Diet-induced Lethality Due to Deletion of the Hdac3 Gene in Heart and Skeletal Muscle

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Many human diseases result from the influence of the nutritional environment on gene expression. The environment interacts with the genome by altering the epigenome, including covalent modification of nucleosomal histones. Here, we report a novel and dramatic influence of diet on the phenotype and survival of mice in which histone deacetylase 3 (Hdac3) is deleted postnatally in heart and skeletal muscle. Although embryonic deletion of myocardial Hdac3 causes major cardiomyopathy that reduces survival, we found that excision of Hdac3 in heart and muscle later in development leads to a much milder phenotype and does not reduce survival when mice are fed normal chow. Remarkably, upon switching to a high fat diet, the mice begin to die within weeks and display signs of severe hypertrophic cardiomyopathy and heart failure. Down-regulation of myocardial mitochondrial bioenergetic genes, specifically those involved in lipid metabolism, precedes the full development of cardiomyopathy, suggesting that HDAC3 is important in maintaining proper mitochondrial function. These data suggest that loss of the epigenomic modifier HDAC3 causes dietary lethality by compromising the ability of cardiac mitochondria to respond to changes of nutritional environment. In addition, this study provides a mouse model for diet-inducible heart failure.

Obesity is strongly associated with cardiac morbidity and mortality (1, 2). A growing body of evidence suggests that derangement of myocardial energy metabolism is a major factor in the pathogenesis of heart diseases (3–5). Metabolism of dietary lipids and carbohydrates is executed by metabolic enzymes that are subject to regulation by environmental factors at the transcriptional level through chromatin remodeling (6, 7). This includes changes in a variety of modifications on DNA and nucleosomal histones, such as histone acetylation (8).

Histone acetylation is governed by histone acetyltransferases and histone deacetylases (HDACs), which are recruited to specific genomic locations by DNA sequence-specific transcription factors (9). In general, histone acetylation is associated with active gene expression, and many transcriptional coactivators contain histone acetyltransferase activity. Conversely, histone deacetylation is associated with gene repression (10, 11). HDACs have been grouped into class I, class II, class IV, and sirtuins based on their sequence homology and catalytic mechanism (12, 13).

Class I HDACs exist in multiprotein nuclear corepressor complexes. HDAC1 and -2 are found in the NuRD (nucleosome remodeling and deacetylating) complex, the Sin3 complex, and the CoREST (corepressor for element-1-silencing transcription factor) complex (12). HDAC3, another class I HDAC, exists in a different set of complexes that contain NCoR (nuclear receptor corepressor) and/or SMRT (silencing mediator of retinoic and thyroid receptors), which function as activating subunits of the HDAC3 enzyme (14–17). Class II HDACs are dynamic in nucleocytoplasmic trafficking and are regulated by several kinase signaling pathways (12). Sirtuins are HDACs that are dependent upon NAD (13).

HDACs have critical roles in cardiac development and function, as revealed by many genetic animal models. For example, mice lacking HDAC5 and HDAC9, class II HDACs, are sensitized to cardiac stress signals and develop severe cardiac hypertrophy in response to pressure overload (18, 19), whereas mice lacking HDAC2, a class I HDAC, are resistant to hypertrophic stress (20). Deficiency of sirtuin 1, sirtuin 3, or sirtuin 7 also results in specific cardiac defects (21, 22). Inactivation of HDAC2 results in resistance to cardiac hypertrophy, whereas transgenic overexpression of HDAC2 induces cardiac hypertrophy (20). In addition, deletion of HDAC1 and HDAC2 together in heart results in cardiac embryonic defects and lethality (23).

A specific function for HDAC3 in cardiac development and function has been suggested by studies in which mid-gestational cardiac-specific deletion resulted in severe hypertrophic cardiomyopathy and lethality by the age of 4 months (24). Embryonic gene inactivation in these studies, however, prevents the discrimination of gestational versus postnatal functions of HDAC3 and obscures the potential contributions of gene-environment interactions. Given the availability of many small molecules that can regulate HDAC enzyme activity and

uretic peptide; BNP, B-type natriuretic peptide; ERR, estrogen-related receptor.
their potential value in treating various diseases, including cancer and heart disease, it is of interest and importance to define how postnatal manipulation of individual HDACs would affect the pathogenesis of diseases (25, 26).

