Functional Cardiac Lipolysis in Mice Critically Depends on Comparative Gene Identification-58*

Efficient catabolism of cellular triacylglycerol (TG) stores requires the TG hydrolytic activity of adipose triglyceride lipase (ATGL). The presence of comparative gene identification-58 (CGI-58) strongly increased ATGL-mediated TG catabolism in cell culture experiments. Mutations in the genes coding for ATGL or CGI-58 in humans cause neutral lipid storage disease characterized by TG accumulation in multiple tissues. ATGL gene mutations cause a severe phenotype especially in cardiac muscle leading to cardiomyopathy that can be lethal. In contrast, CGI-58 gene mutations provoke severe ichthyosis and hepatosteatosis in humans and mice, whereas the role of CGI-58 in muscle energy metabolism is less understood. Here we show that mice lacking CGI-58 exclusively in muscle (CGI-58KOM) developed severe cardiac steatosis and cardiomyopathy linked to impaired TG catabolism and mitochondrial fatty acid oxidation. The marked increase in ATGL protein levels in cardiac muscle of CGI-58KOM mice was unable to compensate the lack of CGI-58. The addition of recombinant CGI-58 to cardiac lysates of CGI-58KOM mice completely reconstituted TG hydrolytic activities. In skeletal muscle, the lack of CGI-58 similarly provoked TG accumulation. The addition of recombinant CGI-58 increased TG hydrolytic activities in control and CGI-58KOM tissue lysates, elucidating the limiting role of CGI-58 in skeletal muscle TG catabolism. Finally, muscle CGI-58 deficiency affected whole body energy homeostasis, which is caused by impaired muscle TG catabolism and increased cardiac glucose uptake. In summary, this study demonstrates that functional muscle lipolysis depends on both CGI-58 and ATGL.

Obesity and type 2 diabetes are often associated with increased fat deposition in cardiac and skeletal muscle (1–3). Commonly, this ectopic accumulation of lipids and lipid metabolites, also referred to as lipotoxicity (4, 5), is regarded as a major cause for cardiac dysfunction and skeletal muscle insulin resistance (6). Cardiomyocytes generate most of their ATP from mitochondrial fatty acid oxidation (FAO), although cardiac muscle (CM) exhibits a high metabolic flexibility, allowing it to switch from preferential FAO to pronounced glucose oxidation in adaptation to physiological and dietary changes (7, 8). Skeletal muscle (SM) uses both carbohydrates and FAs as energy substrates depending on muscle fiber composition and physical activity (9, 10). Skeletal myocytes classified as type I (oxidative, slow) muscle fibers are enriched in mitochondria and exhibit a high oxidative capacity. In contrast, type II (glycolytic, fast) muscle fibers contain less mitochondria and rely on aerobic and anaerobic glycolytic catabolism as the energy source. Changes in energy substrate catabolism in both CM and SM, as often present in obese and/or diabetic individuals, can be linked to myopathy, heart failure, and SM insulin resistance (2, 8).

The cardiac TG pool is very dynamic, and endogenous TG catabolism plays a significant role in the delivery of long chain FA for mitochondrial FAO (11). CM and SM TG catabolism necessitates the TG hydrolytic activity of neutral lipases including ATGL, hormone-sensitive lipase, and monoglyceride lipase (12, 13). Whereas the disruption of hormone-sensitive lipase in

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2 The abbreviations used are: FAO, fatty acid (FA) oxidation; CGI-58, comparative gene identification-58; CGI-58KOM, mice lacking CGI-58 exclusively in cardiac and skeletal muscle; TG, triacylglycerol; ATGL, adipose triglyceride lipase; CM, cardiac muscle; SM, skeletal muscle; NLSD, neutral lipid storage disease; PPAR, peroxisome proliferator-activated receptor; RQ, respiratory quotient; m., musculus; LPL, lipoprotein lipase; CD36, cluster of differentiation 36; CAPS, 3-(cyclohexylamino)propanesulfonic acid; LPA, lysophosphatic acid; DG, deoxyglucose; DGP, 2-deoxyglucose 6-phosphate; LV, left ventricular; CPT1b, carnitine palmitoyltransferase 1β; PGC-1α, peroxisome proliferator-activated receptor γ, coactivator 1α; FFA, free fatty acid.
mice provoked a relatively benign phenotype (14, 15), the global lack of ATGL caused lethal cardiac dysfunction and TG accumulation in multiple organs including the heart, adipose tissue, and liver (16). Impaired cardiac lipolysis in ATGL-deficient mice was tightly linked to defective PPARβ/δ and PGC-1-activated gene expression and mitochondrial respiration, elucidating the close interdependence of lipolysis and mitochondrial gene expression and function (17). Humans affected by ATGL gene mutations develop neutral lipid storage disease (NLSD) with myopathy (18), which in severe cases requires heart transplantation at an early age.

