MCB
Journals.ASM.org

Ablation of ALCAT1 Mitigates Hypertrophic Cardiomyopathy through Effects on Oxidative Stress and Mitophagy

Xiaolei Liu,[a,b] Benlan Ye,[b] Shane Miller,[b] Huijuan Yuan,[b] Hongxiu Zhang,[b] Liang Tian,[b] Jia Nie,[b] Rieko Imae,[c] Hiroyuki Arai,[c] Yuanjian Li,[a] Zeneng Cheng,[a] and Yuguang Shi[b]

Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha, China[a]; Department of Cellular and Molecular Physiology, Pennsylvania State University, College of Medicine, Hershey, Pennsylvania, USA[b]; and Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan[c]

Oxidative stress causes mitochondrial dysfunction and heart failure through unknown mechanisms. Cardiolipin (CL), a mitochondrial membrane phospholipid required for oxidative phosphorylation, plays a pivotal role in cardiac function. The onset of age-related heart diseases is characterized by aberrant CL acyl composition that is highly sensitive to oxidative damage, leading to CL peroxidation and mitochondrial dysfunction. Here we report a key role of ALCAT1, a lysocardiolipin acyltransferase that catalyzes the synthesis of CL with a high peroxidation index, in mitochondrial dysfunction associated with hypertrophic cardiomyopathy. We show that ALCAT1 expression was potently upregulated by the onset of hyperthyroid cardiomyopathy, leading to oxidative stress and mitochondrial dysfunction. Accordingly, overexpression of ALCAT1 in H9c2 cardiac cells caused severe oxidative stress, lipid peroxidation, and mitochondrial DNA (mtDNA) depletion. Conversely, ablation of ALCAT1 prevented the onset of T4-induced cardiomyopathy and cardiac dysfunction. ALCAT1 deficiency also mitigated oxidative stress, insulin resistance, and mitochondrial dysfunction by improving mitochondrial quality control through upregulation of PINK1, a mitochondrial GTPase required for mitochondrial autophagy. Together, these findings implicate a key role of ALCAT1 as the missing link between oxidative stress and mitochondrial dysfunction in the etiology of age-related heart diseases.

Cardiolipin (CL), a mitochondrial phospholipid initially identified in the heart, plays a pivotal role in maintaining normal cardiac function. In mammals, the biological function of CL is determined by the composition of its fatty acyl chains, which are dominated by linoleic acid (18:2), in metabolic tissues, such as heart, liver, and skeletal muscle. This unique acyl composition is believed to support the mitochondrial membrane proton gradient and activities of various mitochondrial enzymes and proteins (7, 36). Consequently, a loss of tetralinoleoyl CL (TLCL), the predominant species in the healthy mammalian heart, occurs during the onset of heart failure in both rodents and humans with dilated cardiomyopathy (39). CL is biosynthesized in a series of steps from phosphatidic acid. Newly synthesized CL must go through a remodeling process that involves phospholipases and acyltransferase or transacylases to incorporate linoleic acid into its fatty acyl chains. Accordingly, defective CL remodeling causes dilated cardiomyopathy in Barth syndrome, an X-linked genetic disorder characterized by TLCL deficiency, mitochondrial dysfunction, growth retardation, and neutropenia (7, 36). Furthermore, aberrant CL acyl composition from pathological CL remodeling has been implicated in the etiology of mitochondrial dysfunction associated with a host of pathophysiological conditions in aging and age-related diseases, including diabetes, obesity, cardiovascular diseases, cancer, and neurodegenerative diseases, all of which are characterized by oxidative stress, CL deficiency, enrichment of docosahexaenoic acid (DHA) in CL, and mitochondrial dysfunction (12, 24, 38, 40).

Cumulative evidence suggests that increased oxidative stress is involved in cardiac hypertrophy and dysfunction (9). Attenuation of oxidative stress prevents left ventricular remodeling and dysfunction (10). Oxidative stress is believed to be a principal causative factor of mitochondrial dysfunction and insulin resistance, which have been implicated in the pathogenesis of cardiomyopathy and cardiac dysfunction (44). Among all the phospholipids, CL is highly sensitive to oxidative damage of its double bonds by reactive oxygen species (ROS), a process known as lipid peroxidation, due to its content rich in polyunsaturated fatty acids and its location near the site of ROS production. Hence, CL is the only phospholipid in mitochondria that undergoes early oxidation during apoptosis (16). Although the molecular mechanisms underlying CL peroxidation remain elusive, it has been shown that increased DHA content in its fatty acyl chains renders CL highly sensitive to oxidative damage, leading to a vicious cycle of lipid peroxidation and mitochondrial dysfunction (13). Consequently, DHA content in CL increases in the aged heart concurrent with oxidative stress (21, 33). Furthermore, it has been noted that ischemia-reperfusion injury of cardiac myocytes causes CL peroxidation, leading to a significant decrease in cytochrome *c* oxidase activity, which can be restored only by exogenously added CL, not by other phospholipids or peroxidized CL (34). In particular, the onset of hyperthyroidism in human is associated with elevated oxidative stress and CL peroxidation, which can be mitigated by euthyroidism (2, 11). Hyperthyroidism stimulates CL remodeling in rodents, leading to a significant increase in polyunsaturated fatty acid and peroxidizability index (6, 11).

ALCAT1 is a lysocardiolipin acyltransferase that catalyzes pathological remodeling of CL in response to oxidative stress in diabetes, obesity, and cardiomyopathy, leading to ROS production, mitochondrial dysfunction, and insulin resistance (5, 6, 24).

Received 8 August 2012 Returned for modification 22 August 2012
Accepted 28 August 2012
Published ahead of print 4 September 2012
Address correspondence to Yuguang Shi, yus11@psu.edu.

doi:10.1128/MCB.01092-12

CL remodeling by ALCAT1 also leads to the synthesis of CL with aberrant acyl composition commonly found in heart diseases, including depletion of linoleic acid and enrichment of DHA content in CL. Consequently, targeted inactivation of ALCAT1 prevents the onset of diet-induced obesity and its related metabolic complications (24). Additionally, ALCAT1 deficiency also improves mitochondrial quality control and mitochondrial DNA (mtDNA) fidelity by preventing the mitochondrial fusion defect commonly associated with age-related metabolic diseases (23). Furthermore, CL remodeling by ALCAT1 is also implicated in the pathogenesis of mitochondrial dysfunction in cardiomyopathy, since ALCAT1 expression in the heart is significantly upregulated by hyperthyroidism and downregulated by hypothyroidism (6). Using mice with hyperthyroidism as a rodent model of oxidative stress and mitochondrial dysfunction, the present study investigated a role of ALCAT1 in mitochondrial dysfunction in hypertrophic cardiomyopathy associated with hyperthyroidism. We have demonstrated for the first time a key role of ALCAT1 in regulating the onset of thyroid hormone-induced cardiac hypertrophy through an effect on oxidative stress and mitochondrial autophagy.

MATERIALS AND METHODS

Animal care. Mice with targeted deletion of the ALCAT1 gene were generated as previously described (24). For induction of hyperthyroid cardiomyopathy, male ALCAT1 knockout (KO) mice and age-matched wild type (WT) control mice (8 to 9 weeks old) were divided into two groups. One group was treated with thyroid hormone (T4, CAS 51-48-9, 1 mg/kg body weight daily by intraperitoneal [i.p.] injection; Sigma) in the vehicle of 0.01N NaOH and 0.9% NaCl for 2 days (acute model) or 28 days (chronic model). Mice in the control group were injected with the same vehicle for the same duration. All animals were maintained in an environmentally controlled facility with a diurnal light cycle and free access to water and a standard rodent chow (Teklad 2018; Harland, Madison, WI). All experiments involving animals were performed in compliance with approved institutional animal care and use protocols according to NIH guidelines (NIH publication no. 86-23 [1985]).

Reagents. Antibodies used in the present studies included polyclonal antibodies to phospho-AKT (Thr308), AKT, phospho-S6K1 (Thr389), S6K1, phospho-S6 (Ser240/244), S6, phospho-glycogen synthase kinase 3 alpha/beta (phospho-Gsk3α/β) (Ser21/9), Gsk3α/β, phospho-4E-BP1 (Thr37/46), and 4E-BP1, all of which were purchased from Cell Signaling Technology (Danvers, MA). Anti-LC3 antibody was purchased from Novus Biologicals (Littleton, CO), anti-p62 antibody was from American Research Products Inc. (Belmont, MA), and L-thyroxine (T4), 3,3′,5-triiodo-L-thyronine sodium salt (T3), and monoclonal anti-Flag antibodies were from Sigma (St. Louis, MO). The PINK1 polyclonal antibody (A01) was purchased from Abnova (Walnut, CA). Donkey anti-rabbit IgG horseradish peroxidase-conjugated antibodies were purchased from GE Healthcare (Piscataway, NJ). Mouse antimyogenin antibodies (F5D) and mouse anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA).

Generation of H9c2 stable cell lines. H9c2 cells were transfected with a Flag-tagged ALCAT1 expression vector or empty vector as a control. The stable transfectants were screened for neomycin resistance by culturing in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with G418 (1 mg/ml), 10% heat-inactivated fetal calf serum, 1% penicillin, and streptomycin and maintained in 95% air plus 5% CO_2 at 37°C. H9c2 cell differentiation was induced by culturing in Dulbecco's modified Eagle's medium (DMEM)–Ham's F-12 (50/50 mix) supplemented with 1% heat-inactivated horse serum (Cellgro, Manassas, VA).

