SIRT3 deficiency decreases oxidative metabolism capacity but increases lifespan in male mice under caloric restriction

Rashpal S. Dhillon1,2 | Yiming (Amy) Qin1,2,3 | Paul R. van Ginkel4 | Vivian X. Fu4 | James M. Vann4 | Alexis J. Lawton1,2 | Cara L. Green5,6 | Fúlia B. Manchado-Gobatto7 | Claudio A. Gobatto7 | Dudley W. Lamming3,5,6 | Tomas A. Prolla4 | John M. Denu1,2,3

1Department of Biomolecular Chemistry, University of Wisconsin-Madison, Madison, Wisconsin, USA
2Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, Wisconsin, USA
3Interdepartmental Graduate Program in Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA
4Department of Genetics and Medical Genetics, University of Wisconsin-Madison, Madison, Wisconsin, USA
5Department of Medicine, SMPH, University of Wisconsin-Madison, Madison, Wisconsin, USA
6William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA
7Laboratory of Applied Sport Physiology, School of Applied Sciences, University of Campinas, Limeira, Brazil

Abstract
Mitochondrial NAD+–dependent protein deacetylase Sirtuin3 (SIRT3) has been proposed to mediate calorie restriction (CR)-dependent metabolic regulation and lifespan extension. Here, we investigated the role of SIRT3 in CR-mediated longevity, mitochondrial function, and aerobic fitness. We report that SIRT3 is required for whole-body aerobic capacity but is dispensable for CR-dependent lifespan extension. Under CR, loss of SIRT3 (Sirt3−/−) yielded a longer overall and maximum lifespan as compared to Sirt3+/+ mice. This unexpected lifespan extension was associated with altered mitochondrial protein acetylation in oxidative metabolic pathways, reduced mitochondrial respiration, and reduced aerobic exercise capacity. Also, Sirt3−/− CR mice exhibit lower spontaneous activity and a trend favoring fatty acid oxidation during the postprandial period. This study shows the uncoupling of lifespan and healthspan parameters (aerobic fitness and spontaneous activity) and provides new insights into SIRT3 function in CR adaptation, fuel utilization, and aging.

Keywords: aerobic fitness, calorie restriction, fatty acid oxidation, fuel switching, lifespan, mitochondrial acetylation, mitochondrial respiration, sirtuins

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Aging Cell published by Anatomical Society and John Wiley & Sons Ltd.
Incidence of non-communicable diseases, such as cardiovascular disease, neurodegeneration, and cancer, increases significantly with age (Balasubramanian et al., 2017). Calorie restriction (CR) robustly delays age-related diseases and extends both health and lifespan in diverse species (Fontana & Partridge, 2015; Mattison et al., 2017). Mitochondria, the center of cellular oxidative metabolism, are vital to cellular health, and mitochondrial dysfunction has been associated with accelerated aging (Jang et al., 2018; Srivastava, 2017). One of the hallmarks of CR is the preservation of mitochondrial function through reducing oxidative stress, enhancing fuel utilization, and maintaining mitochondrial dynamics and integrity (Bruss et al., 2010; Jang et al., 2018; Lanza et al., 2012; Merry, 2004). Previous studies have proposed that Sirtuin3 (SIRT3)-dependent deacetylation may play a major role in modulating mitochondria under CR (Hallows et al., 2011; Qiu et al., 2010; Someya et al., 2010).

Reversible N^2-lysine acetylation is a prominent post-translational modification enriched in mitochondria, with more than 60% of all mitochondrial proteins having at least one acetylated lysine site identified in proteomic studies (Baeza et al., 2016; Liu et al., 2014). Among well-characterized examples, mitochondrial acetylation mostly inhibits enzymatic activity, slows down metabolic pathways, and leads to mitochondrial dysfunction (Hirshey et al., 2010; Still et al., 2013; Vassilopoulos et al., 2014) which has been correlated with diabetes, heart failure, and age-related cellular impairment (Ansari et al., 2017; Fukushima & Lopaschuk, 2016; Horton et al., 2016). SIRT3 is the predominant NAD^+-dependent deacetylase in mitochondria, whose deficiency leads to significant hyperacetylation in mitochondria of various tissues (Dittenhafer-Reed et al., 2015). SIRT3 level is diet-sensitive, and its expression increases under fasting and CR (Palacios et al., 2009; Schwer et al., 2009). We have reported SIRT3-dependent deacetylation of mitochondrial proteins in CR mice and demonstrated that SIRT3 is essential for the prevention of age-related hearing loss in mice fed a CR diet (Someya et al., 2010). These observations have fueled the speculation that SIRT3-dependent control of acetylation may be crucial for CR-induced modulation of aging and lifespan extension. Nonetheless, direct evaluation of the contribution by SIRT3 to CR-dependent lifespan extension and mitochondrial performance during aging is lacking.

Here, using male Sirt3^+/+ (WT) and Sirt3^−/− mice, both in a C57BL/6NJ × C57BL/6J NNT wildtype genetic background, treated with either a control diet (CD, under moderate CR) or CR diet (25% reduction from CD), we found that (1) SIRT3 is essential for whole-body aerobic fitness as assessed by critical velocity, but is not required to mediate CR-dependent longevity, (2) loss of SIRT3 reduces the net OXPHOS respiration when glucose-derived metabolites are utilized, and (3) SIRT3 ablation appears to favor fuel switching from glucose to fatty acids during the postprandial state. We also show that SIRT3 is required for CR-induced increases in spontaneous physical activity (SPA). Collectively, these data reveal that SIRT3 deficiency under CR increases lifespan beyond CR alone and that this condition is associated with lower aerobic fitness, lower spontaneous activity, and altered mitochondrial metabolism favoring fatty acid metabolism.

To investigate the role of SIRT3 in CR-dependent metabolism during aging, we established whole-body Sirt3^−/− mouse models in the Nnt^−/− background by crossing C57BL/J (Nnt^+/−) Sirt3^−/− mice with C57BL/6NJ (Nnt^+/+) Sirt3^−/− mice. This is noteworthy in that many prior aging and SIRT3 studies employed C57BL/6J mice that lack functional mitochondrial nicotinamide nucleotide transhydrogenase (NNT). With increasing evidence demonstrating the importance of NNT in redox control and metabolism (Ronchi et al., 2016; Smith et al., 2020), we generated and used C57BL/6NJ × C57BL/6J background, NNT wild-type Sirt3^−/− and NNT wild-type Sirt3^−/− mice in the current study. From 2 months of age until death, male WT and Sirt3^−/− mice were maintained on a fixed calorie CD (89 kcal/week) or CR diet (67 kcal/week, 25% less calorie intake than CD-fed mice) (Figure 1a). Notably, CD mice in the current study were fed 16% less calories compared to ad libitum mice (105.8 kcal/week) to reduce obesity and related metabolic complications (see Methods for detailed diet design; Pugh et al., 1999). Both CD and CR groups were fed three times/week (Monday, Wednesday, and Friday at ~7 AM). CR mice generally finished food within 12–24 h, and CD mice finished food within 24–36 h. No genotype-dependent food consumption difference was observed. Thus, all study groups were under an intermittent fasting protocol with CR mice experiencing a slightly longer fasting time. Body weight averages increased until ~12 months of age and were maintained until ~27 months of age, after which average weight values declined in both CR and CD groups (Figure 1d). At peak weights, body weights were approximately 25% lower in CR mice (Figure 1d). Since food intake was fixed, variations in body weight in animals during their lifespan likely resulted in alterations in food consumption relative to body weight in individual animals at different ages.

