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Hepatic autophagy contributes to the metabolic response to dietary protein restriction

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Abstract

Autophagy is an essential cellular response which acts to release stored cellular substrates during nutrient restriction, and particularly plays key role in the cellular response to amino acid restriction. However, there has been limited work testing whether the induction of autophagy is required for adaptive metabolic responses to dietary protein restriction in the whole animal. Here, we found that moderate dietary protein restriction led to a series of metabolic changes in rats, including increases in food intake and energy expenditure, the downregulation of hepatic fatty acid synthesis gene expression and reduced markers of hepatic mitochondrial number. Importantly, these effects were also associated with an induction of hepatic autophagy. To determine if the induction of autophagy contributes to these metabolic effects, we tested the metabolic response to dietary protein restriction in BCL2-AAA mice, which bear a genetic mutation that impairs autophagy induction. Interestingly, BCL2-AAA mice exhibit exaggerated responses in terms of both food intake and energy expenditure, whereas the effects of protein restriction on hepatic metabolism were significantly blunted. These data demonstrate that restriction of dietary protein is

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Conflict of Interest

Authors have no conflicts of interest.

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sufficient to trigger hepatic autophagy, and that disruption of autophagy significantly alters both hepatic and whole animal metabolic response to dietary protein restriction.

Keywords

Food intake; metabolism; energy expenditure; autophagy; amino acids

1. INTRODUCTION

The liver plays an essential role in maintaining nutrient homeostasis in response to fluctuations in nutrient intake, altering metabolic flux and mobilizing stored nutrients in order to maintain physiological function during periods of nutrient restriction. Autophagy is an essential cellular process which contributes to both the turnover of cellular components and damaged organelles (including mitochondria), as well as the response to nutrient restriction [1–3]. Hepatic autophagy has recently been linked to disease states, as defects in hepatic autophagy lead to mitochondrial dysfunction, insulin resistance, and lipid accumulation [4, 5]. Conversely, enhancing autophagy improves metabolism and extends lifespan [6, 7]. Thus the induction of hepatic autophagy during nutrient restriction triggers beneficial metabolic responses, while damage to this mechanism may contribute to metabolic dysfunction in the context of obesity and diabetes.

Dietary protein restriction is known to trigger adaptive changes in both feeding behavior and metabolism, and we and others have focused on identifying mechanisms which mediate these adaptive responses [8–10]. Considering autophagy's key role in the cellular response to amino acid restriction *in vitro*, we hypothesized that autophagy might also contribute to the response to protein restriction in the whole animal *in vivo*. Curiously, this question has never been tested. Instead, prior *in vivo* work has principally focused on the induction of autophagy during starvation [2], a setting marked by the acute deprivation of all dietary nutrients and which therefore confounds the restriction protein with the restriction of energy. We therefore assessed the effects of global dietary protein restriction on metabolic responses in rats and mice, and use a unique mouse genetic model, BCL2-AAA mice [11], to test whether the induction of autophagy contributes to the metabolic responses to protein restriction.

2. Methods

2.1 Protein Restriction and Metabolic Phenotyping in Rats

All animal experiments were reviewed and approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee. Male Sprague Dawley (SD) rats (5–7 weeks of age; Harlan Indianapolis, IN) were single housed in standard shoe-box cages with a 12:12 light:dark cycle and constant temperature (22–23°C). Animals were randomly divided into two dietary treatment groups (N=8 per group) and fed either a low protein diet containing 10% casein (LP) or a normal protein control diet containing 20% casein (control) formulated and produced by Research Diets (New Brunswick, NJ) [9, 12]. Diets were made isocaloric via concurrent changes in carbohydrate content while

maintaining a constant fat content. Metabolic phenotyping in rats utilized the PhenoMaster/LabMaster system (TSE Systems, Chesterfield, MO) within the Animal Phenotyping Core at the PBRC. Animals were initially adapted to the control diet in the metabolic chambers for 3 days before half the animals in each group were transitioned to the LP diet. Animals remained in metabolic chambers for the first 7 days of diet exposure, and were then removed to their homecage for the final 7 days. Animals had free access to food and water during this period. Food consumption was monitored daily and body composition was determined via TD-NMR (Bruker Minispec) on the start of experimental diets (Day 0), the final day of metabolic analysis (Day 7) and on the day of sacrifice (Day 14). Animals were euthanized after 14 days of feeding, and tissues were extracted and snap frozen in liquid nitrogen or fixed in 4% paraformaldehyde for later analysis.