Quantitative PCR analysis. Quantitative PCR analyses were carried out as previously described (24). Analysis of mitochondrial copy number in vector and ALCAT1 overexpression H9c2 cells was carried out using mitochondrion-encoded NADH dehydrogenase 1 (ND1) as the mtDNA marker and cyclophilin A as a genomic marker. Quantitative real-time PCR (RT-PCR) analysis was carried out using Fast SYBR green master mix (Applied Biosystems, Foster City, CA) and mtDNA isolated from tissue samples or H9c2 cells pretreated with indicated doses of H_2O_2. The primer pairs used for the RT-PCR analysis of mtDNA include ND1 (forward, 5′-TGACCCATAGCCATAATATGATTT-3′; reverse, 5′-CTCTAC GTTAAACCCTGATACTAA-3′), and cyclophilin A (forward, 5′-ACAC GCCATAATGGCACTCC-3′; reverse, 5′-CAGTCTTGGCAGTGCAGA T-3′). For biomarker analysis, RT-PCR analysis were carried out using cDNAs isolated from tissue samples and primer pairs for β-MHC (forward, 5′-AGGGCGACCTCAACGAGAT-3′; reverse, 5′-CAGCAGACTC TGGAGGCTCTT-3′); BNP (forward, 5′-GCTGCTTTGGGCACAA GATAG-3′; reverse, 5′-GGAGCTCTTCCTACAACAACTT-3′), ANF (forward, 5′-GTGTACAGTGCGGTGTCCAA-3′; reverse, 5′-ACCTCAT CTTCTACCGGATC-3′), ACTA1 (forward, 5′-GTTCGCGCTCTCTCT CCTCA-3′; reverse, 5′-GCAACCACAGCACGATTGTC-3′), collagen I (forward, 5′-GAGCGGAGAGTACTGGATCG-3′; reverse, 5′-GTTCGG GCTGATGTACCAGT-3′), collagen III (forward, 5′-ACCAAAAGGTGA TGCTGGAC-3′; reverse, 5′-GACCTCGTGCTCCAGTTAGC-3′), DIO1 (forward, 5′-GAAGTGCAACATCTGGGATTTC-3′; reverse, 5′-GGAGG CAAAGTCATCTACGAG-3′), DIO2 (forward, 5′-CCTCCTAGATGCC TACAAACAG-3′; reverse, 5′-TGATTCAGGATTGGAGACGTG-3′), and GAPDH (forward, 5′-AATGGTGAAGGTCGGTGTG-3′; reverse, 5′-GTGGAGTCATACTGGAACATGTAG-3′).

Lipid peroxidation assay. Lipid peroxidation products in the cardiac ventricular tissue and H9c2 cells were quantified by measuring the level of thiobarbituric acid-reactive substances (TBARS) using a TBARS kit (catalog no. 10009055; Cayman Chemical Company) according to the manufacturer's instruction.

ROS measurements. The intracellular reactive oxygen species (ROS) generation from isolated mitochondria from H9c2 cells and from ventricular homogenates was detected indirectly by quantitatively measuring hydrogen peroxide (H_2O_2) as previously described (24).

EM analysis of mitochondrial morphology. Mitochondrial ultrastructure in mouse cardiomyocytes was evaluated using electron microscopy (EM). Heart samples were taken at the same site of the left ventricle from three mice in each group to prepare slides. Fragments of heart left ventricle were fixed in 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) with 0.05% $CaCl_2$ for 24 h. After washing in 0.1 M sodium cacodylate buffer, tissues were postfixed overnight in 1% OsO_4 and 0.1 M cacodylate buffer, dehydrated, and embedded in EMbed-812 resin (Electron Microscopy Sciences, Pennsylvania). The sections were stained with 2% uranyl acetate, followed by 0.4% lead citrate, and viewed with a Philips 400 electron microscope.

Echocardiography. After 28 days of thyroid hormone treatment, mice were anesthetized with intraperitoneal injection of sodium pentobarbital (75 μg/g body weight). Transthoracic echocardiography studies were performed using an Acuson Sequoia model 512 echocardiography system with a 14-MHz linear transducer (Siemens, Malvern, PA). The following parameters were measured: interventricular septal wall thickness at the end of diastole (IVSD), end-diastolic dimension of left ventricular (LVEDD), and posterior wall thickness at the end of diastole (LVPWD). Left ventricle fractional shortening (LVFS) was calculated using the formula [(LVEDD − LVESS)/LVEDD] × 100%, where LVESS is left ventricular end systolic stress.

Statistical analysis. Statistical comparisons were done using two-tailed nonpaired *t* tests to evaluate the difference between the two H9c2 cell lines and between WT and ALCAT1 KO mice. For comparisons among more groups, one-way analysis of variance was used, and values were considered statistically significant at *P* values of <0.05. Data were expressed as means ± standard errors of the means (SEM).

RESULTS

ALCAT1 regulates cardiac lipid peroxidation and mtDNA biogenesis. ALCAT1 expression in the heart was recently shown to be

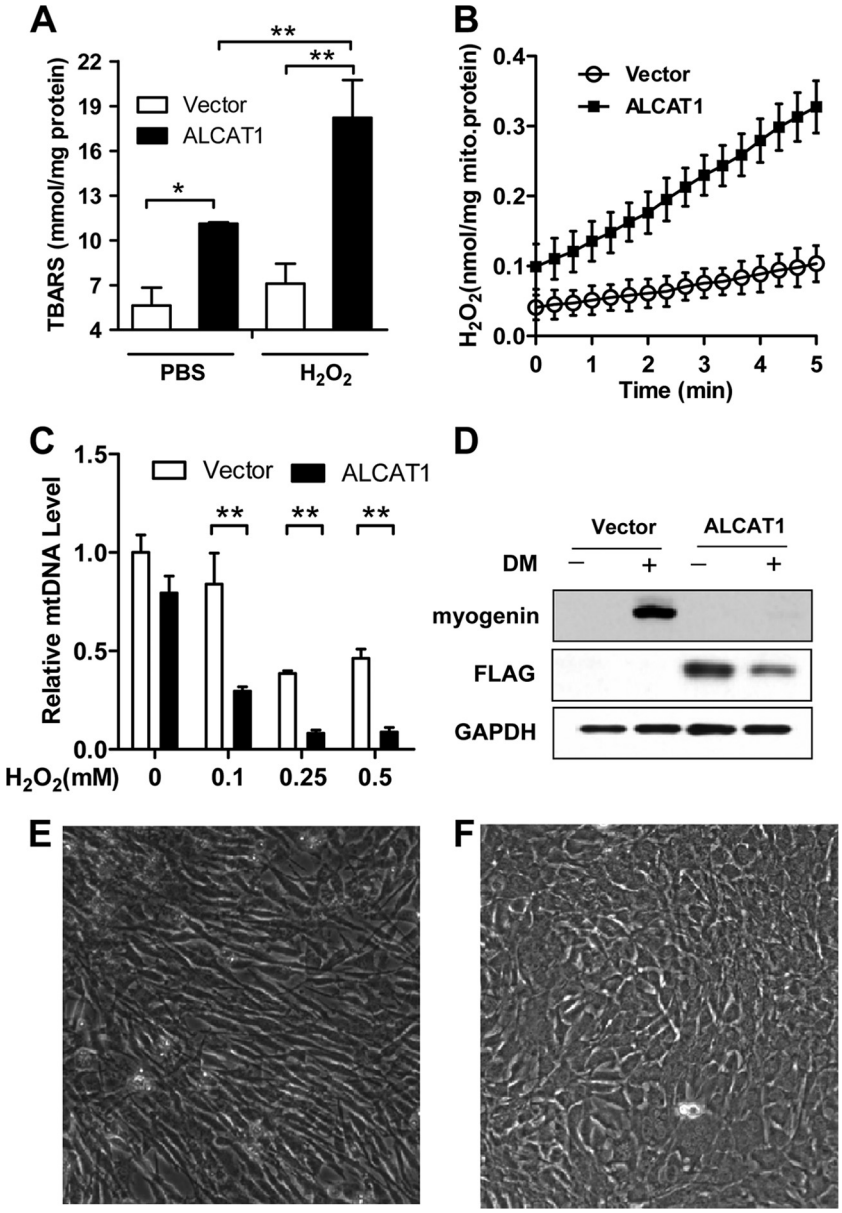

FIG 1 Overexpression of ALCAT1 causes oxidative stress, lipid peroxidation, and mtDNA depletion in H9c2 cardiomyocyte cells. H9c2 cells stably overexpressing ALCAT1 or vector control were analyzed for the intracellular level of *t*hiobar*b*ituric *a*cid *r*eactive *s*ubstances (TBARS), a byproduct of lipid peroxidation, in response to treatment with saline (phosphate-buffered saline [PBS]) or saline plus 2 mM H_2O_2 for 2 h ($n = 5$) (A), real-time production of H_2O_2 from isolated mitochondria ($n = 3$) (B), mtDNA copy number by real-time PCR analysis after treatment with indicated doses of H_2O_2 for 1 h ($n = 3$) (C), expression level of myogenin, a biomarker for H9c2 differentiation into cardiomyotubes (D), or morphology of H9c2 cells stably expressing vector control (E) or ALCAT1 (F) after induction of differentiation to cardiomyotubes by culturing in differentiation medium (DM). All data are expressed as means ± SEM; *, $P < 0.05$; **, $P < 0.01$.

upregulated by oxidative stress and by the onset of hyperthyroid cardiomyopathy (6, 24). Here, we tested the hypothesis that abnormal expression of ALCAT1 plays a causative role in the onset of cardiomyopathy. We first investigated a role of ALCAT1 overexpression in the H9c2 cardiac cell line on cellular morphology and mitochondrial function. To do so, we generated H9c2 cardiac cell lines stably transfected with Flag-tagged ALCAT1 cDNA (ALCAT1) or vector control. The mRNA expression level of ALCAT1 in the stable H9c2 cell line is only 3-fold higher than that in the vector control, which mimics the upregulated level of endogenous ALCAT1 induced by oxidative stress in isolated cardiomyocytes (24). Using the stable H9c2 cell lines as a cell-based model, we first analyzed the effect of ALCAT1 on lipid peroxidation and mitochondrial DNA (mtDNA) copy number. As showed in Fig. 1A, ALCAT1 overexpression significantly increased the intracellular level of *t*hiobar*b*ituric *a*cid *r*eactive *s*ubstances (TBARS), a by-product of lipid peroxidation, compared with that for the vector control. The production of TBARS was further exacerbated in response to treatment with H_2O_2, suggesting a causative role of ALCAT1 in oxidative stress in cardiomyopathy.

