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The influence of Shc proteins and high fat diet on energy metabolism of mice

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Abstract

The purpose of this study was to determine if Shc proteins influence the metabolic response to acute (7 days) feeding of a high fat diet (HFD). To this end, whole animal energy expenditure (EE) and substrate oxidation were measured in the Shc knockout (ShcKO) and wild-type (WT) mice fed a control or HFD. The activities of enzymes of glycolysis, the citric acid cycle, electron transport chain (ETC), and β-oxidation were also investigated in liver and skeletal muscle of ShcKO and WT animals. The study showed that ShcKO increases ($P < 0.05$) EE adjusted for either total body weight or lean mass. This change in EE could contribute to decreases in weight gain in ShcKO versus WT mice fed a HFD. Thus, our results indicate that Shc proteins should be considered as potential targets for developing interventions to mitigate weight gain on HFD by stimulating EE. Although decreased levels of Shc proteins influenced the activity of some enzymes in response to high fat feeding (e.g. increasing the activity of acyl-CoA dehydrogenase), it did not produce concerted changes in enzymes of glycolysis, citric acid cycle or the ETC. The physiological significance of observed changes in select enzyme activities remains to be determined.

Keywords

Beta-oxidation; bioenergetics; energy expenditure; glycolysis; mitochondria

Introduction

The mammalian Shc locus encodes the p46Shc, p52Shc and p66Shc proteins[1]. These adaptor proteins were originally shown to play a role in growth factor signaling[1], although subsequent studies have also shown that these proteins participate in signaling from a wide range of cell surface receptors, including the insulin receptor[2, 3]. This indicates that Shc proteins may be involved in energy metabolism. The p66Shc knockout mouse has been used to investigate the possible roles p66Shc plays in insulin signaling and metabolism. While

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Conflict of Interest

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this mouse model was intended to provide a way to investigate p66Shc depletion alone, it has been shown that these animals also have decreased levels of p46Shc and p52Shc in liver and muscle[4]. In these tissues, the p66Shc knockout mice (referred to as ShcKO hereafter) provides a model for studying the overall decreases in Shc proteins.

It has been reported that body weight was decreased in ShcKO mice, despite the fact that their energy intake did not differ from that of the wild-type (WT) animals[5]. This decrease in body weight was due to the fact that the weights of all fat pads were lower in ShcKO compared to WT mice[5, 6]. Similarly, it has been reported that ShcKO mice show less weight gain on a high fat diet than WT animals[5] and p66Shc knockout in leptin-deficient ob/ob mice decreased weight gain in these obese animals[7]. The results of these studies suggest that Shc proteins play a role in regulating whole animal energy metabolism.

In addition to the whole animal measures of metabolism in the ShcKO mice, it has also been shown that Shc proteins influence the activities of key regulatory enzymes of intermediary metabolism in liver and skeletal muscle[8–10]. The activities of regulatory enzymes of glycolysis (gluckinase/hexokinase, phosphofructokinase-1 and pyruvate kinase) were significantly decreased in liver and skeletal muscle of ShcKO compared to WT mice with fasting and 3 hours following feeding[9, 10]. ShcKO mice also showed a significant increase in the activities of mitochondrial β-oxidation enzymes, although this change in enzyme activities only occurred under fasting conditions[8]. The results of these studies suggest that Shc proteins may play a role in fuel selection. Moreover, the decreased Shc protein levels differentially alters the activities of enzymes involved in the major pathways for glucose versus fatty acid oxidation. However, it is not known if ShcKO mice are better able to alter the activities of enzymes of energy metabolism than WT animals in response to metabolic challenges, such as sustained consumption of a high fat diet.

The purpose of this study was to determine if Shc proteins influence the metabolic response to acute (5–7 days) feeding of a high fat diet (HFD). To this end, the activities of enzymes of glycolysis, the citric acid cycle, electron transport chain, and β-oxidation were investigated in liver and skeletal muscle of ShcKO and WT mice fed a control or HFD. Whole animal energy expenditure and substrate oxidation (respiratory exchange ratio) were also measured in the ShcKO and WT animals consuming either a control or HFD diet.

Material and Methods

Animals and diet

All animal care and use protocols were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC) and are in accordance with the National Institutes of Health (NIH) guidelines for the Care and Use of Laboratory Animals. ShcKO and WT mice came from a breeding colony at UC Davis that was established from mice provided by Dr. Pier Giuseppe Pelicci (Department of Experimental Oncology, European Institute of Oncology, Milan, Italy). The breeding stocks were backcrossed onto C57BL/6J mice to full congenic status, and the homozygous ShcKO and WT lines were generated by mating heterozygous ShcKO mice.

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Male WT and ShcKO mice used for this study were 2 months old and were fed a chow diet (Lab diet 5001) prior to feeding the HFD. At 2 months of age, the mice of both genotypes (WT and ShcKO) were randomly divided and fed either the chow or HFD for an additional 5 (indirect respiration calorimetry study) or 7 days (enzyme study). A total of 8 WT and 8 ShcKO mice were used for the indirect respiration calorimetry study. Separate groups of mice were used for the enzyme study. For the enzyme study, a group of mice were fed the chow diet (10 WT and 8 ShcKO) and another group of mice were fed the HFD (11 WT and 8 ShcKO).

All animals had free access to food and water. The mice were housed at 22–24 °C and 40–60% humidity, with a 12 h light: dark cycle. The nutrient compositions of the diets were as follows: chow lab diet 5001 (% of Kcal; protein = 30%, carbohydrates = 57%, fat = 13%) and HFD (% kcal; protein = 18.3%, carbohydrates = 21.4%, fat = 60.3%). The HFD uses the same macronutrients and vitamin and mineral mixes as the semi-purified AIN93G diet, with the increase in percent of total energy from fat achieved by adding lard and removing carbohydrates from the AIN93G diet.

Indirect respiration calorimetry

Total daily energy expenditure (EE) and respiratory exchange ratio (RER) were measured using whole-body indirect respiration calorimetry through the UC Davis Mouse Metabolic Phenotyping Center. The Oxymax/CLAMS calorimetry system (Columbus Instruments, Columbus, OH) was housed in a room maintained on a 12 hour light/12 hour dark cycle at 22°C. The mice were placed in acclimation cages (calorimetry chambers not connected to the calorimeter) and housed in the Oxymax/CLAMS room for 24 hours. The mice were then transferred to calorimetry chambers contained in a 22°C incubator and calorimetry measurements were then completed over a 7 day period. Mice were fed the control diet for the first two days in the calorimetry chamber, and then all mice were switched to the HFD for the remaining 5 days in the calorimetry chambers.

Calorimetry measurements were completed on the mice on days 1 and 2 (chow diet) and day 7 (HFD). Room air was drawn through the calorimetry chambers at 500 ml/min. Samples of dried room and chamber air were analyzed for oxygen and carbon dioxide content using the Oxymax system. Calorimeter calibration was performed daily prior to the beginning of each 24 hour measurement. A 0.50% CO_2 and 20.50% O_2 (balance nitrogen) calibration gas (Airgas, Sacramento, CA) and dry room air were used to calibrate the analyzers. At the start and end of the experiments, the performance of the entire calorimetry system was validated by bleeding a 20% CO_2 (balance nitrogen) standard (Airgas, Sacramento, CA) into each calorimetry chamber at a regulated flow rate using an OxyVal gas infusion system (Columbus Instruments, Columbus, OH) and measuring recovery of CO_2 and O_2 dilution in the chamber exhaust. Energy expenditure was calculated from oxygen consumption and carbon dioxide production using the Oxymax system and RER was determined as the ratio of the volume of CO_2 produced to the volume of O_2 produced.