2.2 Protein Restriction and Metabolic Phenotyping in Wildtype and BCL2-AAA mice

BCL2-AAA mice were kindly provided by Dr. Beth Levine (UT Southwestern) and were developed as described previously [11]. BCL2-AAA and WT mice were single housed in standard shoe-box cages with a 12:12 light:dark cycle and constant temperature (22–23°C). Animals were randomly divided into two dietary treatment groups (7–8 mice/diet/genotype) and fed either a low protein diet containing 5% casein (LP) or a normal protein control diet containing 20% casein (control) formulated and produced by Research Diets (New Brunswick, NJ) [8]. Mice were initially adapted to the control diet while in metabolic chambers (OxyMax/CLAMS, Columbus Instruments), were transitioned to the LP diet while in the OxyMax, and remained in the OxyMax for the first 7 days of dietary exposure. Afterward mice were returned to standard caging for the final 7 days, and after 14 days on diet were sacrificed and tissue collected as described below. Body weight and food intake were collected 4-times through the 14 day experiment. Body composition was measured via TD-NMR (Bruker Minispec) on the start of experimental diets (Day 0), the final day of OxyMax metabolic analysis (Day 7) and on the day of sacrifice (Day 14).

2.3 Quantitative Real Time RT-PCR

Total mRNA was extracted from frozen liver tissue using TRIzol reagent following the manufacturer's protocol (15596018, Invitrogen, Carlsbad, CA). RNA quality and quantity was determined by spectrophotometry using a NanoDrop (Thermo Scientific, Wilmington, DE). cDNA synthesis was performed with M-MLV reverse transcriptase (M1701, Promega, Madison, WI) and mRNA was quantified on the ABI 7900 platform with TaqMan Universal PCR Master Mix (4304437, Applied Biosystems, Carlsbad, CA), using the default ABI program for amplification conditions. Primer pairs were designed using NCBI Primer-BLAST with at least one primer spanning an exon-exon boundary. Primer sequences for each transcript are provided in Supplemental Table S1. Target gene expression was normalized with cyclophilin as the endogenous control and analyzed using a standard curve.

2.4 Western Blot and Ex vivo autophagy flux

For ATG5/12, whole cell lysates were prepared from liver by homogenizing tissue in whole cell lysate buffer, followed by sonication and centrifugation [13]. The supernatant from centrifugation was used for subsequent Western blotting on a standard 10% SDS-PAGE gel in a Bio-Rad electrophoresis system. In all blots, β -actin (Abcam ab8226; Cambridge, UK)

was used as an internal control. ATG5 (#8540) antibody was obtained from Cell Signaling Technologies (Danvers, MA) and used to detect ATG5 and conjugated ATG5-ATG12 on the same membranes.

To assess autophagy flux, fresh mouse liver tissue from each mouse was minced and divided into wells containing DMEM +10% FBS with or without lysosomal inhibitors (20 mM NH₄Cl; 200 μM leupeptin), and incubated at 37°C in 5% CO₂ atmosphere for 2 hours. After incubation samples were centrifuged (4°C, 5 min, 1000 g) and the supernatant discarded. The pellet was washed with ice-cold PBS and centrifuged (4°C, 5 min, 1000 g) and the supernatant discarded. The tissue was homogenized in 0.25 M sucrose (pH 7.0) with protease inhibitor cocktail tablets (1 tablet/50 ml; Roche) on ice. After centrifugation (4°C, 10 min, 13,000 g), the supernatant was collected and the protein content was quantified with Coomassie Plus (Bradford) Protein Assay Kit (Thermo Scientific) according to the manufacturer's protocol. For SDS-gel electrophoresis, 30 μg protein samples were diluted to 5x Laemmli sample buffer containing 60 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 0.01% (v/v) bromophenol blue. The samples were heated at 95°C for 5 minutes and electrophoresed through a 4–20% precast polyacrylamide gel (Bio-Rad). Proteins were then transferred to 0.2-μm pore size nitrocellulose membranes (BioRad). Membranes were blocked with 3% (w/v) bovine serum albumin in TBST buffer (20 mM Tris/HCl, 0.9% (w/v) NaCl, 0.05% (v/v) Tween-20 (pH 7.6) and incubated with the primary antibody against LC3A/B (Cell Signaling, #12741; dilution: 1:1000) at 4°C for 12 hours. Membranes were then washed with TBST, incubated with the corresponding HRP-conjugated anti-rabbit IgG (1:2000) for 60 minutes at room temperature. After washing with TBST, the membranes were transferred to enhanced chemiluminescence substrate (Western Lightning-ECL; PerkinElmer Inc.) for 2 minutes and exposed to Premium X-Ray Film (F-BX57; Phenix Research Products) for 5 minutes. Bands were scanned and quantified using ImageJ 1.47 (Wayne Rasband, National Institutes of Health). In all blots, β-actin (Abcam ab8226; Cambridge, UK) was used as an internal control. Autophagy flux was calculated with the following equation: sample plus inhibitor (LC3A/B 2 / LC3A/B 1) / sample minus inhibitor (LC3A/B 2 / LC3A/B 1).