Here, we report that mice in which HDAC3 is inactivated postnatally in both cardiac and skeletal muscle survive for over a year without obvious cardiac dysfunction when fed normal chow. However, these mice are exquisitely sensitive to their nutritive environment and exhibit severe hypertrophic cardiomyopathy and heart failure leading to death within weeks after switching to a high fat diet. Our work provides a mouse model for diet-inducible heart failure and suggests that HDAC3 is required for maintaining cardiac metabolic balance under lipid overload conditions.

EXPERIMENTAL PROCEDURES

Animals—To generate mice with conditional HDAC3 null allele, LoxP sites were inserted into Hdac3 gene to flank exons 4–7, which encode a large region required for the catalytic activity of the enzyme. The targeting vector was based on BAC clone from C57BL/6 background, and homologous recombination was performed in C57BL/6 embryonic stem cells. Efficient recombination was confirmed.3 The HDAC3fl/fl mice were cross-bred with MCK-Cre mice (from the Jackson Laboratory) to generate HDAC3fl/fl/MCK-Cre mice that were referred to as MCH3-KO in this study. The control mice were HDAC3fl/wt that were referred to as wild-type (WT) in this study. Mice were housed under the 12-h-light/12-h-dark cycles (lights on at 7 a.m., lights off at 7 p.m.). All mice used in this study were males. High fat diet (HFD) containing 60 kcal % fat was purchased from Research Diets Inc. (D12492i). For fasting studies, fasting was started before the dark cycle. All the animal care and use procedures followed the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Western Blot and Tissue Triglyceride Assay—For Western blot, tissues were lysed in RIPA buffer (1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.2, 2 mm EDTA) containing protease inhibitors (Roche Applied Science) and 0.5 mm DTT. Lysates containing 80 µg of total protein were resolved by Tris-glycine SDS-PAGE, transferred to PVDF membrane, and blotted with anti-HDAC3 antibody (AbCAM) and anti-HSP90 antibody (Cell Signaling). For tissue triglyceride assay, heart samples were homogenized in tissue lysis buffer (140 mm NaCl, 50 mm Tris and 1% Triton X-100, pH 8.0) first by Tissuemixer (Fisher) and then by TissueLyser (Qiagen) with steel beads. Triglyceride concentration in the lysates was then quantified using LiquiColor triglyceride assay kit (StanBio).

Quantitative PCR and Microarray—Total RNA was extracted from tissue samples using the TRIzol (Invitrogen) and High Pure RNA tissue kit (Roche Applied Science). Reverse transcription and quantitative PCR was performed with High Capacity reverse transcription kit, Power SYBR Green PCR MasterMix, and the PRISM 7500 instrument (Applied Biosystems) using absolute quantification method with standard curves. 36B4 (Arbp) was used as the housekeeping control. Primer sequences were as follows: HDAC3, TTGGTATCCCT-GGAGCTGCTT and GACCCGGTCATGAGCTGAGTA; ANP, GCTTCAAGGCCCCATATTGGAGCACAAA and TGACCTCAT-CCTCTACGGGTACCT; BNP, AATGGCCCAGAGACAGC-TCTTGAATTTTTCCAGCTGAGAT; and 36B4, CTTTGAGAAGCAGCTGTAGAT and AGCACTGCGACCTTAACAG.

For microarray (GEO accession number GSE31251), hearts were harvested from four MCH3-KO mice and four control mice at the age of 6 weeks. The total of eight RNA samples was individually processed with the Ambion WT expression kit and GeneChip WT terminal labeling and controls kit (Affymetrix) and hybridized to the Mouse Gene 1.0 ST array (Affymetrix). The array was then read by GCSS0000 laser scanner (Affymetrix), and microarray image analysis was carried out by Penn Microarray Core using Partek Genomics Suite software. Subsequent analysis was carried out using BioConductor. Data from the eight samples were subjected to background subtraction, quantile normalization, log2 transformation, and probe set summarization using the Robust Multichip Average algorithm (27). Only the probe sets that interrogate genes were retained, and those meant for quality control and normalization purposes were excluded from further analysis. The Significance Analysis of Microarrays procedure (28) was then used to obtain multiple test corrected q values for differential expression of genes between the hearts from MCH3-KO mice and control.

