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Original Article

Exercise during weight loss improves hepatic mitophagy

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has recently become a public health concern concurrent with the obesity crisis. Previous work has shown aberrant mitochondrial content/quality and autophagy in models of NAFLD, whereas exercise is known to improve these derangements. The purpose of this study was to examine the effect of different weight-loss modalities on hepatic mitochondrial content, autophagy and mitophagy in NAFLD. Forty-eight male C57BL/6J mice were divided into 1 of 4 groups: low fat diet (LFD, 10% fat, 18 weeks), high fat diet (HFD, 60% fat diet, 18 weeks), weight-loss by diet (D, 60% fat diet for 10 weeks then 10% fat diet for 8 weeks) or weight-loss by diet and physical activity (D/PA, 60% fat diet for 10 weeks, then 10% fat diet plus a running wheel for 8 weeks). Immunoblot data were analyzed by one-way analysis of variance (ANOVA) with significance denoted at $p < 0.05$. COX-IV protein contents were approximately 50% less in HFD compared to LFD. D/PA had 50% more BNIP3 compared to HFD. PINK1 content was 40% higher in D and D/PA compared to LFD. P-PARKIN/PARKIN levels were 40% lower in HFD, D, and D/PA compared to LFD. Whereas p-Ub^{Ser65} was 3-fold higher in HFD. LC3II/I ratio was 50% greater in HFD and D/PA, yet p62 protein content was 2.5 fold higher in HFD. High-fat diet causes disruptions in markers of mitochondrial quality control. Physical activity combined with diet were able to ameliorate these derangements and seemingly improve hepatic mitochondrial quality above control values.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the leading preventable liver ailment in Western society.[1,2] Rates of NAFLD have closely mirrored the obesity epidemic, with an estimated 19%–30% of adults in Western society diagnosed with NAFLD.[1,2] NAFLD can begin with relatively benign fat accumulation within the liver, but with continued lipid overload, can progress to steatosis, inflammation, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and potentially failure.[3] Currently, there are no effective pharmacological interventions for the liver disease once progressed to NASH.[4] As such, it is imperative to halt the progression of NAFLD and subsequent NASH. It is currently understood that lifestyle interventions such as weight-loss, diet and exercise mitigate many of the symptoms associated with NAFLD[5,6] and are therefore a favorable therapeutic modality for halting the progression hepatic pathologies before NAFLD develops.

Mitochondria, the major supplier of ATP (adenosine triphosphate) to the cell, play a major role in the progression of NAFLD.[7] Previous work has demonstrated derangements in mitochondrial quality control mechanisms in various models of hepatic lipid overload.[8–11] Work by Rector et al.[12] has also demonstrated mitochondrial dysfunction preceding hepatic steatosis in a genetic model of NAFLD. To maintain quality control, dysfunctional mitochondria must be removed by mitochondrial specific autophagy, hereafter referred to as mitophagy. Mitochondria undergo multiple mitophagy pathways,[13] with the two predominant pathways including BNIP3 mediated and PINK1/PARKIN mediated mitophagy. BNIP3 mitophagy predominantly occurs under pathological conditions,[14,15] with dysfunctional mitochondria being tagged by BNIP3 and shuttled to an LC3 tagged autophagosome.[14,15] Knockout of BNIP3 results in steatosis in mice fed a normal chow diet, overall suggesting BNIP3-mediated mitophagy is an important contributor to hepatic health.[9] PINK1/PARKIN mitophagy occurs during instances of mitochondrial depolarization,[16–18] with the accumulation of PINK1 causing subsequent phosphorylation of PARKIN and ubiquitin at serine-65, tagging the mitochondria for degradation.[16–18] To our

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Abbreviations

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
ANOVA	Analysis of variance
LFD	Low fat diet group
HFD	High fat diet
D	Diet group
D/PA	Diet + physical activity group
COX-IV	cytochrome *c* oxidase subunit IV
PGC1α	Peroxisome proliferator-activated receptor-gamma coactivator
BNIP3	BCL2 and adenovirus E1B 19-kDa-interacting protein 3
PINK1	PTEN-induced kinase 1
Ub	Ubiquitin
LC3	Microtubule-associated protein1A/1B-light chain 3
p62	ubiquitin-binding protein (also known as Sequestosome-1, SQSTM1)
ATP	Adenosine triphosphate

knowledge, few studies have investigated hepatic PINK1/PARKIN mitophagy during lipid-overload or exercised conditions.

Previous work from our laboratory has suggested increased hepatic autophagy with exercise may provide protective effects from NAFLD despite lipid overload,[19,20] and that Western diet can decrease BNIP3 mediated mitophagy.[19] Yet, it is unclear how physical activity combined with dietary alterations (reduced caloric intake) may differentially affect hepatic mitophagy compared to dietary alterations alone. Furthermore, it is unclear what, if any impact PINK1/PARKIN mitophagy has on hepatic health in NAFLD and with weight-loss interventions to improve NAFLD. Therefore, the purpose of the study was to investigate autophagy and mitophagy regulation after weight-loss by diet or diet combined with physical activity in a murine model of NAFLD.