Kaplan–Meier survival curves (Figure 1b; Table S1a) revealed that as expected, CR significantly extended the average (WTCD: 24.1 months, WTCR: 29.7 months), median (50% survival, WTCD: 25.2 months, WTCR: 30.1 months), and maximum lifespan (WTCD: 35.1 months, WTCR: 39.4 months, calculated as the average lifespan of the top 10% longest-lived mice) of WT mice (Figure 1c). Sirt3^−/− mice exhibited a similar average (WTCD vs. Sirt3^−/−CD: 24.1 vs. 23.5 months, WTCR vs. Sirt3^−/−CR: 29.7 vs. 31.0 months), median lifespan (WTCD vs. Sirt3^−/−CD: 25.2 vs. 24.0 months, WTCR vs. Sirt3^−/−CR: 30.1 vs. 31.5 months) as their diet-matched counterparts under both CD and CR, but unexpectedly Sirt3^−/−CR mice showed an overall increase in lifespan as compared to WTCR mice (p = 0.036, Table S1a). Remarkably, Sirt3^−/−CR mice displayed a 10% increase in
maximum lifespan relative to WTCR (WTCR vs. Sirt3−/−CR: 39.4 vs. 43.8 months, \( p = 0.03 \), Figure 1c). Confirmed with Boschloo’s test, \( p = 0.04 \) at 90% percentile for WTCR versus Sirt3−/−CR, Table S1b. These results show that SIRT3 is not required for the normal life extension benefit of CR and that SIRT3 ablation further extends CR-mediated longevity. Interestingly, the Sirt3−/−-dependent overall lifespan extension benefit was not noticeable until a later age, as the survival curves of WTCR and Sirt3−/−CR mice diverge after the median lifespan of these groups (Figure 1b) and a significant change in 75% percentile in WTCR versus Sirt3−/−CR was observed (\( p = 0.03 \), Table S1b). Lifetime body weight (Figure 1d) and body composition at 25 months of age (Figure S1a–c) showed a diet but not a SIRT3 dependence. Together, these observations revealed an unexpected boost of maximum lifespan in Sirt3−/− mice under CR.

Several reports have highlighted the ability of SIRT3 to preserve mitochondrial integrity and suppress oxidative stress through protein deacetylation (Bause & Haigis, 2013; Kim et al., 2010; Meng et al., 2019; Qiu et al., 2010) including work demonstrating that CR prevents age-related hearing loss through SIRT3-dependent mechanism involving IDH2 activation (Someya et al., 2010; Yu et al., 2012). Recently, Benigni et al. (2019) observed that shortened lifespan in standard chow-fed Sirt3−/− mice is associated with altered cardiac mitochondrial morphology. Given the unexpected longevity in Sirt3−/− CR mice, we investigated whether mitochondrial integrity as examined by transmission electron microscopy was affected at 25 months of age. No morphological differences in gastrocnemius and heart mitochondria were observed between genotypes nor diets (Figure S1d). Similarly, citrate synthase activity, a surrogate marker of mitochondrial density, and its protein expression in the brain, gastrocnemius, heart, and liver were comparable between groups (Figure S1e,f). Moreover, there was no significant link between SIRT3 expression (Figure S1g) and mitochondrial DNA content (mtDNA:nDNA, Figure S1h). NADPH/NADP+ and GSH/GSSG ratios were comparable between Sirt3−/− and WT animals under both diets (Figure S1i,j) in the liver and heart. In summary, these data indicate that loss of SIRT3 has no significant impact on mitochondrial integrity and markers of ROS detoxification in aged C57BL/6NJ x C57BL/6J Nnt−/− mice.
2.2 | SIRT3 opposes hyperacetylation of specific target proteins under CR during aging

Consistent with the role of SIRT3 as a mitochondrial deacetylase, immunoblotting of total mitochondrial protein acetylation in liver from 25-month-old mice displayed an increase with SIRT3 ablation and a combined effect of CR and loss of SIRT3 (Figure S2a). To identify the lysine sites and proteins showing altered acetylation as a function of age, genotype, and diet, we leveraged our recently developed quantitative mass spectrometry workflow (Baeza et al., 2020) (Figure S2b). Site-specific acetylation stoichiometry from enriched liver mitochondrial fractions was determined in 5- and 25-month-old WTCD, WTCR, Sirt3−/−CD, and Sirt3−/−CR mice. From 1854 unique mitochondrial acetyl-lysine sites identified (≤1% false discovery rate), 941 sites on 329 proteins (MitoCarta 2.0, Calvo et al. (2016), Table S2a) were quantified in all eight treatment groups, with acetylation stoichiometry ranging from less than 1% to 99% and a median stoichiometry of ~8.3% among quantified sites.

The effects of diet, age, and genotype were parsed out (Figure S2c-f) to reveal the relative number of changed acetylation sites (density) as a function of change in stoichiometry for those conditions. An age-mediated increase in acetylation stoichiometry in mice fed a CR diet was evident. Both young WT and Sirt3−/−CR mice showed less acetylation alteration and accumulation, suggesting that aged mice are more prone to CR-induced hyperacetylation (Figure S2d). Additionally, the stoichiometry disparities between aged and young mice were more notable when the mice are Sirt3−/− and CR fed, indicating that both CR and Sirt3−/− amplify the age-dependent acetylation alteration (Figure S2e). Lastly, SIRT3-dependent acetylation changes are most significant in aged, CR-treated animals (Figure S2f), with the 25-month Sirt3−/−CR versus 25-month WTCR comparison showing the largest fraction of increased acetylation (Figure 2a) among the various groups. In sum, these results indicate that CR and loss of SIRT3 individually and cooperatively contribute to the global increase in acetylation at old age.

To reveal specific site-level acetylation changes across the various conditions, we plotted the relative stoichiometry of significantly changed sites due to loss of SIRT3 in WTCR versus Sirt3−/−CD mice or WTCR versus Sirt3−/−CR mice at 25 months of age in liver (Figure 2b). As noted in the major acetylation trends displayed in Figure 2d-f, many sites showed increased acetylation in Sirt3−/− mice and an additive effect in Sirt3−/−CR. These sites likely reflect direct SIRT3 targets that become hyper-acetylated under CR. Surprisingly, we identified sites which were hypoacetylated in Sirt3−/− mice versus WT counterparts under both CD and CR (Sirt3−/−CD vs. WTCR: 65 sites, Sirt3−/−CR vs. WTCR: 31 sites), and sites that were hypoacetylated in Sirt3−/−CR versus WTCR mice but not Sirt3−/−CD versus WTCD mice (11 sites) (Table S2b). These sites show opposite behaviors to those expected, but yet their changes in acetylation follow a SIRT3-dependent manner. This is especially interesting for sites that are hypoacetylated only in CR when Sirt3 is absent. These observations reveal a set of acetylation sites that are dependent on CR and SIRT3, but would not be direct substrate targets of SIRT3. The acetyl proteomics indicate major changes to the acetylation status of mitochondrial proteins dependent on age, genotype, and diet, and that the unique set of expected and unexpected changes under both CR and loss of SIRT3 highlights the molecular pathways in Sirt3−/−CR mice associated with increased lifespan.

To identify the mitochondrial processes that are likely perturbed due to Sirt3−/− status and CR at old age, we performed functional cluster analysis of KEGG pathways (Figure 2c, DAVID 6.8) (Dennis et al., 2003). We compared the KEGG pathway enrichment analysis and found many similarities between the enriched pathways in the CD-fed Sirt3−/− vs WT mice and the CR-fed Sirt3−/− vs WT mice comparisons. In both comparisons (Figure 2c) significant enrichment of major metabolic pathways was apparent, including TCA cycle, valine, isoleucine and leucine degradation, and fatty acid degradation. In the most affected pathways (Figure 2d), 52 acetylation sites (>5% stoichiometry change, p < 0.1) were identified in fatty acid oxidation, TCA cycle, electron transport chain (ETC), and branched-chain amino acids (BCAA) metabolism. Among these sites, 23 sites were previously noted in Hebert et al. (2013) and 29 sites are newly identified. In addition to increased acetylated sites due to loss of SIRT3, we observed 44% of sites displaying lower acetylation stoichiometry in Sirt3−/−CD and CR mice relative to their WT counterparts, again suggesting that alterations in acetylation occurred in unexpected combinations of sites that decreased or increased in acetylation. Regardless of the direction change in acetylation, the cluster analysis suggests a functional alteration in oxidative metabolism is likely associated with the extended lifespan observed in Sirt3−/−CR mice (Figure 2c.d).

2.3 | Loss of SIRT3 limits aerobic fitness due to reduced mitochondrial respiration capacity

To understand how altered acetylation of pathways identified in the acetyl-proteomics might affect mitochondrial oxidative metabolism in aged Sirt3−/−CR mice, we first assessed whole-animal bioenergetics through aerobic exercise capacity. At 25 months of age, WTCD, Sirt3−/−CD, WTCR, Sirt3−/−CR mice were subjected to four sessions of treadmill exercise at different velocities until exhaustion, and the critical velocity, a measure of aerobic exercise capacity, was obtained (Scariot et al., 2019). Both Sirt3−/− groups performed poorly, displaying critical velocity for Sirt3−/−CD and Sirt3−/−CR mice that were 24% and 27% slower than that of their diet matched control animals (Figure 3a).