Genes up-regulated or down-regulated in MCH3-KO hearts were selected with a maximum q value of 0.05 and a minimum absolute fold-change of 1.2. Gene ontology analysis was performed in David Informatics Resources 6.7 with GO BP-FAT (29).

FIGURE 1. Efficient postnatal deletion of HDAC3 in cardiac and skeletal muscle by MCK-Cre. A, Western blot analysis of 4-month-old mice. Skeletal muscle is from quadriceps. WAT, white adipose tissue. B, quantitative PCR analysis of HDAC3 mRNA in 4-month-old mice. n = 3. Error bar, S.E., p < 0.05. C, quantitative PCR analysis of HDAC3 in mice at the age of 1 day and at 1, 3, and 6 weeks. n = 4. Error bar, S.E. WT, wild-type control; HDAC3fl/fl, HDAC3 flox/flox; KO (MCH3-KO), HDAC3 flox/flox with MCK-Cre.
Histology—For Oil Red O staining, 5-μm frozen sections were prepared from snap-frozen heart tissues and fixed in 10% buffered formalin for 3 min. The sections were then stained in 0.5% Oil Red O in propylene glycerol and then in hematoxylin for nucleus for 5 s. The procedures were performed by the Morphology Core in the Pennsylvania Center for Molecular Studies in Digestive and Liver Diseases. For H&E and trichrome staining, tissues were fixed in 4% paraformaldehyde for overnight, dehydrated, and paraffin-embedded, and 5-μm sections were prepared. Staining was performed according to standard procedures by Histology and Gene Expression Core in the Pennsylvania Cardiovascular Institute. Wheat germ agglutinin staining was performed on paraffin-embedded cross-sections of ventricles, using tetramethylrhodamine isothiocyanate-conjugated wheat germ agglutinin (20 μg/ml in PBS) (Sigma L5226). Quantification of myocyte diameter was performed in a blinded

FIGURE 2. Mild alterations in HDAC3-deficient heart on normal chow. A and B, Kaplan-Meier survival curves and body weight on normal chow. n = 12–15. C, gross picture of hearts at the age of 4 months. D, heart weight (HW) to tibia length (TL) ratio of 4-month-old mice on normal chow. n = 7–8. Error bar, S.E. *, p < 0.05. E, quantitative PCR analysis of 4-month-old mice for cardiac ANP and BNP, markers for heart failure. n = 3. Error bar, S.E. F, trichrome stain of hearts from 8-month-old mice.
manner using ImageJ software. Myocyte diameter for each heart was calculated based on six random \( \times 20 \) fields of view. The average diameter of the group was calculated from three WT and five KO hearts.

**Grip Strength**—The muscle strength in the forelimbs was measured with a grip meter (TSE; Bad Hamburg, Germany). Briefly, mice were trained to grasp a horizontal metal bar while being pulled by their tail, and the force was detected by a sensor. Ten measurements were determined for each mouse and averaged. The procedure was performed by Mouse Phenotyping, Physiology, and Metabolism Core in the Pennsylvania Diabetes and Endocrinology Research Center.

**Echocardiography**—The Vevo 770 ultrasound system (VisualSonics Inc) was used with an attached Integrated Rail System III for imaging acquisition. Mice were anesthetized with 1–2% isoflurane mixed with 100% oxygen through an inhalation tube. Core body temperature of the mouse was monitored by a rectal temperature probe and maintained at 37–38°C by a tensor lamp throughout the procedure. The chest area was depilated to improve contact for the ultrasound transducer. The electrocardiographic signal was obtained from the electrode platform. Two-dimensional images were obtained at 180 frames/s using a 30-MHz probe (RMV 707B, Visual Sonics) in the parasternal long and short axis views to guide M-mode analysis at the midventricular level. Left ventricular fractional shortening, ejection fraction, and wall dimensions were computed from M-mode measurements using the Vevo 770 standard measurement package. Image measurement and analysis were performed by researchers who were blinded to the mouse genotype.

