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The Influence of acute, late-life calorie restriction on whole body energy metabolism in p66Shc(–/–) mice

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Abstract

It has been proposed that Shc proteins may influence aging by regulating insulin signaling and energy metabolism. Evidence suggests that deletion of p66Shc could partially attenuate weight gain on a high fat diet by increasing energy expenditure. However, the impact of p66Shc on the metabolic response to calorie restriction (CR) has not been determined. Thus, we used indirect respiration calorimetry to determine the impact of CR on energy expenditure (EE) and substrate utilization (RQ) in 18mo p66Shc(–/–) and wild-type (WT) mice. Calorimetry measurements were completed at baseline and following 3d of 40% CR and 2mo of 26% CR. There was no difference ($P>0.10$) in EE and RQ between genotypes, regardless of how EE data was normalized. Both p66Shc(–/–) and WT mice showed decreases ($P<0.001$) in EE normalized for body weight at 2mo of CR. However, the response to 3d of CR was different between genotypes with only the p66Shc(–/–) showing a decrease ($P<0.001$) in 24h EE expressed per mouse or normalized for body weight. The results indicate that p66Shc does not significantly influence EE in 18mo mice at baseline or 2mo of CR, although it may play a role in the EE response to very acute CR.

Keywords

indirect respiration calorimetry; substrate oxidation; energy expenditure; body composition; dietary restriction

1. Introduction

The p66Shc protein is one of three splice variants encoded by the mammalian Shc locus. Shc proteins act as adaptor molecules which specifically bind to phosphorylated tyrosine residues on the cytoplasmic motif of growth factor receptors, thereby serving as mitogenic signaling molecules. It has been proposed that the p66Shc isoform may play a role in the aging process (Migliaccio et al., 1999). Studies have largely focused on p66Shc-induced alterations in oxidative stress and apoptosis as the mechanisms through which this protein could influence aging (Migliaccio et al., 1999; Napoli et al., 2003; Orsini et al., 2006). However, Shc proteins also play a role in insulin signaling (Ravichandran, 2001; Sasaoka and Kobayashi, 2000), and there is some evidence to suggest that Shc may influence aging through alterations in insulin signaling, adiposity and energy metabolism (Berniakovich et

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al., 2008; Tomilov et al., 2011). Nonetheless, the overall impact of p66Shc on energy metabolism is still not entirely clear. It has been reported that body weights are lower in p66Shc(−/−) compared to wild-type (WT) mice when consuming either a standard or high fat diet despite the fact that energy intake is not different between genotypes (Berniakovich et al., 2008). Similarly, deletion of p66Shc in leptin-deficient Ob/Ob mice leads to a decrease in weight gain without altering food intake (Ranieri et al., 2010). The results of these studies indicate that deletion of p66Shc may mitigate weight gain by increasing energy expenditure. In support of this conclusion, it has been reported that oxygen consumption (ml/g body weight) is increased in p66Shc(−/−) compared to wild-type mice (Berniakovich et al., 2008). While there is accumulating evidence that the p66Shc protein may play a role in the metabolic response to over-nutrition, the role of this protein in the metabolic response to calorie restriction (CR) has yet to be determined.

Calorie restriction, without malnutrition, has been shown to increase maximum life span and prevent or delay the onset of age-associated pathophysiological changes in numerous species (Sohal and Weindruch, 1996). Studies have recently identified signaling molecules which play a role in the metabolic adaptation to CR (Bordone and Guarente, 2005; Corton and Brown-Borg, 2005; Houtkooper et al., 2010; Qiu et al., 2010; Smith et al., 2004; Weindruch et al., 2001) but it remains to be determined if p66Shc contributes to the metabolic response to CR. The purpose of the present study was to determine if the energetic response to CR is altered in 18mo old p66Shc(−/−) versus wild-type (WT) mice. There were two reasons for studying mice at this age. First, there is evidence that CR may have beneficial effects on some age-related health outcomes, such as cancer incidence, when initiated as late as 18 months of age in mice (Dhahbi et al., 2004). Second, ad libitum fed C57/B6 mice are near maximum body weight at 18 months of age (Turturro et al., 1999). Similarly, many humans experience weight gain in late middle age and this is a common stage of life to attempt CR for weight loss and improved health. In the current study, we investigated the impact of ad libitum feeding (baseline), 3d of CR and 2mo of CR on EE and RQ in p66Shc(−/−) and WT mice. These CR time points were selected to represent a period of very active weight loss (3d CR) and a period when major weight loss had ceased (2mo CR).