Materials and methods

Animal interventions

All animal work was performed at and approved by the Southern Illinois University at Edwardsville Institutional Animal Care and Use Committee as previously described.[21] Further details of both the animal protocol and other phenotypic outcomes can be found in other works using these same animals.[21] Briefly, male C57BL/6J (n = 48; Jackson Laboratories, Bar Harbor, ME) mice were evenly divided into two groups at 8 weeks of age, one group of 12 animals consumed a low fat diet (LFD, 10% of kcal from fat, Research Diets #D12450J, New Brunwick, NJ) and 36 animals consumed high fat diet to induce obesity (60% of kcal from fat, Research Diets #D12492). Animals consumed diets for 10 weeks, after which, high fat diet-induced obese animals were further divided into three groups, one (HFD, $n = 12$) continued to consume the 60% high fat diet, one group was placed back on the 10% fat diet to induce weight-loss (D, $n = 12$) and the final group was placed on the 10% fat diet and given a freely movable running wheel to provide physical activity to induce weight-loss (D/PA, n = 12). Wheel running activity was monitored daily with the Vital View Data Acquisition System and daily distance travelled was recorded for data analyses (Mini-Mitter, Bend, OR). LFD animals continued consuming a 10% fat diet. Animals continued interventions for an additional 8 weeks. The final groups included: LFD, HFD, D, and D/PA. After interventions, animals were euthanized by an overdose of isoflurane and confirmed by cardiac puncture. Following perfusion with saline (0.9% w/v NaCl), livers were collected and snap-frozen in liquid nitrogen for later analysis. Six hours before tissue

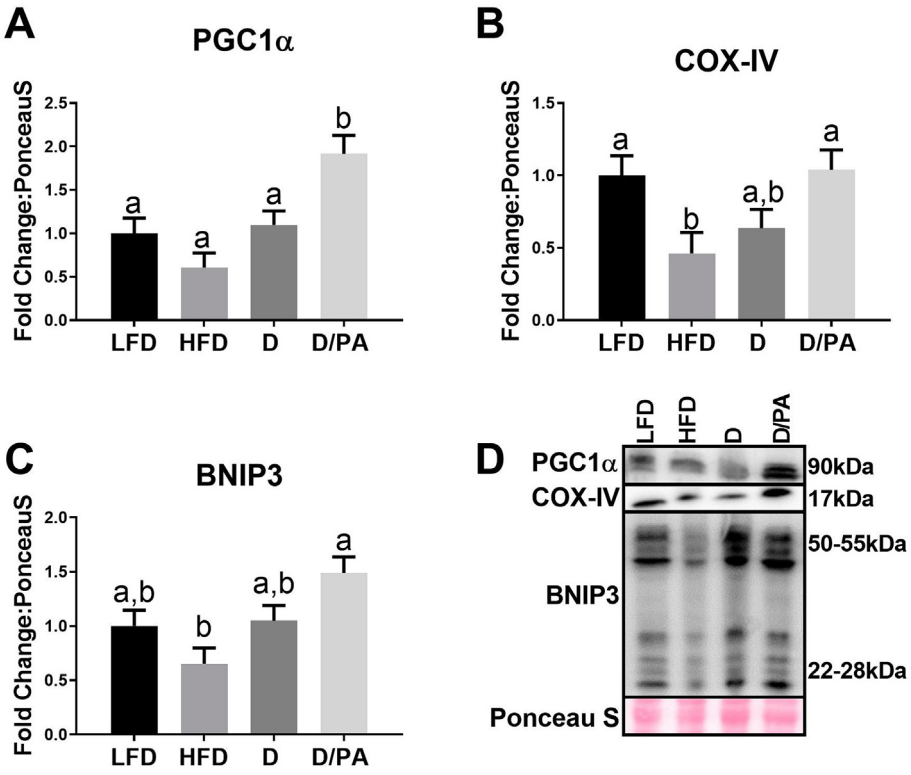

Fig. 1. Mitochondrial biogenesis Western blot data. A.) Western blot analysis for PGC1α content. B.) Western blot analysis for COX-IV content. C.) Western blot analysis for BNIP3 content. D.) Representative Western blot images. n = 8–12/group Different letters represent differences between groups at $p < 0.05$. PGC1α: Peroxisome proliferator-activated receptor-gamma coactivator, COX-IV: cytochrome *c* oxidase subunit IV, BNIP3: BCL2 and adenovirus E1B 19-kDa-interacting protein 3.