In order to uncover the direct effect of ALCAT1 on mitochondria ROS release, we next profiled the mitochondrial ROS produc-

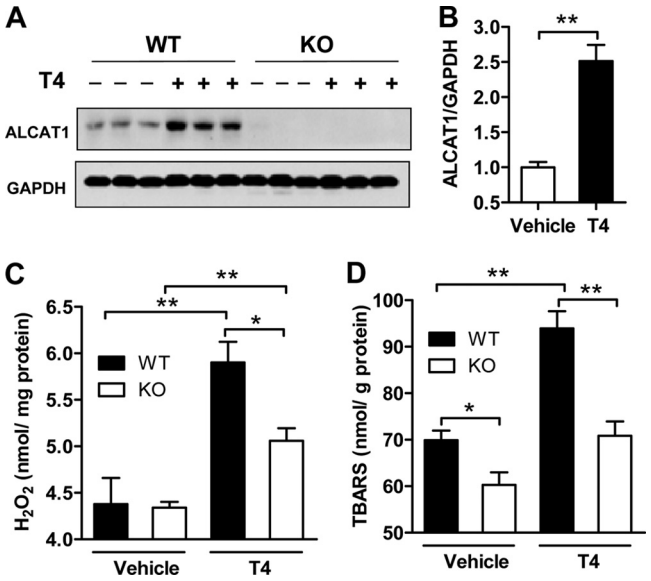

FIG 2 Up-regulated ALCAT1 expression plays a key role in oxidative stress associated with hyperthyroid cardiomyopathy. (A) Wild-type (WT) control mice and ALCAT1 knockout (KO) mice were treated with vehicle or T4 for 28 days, followed by analysis for ALCAT1 protein expression level in the heart by Western blot analysis using anti-ALCAT1 monoclonal antibodies. (B) Quantification of the ALCAT1 protein level in panel A. (C and D) Analysis of oxidative stress (C) or lipid peroxidation (D) of isolated heart tissue from WT and KO mice ($n = 5$). All data are expressed as means ± SEM; *, $P < 0.05$; **, $P < 0.01$.

tion rate in the ALCAT1-expressing H9c2 cell line and the vector control. The H_2O_2 production rate in isolated mitochondria from the H9c2 cells was analyzed at fixed time points after the initiation of the assay. The results show that ALCAT1 overexpression significantly increased the relative ROS release rates by 3.77-fold ($P < 0.01$) (Fig. 1B). Oxidative stress causes mtDNA instability, which has been implicated in mitochondrial dysfunction in age-related metabolic diseases (42). In further support of ALCAT1 as the primary source of oxidative stress, ALCAT1 overexpression led to mtDNA depletion in H9c2 cells in response to treatment with increasing doses of H_2O_2 (Fig. 1C). Additionally, ALCAT1 overexpression also caused hypertrophic growth of H9c2 cells (data not shown). Furthermore, ALCAT1 overexpression caused defective differentiation of H9c2 cells to cardiomyocytes (Fig. 1E and F), as evidenced by a lack of expression of myogenin, a key indicator of differentiated H9c2 cells (Fig. 1D).

ALCAT1 plays a key role in oxidative stress and lipid peroxidation in hyperthyroid cardiomyopathy. Hyperthyroidism causes oxidative stress and lipid peroxidation, which has been shown to stimulate the mRNA expression of ALCAT1 in the heart and in isolated cardiomyocytes (6, 24). To identify a role of ALCAT1 in the pathogenesis of mitochondrial dysfunction in cardiomyopathy, we next analyzed the effect of hyperthyroidism on the expression of the ALCAT1 protein by Western blot analysis using monoclonal anti-ALCAT1 antibodies recently developed in one of our labs (15). In support of a potential role of ALCAT1 in the etiology of hypertrophic cardiomyopathy, ALCAT1 protein expression in the heart was significantly upregulated by the onset of hyperthyroid cardiomyopathy (Fig. 2A; quantified in Fig. 2B). Using ALCAT1 knockout (KO) mice recently generated in our laboratory (24), we next determined a role for ALCAT1 in oxidative stress and lipid peroxidation associated with hyperthyroid cardiomyopathy. The onset of hyperthyroidism significantly increased levels of oxidative stress (Fig. 2C) and lipid peroxidation (Fig. 2D) in the hearts of hyperthyroid mice. Strikingly, targeted inactivation of ALCAT1 significantly mitigated cardiac oxidative stress (Fig. 2C) and lipid peroxidation (Fig. 2D) associated with hypertrophic cardiomyopathy, further implicating a potential causative role of ALCAT1 in mitochondrial dysfunction associated with cardiomyopathy.

Targeted inactivation of ALCAT1 mitigates hyperthyroid cardiomyopathy and ventricular fibrosis. Using the ALCAT1 KO mice, we next investigated a role of the enzyme in the onset of hyperthyroid cardiomyopathy. ALCAT1 KO mice and wild-type (WT) control mice were treated with thyroid hormone (T4) for 28 consecutive days to induce hypertrophic cardiomyopathy, followed by analysis of the effect of ALCAT1 deficiency on heart morphology, cardiac function, and mitochondrial dysfunction. As shown in Fig. 3A, hyperthyroidism caused cardiac hypertrophy in WT mice, as evidenced by marked increases in heart size (Fig. 3A) and the heart-weight-to-body-weight ratio (Fig. 4A). In contrast, ALCAT1 deficiency significantly attenuated the T4-induced cardiac hypertrophy and partially normalized the heart-weight-to-body-weight ratio (Fig. 3B and 4A), suggesting a key role of oxidative stress by ALCAT1 in the pathogenesis of cardiomyopathy induced by T4. These changes are not likely caused by differences between ALCAT1 KO mice and WT mice in the endogenous T3 level or the mRNA expression level of iodothyronine deiodinase 1 (DIO1) and 2 (DIO2), which catalyze the activation of thyroid hormone, because ALCAT1 deficiency did not significantly affect levels of T3 (24), body weight (Fig. 4C), and mRNA expression of DIO1 and DIO2 in multiple metabolic tissues (Fig. 4D). Consistent with these findings, there was no significant difference between the ALCAT1 KO mice and WT mice in heart morphology and the heart-weight-to-body-weight ratio under euthyroid conditions (Fig. 3A and B and 4A).

The development of cardiac hypertrophy often causes structural remodeling of the myocardium, leading to excessive accumulation of collagen type I and III fibers. Ventricular fibrosis is also a major risk factor for the development of heart failure and other cardiac complications (29). To provide further evidence of ALCAT1 as a mediator of cardiomyopathy, we next determined a role of ALCAT1 in regulating deposition of collagen types I and III in the left ventricle. Cardiac fibrosis was analyzed by Masson's trichrome staining of left ventricular sections from ALCAT1 KO mice and the WT mice. As showed in Fig. 3E (quantified in Fig. 3G), chronic hyperthyroidism caused severe ventricular fibrosis as a consequence of cardiomyopathy, as indicated by large areas of blue staining, compared with results for the vehicle control (Fig. 3C). In contrast, ALCAT1 deficiency significantly decreased the level of fibrosis caused by hyperthyroidism (Fig. 3F) compared with findings for the euthyroid condition (Fig. 3D), again implicating a key role of ALCAT1 in cardiac dysfunction caused by hyperthyroid cardiomyopathy. Consistent with the findings on ventricle fibrosis, hyperthyroidism also significantly increased mRNA expression of both collagens I and III in the WT mice but not in the ALCAT1 KO mice (Fig. 3H and I).

ALCAT1 deficiency prevents cardiac dysfunction associated with hypertrophic cardiomyopathy. To provide further evidence for a regulatory role of ALCAT1 in cardiac dysfunction in hyper-

