf2 double-knockout (DKO), and Nrf2 KO mice were either fed with a normal chow diet (Fed) or fasted overnight (Fasted). Liver tissues were harvested and cryo-liver sections were subjected to Oil Red O staining. Representative images are shown. **B:** Mice were treated as in **panel A**, and representative electron microscopic images are shown. The **lower panel** was an enlarged image from the **boxed area** in the **upper panel** image. **Arrows** indicate LDs. **C:** The number of LDs were quantified from **panel B**. Data are expressed as means $\pm$ SEM (**C**). $n = 3$ to 6 mice (**A**); $n = 10$ different images (**C**). * $P < 0.05$ between different treatments, determined by t -test. Scale bar = 2 μ m. N, nucleus.

FASn, acetyl-CoA carboxylase, and mSREBP1, which were all slightly decreased under the fasted conditions. L-Atg5/Nrf2 DKO had higher basal protein levels of FASn, acetyl-CoA carboxylase, and mSREBP1, which were further

increased after fasting. In contrast, Nrf2 KO mice had higher basal protein levels of mSREBP1 but markedly decreased levels under the fasted and refeed conditions. The full-length protein of FASn was not detected in Nrf2 KO mouse livers,

Figure 2 Hepatic and serum lipid changes in wild-type (WT), hepatic autophagy-deficient (L-Atg5) knockout (KO), L-Atg5 KO/nuclear factor-like (Nrf2) double-knockout (DKO), and Nrf2 KO mice under the fed, fasting, and refed conditions. WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were either fed with a normal chow diet (Fed), fasted overnight (Fasted), or refed with chow diet for 6 hours (Refed). **A–D:** Hepatic triglyceride (TG) (**A**) and cholesterol (**B**) levels as well as serum TG (**C**) and cholesterol (**D**) levels were quantified. Pooled plasma samples were used for fast phase liquid chromatographic (FPLC) analysis of lipoprotein profile. **E:** Serum TG profile. **F:** Serum cholesterol profile. **G:** Total serum cholesterol levels were quantified from the pooled FPLC analysis. Data are expressed as means $\pm$ SEM (**A–D**). $n = 3$ to 6 mice (**A–D**); $n = 5$ to 6 mice (**E–G**). * $P < 0.05$ (one-way analysis of variance analysis). HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

but instead a relatively small molecular band of FASN was detected in Nrf2 KO mouse livers and the reasons for these observations were unclear ([Supplemental Figure S4](#)). The hepatic adipose TG lipase protein levels increased under the fasted conditions in all four genotypes of mice. The levels of phosphorylated hormone-sensitive lipase increased in WT and L-Atg5/Nrf2 DKO mice under the fasted conditions but did not change in L-Atg5 KO mice and even decreased in Nrf2 KO mice ([Supplemental Figure S4](#)). Taken together, these data suggested that *de novo* lipogenesis and lipolysis may not be the main factors for the decreased accumulation of LDs in L-Atg5 KO mice after fasting. These data also suggested that deletion of Nrf2 may have aberrant effects on lipid metabolism in mouse livers. Nrf2 deletion may affect the basal hepatic lipogenesis gene expression but it did not affect fasting-induced hepatic lipid accumulation in mice.

The expression of hepatic cholesterol metabolism genes, including *Ldlr* and *Srebp2*, did not change under the fed, fasted, and refed conditions in all four genotypes of mice. The expression of hepatic *Hmgcr* and *Insig1* decreased in all four genotypes of mice under the fasted condition compared with the fed and refed conditions ([Supplemental Figure S5](#)). The expression of several hepatic transporters for sterol and cholesterol secretion, including *Abcg5*, *Abcg8*, and *Abca1*, increased after fasting and decreased after the refed condition in both WT and L-Atg5 KO mice. However, the expression levels of *Abcg5* and *Abcg8* were much higher in L-Atg5 KO mice than in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice under all three conditions (fed, fasted, and refed). In contrast, the hepatic expression of *Abcg5* and *Abcg8* did not change after fasting but decreased under the refed condition in L-Atg5/Nrf2 DKO and Nrf2 KO mice ([Supplemental Figure S6](#)). In addition, the levels of *Scarb1*,

Figure 3 Changes of hepatic lipogenesis gene expression in wild-type (WT), hepatic autophagy-deficient (L-Atg5) knockout (KO), L-Atg5/nuclear factor- κ B (Nrf2) double-knockout (DKO), and Nrf2 KO mice in response to nutrient changes. WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were either fed with a normal chow diet (Fed), fasted overnight (Fasted), or refed with chow diet for 6 hours (Refed). **A** and **B**: Hepatic mRNA was extracted followed by real-time quantitative PCR for fatty acid synthesis genes (**A**) and TG synthesis genes (**B**). Data are expressed as means $\pm$ SEM. $n = 3$ to 6 mice. $*P < 0.05$ (one-way analysis of variance analysis).

a receptor for high-density lipoprotein, did not change significantly in all four genotypes of mice except it increased after fasting in L-Atg5/Nrf2 DKO mice (Supplemental Figure S6).