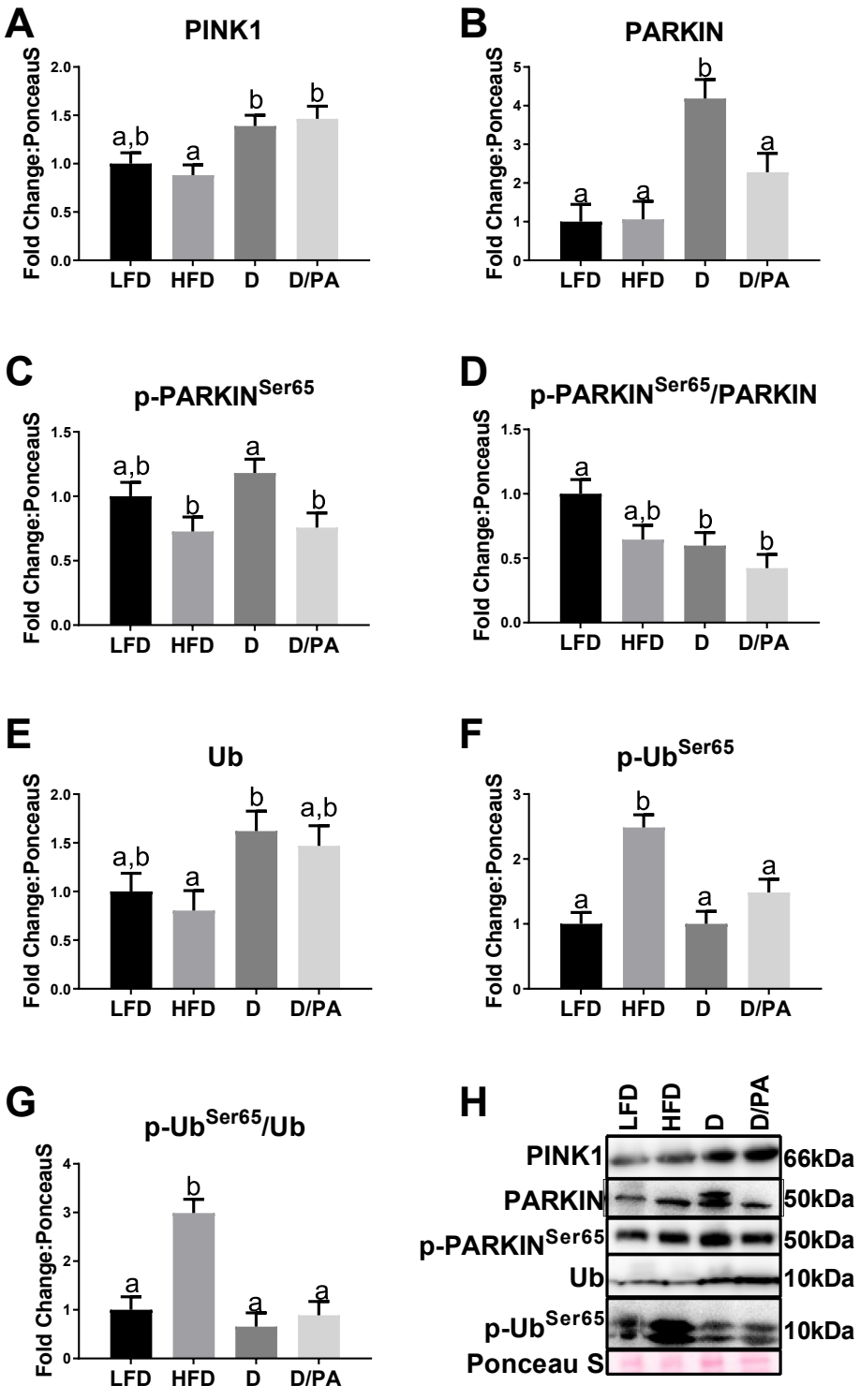

Fig. 2. PINK1 mediated mitophagy Western blot data. A.) Western blot analysis for PINK1. B.) Western blot analysis for PARKIN. C.) Western blot analysis for p-PARKIN^{Ser65}. D.) Western blot analysis p-PARKIN^{Ser65}/PARKIN ratio. E.) Western blot analysis for Ub protein content. F.) Western blot analysis for p-Ub^{Ser65}. G.) Western blot analysis for p-Ub^{Ser65}/Ub. H.) Representative Western blot images. $n = 8–12$/group Different letters represent differences between groups at $p < 0.05$. PINK1: PTEN-induced kinase 1, Ub: Ubiquitin.

harvest, animals' food was removed and 24 h before tissue harvest running wheels were removed from cages of D/PA animals.

As acknowledged in our previous publication,[21] it is possible animals exhibited excessive energy expenditure due to housing temperatures between 22.5 °C and 23.5 °C.[22] Due to males being more physiologically susceptible to lipid-induced hepatic alterations,[23] and epidemiological data implying males have slightly greater rates of NAFLD compared to females,[24] only males were used in this investigation.

Isolation of protein and immunoblotting

Isolation of protein and immunoblotting were performed as we have previously described.[19,25] For analysis at least one sample from every group was run on each membrane. Membranes were imaged using a FlourChem M (Protein Simple, San Jose, CA) and protein content normalized to *Ponceau S*. Primary antibodies included: PGC1α (Santa Cruz, CAT# sc-13067), COX-IV (Cell Signaling, CAT# 4844S), BNIP3

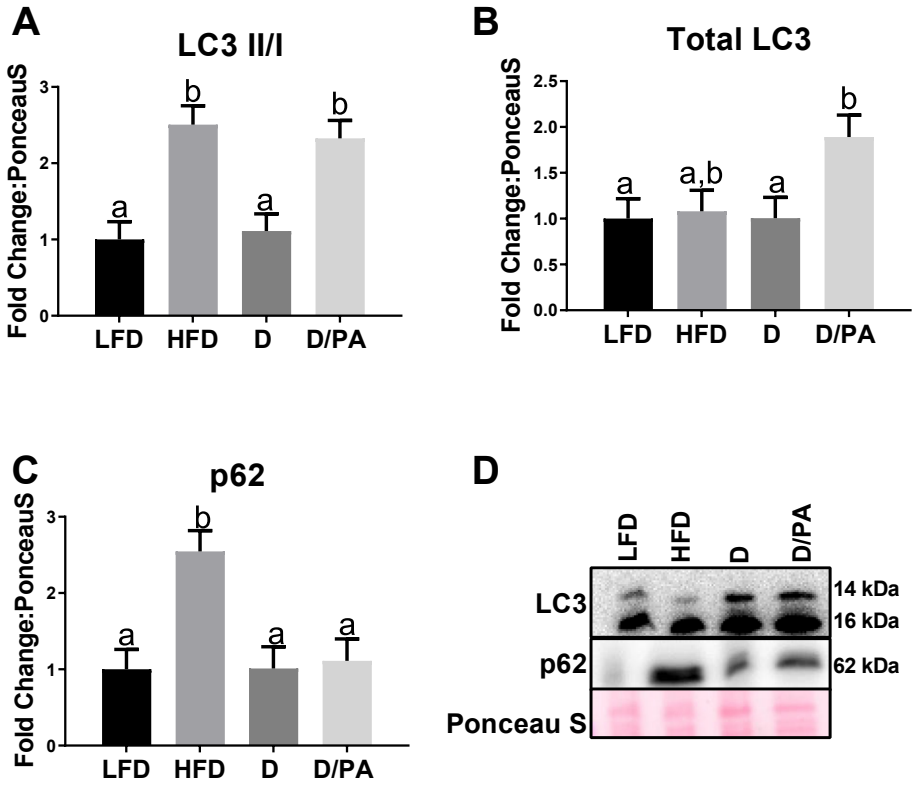

Fig. 3. Macroautophagy Western blot data. A.) Western blot analysis for LC3II/I. B.) Western blot analysis for total LC3 content. C.) Western blot analysis for p62 protein content. D. Representative Western blot images. n = 8–12/group Different letters represent differences between groups at $p < 0.05$. LC3: Microtubule-associated protein 1A/1B-light chain 3, p62: ubiquitin-binding protein.