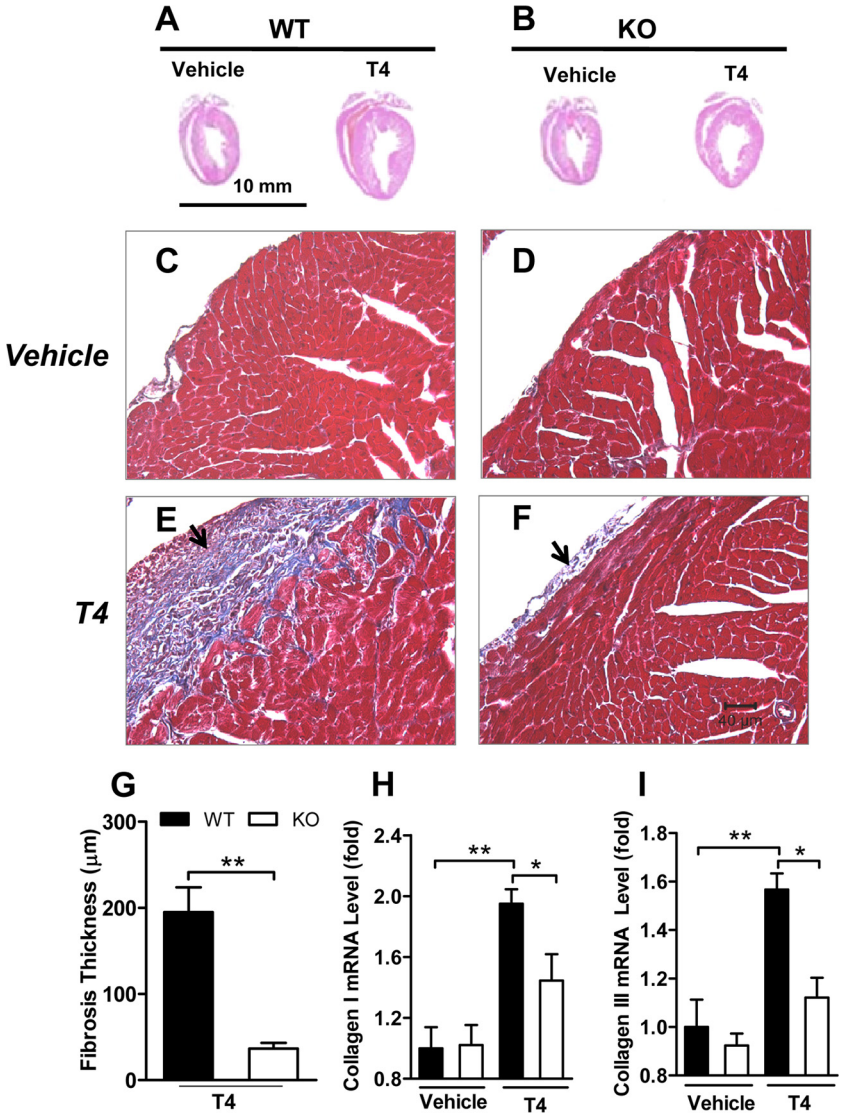

FIG 3 Targeted deletion of ALCAT1 mitigates hyperthyroid cardiomyopathy and ventricular fibrosis. WT and KO mice were treated with vehicle or T4 for 28 days and were analyzed for morphology of the whole heart sections by H&E staining (A and B), ventricular fibrosis by Masson's trichrome staining of the left ventricle from WT (C and E) or KO (D and F) mice treated with vehicle (C and D) or T4 (E and F), respectively. Fibrosis areas, which exhibit blue staining, are highlighted by arrows. (G) Quantification of left ventricle epicardial fibrosis thickness by morphometric analysis of Masson's trichrome-stained sections from WT and KO mice with hyperthyroidism. (H and I) RT-PCR analysis of mRNA expression levels of fibrosis biomarkers, including collagen I and collagen III, from the same group of mice as that used for panels C to F ($n = 5$). All data are expressed as means ± SEM; *, $P < 0.05$; **, $P < 0.01$.

trophic cardiomyopathy, we next determined the effect of ALCAT1 deficiency on echocardiographic parameters, including heart rate, interventricular septal defect (IVSD), left ventricular end diastolic diameter (LVEDD), left ventricular posterior wall dimensions (LVPWD), and left ventricular fractional shortening (LVFS). The onset of cardiomyopathy is associated with significant echocardiographic abnormalities, including arrhythmia (Fig. 4B) and increased levels of IVSD, LVEDD, and LVPWD in WT control mice (Fig. 5A to C). The development of left ventricular hypertrophy is believed to be an adaptive response to pressure overload, eventually leading to heart failure (25). In support of a causative role of ALCAT1 in cardiac dysfunction, ALCAT1 deficiency significantly attenuated these defects. Furthermore, the onset of hyperthyroid cardiomyopathy is associated with left ventricular dysfunction, as evidenced by a significant decrease in LVFS (Fig. 5D). Remarkably, ALCAT1 deficiency also significantly increased LVFS under both euthyroid and hyperthyroid conditions, implicating a key role of ALCAT1 in left ventricular systolic dysfunction.

Ablation of ALCAT1 mitigates T4-induced hypertrophic growth of cardiomyocytes. Cardiac hypertrophy is characterized by an increased size of terminally differentiated cardiomyocytes as an adaptive response to various physiological and pathophysiological stimuli. Since ALCAT1 overexpression caused hypertrophic growth of H9c2 cells, we next determined whether ALCAT1 deficiency would prevent T4-induced hypertrophic growth of cardiomyocytes by morphometric analysis of cardiomyocytes in sections of left ventricles stained with hematoxylin and eosin (H&E).

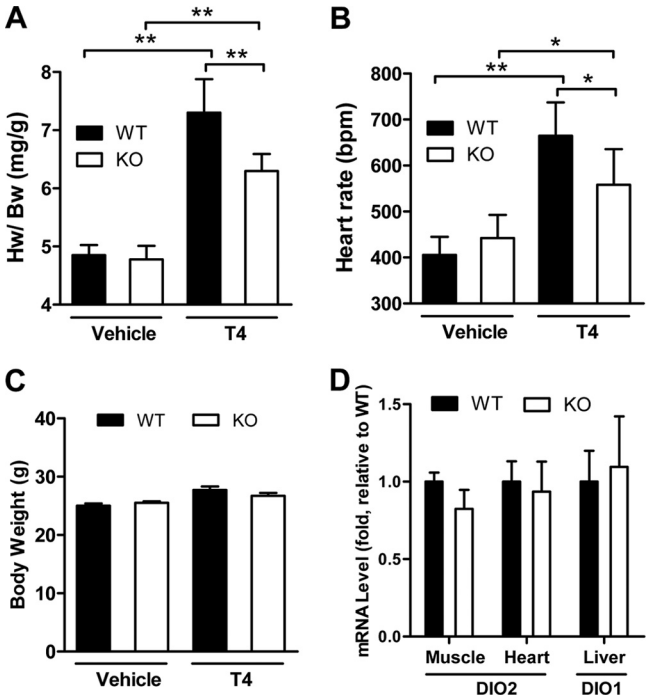

FIG 4 Ablation of ALCAT1 significantly attenuated cardiac hypertrophy and arrhythmia associated with hyperthyroidism. (A to D) WT and KO mice were treated with vehicle or T4 for 28 days and were analyzed for changes of heart-weight (Hw)-to-body-weight (Bw) ratio (A), heart rate (in beats per min [bpm]) (B), body weight (C), or mRNA expression levels of iodothyronine deiodinases 1 (DIO1) and 2 (DIO2) from heart, liver, and skeletal muscle by RT-PCR analysis. Means ± SEM ($n = 6$ to 8); *, $P < 0.05$; **, $P < 0.01$.

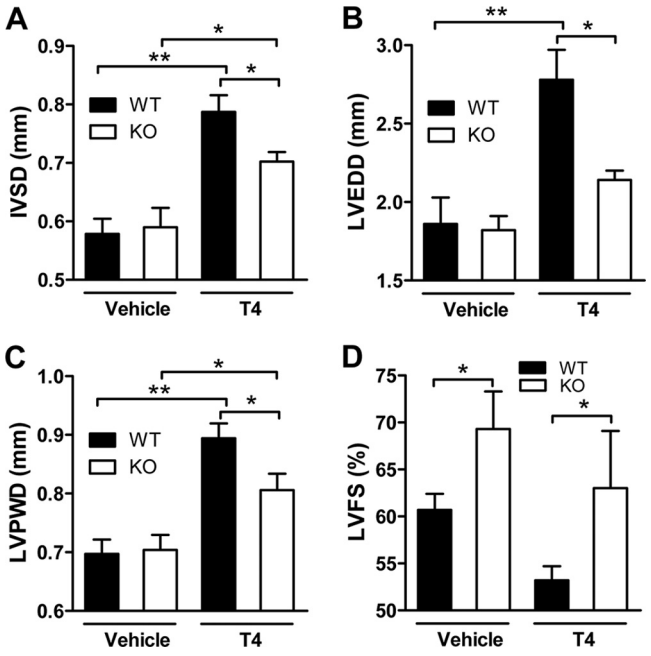

FIG 5 ALCAT1 deficiency prevents cardiac dysfunction associated with hypertrophic cardiomyopathy. (A to D) WT and KO mice used for Fig. 4 were analyzed for changes in echocardiographic parameters, including interventricular septal defect (IVSD), left ventricular end diastolic diameter (LVEDD), left ventricular posterior wall dimensions (LVPWD), and left ventricular fractional shortening (LVFS), to evaluate left ventricle hypertrophy and dysfunction. Means ± SEM ($n = 6$ to 8); *, $P<0.05$; **, $P<0.01$.

As shown in Fig. 6, there was no significant difference between ALCAT1 KO mice and WT mice in cardiomyocyte size under euthyroid conditions. Consistent with hypertrophic growth, the onset of cardiomyopathy significantly increased the size of cardiomyocytes in WT mice (Fig. 6C) relative to findings for the euthyroid control (Fig. 6A). In contrast, the hypertrophic growth of cardiomyocytes was significantly attenuated in ALCAT1 KO mice (Fig. 6D) relative to results for the euthyroid condition (Fig. 6B), compared with WT mice with hyperthyroidism. These observations are further supported by a shift in cardiomyocyte size distribution (Fig. 6E) and a significant increase in mean cardiomyocyte size (Fig. 6F) in WT control mice relative to results for ALCAT1-deficient mice.

ALCAT1 inactivation normalizes the expression of biomarkers associated with cardiomyopathy. Persistent hypertrophy induced by pathological conditions, such as hyperthyroidism, eventually leads to heart failure, a major cause of death in industrialized nations. An elevated brain natriuretic peptide (BNP) is a specific test indicative of heart failure (19). BNP is a cardiac neurohormone specifically secreted from the cardiac ventricles in response to ventricular volume expansion, pressure overload, and increased wall tension. The onset of hypertrophic cardiomyopathy is also associated with induction of a subset of fetal genes, including β-myosin heavy chain (β-MHC), atrial natriuretic factor (ANF), and skeletal muscle α-actin (ACTA1) (28). To identify a role of ALCAT1 in the progression of cardiac hypertrophy, we next analyzed mRNA expression of these hallmarks of cardiac hypertrophy and heart failure. Chronic hyperthyroidism significantly increased mRNA expression of all hypertrophic biomarkers in WT mice (Fig. 7). Consistent with a lack of hypertrophic cardiomyopathy in ALCAT1 KO mice, ALCAT1 deficiency significantly attenuated the expression of the biomarkers compared with that in WT mice. In contrast, there was no significant difference in mRNA expression of these biomarkers between WT and ALCAT1 KO mice when they were treated with vehicle.