2.5 Immunostaining and Immunohistochemistry

For immunohistochemistry (IHC), liver was fixed in 4% paraformaldehyde overnight, paraffin embedded and sectioned at 5μm. Samples were deparaffinized and treated with 0.01M sodium citrate buffer with 0.2% Triton-X100 for antigen retrieval. For colocalization of autophagy and mitochondrial markers, light chain 3B (LC3B; Abcam ab64781) and cytochrome c oxidase subunit IV (COX IV; Abcam ab14744) with goat anti-rabbit IgG FITC (Santa Cruz sc-2012) and goat anti-mouse IgG TR (Abcam ab6787) secondary antibodies, respectively, were used with the primary antibodies incubated overnight at 4°C and secondaries incubated for 2h at room temperature. For oil red O staining, frozen liver was sectioned at 10μm, dried at room temperature for 1 h and stained using a NovaUltra Oil Red O Stain Kit per the manufacturer's protocol (IW-3008, IHCWorld, Woodstock, MD). All sections were mounted with Vectashield mounting media containing DAPI (H-1200, Vector Labs, Burlingame, CA) for visualization of nuclei. Images were captured using a Zeiss Axioplan 2 upright microscope (Intelligent Imaging Innovations, Inc., Denver, CO) and

Slidebook Software v2.0 (Intelligent Imaging Innovations, Inc., Denver, CO) for Oil Red O stained sections, a Leica TCS SP5 AOBS Resonant Scanning Multiphoton Confocal microscope and Leica LAS AF Lite 2.0.2 Build 2038 (Leica Microsystems, Buffalo Grove, IL) for colocalization IHC, all housed in the Cell Biology and Bioimaging Core at the PBRC. Image analyses were conducted using ImageJ software (MacBiophotonics, Bethesda, Maryland, USA) using the count plugin, analyze particles plugin or threshold functions, as necessary.

2.6 Statistical Analysis

Data were analyzed using the SAS software package (SAS V9, SAS Institute) using one-way or two-way ANOVA, or repeated measures ANOVA, using the general linear model procedure. When experiment-wide tests were significant, post-hoc comparisons were made using the LSMEANS statement with the PDIF option, and represent least significant differences tests for pre-planned comparisons. Energy expenditure was first normalized to body weight and then assessed via repeated measures ANOVA. EE was also analyzed via analysis of covariance (ANCOVA) with body weight as the covariate using the general linear model procedure of SAS, with results validated against the MMPC.org ANCOVA data analysis tool. All data are expressed as mean $\pm$ SEM, with a probability value of 0.05 considered statistically significant.

3. Results

3.1 Protein restriction increases food intake and energy expenditure without influencing weight gain

Rats placed on a low protein (LP) diet exhibited an abrupt and significant increase in food intake that persisted for the duration of the experiment (Fig 1A). This increase in food intake was matched with a significant increase in energy expenditure (EE) but no difference in respiratory exchange ratio (Control: 0.95 ± 0.01 vs LP: 0.96 ± 0.01 ; $P = 0.55$). Increased energy expenditure was apparent regardless of whether the data were normalized to total body mass (Fig 1C, D), analyzed via analysis of covariance with body weight as the covariate (Fig 1E,F), or expressed on a per animal basis (data not shown). The concomitant increase in both food intake and energy expenditure resulted in no difference in body weight gain (Fig 1G) between control and LP rats over the 14 day period, although there was a significant increase in fat and fluid but not lean mass gain in LP compared to control (Fig 1H,I,J).

