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Conditional deletion of Hdac3 in osteoprogenitor cells attenuates diet-induced systemic metabolic dysfunction

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Abstract

Obesity is a major health epidemic in the United States and a leading cause of preventable diseases including type 2 diabetes. A growing body of evidence indicates that the skeleton influences whole body metabolism and suggests a new avenue for developing novel therapeutic agents, but the underlying mechanisms are not well understood. Here, it is demonstrated that conditional deletion of an epigenetic regulator, Hdac3, in osteoblast progenitor cells abrogates high fat diet-induced insulin resistance and hepatic steatosis. These Hdac3-deficient mice have reduced bone formation and lower circulating levels of total and undercarboxylated osteocalcin, coupled with decreased bone resorption activity. They also maintain lower body fat and fasting glucose levels on normal and high fat chow diets. The mechanisms by which Hdac3 controls systemic energy homeostasis from within osteoblasts have not yet been fully realized, but the current study suggests that it does not involve elevated levels of circulating osteocalcin. Thus, Hdac3 is a new player in the emerging paradigm that the skeleton influences systemic energy metabolism.

Keywords

Hdac3; insulin resistance; high fat diet; Bglap; hepatic steatosis

1. Introduction

Chronic illnesses linked to obesity, including cardiovascular disease, type 2 diabetes and cancer, are rising in association with the global spread of a western-style diet that is high in fats and carbohydrates (1–3). In the United States, type 2 diabetes mellitus affects over 23

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million Americans, approximately 80 to 90% of whom are obese, and causes significant morbidity and mortality at a cost of ~\$170 billion/year. Another 19 million Americans have prediabetes and have a high risk of developing type 2 diabetes (4). Diabetes arises from defects in both insulin secretion and insulin action, and frank diabetes occurs when insulin secretion is unable to overcome defects in insulin signaling. Other than lifestyle modification and weight loss, the current interventions used to treat prediabetes lack the ability to alter the natural history of the disease (5). Thus, there is an urgent clinical need for novel therapies to treat and prevent diabetes. Bone and osteoblast-derived factors were recently identified as endocrine regulators of metabolic homeostasis, and provide new opportunities to develop novel diabetes treatments. The skeleton is an insulin sensitive organ (6,7), and recent research suggests that bone may release factors that regulate systemic glucose and energy metabolism (8–11). For example, osteoblast suppression is linked to glucocorticoid-induced metabolic dysfunction and weight-gain in mice (8), and ablation of osteoblasts in adult mice causes insulin insensitivity (12).

Histone deacetylases (Hdacs) are important epigenetic modulators in both bone and energy metabolism, and may represent an avenue by which to target osteoblastic control of systemic energy regulation. Hdacs remove acetyl groups from lysine side chains in histones and other proteins (e.g., transcription factors) to epigenetically regulate gene transcription and affect bone development and maintenance. They also control inflammation, metabolic control, and transcriptional reprogramming (13,14). With regards to metabolism, small molecule inhibitors of Hdacs reduce body weight, glucose, and insulin levels in diabetic models by enhancing oxidative metabolism in adipose tissue and skeletal muscle (15). Hdac3 was implicated as a likely mediator of these phenotypes (16–20). Hdac3 regulates the metabolic activity of several tissues, including liver and skeletal muscle, two of the major organs controlling systematic energy metabolism, by altering gene expression networks (16,17,19–21). The role of Hdac3 in regulating energy homeostasis from bone and within osteoblasts has not yet been determined, but Hdac3 is essential for the proper development and maintenance of the skeleton, as shown by previous characterizations of the osteopenic skeletal phenotype of conditional knockout (CKO) mice lacking Hdac3 in osteoblast lineage cells (22,23). Interestingly, gross morphology at necropsy of the animals lacking Hdac3 in osteoprogenitor cells (via conditional deletion with *Osx1-Cre*) revealed a lean body composition with minimal visible abdominal fat. To better understand this phenomenon, we sought to characterize the metabolic phenotype of these Hdac3-insufficient mice and to determine whether any resulting characteristics would have a protective effect upon a metabolic challenge induced by long-term administration of a high fat diet.