ALCAT1 deficiency prevents mitochondrial swelling and defective mitophagy. Selective mitochondrial autophagy, also known as mitophagy, contributes to the maintenance of mitochondrial quality by eliminating damaged mitochondria from oxidative stress. Mitophagy also plays an essential role in maintaining mitochondrial quantity and quality by reducing mitochondrial ROS production and mutation of mtDNA (20). Hence, cardiac-specific ablation of autophagy causes cardiomyopathy (27). Using electron microscopic (EM) analysis of ventricle sections, we next determined a role of ALCAT1 in mitochondrial morphology and autophagy associated with hyperthyroid cardiomyopathy. Consistent with severe oxidative stress in hyperthyroidism, the onset of hyperthyroid cardiomyopathy is associated with mitochondrial swelling, disorganized cristae, and abnormal ultrastructure of the mitochondria in WT mice (Fig. 8A, highlighted in 8B). In contrast, these damages were largely mitigated by ALCAT1 deficiency (Fig. 8C, highlighted in 8D). As a compensatory response to damaged mitochondria, the expression level of cardiac LC3-II, an autophagosome marker, was significantly upregulated by hyperthyroidism in WT mice (Fig. 8E, quantified in 8F). Furthermore, the expression of the p62 protein, which is negatively correlated with autophagy, was significantly lower in WT mice.

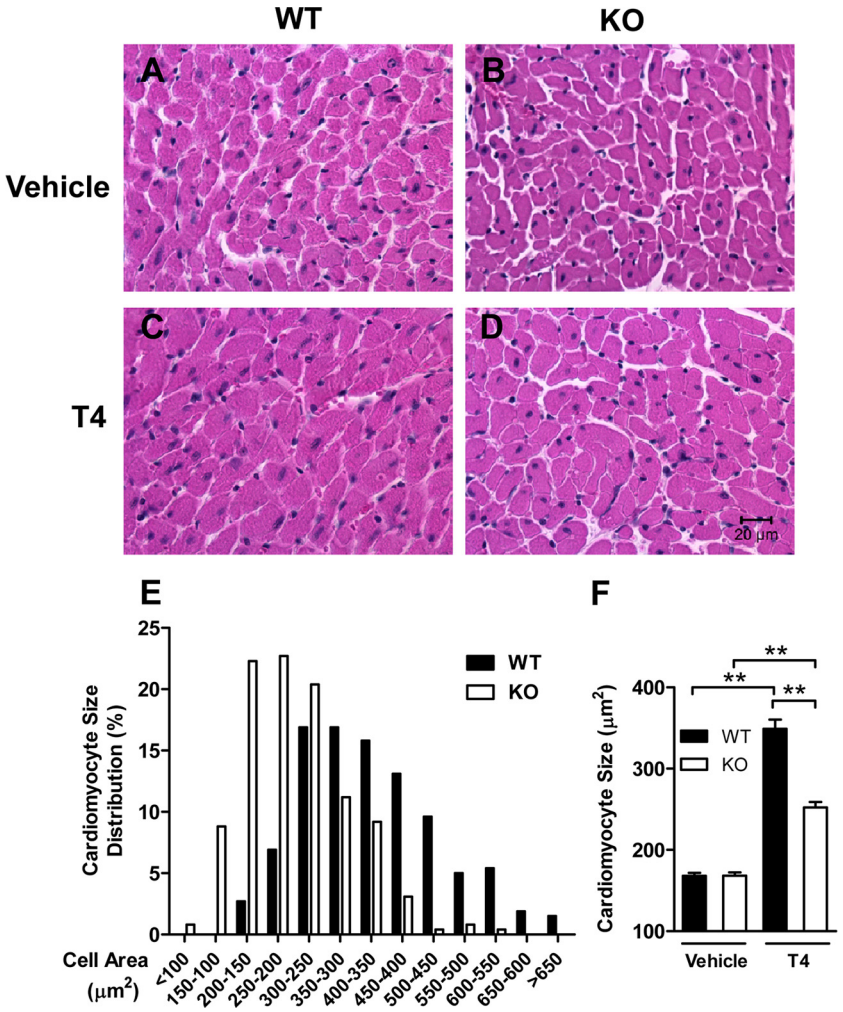

FIG 6 Ablation of ALCAT1 mitigates T4-induced hypertrophy of cardiomyocytes. WT and KO mice were treated with vehicle or T4 for 28 days and were analyzed for cardiomyocyte morphology by H&E staining (A to D), cardiomyocyte size distribution (E), and cardiomyocyte size (quantitative analysis) (F). Means ± SEM ($n = 250$); **, $P < 0.01$.

PTEN-induced putative kinase 1 (PINK1) is a serine/threonine protein kinase that protects against mitochondrial dysfunction during cellular stress by promoting the clearance of damaged mitochondria via mitophagy. PINK1 deficiency causes oxidative stress and mitophagy, leading to cardiomyopathy in mice (3, 8, 37). In further support of a causative role of ALCAT1 in mitochondrial dysfunction, cardiac PINK1 protein expression was significantly upregulated by ALCAT1 deficiency (Fig. 8E, quantified in 8G), which is consistent with decreased levels of oxidative stress and mitophagy in the ALCAT1 KO mice. In further support of these observations, the number of autophagic mitochondria was significantly higher in WT mice in response to the onset of hyperthyroidism than in the ALCAT1 KO mice (Fig. 9). These findings suggest that oxidative stress elicited by ALCAT1 plays a key role in mitochondrial dysfunction and mitophagy associated with hyperthyroid cardiomyopathy.

Oxidative stress by ALCAT1 causes insulin resistance and attenuates Akt-mTOR signaling. Hyperthyroidism leads to cardiac hypertrophy by stimulating protein synthesis in the cardiomyocytes. To identify molecular mechanisms underlying a role of ALCAT1 in hypertrophic growth, we analyzed the effects of ALCAT1 overexpression and deficiency on signal transduction pathways involved in cellular growth and proliferation, including phosphorylation of Akt, Erk, S6K1, and 4E-BP1, in H9c2 cells and in mice with hyperthyroidism. It has been reported that increased ROS levels lead to insulin resistance, which plays a causative role in cardiac dysfunction (44). As shown in Fig. 10A, treatment of H9c2 cells with insulin dose-dependently stimulated phosphorylation of Akt and Erk in vector control cells. In contrast, ALCAT1 overexpression significantly impaired insulin-stimulated Akt and Erk phosphorylation, suggesting severe insulin resistance (Fig. 10A). Furthermore, chronic oxidative stress by ALCAT1 also dramatically impaired T3-induced activation of Akt, S6K1, and 4E-BP1 signaling pathways in H9c2 cells (Fig. 10B). In support of the findings in H9c2 cells, acute hyperthyroidism significantly stimulated cardiac Akt-mTOR phosphorylation in both ALCAT1 KO mice and WT mice (Fig. 10C). However, chronic hyperthyroidism caused significant downregulation of Akt and mTOR signaling pathways, as evidenced by significantly lower phosphorylation of Akt and 4E-BP1 in the heart tissue of WT mice (Fig. 10D). Hyper-

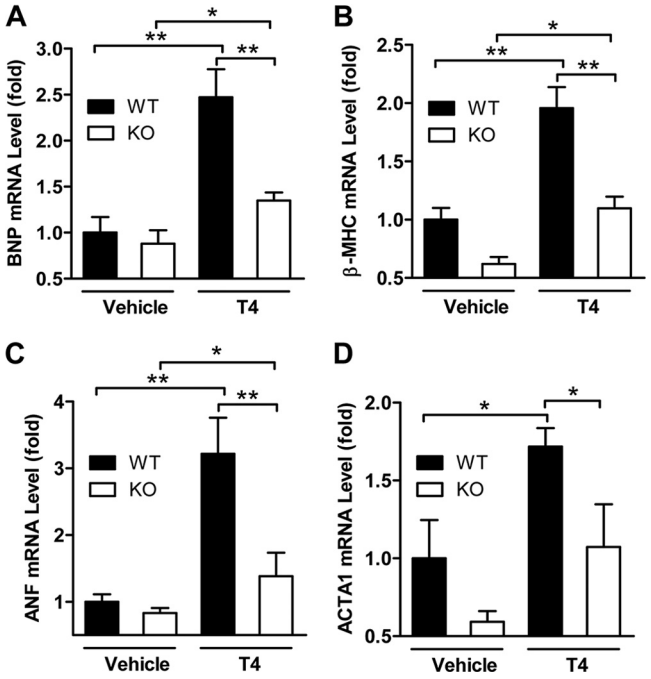

FIG 7 Targeted deletion of ALCAT1 normalizes mRNA expression of biomarkers associated with cardiomyopathy. (A to D) WT and KO mice were treated with vehicle or T4 treatment for 28 days, followed by RT-PCR analysis of mRNA expression levels of key biomarkers associated with left ventricular hypertrophy and heart failure, including brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), atrial natriuretic factor (ANF), and skeletal muscle α-actin (ACTA1). Means ± SEM ($n = 6$ to 8); *, $P < 0.05$; **, $P < 0.01$.

thyroidism also inhibited phosphorylation of Gsk3, whose deficiency causes cardiomyopathy (18). Consistent with a lack of cardiac hypertrophy in ALCAT1 KO mice, ALCAT1 deficiency arrested the downregulation of phosphorylation of these signaling proteins induced by chronic hyperthyroidism (Fig. 10D). These results are consistent with previous reports that acute treatment with thyroid hormone increases Akt-mTOR activation, whereas chronic hyperthyroidism leads to downregulation of Akt-mTOR signaling as a compensatory response to cardiomyopathy (17, 41).