For full enzyme activity, ATGL requires a cofactor designated as CGI-58 (19). CGI-58 deficiency also causes NLSD, but the clinical presentations of ATGL deficiency and CGI-58 deficiency differ in some aspects as follows. (a) The development of cardiomyopathy is always present in humans (and mice) harboring mutated ATGL alleles, whereas cardiomyopathy has not been described for humans carrying mutated CGI-58 alleles (20). (b) CGI-58 mutations cause a severe skin barrier defect in humans and mice (21, 22), and accordingly, the disease was designated as NLSD with ichthyosis (23). Nevertheless, muscle weakness is described in some NLSD with ichthyosis case reports. These clinical findings suggest a less prominent role for CGI-58 in muscle lipid catabolism than for ATGL. Considering the pivotal role of ATGL in adipose tissue TG mobilization and the supply of FA as oxidative fuel for CM and SM, it cannot be excluded that cardiac dysfunction in humans and mice globally lacking ATGL involves an inadequate FA delivery from the circulation.

To address the role of CGI-58 in muscle energy metabolism and to exclude phenotypic changes caused by generalized metabolic derangements, we generated and characterized a mouse model lacking CGI-58 exclusively in CM and SM. The present study reveals a critical role for CGI-58 in muscle lipolysis. Besides, the lack of CGI-58 in CM caused profound cardiovascular derangements.

**EXPERIMENTAL PROCEDURES**

**Animals**—Mice were housed in a specific pathogen-free animal facility on a regular light/dark cycle (12 h/12 h) and had ad libitum access to water and a standard chow diet (4.5% w/w fat; Ssniff, Soest, Germany). Animals were anesthetized with IsoFlur®/isoflurane (Abbott) and euthanized by cervical dislocation. The study was approved by the Austrian ethics committee and is in accordance with the council of Europe Convention.

**Generation of Mice with Muscle-specific CGI-58 Deficiency (CGI-58KOM)**—Targeted ES cells harboring a single loxP site within intron 4 of the mouse CGI-58 gene and a loxP flanked CRE-expressing plasmid for loxP-mediated recombination. ES cells showing recombination at the loxP sites of the NEO cassette were used to generate chimeric mice. Animals carrying the floxed CGI-58 allele transmitted this locus through the germ line and yielded mice heterozygous for the targeted allele. Mice heterozygous for the floxed CGI-58 allele were back-crossed six to eight times onto the C57BL/6 background before breeding the animals with transgenic mice expressing the Cre-recombinase under the control of the muscle-specific muscle creatine kinase promoter (24). Mice homozygous for the CGI-58 floxed allele (flox/flox) showed no significant differences in body weight, muscle TG levels, and plasma parameters compared with C57BL/6 wild type mice (data not shown) and were used as controls. Mice homozygous for the floxed CGI-58 allele and heterozygous for the muscle-specific muscle creatine kinase-Cre transgene were used for the generation of muscle-specific CGI-58-deficient (CGI-58KOM) mice.

**Tissue TG Hydrolase Assay**—Determination of tissue neutral TG hydrolytic activity in the absence and presence of recombinant GST-tagged CGI-58 was performed as previously described (16, 19). For musculus (m.) soleus, TG hydrolytic activities were measured in at least 2 pools per genotype, each containing tissue preparations of 2–3 mice. Determination of cardiac lipoprotein lipase (LPL) hydrolytic activity was carried out as described before (17).

**Quantitative RT-quantitative PCR-based Gene Expression**—Total RNA was extracted with TRIzol reagent (Invitrogen) and treated with DNase I (Invitrogen). For first-strand cDNA synthesis, 1 µg of total RNA was reverse-transcribed at 37 °C for 2 h using random hexamer primers and the MultiScribe High Capacity cDNA Reverse Transcription kit from Applied Biosystems. Primers used for RT-quantitative PCR were designed using ABI Primer Express® and/or Primer3. Primers were designed to span exon–intron boundaries with an ampiclon size of less than 150 bp and BLASTed for specificity. RT-quantitative PCR reactions (20 µl) contained 8 ng of cDNA, 10 pmol of each primer, and 10 µl of SYBR Green Master mix (Fermentas, Thermo Scientific, St. Leon-Rot, Germany) and were carried out using the StepOne™ Plus real time PCR system (Applied Biosystems). Relative mRNA levels were quantified using the comparative ΔΔCt method, normalized to β-actin. The following primer sequences were used for RT-PCR: CGI-58 forward (5’-GGT TAA GTG TAG TGC AGC-3’) and reverse (5’-AAG CTG TCT CAC CAC TTG-3’); ATGL forward (5’-GAG ACC AAG TGG AAC AAT-3’) and reverse (5’-GTA GAT GTG AGT GGC GTC-3’); MCAD (acyl-coenzyme A dehydrogenase, long chain) forward (5’-TTT CCG GGA GAG TGT AAG GA-3’) and reverse (5’-ACT TCT CCA GCT TTC CCA-3’); AOX (acyl-coenzyme A oxidase 1, palmitoyl) forward (5’-GGG AGT GCT ACG GGT TAC ATG-3’) and reverse (5’-CCG ATA TCC CCA ACA GTG ATG-3’); LDH (lactate dehydrogenase, muscle A form) forward (5’-GCC CGG CAG CAG TAT ACG-3’) and reverse (5’-TGG TCA ACC TCT CCT TCC AAT-3’); PDK4 (pyruvate dehydrogenase kinase, isoenzyme 4) forward (5’-ATC TAA CAT CCG CAG AAT TAA ACC-3’) and reverse (5’-GGG AGT GCT ACG GGT TAC ATG-3’); PGC-1α (peroxisome proliferator-activated receptor γ, coactivator 1α) forward (5’-CCC TGG CAT TGT TAA GAC-3’) and reverse (5’-GTG TGC TGT TCC TGT CTC C-3’); CPT1b (carnitine palmitoyltransferase 1b) forward (5’-GCC ACC TCT TCT GCC TTT TCC AAT-3’) and reverse (5’-AGG TCA AAC ATG CAT GAT AT-3’); LPL forward (5’-TCC AGC CAG GAT GCA ACA-3’) and reverse (5’-CCA GGT CTC CGA GTC TCT TCT-3’); CD36 (cluster of differentiation 36) forward (5’-GAA CCT ATT GAA GCC TTA CAT CC-3’).
After perfusion with 0.9% NaCl for removal of blood lipids, insulin ELISA kit (ChrystalChem) in re-fed mice. Plasma insulin levels were determined with a mouse/rat insulin ELISA kit (IKA GmbH, Staufen, Germany). The homogenates were centrifuged (40,000 × g at 4°C for 30 min). The infranatants were collected and used for Western blot analysis. Proteins were separated by 10% SDS-PAGE followed by Western blot analysis using PVDF membranes (Carl Roth GmbH, Karlsruhe, Germany) and CAPS buffer (10 mM 3-cyclohexylamino-1-propanesulfonic acid, 10% methanol) for protein transfer. Blots were probed using either a rabbit polyclonal antiserum raised against murine CGI-58 (kindly provided by Dr. Dawn L. Brasaemle) or specific antibodies against ATGL (Cell Signaling, Boston, MA), CGI-58 (designated as ABHD5, Abnova, Heidelberg), or GAPDH (Cell Signaling, Boston, MA). Specifically bound immunoglobulins were detected in a second reaction using horseradish peroxidase-conjugated anti-rabbit or antiserum IgG antibody and visualized by enhanced chemiluminescence detection (GE Healthcare).