2. Methods

2.1 Animal model and diets

All mice were maintained on a C57BL/6 background and genotyped as previously described (22,23). Hdac3^{fl/fl} mice were bred with mice expressing Cre recombinase under control of the osterix (*Osx1*) promoter, eventually yielding two groups of progeny that were studied: Hdac3-conditional knockout (Hdac3 CKO) animals (Hdac3^{fl/fl} : *Osx1-Cre*+) and control littermates (Hdac3^{+/+} and Hdac3^{fl/fl}, all *Osx1-Cre*-). In an additional set of experiments

designed to assess tissue specificity of the *Osx1*-Cre transgene, *Osx1*-Cre expressing mice were bred to Rosa26 reporter mice (R26R, B6.129S4-Gt(ROSA)26Sortm1Sor/J; The Jackson Laboratory, Strain #003474). Mice were weaned onto a normal diet (ND: 12% fat, 24% protein, 64% carbohydrate) or high fat diet (HFD: 60% fat, 20% protein, 20% carbohydrate; Research Diets D12492) by 4 weeks of age. Both males and females were tested; results were considered separately for each sex during data analysis. Mice on normal diets were sacrificed at 10 weeks of age by carbon dioxide asphyxiation, whereas mice receiving HFD were sacrificed at 26 weeks of age (i.e., following approximately 6 months on a HFD). Blood was collected via cardiac puncture and serum was stored at -80°C . For gene expression studies, tissues were immediately flash frozen in liquid nitrogen. For paraffin embedding, tissues were fixed for 24 hours in 10% formalin and transferred to 70% ethanol for storage. For studies of *lacZ* expression with the Rosa26 reporter mice, tissues were fixed in 0.2% glutaraldehyde at 4°C for 7 days (mineralized tissues) or 24 hours (soft tissues).

2.2 Body composition measurements

Body composition of individual mice was quantified via dual-energy X-ray absorptiometry (DXA) scanning (PIXImus, GE Healthcare) at a resolution of 0.18×0.18 mm pixels, permitting determination of lean mass, fat mass, and bone mineral density in a compartment-specific manner. Scans were performed between 7 to 10 wks of age (ND mice) or between 24 to 25 weeks of age (HFD mice). Mice were anesthetized by isoflurane inhalation during scanning. X-ray absorptiometry data from the posterior body (defined as a region of interest extending from the posterior aspect of the ribs to the feet, including the lumbar spine, pelvis, and hindquarters) were processed with manufacturer-supplied software. Body fat percentage for each mouse was normalized to the average of sex-matched littermate controls.

2.3 Fasting glucose, glucose and insulin tolerance tests

Fasting blood glucose levels were assessed in ND mice at 8 weeks of age and HFD mice at 24 weeks of age. For these studies, food was withdrawn for 6 hours, blood was obtained via needle puncture of the tail, and glucose levels were measured with a handheld glucometer. Insulin sensitivity was measured in the HFD group at 24 wks of age following a 4 hour fast by measuring glucose concentrations before (time 0) and 15, 30, 60, 90, and 120 minutes after an intraperitoneal bolus of insulin (0.50 mU/kg). Following a recovery period of at least 2 days, glucose tolerance was assessed in HFD mice after a 6 hour fast by measuring blood glucose concentrations before (time 0) and 15, 30, 60, 90, and 120 minutes after administration of an intraperitoneal bolus of glucose (1g/kg),

2.4 Metabolic activity

Ambulatory activity, food consumption, oxygen consumption (VO_2) and carbon dioxide production (VCO_2) of individual mice were monitored over a 48-hour period (24 hours fed and 24 hours fasted) using a comprehensive laboratory animal monitoring system equipped with photocells (CLAMS equipped with an Oxymax Open Circuit Calorimeter System; Columbus Instruments). Activity was analyzed for light and dark periods under both fed and fasted conditions. VO_2 and VCO_2 values were used to calculate the respiratory exchange

ratio (RER), and VO_2 and RER values were used to determine the metabolic rate (kcal/kg/h).

2.5 Liver histology

One lobe of each liver was dehydrated and paraffin embedded, after which thin (8 μm) sections were collected and prepared with hematoxylin and eosin staining according to standard procedures (24) for observation of tissue microstructure and morphology.