The expression of hepatic TG synthesis genes was also determined under the fed, fasted, and refed conditions in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice. The expression of *Gyk*, *Gapdhm*, *Dgat1*, and *Dgat2* all increased in response to fasting and declined after the refed condition in all four genotypes of mice. These data suggested that hepatic TG synthesis may not be the factor that contributed to the decreased hepatic TGs in L-Atg5 mice after fasting (Figure 3B).

To determine whether decreased accumulation of hepatic LDs would be due to increased secretion of VLDL-TG from hepatocytes in L-Atg5 KO mice after fasting, hepatic TG secretion assay was performed in mice. No difference was found for TG secretion between WT and L-Atg5 KO mice after fasting (Supplemental Figure S7A). The hepatic expression of microsomal triglyceride transfer protein, an important protein for hepatic lipoprotein assembly and secretion, increased in WT, L-Atg5/Nrf2 DKO, and Nrf2

KO mice but did not change in L-Atg5 KO mice after fasting (Supplemental Figure S7B).

The hepatic expression of FA β -oxidation-related genes was next determined. The hepatic expression of *Ppara*, *Cpt1a*, *Acox*, *Crot*, and *Acadm* increased significantly in all four genotypes of mice under the fasted condition compared with the fed and refed conditions. However, the induction of these genes was much lower in L-Atg5 KO mice than in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice (Figure 4A). It is well known that fasting increases serum levels of ketone bodies, including β -HB.⁷ Indeed, serum levels of β -HB increased in all four genotypes of mice after fasting. Although the levels of serum of β -HB were comparable after fasting among all four genotypes of mice, the basal fed levels of serum of β -HB were much higher in L-Atg5 KO, L-Atg5 KO/Nrf2 DKO, and Nrf2 KO mice. Such a high level of serum β -HB persisted even after the refed condition in L-Atg5 KO mice (Figure 4B). Taken together, these data suggested that the lack of accumulation of LDs in L-Atg5 KO mice after fasting was less likely due to the changes of *de novo* lipogenesis, secretion of VLDL-TG, and β -oxidation of FA.

Figure 4 Changes of hepatic lipid metabolism gene expression in wild-type (WT), hepatic autophagy-deficient (L-Atg5) knockout (KO), L-Atg5/nuclear factor-like (Nrf2) double-knockout (DKO), and Nrf2 KO mice in response to nutrient changes. WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were either fed with a normal chow diet (Fed), fasted overnight (Fasted), or refed with chow diet for 6 hours (Refed). **A:** Hepatic mRNA was extracted, followed by real-time quantitative PCR for fatty acid β-oxidation synthesis genes. **B:** The serum β-hydroxybutyrate levels were measured. Data are expressed as means ± SEM. $n = 3$ to 6 mice. $*P < 0.05$ (one-way analysis of variance analysis).

Decreased Serum NEFAs after Fasting and Increased Hepatic CD36 in L-Atg5 KO Mice

The serum levels of nonesterified FAs (NEFAs) increased almost fourfold compared with the fed or refed condition in WT mice. In response to fasting, the serum levels of NEFAs also increased in L-Atg5/Nrf2 DKO and Nrf2 KO mice, although the levels were lower than in WT mice. In contrast, the basal (fed) levels of serum NEFAs were relatively higher in L-Atg5 KO mice than in WT mice, and the serum NEFA levels did not change after fasting in L-Atg5 KO mice (Figure 5A). Similar to the changes of serum NEFA, the levels of serum FGF21 significantly increased under the fasted condition compared with the fed and refed conditions in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice. In contrast, the basal (fed) levels of serum FGF21 in L-Atg5 KO mice was already significantly higher than either WT, L-Atg5/Nrf2 DKO, or Nrf2 KO mice, which remained almost at the same levels when the mice were either fasted or refed (Figure 5B). Consistent with the serum FGF21 changes, the hepatic mRNA levels of *Fgf21* markedly increased under the fasted condition and declined under the refed condition in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice. In contrast, the hepatic mRNA levels of *Fgf21* were already higher at the fed (basal) condition in L-Atg5 KO mice compared with WT mice, which further