3.2 Protein restriction induces hepatic autophagy

Following 14 days of control or LP diet, rats were sacrificed and livers collected to assess metabolic endpoints. To test whether LP induces hepatic autophagy, we first measured hepatic ATG5 protein and ATG5-ATG12 conjugation. Although there was no significant increase in ATG5 at day 14 of feeding, a significant increase in the ATG5-ATG12 complex, a marker of autophagosome formation, was observed in LP compared to control (Fig. 2A). LP livers also exhibited increased levels of LC3B, an additional marker of autophagy (Fig 2B). Interestingly within the liver of LP rats, LC3B protein colocalized with the

mitochondrial marker COX IV (Fig. 2C), suggesting that mitochondria may be specifically targeted by LP-induced autophagy.

3.3 Protein restriction alters hepatic metabolism

Autophagy is associated with both the turnover of mitochondria and the regulation of lipid metabolism [4, 7]. In assessing the expression of several metabolic genes in the liver, we detected a LP-induced reduction in genes associated with lipogenesis, most notably *Scd-1*, *Fas* and *Srebp1c* (Fig 3A). This reduction in lipogenic gene expression was associated with a trend toward reduced hepatic lipid content as measured by oil red O staining (Fig 3B) or total liver triglyceride (Fig 3C), although these changes did not reach statistical significance. Finally, LP induced a significant reduction in mitochondrial DNA content (Fig 3D), consistent with evidence that autophagy promotes the turnover of mitochondria and our evidence for increased colocalization of LC3B with the mitochondrial marker COX IV.

3.4 Induction of hepatic autophagy during protein restriction is impaired in BCL2-AAA mice

Since dietary protein restriction induced autophagy in association with alterations in metabolic parameters, we next tested whether LP-induced autophagy was required for the metabolic responses to protein restriction. BCL2 plays a key role in the induction of autophagy in response to various stimuli such as starvation, and BCL2-AAA mice bear a series of mutations within BCL2 that prevent its phosphorylation [11]. Thus baseline autophagy is intact in these mice, but the induction to autophagy in response to starvation or exercise is impaired. To first confirm that the BCL2-AAA mutation impairs the LP-induced increase in hepatic autophagy, we placed wildtype (WT) or BCL2-AAA mice on control or LP diet for 14 days and collected liver to assess autophagy induction via an autophagy flux assay. Fresh liver samples were incubated with lysosomal inhibitors (NH_4Cl and leupeptin) for 2hrs, and the accumulation of LC3 with addition of the lysosomal inhibitor was used as a marker of autophagy flux (Fig 4A and 4B). In WT mice, there was no consistent change in LC3 accumulation in mice consuming control diet, but in the WT-LP group every animal exhibited increases in LC3 content in liver samples incubated with lysosomal inhibitor, strongly suggesting that autophagy flux was increased by LP diet (Fig 4C). Importantly, this LP-induced increase in hepatic autophagy was absent in the BCL2-AAA mice consuming LP diet, indicating that the BCL2-AAA mutation effectively attenuates the induction of autophagy during dietary protein restriction.

3.5 Impaired autophagy potentiates the effect of LP diet on EE and food intake

Consistent with prior observations in rats (Fig 1) and mice [8], the LP diet induced a significant increase in EE (Fig 5A), with this increase occurring approximately 3 days following dietary exposure. Unexpectedly, this LP-induced increase in EE was exaggerated in BCL2-AAA mice (Fig 5B and 5C). Consistent with the changes in EE, food intake was also increased by the LP diet in WT mice, and this effect was again exaggerated in BCL2-AAA mice on LP diet compared to BCL2-AAA on control diet (Fig 6A). As a result, the effect of LP on body weight gain was similar between WT and BCL2-AAA mice, as LP diet reduced body weight and lean gain but not fat gain in both WT and BCL2-AAA equally when compared to control animals (Fig 6B, C and D). Collectively, these data suggest that

the lack of LP-induced autophagy in BCL2-AAA mice exaggerates the adaptive changes in food intake and EE in response to protein restriction.

3.6 Impaired autophagy blocks the effect of LP diet on hepatic lipogenic expression and mitochondrial DNA

Because protein restriction in rats reduced hepatic lipogenic gene expression and hepatic mitochondrial DNA content, we next tested whether these effects would be altered in the absence of LP-induced autophagy. Consistent with our prior work, the LP diet reduced hepatic lipogenic gene expression and mitochondrial DNA content in WT mice (Fig 7); however, these effects were largely abolished in BCL2-AAA mice. These data suggest that autophagy plays a key role in regulating mitochondrial turnover and lipogenic gene expression in the liver during dietary protein restriction.