Muscle Lipid Measurement and Plasma Chemistry—Tissue lipids were extracted according to the method of Folch et al. (25). Tissue lipids were dried in a stream of nitrogen and redissolved by brief sonication in 2% Triton X-100. TG concentrations were measured using the Infinity Triglycerides Reagent (Thermo Electron Corp., Scoresby, Victoria, Australia). For tissue protein determination, the remaining protein pellets were lysed in lysis buffer (0.3 M NaOH, 0.1 M SDS), and protein content was measured subsequently using the BCA reagent (Thermo Scientific, Pierce). Muscle tissue acetyl- and acyl-CoA levels were determined by online solid phase extraction-LC-MS according to the method of Magnes et al. (26).

For the analysis of plasma parameters, non-fasted or 14 h fasted animals were briefly anesthetized, and blood samples were collected by retro-orbital puncture. Plasma levels of TG, glycerol, free FAs, and total cholesterol were determined using commercial kits (Thermo Electron Corp., Sigma; Wako Chemicals, Neuss, Germany; Roche Diagnostics). Blood glucose was determined using a glucose analyzer (Olympus, Tokyo, Japan). Plasma insulin levels were determined with a mouse/rat insulin ELISA kit (ChrystalChem) in re-fed mice.

Histology and Electron Microscopy—To visualize neutral lipids, SM and hearts were instantly frozen in liquid nitrogen. After perfusion with 0.9% NaCl for removal of blood lipids, cryosections (3 µm) were stained with Sudan III (Sigma), and nuclei were stained with hematoxylin following a standard protocol. Electron microscopy was performed as previously described (17).

Cardiac Oxygen Consumption and Muscle Tissue FAO—CM oxygen uptake was determined in cardiac tissue slices as previously described (17). FAO in CM and SM (pools of m. gastrocnemius and m. soleus) homogenates was performed according to the protocol of Hirshey et al. (27) with small adaptations. In brief, tissues from fasted mice were homogenized in chilled STE buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA) using an Ultra Turrax® (IKA, Staufen, Germany). Homogenates were centrifuged at 420 × g at 4°C for 10 min to pellet nuclei and cell debris. The supernatant was subsequently centrifuged at 15,000 × g at 4°C for 15 min to obtain a mitochondria-enriched fraction. Pellets were washed and suspended in STE buffer. After protein determination, 20 µl of the suspension was incubated with 400 µl of reaction mix (100 mM sucrose, 10 mM Tris, 5 mM KH2PO4, 0.2 mM EDTA, 0.3% BSA, 80 mM KCl, 1 mM MgCl2, 2 mM carnitine, 0.1 mM malate, 0.05 mM CoA, 2 mM ATP, 1 mM DTT, 100 µM oleic acid complexed to BSA, 0.1 µCi/reaction [14C]oleic acid) for 30 min at 37°C. The reaction was terminated by transferring the reaction mix in fresh tubes containing 200 µl of 1 M HClO4. For CO2 trapping, the tube caps were equipped with a piece of filter paper soaked with 20 µl of 10 N NaOH. The closed tubes were incubated at 37°C for 1 h. Afterward the filter papers were subjected to liquid scintillation counting.