increased significantly after fasting and declined after the refed condition (Supplemental Figure S8A). The hepatic mRNA levels of *Pgc1a* increased in all four genotypes of mice in response to fasting and declined to the levels of the fed condition after the mice were refed, except that the mRNA levels of *Pgc1a* were much higher at the fed (basal) condition of Nrf2 KO mice than that of WT, L-Atg5 KO, and L-Atg5/Nrf2 DKO mice (Supplemental Figure S8B). The serum levels of glycerol did not change in WT and L-Atg5/Nrf2 DKO mice under the fed, fasted, and refed conditions. The level of serum glycerol was almost doubled under the fed condition in L-Atg5 KO mice compared with WT mice, but it decreased to the same levels of WT mice under the fasted and refed conditions. Under the fasted condition, Nrf2 KO mice had higher serum glycerol levels (almost twofold higher) than WT, L-Atg5 KO, and L-Atg5/Nrf2 DKO mice (Figure 5C). The hepatic mRNA levels of *Cd36*, a gene that encodes an integral membrane protein that imports hepatic FAs into cells, increased in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice under the fasted condition compared with the fed condition, which declined under the refed condition in WT and Nrf2 KO mice but not in L-Atg5/Nrf2 DKO mice. In contrast, the hepatic mRNA levels of *Cd36* were approximately 10-fold higher in L-Atg5 KO mice than in WT mice under the fed condition. The levels of *Cd36* expression further increased under the fasted condition and

Figure 5 The serum levels of nonesterified fatty acids (NEFAs), glycerol, and fibroblast growth factor (FGF)21 in wild-type (WT), hepatic autophagy-deficient (L-Atg5) knockout (KO), L-Atg5/nuclear factor-like (Nrf) 2 double-knockout (DKO), and Nrf2 KO mice in response to nutrient changes. WT, L-Atg5 KO, L-Atg5/Nrf2 DKO, and Nrf2 KO mice were either fed with a normal chow diet (Fed, FD), fasted overnight (Fasted, FT), or refed with chow diet for 6 hours (Refed, RF). **A–C:** The serum levels of NEFAs (**A**), free glycerol (**B**), and FGF21 (**C**) were measured. **D:** Hepatic mRNA was extracted, followed by real-time quantitative PCR for *Cd36* (**D**). **E:** Total liver lysates were prepared, and pooled liver lysates were subjected to Western blot analysis. Data are expressed as means $\pm$ SEM (**A–D**). $n = 3$ to 6 mice. $*P < 0.05$ (one-way analysis of variance analysis). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; L-Exp, longer exposure; S-Exp, shorter exposure.

slightly declined under the refed condition in L-Atg5 KO mice (Figure 5D). Consistent with the mRNA data, Western blot analysis also revealed higher CD36 protein levels in L-Atg5 KO mice than in WT, L-Atg5/Nrf2 DKO, and Nrf2 KO mice (Figure 5E). Together these data indicated that L-Atg5 KO mice have increased basal levels of serum NEFAs, FGF21, and glycerol, which are not altered under the fasting conditions.

No Difference in the Uptake of OA and Formation of LDs between Atg5 KO Hepatocytes and WT Hepatocytes

A previous study reported that LC3-phosphatidylethanolamine (PE) conjugation was required for the biogenesis of LDs.⁴¹ To further determine whether autophagy-deficient cells would have defects for uptake of FFAs and biogenesis of LDs, hepatocytes were isolated from L-Atg5 KO and WT mice, and these cells were loaded with OA. The number of LDs markedly increased in the cytoplasm of OA-loaded WT hepatocytes based on the Bodipy staining and electron microscopic studies (Figure 6, A–C). Atg5 KO hepatocytes already had a higher number of LDs than that of WT hepatocytes even when they were cultured in the regular medium without OA. Addition of OA further increased the number of LDs in Atg5 KO hepatocytes (Figure 6, A–C), suggesting that deletion of Atg5 in hepatocytes may not affect the uptake of OA and biogenesis of LDs. Western blot analysis showed the lack of LC3-II forms

and increased p62 proteins in Atg5 KO hepatocytes (Figure 6D), supporting the lack of autophagy in Atg5 KO hepatocytes. Similar to primary hepatocytes, increased accumulation of LDs was also observed in WT, Atg5 KO, and Atg7 KO MEFs when they were loaded with OA (Supplemental Figure S9A). No LC3-II forms were detected in Atg5 or Atg7 KO MEFs (Supplemental Figure S9B). These data not only confirmed the lack of autophagy but also suggested that LC3-II was dispensable for the formation/biogenesis of LDs in Atg5 KO and Atg7 KO cells. Taken together, these data suggested that cells lacking essential autophagy proteins such as Atg5 or Atg7 did not compromise the uptake of FAs and the formation/biogenesis of LDs in these autophagy-deficient cells.