2. Materials and Methods

2.1 Animals and Diet

All mice in this study were on a C57/B6J background and have been previously described (Migliaccio et al., 1999). Heterozygous crosses were used to generate the founder lines of p66Shc(−/−) and WT animals. All wild-type (WT) and p66Shc(−/−) mice (n=8 per genotype) were 18 months of age at the start of the study. Prior to collection of indirect respiration calorimetry data, food intake and body weight was monitored for 7 days. All animals were housed in individual cages in a light (12-h light/12-h dark cycle) and temperature (22°C) controlled conventional vivarium at the University of California-Davis (UCD). This study was approved by the UCD Animal Care and Use Committee. All mice were fed a commercial diet (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan USA). Sentinel mice were housed on the same rack as the study mice and exposed to bedding from the cages of the study animals. Two sentinel mice were euthanized every three months for serology. All tests from these mice were negative.

2.2 Energy intake

Ad libitum food intake was measured by weighing the amount of food remaining in the hopper at the same time daily, while accounting for any spillage by sifting bedding and weighing any food particles remaining in the cages. The calculated metabolizable energy (ME) of the diet (14.27 kJ/g) was used to determine ME intake.

2.3 Indirect Respiration Calorimetry

Total daily EE was measured using whole-body indirect respiration calorimetry. Calorimetry measurements were completed for each animal on three individual 24h data collection periods; baseline (ad libitum intake), day 3 of CR, and 2mo of CR. Chambers had the same dimensions and shape as the animals' home cage (Paige Instruments, Woodland, CA). Room air was drawn through the chambers at 400 mL/min. This flow rate was controlled and measured with a mass flow controller (MFS-5, Sable Systems International, Las Vegas, NV). Samples of room and chamber air were dried by a Peltier condenser (PC-4, Sable Systems) before entering Oxygen and CO₂ analyzers. Oxygen content was measured by a fuel cell oxygen analyzer (FC-10, Sable Systems) and CO₂ content was measured by an infrared CO₂ analyzer (CA-10, Sable Systems). Calorimeter calibration was performed daily prior to beginning each 24h measurement. A 1.9% CO₂ reference gas, 100% industrial grade Nitrogen gas, and dry room air were used to calibrate CO₂ and Oxygen analyzers. Data from the mass flow controllers and gas analyzers were collected using a data acquisition system (UI2, Sable systems) with a PC using Expedata software (Version 1.3.0.12, Sable Systems). EE was calculated using the following modified Weir equation.

$$EE(kJ)=(16.5kJ/L \times V_{O_2})+(4.63kJ/L \times V_{CO_2}) \text{ (Weir, 1949)}$$

RQ was calculated as the ratio of volume of CO₂ produced to the volume of O₂ consumed. A food quotient of 0.87 was calculated from the proportions of protein, fat, and carbohydrates in the diet. The ratio of dark EE to light EE was used to provide an indication of the magnitude of diurnal changes in EE.

2.4 Calorie Restriction

Immediately following indirect respiration calorimetry measurements at baseline, mice were given 60% of food intake measured during ad libitum feeding. This acute phase of CR was maintained for three days. Upon completion of day 3 of CR, animals were maintained for 2 months on a calorie restriction diet that represented 74% of ad libitum intake. The purpose of this study was to determine if energy expenditure was different in p66Shc(−/−) and wild-type mice during a period of acute, active weight loss (40% CR for 3 days) and during a period when major weight loss had ceased (26% CR for 2 months). The reason for the change in level of CR is that an initial pilot project showed that 40% CR in 18 month old mice produced body weights below 20 g. In contrast, we noticed that body weight, through middle age, stabilized near 24 g in a parallel life span study in p66Shc(−/−) and WT mice started on 40% CR at 4 months of age. We did not wish to produce major differences in body weight between these studies, so the amount of food provided was adjusted to the level of energy intake in the lifespan study. However, we chose to use 40% CR for our very acute (3 day CR) measurements for two reasons. First, we wanted to use a relatively severe level of CR to induce very clear weight loss in all animals over a 3 day period. Second, we wanted to use a level of CR that is commonly used in CR and aging studies. During all CR periods animals were fed daily at 9:00 PM.