(Cell Signaling, CAT# 3769), LC3 (Cell Signaling, CAT# 4108), p62/SQSTM1 (Cell Signaling, CAT# 5114s), PINK1 (Santa Cruz, CAT# sc-33796), PARKIN (Cell Signaling, CAT# 42115), p-PARKIN Ser65 (Abcam, CAT# ab154995), Ubiquitin (Cell Signaling, CAT#3933), and P-Ubiquitin^{Ser65} (R & D Systems, CAT# A-110). All antibodies were diluted with 1:1000 or 1:500 per manufacture recommendations.

Statistical analysis

Independent variables were the interventions (LFD v. HFD v. D v. D/PA). Results were analyzed by one-way analysis of variance (ANOVA), with α set at 0.05. If the global F test was significant, Tukey's post-hoc-adjustment was used to determine differences between groups. All data were analyzed using the Statistical Analysis System (SAS, version 9.3, Cary, NC) and expressed as mean ± standard error of the mean (*SEM*). All statistical code and data are available upon request to the corresponding author.

Results

HFD lowered mitochondrial content, D/PA restored multiple components of mitochondrial turnover

Other phenotypic data on these animals has previously been reported.[21] In brief, HFD animals had significantly larger livers compared to LFD, D, or D/PA (~3 g v. ~1 g). Correspondingly, HFD also had significantly greater hepatic lipid mass and triglyceride concentrations compared to LFD, D, or D/PA (~24% total lipid mass compared to 5%–10% lipid mass in other groups and ~25 μg/mg tissue compared with 5–11 μg/mg in other groups).[21] Finally, as reported in the prior study,[21] D/PA animals ran ~8 km/day during the 8-week running wheel phase of the study, corresponding to an additional estimated energy expenditure of ~25kCal/week in D/PA animals compared to D.[21] In this study, HFD did not result in lower PGC1α content ($p = 0.383$); however, D/PA had ~1.5-fold greater PGC1α content compared to HFD ($p < 0.0011$), which also was ~1-fold greater than LFD animals ($p = 0.010$) and ~1-fold greater than D animals ($p = 0.019$, Fig. 1A and D). COX-IV content, a common surrogate marker of mitochondrial content,[25,26] was approximately 50% lower in HFD compared to LFD animals ($p = 0.049$, Fig. 1B and D). COX-IV content in D animals was not different from either HFD ($p = 0.811$), LFD ($p = 0.237$) or D/PA ($p = 0.163$, Fig. 1B and D). D/PA appeared to restore COX-IV content, with no difference between D/PA and LFD ($p = 0.997$) and ~50% greater COX-IV content compared to HFD (Fig. 2B and D, $p = 0.031$). Similar to PGC1α content, BNIP3 content was not statistically different between LFD and HFD ($p = 0.348$, Fig. 1C and D). However, D/PA had ~84% greater BNIP3 content compared to HFD ($p = 0.001$). D was not significantly different compared to all groups (p = 0.147–0.994, Fig. 1C and D).

PINK1/PARKIN-mediated mitophagy was altered at different regulatory points in all three experimental conditions

PINK1 protein content was unaffected by HFD ($p = 0.865$, Fig. 2A and H), whereas, D and D/PA each resulted in approximately 50% greater PINK1 compared to HFD ($p = 0.011$ & $p = 0.048$, Fig. 2A and H). PARKIN content was not different between LFD and HFD animals ($p = 0.997$, Fig. 2B and H). However, PARKIN content was ~3–4 fold greater in D animals compared to LFD ($p < 0.001$), HFD ($p < 0.001$), and D/PA (p = 0.043, Fig. 2B and H). With regards to p-PARKIN^{Ser65} no interventions were statistically different from LFD ($p = 0.312$–0.643). However, D had ~45% greater p-PARKIN^{Ser65} compared to both HFD ($p = 0.029$) and D/PA ($p = 0.047$, Fig. 2C and H). Comparing p-PARKIN^{Ser65}/PARKIN ratios, both intervention groups (D and D/PA) were roughly 45% lower compared to LFD ($p = 0.050$ & $p = 0.002$, Fig. 2C and H).

Total Ubiquitin (Ub) protein content was unaffected by HFD compared to LFD ($p = 0.893$, Fig. 2D and H). D had ~80% greater Ub compared to HFD ($p = 0.038$, Fig. 2D and H). p-Ubiquitin^{Ser65} was ~100%–150% greater in HFD compared to LFD, D, and D/PA ($p < 0.001$, $p < 0.001$, and $p = 0.006$, Fig. 2F and H), with no other differences noted between groups. Additionally, p-Ubiquitin^{Ser65}/Ubiquitin was ~1.5-fold greater in HFD animals compared to LFD, D, and D/PA ($p < 0.001$, $p < 0.001$, & $p < 0.001$ respectively, Fig. 2G and H), with no other differences between groups.

Macroautophagy resolution was altered in HFD animals and rescued with weight-loss interventions

LC3II/I ratio was approximately 1.5-fold greater in HFD and D/PA animals compared to LFD ($p < 0.001$ & $p < 0.001$, Fig. 3A and D), with no other differences noted. Total LC3, found by adding the density of both the LC3I and LC3II bands, was ~80% greater in D/PA animals compared LFD and D ($p = 0.050$ & $p = 0.043$); additionally the ~80% differences between D/PA and HFD approached statistical significance ($p = 0.085$, Fig. 3B and D). p62 content was ~1.5-fold greater in HFD compared to LFD, D, or D/PA ($p = 0.001$, $p = 0.002$, & $p = 0.004$, Fig. 3C and D), there were no other differences between groups.