Discussion

The main finding of the present study was that mice with defective hepatic autophagy (L-Atg5 KO mice) had impaired adaptation to fasting-induced hepatic accumulation of LDs, which could be rescued by further deletion of Nrf2. The maladaptation to fasting-induced hepatic accumulation of LDs was not due to changes of *de novo* lipogenesis, secretion of hepatic VLDL, or increase in FA β -oxidation.

Previous reports show that the LC3-PE conjugated form is associated with LDs, and it has been suggested that LC3-PE conjugation is required for the biogenesis of LD.⁴¹ However, this notion is largely based on the observation of decreased

Figure 6 Lipid droplet (LD) formation in primary cultured wild-type (WT) and hepatic autophagy-deficient (Atg5) knockout (KO) hepatocytes. Primary cultured hepatocytes that were isolated from WT and L-Atg5 KO mice were loaded with either bovine serum albumin or 250 $\mu\text{mol/L}$ oleic acid (OA) for 6 hours. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline. Cells were further stained with 1 $\mu\text{mol/L}$ Bodipy 493/503 and 1 $\mu\text{mol/L}$ Hoechst 33342, followed by fluorescence microscopy. **A:** Representative images of Bodipy and Hoechst 33342 staining are shown. **B:** Some cells were fixed with 2.5% glutaraldehyde, followed by electron microscopic (EM) analysis. Representative EM images are shown. **C:** The number of LDs was quantified from panel B. **D:** Total cell lysates were extracted and subjected to Western blot analysis. Data are expressed as means $\pm$ SEM (**C**). $n = 15$ to 20 images (**C**). $*P < 0.05$ (one-way analysis of variance analysis). Scale bars: 20 μm (**A**); 0.5 μm (**B**). LC3, light chain 3; M, mitochondria; N, nucleus.

LD biogenesis in Atg7-deficient mouse livers after fasting. In the present study, both WT and Atg5 KO hepatocytes and MEFs increased the number of LD formation when they were loaded with OA in culture. Because both Atg5 KO hepatocytes and MEFs lack LC3-PE conjugation, these results suggest that LC3-PE conjugation is dispensable for the biogenesis of LD.

In cultured cells, deprivation of amino acids, glucose, and serum increases the number of intracellular LDs accompanied with increased autophagy. Increased accumulation of

LDs in these starved cells has been suggested to be due to autophagic degradation of intracellular membranes, which generates FFAs. Increased LD formation is to channel the FFAs as an adaptive protective mechanism against FFA accumulation and lipotoxicity.^{17,19} Although increased numbers of AVs and lipophagy-like structures were found in fasted WT mouse livers, the number of LDs was markedly increased compared with fed mice. These results suggest that autophagy-mediated degradation of LDs (lipophagy) is likely not sufficient to counteract the

formation/biogenesis of LDs that convert from FFAs under starvation/fasting conditions. In addition, although the phenotype of increased LD formation is quite similar in starved cells and fasted mouse livers, the mechanisms for the accumulation of the intracellular LDs could be different *in vivo* versus *in vitro*. As discussed in the introduction, in response to starvation, mammals, including humans, increase the mobilization of FFAs from adipose tissues to the liver that contributes to the accumulation of LDs in the fasted liver, which is different from increased autophagic degradation of membranes in cultured cells under starvation conditions.