2.5 Organ weights and body composition

Subsequent to collection of calorimetry data at the 2 month time point, animals were sacrificed via CO₂ inhalation and cervical dislocation. Immediately following euthanasia, organs were collected, weighed, and returned to the carcass at which time the carcass was weighed and stored at −20C for preparation of total body water (TBW) analysis. Carcasses were then freeze-dried for 7 days to remove all fluids (Virtis Sublimator). Total body water was determined by subtracting the freeze dried weight from the carcass weight. Lean body

mass was determined by the following equation previously described (Krzywicki et al., 1974; Ramsey et al., 1996; Widdowson, 1968).

$$\text{Lean body mass} = \text{TBW} / 0.73$$

2.6 Statistical Analyses

Independent t-tests were used to determine if EI, BW, EE, PA and RQ differed between genotypes at baseline ad libitum conditions. EE is expressed as kJ/min/mouse (kJ per min per mouse), kJ/g BW/min (kJ per gram BW per min), and kJ/min using BW as a covariate in the model. Analyses were performed separately by light/dark cycle. To investigate possible differences in the energetic response over time to CR (3d and 2mo), individual trajectories of changes in EE, PA, and RQ over time were compared between genotypes by a repeated measures analysis of variance (ANOVA) using linear random-effects models. Each response level was entered as the dependent variable. Genotype, time, and genotype*time interaction terms were entered as independent variables. To account for between subject heterogeneity in the changes of response levels, intercept and time were modeled as random effects. Multiple comparisons were controlled by the Bonferroni correction method where appropriate. Differences in organ weights and LBM at the completion of long term CR treatment were determined using independent t-tests. Additionally, EE at the 2mo CR time point was analyzed including LBM and crude organ weight as covariates in the model. Significance was defined as a two-sided $P < 0.05$. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc).

3. Results

3.1 Energy intake

Mean energy intake during the 24 hour baseline calorimetry period did not differ significantly ($P > 0.10$) between p66Shc(−/−) and WT mice ($50.66 \text{ kJ} \pm 3.14$ and $45.66 \text{ kJ} \pm 1.00$, respectively).

3.2 Body Weight and Organ Weights

Body weight did not differ significantly between 18 month old p66Shc(−/−) and WT mice at baseline ($31.48 \text{ g} \pm 1.07$ and $34.30 \text{ g} \pm 1.58$, respectively). As expected, there was a decrease ($P < 0.001$) in body weight in both the p66Shc(−/−) and WT mice following 3d of CR. A further decrease ($P < 0.001$) in body weight was observed between 3d and 2mo of CR in both groups of mice (Figure 1). There were no significant differences in body weight between genotypes at either 3d or 2mo of CR (Figure 1).

Following 2mo of CR, the mice were euthanized and lean body mass and organ weights were collected (Table 1). There were no differences in organ weights between genotypes, with the exception of liver weight which was decreased ($P < 0.01$) in the p66Shc(−/−) mice. Crude organ weight was also decreased ($P < 0.05$) in the p66Shc(−/−) mice, although this appeared to be due primarily to the low liver weight in these animals. There was also a trend towards a decrease ($P = 0.08$) in lean body mass and lung weight in the p66Shc(−/−) mice.

3.3 Energy Expenditure

EE was measured at baseline, 3d of CR and 2mo of CR in p66Shc(−/−) and WT mice (Table 2). There was no significant overall effect ($P > 0.10$) of genotype on EE expressed as kJ/min/mouse or normalized for body weight using ANCOVA. However, the p66Shc(−/−) and WT mice did show different responses to 3d of CR. In the p66Shc(−/−) mice, there was a

decrease ($P<0.001$) in 24 hour EE, regardless of how EE was expressed, at 3d of CR compared to baseline. This was due to a decrease ($P<0.01$) in EE during the light cycle, since no significant change in EE was observed in the p66Shc(-/-) mice during the dark cycle. In contrast, there was no significant difference in 24 hour EE, regardless how EE was expressed, between 3d of CR and baseline in the WT mice. Similar to the p66Shc(-/-) animals, there was a decrease ($P<0.001$) in EE during the light cycle in the WT mice but the magnitude of this change was not sufficient to induce a significant decrease in 24 hour EE when coupled with dark cycle EE.