Next, we investigated whether reduced aerobic fitness in Sirt3−/− mice can be explained by compromised mitochondrial respiration given that subunits of ETC complexes were one of the most prominent SIRT3 target groups and found to be inhibited under hyperacetylation induced by SIRT3 ablation (Ahn et al., 2008; Bao et al., 2010; Horton et al., 2016; Parodi-Rullán et al., 2018) (Table S2). To this end, we measured ex-vivo mitochondrial respiration in permeabilized brain, gastrocnemius, heart,
and liver from 25-month-old treatment groups. Using high resolution respirometry with pyruvate, glutamate, malate, and succinate as substrates, we found that WTCR mice exhibited the highest electron transport capacity in heart (Figure S3a) and the highest percentage of net OXPHOS respiration relative to total respiration in heart and liver (Figure 3b, see Figure S3b for statistics) among

FIGURE 2 SIRT3 opposes hyperacetylation of its targets under CR during aging. (a–d) Liver mitochondrial acetylome analysis using stoichiometry-based MS quantification. See Table S2 for all identified acetyl-lysine sites and statistical analysis, n = 4 per group. (a) Percentage of significantly changed acetyl-lysine residues that show increased stoichiometry due to Sirt3−/− status, calculated by (the number of acetyl-lysine sites showing increased stoichiometry)/(the number of significantly changed acetyl-lysine sites, p < 0.05 ×100%. 5: 5 months old; 25: 25 months old. (b) Heat map of significantly changed lysine sites p < 0.05 in 25 month-old mice that are a response to loss of SIRT3. Plotted sites are significantly changed (p < 0.1) in either Sirt3−/−CD versus WTCD or Sirt3−/−CR versus WTCR comparison. Values are colored based on relative acetylation stoichiometry, normalized to the median value of each site in all four groups, scaling ranging from -0.8 to 0.8 (×100%). (c) Functional cluster analysis of KEGG pathways (DAVID 6.8). Significantly enriched (−log10(p value)) >1.5) pathways are indicated, with 25 month-old Sirt3−/−CD versus WTCD in orange and 25 month-old Sirt3−/−CR versus WTCR in blue. (d) Acetylation sites in FAO and BACC metabolism, TCA cycle, and ETC that displayed larger than 5% stoichiometry (p < 0.1) for 25 month-old Sirt3−/−CD versus WTCD (orange colored) and 25 month-old Sirt3−/−CR versus WTCR comparison (blue colored). CD, control diet; CR, calorie restriction; MS, mass spectrometry; SIRT3, sirtuin3
four treatment groups, consistent with previous reports that CR preserves respiration efficiency in aged mice (Lanza et al., 2012; Weindruch et al., 1980). In the CD group, net OXPHOS respiration (Figure S3a) and its percentage to total respiration (Figure 3b) are largely comparable in gastrocnemius muscle, heart, and liver between genotypes, with a ~20% reduction ($p = 0.042$) in percentage of net OXPHOS respiration found in brain mitochondria from Sirt3$^{-/-}$ relative to WT mice. In contrast, under CR, we observed pronounced reductions in net OXPHOS respiration (Figure 3b) and its percentage relative to total respiration capacity (Figure S3a) in brain, heart and liver in Sirt3$^{-/-}$CR compared to WTCR mice. These respiration phenotypes suggest that both SIRT3 and CR can play a role in preserving mitochondrial respiration capacity and bioenergetics in old age and that reduced net OXPHOS respiration is a consistent feature of Sirt3$^{-/-}$ CR mice.

To pinpoint the affected respiration pathway(s), we parsed out substrate-dependent respiration and conducted enzymatic activity assays for the 25-month-old treatment groups. No significant difference in NADH-linked substrates (pyruvate, glutamate, and malate) coupled respiration between genotypes nor between diets was observed (Figure S3c). Succinate-dependent respiration capacity, however, was significantly reduced in Sirt3$^{-/-}$CR liver and heart relative to WTCR (Figure S3c). In CD-treated groups, succinate-dependent respiration capacity showed minor difference in heart between WT and Sirt3$^{-/-}$. Enzymatic activity assays revealed that SIRT3 and CR indeed had the more prominent impact on Complex II activity compared to that of Complex I (Figure S3d,e). Moreover, we determined if fatty acid oxidation (FAO)-dependent respiration was limited in permeabilized liver and heart from Sirt3$^{-/-}$ animals using palmitoylcarnitine and malate as substrates. Interestingly, palmitoylcarnitine-dependent coupled respiration (Figure 3d) and its net OXPHOS control efficiency (Figure S3f) was similar between WTCR and Sirt3$^{-/-}$CR mice in both liver and heart. In contrast to mice on the CD diet, palmitoylcarnitine-dependent OXPHOS control efficiency (Figure S3f) was significantly lower in Sirt3$^{-/-}$ mouse heart relative to WT. These results suggest that the reduced Complex II respiration in SIRT3-deficient mice provides a molecular basis for lowered whole-body aerobic exercise capacity. But notably, CR treatment allows Sirt3$^{-/-}$ mice to maintain FAO-dependent respiration.

2.4 | Sirt3$^{-/-}$ mice under CR display trends in metabolic parameters that are consistent with faster switching from glucose to fatty acid oxidation during the postprandial period

In light of the critical velocity and substrate-dependent respiration phenotypes obtained from the four treatment groups, we speculated that Sirt3$^{-/-}$CR mice display unique metabolic features. The four groups of 25-month-old mice were subjected to a metabolic chamber experiment that consisted of a 24-h fasting period followed by a re-feeding period with 8 h of food provision (Figure 4a,b; Figure S4b,c). Diet-matched groups consumed a similar amount of food during the 8-hour food provision period (Figure 4c). During the fasting period, the respiratory exchange ratio (RER) was not significantly different among the treatment groups, with RERs ranging between 0.7 and 0.8, indicating that fatty acids were the preferred energy source. Upon feeding, RER increased to $\geq 1$ for all groups, reflecting a fuel switching to the predominant use of carbohydrate as an energy source, accompanied by fatty acid synthesis before returning to FAO in the fasted state (Bruss et al., 2010; Mitchell et al., 2019). We observed a trend of smaller amplitudes of RER elevation upon feeding in Sirt3$^{-/-}$CR mice and a shorter duration of the RER $\geq 1$ period as compared to that of WTCR mice, whereas the comparison of WTCD and Sirt3$^{-/-}$CD mice showed no such trend. Notably, the shape of the RER curve for CR mice was different from that of CD mice. Instead of a RER drop to baseline (fasted state RER), CR mice maintained a RER ~0.8–0.9 for 6 h, suggesting a longer period of mixed fuel utilization compared to CD mice. We noted that CR mice consumed significantly more food in the feeding period (Figure 4c), which may have contributed to this observation. We also noted that food consumption of KOCR mice was trending lower, which may play a role in the earlier reduction in RER compared to WTCR. Energy expenditure under both fast and fed states was calculated (Figure S4d,e), along with analysis of
volume of O$_2$ consumption and CO$_2$ generation (Figure S4f,g). From RER and energy expenditure measurements, fed and fasted state FAO were calculated (Figure 4d,e). We observed a trend of increased FAO in Sirt3$^{-/-}$CR mice compared to WTCR mice. To control for the confounding impact of weight on energy expenditure, we measured the impact of genotype (WT vs. Sirt3$^{-/-}$) and diet (CD vs. CR) on energy expenditure using analysis of covariance (ANCOVA) with body weight as a covariate. We split mice into fed/fasted and dark/light groups, and performed ANCOVAs on each set. For fed mice at both dark and light times of day, energy expenditure was not significantly affected by diet or genotype (Table S4). For mice fasted during the dark period, this was also true, although body weight did have a significant impact on energy expenditure, with higher body weights resulting in higher energy expenditures. For fasted mice in the light phase, while controlling for body weight, energy expenditure was significantly different between genotypes, with energy expenditure significantly increased in the KO group, although there was no effect of diet.

Given the every-other-day feeding protocol in the current lifespan experiment for both CD and CR animals (Pugh et al., 1999), CR
animals generally consumed their daily allotment of food within 12–16 h without altered eating behavior observed between genotypes. To capture daily RER fluctuations and estimate the duration of the predominant carbohydrate utilization period in CR mice, we repeated the metabolic chamber experiments using a different set of CR animals with 24 h of fasting followed by a refeeding period in which the normal daily food allotment used in the lifespan study was provided, and no food was removed during the experiment. These conditions mimic the normal food intake routine of this cohort of animals throughout their lifespan. Again, a trend of smaller amplitude of RER in response to food intake and a shorter duration of RER ≥1 were observed in Sirt3−/−CR relative to WTCR mice (Figure 4f; Figure S4h,i), consistent with the RER pattern observed in Figure 4a,b. Energy expenditure (Figure S4j,k), O2 consumption, and CO2 generation (Figure S4l,m) were comparable between WTCR and Sirt3−/−CR mice, and fasted state FAO trended higher in Sirt3−/−CR mice (Figure S4n). Our observations suggest that Sirt3−/−CR mice are able to switch to carbohydrates as the predominant fuel source upon feeding, but may be less capable of maintaining carbohydrate utilization during the fed state. Possibly, Sirt3−/−CR animals experience an earlier switch to FAO-dependent metabolism (fasting state) compared to WTCR.