The calorimetry chambers were also fitted with an infrared photocell system (Columbus Instruments, Columbus, OH) which measured activity as "counts" each time an infrared beam break occurred. Activity was measured in the horizontal (x) and vertical (z) plane.

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Immediately following calorimetry measurements, the mice were anesthetized with 2–4% isoflurane and body composition (lean and fat mass) were measured using a dual energy X-ray absoptiometry (DEXA) machine (PixiMus, Fitchburg, WI). Each mouse was scanned in less than 5 minutes, and body composition was determined using the PixiMus software.

Chemicals

The chemicals and reagents were purchased from MilliporeSigma (St. Louis, MO), except bovine serum albumin (BSA, MP Biochemicals, Santa Ana, CA), Bio-Rad protein assay dye (BioRad, Hercules, CA) and NAD, NADH and ATP (Roche Diagnostics, Indianapolis, IN). Auxiliary enzymes used in the assays were from Roche Diagnostics (Indianapolis, IN), MilliporeSigma (St Louis, MO) and Megazyme (Bray, Ireland).

Sample preparation

For the enzyme studies, food was removed from the cage at 8 AM and mice were sacrificed by cervical dislocation between 9–10 am. Body weight (BW) was recorded and liver and hindlimb skeletal muscles were rapidly removed, weighed immediately and frozen in liquid nitrogen. The visible fat and connective tissue were trimmed from the muscle samples prior to freezing. In addition to BW, organs (heart, kidneys, and spleen) and epididymal fat pad weights were also recorded. Frozen liver and muscles were then powdered in a porcelain mortar and pestle maintained under liquid nitrogen. All tissue powders were stored under liquid nitrogen until used in enzyme assays. The powders were homogenized at a 1:10 ratio (w/v) in buffer containing 10 mM HEPES, 250 mM sucrose, 0.5 mM EDTA, 100 mM KCl and 10% glycerol (pH = 7.4 at 4° C). The homogenates were centrifuged at 600 g for 10 minutes at 4° C and the supernatants carefully collected and stored on ice.

Enzyme assays

All assays were performed in a 1 ml final volume using a Perkin Elmer Lambda 25 UV/Vis spectrophotometer equipped with a Peltier heating control system and 9 cell changer (Perkin Elmer, Shelton, CT). Enzyme activities were expressed as μmol/min/mg protein using the respective extinction coefficient (**ε**), and presented as mean ± SEM determined from at least eight animals per group.

Glycolytic enzymes

The activities of the glycolytic enzymes glucokinase/hexokinase (GK/HK, EC 2.7.1.1), phosphofructokinase-1 (PFK-1, EC 2.7.1.11) and pyruvate kinase (PK, EC 2.7.1.40) were measured in both liver and muscle samples of WT and ShcKO animals, at 340nm (**ε** = 6.22 mM−1.cm−1), as previously described[11]. GK activity was determined in the presence of 0.5 and 100 mM glucose and taken as the activity at 100 mM glucose minus the activity at 0.5 mM glucose. HK activity in muscle samples was determined using 10 mM glucose. Lactate dehydrogenase activity (LDH, EC 1.1.1.27) was also measured in both liver and muscle samples, at 340 nm (**ε** = 6.22 mM−1.cm−1) as previously described[8].

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Citric acid cycle and electron transport chain (ETC) enzymes

The activities of citrate synthase (CS, EC 2.3.3.1) and aconitase (AC, EC 4.2.1.3) were measured in both liver and muscle samples of WT and ShcKO animals. CS and AC were determined as previously described[12], at 412 nm (ε = 13.6 mM−1.cm−1) and 240nm (ε = 3.6 mM−1.cm−1), respectively. For the ETC enzymes, complex I (NADH: Ubiquinone oxidoreductase, EC 1.6.5.3) and complex IV (Cytochrome C oxidase, EC 1.9.3.1) activities were measured at 340nm (ε = 6.22 mM−1.cm−1) and 550nm (ε = 28 mM−1.cm−1), respectively, as previously described[13].

β-oxidation and ketone body metabolism enzymes

Activities of acyl-CoA dehydrogenase [EC 1.3.8.8] was measured in skeletal muscle and liver as previously described[8]. Acyl-CoA dehydrogenase activity was measured at 600nm (ε =21 mM−1cm−1), using n-palmitoyl-CoA as substrate. For ketone body synthesis, the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA synthase, EC 2.3.3.10), was determined at 303 nm (ε =12.2 mM−1cm−1), as previously reported[14].

Protein assays

Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA), with BSA as the standard.

Statistical analysis

Mean of body weights of WT and ShcKO mice in each diet group were compared using two-tailed t-tests. A two-way ANOVA was used considering the mains effects of diets (chow or HFD) and genotypes (WT or ShcKO). Both factors and their interaction (diet*genotype) were evaluated. All analyses were conducted with SAS software (version 9.3) and for interaction effect ($P < 0.05$), Tukey's multiple comparison procedure was used to identify which diet*genotype groups differed significantly. To control for multiple testing, a Bonferroni adjustment or false discovery rating (FDR) methods were used to maintain the family-wise error rate at 0.05 for the pairwise comparisons. For the variables not normally distributed, log transformation was applied and then used as log-transformed data for ANOVA tests. When this procedure did not work, Friedman's two-way nonparametric ANOVA was applied. For energy expenditure data, analysis of covariance (ANCOVA) was used with either BW or lean mass as a covariate in the model.

Results

Body, organ and epididymal fat weights

Final body weights, weight change, and organ weights are summarized in Table 1. No weight change was observed in ShcKO mice on chow ($P = 0.370$), while a significant increase ($P < 0.05$) in body weight was observed in WT animals after consuming the HFD for one week. Changes in body weight following consumption of the HFD also reach statistical significance ($P < 0.01$) in the ShcKO mice after consuming the HFD for 7 days. However, changes in body weight trended ($P = 0.075$) to be lower in ShcKO compared to WT mice on HFD. There were no differences in final body weight ($P = 0.446$) between the

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WT or ShcKO mice on HFD, reflecting the fact that the two genotypes had similar body weights on chow ($P = 0.370$) and any body weight changes with the HFD over the one week period were relatively small. In both genotypes, the HFD increases ($P < 0.01$) the amounts of epididymal fat compared to their counterparts on chow diet. Also, WT and ShcKO mice fed a HFD showed similar results to organs weights such as heart, kidneys, spleen and epididymal fat.

Indirect respiration calorimetry

No differences between initial BW ($P = 0.914$) and final BW ($P = 0.678$) of ShcKO and WT mice were detected during calorimetry measurements. The initial BW of the ShcKO and WT mice when they were placed in the calorimetry chamber (CLAMS) was 24.477 ± 0.665 g and 24.570 ± 0.518 g, respectively. In addition, the final BW of the ShcKO and WT when they were removed from CLAMS was 25.904 ± 0.565 and 26.269 ± 0.643 g, respectively. The lean body mass (LBM), measured following completion of the calorimetry study in the CLAMS, was also similar ($P = 0.572$) between ShcKO and WT mice, and the mean LBM 19.584 ± 0.586 and 19.221 ± 0.216 g, respectively.

Whole animal energy expenditure (EE) data of WT and ShcKO mice are summarized in Table 2. No interaction between genotype and diet was observed ($P > 0.05$) for EE expressed as kJ/min or normalized for BW or LBM using ANCOVA (EEBW and EELBM, respectively). However, there is an effect ($P < 0.05$) of genotype, whereby ShcKO mice showed increased ($P < 0.05$) in 24h EE compared to WT animals fed on chow or HFD. No effects of genotype ($P > 0.05$) were observed for daily respiratory exchange ratio (RER). As expected, there is an overall effect of HFD ($P < 0.01$) increasing EE and decreasing RER of WT and ShcKO mice (Figure 1).