Echocardiographic Analyses—Mouse echocardiography (28) was performed under light isoflurane anesthesia. Mice were allowed to breathe spontaneously. Two-dimensional guided M-mode echoes were obtained from short- and long-axis views at the level of the largest left ventricular (LV) diameter using a VS-VEVO 770 high resolution imaging system (Visualsonics, Toronto, Canada) equipped with a real-time microvisualization scanhead (30 MHz). LV end-diastolic and end-systolic dimensions were measured from original tracings by using the leading edge convention of the American Society of Echocardiography. All measurements and calculations were done in triplicate. Percent fractional shortening, ejection fraction, and LV mass were calculated as previously described (28).

Determination of Enzymatic Activities—CPT1 activity of isolated mitochondria prepared from cardiac muscle tissue was determined according to established protocols (29, 30). Lyso-phosphatidic acid (LPA) acyltransferase activity was determined in CM microsomal fractions by measuring acylation of LPA using [1-14C]oleoyl-CoA as a radioactive tracer (31). Formation of radioactively labeled phosphatidic acid was measured in lipid extracts that were separated by TLC. Spots comigrating with the phosphatidic acid standard were cut and quantified by scintillation counting. CM and SM LPL activities were performed as previously described (32). Dioleoylglycerol acyltransferase enzymatic activity was measured as described by Cases et al. (33). In brief, 10 µg of cardiac muscle microsomal fractions (in 250 mM sucrose, 50 mM Tris, pH 7.4, supplemented with protease inhibitor mixture) were incubated for 10 min at 37°C with 200 µM dioleoylglycerol (emulsified with phosphatidylcholine, molar ratio 0.2) and 25 µM [1-14C]oleoyl-CoA (ARC, St. Louis, MO). The reaction was stopped with 1.2 ml of chloroform:methanol (2:1), and extracted lipids were dried and separated by TLC using hexane-diethyl ether-acetic acid, 70:29:1, as solvent system. TG formation was quantified by scintillation counting.

Glucose and Insulin Tolerance Tests—Glucose tolerance and insulin tolerance tests were performed with mice fasted for 6 and 4 h, respectively. Tests were performed as previously described (16).

Determination of Tissue Glucose Uptake—Tissue specific glucose uptake was determined using [2-3H]deoxyglucose (DG)
for 5 min, 800 µl of HClO₄ and centrifuged for 20 min at 2000 × g. Then, 1 ml of supernatant was neutralized using 50 µl of 1 M potassium phosphate, pH 7.0, and 50 µl of 10% KOH. After centrifugation for 10 min, the radioactivity was determined in 2 ml of 0.5% HClO₄ and centrifuged for 20 min at 2000 × g. Then, 1 ml of supernatant was neutralized using 50 µl of 1 M potassium phosphate, pH 7.0, and 50 µl of 10% KOH. After centrifugation for 10 min, the radioactivity was determined in an aliquot of 400 µl by scintillation counting, representing total tissue counts of [2-3H]DG and [2-3H]DGP. In the remainder (500 µl), 2-DGP was precipitated with 250 µl of 0.15 M Ba(OH)₂ and 250 µl of 0.15 M ZnSO₄. After centrifugation at 20,000 × g for 5 min, 800 µl of the supernatant were measured to determine [2-3H]DG. Tissue [2-3H]DGP counts were calculated as the difference between total tissue counts and [2-3H]DG counts. For analyses of protein content, pellets obtained after tissue homogenization were lysed overnight at 55 °C in 0.3 N NaOH, 0.1% SDS.

**Statistical Analysis**—Statistical significance was determined by Student’s unpaired two-tailed t test. Group differences considered significant: p < 0.05 (*), p < 0.01 (**), and p < 0.001 (***).

### RESULTS

**Generation of CGI-58KOM Mice**—To delete CGI-58 exclusively in CM and SM (CGI-58KOM), we crossed mice carrying a muscle creatine kinase promoter-driven Cre recombinase transgene (24) onto the CGI-58-floxed background (22). Recombination generated mice with extremely low CGI-58 mRNA levels in CM (~86%), m. soleus (~82%), m. tibialis (~99%), and m. gastrocnemius (~84%) compared with levels of flox/flox muscle tissues (Fig. 1a). CGI-58 protein signals were absent in CMs of CGI-58KOM mice demonstrating that CGI-58 was efficiently deleted in the heart (Fig. 1b). CGI-58 protein concentration in SM of CGI-58KOM mice could not be analyzed due to the already low CGI-58 expression levels in control mice. In the liver we observed a moderate increase in

**Normal Body Weight but Decreased Plasma Glucose Levels in Fasted CGI-58KOM Mice**—Body weights of CGI-58KOM mice were comparable with weights of flox/flox mice (27.7 ± 2.4 g for 14–16-week-old male flox/flox mice versus 30.8 ± 2.7 g for CGI-58KOM mice). Plasma concentrations of TG, non-esterified FA, glycerol, and total cholesterol levels were similar in CGI-58KOM mice compared with flox/flox mice (Table 1). Glucose concentrations were decreased in blood samples of fasted CGI-58KOM mice.