In both genotypes, 24 hour EE was decreased ($P<0.001$) at 2mo of CR compared to baseline or 3d of CR. This occurred regardless of how EE was expressed. This reduction in 24 hour EE was due to decreases ($P<0.001$) in dark and light cycle EE in both the p66Shc(-/-) and WT mice. The availability of body composition data at 2mo of CR allowed comparisons between genotypes of energy expenditure normalized for fat free mass (FFM) or organ weights. There were no significant differences in 24 hour, light or dark EE between p66Shc(-/-) and WT mice when energy expenditure was normalized for either FFM or organ weights.

Diurnal patterns of energy expenditure for both genotypes are shown in Figure 2. There was an increase ($P<0.05$) in dark compared to light cycle energy expenditure in p66Shc(-/-) and WT mice at baseline (Fig. 3A), 3d of CR and 2mo of CR. The ratio of dark to light EE was used to provide an indication of the magnitude of diurnal changes. There was no difference between genotypes in the ratio of dark to light EE. Both p66Shc(-/-) and wild-type mice showed increases ($P<0.001$) in dark to light EE with 3d or 2mo of CR compared to baseline (Table 3).

3.4 Respiratory quotient (RQ)

There were no differences in 24 hour, light cycle or dark cycle RQ between p66Shc(-/-) and WT mice (Table 2). Both genotypes showed a decrease ($P<0.001$) in RQ with 3d of CR compared to either baseline or 2mo of CR. There was no difference in RQ between baseline and 2mo of CR for either genotype. Figure 2 shows diurnal RQ patterns for both p66Shc(-/-) and WT mice. For both genotypes, there was an increase ($P<0.001$) in dark versus light RQ at baseline, 3d of CR and 2mo of CR.

4. Discussion

CR, without malnutrition, has been shown to increase maximum life span and prevent or delay the onset of age-associated pathophysiological changes in many species of animals (Sohal and Weindruch, 1996). However, the mechanism by which such benefits are obtained is not entirely known. Alterations in the level or activity of AMPK, SIRT1/SIRT3, and PGC-1 α all appear to play a role in the metabolic adaptation to CR (Bordone and Guarente, 2005; Corton and Brown-Borg, 2005; Houtkooper et al., 2010; Qiu et al., 2010; Smith et al., 2004; Weindruch et al., 2001), but it is possible that p66Shc is also a contributor. It has been shown that Shc proteins play a role in the metabolic response to over-nutrition, and prevent weight gain on high fat diets (Berniakovich et al., 2008; Tomilov et al., 2011). However, there is little information about the influence of p66Shc on the metabolic response to CR. The results of the present study indicate that p66Shc does not impact whole animal energetic response to 2mo of CR, but it may influence the response to 3d of CR.

It has been previously demonstrated that body weight is reduced in ad libitum fed young (2–5 month old) p66Shc(-/-) mice compared to WT controls, and this decrease in body weight is primarily due to lower fat pad weights in the p66Shc(-/-) animals (Berniakovich et al., 2008; Tomilov et al., 2011). In contrast, the present study shows that body mass under ad

libitum/baseline conditions did not differ significantly between 18 month old p66Shc(-/-) and WT mice. These results suggest that aging does not exacerbate differences in body weight between p66Shc(-/-) and WT mice, and may actually mitigate or eliminate these differences by late middle age. It was not possible in the present study to measure body composition in the mice at baseline, so we do not know if the decreases in fat pad weights observed in young p66Shc(-/-) mice (Berniakovich et al., 2008; Tomilov et al., 2011) persist through late middle age. Additional studies are needed to further determine the influence of aging on body composition in p66Shc(-/-) mice.