DISCUSSION

CL remodeling plays an important role in regulating function of the heart, a tissue with perpetually high energy demand from oxidative phosphorylation (40). The biological function of CL is determined by the structure of its fatty acyl chains. In the heart, functional CL is enriched with linoleic acid, which is important in supporting the activities of mitochondrial enzymes and proteins (7). Consequently, a loss of TLCL, the signature CL in the heart, as a consequence of pathological remodeling, has been implicated in the etiology of cardiomyopathy and heart failure (38, 40). However, the enzyme(s) responsible for pathological remodeling of CL in heart diseases remains elusive. ALCAT1 is a lysocardiolipin acyltransferase that catalyzes deleterious remodeling of CL, leading to the production of aberrant CL species commonly found in heart diseases (24). The present studies investigated a role of ALCAT1 gain and loss of function in regulating the onset of hypertrophic cardiomyopathy-associated hyperthyroidism. Our results identify for the first time a key role of ALCAT1 in regulating the onset of hypertrophic cardiomyopathy. Accordingly, overexpression of ALCAT1 caused hypertrophic growth of H9c2 cells, whereas ablation of ALCAT1 prevented the onset of T4-induced cardiomyopathy and its related cardiac dysfunction, including ventricular hypertrophy, fibrosis, and elevated expression of collagen types I and III. Additionally, ALCAT1 deficiency also normalized the expression of hypertrophic biomarkers, including BNP, β-MHC, ANF, and ACTA1, which are commonly upregulated by the onset of cardiomyopathy. In support of a potential causative role of ALCAT1 in the etiology of hypertrophic cardiomyopathy, ALCAT1 mRNA and protein expression is significantly upregulated by the onset of cardiomyopathy in the heart (6). Furthermore, CL remodeling by ALCAT1 also causes TLCL deficiency, which was identified as the primary cause of cardiomyopathy in Barth syndrome (7).

Hypothyroidism significantly increases oxidative stress, which is known to cause CL peroxidation and heart failure (9, 22, 32). Pathological CL remodeling in age-related diseases is believed to exacerbate ROS production through enrichment of DHA content in CL (21, 33, 39). In support of this hypothesis, DHA content in CL significantly increases the lipid peroxidation index, which has been implicated in mitochondrial dysfunction in aging and age-related diseases (12, 31, 38, 40, 43). Accordingly, the onset of aging is also associated with depletion of cardiac TLCL with concurrent enrichment of DHA in CL (21, 33, 39). Additionally, mitochondrial membrane DHA content is negatively correlated with life span and positively correlated with ROS production and the lipid peroxidation index (13, 30). Hence, increased DHA content and CL peroxidation rate have been identified as common defects associated with cardiac abnormality in hyperthyroidism (11), diabetes (12, 39), ischemia-reperfusion injury (22, 32), and heart failure (40). In support of ALCAT1 as a key mediator of oxidative stress in T4-induced cardiomyopathy, CL remodeling by ALCAT1 increases DHA content in CL, whereas targeted deletion of ALCAT1 significantly increases the cardiac level of TLCL in the ALCAT1 KO mice (24). Consistent with these findings, we showed in the present study that overexpression of ALCAT1 caused severe oxidative stress, lipid peroxidation, and mtDNA depletion in H9c2 cardiac cells, leading to impaired differentiation of these cells into cardiomyotubes. In direct support of ALCAT1 as the primary mediator of oxidative stress in hyperthyroidism, ablation of ALCAT1 expression completely prevented cardiac oxidative stress and lipid peroxidation caused by hyperthyroidism.

Mitophagy is a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Cardiac mitophagy is upregulated by the onset of cardiac hypertrophy and heart failure in humans and rodents (45). Excessive autophagy is associated with cardiomyopathy and heart failure (35), whereas loss of autophagy causes severe oxidative stress in yeast and cardiomyopathy in mice (20, 27). PINK1 is a key regulator of mitophagy and when mutated causes Parkinson's disease. PINK1 also plays an essential role in normal cardiac function. PINK1 protects against mitochondrial dysfunction and oxidative stress by promoting the clearance of damaged mitochondria through upregulation of mitophagy. Accordingly, PINK1 expression is downregulated in end-stage human heart failure (3), whereas PINK1 deficiency in mice causes oxidative stress, mitophagy, and cardiomyopathy (3, 8, 37). In further support of a causative role of ALCAT1 in the pathogenesis of cardiomyopathy, the present study identified ALCAT1 as a key regulator of mitophagy and PINK1 expression in the

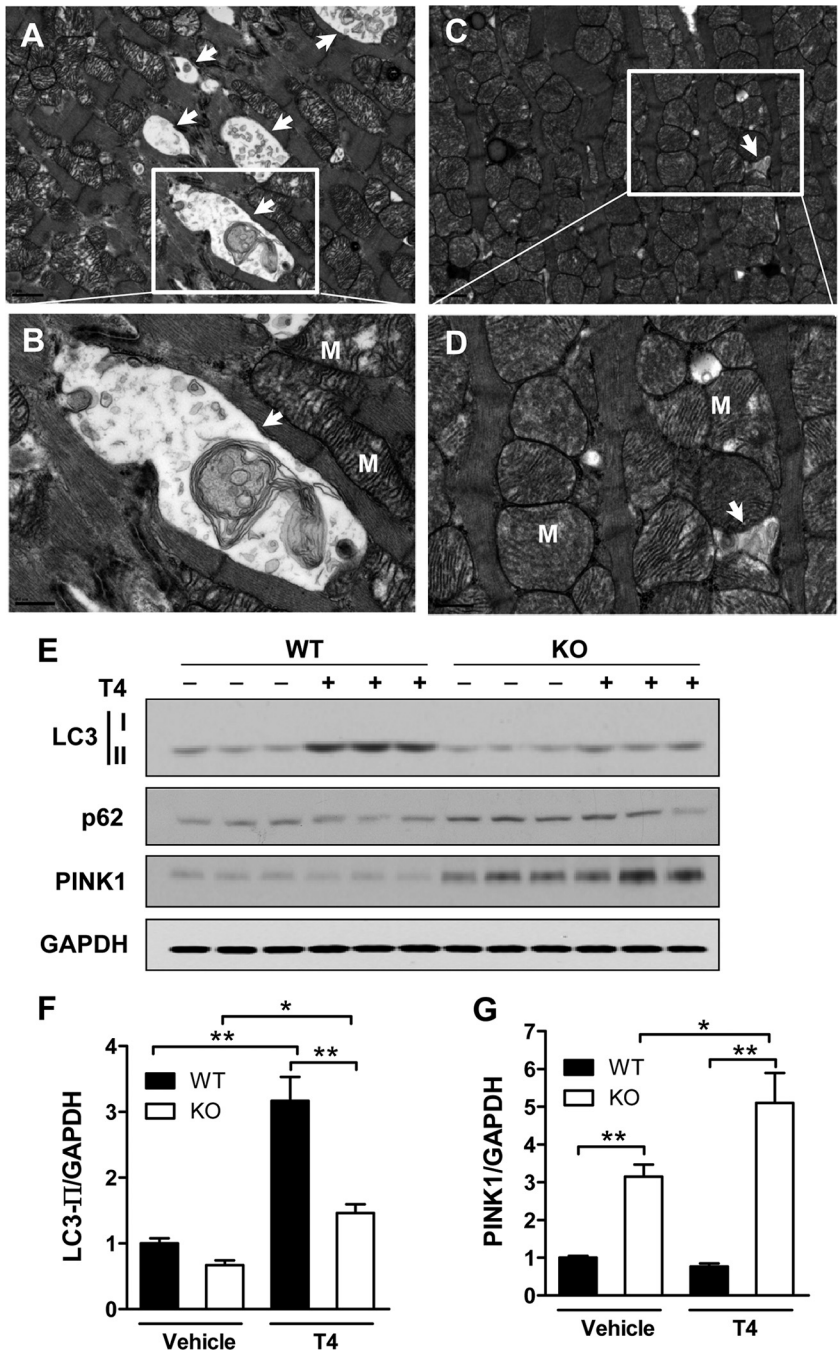

FIG 8 T4-induced mitochondrial swelling and mitophagy are attenuated by ALCAT1 deficiency. (A to D) Representative EM micrographs of cardiomyocytes from WT (A and B) or KO (C and D) mice after T4 treatment for 28 days. Arrows highlight mitochondria which exhibit a damaged structure and abnormal morphology. Boxed areas in panels A and C are enlarged in panels B and D. Scale bars represent 1 μm. (E) Western blot analysis of the autophagic biomarkers LC3-I/II, p62, and PINK1 in isolated ventricular tissues from individual WT and KO mice using GAPDH as an internal control. (F and G) Quantitative analysis of expression levels of the LC3-II (F) or PINK1 (G) protein shown in panel E. Mean ± SEM ($n = 3$); *, $P < 0.05$; **, $P < 0.01$.

heart. We demonstrate that the onset of cardiomyopathy dramatically increased the number of mitophagic mitochondria, which is supported by the changes in autophagy biomarkers, including the expression of LC3 and p62 proteins. In contrast, ablation of ALCAT1 protected mitochondria from oxidative damage associated with hyperthyroid cardiomyopathy. ALCAT1 deficiency also attenuated excessive autophagy associated with the onset of cardiomyopathy through downregulation of LC3 concurrent with increased expression of the p62 protein. Our results are corroborated by previous reports that autophagy is stimulated by myocardial ischemia, and excessive autophagy is a maladaptive response to the onset of cardiomyopathy and heart failure (26, 35, 47). Strikingly, ALCAT1 deficiency dramatically increased the expression of PINK1, which is consistent with a