Discussion

To our knowledge, our group is the first to report on PINK1/PARKIN-mediated mitophagy in high-fat diet-induced NAFLD in addition to utilizing weight-loss interventions to lessen NAFLD symptoms. Our results demonstrate concurrent diet and physical activity during weight-loss provides greater benefits on aspects of mitochondrial biogenesis and content compared to diet alone as a treatment for NAFLD. Furthermore, D/PA appears to provide benefits to autophagy and mitophagy, whereas diet does not appear to have as prominent effects. Our results suggest that in NAFLD hepatocytes attempt to initiate mitophagy without resolution of the process, allowing dysfunctional mitochondria to remain and potentially exacerbating the disease.

First, our high fat diet was sufficient to induce hepatic lipid overload,[21] a known precursor to the development of NAFLD and similar to previous research.[6,12,27,28] Our data follows our previous findings with reductions in mitochondrial content.[19] Overall, D had a limited effect on all measures of both mitochondrial content and biogenesis. Contrastingly, D/PA increased PGC1α content above baseline and completely restored mitochondrial content as measured by COX-IV. While PGC1α content does not specifically measure mitochondrial biogenesis, as the major regulator of mitochondrial biogenesis,[29] PGC1α content provides valuable insight into the promotion of mitochondrial biogenesis in this model. Additionally, previous studies have corroborated our findings of reduced mitochondrial content in NAFLD utilizing multiple methods.[10,28,30] Taken together, these data demonstrate diet combined with physical activity is more effective for treating disrupted hepatic mitochondrial content compared to diet alone in murine models of NAFLD.

HFD appeared to attenuate the hepatocyte's capacity for BNIP3-mediated mitophagy, as measured by reduced BNIP3 content. As directly measuring the mitophagy process was not a viable option for this study, we interpret alterations in BNIP3 protein content as reflective of the hepatocyte's capacity for mitophagy. While HFD reduced BNIP3-mediated mitophagy, D was not different from either HFD or LFD animals, thus the total impacts of D on BNIP3-mediated mitophagy are inconclusive. Yet, D/PA increased BNIP3-mediated mitophagy capacity compared to HFD. Our findings align with previous research in murine skeletal muscle demonstrating increased mitophagy markers (suggestive of increased mitochondrial turnover) in exercised animals.[31,32] Recent reports have also suggested that increased mitophagy is necessary for increased mitochondrial biogenesis,[33,34] therefore it is unsurprising that BNIP3 content mirrored PGC1α content in exercised animals. Taken together, our findings suggest that D/PA is more effective in restoring and improving BNIP3-mediated mitophagy.

Interestingly, while BNIP3 increased in D/PA animals, the PINK1/PARKIN pathway is not as easily interpreted. While D or D/PA appear to increase capacity for mitophagy through this mechanism as evidenced by greater PINK1 content, the entire PINK1/PARKIN mitophagy process may be attenuated in HFD, D, and D/PA, as evidenced by lower p-PARKIN/PARKIN content. Yet, p-Ub^{Ser65} appears to suggest the opposite in HFD animals, with p-Ub^{Ser65}/Ub ratios greatly increased compared to all other groups. The complicated interplay between p-Ub^{Ser65}, PARKIN and PINK1 is not yet entirely understood, but current literature suggests that for optimal activation of PARKIN, PINK1 needs to phosphorylate both ubiquitin and PARKIN at the corresponding Serine 65 of each target.[35] In addition to phosphorylation by PINK1, PARKIN is further activated by p-Ub^{Ser65}.[36,37] Specifically, p-Ub^{Ser65} binds to p-PARKIN^{Ser65} leading to a conformational shift,[38–40] allowing PARKIN to mediate ubiquitination of the outer mitochondrial membrane proteins, and tagging the mitochondria for degradation.[41,42] Currently, PINK1 is the only known protein to phosphorylate Ubiquitin at Ser65, suggesting that p-Ub^{Ser65} is specific to PINK1 activity; although it is possible that another protein may perform the same function.[35,43] We noted increased p-Ub^{Ser65} with a concurrent reduction in P-PARKIN/PARKIN ratio. p-Ub^{Ser65} is currently known to be predominantly utilized for mitophagy[17] and p-Ub^{Ser65} binds to PARKIN to trigger E3 ligase activity.[38,44,45] This may suggest that in NAFLD, hepatocytes may attempt to increase mitophagy through the accumulation of p-Ub^{Ser65} in order to dispose of damaged mitochondria and/or replace damaged mitochondria with new mitochondria. Yet, due to decreased mitochondrial biogenesis, the cell may forgo the resolution of PINK1/PARKIN-mediated mitophagy in an effort to maintain some of the mitochondrial networks, regardless of the mitochondria's functionality. Or there may be some unknown dysfunction in the signaling between p-Ub^{Ser65} and p-PARKIN^{Ser65}, resulting in an accumulation of p-Ub^{Ser65} without the synchronized increase in p-PARKIN^{Ser65} activity. Correspondingly, mitochondria in D or D/PA animals, although having a greater capacity for PINK1/PARKIN mitophagy, may forgo PINK1/PARKIN mitophagy in favor of BNIP3-mediated mitophagy. Although future research investigating total mitochondrial density and function is required to substantiate these claims. We should note that our data are collected in a relatively basal state as running wheels were removed 24 h prior to harvest and food removed 6 h before to remove the effects of acute PA and feeding. Considering this we believe, our examination is of basal phosphorylation and content of these targets which may impact interpretations of the effects of these stimuli.

Corroborating mitophagy specific markers, markers of macroautophagy suggested a slightly greater capacity for autophagosome formation in D/PA animals, as evidenced by increased total LC3 content.[46] Interestingly, HFD animals had a greater LC3II/I ratio compared to LFD and D, potentially suggesting enhanced autophagy initiation through increased autophagosome formation. Yet HFD had significantly greater p62 levels, compared to all other groups, implying an impaired resolution of autophagy via accumulation of p62. Taken together, these markers suggest an attempted increase in macroautophagy flux but a decreased resolution in high fat diet-induced NAFLD.