In contrast to studies that have found differences in body weight change in response to over nutrition between p66Shc(-/-) and WT mice (Berniakovich et al., 2008; Ranieri et al., 2010), the present study demonstrates that p66Shc does not modulate changes in body mass induced by 3d or 2mo of calorie restriction initiated in 18 month old animals. Three days of CR induced an 11% decrease in body mass in p66Shc(-/-) animals and a 10% decrease in that of WT animals. Similarly, genotype did not impact body mass change in response to 2mo of CR, as demonstrated by a 25% and 24% decrease from baseline in p66Shc(-/-) and WT animals, respectively. A decrease in body mass is the most obvious physiological response to CR treatment. However, the degree of this decrease varies greatly among studies and is dependent on the extremity and duration of the restriction, as well as age, sex, and strain of the animal (Boily et al., 2008; Rikke et al., 2006; Sohal et al., 2009). Because most studies published thus far have been designed to examine the impact of CR induced at a relatively young age in mice, the expected severity of weight loss in 18mo mice were unknown. Interestingly, several of these studies using older animals did not report body mass or body composition data (Dhahbi et al., 2001; Wu et al., 2009). It has recently been reported that body mass is decreased by 17% in 14mo C57Bl/6 mice fed a 26% CR diet for 2mo (Cameron et al., 2011). While the magnitude of weight loss demonstrated in this study is lower than that of our findings, it is important to note that the mean body mass of the animals before the initiation of CR was close to 40g. In contrast, the mean body mass in the present study was 31 and 34g in p66Shc(-/-) and WT mice, respectively. This difference between studies could be due to the weight and age of the animals at the start of the study, or differences in the CR protocols. Nonetheless, our study indicates that magnitude of weight loss with CR is comparable between 18 month old p66Shc(-/-) and WT mice.

Because our measurements of lean body mass and organ mass required termination of the animals, our analyses of these variables were limited to a comparison between genotypes at 2mo of CR. Thus, we cannot determine if changes in body composition or organ size in response to CR is modulated by p66Shc. Of particular interest, however, is our finding that liver weights of p66shc(-/-) animals were significantly smaller than that of WT mice. A decrease in liver mass has consistently been reported in CR studies. It has been shown that the individual weights of heart, kidney, and liver were significantly lower in 6mo and 23mo C57BL/6 mice when subjected to 2mo of 40% CR compared to that of ad libitum fed age-matched controls (Sohal et al., 2009). Recently, Cameron et al. investigated the energetic impact of a 26% CR diet for 2mo in 14mo C57Bl/6 mice and found that the weights of liver, spleen, kidneys, and thymus were all significantly smaller in the CR group compared to ad libitum-fed control mice (Cameron et al., 2011). Because we do not know if liver mass in p66Shc(-/-) mice was lower than that of WT during baseline, it is not clear whether or not the smaller liver mass observed after long term CR treatment was a result of p66Shc related modulations to changes in organ mass in response to CR. It has been previously reported that liver weight is significantly lower in young (3–5 month old) ad libitum fed p66Shc(-/-) compared to WT mice (Tomilov et al., 2011). It remains to be determined if liver weights are different in ad libitum fed older p66Shc(-/-) compared to WT mice.

Previous studies which reported that young p66Shc(-/-) mice have significantly lower body weights but the same level of energy intake as WT mice provide indirect evidence that EE may be increased in the p66Shc(-/-) animals. Support for this conclusion is provided by one study which found that oxygen consumption divided by body weight is increased in p66Shc(-/-) compared to WT mice (Berniakovich et al., 2008). This study also indicated that an increase in brown adipose tissue (BAT) mitochondrial proton leak may be a mechanism for the increased EE in the p66Shc(-/-) mice. In contrast to these findings, there was no significant difference in 24 hour, light cycle or dark cycle EE between 18 month old p66Shc(-/-) and WT mice, regardless of whether EE was expressed as kJ/mouse or normalized for body weight, lean mass or organ weights using ANCOVA. There are at least two possible reasons for the differences in EE results between p66Shc studies. First, the ages of the mice used in the studies are different. The Berniakovich et al. (Berniakovich et al., 2008) study used young mice (<6 months of age) while the present study used 18–20 month old animals. It is possible that physiological mechanisms, like BAT mitochondrial proton leak, which stimulate EE in younger animals were not active in the older mice. Second, the statistical methods used to normalize EE (or oxygen consumption) for body weight were different between studies. Expressing energy expenditure per unit of body mass as a ratio has long been criticized in the literature and is not considered to be appropriate for analyzing and comparing energy expenditure data (Allison et al., 1995). Furthermore, it has been well documented that the use of ANCOVA with covariates known to impact energy expenditure (i.e: body mass, lean body mass, and organ mass) is the most appropriate statistical method to interpret energy expenditure data (Tschop et al., 2011). There is debate about which covariate should be used for these comparisons, although in the present study no differences in EE between genotypes were observed regardless of the covariate used.