To further investigate whether Sirt3−/−CR mice have compromised glucose oxidation during the fed state, we performed liquid chromatography–mass spectrometry-based metabolite profiling in liver, heart, and muscle of 6-h refed mice. Compared to WTCR mice, the Sirt3−/−CR group exhibited a trend of reduced levels of ATP (p = 0.07), acetyl-CoA (p = 0.08), and citrate (p = 0.08) accompanied with a significant accumulation of alanine and asparagine in liver (Figure 4g). We also observed a significant reduction of citrate and a trend of accumulation of aspartate (p = 0.06) and asparagine (p = 0.06) in the heart (Figure S4p). Reduced pyruvate and alpha ketoglutarate were observed in the heart of Sirt3−/−CD mice compared to WTCD mice (Figure S4r), and no difference was detected in liver (Figure S4q). No difference in TCA metabolites was found in gastrocnemius among all groups except increased pyruvate noted in CR groups (Figure S4s). Next, we examined whether FAO genes were upregulated at an earlier time upon feeding, which could explain the decline of the RER in Sirt3−/−CR mice. We measured transcript levels of key FAO genes, including Cpt1α, Lcad, Etfδh, and Ppara in 6-h refed liver from 25-month-old WTCR and Sirt3−/−CR mice (Figure 4h). After 6 h of food provision, Lcad transcripts were significantly increased in Sirt3−/−CR mice and a trend for Cpt1α increase was observed (p = 0.07), consistent with the Sirt3−/−CR mice exhibited further switching from carbohydrates to fatty acid oxidation during the postprandial period.

3 | DISCUSSION

Caloric restriction is a widely studied regimen in mice that robustly extends both healthspan and lifespan (Fontana & Partridge, 2015; Mitchell et al., 2019; Weindruch et al., 1986; Zhang et al., 2013). Previous work revealed that CR can stimulate SIRT3 expression (Palacios et al., 2009; Schwer et al., 2009), and through deacetylation of mitochondrial enzymes enhances metabolic flux of pathways often dysregulated in aging and age-related disorders (Ansari et al., 2017; Kincaid & Bossy-Wetzel, 2013; McDonnell et al., 2015). Here, we set out to investigate the importance of SIRT3 in CR-mediated longevity. We report that SIRT3 is dispensable for CR-dependent longevity and unexpectedly find that SIRT3 ablation further extends maximum lifespan under CR. Maximum lifespan is a parameter thought to correlate better with rates of aging as compared to average
lifespan, since it is less likely to be influenced by strain-specific diseases that can shorten life (Weindruch & Sohal, 1997). Though it is unclear if the extended maximum lifespan of Sirt3−/− CR mice is due to a retardation of the aging process, lifelong metabolic adaptations in Sirt3−/− CR mice may confer stress resistance late in life. SIRT3 deficiency attenuates succinate-dependent respiration but preserves palmitate-dependent respiration in heart and liver of aged CR-treated mice. Consistent with the ex vivo respiration analyses, Sirt3−/− CR mice were less able to maintain carbohydrate-derived energy production and displayed faster fuel switching to FAO during the postprandial period, leading to a longer fasting state relative to WTCR animals. We note that our results derived from metabolic cage experiments were limited by the number of mice available at the end of this study, but collectively with other analyses are consistent with a fuel-switching difference between Sirt3−/− CR versus WTCR groups. Also, we acknowledge that only male mice were utilized in this study and that sex-specific phenotypic differences are often observed in such studies. Despite longer maximum lifespan, Sirt3−/− CR mice display altered fuel utilization, reduced exercise capacity, and reduced spontaneous activity.

Managing ROS production and detoxification has been proposed as a major SIRT3 function (Bause & Haigis, 2013; Liu et al., 2017; Zeng et al., 2014). In the current study, we found no compelling evidence that Sirt3 deletion leads to ROS-dependent dysfunction. It is important to note that most prior aging and SIRT3 studies used C57BL/6J mice that lack functional NNT, a major contributor to NADPH production. The present study was conducted using the C57BL/6NJ×C57BL/6J Nnt+/− genetic background. Likely, loss of both SIRT3 and NNT in previous studies generated a more severe mitochondrial phenotype in which levels of NADPH and reduced glutathione (GSH) were significantly reduced (Qiu et al., 2010; Someya et al., 2010). We found that in the presence of functional NNT, NADPH and GSH levels were maintained in all treatment groups, strongly suggesting that the aging and metabolic phenotypes in the current study are independent of oxidative stress. Another important difference in our experimental design is the choice of a CD that provides a fixed amount of 16% less calories as compared to ad libitum food intake. This CD minimizes obesity-induced complications and allows a better comparison of the health benefits of CR relative to a non-pathological diet (Pugh et al., 1999).

Loss of Sirt3 leads to a composite set of acetylation trends on mitochondrial proteins. While CR, age, and loss of Sirt3 combined led to more sites of hyperacetylation, a detailed clustering analysis revealed sub-groups of sites in metabolic proteins that were dependent on both CR and Sirt3, but displayed decreased acetylation in Sirt3−/− CR mice. Fasting and CR increase protein acetylation in a tissue-dependent manner (Dittenhafer-Reed et al., 2015; Schwer et al., 2009), likely by promoting FAO that increases mitochondrial acetyl-CoA production and consequently protein acetylation (Mezhnikina et al., 2020; Pougovkina et al., 2014). Aging was also associated with elevated mitochondrial acetylation in various tissues (Joseph et al., 2012; Zeng et al., 2014). In current study, CR, aging, and SIRT3 ablation increase overall liver mitochondrial acetylation. However, detailed analysis of our acetyl-proteomics suggests that each variable contributes to acetylation sites to different extents and that these lysine sites can be independent or summations of the variables. Compared to CR and aging, loss of SIRT3 yielded the fewest number of lysine sites that showed increased stoichiometry (Figure S2a). This result is not surprising given that CR and aging have broader impacts that affect acetyl-CoA level, mitochondrial integrity, and metabolism in general. In addition, we employed a stoichiometry-based MS approach that does not introduce possible bias of overestimation from use of acetyl-antibody enrichment. SIRT3-dependent acetylation sites are primarily enriched with proteins from oxidative metabolism pathways, and appear to counteract a rise in protein acetylation in a subset of mitochondrial proteins under metabolic stress. KEGG pathway enrichment analysis of hyperacetylated proteins in old Sirt3−/− CR mice demonstrated that BCAA degradation and fatty acid degradation were highly enriched within this particular subset. Some of the BCAA pathway acetylation sites with the largest increase (Table S2) in the old Sirt3−/− CR mice compared to the baseline condition, the same age WTCD mice, included ACAD8 K220 (37% increase), ACAT1 K178 (16% increase), AUH K186 (10% increase), and ALDH7A1 K462 (50% increase). These sites were of particular interest because structural analyses indicate these sites are in close proximity to active sites or dimerization interfaces. For example, ACAD8 K220, ACAD8 K178, and AUH K186 are all adjacent to nearby NAD+, FAD+, and enoyl-CoA binding pockets, respectively (Kurimoto et al., 2001; Battaile et al., 2004, PDB2F2S). ALDH7A1 is a homotetramer, and K462 is found at the interface between the dimers of dimers. Others have suggested that NAD+ binding promotes the tetramerization of ALDH7A1 and its activation; therefore, acetylation at this interface could be an important regulator of ALDH7A1 dehydrogenase activity (Korasics et al., 2018; Luo et al., 2015). Recent studies (Richardson et al., 2021; Yu et al., 2021) have shown that manipulation of BCAA level and its metabolism may lead to a profound impact on metabolic parameters, including glucose control, adiposity, and longevity. Future studies are needed to determine the molecular and phenotypic consequence of SIRT3 loss in BCAA metabolism to resolve the influence of SIRT3 on aging mechanisms.