2.6 β -gal staining

To trace expression and activity of the *Osx1*-driven Cre with the Rosa26 reporter mouse, bone and liver specimens were incubated in 30% sucrose (dissolved in phosphate buffered saline (PBS), pH 7.4) at 4 deg C for 48 hours, frozen in embedding medium (Tissue-Tek O.C.T.), and sectioned at 8 micron thickness on a cryotome using the Cryofilm IIc system as previously described (25). Sections were incubated in X-gal reaction buffer (26) overnight, counterstained with eosin, dehydrated through graded ethanols and xylenes, and mounted with Permount medium on glass slides.

2.7 Gene expression

For mRNA analyses of tissue-level gene expression, humerus or liver samples from each mouse were homogenized in TRIzol using a high-speed disperser (Ultra-Turrax T25, IKA). RNA was extracted and purified from the ground tissue with TRIzol reagent (Invitrogen) and was reverse transcribed into cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen). Relative expression levels of mRNAs for *Hdac3* and Cre were measured by real-time PCR (qPCR). Reactions were performed using 37.5 ng of cDNA per 15 μl with Bio-Rad iQ SYBR Green Supermix and the Bio-Rad MyiQ Single Color Real-Time PCR Detection System. Transcript levels were normalized to the reference gene *Gapdh*. Gene expression levels were quantified using the $2^{-\text{Ct}}$ method (27); primer sequences were as follows: *Gapdh* F: 5'-GGGAAGCCCATCACCATCTT-3', *Gapdh* R: 5'-GCCTCACCCCATTTGATGTT-3', *Hdac3* F: 5'-CCCGCATCGAGAATCAGAAC-3', *Hdac3* R: 5'-TCAAAGATTGTCTGGCGGATCT-3'; Cre F: 5'-ACCAGCCAGCTATCAACTCG-3', Cre R: 5'-TTACATTGGTCCAGCCACC-3'.

2.8 Serum bone remodeling markers

Circulating levels of procollagen type 1 amino-terminal propeptide (P1NP; bone formation marker), tartrate-resistant acid phosphatase 5b (TRAcP5b; bone resorption marker) and total osteocalcin were measured with colorimetric assays (Rat/Mouse P1NP EIA #AC-33F1, Immunodiagnostic Systems, Fountain Hills AZ; Mouse TRAP ELISA #SB-TR103, Immunodiagnostic Systems, Fountain Hills AZ; Mouse Osteocalcin ELISA #60-1305, Immutopics International, San Clemente CA). Undercarboxylated osteocalcin was quantified according to previously described methods (8); osteocalcin-free serum used in the assay was a generous gift from Dr. Caren Gundberg. All samples were tested in duplicate within each assay.

2.9 Statistics

Statistics were performed with JMP 11 statistical analysis software (SAS Institute Inc., Cary, NC). Data were compared between groups within each experiment with Student's t-tests, where a significance level of $p = 0.05$ was used for all comparisons.

3. Results

3.1 Hdac3 CKO mice on a normal chow diet are leaner and have low fasting glucose levels

While defining the skeletal phenotype of Hdac3 CKO mice (22), we observed that the CKO animals had less abdominal fat compared to control littermates. To investigate this phenotype, posterior body composition (defined as a region of interest extending from the posterior aspect of the ribs to the feet, including the lumbar spine, pelvis, and hindquarters) was quantified by DXA in mice on a normal diet. Consistent with initial observations, average body fat percentage was 16% lower in the Hdac3 CKO as compared to sex-matched control littermates (Figure 1A). Fasting glucose levels were also reduced (−28% females, −29% males) in Hdac3 CKO as compared to control littermates on normal diets (Figure 1B).

3.2 Food intake and respiratory exchange ratio are unchanged in Hdac3 CKO mice on a normal diet

The lean body composition and low fasting glucose levels in Hdac3 CKO mice could result from many causes, including increased metabolic rate, decreased food consumption, or altered use of metabolic fuel sources. To better define the metabolic phenotype of the Hdac3 CKO mice on a normal diet, male animals were subjected to comprehensive monitoring in CLAMS units. Hdac3 CKO mice consumed as much normal chow on a per-bodyweight basis as control littermates (Figure 2A). Similarly, no differences were observed in respiratory exchange ratio ($p > 0.215$, Figure 2B). Daytime metabolic rate was slightly elevated during both fasting and fed conditions (+24% fed, +19% fasted) in the male Hdac3 CKO relative to control mice, but was not different between groups during nighttime fasting or fed conditions ($p > 0.122$) (Figure 2C). Thus, the lean phenotype and lower fasting glucose levels of Hdac3 CKO mice were not caused by improperly low food intake or selective fat metabolism, but were associated with a slight increase in daytime metabolic rate.