**Statistics**—For all the analysis except microarray, Student's \( t \) test was performed to determine significance of differences between two groups with each containing multiple samples from different individual mice.

## RESULTS

**Efficient Postnatal Deletion of HDAC3 in Heart and Skeletal Muscle**—A previous study utilized myosin heavy chain \( \alpha \) (MHC\( \alpha \))-Cre to delete cardiac HDAC3 at approximately

![Figure 3](image_url)  
**FIGURE 3.** Echocardiography analysis of cardiac structure and function on 4-month-old mice on normal chow. **A**, cardiac structure data. **B**, contractile function data. \( n = 4–5 \). Error bar, S.E. *, \( p < 0.05 \). FS, left ventricular fractional shortening; EF, left ventricular ejection fraction; IVRT, isovolumic relaxation time; LVIDd, left ventricular diameter during diastole; LVIDs, left ventricular diameter during systole; LA, left atrium area; LVPW, left ventricular posterior wall thickness during diastole; RAVW, right ventricular anterior wall thickness; IVS, interventricular septal thickness during diastole; HR, heart rate.

![Figure 4](image_url)  
**FIGURE 4.** No significant alteration of function or morphology of HDAC3-deficient skeletal muscles. **A**, grip strength measurement on 8-month-old mice. \( n = 5 \). Extensor digitorum longus (EDL) and soleus (Sol) muscle weight to tibia length ratio of 8-month-old mice. \( n = 5 \). Error bar, S.E. C, H&E stain of gastrocnemius (Gastroc) muscle from 8-month-old mice.
TABLE 1
Genes differentially expressed in KO versus WT myocardium