4. DISCUSSION

The data presented here are consistent with previous work demonstrating that dietary protein restriction induces a series of behavioral and metabolic changes, including increases in food intake, increases in energy expenditure, and alterations in hepatic metabolism [8, 9, 14, 15]. Importantly, these metabolic changes are associated with an increase in hepatic autophagy. It is well known that amino acid restriction induces autophagy *in vitro* [1–3], and that autophagy in the liver is induced by acute amino acid deprivation [16]. However, few studies have specifically tested whether autophagy is required for the metabolic response to protein restriction, particularly changes in whole animal feeding behavior and energy expenditure. The data presented here not only demonstrate that dietary protein restriction in the absence of energy restriction is sufficient to induce hepatic autophagy, these data also indicate that autophagy is a mediator of the metabolic effects of protein restriction.

Our work uses several approaches to demonstrate LP-induced hepatic autophagy. First, we demonstrate that protein restriction promotes the conjugation of ATG5 and ATG12. This ubiquitin-like conjugation event is required for autophagosome formation, and deletion or overexpression of ATG5 blocks or activates autophagy, respectively [6, 7, 17]. Second, we demonstrate the accumulation of LC3B protein, another marker of autophagosome accumulation, and in addition provide evidence that protein restriction promotes the subcellular colocalization of LC3B with the mitochondrial marker Cox IV. We also measure autophagy flux by testing the differential accumulation of LC3 in the presence or absence of lysosomal inhibitors *ex vivo* [18]. Importantly, this LP-induced increase in autophagic flux is lost in BCL2-AAA mice. The phosphorylation of BCL2 contributes to the induction of autophagy in response to various stimuli, and it has been previously shown that starvation or exercise-induced autophagy is impaired in these mice [11]. Here we demonstrate the failure of dietary protein restriction to induce hepatic autophagy within this model as well. These mice therefore provide a very unique model to test the effects of protein restriction on metabolic outcomes, because baseline autophagy is intact but the induction of autophagy in response to stimuli such as protein restriction is impaired.

Consistent with previous work by our group and others [8, 9, 12, 14, 15, 19–22], we observed rapid and persistent increases in food intake and energy expenditure when mice or

rats were placed on a LP diet. Interestingly, these effects were not lost but instead enhanced in the autophagy-deficient BCL2-AAA mice. Currently it is unclear why inhibition of autophagy produced this enhanced response. Autophagy provides a rapid and efficient mechanism to release cellular amino acids in response to protein restriction, and therefore one possibility is that more energy intensive metabolic pathways (such as proteasome-dependent protein turnover) were recruited to meet physiological demands in the absence of autophagy, ultimately leading to increased energy expenditure which was offset by an increase in food intake. Alternatively, it is possible that the loss of autophagy triggered adaptive increases in food intake directly. For instance, it is possible that impaired autophagy lead to alterations in circulating amino acid concentrations, with these changes being sensed by the brain and promoting hyperphagia. It is well known that increases in leucine signaling within the brain are sufficient to suppress food intake and may contribute to reductions in food intake on a high protein diet [9, 12, 23–27]. However, we have tested whether fluctuations in circulating or brain amino acid signaling are necessary for LP-induced hyperphagia, and our results suggest that a fall of circulating leucine or BCAAs is not required for the observed hyperphagia [9].

These changes in whole body energy metabolism were associated with metabolic effects in the liver, including reduced hepatic lipogenic gene expression and a trend toward reduced hepatic lipid content. We emphasize that the control group consisted of young, low fat-fed rats with low hepatic lipid content, and thus our ability to detect further reductions in lipid content was likely limited. It will be interesting in future studies to test whether protein restriction reduces hepatic lipid content in the context of obesity, and it is noteworthy that a model of dietary methionine restriction produces similar reductions in hepatic lipogenic gene expression and hepatic lipid content [28, 29]. Importantly, our data also demonstrate that LP-induced autophagy contributes to several of these metabolic responses, as LP-induced reductions in hepatic lipogenic gene expression and mitochondrial DNA content were lost in the autophagy defective BCL2-AAA mice. These observations are consistent with evidence that autophagy is essential for the turnover of organelles, including mitochondria [30, 31], as well as evidence linking autophagy with fatty acid synthesis and lipid accumulation in the liver [4, 5, 7, 32, 33]. Considering that mitochondria play a critical role in balancing lipid synthesis and oxidation and that mitochondrial dysfunction may contribute to the onset and progression of obesity and insulin resistance [34, 35], these data suggest that the autophagy-induced turnover of mitochondria within the liver may be a key effect of dietary protein restriction.