To our knowledge, no previous studies have investigated the influence of p66Shc on EE in CR animals. We observed that there is a difference in the response to 3d but not 2mo CR between the p66Shc(-/-) and WT animals. While p66Shc(-/-) mice demonstrate a significant decrease (11%) in 24h EE from ad libitum conditions to acute CR, WT mice demonstrated a mere 3% decrease in 24h EE in response to acute CR. However, both groups significantly decreased 24h EE in response to 2mo CR. Expressing energy expenditure per unit of body mass as a ratio has long been criticized in the literature and is not considered to be appropriate for analyzing and comparing energy expenditure data (Allison et al., 1995; Arch et al., 2006; Tschop et al., 2012). Thus, we used BW as a covariate in the model when analyzing EE using ANCOVA. When looking at 24h EE adjusted for BW, we found that EE decreased significantly from ad libitum to 2mo CR conditions in both groups of animals. However, only the p66Shc(-/-) animals significantly decreased 24h EE in response to 3d CR. This decrease in EE in the p66Shc(-/-) mice would be expected to mitigate loss of body energy reserves during very acute CR and may help the animals adapt to periods of CR. However, the physiological consequences of these changes in EE in response to 3d CR remain to be determined. While 24h EE provides us with an overview of the energetic adaptation to CR, partitioning our analyses further by light and dark cycle allows us to observe differences in diurnal patterns of EE. The observation that, in both groups of animals, light cycle EE decreased significantly in response to both 3d and 2mo CR, while dark cycle EE only decreased significantly in response to long term CR could be explained by several factors that impact dark cycle EE in mice. Because mice are nocturnal animals, an increase in activity or food-seeking behavior in response to 3d but not 2mo CR could provide an explanation for this discrepancy. An increase in physical activity in rodents in response to CR has been previously reported (Duffy et al., 1997; McCarter et al., 1997). However, some studies have noted no change in overall daily physical activity with only an increase in physical activity during times of feeding in response to CR (Cameron et al., 2011). We utilized the ratio of dark to light EE in order to estimate magnitude of diurnal changes in response to CR. While it is possible that diet induced thermogenesis could

contribute to such diurnal variation in EE, this is unlikely because animals in this study were calorie restricted and thus would be expected to have a decrease in diet induced thermogenesis. For this reason, it is likely that increases in physical activity during the dark cycle, decreases in physical activity during the light cycle, or both would primarily account for an increase in this ratio with CR. Future studies utilizing a more specific method of activity detection will explore the possible impact of the p66Shc protein on CR induced changes in physical activity.

CR induced changes in substrate utilization showed similar patterns in both p66Shc(−/−) and WT animals. This pattern, a decrease in RQ at 3d of CR followed by a return to baseline RQ at 2mo of CR, is consistent with the idea that animals in both groups returned to weight stable conditions at 2mo of CR. In contrast, 3d CR conditions represented a state of active weight loss for both groups of mice. It is of considerable interest that under 2mo but not 3d CR conditions, both groups of animals responded to feeding with an increase in RQ that exceeded 1.0, indicating a temporary state of lipogenesis. These findings are consistent with that of a previous study which reported periods of elevated fatty acid synthesis which alternated with periods of fatty acid oxidation in response to a 30% calorie restriction diet (Bruss et al., 2011).