| Process                      | Gene                          | Gene name                                                                 | Fold-change KO vs. WT | q value |
|------------------------------|-------------------------------|----------------------------------------------------------------------------|-----------------------|---------|
| Electron transport chain and ATP synthesis | Atp5g1 | ATP synthase, H^+ -transporting, mitochondrial Fe complex, subunit C1 | -1.55 | 0.022 |
|                              | Atp5g2 | ATP synthase, H^+ -transporting, mitochondrial Fe complex, subunit C2 | -1.31 | 0.036 |
|                              | Atp5l | ATP synthase, H^+ -transporting, mitochondrial Fe complex, subunit g | -1.28 | 0.025 |
|                              | Atp5s | ATP synthase, H^+ -transporting, mitochondrial Fe complex, subunit s | -1.21 | 0.044 |
|                              | Cox15 | COX15 homolog, cytochrome c oxidase assembly protein (yeast) | -1.30 | 0.024 |
|                              | Cox19 | COX19 cytochrome c oxidase assembly homolog (Saccharomyces cerevisiae) | -1.25 | 0.030 |
|                              | Ndufa5f | NADH dehydrogenase (ubiquinone) Fe-5 protein 1 | -1.30 | 0.021 |
|                              | Ndufa5f4 | NADH dehydrogenase (ubiquinone) 1x subcomplex, αf 4 | -1.26 | 0.027 |
|                              | Ndufa5f2 | NADH dehydrogenase (ubiquinone) Fe-5 protein 2 | -1.24 | 0.025 |
|                              | Ndufa5f8 | NADH dehydrogenase (ubiquinone) Fe-5 protein 8 | -1.23 | 0.029 |
|                              | Ndufa5f1 | NADH dehydrogenase (ubiquinone) flavoprotein 1 | -1.20 | 0.040 |
| TCA cycle                    | Aco1 | Acacitase 1 | -1.31 | 0.022 |
|                              | Fh1 | Fumarate hydratase 1 | -1.31 | 0.021 |
|                              | Idh1 | Isocitrate dehydrogenase 1 (NADP^+), soluble | -1.21 | 0.042 |
|                              | Mdh2 | Malate dehydrogenase 2, NAD (mitochondrial) | -1.29 | 0.024 |
|                              | Ogdh1 | Oxoglutathione dehydrogenase-like | -3.67 | 0.019 |
|                              | Phdha | Pyruvate dehydrogenase E1x1 | -1.21 | 0.027 |
|                              | Sdhb | Succinate dehydrogenase complex, subunit B, iron sulfur (lp) | -1.20 | 0.027 |
|                              | Sdhα | Succinate dehydrogenase complex, subunit A, flavoprotein (Fp) | -1.19 | 0.029 |
| Fatty acid metabolism        | Cpt1b | Carnitine palmitoyltransferase 1b, muscle | -1.49 | 0.020 |
|                              | Ehhadh | Enoyl-coenzyme A dehydrogenase, short chain | -1.30 | 0.020 |
|                              | Acad | Acyl-coenzyme A dehydrogenase, short chain | -1.30 | 0.027 |
|                              | Acad8 | Acyl-coenzyme A dehydrogenase family, member 8 | -1.36 | 0.021 |
|                              | Crot | Carnitine O-octanoyltransferase | -1.33 | 0.023 |
|                              | Peci | Peroxisomal Δ3,Δ2-enoyl-coenzyme A isomerase | -1.27 | 0.026 |
|                              | Acsl1 | Acyl-CoA synthetase short chain family member 1 | -1.38 | 0.021 |
|                              | Acsl5 | Acyl-CoA synthetase long-chain family member 5 | +1.34 | 0.026 |
|                              | Aco2 | Acyl-CoA thioesterase 2 | -1.55 | 0.020 |
| Glycerolipid metabolism      | Agpat9 | 1-Acetylglcopol-3-phosphate O-acetyltransferase 9 | -1.34 | 0.022 |
|                              | Akr1b10 | Aldo-keto reductase family 1, member B10 (aldose reductase) | -2.20 | 0.019 |
|                              | Dgat1 | Diacylglycerol O-acetyltransferase 1 | -1.58 | 0.020 |
|                              | Gk | Glycolipid kinase | -1.39 | 0.022 |
| Other metabolic process       | Ucp2 | Uncoupling protein 2 (mitochondrial, proton carrier) | +2.06 | 0.019 |
|                              | Ucp3 | Uncoupling protein 3 (mitochondrial, proton carrier) | +1.96 | 0.020 |
|                              | Glpdx | Glucose-6-phosphate dehydrogenase X-linked | +1.41 | 0.022 |
|                              | Me3 | Malic enzyme 3, NADP^+ -dependent, mitochondrial | -1.19 | 0.033 |
|                              | Pdk1 | Pyruvate dehydrogenase kinase, isoenzyme 1 | -1.26 | 0.027 |
|                              | Pfkfb1 | 6-Phosphofructo-2-kinase | -2.06 | 0.019 |
| Transcription regulation      | Ppargc1b | Peroxisome proliferative-activated receptor, γ, coactivator 1β | -1.26 | 0.044 |
|                              | Mixipl | MLX interacting protein-like | -1.53 | 0.020 |
|                              | Irx2 | Iron-responsive element-binding protein 2 | -1.21 | 0.029 |

embryonic day 9.5 (E9.5) (24, 30, 31). To conditionally delete HDAC3 in muscle tissues at later developmental stages, HDAC3^{−/−} mice on C57BL6 background (32) were bred to transgenic C57BL6 mice expressing Cre recombinase under the control of the muscle creatine kinase (MCK) promoter (33). This resulted in efficient deletion of HDAC3 from both heart and skeletal muscle of adult mice (Fig. 1, A and B). Consistent with a previous report (34), the recombination mediated by MCK-Cre did not occur until ~7 days after birth (Fig. 1C). To distinguish these mice from the mice in which HDAC3 was deleted using MHCα-Cre (24), the HDAC3^{−/−}/MCK-Cre mice will be referred to as MCH3-KO.