To determine the role of autophagy in hepatic biogenesis of LDs in response to fasting, mice that have impaired hepatic autophagy would be helpful tools. Indeed, L-Atg5 KO mice failed to increase the accumulation of hepatic LDs in response to overnight fasting compared with their matched WT mice. At first glance, these *in vivo* data from L-Atg5 KO mice would greatly support the notion derived from the cell culture studies that autophagy is important for the biogenesis of LDs. However, further deletion of Nrf2 in L-Atg5 KO mice almost completely corrected the lack of accumulation of hepatic LDs in fasted L-Atg5 mouse livers, suggesting that the lack of accumulation of LDs in fasted L-Atg5 KO mouse livers is most likely due to secondary effects in L-Atg5 KO mice. Mice with defective hepatic autophagy have increased liver cell death/injury, hepatomegaly, inflammation, and fibrosis and eventually develop benign adenoma in aged mice.^{26,27,30} It is highly likely that the pathologic changes in the L-Atg5 KO mice liver may influence hepatic lipid metabolism and likely affect the whole body metabolic status in L-Atg5 KO mice. Indeed, at the basal fed conditions, L-Atg5 KO mice had increased serum levels of FFAs, β -HB, and FGF21 compared with their matched WT mice. Because all of these markers generally increased under fasting/starvation conditions, it is unclear whether L-Atg5 KO mice may constantly be under a chronic starvation condition even when they are free to access chow diet. This would help to explain why L-Atg5 KO mice had diminished induction of hepatic PPAR α and PPAR α -mediated expression of FA oxidation genes. As a result, white adipose tissue lipolysis may also constantly increase, resulting in the high serum levels of FFAs to meet the need of the chronic starvation in L-Atg5 KO mice.

Interestingly, accumulating evidence suggests that Nrf2 also regulates lipid metabolism and plays a role in fatty liver diseases although conflicting observations have been reported. Nrf2 KO mice have exacerbated, whereas Keap1 knockdown mice have less hepatic steatosis when these mice are fed with a methionine- and choline-deficient diet.^{35,44} Moreover, pharmacologic activation of Nrf2 attenuates diet-induced hepatic steatosis.⁴⁵ In contrast, Nrf2 KO mice are partially protected against diet-induced fatty liver and obesity likely due to the induction of FGF21 in the adipose and liver tissues, suggesting that Nrf2 is a negative regulator of FGF21.³³ These controversial results are likely due to

different diets and feeding periods in these studies, but it implies a very complex role of Nrf2 in regulating lipid metabolism. Interestingly, Keap1 knockdown mice show decreased hepatic accumulation of LDs when these mice are fasted for 24 hours, which is similar to the L-Atg5 KO mice in our present study. Both Keap1 knockdown mice and L-Atg5 KO mice all have persistent Nrf2 activation, implying that activation of Nrf2 may contribute to the lack of hepatic LD accumulation after fasting. However, there were also some fundamental differences among Keap1 knockdown mice and L-Atg5 KO mice in response to fasting. Xu et al³² found that the gene expression of *de novo* lipogenesis and FA transporters decreased in Keap1 knockdown mice. However, the expression of *de novo* lipogenesis genes decreased in both WT and L-Atg5 KO mice, suggesting that *de novo* lipogenesis may not be important for the accumulation of LDs in response to fasting. In contrast to the Keap1 knockdown mice, the hepatic levels of CD36 are much higher in the L-Atg5 KO mice even at the basal fed condition. Despite that both Keap1 knockdown and L-Atg5 KO mice have persistent Nrf2 activation, no obvious liver injury and other pathogenesis are observed in Keap1 knockdown/KO mice. Therefore, some of the differences between Keap1 knockdown and L-Atg5 KO mice may be due to the secondary effects in the L-Atg5 mouse livers as results of lack of autophagy in addition to Nrf2 activation.

Notably, our results that decreased hepatic LD accumulation in L-Atg5 KO mice after fasting are consistent with some previous reports that used liver-specific Atg7 or FIP200 KO mice.^{20,21} Note that liver-specific Atg7 and FIP200 KO mice have similar liver pathogenesis, including hepatocyte death, inflammation, fibrosis, and benign liver tumors, similar to L-Atg5 KO mice. Although Nrf2 activation has not been examined in L-FIP200 KO mice, further deletion of Nrf2 also corrected the liver pathologic conditions in L-Atg7 KO mice. Therefore, the mechanisms that contribute to the lack of adaptive hepatic biogenesis of LD in L-Atg5 mice in response to fasting that we identified in the present study may be generally true for other mice that also have defective hepatic autophagy.

Conclusions

Here it was shown that hepatic autophagy-deficient mice (L-Atg5 KO mice) had impaired adaptation to fasting-induced hepatic steatosis. The secondary effects (liver injury and Nrf2 activation) rather than the direct lack of hepatic autophagy may be the key contributors for the decreased hepatic LD accumulation in autophagy-deficient livers in response to fasting.

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Supplemental Data

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