The interaction between BW and genotype was significant ($P < 0.05$) for a few comparisons. Whenever this occurred the Johnson-Neyman technique[15, 16] was used to investigate possible differences between experimental groups. This technique provides cut-offs which show which body weights showed differences between groups (see Table 2). Thus, the HFD comparison between the WT and ShcKO mice showed a significant ($P < 0.05$) interaction between BW and genotype, whereby there is a significant increase in EEBW 24h in ShcKO mice at BW 18.12 or 23.69 g. Additionally, on chow diet comparison, there is a significant ($P < 0.05$) increase in EEBW in dark cycle of ShcKO animals at BW 24.44 g.

Activities of glycolytic enzymes

The activities of the enzymes GK, PFK-1, PK and LDH from hepatic tissues of WT and ShcKO mice fed chow or a HFD are summarized in Figure 2. Interactions between diet and genotype ($P < 0.05$) were observed for PK and GK. In contrast to the WT animals, ShcKO mice fed a HFD showed an increase in GK acitivity ($P < 0.05$) compared to the chow diet. On a HFD, ShcKO mice also showed higher GK activity ($P < 0.05$) when compared with WT animals. Similarly, ShcKO mice fed a HFD showed higher ($P < 0.05$) PK activity when compared to chow diet while diet did not significantly alter PK activity in the WT animals. PK, PFK-1 and LDH activities decreased ($P < 0.05$) in ShcKO compared to WT mice on the chow diet while no differences between the genotypes were observed for these enzymes with

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the HFD. Additionally, LDH activity was increased ($P < 0.05$) in both genotypes with the HFD.

The glycolytic enzymes HK, PFK1, PK, and LDH were also assayed in skeletal muscle of WT and ShcKO animals (Figure 3). No interaction between the diets and genotypes was observed. No effects of either diet or genotype were observed on the activities of HK and LDH. In both genotypes, PFK-1 activity tended to be higher on the HFD with this increase being significant ($P < 0.05$) in the WT mice. PFK-1 activity was also increased ($P < 0.05$) in the ShcKO versus WT mice on the chow diet. PK activity was also decreased ($P < 0.05$) in the ShcKO compared to WT mice on both diet, and PK activity was significantly increased ($P < 0.05$) with HFD feeding in the ShcKO animals.

Activity of citric acid cycle and ETC enzymes

The activities of the citric acid cycle (CS and AC) and electron transport chain (Complex I and IV) enzymes in liver and muscle are summarized in Figures 4 and 5, respectively. An Interaction between diet and genotype was observed for liver CS activity ($P < 0.05$) with the ShcKO mice showing a trend toward an increase ($P < 0.10$) in CS activity with the HFD while no change was observed with diet in the WT animals. For liver AC, diet did not alter enzyme activity in either genotype while AC activity was decreased ($P < 0.05$) in the ShcKO compared to WT mice on both diets.

The interaction between diet and genotype was observed ($P < 0.05$) for both ETC enzymes (Complexes I and IV) in liver. ShcKO showed lower activities ($P < 0.05$) of complexes I and IV on a HFD compared to the chow diet, while no differences with diet were observed for the WT animals. The ShcKO mice also had higher ($P < 0.05$) Complex IV activities than the WT mice when consuming chow, but no differences were observed between genotypes with the HFD.

No diet and genotype interactions were observed for any of the mitochondrial enzymes in skeletal muscle. Both WT and ShcKO mice showed an increase ($P < 0.05$) in CS in response to feeding a HFD. Diet had no impact on the activities of aconitase, Complex I or Complex IV in either genotype. However, similar to hepatic tissue, aconitase activity was decreased ($P < 0.05$) in ShcKO compared to WT mice on both chow and HFD.

Activities of β-oxidation and ketone body metabolism enzymes

The activities of Acyl-CoA dehydrogenase (ACDH) in liver and skeletal muscle and HMG-CoA synthase in liver of WT and ShcKO animals are summarized in Figures 6 and 7. An Interaction between diet and genotype was observed for ACDH activity ($P < 0.05$) in both liver and skeletal muscle with only the ShcKO mice showing an increase ($P < 0.05$) in ACDH activity on the HFD versus chow diet. Also, ShcKO mice on the HFD showed higher ACDH activity ($P < 0.05$) than WT mice in both liver and skeletal muscle. In liver, no effects for diet, genotype or interaction were obtained for HMG-CoA synthase (Figure 7).

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Discussion

This study used ShcKO mice to determine the influence of Shc proteins on the metabolic response to acute feeding of a HFD. The study showed that ShcKO increases ($P < 0.05$) energy expenditure adjusted for either total body weight or lean mass (Table 2). This change in energy expenditure could contribute to the previously reported decrease in weight gain in ShcKO versus WT mice fed a HFD[5]. Moreover, the decrease in weight and fat mass gain previously reported in ShcKO mice following feeding of a HFD could be due to better ability to alter substrate oxidation in response to a shift in diet composition and/or increased energy expenditure. However, the study did not find shifts in metabolic pathways similar to those observed in ShcKO mice with fasting. Although decreased levels of Shc proteins influenced the activity of some enzymes in response to high fat feeding, such as increasing the activity of acyl-CoA dehydrogenase, it did not produce concerted changes in enzymes of glycolysis, citric acid cycle or the ETC. Furthermore, low Shc levels did not alter whole animal substrate oxidation, as shown by RER, with acute (5–7 days) high fat feeding.

Energy expenditure

In order to study the substrate oxidation in ShcKO mice, we investigated this by measuring RER and found no evidence that Shc proteins alter whole animal substrate oxidation in response to short-term feeding of a HFD (Figure 1 B). A study showed that ShcKO mice are resistant to weight gain on a HFD despite consuming the same kcal as controls[5]. Also, ShcKO mice on a leptin-deficient ob/ob mouse background are also resistant to weight gain compared to ob/ob WT mice, despite the fact that they consumed the same amount of energy per day[17]. An increase in energy expenditure is one mechanism that could explain this resistance to weight gain. The Berniakovich et al. paper[5] suggests that energy expenditure may be increased in the ShcKO mice, but they do not provide calorimetry data from mice consuming a HFD. Previous work has indicated that energy expenditure adjusted for either body weight or lean mass is not increased in ShcKO mice maintained on a standard rodent chow diet or subjected to calorie restriction on the same chow diet[18–20]. However, there is evidence that ShcKO mice can show increased activation of brown adipose tissue[21], suggesting that energy expenditure could be increased in these mice under certain circumstances.

The results of the present study indicate that energy expenditure is increased in ShcKO versus WT mice with high fat feeding. It remains to be determined if brown adipose tissue, or other tissues, are responsible for this increase in energy expenditure. Our results indicate that Shc proteins should be considered as potential targets for developing interventions to mitigate weight gain on HFD by stimulating energy expenditure. Additionally, an increase in mass-adjusted energy expenditure may be a mechanism that contributes to the decreased weight gain previously reported in ShcKO mice fed a HFD. This would fit with previous studies indicating that brown fat activity is increased in ShcKO mice[21]. Future studies should investigate brown fat in ShcKO mice to determine if this is the main mechanism that contributes to the metabolic response to a HFD in these mice.

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Glycolysis

Decreased levels of Shc proteins have been shown to produce concerted shifts in the activities of enzymes of intermediary metabolism toward an increase in capacity for fatty acid oxidation and a decrease in capacity for glycolysis[9–10]. These changes are most pronounced with fasting. Similar to fasting, adaptation to a HFD requires a shift in metabolism toward fatty acid oxidation and away from glucose. However, the present study does not provide support for Shc proteins inducing the same decrease in capacity for glycolysis observed with fasting in the HFD fed mice. In liver, ShcKO differed from WT mice in that the activities of GK and PK were increased with a HFD and the activity of PFK-1 was not decreased in these animals. Thus, the change in glycolysis is nearly opposite of what was observed in the liver of fasted ShcKO mice and ShcKO mice shortly after feeding (3 hrs)[9].