**MCH3-KO Mice Are Viable on Normal Chow**—MCH3-KO mice were born in Mendelian ratios and exhibited no obvious abnormalities when fed normal chow. They survived in equal numbers with control littersmates to at least the age of 14 months and had normal body weight (Fig. 2, A and B). This is in contrast to mice in which HDAC3 was deleted from the heart in mid-gestation, which resulted in lethality by the age of 4 months (24). Indeed, at 4 months of age, MCH3-KO hearts did not show obvious abnormalities by gross appearance (Fig. 2C), although heart weight to tibia length ratios were modestly increased, suggesting mild cardiac hypertrophy (Fig. 2D). Cardiac expression of ANP and BNP, markers of heart failure, were unchanged relative to control HDAC3^{−/−} mice (Fig. 2E). Histology of MCH3-KO hearts appeared normal at the age of 4 months (supplemental Fig. S1A) and electrocardiography (ECG) revealed no evidence of arrhythmia (supplemental Fig. S1B). By 8 months of age, MCH3-KO hearts exhibited only very mild fatty infiltration (Fig. 2F).

To functionally characterize hearts of the MCH3-KO mice, echocardiography was performed on 4-month-old mice. MCH3-KO hearts showed thickening of ventricular walls and interventricular septae, as well as enlarged atria, but cardiac contractile function was preserved (Fig. 3A). MCH3-KO hearts exhibited slightly enhanced systolic function, as evidenced by increased fraction shortening and ejection fraction (Fig. 3B). In addition, skeletal muscle from MCH3-KO mice had normal grip strength and weight (Fig. 4, A and B), with no overt histological abnormalities at 8 months (Fig. 4C). Thus, postnatal muscle-specific deletion of HDAC3 did not reduce survival when mice were fed normal chow. Cardiac contractile function was preserved with only mild cardiohypertrophy, and skeletal muscles show no obvious alterations.
Dietary Lethality in Mice Lacking Cardiac HDAC3

Abnormal Mitochondrial Gene Expression and Lipid Metabolism in HDAC3-deficient Myocardium—We performed microarray analysis of gene expression in myocardium from 6-week-old mice prior to the development of cardiac hypertrophy (supplemental Fig. S2 and supplemental Table S1). Gene ontology analysis showed that genes down-regulated in MCH3-KO myocardium were substantially enriched in mitochondrial bioenergetic processes and lipid metabolism, whereas genes up-regulated in MCH3-KO myocardium were enriched in immune responses (supplemental Fig. S3). Further pathway analysis showed that many differentially expressed genes encode enzymes that are involved in mitochondrial electron transport, ATP synthesis, tricarboxylic acid cycle, fatty acid metabolism, glycerolipid, and phospholipid metabolism (Table 1). Myocardial triglycerides were significantly elevated in the MCH3-KO hearts after 24 h of fasting (Fig. 5, A and B), consistent with abnormal lipid metabolism and bioenergetics in the HDAC3-deficient myocardium.

High Fat Diet Is Lethal to MCH3-KO Mice—The altered gene expression profiles of MCH3-KO hearts led us to test the hypothesis that MCH3-KO mice might be susceptible to cardiomyopathy when fed a HFD. Remarkably, when placed on a diet containing 60% fat by kilocalorie at 1 month of age, MCH3-KO mice began to die within weeks, with most dying between 3 and 4 months on the HFD and none surviving beyond the age of 6 months (Fig. 6A). By contrast, 100% of control HDAC3−/− mice were alive on the same dietary regimen (Fig. 6A). Until shortly before death, MCH3-KO and control HDAC3−/− mice gained similar amounts of weight (Fig. 6B and data not shown).

We next characterized cardiac morphology, architecture, and function of 4-month-old mice fed for 3 months on HFD. Hearts from HFD-fed MCH3-KO hearts were enlarged with significantly elevated heart weight to tibia length ratios when compared with control HDAC3−/− mice (Fig. 6, C and D). Myocardial expression of ANP and BNP was significantly elevated, suggesting the existence of heart failure (Fig. 6E). Moreover, cardiac histology and trichrome staining revealed widespread fibrosis throughout the ventricles (Fig. 6F). Wheat germ agglutinin staining of cross-sections of ventricles showed significantly increased cardiomyocyte diameter in MCH3-KO hearts, suggesting that myocyte hypertrophy, rather than hyperplasia, underlies the cardiohypertrophy (Fig. 6G).