Maintaining dysfunctional hepatic mitochondria via decreased mitophagy at the BNIP3 and PINK1/PARKIN levels, may lead to the accumulation of reactive oxygen species (ROS). Unrestrained ROS production has been associated with decreased liver health[47] and the possible progression of fatty liver disease.[47,48] Therefore, it is possible that decreased mitophagy through high fat diet-induced NAFLD may be a major regulatory point for the progression of NAFLD. As such, therapeutics to promote mitophagy in NAFLD may be possible treatment options.

We should acknowledge some limitations to the present study. As noted in the previous study using these animals,[21] although the high fat diet interventions were sufficient to induce greater hepatic lipid and triglyceride content, histological confirmation of NAFLD or NASH was

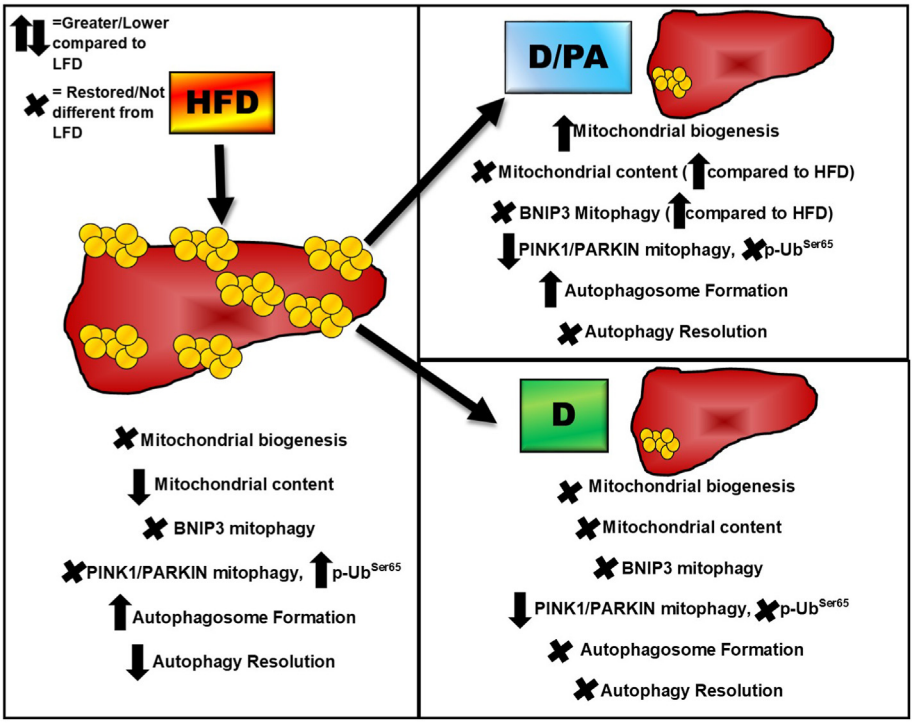

Fig. 4. Pictorial summary of the data from the current study. LFD: Low fat diet group, HFD: high fat diet group, D: diet group, D/PA: diet + physical activity group.

not possible in this study. Therefore, the present work should be interpreted as lipid-induced alterations that would precede the development of diagnosable NAFLD or NASH. Moreover, we were not able to delineate the potential interactions between HFD consumption when performed in conjunction with physical activity. However, given our previous work in animals fed a 45% Western Diet and given access to a running wheel,[19] we anticipate the relatively greater volumes of physical activity in the present study (~500 m/day v. ~8 800 m/day) would be sufficient to confer similar hepatic protections noted in the previous study.

Finally, we also were only able to conduct this study on male rodents. Recent works have begun to elucidate the nuances of hepatic health in relation to biological sex.[24,49,50] For example, epidemiologically, females tend to have a lower incidence of NAFLD.[24] Mechanistically, given the same HFD stimulus, females have lower hepatic H_2O_2 emission compared to males,[49,50] and almost no difference in H_2O_2 measurements compared to low fat fed females.[50] Additionally, given access to a running wheel, females have a greater response in mitochondrial maximal respiratory capacity compared to males.[50] Therefore, given the results of prior research, it is likely females would have a slightly different (likely greater) mitochondrial response to physical activity and diet interventions. However, this hypothesis would require further validation along with data collection collected in a more thermoneutral (i.e. 30 °C) environments.[22]

Taken together, our research demonstrates fluctuating mitophagy responses to HFD-induced NAFLD as well as weight-loss therapeutic interventions. PINK1/PARKIN mitophagy appears to be induced but not fully resolved in NAFLD. D and D/PA both appear to restore some aspects of mito/macroautophagy, with an overall greater effect of D/PA. Increasing autophagy may partially ameliorate symptoms of NAFLD, though more research is necessary to substantiate these findings. These conclusions are summarized in Fig. 4. With no current FDA approved pharmacological methods to specifically target NAFLD, it appears lifestyle interventions incorporating both diet and physical activity are the best available option to increase hepatic autophagy and thereby improve symptoms of NAFLD, with an emphasis on physical activity in addition to the diet.

Submission statement

All authors have read and agree with manuscript content. While this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Ethical approval statement

All animals were housed at 22.5 °C and 23.5 °C temperatures and a 12 h dark and 12 h light cycle. Food and water were provided *ad libitum* during experimental period. All procedures were performed in accordance with the guidelines established by Animal Care and Committee Guidelines of Southern Illinois University at Edwardsville.

Authors' contributions

Assistance with animal experiments—KEP, AS, MPH, JSW, Collection and Analysis of data—MER, KEP, AS, MPH, JSW. Data Analysis and Interpretation—MER, NPG, JSW. Editing/Revising of Manuscipt—MER, KEP, AS, MPH, NPG, JSW.

Conflict of interest

The authors declare no financial or other conflicts of interest that could influence the interpretations of this work.