In contrast, the activities of glycolytic enzymes, with the exception of PK, were not changed with a HFD in skeletal muscle of ShcKO mice. Thus, Shc proteins appear to play distinct roles in regulating glycolytic enzyme activities depending on either physiological state (fed versus fasted) or diet (chow versus HFD). Therefore, unlike fasting, ShcKO did not change enzymes activities of glycolysis. Response to a HFD clearly shows tissue differences in the ShcKO animals and there is no evidence in either skeletal muscle or liver that ShcKO decreases capacity for glycolysis with high fat feeding. More studies are needed to determine how Shc proteins specifically change the activities of glycolytic enzymes in response to a HFD.

Fatty acid oxidation and ketogenesis

With sustained high fat feeding, there is evidence for increased activity of the β-oxidation enzyme acyl-CoA dehydrogenase in both skeletal muscle[22, 23] and liver[24, 25]. Such a change could be beneficial for increasing capacity for fatty acid β-oxidation. The present study indicates that ShcKO dramatically increases the activity of skeletal muscle and liver acyl-CoA dehydrogenase in response to high fat feeding. This results suggest that Shc proteins influence β-oxidation pathway and may contribute to the low body fat in ShcKO mice. However, in the present study ShcKO mice do not increased the activity of HMG-CoA synthase, a key enzyme in ketogenesis. Thus, ShcKO does not have uniform impact on all enzymes involved in lipid metabolism and it remains to be determined if ShcKO stimulates the activities of other enzymes involved in fatty acid oxidation. Previous work indicated that the low Shc levels result in increased liver and muscle β-oxidation enzyme activities in response to fasting and induce chronic increases in the activity of liver ketogenic enzymes. The activities of acyl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and ketoacyl-CoA thiolase were increased in fasted ShcKO compared to WT mice[8]. However, additional studies are needed to determine how Shc proteins change the activities of enzymes involved in lipid metabolism and ketogenesis in mice fed a HFD.

Mitochondrial enzymes (citric acid cycle and ETC)

Maintenance of mitochondrial content is important with high fat feeding, since mitochondria are a major site of lipid β-oxidation. However, the impact of high fat feeding on mitochondrial enzyme activities is complex. Citrate synthase (CS), an enzyme frequently

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used as a marker of mitochondrial content[26], has been previously reported in skeletal muscle to show either no change[27–30] or an increase[22, 23, 28, 30–32] in activity with consumption of a HFD. In liver, CS activity has often been found to not change with sustained feeding of a HFD[25, 29, 33, 34]. In the present study, we found no impact of ShcKO on changes in CS activity in response to a HFD with both genotypes showing an increase in CS activity in skeletal muscle and no change in the activity of this enzyme in liver.

Caution should be taken, however, in relying solely on CS activity as an indicator of mitochondrial content or changes in the major energy metabolism pathways in mitochondria. In our study, the activities of Complexes I and IV (NADH: Ubiquinone oxidoreductase and Cytochrome C oxidase, respectively) did not mirror the activities of CS in response to a HFD in the ShcKO mice for either liver or muscle. These results suggest that Shc proteins may play a role in modulating ETC enzyme activity in response to a HFD. Aconitase activity was also decreased in the ShcKO compared to WT mice for both the chow and HFD. ShcKO is considered to decrease oxidative stress[35, 36], and thus, it seems unlikely that the decreased aconitase activities in young ShcKO mice consuming chow is due to oxidative damage. Here, the effect of genotype was clear on the aconitase activity and, therefore, ShcKO mice may decrease the flow of electrons to oxygen, leading to decreased oxidative phosphorylation and this result indicates the importance of Shc proteins to prevent oxidative stress[34, 35]. However, future studies are also needed to determine if Shc proteins are an important regulator of aconitase.

Limitations

With the ShcKO mouse, it is not possible to determine which specific Shc isoform is responsible for observed changes in enzyme activities or energy expenditure. Additional models that allow controlled expression of specific Shc isoforms are needed to further dissect the contribution of the individual isoforms to changes in metabolism following consumption of a HFD. In addition, the extremely low Shc expression may alter the whole system to a dysfunctional state, which is possible in knockout models. The present study only investigated changes in metabolism in response to consumption of a HFD for 7 days. This period of time is sufficient for changes in the expression of most proteins and enzymes, however, our study is not capable of determining the influence of Shc proteins on the metabolic response to long term consumption of a HFD.

Conclusion

ShcKO increases energy expenditure adjusted for either body weight or lean mass in response to feeding a HFD. It is possible that this increase in energy expenditure contributes to the decrease in weight gain previously reported for these mice when consuming a HFD[5]. ShcKO also influenced, in a tissue specific manner, the activities of some enzymes of intermediary metabolism in response to consumption of a HFD. Thus, short-term feeding of HFD was enough to partially alter enzyme activities in liver and muscle of ShcKO mice, potentially leading to changes in energy metabolism. However, the physiological significance of these changes in specific enzyme activities remains to be determined.

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Significance of the study

We report higher energy expenditure in ShcKO versus WT mice when consuming the high fat diet. Although decreased levels of Shc proteins influenced the activity of a central enzyme of β-oxidation in response to high fat feeding, it did not produce concerted changes in enzymes of glycolysis, citric acid cycle or the electron transport chain. Thus, an increase in energy expenditure in response to consumption of a high fat diet may be a mechanism that leads to decreased weight gain previously reported in ShcKO mice with long-term consumption of a high fat diet.

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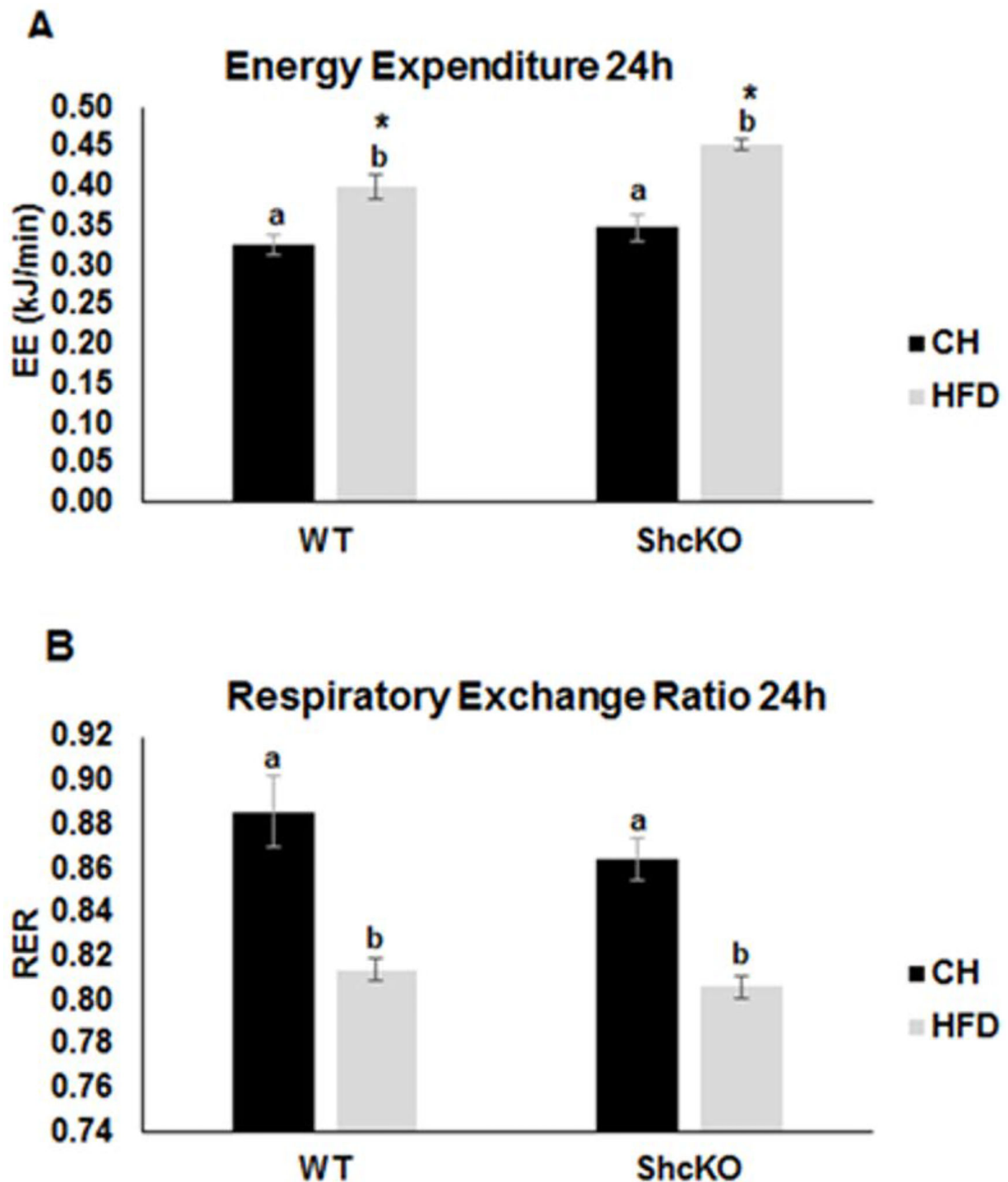