**FIGURE 1. Examination of CGI-58 expression levels in tissues of CGI-58KOM (KOM) and flox/flox mice. a, shown are CGI-58 mRNA expression levels in CM, m. soleus, m. tibialis, and m. gastrocnemius of male 9–10-week-old CGI-58KOM and flox/flox mice, respectively (n = 4). b, Western blot analysis of CGI-58 protein levels in CM, liver, and white and brown adipose tissue (WAT and BAT, respectively) of CGI-58KOM and flox/flox mice, respectively. Western blots are representative for two individual tissue preparations. Arrows indicate CGI-58-specific signals. GAPDH was used as loading control. Data are shown as means ± S.D. ***,***, p < 0.001 versus flox/flox mice.**
Role of CGI-58 in Muscle Lipolysis

TABLE 1
Plasma energy metabolites in non-fasted and fasted mice

Plasma parameters of 11–12-week-old non-fasted or 14 h fasted male mice received a standard chow diet (n = 5 - 7). Data are shown as the mean ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-fasted</th>
<th>CGI-58KOM</th>
<th>Fasted</th>
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<tr>
<td></td>
<td>flox/flox</td>
<td>CGI-58KOM</td>
<td>flox/flox</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>164.3 ± 18.1</td>
<td>145.67 ± 24.2</td>
<td>95.7 ± 8.2</td>
</tr>
<tr>
<td>FFA (mmol/liter)</td>
<td>0.58 ± 0.10</td>
<td>0.49 ± 0.27</td>
<td>0.87 ± 0.24</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>101.4 ± 44.9</td>
<td>139.3 ± 47.5</td>
<td>88.3 ± 25.4</td>
</tr>
<tr>
<td>Glycerol (mmol/liter)</td>
<td>0.35 ± 0.22</td>
<td>0.34 ± 0.10</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>102.6 ± 28.3</td>
<td>99.4 ± 18.7</td>
<td>123.8 ± 40.7</td>
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* p < 0.01 versus flox/flox mice.

Muscle CGI-58 Deficiency Causes Severe Cardiac Steatosis, Heart Dysfunction, and Shortens Life Span—Hearts from CGI-58KOM mice were increased in mass (~1.6-fold) and exhibited a yellowish color (Fig. 2a). Sudan III staining of neutral lipids indicated massive fat accumulation in CM sections of CGI-58KOM mice (Fig. 2b). Electron microscopy of CGI-58-deficient cardiomyocytes revealed a marked increase in lipid droplet size and number (Fig. 2c), and lipid droplets were abundantly localized within the intermyofibrillar network. Mitochondrial morphology appeared normal, exhibiting the typical cristae structure. The presence of large lipid droplets in CGI-58KOM cardiomyocytes hindered the chain-like clustering of mitochondria. CM TG levels of non-fasted and fasted CGI-58KOM mice (Fig. 2d) were increased 43- and 15-fold, respectively.

Echocardiography (Fig. 2e) revealed an impaired LV systolic function (Table 2) and a concentric cardiac hypertrophy in CGI-58KOM mice. The defect in LV systolic function was characterized by an 1.5-fold increase in the LV end-systolic dimension and a robust decrease in LV fractional shortening (Table 2). The marked increase in the LV mass index (+163%) of CGI-58KOM hearts was largely a consequence of septal and posterior wall thickening. Consistent with the observed heart dysfunction, the life span of CGI-58KOM mice was shortened (Fig. 2f). Interestingly, CGI-58KOM mice exhibited more than twice the life span of mice globally lacking ATGL.

Impaired Lipolysis Despite a Marked Increase in Cardiac ATGL Protein Levels—Next we examined the underlying mechanism(s) that provokes fat accumulation in CM of CGI-58KOM mice. CM TG hydrolytic activities were decreased in CM homogenates of non-fasted (~24%; p = 0.058) and overnight fasted (~49%) CGI-58KOM mice when compared with flox/flox tissue activities (Fig. 3a). The addition of recombinant glutathione S-transferase-tagged CGI-58 (GST-CGI-58) strongly increased TG hydrolytic activities in CM homogenates of non-fasted (3.3-fold) and overnight fasted (5.3-fold) CGI-58KOM mice but not those of flox/flox control mice. Notably, the addition of GST-CGI-58 increased TG hydrolytic activities in CGI-58KOM cardiac homogenates to a larger extent than those of flox/flox control homogenates (2.5- and 2.7-fold, respectively). To test whether the increase of cardiac TG hydrolysis above flox/flox activity was caused by augmented ATGL expression, we measured cardiac ATGL mRNA and protein levels by RT-quantitative PCR and Western blotting, respectively. We found that ATGL mRNA expression (Fig. 3b) was reduced (~54%), whereas the ATGL protein level (Fig. 3c) was substantially increased (6.6-fold increase in signal intensity) in CMs of CGI-58KOM mice. We conclude that this increase in the ATGL protein levels in CM derived from CGI-58KOM mice is responsible for the significant elevation of TG hydrolytic activity upon the addition of recombinant CGI-58.
TABLE 2