3.4 Hdac3 CKO mice maintain a leaner body phenotype on a long-term high fat diet

To test the robustness of the Hdac3 CKO mouse metabolic phenotype, animals were metabolically challenged with a HFD beginning at four weeks of age until sacrifice at 26 weeks of age. Differences in body size and fat abundance between control and Hdac3 CKO mice were evident upon examination (Figure 3A). Compared to their control littermates, female Hdac3 CKO mice maintained a leaner body composition on the HFD (Figure 3B). A male Hdac3 CKO mouse also demonstrated a lower body fat percentage as compared to male controls (Figure 3B; note DXA scans for other male Hdac3 CKO mice were lost during processing, and therefore male body composition data are presented for qualitative assessment only). Both male and female HFD-fed Hdac3 CKO mice tended to maintain lower fasting glucose levels as compared to control mice (−19% females, −25% males),

although differences in neither sex achieved statistical significance ($p = 0.169$ females, $p = 0.115$ males) (Figure 3C).

Mice were analyzed in metabolic chambers approximately 12 weeks after beginning the HFD. Hdac3 CKO mice consumed as much or more chow per kg of bodyweight as compared to control littermates (Figure 4A), indicating that the leaner body phenotype was not due to insufficient dietary intake. Respiratory exchange ratio was lower in HFD-fed mice as compared to mice fed a normal diet, but no differences in respiratory exchange ratio were found between control and Hdac3 CKO mice ($p > 0.448$, Figure 4B). Metabolic rate remained mildly elevated under varying conditions in both male and female Hdac3 CKO animals relative to control mice (Figure 4C). Thus, as noted in mice on normal diet, the leaner phenotype of Hdac3 CKO mice on HFD was associated with a higher metabolic rate but not lower food intake or preferential utilization of lipids.

3.5 Hdac3 CKO mice maintain insulin sensitivity on a prolonged high fat diet

Insulin and glucose sensitivity were assessed via insulin and glucose tolerance testing. Compared to control animals on a HFD, both male and female HFD-fed Hdac3 CKO mice demonstrated greater reductions in blood glucose after a bolus injection of insulin, indicating a preservation of insulin sensitivity while on a HFD (Figure 5A,B). Hdac3 CKO mice also quickly cleared an injected bolus of glucose (in a glucose tolerance test), showing a greater recovery towards baseline fasting glucose levels by the conclusion of the test as compared to control animals (Figure 5C,D). These results indicate that deletion of Hdac3 in osteoblast progenitors alters systemic metabolism, impeding HFD-induced weight gain and onset of metabolic dysfunction.

3.6 Hdac3 CKO mice prevent hepatic steatosis while on a high fat diet

One of the earliest changes observed in the progression to frank type 2 diabetes is the onset of hepatic insulin resistance (28), often related to hepatic steatosis, or fatty liver development. Livers from male and female mice fed a HFD were examined during tissue harvest and histologically prepared for observation of lipid abundance. Whereas control mice demonstrated severe liver hypertrophy and steatosis, both male and female Hdac3 CKO animals had relatively normal livers (Figure 6). Thus, conditional deletion of Hdac3 in osteoprogenitor cells negated HFD-induced development of hepatic hypertrophy and steatosis.

The striking liver phenotype of HFD-fed Hdac3 CKO mice led us to consider whether the metabolic phenotype might be influenced by aberrant expression of the *Osx1*-Cre transgene in hepatocytes. However, while cortical and trabecular bone from *Osx1*-Cre mice crossed to *Rosa26* reporter animals showed abundant blue staining (indicative of *lacZ* expression), no such staining was observed in the liver (Figure 7A), indicating that the *Osx1*-Cre transgene was not active in this location. Similarly, HFD-fed Hdac3 CKO mice demonstrated a robust Cre mRNA signal and a 50% reduction in gene expression of Hdac3 in the humerus, whereas Hdac3 expression was unaffected and little to no Cre signal was detected in the liver of the same HFD-fed Hdac3 CKO animals (Figure 7B). Thus, the metabolic phenotype

of bone-specific Hdac3 CKO mice was not likely due to aberrant expression of Cre in the liver.