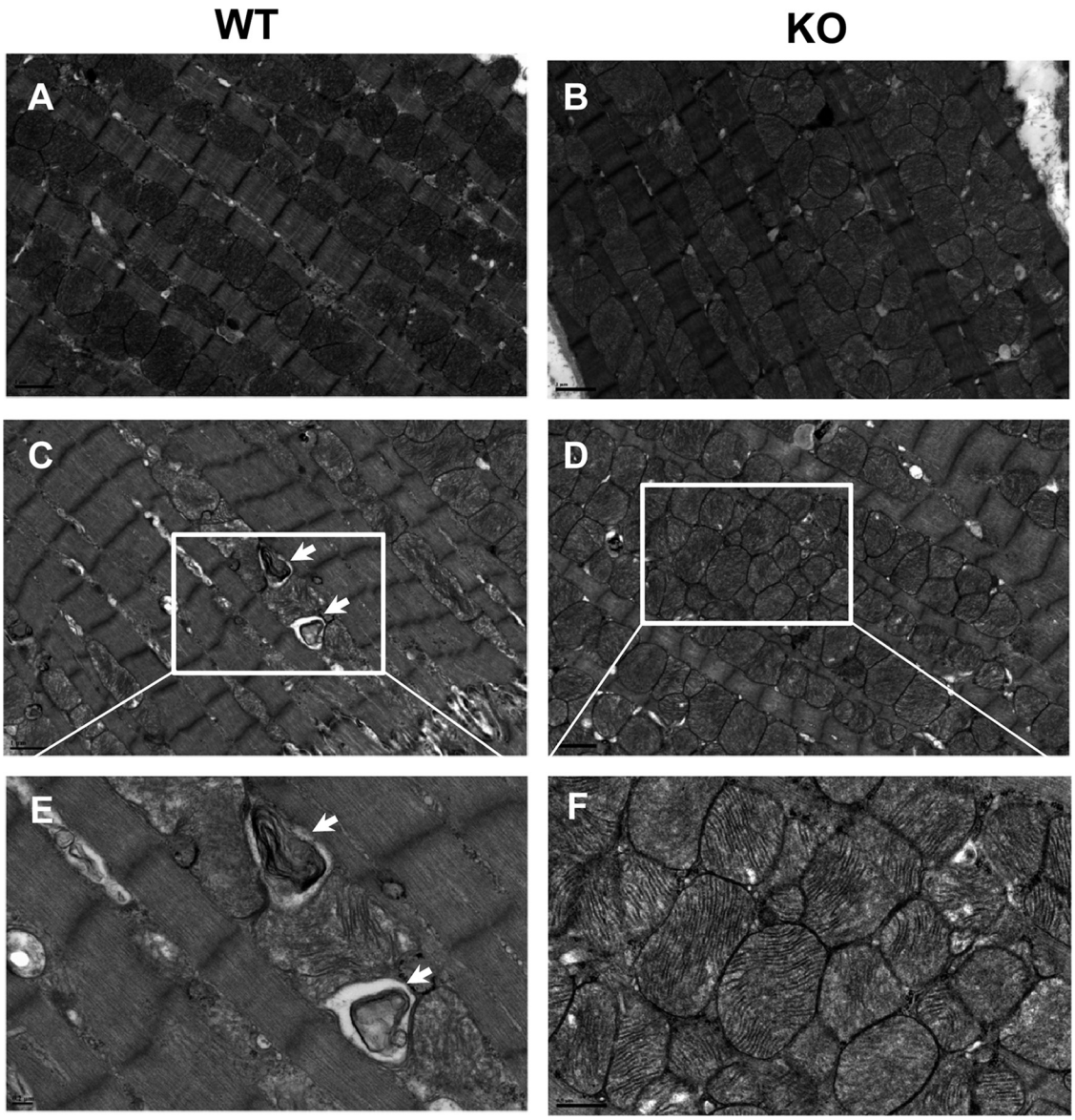

FIG 9 Ablation of ALCAT1 prevents mitochondrial damage and mitophagy associated with cardiomyopathy. The WT and KO mice were treated with vehicle or T4 for 28 days, followed by electron micrographic analysis of longitudinal sections of cardiac ventricular muscle. (A and B) Representative electron micrographs of cardiomyocytes from vehicle-treated WT (A) or KO (B) mice. (C to F) Hyperthyroidism caused a marked increase in the number of damaged mitochondria in WT mice (C) compared with that in KO mice (D). Boxed areas in panels C and D are enlarged in panels E and F, which highlight mitochondria that were undergoing mitophagy, as indicated by arrows. Scale bars, 1 μm.

protective role of PINK1 against oxidative stress and cardiomyopathy. Our findings are further supported by elevated levels of mitophagy and cardiomyopathy in mice with targeted deletion of the *TAZ* gene, whose mutation causes TLCL deficiency in Barth syndrome (1).

The onset of cardiomyopathy stimulates protein synthesis in the cardiomyocytes by activating the Akt-mTOR and Erk pathways (17). Activation of Akt signaling pathways protect cells against oxidative stress-induced apoptosis, whereas mTOR activation is required for hypertrophic growth of cardiomyocytes (14, 46). However, chronic hyperthyroidism causes significant downregulation of Erk, Akt, and mTOR pathways in the late stage of cardiac failure (17, 41). Accordingly, treatment with rapamycin prevents the onset of T4-induced cardiomyopathy, whereas targeted inactivation of mTOR in mice leads to severe dilated cardiomyopathy, characterized by apoptosis, mitophagy, and mitochondrial swelling (46). In further support of a role of ALCAT1 in oxidative stress in cardiomyopathy, our results show that overexpression of ALCAT1 in H9c2 cells or ALCAT1 expression upregulated by hyperthyroidism in mice significantly impaired T4-induced phosphorylation of Akt and downstream signaling components, such as GSK-3β, mTOR, and S6 kinase. These defects are completely normalized by ALCAT1 deficiency in ALCAT1 KO mice. Furthermore, ALCAT1 deficiency also ameliorated insulin resistance, a major contributing factor for the pathogenesis of cardiomyopathy (4).

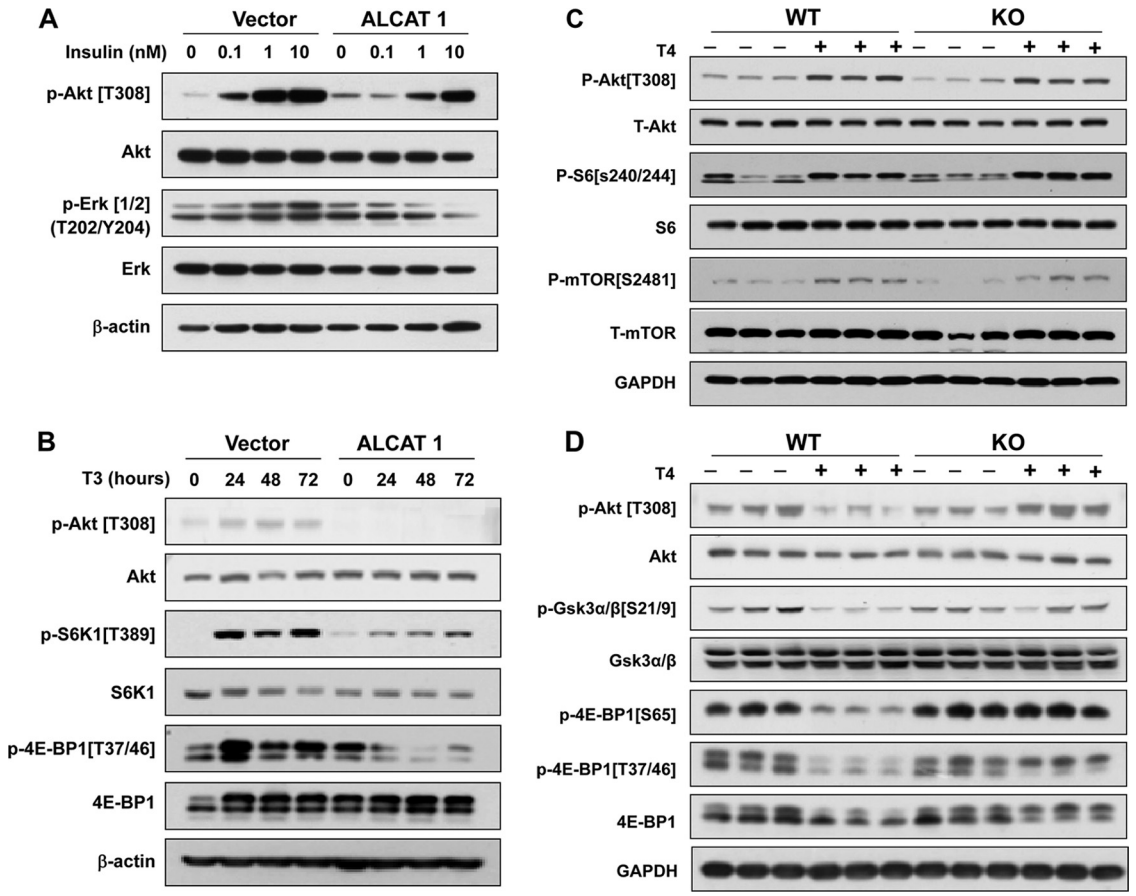

FIG 10 Oxidative stress by ALCAT1 causes insulin resistance by attenuating Akt-mTOR signaling in cardiomyocytes. (A and B) H9c2 cardiac cells stably expressing ALCAT1 or vector control were stimulated with indicated doses of insulin for 10 min (A) or with 100 nM of T3 for the indicated time (B), followed by analysis of phosphorylation of Akt, Erk, S6K1, and 4E-BP1 by Western blotting using β-actin as an internal control. (C) WT and KO mice were treated with vehicle or T4 for 2 days, followed by Western blot analysis of phosphorylation of Akt, S6, and mTOR using GAPDH as an internal control for protein loading. (D) WT and KO mice were treated with vehicle or T4 for 28 days, followed by Western blot analysis of phosphorylation of Akt, Gsk3α/β, and 4E-BP1 in cardiomyocytes using GAPDH as an internal control.