Figure 1.
Energy expenditure (**A**) and respiratory exchange ratio (**B**) of wild-type (WT) and Shc knockout (ShcKO) male mice over 24h fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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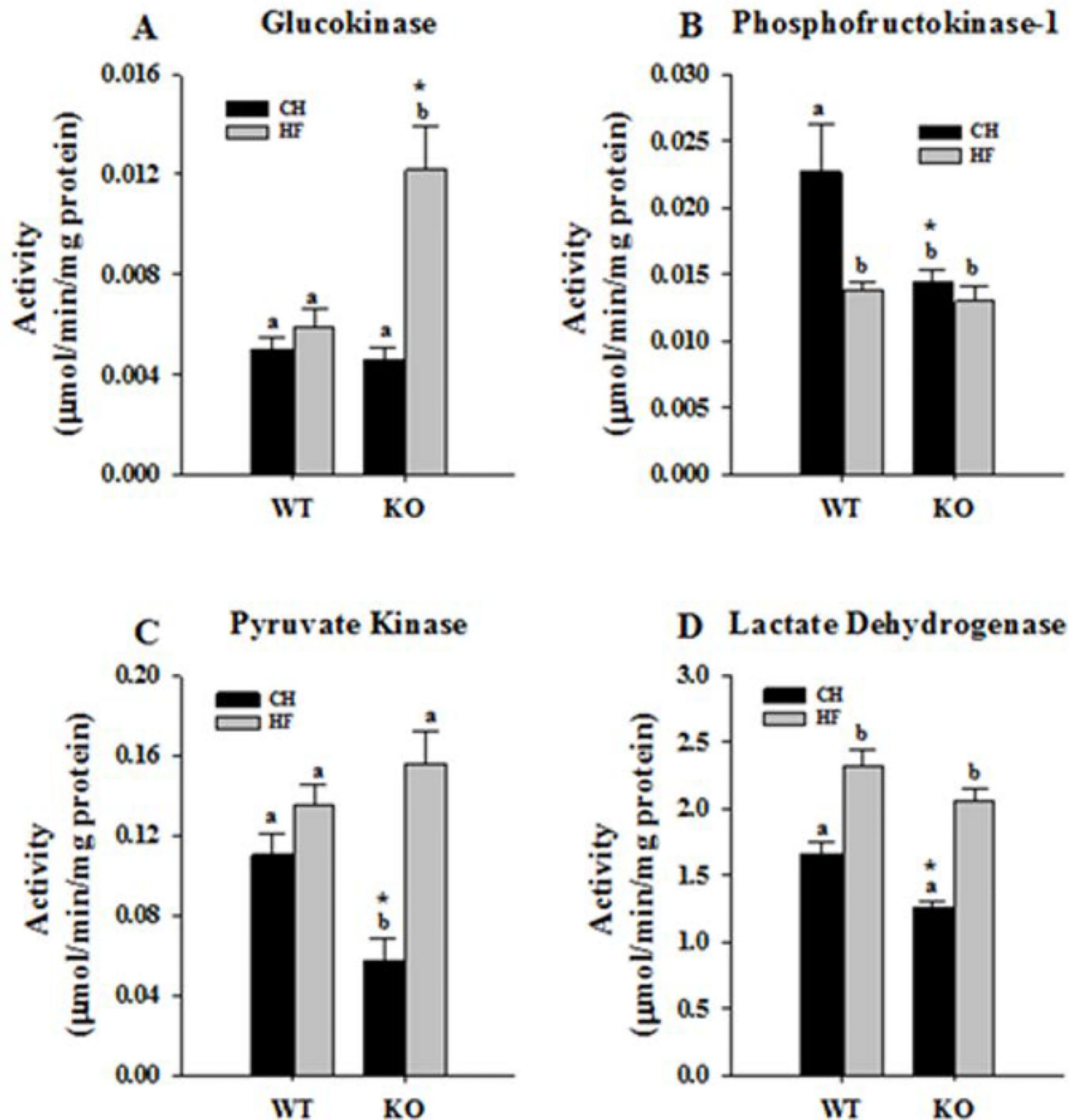

Figure 2.