3.7. Bone resorption and formation activity are suppressed in Hdac3 CKO mice on a high fat diet

Recent studies demonstrated that molecules released during bone remodeling could regulate systemic metabolism (7,9,29,30). Most notable is osteocalcin (bone gamma-carboxyglutamic acid-containing protein, Bglap), which is a bone-derived hormone that affects other metabolic tissues such as the pancreas and liver. Osteocalcin is produced by osteoblasts during bone formation and is stored in the bone matrix. During bone resorption, osteocalcin is decarboxylated and released from the bone matrix into the circulation (9,30). Circulating levels of total osteocalcin, undercarboxylated osteocalcin, P1NP (a bone formation marker), and TRAcP5b (a bone resorption / osteoclast number marker) were quantified in serum from Hdac3 CKO and control mice fed a HFD. Both P1NP and TRAcP5b levels were lower in the Hdac3 CKO females (–50% P1NP, –48% TRAcP5b; Figure 8A, B) as compared to control mice, reflecting the osteopenic phenotype of the Hdac3 CKO mice (22) and a balanced decrease in bone resorption. In accordance with lower bone formation and resorption rates, both total and undercarboxylated osteocalcin levels were lower (–43% total osteocalcin, –35% undercarboxylated osteocalcin) in Hdac3 CKO as compared to control female mice (Figure 8C,D). Thus, loss of Hdac3 in osteoprogenitor cells confers a protective metabolic phenotype with a higher sensitivity to insulin despite low bone remodeling activity.

4. Discussion

Recent studies showing that the skeleton can impact energy metabolism raise the intriguing possibility that bone-derived proteins might be able to treat or prevent type 2 diabetes. This study identified Hdac3 as a crucial factor in osteoblasts as its conditional deletion in the osteoprogenitor cell population protected systemic metabolism. Hdac3 CKO animals maintained lower body fat and fasting glucose levels on both normal and high fat diets. Importantly, this phenotype prevented insulin resistance and hepatic steatosis in mice on a HFD. These data provide new insights into the mechanistic link between the skeleton, namely osteoblasts, and whole body metabolism.

Hdac3 is essential for proper bone formation and osteoprogenitor cell survival (22–24,31). We previously showed that Hdac3 binds Runx2 and represses transcription of the minimal osteocalcin promoter (31). Osteocalcin (Bglap) is an osteoblast-derived hormone that, in its undercarboxylated form, regulates systemic metabolism by increasing insulin secretion in beta cells of the pancreas (9). HFDs promote ubiquitination and degradation of the insulin receptor in osteoblasts, ultimately leading to bone-specific insulin resistance, decreased bone resorption, and decreased activation of osteocalcin which contributes to the development of systemic insulin insensitivity (30). Osteocalcin knockout mice are obese, glucose intolerant and insulin resistant (11,32), and osteocalcin heterozygous animals are less sensitive to insulin while on a HFD as compared to wildtype mice (30). Conversely, increasing circulating levels of undercarboxylated osteocalcin reduces peripheral adiposity under HFD conditions (7,30,33). Given this well-defined role for bone-derived osteocalcin in systemic

metabolism and its repression by Hdac3, we quantified circulating levels of total and undercarboxylated osteocalcin in HFD-fed Hdac3 CKO animals as compared to control littermates. Circulating osteocalcin levels were lower in Hdac3 CKO mice as compared to controls, despite their ability to maintain greater insulin sensitivity on a HFD, likely due to a combined reduction in both bone formation and bone resorption. At present, it is unclear what role circulating osteocalcin plays in the metabolic physiology of these animals. Recent evidence suggests that other osteoblast-derived factors may synergize with osteocalcin to regulate energy homeostasis, as exogenous administration of undercarboxylated osteocalcin was not capable of fully rescuing insulin insensitivity in osteoblast-ablated adult mice (12). Our small sample sizes in some groups (particularly the male Hdac3 CKO mice on high fat diet) make it difficult to conclusively state the role of osteocalcin in the metabolic phenotype of Hdac3 CKO animals. Future studies of Hdac3 CKO mice will decipher the systemic, cellular, and epigenetic mechanisms by which Hdac3-depletion in osteoblast-lineage cells prevents HFD-induced glucose intolerance and insulin resistance.