Unexpectedly, we identified a subgroup of acetylation sites that showed hypoacetylation in SIRT3-deficient animals on the CR diet. Though regulated by SIRT3 under CR, these sites are unlikely to be direct deacetylation substrates of SIRT3. The acetylation status of these sites could reflect the metabolic adaptation due to loss of SIRT3, leading to the unique phenotypes observed in Sirt3−/− CR mice. It is intriguing to speculate that acetylation of these sites is linked to acetyl-CoA availability, which could be positively regulated by SIRT3, for example, through PDH (Jing et al., 2013). Loss of SIRT3 leads to downregulation of Pyruvate Dehydrogenase (PDH) activity and reduced protein acetylation of these acetyl-CoA-sensitive sites, which include the site K304 on ACAT1 that was one of the most reactive lysine residues during non-enzymatic acetylation with acetyl-CoA (Baeza et al., 2016). Consistent with this idea, Sirt3−/− CR compared to WTCR mice show decreased levels of acetyl-CoA and
citrate. Among this group of hypoacetylated sites in the Sirt3−/−CR condition, additional FAO pathway proteins include HADHA, ACAA2, and EC11. Such protein acetylation sites may serve as an acetyl-CoA sensor and contribute to the FAO metabolic adaptation observed in Sirt3−/−CR mice.

Manipulation of SIRT3 expression results in clear changes in respiration phenotypes, with significant differences observed between the CR-treated groups. SIRT3 ablation blunted CR-mediated respiration preservation and limited net OXPHOS respiration in Sirt3−/− CR animals, suggesting that both CR and SIRT3 are needed to maintain maximal bioenergetic capacity in aged mice. Notably, this CR and SIRT3-dependent respiratory phenotype is tissue-dependent. Liver and heart displayed additive effects, whereas the brain showed only a genotype effect, and muscle showed neither genotype nor diet-induced effects. We also identified Complex II as the major contributor of reduced respiration in Sirt3−/−CR mice, consistent with studies (Cimen et al., 2010; Finley et al., 2011; Horton et al., 2016) showing that SIRT3 directly targets Complex II and restores activity through deacetylation. Several studies have also reported reduced Complex I activity in response to SIRT3 deletion (Ahn et al., 2008; Lantier et al., 2015; Williams et al., 2019). Although Complex I displayed altered function, compared to Complex II, Complex I activity and NADH-linked respiration were less affected in all tissues tested, suggesting Complex II-dependent respiration is more prone to dysfunction under current experimental settings. Intriguingly, despite a significant reduction in respiration (heart and liver) from Sirt3−/−CR animals using carbohydrate-derived substrates (pyruvate, malate, succinate), these mice maintained comparable palmitoylcarnitine, malate-dependent coupled respiration, indicating FAO is not limited.

Recent studies (Lantier et al., 2015; Williams et al., 2019) report an enhanced palmitoylcarnitine/malate-dependent respiration phenotype in muscle of high-fat diet-fed Sirt3−/− mice. While these are dramatically different dietary models (high-fat diet vs. CR), the findings demonstrate that Sirt3−/− mice can preserve FAO capacity under both diets despite altered mitochondrial acetylation. Together, maintained/enhanced FAO respiration under CR or a high-fat diet, and the composite trends in acetylation challenge the generalized idea that mitochondrial hyperacetylation limits FAO (Hallows et al., 2011; Hirschey et al., 2010; Tsuda et al., 2018) and instead highlights the importance of the dietary regime in the context of SIRT3-dependent mitochondrial regulation.

Sirt3−/− CR mice displayed faster fuel switching from glucose to FA compared to WTCR animals during the postprandial period. This was evident in RER and amount of FAO (Figure 4a–f) showing a trend of increased FAO during the postprandial period in Sirt3−/− CR relative to WTCR mice. This may be part due to limited Complex II function and partial redirected carbon flux to alanine and asparagine, indicating reduced glucose oxidation-dependent energy production. Unlike glucose oxidation, FAO requires an extra ETC component, the flavoprotein-ubiquinone oxido.reductase (ETF-QO, or ETFDH), to oxidize FADH2 harvested from beta-oxidation (Gnaiger & MitoEagle Task Group, 2020; Goetzman, 2011). During acyl-CoA chain-shortening cycles, NADH and FADH2 are produced and further oxidized by Complex I and ETFDH, respectively. Switching to FAO may lead to more ETFDH-dependent electron transfer rather than transfer through Complex II. A recent study (Klučková et al., 2020) reported that SDH-deficient murine chromaffin cells could maintain efficient FAO respiration that are higher than wildtype cells when palmitoylcarnitine is provided. Consistent with these observations, HepG2 cells treated with SDH inhibitor 3-NPA blocks succinate-dependent respiration completely but affects fatty acid oxidation minimally (Y. Qin, J. M. Denu, unpublished observation). With only weakly affected Complex I observed in our study, FAO could be preserved in Sirt3−/− CR mice by increased flux through ETFDH, serving as a compensatory mechanism to drive FAO-dependent energy generation. Future studies are needed to determine whether compromised Complex II plays a role in fuel switching.

The early shift to FAO may lead to a longer overall FAO time and ultimately a longer fasting time in Sirt3−/− CR compared to WTCR mice. An increased fasting period has been a feature of meal-fed CR models, in which CR animals quickly consume their food allotment and fast until the next meal (Acosta-Rodríguez et al., 2017). Intriguingly, Pak et al. (2021) found that even without CR, increasing fasting time alone can promote CR-like metabolic phenotypes, highlighting the importance of fasting time towards CR benefits. Both alternate day feeding and daily fasting increase longevity in mice, and the beneficial effects of these interventions have been shown to be independent of caloric intake (Mattson et al., 2014; Mitchell et al., 2019). Fasting is also associated with improved intestinal stem cell function in aging (Mihaylova et al., 2018). Interestingly, the long-lived Ames dwarf and GHR-knockout mice display increased reliance on fatty acid oxidation as a fuel source (Barthe & Westbrook, 2012). Thus, existing studies have linked increased fasting or increased fatty acid oxidation with health benefits in rodents. We speculate that the additional fatty acid oxidation dominant period may play a role in extending the maximum lifespan of Sirt3−/− CR mice. Also, the ability to maintain similar fat content among the Sirt3−/− CR and WTCR groups might be explained by the lower SPA in Sirt3−/− CR which could spare fat mass consumption over their lifetime. While the difference in SPA is significant at 24.5%, this difference would be too small to capture in the energy expenditure (EE) estimates from the metabolic cage experiments. Estimates from literature put SPA as only 5%–20% of total EE. The feeding protocol used in the current study involves an every-other-day feeding schedule for both CD and CR animals. This design increases fasting time between feedings and provides a more rigorous examination on the effects of a CR regime, as compared to studies that use ad libitum feeding for controls. Despite increased lifespan, Sirt3−/− CR mice are less aerobically fit compared to WTCR mice due to their inability to perform full glucose utilization (Seiler et al., 2015), emphasizing the role of SIRT3 in preserving aerobic capacity.

Taken together, our findings reveal that SIRT3 is required for optimal aerobic fitness during aging but not for CR-mediated longevity. The lack of observable skeletal muscle differences in molecular analyses suggests that other tissues (i.e., brain, heart and/or liver), which displayed clear effects with SIRT3 loss, are
more likely to be responsible for the whole-animal phenotypic differences. These results highlight the uncoupling of lifespan and healthspan parameters such as aerobic fitness and SPA and address the need to comprehensively assess the factors that contribute to CR-dependent phenotypes. Possibly, some CR-induced factors contribute to lifespan extension, while having no impact on healthspan.

AUTHOR CONTRIBUTIONS

John M. Denu and Tomas A. Prolla: Conceptualization. Rashpal S. Dhillon, Yiming (Amy) Qin, Paul R. van Ginkel, Vivian X. Fu, Alexis J. Lawton, Cara L. Green, Fúlvia B. Manchado-Gobatto, and Claudio A. Gobatto: Methodology, investigation, and data collection. Yiming (Amy) Qin, Rashpal S. Dhillon, Alexis J. Lawton, Cara L. Green, Fúlvia B. Manchado-Gobatto, and Claudio A. Gobatto: Data analysis and figure generation. Yiming (Amy) Qin, John M. Denu, Tomas A. Prolla, Rashpal S. Dhillon, and Alexis J. Lawton: Writing—Original Draft. Yiming (Amy) Qin, John M. Denu, Tomas A. Prolla, and Cara L. Green: Writing—Review & Editing. John M. Denu, Tomas A. Prolla, Dudley W. Lamming, Cara L. Green, Fúlvia B. Manchado-Gobatto, and Claudio A. Gobatto: Resources. Vivian X. Fu and James M. Vann: Animal breeding and care. John M. Denu, Tomas A. Prolla, Dudley W. Lamming, Fúlvia B. Manchado-Gobatto, and Claudio A. Gobatto: Funding acquisition.