In conclusion, the results of this study indicate that whole body energy expenditure is not altered in 18mo p66Shc(−/−) compared to WT mice. However, the response to CR was different between 18 mo p66Shc(−/−) and WT mice. While both p66Shc(−/−) and WT mice showed a decrease in EE at 2mo of CR, only the p66Shc(−/−) showed a decrease in EE at 3 day of CR. Thus, p66Shc may play a role in the energetic response to very acute CR in 18 month old mice. Future studies are needed to determine the impact of p66Shc on the energetic response to CR induced at various stages of life.

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Highlights

- Energy expenditure was studied in p66Shc(-/-) and wild-type mice
- Energy expenditure was measured under ad libitum and calorie restricted conditions
- Energy expenditure was decreased in p66Shc(-/-) mice at 3 d of calorie restriction
- Respiratory quotient was not altered in the p66Shc(-/-) mice
- p66Shc may play a role in the energetic response to calorie restriction

Fig. 1. Effect of 3d and 2mo CR on body mass in p66Shc(-/-) and WT mice. Bars that do not contain the same letter differ ($P < 0.01$).

Fig. 2. 24h Energy Expenditure in (A) p66Shc(-/-) and (B) WT mice and RQ in (C) p66Shc(-/-) and (D) WT mice in under ad libitum, acute, and longer CR conditions. Data are presented as means $\pm$ SEM.

Fig. 3. Mean EE (A) and RQ (B) under ad libitum conditions during light and dark cycle. Data are presented as means $\pm$ SEM. Superscripts that differ indicate significance ($P < 0.05$).

Table 1Body composition and organ weights at completion of 2mo calorie restriction in p66Shc(-/-) and WT mice¹

	p66Shc(-/-) (n=7)	WT (n=7)
	<i>g</i>	<i>g</i>
Body Weight	23.82 ± 0.448	25.74 ± 0.814
Lean Body Mass	20.67 ± 0.154	22.78 ± 1.005
Liver	0.976 ± 5.37E-2 *	1.257 ± 5.59E-2
Spleen	0.034 ± 2.38E-3	0.032 ± 2.32E-3
Kidneys	0.376 ± 2.26E-2	0.364 ± 2.84E-2
Lungs	0.152 ± 1.03E-2	0.175 ± 6.52E-3
Heart	0.158 ± 9.37E-3	0.150 ± 4.10E-3
Brain	0.430 ± 1.15E-2	0.430 ± 8.16E-3
Crude Organ Weight	1.940 ± 3.69E-2 *	2.202 ± 6.88E-2

¹Data are presented as means ± SEM;[†]Independent t-test;* p66Shc(-/-) differ from WT, *P*<0.05; Crude organ weight represents the sum of liver, kidneys, heart, and brain.

Table 2

Energy expenditure and respiratory quotient in response to ad libitum feeding, acute, and long term calorie restriction in WT and p66Shc(-/-) mice.¹