The retention of insulin sensitivity in Hdac3 CKO mice fed a HFD was associated with resistance to hepatic steatosis. Hdac3 is crucial for lipid metabolism in the liver, where it interacts with the nuclear receptor co-repressors (NCoR1/2) (34) to regulate the expression of genes controlling lipid synthesis and sequestration. The association of Hdac3 with hepatocyte chromatin follows a circadian pattern, where Hdac3 is recruited during periods of fasting, promoting a closed chromatin conformation, and released during periods of active feeding, favoring chromatin with acetylated histone proteins and an open conformation (19). By crossing the *Osx1-Cre* mice to *Rosa26* reporter animals we verified that the *Osx1-Cre* transgene was not active in the liver, and we also did not detect *Cre* mRNA in livers of Hdac3 CKO mice on HFDs. It is notable that genetic deletion of Hdac3 in the liver (via adeno-associated virus (AAV)-*Cre*, driven by the hepatocytic thyroxine-binding globulin (*Tbg*) promoter) lowers fasting glucose levels and raises insulin sensitivity (20), but also promotes hepatic hypertrophy and steatosis due to increased hepatic lipid synthesis and sequestration (17,20), which is the opposite hepatic morphology of the *Osx1-Cre:Hdac3* CKO mice. Thus, the observation that bone-specific Hdac3 CKO animals are resistant to hepatic steatosis while on a HFD, coupled with a lack of an *Osx1-Cre* transgene signal in the liver, demonstrates that the metabolic phenotype of these *Osx1-Cre:Hdac3* CKO mice is not due to off-target *Cre* effects. The mechanisms behind the protective metabolic phenotype caused by deletion of Hdac3 in osteoprogenitor cells are not yet known, but may be related to the mild elevation of metabolic rate observed in both ND and HFD-fed mice, or synthesis and secretion of osteokines that could benefit liver health.

In conclusion, conditional deletion of Hdac3 in the osteoprogenitor cell population altered systemic metabolism to reduce body fat and fasting glucose levels. Importantly, this phenotype persisted during a metabolic challenge, as Hdac3 CKO animals maintained insulin sensitivity despite prolonged administration of a HFD. Hdac3 contributes to metabolic control and transcriptional reprogramming in other organs like the liver by virtue of its ability to bind transcription factors, deacetylate histones, and control gene expression (13,14). This metabolic role of Hdac3 in regard to bone cells has not yet been fully realized, but the current study suggests that Hdac3 likely has an important function in the newly emerging paradigm of skeletal regulation of energy metabolism.

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Highlights

- Osteoblastic expression of Hdac3 is required for normal bone maintenance
- Osteoprogenitor-specific Hdac3 CKO mice were leaner with low fasting glucose levels
- Hdac3 CKO mice maintained insulin sensitivity on a prolonged high fat diet
- Hdac3 CKO mice prevented hepatic steatosis on a prolonged high fat diet
- Hdac3 is involved in the paradigm of skeletal regulation of energy metabolism

Figure 1.

Body fat and fasting glucose levels are lower in Hdac3 CKO mice on a normal diet. A) Percent body fat (fat mass / total mass) was quantified by DXA in the posterior body (lumbar vertebrae, pelvis, and hindquarters) of mice on a normal diet between 7 to 10 weeks of age and normalized for each mouse to the sex-matched control group within each litter to reduce between-litter variability. Sample sizes: n = 5 female control, 7 female Hdac3 CKO, 4 male control, 5 male Hdac3 CKO. B) Blood glucose levels were measured at the conclusion of a 6 hour fast in control and Hdac3 CKO mice. Sample sizes: n = 7 female control, 4 female Hdac3 CKO, 6 male control, 5 male Hdac3 CKO. Means $\pm$ SEM are presented. * p < 0.05, # p < 0.07 vs. sex-matched control mice.

Figure 2.

Metabolic changes in Hdac3 CKO mice are not due to insufficient dietary intake or a change in fuel utilization. A) Food intake over a 24-hour period (day = 12 hours light, night = 12 hours dark) was measured in male mice on a normal diet at 8 weeks of age and normalized to body mass to account for baseline differences in animal size. B) Respiratory exchange ratio (RER) was calculated as the ratio of VCO_2/VO_2 over a 48-hour period (24 hours fed, 24 hours fasted). C) Metabolic rate was calculated over a 48-hour period (24 hours fed, 24 hours fasted). Sample sizes: $n = 6$ male control, $n = 4$ male Hdac3 CKO. Means $\pm$ SEM are presented. * $p < 0.05$ vs. sex-matched control mice.