Activities of glucokinase (**A**), phosphofructokinase-1 (**B**), pyruvate kinase (**C**), and lactate dehydrogenase (**D**) in hepatic tissue of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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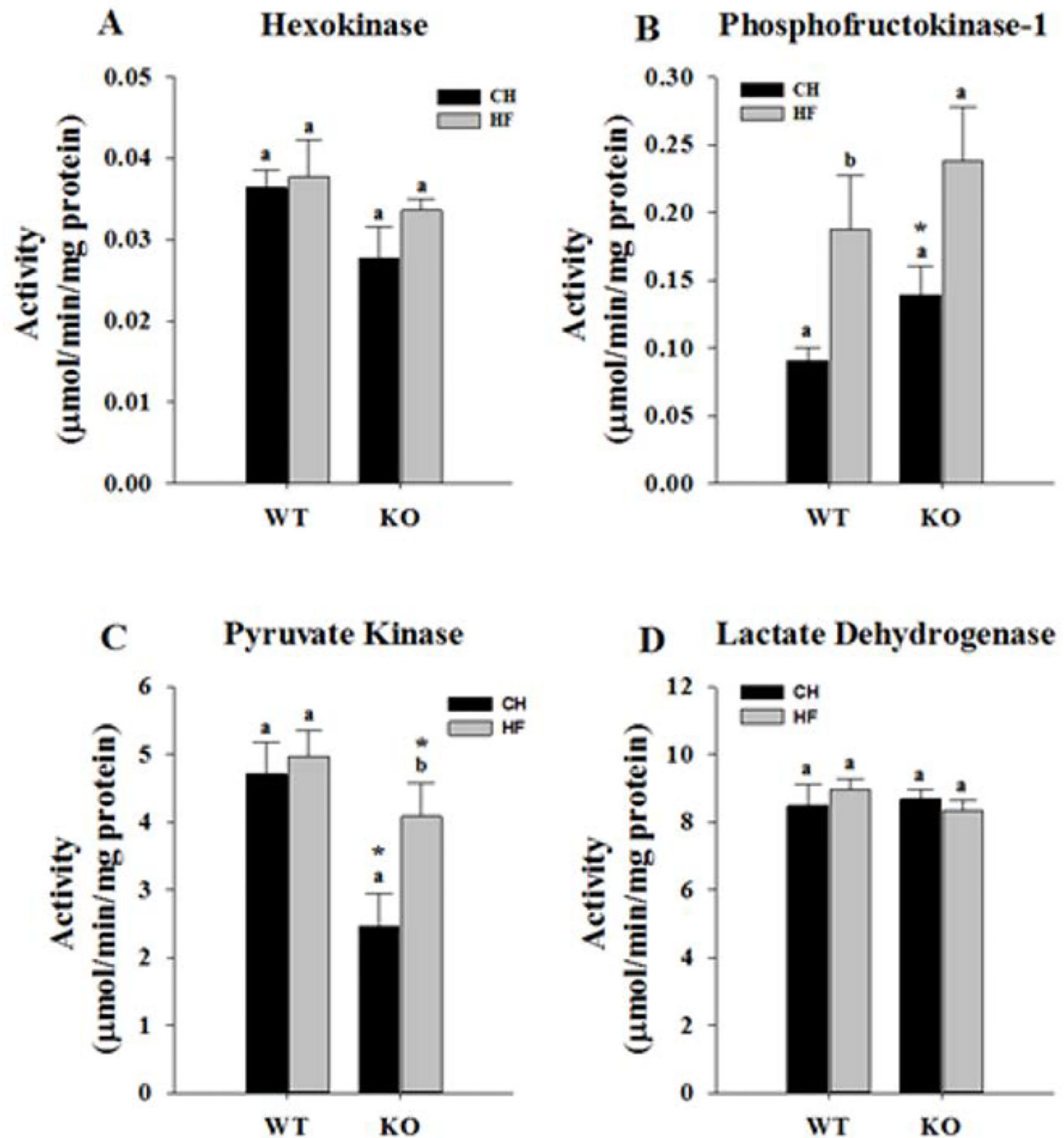

Figure 3.

Activities of Hexokinase (**A**), phosphofructokinase-1 (**B**), pyruvate kinase (**C**) and lactate dehydrogenase (**D**) in skeletal muscle of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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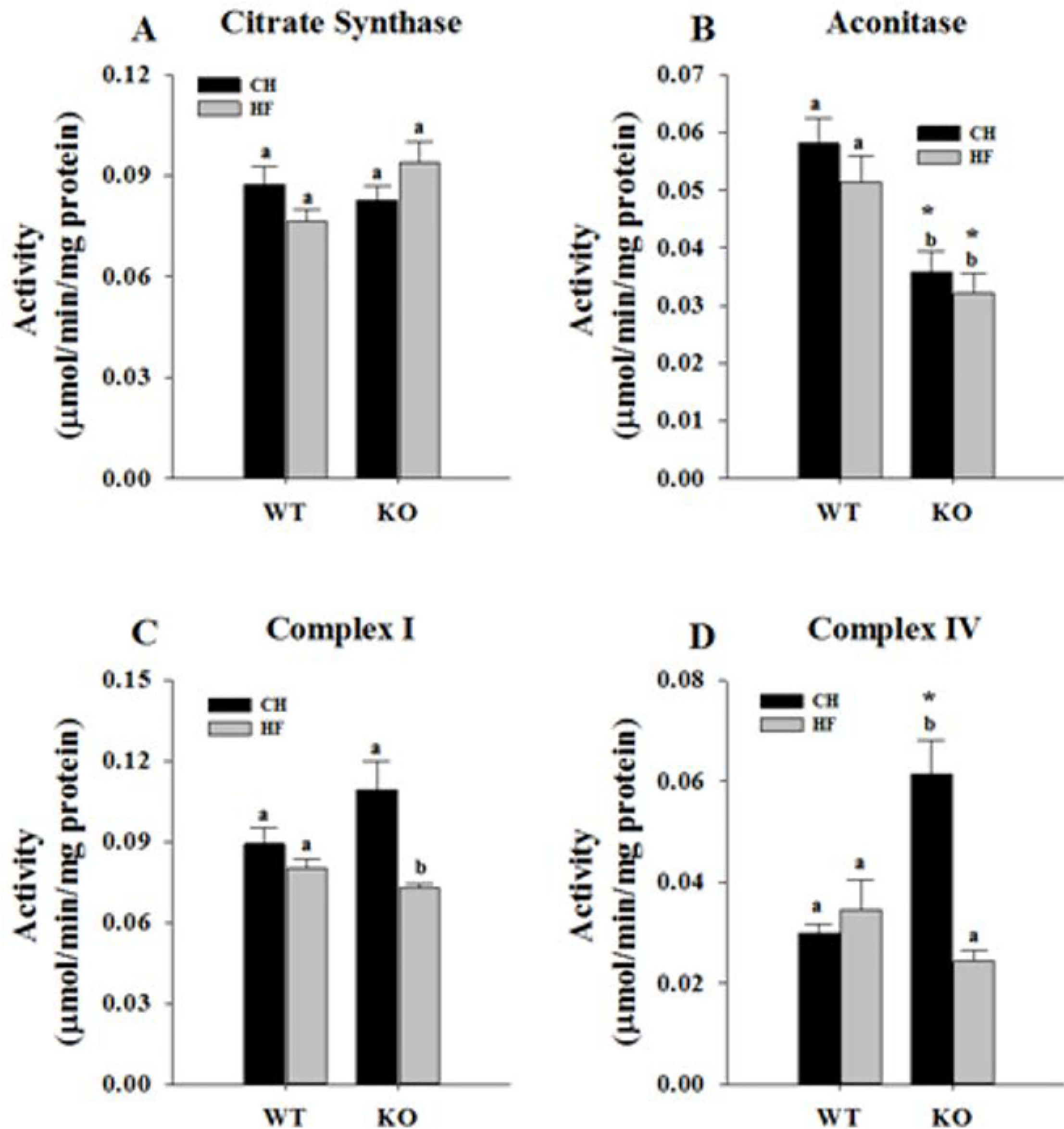

Figure 4.