Echocardiography revealed impaired LV systolic function in CGI-58-KOM mice

Echocardiography was performed with non-fasted 12–14-week-old male CGI-58-KOM and flox/flox mice (n = 5–6). LV fractional shortening (LVFS), LV ejection fraction (LV EF), and LV mass were calculated from original tracings. Interventricular septal wall thickness (IVSd), LV enddiastolic diameter (LVEDD), and LV endystolic diameter (LVEDd), and LV posterior wall thickness (LVPWd) were determined from echocardiographic tracings. Data are shown as the mean ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>flox/flox</th>
<th>CGI-58KOM</th>
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<tbody>
<tr>
<td>LVFS (%)</td>
<td>40.6 ± 0.7</td>
<td>21.4 ± 7.8*</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>119.9 ± 16.2</td>
<td>315 ± 80.0*</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>0.78 ± 0.04</td>
<td>1.51 ± 0.20*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>3.92 ± 0.32</td>
<td>4.21 ± 0.51</td>
</tr>
<tr>
<td>LVEDd (mm)</td>
<td>2.32 ± 0.21</td>
<td>3.60 ± 0.67*</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>0.69 ± 0.07</td>
<td>1.43 ± 0.19*</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>64.66 ± 0.82</td>
<td>50.03 ± 16.59</td>
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a p < 0.001 versus flox/flox mice.

b p < 0.01 versus flox/flox mice.

** FIGURE 3. Impaired cardiac lipolysis despite increased ATGL protein levels in CM of CGI-58KOM mice.** a, shown are TG hydrolytic activities in CM homogenates of female 14–15-week-old non-fasted and 14 h fasted CGI-58-KOM and flox/flox mice, respectively (n = 4–5). The addition of recombinant GST-tagged CGI-58 markedly increased the TG hydrolytic activity in CGI-58KOM cardiac homogenates but not that of control mice. ATGL mRNA levels were significantly reduced in 14 h fasted female CGI-58KOM mice (n = 4) (b), whereas ATGL protein concentrations were inversely increased in the CGI-58 deficient CM of 14 h fasted mice (c). The arrow indicates ATGL-specific signals. Western blot is representative for two individual tissue preparations. GAPDH served as loading control. Data are shown as mean ± S.D. *, p < 0.05; **, p < 0.01 and ***p < 0.001 versus flox/flox mice.

Reduced Lipoprotein Lipase Activity but Mildly Increased LPA Acyltransferase Activity in CM of CGI-58KOM Mice—To address whether additional metabolic changes could interfere with cardiac TG houseostasis in CGI-58KOM mice, we examined mRNA expression levels and enzyme activities of genes involved in heart FA uptake and lipid synthesis. CM acquires the vast bulk of FAs via LPL-catalyzed hydrolysis of TG-rich lipoproteins or from the CD36-mediated uptake of non-esterified FAs bound to albumin (35). LPL and CD36 mRNA expression levels (Fig. 4a) were markedly decreased (−38 and −52%, respectively) in CGI-58-deficient CM together with a marked reduction in CM LPL activity (Fig. 4b) of fasted CGI-58KOM mice (−73%).

Recombinant human or murine CGI-58 exhibits intrinsic LPA acyltransferase activity and generates phosphatidic acid (31, 37), which is an intermediate for TG and phospholipid synthesis. LPA acyltransferase activity was moderately increased in CM of CGI-58KOM mice (+23%) compared with cardiac LPA acyltransferase activity of flox/flox mice (Fig. 4c).

**FIGURE 4. mRNA levels of LPL and CD36 as well as determination of LPL and acyltransferase activities in cardiac preparations of CGI-58KOM and flox/flox mice.** a, LPL and CD36 mRNA levels were determined by qRT-PCR in CM of fasted CGI-58-KOM and flox/flox mice (n = 4), b, shown are TG hydrolytic activities in cardiac preparations of 3-month-old fasted CGI-58-KOM and control mice, respectively (n = 5). c, LPA acyltransferase activity was determined in CM microsomal fractions of non-fasted mice. The formation of radioactively labeled phosphatidic acid (PA) derived from LPA acylation was analyzed using 14C-labeled oleoyl-CoA as a radioactive tracer (n = 4), d, microsomal diacylglycerol acyltransferase activity in CM of non-fasted mice was determined by using a substrate containing dioleoylglycerol (emulsified with phosphatidylcholine) and 14C-labeled oleoyl-CoA (n = 4). Data are shown as the mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus flox/flox mice.

**FIGURE 4. mRNA levels of LPL and CD36 as well as determination of LPL and acyltransferase activities in cardiac preparations of CGI-58KOM and flox/flox mice.** a, LPL and CD36 mRNA levels were determined by qRT-PCR in CM of fasted CGI-58-KOM and flox/flox mice (n = 4), b, shown are TG hydrolytic activities in cardiac preparations of 3-month-old fasted CGI-58-KOM and control mice, respectively (n = 5). c, LPA acyltransferase activity was determined in CM microsomal fractions of non-fasted mice. The formation of radioactively labeled phosphatidic acid (PA) derived from LPA acylation was analyzed using 14C-labeled oleoyl-CoA as a radioactive tracer (n = 4), d, microsomal diacylglycerol acyltransferase activity in CM of non-fasted mice was determined by using a substrate containing dioleoylglycerol (emulsified with phosphatidylcholine) and 14C-labeled oleoyl-CoA (n = 4). Data are shown as the mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus flox/flox mice.