In addition to changes in hepatic metabolism, it should be noted that the BCL2-AAA mutation is not specific for the liver in this mouse line. As such, it is possible that alterations in autophagy and/or metabolism within tissues such as adipose tissue or muscle may also contribute to the phenotype of BCL2-AAA mice on the LP diet. It is also possible that other signaling pathways may play an important role in the adaptive response to protein restriction. For instance, mTOR signaling is an important sensor of amino acid availability and a well-known regulator of autophagy, while protein restriction also activates the integrated stress response via the kinase GCN2 [8, 36, 37]. Considering that these pathways are highly integrated within the cell, it seems likely that additional signaling systems beyond hepatic

autophagy are contributing to the diverse metabolic responses to protein restriction [1–3, 38–40]

Our data provide clear evidence that dietary protein restriction induces significant changes in both whole body and liver metabolism, that hepatic autophagy can be triggered by dietary protein restriction, and that this induction of autophagy contributes to the metabolic responses to protein restriction. Autophagy is known to promote metabolic health and longevity [2, 6, 41], while protein restriction similarly triggers metabolic adaptations and promotes longevity [42]. When taken together, these data indicate that the induction of autophagy is an important component to the metabolic response to dietary protein restriction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

1. Protein restriction induces adaptive changes in feeding behavior and metabolism.
2. Protein restriction induces autophagy within the liver.
3. Disruption of autophagy alters the metabolic response to protein restriction.

Figure 1. Protein restriction increases food intake and EE in rats

Male rats were placed on control or low protein (LP) diets for 14 days. Food intake (**A, B**) was measured daily, while EE was measured in metabolic chambers for the first 7 days on diet (**C**). Average EE on days 5–7 was normalized to body weight (**D**) or analyzed via analysis of covariance (ANCOVA) with BW as the covariate (**E, F**). Body weight (**G**), fat (**H**), lean (**I**), and fluid (**J**) gain over the 14 day period. N=8. *P < 0.05 vs. Control.

Figure 2. Protein restriction induces hepatic autophagy

Rat liver samples were collected following 14 days of control or LP diet. Induction of autophagy was determined via Western blot analysis of the conjugation of ATG5 to ATG12 (**A**) and the increase of LC3B (**B**). The colocalization of LC3B with CoxIV was also increased by LP, suggesting the targeting of mitochondria to the autophagosome. N=8. *P < 0.05 vs. Control.

Figure 3. Protein restriction alters hepatic metabolism

Rat liver samples were collected following 14 days of control or LP diet to assess metabolic gene expression (**A**), oil-red O staining (**B**) triglyceride content (**C**) and mitochondrial DNA content (**D**). N=8. *P < 0.05 vs. Control.

Figure 4. Induction of hepatic autophagy in response to LP diet is impaired in BCL2-AAA mice
 Wildtype and BCL2-AAA mice were placed on control or LP diet for 14 days. At sacrifice, liver samples were collected and assayed for autophagic flux. Representative blots of LC3A/B staining in the presence and absence of lysosomal inhibitors (**A**). Effect of inhibitor treatment on LC3A/B staining (**B**). Quantification of autophagy flux (fold induction with inhibitor), demonstrating loss of LP-induced hepatic autophagy in BCL2-AAA mice (**C**). N=7–8. *P < 0.05 vs. Control.

Figure 5. Impaired autophagy potentiates the effect of LP diet on EE

Wildtype and BCL2-AAA mice were placed on control or LP diet for 14 days, with EE measured the first 7 days. LP increased EE in WT mice (**A, C, D**), and this effect was enhanced in BCL2-AAA mice (**B, C, E**). N=7–8. *P < 0.05 vs. Control. #P < 0.05 vs WT-LP.

Figure 6. Effects of LP diet on food intake and body weight gain

Wildtype and BCL2-AAA mice were placed on control or LP diet for 14 days. Food intake (A) throughout the 14 day experiment. Body weight gain (B), fat gain (C) and lean gain (D) were measured at day 14. N=7-8. *P < 0.05 vs. Control. #P < 0.05 vs. WT-LP.

Figure 7. Impaired autophagy blocks the hepatic response to LP diet

Wildtype and BCL2-AAA mice were placed on control or LP diet for 14 days. Loss of autophagy attenuated the effect of LP diet on liver gene expression (**A**), and also blocked the reduction of mitochondrial DNA content (**B**). N=7–8. *P < 0.05 vs. Control