ACKNOWLEDGMENTS

This work was supported by NIA grant AG038679 and GM65386 to T.A.P and J.M.D. The Lamming lab is supported in part by the NIA (AG056771, AG062328, and AG061635), the NIDDK (DK125859), and startup funds from UW-Madison to D.W.L. C.L.G. was supported in part a Glenn/AFAR Postdoctoral Fellowship from the Glenn Foundation for Medical Research. Support for this research was provided by the University of Wisconsin–Madison Office of the Vice Chancellor for Research and Graduate Education with funding from the Wisconsin Alumni Research Foundation. The Lamming lab is supported in part by the U.S. Department of Veterans Affairs (I01-BX004031), and this work was supported using facilities and resources from the William S. Middleton Memorial Veterans Hospital. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work does not represent the views of the Department of Veterans Affairs or the United States Government F.B.M.-G. and C.A.G. were supported by FAPESP (#2015/00272-6, #2015/01362-9) for their working period at University of Wisconsin-Madison. We would like to thank Randall Massey at the Medical School Electron Microscope Facility for his help in transmission electron microscopy. We thank Dr. Melissa Skala, Dr. Alex Walsh, and Kelsey Tweed for the insightful discussions and comments. We also thank Eric Armstrong for his help with the metabolomic analysis.

CONFLICT OF INTEREST

J.M.D. is a consultant for Evrys Bio and co-founder of Galilei BioSciences. T.A.P. is a co-founder of Lifegen Technologies and a scientific advisory board member of Nu Skin International Inc. and CyteGen Corporation. D.W.L. has received funding from and is a scientific advisory board member of Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases. The remaining authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The raw data, processed data, spectral library, and the analysis logs describing the settings for the Spectronaut analyses have been deposited to the ProteomeXchange Consortium via the MassIVE partner repository with the dataset identifier MSV000087085 and PXD024961 (DOI: 10.25345/C59Z2Q). The data were processed and cleaned using an in-house R script, which can be accessed through the GitHub link: (DOI: 10.5281/zenodo.3360892). Other data are available upon request.

ORCID

Yiming (Amy) Qin https://orcid.org/0000-0002-2413-0024

REFERENCES

Acosta-Rodriguez, V. A., de Groot, M. H. M., Rijo-Ferreira, F., Green, C. B., & Takahashi, J. S. (2017). Mice under caloric restriction self-impose a temporal restriction of food intake as revealed by an automated feeder system. Cell Metabolism, 26(1), 267–277.e2. https://doi.org/10.1016/j.cmet.2017.06.007

Ahn, B. H., Kim, H. S., Song, S. Lee, I. H., Liu, J., Vassilopoulos, A., Deng, C. X., & Finkel, T. (2008). A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proceedings of the National Academy of Sciences of the United States of America, 105(38), 14447–14452. https://doi.org/10.1073/pnas.0803790105

Ansari, A., Rahman, M. S., Saha, S. K., Saikot, F. K., Deep, A., & Kim, K. H. (2017). Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. Aging Cell, 16, 4–16. https://doi.org/10.1111/acel.12538

Baeza, J., Lawton, A. J., Fan, J., Smallegan, M. J., Lienert, I., Gandhi, T., Bermhardt, O. M., Reiter, L., & Denu, J. M. (2020). Revealing dynamic protein acetylation across subcellular compartments. Journal of Proteome Research, 19(6), 2404–2418. https://doi.org/10.1021/acs.jproteome.0c00088

Baeza, J., Smallegan, M. J., & Denu, J. M. (2016). Mechanisms and dynamics of protein acetylation in mitochondria. Trends in Biochemical Sciences, 41(3), 231–244. https://doi.org/10.1016/j.tibs.2015.12.006

Balasubramanian, P., Howell, P. R., & Anderson, R. M. (2017). Aging and caloric restriction research: A biological perspective with translational potential. EBioMedicine, 21, 37–44. https://doi.org/10.1016/j.ebiom.2017.06.015

Bao, J., Scott, I., Lu, Z., Pang, L., Dimond, C. C., Gius, D., & Sack, M. N. (2010). SIRT3 is regulated by nutrient excess and modulates hepatic susceptibility to lipotoxicity. Free Radical Biology and Medicine, 49(7), 1230–1237. https://doi.org/10.1016/J.FREERADBIO MED.2010.07.009

Bartke, A., & Westbrook, R. (2012). Metabolic characteristics of long-lived mice. Frontiers in Genetics, 3, 1–6. https://doi.org/10.3389/fgene.2012.00288

Battaille, K. P., Nguyen, T. V., Vockley, J., & Kim, J. J. P. (2004). Structures of isobutyryl-CoA dehydrogenase and enzyme-product complex: Comparison with isovaleryl- and short-chain acyl-coa
dehydrogenases. Journal of Biological Chemistry, 279(16), 16526–16534. https://doi.org/10.1074/jbc.M400034200

Bause, A. S., & Haigis, M. C. (2013). SIRT3 regulation of mitochondrial oxidative stress. Experimental Gerontology, 48, 634–639. https://doi.org/10.1016/j.exger.2012.08.007

Benigni, A., Cassis, P., Conti, S., Perico, L., Corna, D., Cerullo, D., Zentilin, L., Zoja, C., Perna, A., Lionetti, V., Giacca, M., Trionfini, P., Tomasoni, S., & Remuzzi, G. (2019). Sirt3 deficiency shortens life span and impairs cardiac mitochondrial function rescued by Opa1 gene transfer. Antioxidants and Redox Signaling, 31(17), 1255–1271. https://doi.org/10.1089/ars.2018.7703

Bruss, M. D., Khambatta, C. F., Ruby, M. A., Aggarwal, I., & Hellerstein, M. K. (2010). Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. American Journal of Physiology-Endocrinology and Metabolism, 298(1), E108–E116. https://doi.org/10.1152/ajpendo.00524.2009

Calvo, S. E., Clauser, K. R., & Mootha, V. K. (2016). MitoCarta2.0: An updated inventory of mammalian mitochondrial proteins. Nucleic Acids Research, 44(D1), D1251–D1257. https://doi.org/10.1093/nar/gkv1003

Cimen, H., Han, M. J., Yang, Y., Tong, Q., Koc, H., & Koc, E. C. (2010). Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria. Biochemistry, 49(2), 304–311. https://doi.org/10.1021/bi901627u

Dennis, G., Shermar, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., & Lempicki, R. A. (2003). DAVID: Database for annotation, visualization, and integrated discovery. Genome Biology, 4(5), 1–11. https://doi.org/10.1186/gb-2003-4-9-r60

Dittenhafer-Reed, K. E., Richards, A. L., Fan, J., Smallegan, M. J., Fotuhi, A., Balloon, A. J., Westphall, M. S., Pagliarini, D. J., Prolla, T. A., Assadi-Porter, F., Roy, S., Denu, J. M., & Coon, J. J. (2013). Calorie Restriction and SIRT3 Trigger Global Reprogramming of the Mitochondrial Protein Acylome. Molecular Cell, 49(1), 186–199. https://doi.org/10.1016/j.molcel.2012.10.024

Hirschy, M. D., Shimagu, T., Goetzman, E., Jie, E., Schwor, B., Lombard, D. B., Grueter, C. A., Harris, C., Bidding, S., Ilkayeva, O. R., Stevens, R. D., Li, Y., Saha, A. K., Ruderman, N. B., Bai, J. R., Newgarc, C. B., Farese, R. V., Jr., Alt, F. W., Kahn, C. R., & Verdin, E. (2010). SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature, 464(7285), 121–125. https://doi.org/10.1038/nature08778

Horton, J. L., Martin, O. J., Lai, L., Riley, N. M., Richards, A. L., Vega, R. B., Leone, T. C., Pagliarini, D. J., Muoio, D. M., Bedi, K. C., Jr., Margulies, K. B., Coon, J. J., & Kelly, D. P. (2016). Mitochondrial protein hyper-acetylation in the failing heart. JCI Insight, 2(1), e84897. https://doi.org/10.1172/jci.insight.84897

Jang, J. Y., Blum, A., Liu, J., & Finkel, T. (2018). The role of mitochondria in aging. Journal of Clinical Investigation, 128, 3662–3670. https://doi.org/10.1172/JCI120842