Impaired Cardiac Respiration and FAO Coupled to Low Expression Levels of PPARα and β/δ Targets in CGI-58-KOM Mice—In mice systemically lacking ATGL, impaired PPARα and PGC-1α signaling causes a defect in heart TG catabolism, reduced cardiomyocyte mitochondrial respiration, and heart dysfunction (17). Oxygen consumption was decreased in CMs of CGI-58KOM mice (Fig. 5a) compared with flox/flox CM under basal conditions (−50%) and upon the addition of succinate (−51%), indicating that the lack of CGI-58 could provoke cardiometabolic derangements similar to those observed in ATGL-deficient mice. To assess whether CGI-58 deficiency in CM also causes defective oxidative metabolism, we analyzed cardiac mRNA expression of genes involved in mitochondrial substrate oxidation and function. We found that mRNA levels of established PPARα and β/δ target genes including acyl-CoA oxidase (AOX), medium-chain acyl-CoA oxidase (MCAD), long-chain acyl-CoA oxidase (LCAD), carnitine palmitoyltransferase-1b (CPT1b), and pyruvate dehydrogenase kinase-4 (PDK4) were markedly decreased (ranging
from $-45$ to $-82\%$) in CMs of fasted CGI-58KOM mice (Fig. 5b) compared with cardiac mRNA levels of flox/flox mice. Moreover, mRNA concentration of PGC-1α, a nuclear transcriptional co-activator that initiates mitochondrial biogenesis and co-activates PPARα, was significantly reduced ($-64\%$) in CGI-58KOM CM (Fig. 5b). We also examined whether the reduction of FAO gene expression levels adversely impacts mitochondrial FA import and oxidation. CPT1 enzyme activity and oxidation of $^{14}$C-labeled oleic acid were significantly decreased ($-39\%$ and $-65\%$, respectively) in mitochondria prepared from CGI-58KOM CM compared with flox/flox preparations (Fig. 5c and d). In accordance with impaired FAO, acetyl- and acyl-CoA levels were significantly decreased in CM of CGI-58KOM mice compared with levels of flox/flox mice (Fig. 5e).

**Increased TG Deposition in SM of CGI-58KOM Mice but Unchanged in Vitro TG Hydrolytic Activities**—The efficient dual knockdown of CGI-58 in both CM and SM prompted us to examine the impact of muscle CGI-58 deficiency on lipid and energy catabolism in SM. The lack of CGI-58 significantly increased TG levels (ranging from 2.1- up to 11.1-fold) in all investigated SM types (Fig. 6a). However, in vitro TG hydrolytic activities of CGI-58KOM SM homogenates were comparable with those of flox/flox mice (Fig. 6b). Furthermore, the addition of recombinant GST-CGI-58 markedly increased lipolytic activities in both genotypes, and this stimulatory effect was most pronounced in m. soleus and m. tibialis of fasted CGI-58KOM mice. ATGL protein levels were increased in m. soleus of fasted CGI-58KOM mice, where the highest TG hydrolytic activities were measured (Fig. 6c). In contrast, ATGL protein content was mildly increased in m. tibialis of fasted CGI-58KOM mice compared with controls, whereas ATGL protein levels were similar in m. gastrocnemius of both genotypes (Fig. 6c).

**FAO Gene Expression Levels and Mitochondrial FA Oxidation Were Unchanged in SM of CGI-58KOM Mice**—In analogy to unchanged TG hydrolytic activities, mRNA levels of established PPARα and β/δ target genes (Fig. 7a) and mitochondrial oxidation of $^{14}$C-labeled oleic acid (Fig. 7b) were similar in SM preparations of CGI-58KOM and flox/flox mice, respectively.
determined after 30 min of exercise and compared with samples derived from resting (non-fasted) mice. Plasma TG and FA concentrations were reduced in exercised CGI-58KOM mice compared with exercised flox/flox controls (106.9 ± 0.2 TG mg/dl for flox/flox mice versus 79.7 ± 0.1 mg/dl for CGI-58KOM, p < 0.057, n = 4–5; 0.65 ± 0.08 free fatty acid mmol/liter for flox/flox mice versus 0.47 ± 0.13 mmol/liter for CGI-58KOM mice, p < 0.040, n = 4–5). Blood glucose levels were moderately decreased in CGI-58KOM mice (−19%), although levels did not reach statistical significance (data not shown). Exercise provoked a significant decrease in SM TG levels of flox/flox mice (−61%) compared with levels of resting mice, whereas TG levels remained unchanged in CGI-58KOM mice (Fig. 8a). However, TG hydrolytic activities were similar in SM lysates of both genotypes independent of whether tissues were derived from resting, trained, or fasted mice (Fig. 8b). Yet ATGL protein levels were increased in SMs of resting or exercised CGI-KOM mice compared with controls (Fig. 8c). Analysis of mRNA levels of FAO genes revealed no significant differences in SMs derived from exercised CGI-58KOM mice compared with controls. In contrast, LPL and CD36 mRNA levels were significantly increased (2.2- and 2.4-fold, respectively) in SMs of exercised CGI-58KOM mice compared with exercised flox/flox mice (Fig. 8d). Together, data indicate a defect in SM TG catabolism of CGI-58KOM mice, which is even more evident in exercising animals. As a compensatory measure, LPL and CD36 expression is up-regulated.