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References

1. Bellentani S, Scaglioni F, Marino M, et al. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis*. 2010;28(1):155–161. https://doi.org/10.1159/000282080.
2. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the third national health and nutrition examination survey, 1988-1994. *Am J Epidemiol*. 2013;178(1):38–45. https://doi.org/10.1093/aje/kws448.
3. Lavallard VJ, Gual P. Autophagy and non-alcoholic fatty liver disease. *BioMed Res Int*. 2014;2014:120179. https://doi.org/10.1155/2014/120179.
4. Filozof C, Goldstein BJ, Williams RN, et al. Non-alcoholic steatohepatitis: limited available treatment options but promising drugs in development and recent progress towards a regulatory approval pathway. *Drugs*. 2015;75(12):1373–1392. https://doi.org/10.1007/s40265-015-0437-3.
5. Alex S, Boss A, Heerschap A, et al. Exercise training improves liver steatosis in mice. *Nutr Metab*. 2015;12:29. https://doi.org/10.1186/s12986-015-0026-1.
6. Rector RS, Uptergrove GM, Morris EM, et al. Daily exercise vs. caloric restriction for prevention of nonalcoholic fatty liver disease in the OLETF rat model. *Am J Physiol Gastrointest Liver Physiol*. 2011;300(5):G874–G883. https://doi.org/10.1152/ajpgi.00510.2010.
7. Di Ciaula A, Passarella S, Shanmugam H, et al. Nonalcoholic fatty liver disease (NAFLD). Mitochondria as players and targets of therapies? *Int J Mol Sci*. 2021;22(10):5375. https://doi.org/10.3390/ijms22105375.
8. Galloway CALH, 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*. 2014;307(6):G632–G641. https://doi.org/10.1152/ajpgi.00182.2014.
9. Glick D, Zhang W, Beaton M, et al. BNip3 regulates mitochondrial function and lipid metabolism in the liver. *Mol Cell Biol*. 2012;32(13):2570–2584. https://doi.org/10.1128/MCB.00167-12.
10. Kuo JJ, Chang HH, Tsai TH, et al. Positive effect of curcumin on inflammation and mitochondrial dysfunction in obese mice with liver steatosis. *Int J Mol Med*. 2012;30(3):673–679. https://doi.org/10.3892/ijmm.2012.1049.
11. Xu J, Cao K, Li Y, et al. Bitter gourd inhibits the development of obesity-associated fatty liver in C57BL/6 mice fed a high-fat diet. *J Nutr*. 2014;144(4):475–483. https://doi.org/10.3945/jn.113.187450.
12. Rector RS, Thyfault JP, Uptergrove GM, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *J Hepatol*. 2010;52(5):727–736. https://doi.org/10.1016/j.jhep.2009.11.030.
13. Nguyen TN, Padman BS, Lazarou M. Deciphering the molecular signals of PINK1/parkin mitophagy. *Trends Cell Biol*. 2016;26(10):733–744. https://doi.org/10.1016/j.tcb.2016.05.008.
14. Novak I, Kirkin V, McEwan DG, et al. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep*. 2010;11(1):45–51. https://doi.org/10.1038/embor.2009.256.
15. Hamacher-Brady A, Brady NR. Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. *Cell Mol Life Sci*. 2016;73(4):775–795. https://doi.org/10.1007/s00018-015-2087-8.
16. Kondapalli C, Kazlauskaite A, Zhang N, et al. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol*. 2012;2(5):120080. https://doi.org/10.1098/rsob.120080.
17. Kane LA, Lazarou M, Fogel AI, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol*. 2014;205(2):143–153. https://doi.org/10.1083/jcb.201402104.
18. Kazlauskaite A, Kondapalli C, Gourlay R, et al. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J*. 2014;460(1):127–139. https://doi.org/10.1042/BJ20140334.
19. Rosa-Caldwell ME, Lee DE, Brown JL, et al. Moderate physical activity promotes basal hepatic autophagy in diet-induced obese mice. *Appl Physiol Nutr Metabol*. 2017;42(2):148–156. https://doi.org/10.1139/apnm-2016-0280.
20. Rosa-Caldwell ME, Jansen LT, Lim S, et al. Neither autophagy nor exercise training mode affect exercise-induced beneficial adaptations in high fat-fed mice. *Sports Medicine and Health Science*. 2020;2(1):44–53. https://doi.org/10.1016/j.smhs.2020.03.003.
21. Wooten JS, Poole KE, Harris MP, et al. The effects of voluntary wheel running during weight-loss on biomarkers of hepatic lipid metabolism and inflammation in C57Bl/6J mice. *Curr Res Physiol*. 2022;5:63–72. https://doi.org/10.1016/j.crphys.2022.01.003.
22. Fuller KNZ, Thyfault JP. Barriers in translating preclinical rodent exercise metabolism findings to human health. *J Appl Physiol*. 1985;130(1):182–192. https://doi.org/10.1152/japplphysiol.00683.2020.
23. González-Granillo M, Helguero LA, Alves E, et al. Sex-specific lipid molecular signatures in obesity-associated metabolic dysfunctions revealed by lipidomic characterization in ob/ob mouse. *Biol Sex Differ*. 2019;10(1):11. https://doi.org/10.1186/s13293-019-0225-y.
24. Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;67(1):123–133. https://doi.org/10.1002/hep.29466.
25. Greene NP, Lee DE, Brown JL, et al. Mitochondrial quality control, driven by PGC-1α, is dysregulated by Western Diet-induced obesity and partially restored by moderate physical activity in mice. *Phys Rep*. 2015;3(7):e12470. https://doi.org/10.14814/phy2.12470.