Jing, E., Onelli, B. T., Rardin, M. J., Kleinidders, A., Ilkayeva, O. R., Ussar, S., Bain, J. R., Lee, K. Y., Verdin, E. M., Newgard, C. B., Gibson, B. W., & Kahn, C. R. (2013). Sirt3 regulates metabolic flexibility of skeletal muscle through reversible enzymatic deacetylation. Diabetes, 62(10), 3404–3417. https://doi.org/10.23736/s0012-1867-16-5039-8

Joseph, A. M., Adhitye, P. J., Buford, T. W., Wohlgemuth, S. E., Lees, H. A., Nguyen, L. M. D., Aranda, J. M., Sandesa, B. D., Pahor, M., Manini, T. M., Marzetti, E., & Leeuwenburgh, C. (2012). The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Aging Cell, 11(5), 801–809. https://doi.org/10.1111/j.1474-9726.2012.00844.x

Kim, H. S., Patel, K., Muldoon-Jacobs, K., Bisht, K. S., Aycin-Burns, N., Pennington, J. D., van der Meer, R., Nguyen, P., Savage, J., Owens, K. M., Vassilopoulos, A., Oudenn, O., Park, S. H., Singh, K. K., Abdulkadir, S. A., Spitz, D. R., Deng, C. X., & Gius, D. (2010). SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell, 17(1), 41–52. https://doi.org/10.1016/j.ccr.2009.11.023

Kincaid, B., & Bossy-Wetzel, E. (2013). Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. Frontiers in Aging Neuroscience, 5, 48. https://doi.org/10.3389/fnagi.2013.00048

Kluccková, K., Thakker, A., Vettore, L., Escibano-Gonzalez, C., Hindshaw, R. L., Lear, J. E. L., Goncalves, J., Kaull, B., Lavery, G. G., Favier, J., & Tennant, D. A. (2020). Succinate dehydrogenase deficiency in a chromaffin cell model retains metabolic fitness through the maintenance of mitochondrial NADH oxidoreductase function. The FASEB Journal, 34(1), 303–315. https://doi.org/10.1096/fj.201901456R

Korasic, D. A., White, T. A., Chakravarthy, S., & Tanner, J. J. (2018). NAD+ promotes assembly of the active tetramer of aldehyde dehydrogenase 7A1. FEBS Letters, 592(19), 3229–3238. https://doi.org/10.1002/1742-4658.13238

Kurimoto, K., Fukai, S., Nureki, O., Muto, Y., & Yokoyama, S. (2001). Crystal structure of human AHH protein, a single-stranded RNA binding homolog of enoyl-CoA hydratase. Structure, 9(12), 1253–1263. https://doi.org/10.1016/S0969-2126(01)00686-4

Lantier, L., Williams, A. S., Williams, I. M., Yang, K. K., Bracy, D. P., Goelzer, M., James, F. D., Gius, D., & Wasserman, D. H. (2015). SIRT3 is crucial for maintaining skeletal muscle insulin action and protects against severe insulin resistance in high-fat-fed mice. Diabetes, 64(9), 3081–3092. https://doi.org/10.2337/db14-1810

Lanza, I. R., Zabielski, P., Klaus, K. A., Morse, D. M., Heppelmann, C. J., Bergen, H. R., Ills, Dasiari, S., Walrand, S., Short, K. R., Johnson, M. L., Robinson, M. M., Schmikle, J. M., Jakaitis, D. R., Asmann, Y. W., Sun, Z., & Nair, K. S. (2012). Chronic caloric restriction preserves
mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metabolism, 16(6), 777-788. https://doi.org/10.1016/j.cmet.2012.11.003

Lieberman, D. E., Kistner, T. M., Richard, D., Lee, I. M., & Baggish, A. L. (2021). The active grandparent hypothesis: Physical activity and the evolution of extended human healthspans and lifespans. Proceedings of the National Academy of Sciences of the United States of America, 118(50), e2107621118. https://doi.org/10.1073/pnas.2107621118

Liu, J., Li, D., Zhang, T., Tong, Q., Ye, R. D., & Lin, L. (2017). Sirt3 protects hepatocytes from oxidative injury by enhancing ROS scavenging and mitochondrial integrity. Cell Death and Disease, 8(10), e3158. https://doi.org/10.1038/cddis.2017.564

Liu, Z., Wang, Y., Gao, T., Pan, Z., Cheng, H., Yang, Q., Cheng, Z., Guo, A., Ren, J., & Xue, Y. (2014). CPLM: A database of protein lysine modifications. Nucleic Acids Research, 42(D1), D531-D536. https://doi.org/10.1093/nar/gkt1093

Luo, M., Gates, K. S., Henzl, M. T., & Tanner, J. J. (2015). Diethylenobenzaldehyde is a covalent, irreversible inactivator of diaphorase to peroxide detoxification is dependent on the respiratory complexes. Cell Metabolism, 16, 486–492. https://doi.org/10.1016/j.cmet.2015.06.001

Meng, H., Yan, W. Y., Lei, Y. H., Wan, Z., Hou, Y. Y., Sun, L. K., & Zhou, J. P. (2019). SIRT3 regulates progression and development of diseases of aging. Trends in Endocrinology and Metabolism, 26, 486–492. https://doi.org/10.1016/j.tem.2015.06.013

Merr, B. J. (2004). Oxidative stress and mitochondrial function with aging - The effects of calorie restriction. Aging Cell, 3, 7-12. https://doi.org/10.1046/j.1474-7968.2003.00074.x

Mezhina, V., Pearce, R., Poe, A., Velingkaar, N., Astafev, A., Ebeiige, O. P., Makwana, K., Sanders, Y., & Kondratov, R. V. (2020). CR reprograms acetyl-CoA metabolism and induces long-chain acyl-CoA dehydrogenase and CrAT expression. Aging Cell, 19(11), e13266. https://doi.org/10.1111/acel.13266

Mihaylova, M. M., Cheng, C. W., Cao, A. Q., Tripathi, S., Mana, M. D., Bauer-Rowe, K. E., Abu-Remaileh, M., Clavain, L., Erdemir, A., Lewis, C. A., Freinkman, E., Dickey, A. S., Is Paada, A. R., Huang, Y., Bell, G. W., Deshpande, V., Carmeliet, P., Katajisto, P., Sabatini, D. M., & Yilmaz, Ö. H. (2018). Fasting activates fatty acid oxidation to enhance intestinal stem cell function during homeostasis and aging. Cell Stem Cell, 22(5), 769–778.e4. https://doi.org/10.1016/j.stem.2018.04.001

Mitchell, S. J., Bernier, M., Mattison, J. A., Aon, M. A., Kaiser, T. A., Anson, R. M., Ikeno, Y., Anderson, R. M., Ingram, D. K., & de Cabo, R. (2019). Daily fasting improves health and survival in male mice independent of diet composition and calories. Cell Metabolism, 29, 221–228. https://doi.org/10.1016/j.cmet.2018.08.011

Pak, H. H., Hays, S. A., Green, C. L., Koller, M., Lavarias, M. T., Richardson, N. E., Yang, S. E., Dumas, S. N., Sonsalla, M., Bray, L., Johnson, M., Barnes, S., Darley-Usmar, V., Zhang, J., Yen, C. L. E., Denu, J. M., & Lamming, D. W. (2021). Distinct roles of fasting and calories in the metabolic, molecular, and geroprotective effects of a calorie restricted diet. Nature Metabolism, 3, 1327–1341. https://doi.org/10.1038/s42255-021-00466-9

Palacios, O. M., Carmona, J. J., Michan, S., Chen, K. Y., Manabe, Y., Ward, J. L., 3rd, Goodyear, L. J., & Tong, Q. (2009). Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. Aging, 1(9), 771–783. https://doi.org/10.18632/aging.100075

Parodi-Rullán, R. M., Chapa-Dubocq, X. R., & Javadov, S. (2018). Acetylation of mitochondrial proteins in the heart: The role of SIRT3. Frontiers in Physiology, 9, 1094. https://doi.org/10.3389/fphys.2018.01094

Pougoukina, O., te Brinke, H., Ofman, R., van Cruchten, A. G., Kulik, W., Wanders, R. J. A., Houten, S. M., & de Boer, V. C. J. (2014). Mitochondrial protein acetylation is driven by acetyl-CoA from fatty acid oxidation. Human Molecular Genetics, 23(13), 3513–3522. https://doi.org/10.1093/hmg/ddu059