Importantly, the present findings have additional implications for future studies to uncover molecular mechanisms underlying the causes of other forms of cardiovascular diseases, such as diabetic cardiomyopathy, ischemic reperfusion, and heart failure, since oxidative stress and pathological CL remodeling have been implicated in the etiology of these pathological conditions (38, 40). In support of this hypothesis, we have recently shown that ALCAT1 is upregulated by oxidative stress and by the onset of diabetes and obesity (24). Targeted inactivation of ALCAT1 prevents mitochondrial dysfunction and the onset of obesity, which is a major causative factor for type 2 diabetes and cardiovascular diseases (4). Additionally, we have recently demonstrated a key role of oxidative stress caused by ALCAT1 in mitochondrial fragmentation, a common defect associated with mitochondrial dysfunction in age-related metabolic diseases (23). Together, these findings suggest that development of inhibitors of ALCAT1 may provide novel treatments for cardiac hypertrophy and other heart diseases, which comprise the major cause of fatality in developed countries.

ACKNOWLEDGMENTS

We thank Roland Myers for EM analysis and Lenard J. Jefferson for critically reading the manuscript.

This study was supported in part by grants from NIH (DK076685; to Y.S.) and Pennsylvania Department of Health (10-K-273; to Y.S.).

We have no financial conflict of interest to disclose.

REFERENCES

1. **Acehan D, et al.** 2011. Cardiac and skeletal muscle defects in a mouse model of human Barth syndrome. J. Biol. Chem. **286**:899–908.
2. **Bianchi G, et al.** 1999. Oxidative stress and anti-oxidant metabolites in patients with hyperthyroidism: effect of treatment. Horm. Metab. Res. **31**:620–624.
3. **Billia F, et al.** 2011. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. Proc. Natl. Acad. Sci. U. S. A. **108**: 9572–9577.
4. **Boudina S, Abel ED.** 2007. Diabetic cardiomyopathy revisited. Circulation **115**:3213–3223.
5. **Cao J, Liu Y, Lockwood J, Burn P, Shi Y.** 2004. A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. J. Biol. Chem. **279**:31727–31734.
6. **Cao J, Shen W, Chang Z, Shi Y.** 2009. ALCAT1 is a polyglycerophospholipid acyltransferase potently regulated by adenine nucleotide and thyroid status. Am. J. Physiol. Endocrinol. Metab. **296**:E647–E653.
7. **Claypool SM, Koehler CM.** 2012. The complexity of cardiolipin in health and disease. Trends Biochem. Sci. **37**:32–41.
8. **Dagda RK, et al.** 2009. Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. J. Biol. Chem. **284**:13843–13855.

9. **Drummond GR, Selemidis S, Griendling KK, Sobey CG.** 2011. Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. Nat. Rev. Drug Discov. **10**:453–471.
10. **Engberding N, et al.** 2004. Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? Circulation **110**:2175–2179.
11. **Gredilla R, Lopez Torres M, Portero-Otin M, Pamplona R, Barja G.** 2001. Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. Mol. Cell Biochem. **221**:41–48.
12. **Han X, et al.** 2007. Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. Biochemistry **46**:6417–6428.
13. **Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA.** 2007. Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. **87**:1175–1213.
14. **Ikeda Y, et al.** 2009. Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction. J. Biol. Chem. **284**:35839–35849.
15. **Imae R, et al.** 2012. LYCAT, a homologue of C. elegans acl-8, acl-9, and acl-10, determines the fatty acid composition of phosphatidylinositol in mice. J. Lipid Res. **53**:335–347.
16. **Kagan VE, et al.** 2005. Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. Nat. Chem. Biol. **1**:223–232.
17. **Kenessey A, Ojamaa K.** 2006. Thyroid hormone stimulates protein synthesis in the cardiomyocyte by activating the Akt-mTOR and p70S6K pathways. J. Biol. Chem. **281**:20666–20672.
18. **Kerkela R, et al.** 2008. Deletion of GSK-3β in mice leads to hypertrophic cardiomyopathy secondary to cardiomyoblast hyperproliferation. J. Clin. Invest. **118**:3609–3618.
19. **Kim HN, Januzzi JL, Jr.** 2011. Natriuretic peptide testing in heart failure. Circulation **123**:2015–2019.
20. **Kurihara Y, et al.** 2012. Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. J. Biol. Chem. **287**:3265–3272.
21. **Lee H-J, Mayette J, Rapoport SI, Bazinet RP.** 2006. Selective remodeling of cardiolipin fatty acids in the aged rat heart. Lipids Health Dis. **5**:2.
22. **Lesnefsky EJ, et al.** 2004. Ischemia, rather than reperfusion, inhibits respiration through cytochrome oxidase in the isolated, perfused rabbit heart: role of cardiolipin. Am. J. Physiol. Heart Circ. Physiol. **287**:H258–H267.
23. **Li J, et al.** 2012. Lysocardiolipin acyltransferase 1 (ALCAT1) controls mitochondrial DNA fidelity and biogenesis through modulation of MFN2 expression. Proc. Natl. Acad. Sci. U. S. A. **109**:6975–6980.
24. **Li J, et al.** 2010. Cardiolipin remodeling by ALCAT1 links oxidative stress and mitochondrial dysfunction to obesity. Cell Metab. **12**:154–165.
25. **Litwin SE, et al.** 1995. Serial echocardiographic-Doppler assessment of left ventricular geometry and function in rats with pressure-overload hypertrophy. Chronic angiotensin-converting enzyme inhibition attenuates the transition to heart failure. Circulation **91**:2642–2654.
26. **Matsui Y, et al.** 2007. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ. Res. **100**:914–922.
27. **Nakai A, et al.** 2007. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nat. Med. **13**:619–624.
28. **Nakao K, Minobe W, Roden R, Bristow MR, Leinwand LA.** 1997. Myosin heavy chain gene expression in human heart failure. J. Clin. Invest. **100**:2362–2370.
29. **Ohtani K, et al.** 1995. High prevalence of atrial fibrosis in patients with dilated cardiomyopathy. J. Am. Coll. Cardiol. **25**:1162–1169.
30. **Pamplona R, et al.** 1998. Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. J. Lipid Res. **39**:1989–1994.
31. **Paradies G, Petrosillo G, Paradies V, Ruggiero FM.** 2010. Oxidative stress, mitochondrial bioenergetics, and cardiolipin in aging. Free Radic. Biol. Med. **48**:1286–1295.
32. **Paradies G, et al.** 1999. Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion. Free Radic. Biol. Med. **27**:42–50.
33. **Paradies G, Ruggiero FM.** 1990. Age-related changes in the activity of the pyruvate carrier and in the lipid composition in rat-heart mitochondria. Biochim. Biophys. Acta **1016**:207–212.
34. **Petrosillo G, Ruggiero FM, Di Venosa N, Paradies G.** 2003. Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin. FASEB J. **17**:714–716.
35. **Rothermel BA, Hill JA.** 2008. Autophagy in load-induced heart disease. Circ. Res. **103**:1363–1369.
36. **Schlame M.** 2008. Cardiolipin synthesis for the assembly of bacterial and mitochondrial membranes. J. Lipid Res. **49**:1607–1620.
37. **Sengupta A, Molkentin JD, Paik JH, DePinho RA, Yutzey KE.** 2011. FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress. J. Biol. Chem. **286**:7468–7478.
38. **Shi Y.** 2010. Emerging roles of cardiolipin remodeling in mitochondrial dysfunction associated with diabetes, obesity, and cardiovascular diseases. J. Biomed. Res. **24**:6–15.
39. **Sparagna GC, et al.** 2007. Loss of cardiac tetralinoleoyl cardiolipin in human and experimental heart failure. J. Lipid Res. **48**:1559–1570.
40. **Sparagna GC, Lesnefsky EJ.** 2009. Cardiolipin remodeling in the heart. J. Cardiovasc. Pharmacol. **53**:290–301.
41. **Suarez J, Scott BT, Suarez-Ramirez JA, Chavira CV, Dillmann WH.** 2010. Thyroid hormone inhibits ERK phosphorylation in pressure overload-induced hypertrophied mouse hearts through a receptor-mediated mechanism. Am. J. Physiol. Cell Physiol. **299**:C1524–C1529.
42. **Wallace DC.** 2005. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. **39**:359–407.
43. **Watkins SM, Carter LC, German JB.** 1998. Docosahexaenoic acid accumulates in cardiolipin and enhances HT-29 cell oxidant production. J. Lipid Res. **39**:1583–1588.
44. **Wong C, Marwick TH.** 2007. Obesity cardiomyopathy: pathogenesis and pathophysiology. Nat. Clin. Pract. Cardiovasc. Med. **4**:436–443.
45. **Yan L, et al.** 2005. Autophagy in chronically ischemic myocardium. Proc. Natl. Acad. Sci. U. S. A. **102**:13807–13812.
46. **Zhang D, et al.** 2010. MTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice. J. Clin. Invest. **120**:2805–2816.
47. **Zhu H, et al.** 2007. Cardiac autophagy is a maladaptive response to hemodynamic stress. J. Clin. Invest. **117**:1782–1793.