	WT			p66Shc(-/-)			P Value	
	Baseline	3d CR	2mo CR	Baseline	3d CR	2mo CR	Geno	Treatment
							Geno	Geno*Treatment
24 hour								
RQ	0.90 ± 2.39E-2 ^a	0.83 ± 4.73E-3 ^b	0.91 ± 9.80E-3 ^a	0.91 ± 1.92E-2 ^a	0.84 ± 5.45E-3 ^b	0.92 ± 7.01E-3 ^a	0.814	<0.0001
EE (kJ/min/mouse)	0.030 ± 9.79E-4 ^a	0.0290 ± 2.88E-3 ^a	0.0221 ± 6.27E-4 ^b	0.031 ± 6.58E-4 ^a	0.0275 ± 2.44E-3 ^b	0.0222 ± 6.91E-4 ^c	0.883	<0.0001
EE _{BW} (kJ/min) [†]	0.0292 ± 1.31E-3 ^a	0.0287 ± 1.01E-3 ^a	0.0228 ± 8.45E-4 ^b	0.0307 ± 6.75E-4 ^a	0.0277 ± 9.21E-4 ^b	0.0232 ± 9.75E-4 ^c	0.777	<0.0001
EE _{FFM} (kJ/min) [§]	-	-	0.0225 ± 6.22E-4	-	-	0.0248 ± 1.69E-3	0.239	-
EE _{ORGAN} (kJ/min) [†]	-	-	0.0229 ± 7.96E-4	-	-	0.0227 ± 1.11E-3	0.914	-
Light								
RQ	0.88 ± 2.39E-2 ^a	0.77 ± 1.89E-3 ^b	0.86 ± 1.19E-2 ^a	0.88 ± 2.10E-2 ^a	0.78 ± 5.06E-3 ^b	0.86 ± 1.14E-2 ^a	0.66	<0.0001
EE (kJ/min/mouse)	0.0274 ± 9.15E-4 ^a	0.0229 ± 8.36E-4 ^b	0.0166 ± 7.62E-4 ^c	0.0275 ± 8.16E-4 ^a	0.0222 ± 5.44E-4 ^b	0.0169 ± 7.43E-4 ^c	0.89	<0.0001
EE _{BW} (kJ/min) [†]	0.0264 ± 1.18E-3 ^a	0.0226 ± 8.15E-4 ^b	0.0173 ± 8.92E-4 ^c	0.0271 ± 7.35E-4 ^a	0.0224 ± 5.63E-4 ^b	0.0181 ± 8.91E-4 ^c	0.629	<0.0001
EE _{FFM} (kJ/min) [§]	-	-	0.0177 ± 6.53E-4	-	-	0.0185 ± 1.78E-3	0.531	-
EE _{ORGAN} (kJ/min) [†]	-	-	0.0179 ± 7.24E-4	-	-	0.0181 ± 1.01E-3	0.923	-
Dark								
RQ	0.93 ± 2.44E-2 ^a	0.88 ± 6.31E-3 ^b	0.97 ± 4.27E-3 ^a	0.94 ± 2.14E-2 ^a	0.89 ± 6.43E-3 ^b	0.96 ± 8.47E-3 ^a	0.87	<0.0001
EE (kJ/min/mouse)	0.0328 ± 1.09E-3 ^a	0.0345 ± 1.30E-3 ^a	0.0275 ± 7.99E-4 ^b	0.0347 ± 7.23E-4 ^a	0.0327 ± 1.25E-3 ^a	0.0274 ± 7.26E-4 ^b	0.95	<0.0001
EE _{BW} (kJ/min) [†]	0.0326 ± 1.38E-3 ^a	0.0344 ± 1.32E-3 ^a	0.028 ± 9.95E-4 ^b	0.0345 ± 8.10E-4 ^a	0.0327 ± 1.28E-3 ^a	0.027 ± 9.95E-4 ^b	0.971	<0.0001
EE _{FFM} (kJ/min) [§]	-	-	0.0276 ± 7.85E-4	-	-	0.0309 ± 2.14E-3	0.182	-
EE _{ORGAN} (kJ/min) [†]	-	-	0.0278 ± 1.10E-3	-	-	0.0274 ± 1.45E-3	0.821	-

BW, body weight; FFM, fat-free mass; ORGAN, crude organ mass (sum of liver, kidney, heart, and brain mass); EE is expressed as EE kJ/min/mouse (kJ per min per mouse) and EE_{BW}, FFM, ORGAN (kJ/min normalized by BW, FFM, and ORGAN)

¹Data is presented as means ± SEM unless otherwise indicated; superscript letters that differ indicate differences within genotype between treatment days, Bonferroni corrected $P < 0.001$;

[†], [§], [†] values are presented as least square mean ± SEM, adjusted for BW, FFM, and ORGAN, respectively. Treatment refers to baseline, 3d CR, and 2mo CR.

Ratio of dark to light energy expenditure in response to ad libitum feeding, acute, and long term calorie restriction in WT and p66Shc(-/-) mice.[/]

Table 3

	WT			p66Shc(-/-)			P Value	
	Baseline	3d CR	2mo CR	Baseline	3d CR	2mo CR	Geno	Day
D:L Ratio	1.198 ± 0.017 ^a	1.507 ± 0.0146 ^b	1.679 ± 0.091 ^b	1.266 ± 0.033 ^a	1.469 ± 0.035 ^b	1.626 ± 0.045 ^c	0.755	<0.0001
								0.122

[/]Data is presented as means ± SEM; superscript letters that differ indicate differences within genotype between treatment days, Bonferroni corrected *P*<0.001.