26. Greene NPNM, Washington TA, Lee DE, et al. Impaired exercise-induced mitochondrial biogenesis in the obese Zucker rat, despite PGC-1α induction, is due to compromised mitochondrial translation elongation. *Am J Physiol Endocrinol Metab*. 2014:E503–E511. https://doi.org/10.1152/ajpendo.00671.2013.
27. Chen HL, Tung YT, Tsai CL, et al. Kefir improves fatty liver syndrome by inhibiting the lipogenesis pathway in leptin-deficient ob/ob knockout mice. *Int J Obes*. 2014;38(9):1172–1179. https://doi.org/10.1038/ijo.2013.236.
28. Perfield JW, Ortinau LC, Pickering RT, et al. Altered hepatic lipid metabolism contributes to nonalcoholic fatty liver disease in leptin-deficient Ob/Ob mice. *J Obes*. 2013;2013:296537. https://doi.org/10.1155/2013/296537.
29. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell Market*. 1998;92(6):829–839. https://doi.org/10.1016/s0092-8674(00)81410-5.
30. Aharoni-Simon M, Hann-Obercyger M, Pen S, et al. Fatty liver is associated with impaired activity of PPARγ-coactivator 1α (PGC1α) and mitochondrial biogenesis in mice. *Lab Invest*. 2011;91(7):1018–1028. https://doi.org/10.1038/labinvest.2011.55.
31. Jamart C, Naslain D, Gilson H, et al. Higher activation of autophagy in skeletal muscle of mice during endurance exercise in the fasted state. *Am J Physiol Endocrinol Metab*. 2013;305(8):E964–E974. https://doi.org/10.1152/ajpendo.00270.2013.
32. Lira VA, Okutsu M, Zhang M, et al. Autophagy is required for exercise training-induced skeletal muscle adaptation and improvement of physical performance. *Faseb J*. 2013;27(10):4184–4193. https://doi.org/10.1096/fj.13-228486.
33. Ivankovic D, Chau KY, Schapira AH, et al. Mitochondrial and lysosomal biogenesis are activated following PINK1/parkin-mediated mitophagy. *J Neurochem*. 2016;136(2):388–402. https://doi.org/10.1111/jnc.13412.
34. Sin J, Andres AM, Taylor DJ, et al. Mitophagy is required for mitochondrial biogenesis and myogenic differentiation of C2C12 myoblasts. *Autophagy*. 2016;12(2):369–380. https://doi.org/10.1080/15548627.2015.1115172.
35. Herhaus L, Dikic I. Expanding the ubiquitin code through post-translational modification. *EMBO Rep*. 2015;16(9):1071–1083. https://doi.org/10.15252/embr.201540891.
36. Ordureau A, Sarraf SA, Duda DM, et al. Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. *Mol Cell*. 2014;56(3):360–375. https://doi.org/10.1016/j.molcel.2014.09.007.
37. Wauer T, Swatek KN, Wagstaff JL, et al. Ubiquitin Ser65 phosphorylation affects ubiquitin structure, chain assembly and hydrolysis. *EMBO J*. 2015;34(3):307–325. https://doi.org/10.15252/embj.201489847.
38. Kazlauskaite A, Martínez-Torres RJ, Wilkie S, et al. Binding to serine 65-phosphorylated ubiquitin primes Parkin for optimal PINK1-dependent phosphorylation and activation. *EMBO Rep*. 2015;16(8):939–954. https://doi.org/10.15252/embr.201540352.
39. Wauer T, Simicek M, Schubert A, et al. Mechanism of phospho-ubiquitin-induced PARKIN activation. *Nature*. 2015;524(7565):370–374. https://doi.org/10.1038/nature14879.
40. Sauvé V, Lilov A, Seirafi M, et al. A Ubl/ubiquitin switch in the activation of Parkin. *EMBO J*. 2015;34(20):2492–2505. https://doi.org/10.15252/embj.201592237.
41. Narendra D, Walker JE, Youle R. Mitochondrial quality control mediated by PINK1 and Parkin: links to parkinsonism. *Cold Spring Harbor Perspect Biol*. 2012;4(11):a011338.. https://doi.org/10.1101/cshperspect.a011338.
42. Sarraf SA, Raman M, Guarani-Pereira V, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature*. 2013;496(7445):372–376. https://doi.org/10.1038/nature12043.
43. Swaney DL, Rodríguez-Mias RA, Villén J. Phosphorylation of ubiquitin at Ser65 affects its polymerization, targets, and proteome-wide turnover. *EMBO Rep*. 2015;16(9):1131–1144. https://doi.org/10.15252/embr.201540298.
44. Koyano F, Okatsu K, Kosako H, et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature*. 2014;510(7503):162–166. https://doi.org/10.1038/nature13392.
45. Ordureau A, Heo JM, Duda DM, et al. Defining roles of PARKIN and ubiquitin phosphorylation by PINK1 in mitochondrial quality control using a ubiquitin replacement strategy. *Proc Natl Acad Sci U S A*. 2015;112(21):6637–6642. https://doi.org/10.1073/pnas.1506593112.
46. Klionsky DJ, Abdalla FC, Abeliovich H, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*. 2012;8(4):445–544. https://doi.org/10.4161/auto.19496.
47. Nassir F, Ibdah JA. Role of mitochondria in nonalcoholic fatty liver disease. *Int J Mol Sci*. 2014;15(5):8713–8742. https://doi.org/10.3390/ijms15058713.
48. Gusdon AM, Song KX, Qu S. Nonalcoholic Fatty liver disease: pathogenesis and therapeutics from a mitochondria-centric perspective. *Oxid Med Cell Longev*. 2014;2014:637027. https://doi.org/10.1155/2014/637027.
49. Fuller KNZ, McCoin CS, Allen J, et al. Sex and BNIP3 genotype, rather than acute lipid injection, modulate hepatic mitochondrial function and steatosis risk in mice. *J Appl Physiol (1985)*. 2020;128(5):1251–1261. https://doi.org/10.1152/japplphysiol.00035.2020.
50. McCoin CS, Von Schulze A, Allen J, et al. Sex modulates hepatic mitochondrial adaptations to high-fat diet and physical activity. *Am J Physiol Endocrinol Metab*. 2019;317(2):E298–E311. https://doi.org/10.1152/ajpendo.00098.2019.