Pugh, T. D., Klopp, R. G., & Weindruch, R. (1999). Controlling calorific consumption: Protocols for rodents and rhesus monkeys. Neurobiology of Aging, 20(2), 157–165. https://doi.org/10.1016/S0197-4580(99)00043-3

Qiu, X., Brown, K., Hirschey, M. D., Verdin, E., & Chen, D. (2010). Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metabolism, 12(6), 662–667. https://doi.org/10.1016/j.cmet.2010.11.015

Richardson, N. E., Konon, E. N., Schuster, H. S., Mitchell, A. T., Boyle, C., Rodgers, A. C., Finke, M., Haider, L. R., Yu, D., Flores, V., Pak, H. H., Ahmad, S., Ahmed, S., Radcliff, A., Wu, J., Williams, E. M., Abdi, L., Sherman, D. S., Hacker, T., & Lamming, D. W. (2021). Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and life span in mice. Nature Aging, 1(1), 73–86. https://doi.org/10.1038/s43587-020-00006-2

Ronchi, I. A., Francisco, A., Passos, L. A. C., Figueira, T. R., & Castilho, R. F. (2016). The contribution of nicotinamide nucleotide transhydrogenase to peroxide detoxification is dependent on the respiratory state and counterbalanced by other sources of NADPH in liver mitochondria. Journal of Biological Chemistry, 291(38), 20173–20187. https://doi.org/10.1074/jbc.M116.730473

Scariot, P. P. M., Manchado-Gobatto, F. B., Prolla, T. A., Masselli dos Reis, I. G., & Gobatto, C. A. (2019). Housing conditions modulate spontaneous physical activity, feeding behavior, aerobic running capacity and adiposity in C57BL/6J mice. Hormones and Behavior, 115, 104556. https://doi.org/10.1016/j.yhbeh.2019.07.004

Schwer, B., Eckersdorff, M., Li, Y., Silva, J. C., Ferrin, D., Kurtev, M. V., Giallourakis, C., Comb, M. J., Alt, F.W., & Lombard, D. B. (2009). Calorie restriction alters mitochondrial protein acetylation. Aging Cell, 8, 604–606. https://doi.org/10.1111/j.1474-9726.2009.00503.x

Seiler, S. E., Koves, T. R., Gooding, J. R., Wong, K. E., Stevens, R. D., Ilkayeva, O. R., Wittmann, A. H., DeBalsi, K. L., Davies, M. N., Lindeboom, L., Schrauwen, P., Schrauwen-Hinderling, V. B., & Muoio, D. M. (2015). Carnitine acetyltransferase mitigates metabolic inertia and muscle fatigue during exercise. Cell Metabolism, 22(1), 65–76. https://doi.org/10.1016/j.cmet.2015.06.003

Smith, C. D., Schmidt, C. A., Lin, C. T., Fisher-Wellman, K. H., & Neufer, P. D. (2020). Flux through mitochondrial redox circuits linked to nicotinamide nucleotide transhydrogenase generates counterbalance changes in energy expenditure. Journal of Biological Chemistry, 295(48), 16207–16216. https://doi.org/10.1074/jbc.RA120.013899

Someya, S., Wu, Y., Hallows, W. C., Xu, J., Vann, J. M., Leeuwenburgh, C., Tanokura, M., Denu, J. M., & Prolla, T. A. (2010). Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell, 143(5), 802–812. https://doi.org/10.1016/j.cell.2010.10.002

Srivastava, S. (2017). The mitochondrial basis of aging and age-related disorders. Genes, 8, 398. https://doi.org/10.3390 Genes8120398
Still, A. J., Floyd, B. J., Hebert, A. S., Bingman, C. A., Carson, J. J., Gunderson, D. R., Dolan, B. K., Grimsrud, P. A., Dittenhafer-Reed, K. E., Stapleton, D. S., Keller, M. P., Westphall, M. S., Denu, J. M., Attie, A. D., Coon, J. J., & Pagliarini, D. J. (2013). Quantification of mitochondrial acetylation dynamics highlights prominent sites of metabolic regulation. The Journal of Biological Chemistry, 288(36), 26209–26219. https://doi.org/10.1074/jbc.M113.483396

Tsuda, M., Fukushima, A., Matsumoto, J., Takada, S., Kakutani, N., Nambu, H., Yamanashi, K., Furihata, T., Yokota, T., Okita, K., Kinugawa, S., & Anzai, T. (2018). Protein acetylation in skeletal muscle mitochondria is involved in impaired fatty acid oxidation and exercise intolerance in heart failure. Journal of Cachexia, Sarcopenia and Muscle, 9(5), 844–859. https://doi.org/10.1002/jcsm.12322

Vassilopoulos, A., Pennington, J. D., Andresson, T., Rees, D. M., Bosley, A. D., Fearnley, I. M., Ham, A., Flynn, C. R., Hill, S., Rose, K. L., Kim, H. S., Deng, C. X., Walker, J. E., & Gius, D. (2014). SIRT3 deacetylates ATP synthase F1 complex proteins in response to nutrient- and exercise-induced stress. Antioxidants and Redox Signaling, 21(4), 551–564. https://doi.org/10.1089/ars.2013.5420

Weindruch, R., & Sohal, R. S. (1997). Caloric intake and aging. New England Journal of Medicine, 337(14), 986–994. https://doi.org/10.1056/nejm199710023371407

Weindruch, R., Walford, R. L., Fligiel, S., & Guthrie, D. (1986). The retardation of aging in mice by dietary restriction: Longevity, cancer, immunity and lifetime energy intake. Journal of Nutrition, 116(4), 641–654. https://doi.org/10.1093/jn/116.4.641

Weindruch, R. H., Cheung, M. K., Anthony Verity, M., & Walford, R. L. (1980). Modification of mitochondrial respiration by aging and dietary restriction. Mechanisms of Ageing and Development, 12(4), 375–392. https://doi.org/10.1016/0047-6374(80)90070-6

Williams, A. S., Koves, T. R., Davidson, M. T., Crown, S. B., Fisher-Wellman, K. H., Torres, M. J., Draper, J. A., Narowski, T. M., Slentz, D. H., Lantier, L., Wasserman, D. H., Grimsrud, P. A., & Muoio, D. M. (2019). Disruption of acetyl-lysine turnover in muscle mitochondria promotes insulin resistance and redox stress without overt respiratory dysfunction. Cell Metabolism, 31(1), 131-147.e11. https://doi.org/10.1016/j.cmet.2019.11.003

Yu, D., Richardson, N. E., Green, C. L., Spicer, A. B., Murphy, M. E., Flores, V., Jang, C., Kasza, I., Nikodemova, M., Wakai, M. H., Tomasiewicz, J. L., Yang, S. E., Miller, B. R., Pak, H. H., Brinkman, J. A., Rojas, J. M., Quinn, W. J., Ill, Cheng, E. P., Konon, E. N., ... Lamming, D. W. (2021). The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metabolism, 33(5), 905–922. e6. https://doi.org/10.1016/j.cmet.2021.03.025

Yu, W., Dittenhafer-Reed, K. E., & Denu, J. M. (2012). SIRT3 protein deacetylases iso-citrate dehydrogenase 2 (IDH2) and regulates mitochondrial redox status. Journal of Biological Chemistry, 287(17), 14078–14086. https://doi.org/10.1074/jbc.M112.355206

Zeng, L., Yang, Y., Hu, Y., Sun, Y., du, Z., Xie, Z., Zhou, T., & Kong, W. (2014). Age-related decrease in the mitochondrial sirtuin deacetylase sirt3 expression associated with ROS accumulation in the auditory cortex of the mimetic aging rat model. PLoS ONE, 9(2), e88019. https://doi.org/10.1371/journal.pone.0088019

Zhang, C., Li, S., Yang, L., Huang, P., Li, W., Wang, S., Zhao, G., Zhang, M., Pang, X., Yan, Z., Liu, Y., & Zhao, L. (2013). Structural modulation of gut microbiota in life-long calorie-restricted mice. Nature Communications, 4(1), 1–10. https://doi.org/10.1038/ncomms3163

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dhillon, R. S., Qin, Y., van Ginkel, P. R., Fu, V. X., Vann, J. M., Lawton, A. J., Green, C. L., Manchado-Gobatto, F. B., Gobatto, C. A., Lamming, D. W., Prolla, T. A., & Denu, J. M. (2022). SIRT3 deficiency decreases oxidative metabolism capacity but increases lifespan in male mice under caloric restriction. Aging Cell, 21, e13721. https://doi.org/10.1111/acel.13721