Activities of citrate synthase (**A**), aconitase (**B**) and electron transport chain enzymes (ETC) complex I (**C**) and complex IV (**D**) in hepatic tissue of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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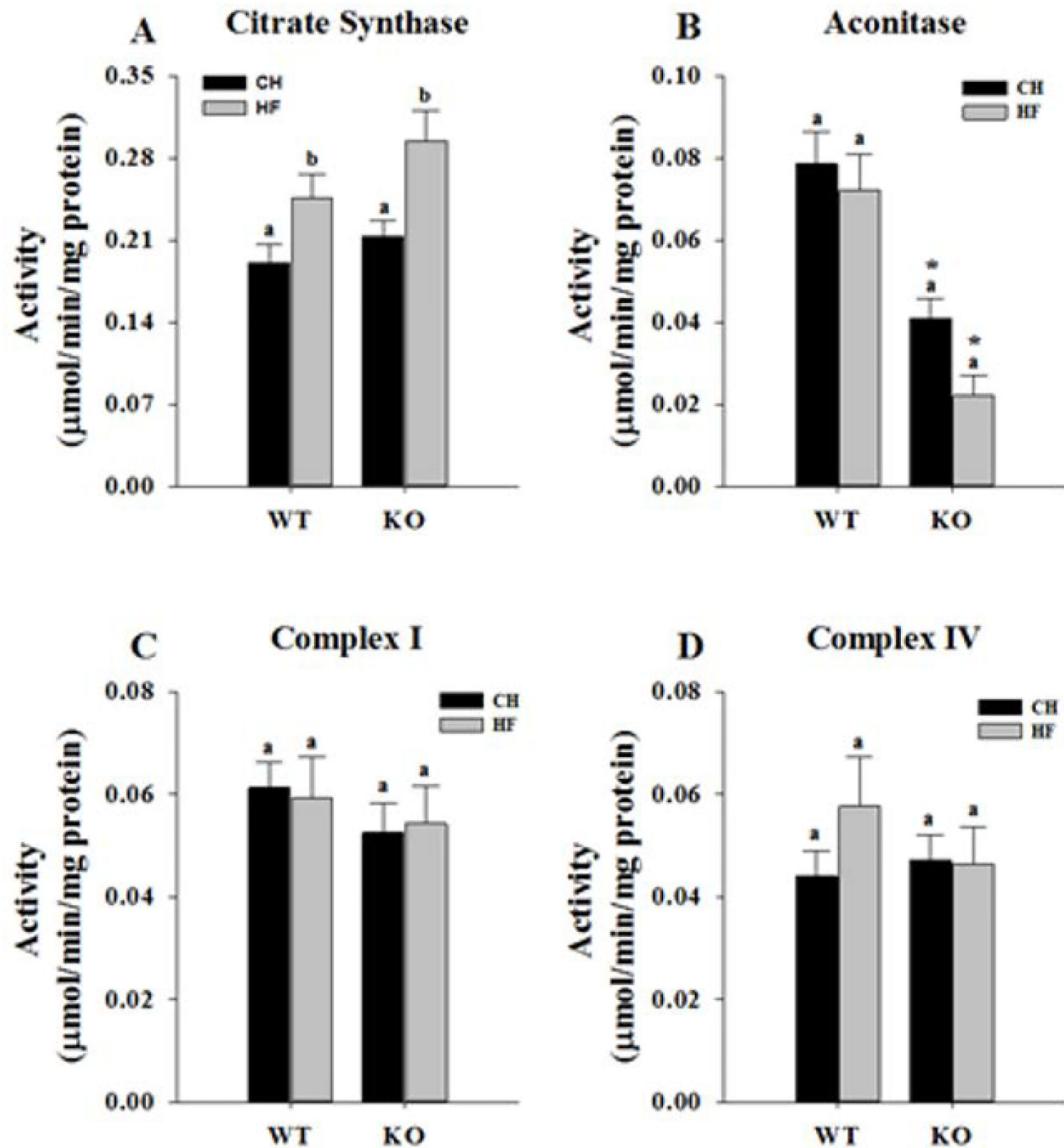

Figure 5.

Activities of citrate synthase (**A**), aconitase (**B**) and electron transport chain enzymes (ETC) complex I (**C**) and complex IV (**D**) in skeletal muscle of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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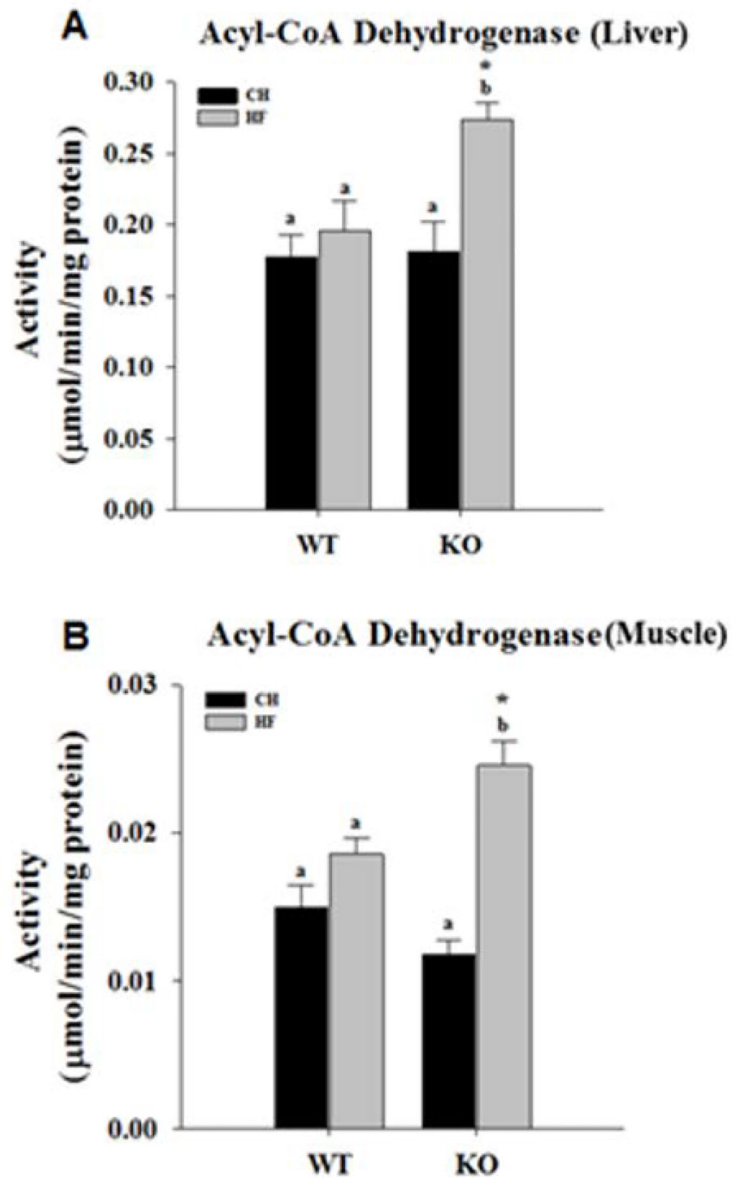

Figure 6.

Activities of acyl-CoA dehydrogenase in liver (**A**) and muscle (**B**) of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P <$ 0.05).