Figure 3.

Body fat is lower in Hdac3 CKO mice on a prolonged HFD. A) Photographs of the HFD-fed control and Hdac3 CKO mice at 26 weeks of age. B) Percent body fat (fat mass / total mass) was quantified by dual x-ray absorptiometry (DXA) in the posterior body (lumbar vertebrae, pelvis, and hindquarters) of mice on a HFD between 24 to 25 weeks of age (i.e., after 21 to 22 weeks on a HFD) and normalized for each mouse to the sex-matched control group within each litter to reduce between-litter variability. Sample sizes: n = 7 female control, 4 female Hdac3 CKO, 8 male control, 1 male Hdac3 CKO. C) Blood glucose levels, measured at the conclusion of a 6-hour fast, in control and Hdac3 CKO mice on a HFD at 24 weeks of age (i.e., after 21 weeks on a HFD). Sample sizes: n = 8 female control, 5 female Hdac3 CKO, 10 male control, 2 male Hdac3 CKO. Means $\pm$ SEM are presented. * p $\leq$ 0.05 vs. sex-matched control mice.

Figure 4.

Metabolic changes in Hdac3 CKO mice on a HFD are not due to insufficient dietary intake or a change in fuel utilization. A) Food intake over a 24-hour period (day = 12 hours light, night = 12 hours dark) was measured in female and male mice on a HFD at 15 weeks of age (i.e., 12 weeks of HFD) and normalized to body mass to account for baseline differences in animal size. B) Respiratory exchange ratio (RER) was calculated as the ratio of VCO_2/VO_2 over a 48-hour period (24 hours fed, 24 hours fasted). C) Metabolic rate was calculated over a 48-hour period (24 hours fed, 24 hours fasted). Sample sizes: n = 3 female control, 5 female Hdac3 CKO, 2 male control, 2 male Hdac3 CKO. Means $\pm$ SEM are presented. * p 0.05, #p 0.09 vs. sex-matched control mice.

Figure 5.

Hdac3 CKO mice maintain greater insulin sensitivity on a HFD. In insulin tolerance tests, female (A) and male (B) Hdac3 CKO mice were injected with a bolus of insulin and blood glucose levels were measured for the next 2 hrs. For glucose tolerance tests, female (C) and male (D) Hdac3 CKO mice were injected bolus of glucose and blood glucose levels were measure at the indicated times over the next 2 hours. Sample sizes: n = 8 female control, 5 female Hdac3 CKO, 10 male control, 2 male Hdac3 CKO. Means $\pm$ SEM are presented. # p 0.08, * p 0.05.

Figure 6. Hdac3 CKO mice do not develop liver hypertrophy and steatosis while on a HFD. A) Macroscopic views of harvested livers. B–C) Microscopic images of H&E stained sections of livers from Hdac3 CKO and sex-matched control mice. (B: 100X, C: 400X)

Figure 7.

The *Osx1-Cre* transgene is not expressed in the liver. A) Bone and liver tissues from *Osx1-Cre+* and *R26R+; Osx1-Cre+* mice were incubated with X-gal reaction buffer where blue staining indicates *lacZ* activity. Images were taken at the indicated magnification. B) *Cre* and *Hdac3* transcript expression was measured in liver and bone tissue from male HFD-fed mice by qPCR. Sample sizes: $n = 2$ male control, 2 male *Hdac3* CKO. Means $\pm$ SEM are presented.

Figure 8.

Serum markers of bone turnover in control and Hdac3 CKO mice on a HFD. Circulating levels of the bone formation marker P1NP (A) and the osteoclast bone resorption marker TRAcP5b (B) were measured by ELISA. Sample sizes: n = 8 female control, 4 female Hdac3 CKO, 9 male control, 2 male Hdac3 CKO. C and D. Total osteocalcin (Ocn) and undercarboxylated osteocalcin (ucOcn) levels were measured by ELISA. Sample sizes: n = 8 female control, 5 female Hdac3 CKO, 8 male control, 2 male Hdac3 CKO. Means $\pm$ SEM are presented. * p < 0.05 vs. sex-matched control mice.