Echocardiography demonstrated severe hypertrophic cardiomyopathy, with marked thickening of left and right ventricular walls and the interventricular septum (Fig. 7, A and C). MCH3-KO hearts at 4 months after HFD exhibited significant ventricular systolic dysfunction, as evidenced by impaired fractional shortening and ejection fraction (Fig. 7, B and C). Increased isovolumic relaxation time and the markedly enlarged left atrium suggest that diastolic function of the left ventricle was also substantially impaired (Fig. 7, A and B). Short axis echocardiographic video clips showed obvious motion abnormality of the ventricle wall (supplemental video clips A and B). Taken together, loss of cardiac HDAC3 in the presence of lipid overload and obesity caused severe cardiac contractile dysfunction and hypertrophic cardiomyopathy that lead to heart failure and lethality.

DISCUSSION

We have shown that MCH3-KO mice, with postnatal cardiac and skeletal muscle-specific deletion of HDAC3, do not exhibit significant myocardial dysfunction on normal chow, but develop severe hypertrophic cardiomyopathy, fibrosis, and heart failure leading to death when fed a high fat diet. These findings underscore the importance of gene-diet interactions that can powerfully impact cardiovascular health.

It is of great interest to compare our results with the report of Montgomery et al. (24) that described heart failure and complete lethality by the age of 4 months in normal chow-fed mice whose cardiac HDAC3 was deleted using αMHC-Cre that is active during mid-gestation (30, 31). By contrast, we find that mice with postnatal cardiac HDAC3 deletion survive normally on normal chow. In this fundamentally different model using MCK-Cre, which is not fully active until after birth (34), adult mice lacking HDAC3 are exquisitely sensitive to their nutritive environment, such that exposure to a high fat diet that is well tolerated by wild-type mice is lethal in the absence of HDAC3.

The more severe phenotype produced by earlier deletion suggests that HDAC3 plays an important role during cardiac development (24). Indeed, several critical cardiac development events occur between the time that the Cre recombinase is expressed in the two models. 1) The ventricular chamber starts...
to form at E8–9 and finishes at E10.5. 2) Atrial MHC quickly decreases from E8 to E9.5, such that MHC is restricted to the ventricles until birth when it is replaced by MHC. 3) Ventricular MHC starts to gradually decrease at E10.5 until birth, when its transcription starts to increase until it become predominant by day 7 after birth (35–38). Thyroid hormone is the most potent known regulator of MHC isoform transcription, acting through its nuclear receptors to up-regulate ventricular MHC and repress MHC (37, 39). Because HDAC3 is a component of NCoR and SMRT complex and is required for thyroid hormone receptor-mediated transcriptional repression (14, 16, 40, 41), it is conceivable that MHCα-Cre-mediated deletion of HDAC3 would disrupt the temporal expression patterns of ventricular MHCα and MHCβ during embryonic and early neonatal heart development, which explains the more severe cardiomyopathy in HDAC3/MHCα-Cre mice. In light of the promising therapeutic effects of class I HDAC inhibitors in treating various diseases, our study suggests that HDAC3 inhibition may not pose cardiac toxicity that is as detrimental as previously suggested by Montgomery et al. (24), as long as diets and obesity conditions of the patients are carefully controlled.

The high fat diet-dependent lethality and heart failure in MCH3-KO mice is unexpected, and it is likely to be explained on the basis of myocardial energy starvation when the heart is forced to use fatty acids as the major fuel source. The high energy demand of myocardium is supplied by mitochondrial bioenergetics machinery, including the fatty acid oxidation process, tricarboxylic acid cycle (TCA), and the oxidative phosphorylation pathway. Dysfunction of this bioenergetics system and the subsequent energy starvation was believed to be one of.