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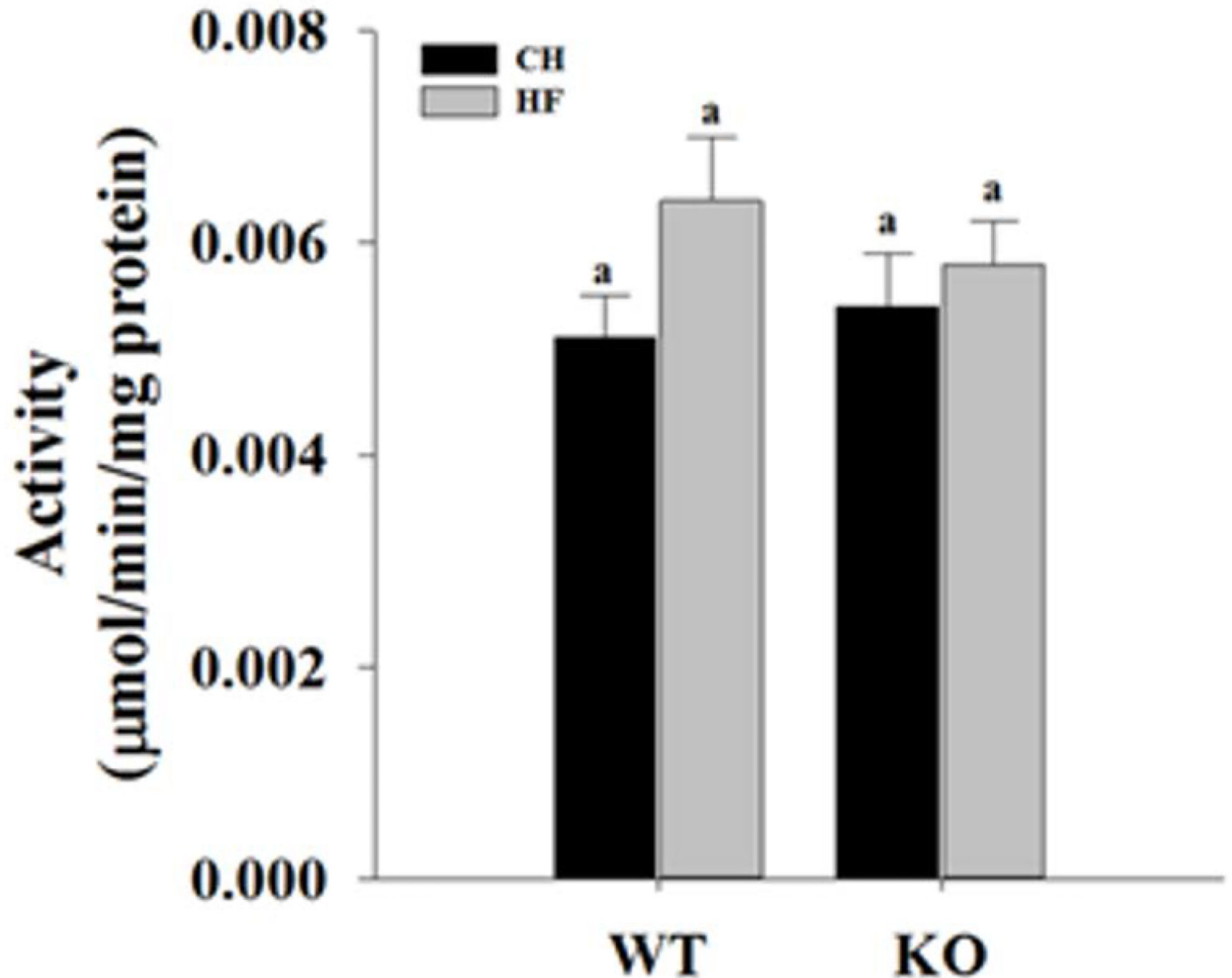

Figure 7.
The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA synthase) in the liver of wild-type (WT) and Shc knockout (KO) male mice fed on chow (CH) or high fat diet (HFD). All values are mean ± SEM (n = 8 – 11). Different letters are used to indicate a diet effect ($P < 0.05$) within a genotype. The symbol (*) indicated the difference between diets across genotypes ($P < 0.05$).

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Table 1

Effects of 7 days on chow (CH) or high fat diet (HFD) on body, epididymal fat and organs weights of wild-type and ShcKO (deletion of Shc) male mice.

Variable[1]	Wild-type (WT)		ShcKO		P value			
	CH	HFD	CH	HFD	Diet effect		Genotype effect	
	n = 10	n = 11	n = 8	n = 8	WT	ShcKO	CH	HFD
	Mean ± SEM				CH vs HFD	CH vs. HFD	WT vs. ShcKO	WT vs. ShcKO
Final BW	22.501 ± 0.570	26.356 ± 0.371	23.770 ± 0.322	26.476 ± 0.779	<0.01	<0.01	0.370	0.446
Change in BW (%)	2.063 ± 0.604	10.916 ± 1.720	1.645 ± 1.166	7.560 ± 1.084	<0.01	<0.01	0.370	0.075
Liver	1.104 ± 0.049	1.131 ± 0.030	1.001 ± 0.021	1.021 ± 0.047	0.972	0.703	0.038	0.027
Skeletal Muscle	1.049 ± 0.043	1.130 ± 0.029	1.078 ± 0.044	1.200 ± 0.039	0.131	0.057	0.327	0.080
Heart	0.113 ± 0.003	0.119 ± 0.003	0.114 ± 0.005	0.121 ± 0.005	0.181	0.329	0.449	0.355
Kidneys	0.310 ± 0.008	0.337 ± 0.008	0.329 ± 0.010	0.359 ± 0.019	0.029	0.179	0.078	0.158
Spleen	0.069 ± 0.003	0.074 ± 0.005	0.074 ± 0.005	0.076 ± 0.003	0.626	0.680	0.196	0.335
Ep.fat	0.356 ± 0.018	0.742 ± 0.045	0.324 ± 0.014	0.740 ± 0.057	<0.01	<0.01	0.096	0.490

[1] BW = body weight; Ep.fat = epididymal fat. All weights in g.

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Table 2

Energy expenditure (EE) and respiratory exchange ratio (RER) in the wild-type (WT) and Shc knockout (ShcKO) animals consuming either a control chow (CH) or high fat diet (HFD).

Variables[1]	Wild-type (WT)		Shc knockout (ShcKO)		P value			
					Diet effect		Genotype effect	
	CH	HFD	CH	HFD	WT	ShcKO	CH	HFD
	Mean ± SEM (n = 7)				CH vs. HFD	CH vs. HFD	WT vs. ShcKO	WT vs. ShcKO
24h								
EE (kcal/hr)	0.364 ± 0.008	0.443 ± 0.008	0.389 ± 0.019	0.487 ± 0.013	<0.001	<0.001	0.261	0.015
EE_{BW}	0.360 ± 0.009	0.440 ± 0.009 *	0.390 ± 0.009	0.490 ± 0.009 *	<0.001	<0.001	0.098	0.001
EE_{LBM}	0.370 ± 0.008	0.450 ± 0.008	0.380 ± 0.009	0.480 ± 0.009	<0.001	<0.001	0.304	0.003
RER	0.909 ± 0.026	0.808 ± 0.009	0.898 ± 0.021	0.802 ± 0.007	<0.001	<0.001	0.189	0.189
Light								
EE (kcal/hr)	0.326 ± 0.013	0.399 ± 0.015	0.347 ± 0.018	0.453 ± 0.008	<0.001	<0.001	0.012	0.012
EE_{BW}	0.330 ± 0.014	0.400 ± 0.014	0.350 ± 0.010	0.450 ± 0.010	0.003	<0.001	0.207	0.006
EE_{LBM}	0.340 ± 0.014	0.410 ± 0.014	0.340 ± 0.010	0.440 ± 0.010	0.004	<0.001	0.484	0.009
RER	0.886 ± 0.016	0.814 ± 0.005	0.864 ± 0.009	0.806 ± 0.005	<0.001	<0.001	0.138	0.138
Dark								
EE (kcal/hr)	0.403 ± 0.005	0.488 ± 0.009	0.431 ± 0.021	0.522 ± 0.020	<0.001	<0.001	0.053	0.053
EE_{BW}	0.400 ± 0.008 ¥	0.490 ± 0.008	0.430 ± 0.009 ¥	0.520 ± 0.009	<0.001	<0.001	0.038	0.015
EE_{LBM}	0.410 ± 0.014	0.500 ± 0.014	0.420 ± 0.009	0.510 ± 0.009	<0.001	<0.001	0.155	0.079
RER	0.932 ± 0.005	0.803 ± 0.004	0.932 ± 0.008	0.798 ± 0.004	<0.001	<0.001	0.62	0.62

[1] EE_{BW} = energy expenditure (kcal/hr) normalized for body weight; EE_{LBM} = energy expenditure (kcal/hr) normalized for lean body mass.

[*] Interaction between BW and genotype was significant ($P < 0.05$) and Johnson-Neyman technique was used to compare the experimental groups (WT HFD vs. ShcKO HFD). Johnson-Neyman Cutoffs: 18.12 BW 23.69 g.

[¥] Interaction between BW and genotype was significant ($P < 0.05$) and Johnson-Neyman technique was used to compare the experimental groups (WT CH vs. ShcKO CH). Johnson-Neyman Cutoffs: 8.108 BW 24.44 g.