**FIGURE 6.** Dietary lipid overload induced severe cardiac defects in mice lacking cardiac HDAC3. A, Kaplan-Meier survival curves on high fat diet (HFD), n = 10 for WT and n = 12 for KO. B, KO mice have normal weight gain on HFD. C, gross picture of hearts from 4-month-old mice after feeding HFD for 3 months. D, heart weight (HW) to tibia length (TL) ratio of 4-month-old mice on HFD. n = 4–5. Error bar, S.E., p < 0.05. E, quantitative PCR analysis of myocardial ANP and BNP from 4-month-old mice. n = 4–5. Error bar, S.E., p < 0.05. F, trichrome stain of hearts from 4-month-old mice on HFD. G, wheat germ agglutinin (WGA) staining of cross-sections of ventricles from 4-month-old mice on HFD. Myocyte diameter was quantified on six random ×20 fields of view from each heart. n = 3 for WT and n = 5 for KO. Error bar, S.E., p < 0.05.
the major causative factors of cardiomyopathy and heart failure (5). Myocardial mitochondria undergo several metabolic derangements in the presence of obesity and excess lipids, including decreased oxidative capacity, reduced respiratory chain complexes, and increased mitochondrial uncoupling (42, 43). Coincidently, MCH3-KO mice show markedly decreased expression of many respiratory chain complexes, TCA cycle enzymes, and fatty acid oxidation enzymes but increased uncoupling proteins even when fed normal chow (Table 1). It is likely that diet-induced obesity and HDAC3 deficiency have additive effects in causing profound decrease in oxidative capacity of myocardial mitochondria, thus leading to significant energy starvation and heart failure. It is also possible that the mild phenotype of MCH3-KO on normal chow is because of metabolic compensation or cardiac remodeling and that such compensation failed to keep up with the higher energy demand because of obesity.

The balance between lipids and carbohydrates as the myocardial fuel sources and the flexibility on fuel selection is believed to be essential for maintaining normal cardiac function (44). Dietary lipid overload and increased availability of circulating fatty acids enhances cardiac reliance on fatty acids and reduced utilization of carbohydrates (4, 45, 46). The significance of this fuel switch has been debated; however, cardiac specific overexpression of peroxisome proliferator-activated receptor α in mice causes a similar fuel switch and results in hypertrophic cardiomyopathy, suggesting that increased cardiac fatty acid uptake causes deleterious effects (47). When cardiac fatty acid uptake exceeds the capacity of myocyte for complete fatty acid oxidation, intramyocardial lipid accumulation and elevated reactive oxygen species result in mitochondrial uncoupling and cardiac dysfunction (48). Alternatively, deficiency in mitochondrial fatty acid oxidation and increased reliance on glucose oxidation could also passively cause lipid accumulation and results in cardiac lipotoxicity (3, 4, 49). This notion was supported by the observation that mice deficient in peroxisome proliferator-activated receptor β/δ or PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1) showed the aforementioned pattern of fuel switch and developed cardiac dysfunction (50–52). In this study, myocardial HDAC3 deletion markedly reduced the expression of several genes encoding key enzymes in fatty acid oxidation and lipid metabolism, such as CPT-1b (carnitine palmitoyltransferase 1b) and enoyl-Coenzyme A hydratase (Table 1). MCH3-KO mice developed severe cardiomyopathy in the presence of dietary lipid overload, suggesting that efficient fatty acid oxidation is essential for maintaining the myocardial metabolic balance and normal contractile function, especially when fatty acids are the main fuel sources.

It is intriguing to consider the molecular mechanisms by which HDAC3 regulates expression of cardiac mitochondrial bioenergetics genes. Unexpectedly, the loss of HDAC3, whose most well understood function is in transcriptional repression, led to down-regulation of many cardiac metabolic genes. Future investigation is needed to address the underlying mechanism. Interestingly, many mitochondrial oxidative phosphorylation components and TCA cycle enzymes that were down-regulated in MCH3-KO myocardium are known targets of estrogen-related receptors (ERRs) and their potent PGC-1 family members, cytochrome c oxidase subunits, NADH dehydrogenase subcomplexes, and succinate dehydrogenase subunits (Table 1) (53, 56). Indeed, PGC-1β itself was found to be down-regulated in HDAC3-deficient heart (Table 1). Because deletion of either ERRα or ERRγ causes cardiac contractile defects (55, 57), loss of
HDAC3 may interfere with the ERR/PGC-1 signaling pathway in the pathogenesis of cardiomyopathy.

Given the association between obesity and heart failure in human (1–3), the MCH3-KO mouse model may be useful for clarifying the complex interplay between obesity and heart diseases. Moreover, HDAC inhibitors are being developed and clinically tested for the treatment of many diseases, especially various forms of cancer (58). The diet dependence of the ERR/PGC-1 signaling pathway suggests a potential role in cardiomyopathy.

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