**Improved Glucose Tolerance, Normal Insulin Sensitivity, and Substantially Increased Cardiac Glucose Uptake in CGI-58-KOM Mice**—Ectopic accumulation of lipids and lipid metabolites as a consequence of increased FA uptake, lipid synthesis, or impaired fat catabolism are often associated with the development of insulin resistance and type 2 diabetes (38). Fat accumulation in the CM and SM of CGI-58KOM mice despite normal circulating FA levels prompted us to examine whether ectopic fat accumulation alone impairs insulin sensitivity and glucose homeostasis. Blood glucose and plasma insulin levels (Fig. 9, a and b) were significantly lower (−18% and −46%, respectively) in re-fed CGI-58KOM mice compared with flox/flox mice, indicating an increased insulin sensitivity of CGI-58KOM mice. The clearance of glucose from the circulation was enhanced in CGI-58KOM mice (Fig. 9c) compared with flox/flox mice. The glucose response to insulin was similar in CGI-58KOM mice and control mice (Fig. 9d). To determine whether the improved glucose tolerance originates from a tissue-specific increase in glucose uptake, we injected glucose together with [2-3H]deoxyglucose as radioactive tracer and examined the formation of [2-3H]deoxyglucose-6-phosphate in several tissues. CGI-58KOM mice showed a 6.7-fold increase in CM glucose uptake (Fig. 9e), whereas glucose uptake was similar in SM (m. quadriceps), liver, and adipose tissue of both genotypes. To address the consequences of muscle CGI-58 deficiency on whole body energy catabolism, we kept mice in metabolic cages and monitored oxygen consumption and carbon dioxide output for calculating RQ values. A RQ value close to 1 reflects mainly glucose utilization as oxidative substrate, whereas a drop indicates a switch toward more FA oxidation. In accordance with decreased plasma glucose concentrations (Table 1), the
increased RQ values of CGI-58KOM mice during the light and dark phase (Fig. 9f) indicated a shift toward preferential glucose utilization as oxidative fuel.

Finally, we investigated the impact of muscle CGI-58 deficiency on TG homeostasis in non-muscle tissue that may additionally interfere with whole body energy metabolism. Body mass composition of CGI-58KOM mice was comparable to flox/flox mice (Table 3). Accordingly, weights of white (gonadal) and brown adipose tissue and liver were similar in both genotypes (Table 3). Nonetheless, liver TG levels were moderately but significantly decreased (−24%) in fasted CGI-58KOM mice (Table 3), which prompted us to examine whether muscle CGI-58 deficiency secondarily affects TG catabolism in peripheral organs. TG hydrolytic activities were similar in adipose tissue and liver lysates of fasted CGI-58KOM mice compared with controls (Table 3). Furthermore, ATGL protein expression levels in gonadal and brown adipose tissue and liver were unchanged in CGI-58KOM mice compared with controls (data not shown). In summary, these findings demonstrate that muscle TG catabolism critically depends on both CGI-58 and ATGL and that impaired muscle TG catabolism via the lack of CGI-58 affects whole body lipid and glucose homeostasis.

DISCUSSION

The generation of a mouse model lacking CGI-58 exclusively in CM and SM allowed us to examine the muscle-specific role of CGI-58 in energy metabolism and to exclude phenotypic effects caused by the global lack of CGI-58 as evident in mice lacking ATGL (17). We show that CGI-58 is an absolute requi-
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site for ATGL-mediated TG catabolism in CM and that the lack of CGI-58 in CM cannot be compensated by increased ATGL protein levels. The functional role of CGI-58 as an ATGL co-activator is particularly apparent by the substantial increase in TG hydrolytic activity of CGI-58-deficient CM homogenates upon the addition of recombinant CGI-58. The marked increase in ATGL protein levels in CM of CGI-58KOM mice did not correlate with mRNA expression levels, which were actually reduced. ATGL is a PPARγ-regulated gene, and mice lacking PPARγ show a strong defect in the induction of ATGL mRNA expression upon PPARγ agonist treatment (39). Therefore, it is very likely that the reduction in ATGL mRNA expression levels is a consequence of impaired PPARγ-activated gene expression, which is in accordance with the robust decrease of mRNA levels of established PPARγ-target genes in CM of CGI-58KOM mice. This conclusion implies that the increase in ATGL protein levels of CGI-58KOM mice is caused by a post-translational mechanism leading to increased protein stability.

Furthermore, muscle CGI-58 deficiency provoked heart dysfunction associated with massive TG accumulation, decreased FAO gene expression levels, impaired mitochondrial FAO, and reduced oxygen consumption in CM. All these phenotypic changes are also present in ATGL-deficient mice (17), strongly suggesting that the cardio-metabolic derangements of CGI-58KOM mice primarily originate from defective cardiac lipolysis caused by diminished stimulation of ATGL-mediated TG catabolism in the absence of CGI-58.

Excess TG accumulation in CM is associated with the development of cardiomyopathy in humans and animal models (4). It is generally assumed that metabolic derangements including defective or increased FAO and/or the accumulation of toxic lipids are causative for the development of heart dysfunction. Nevertheless, fat accumulation per se and the occurrence of large lipid droplets may disturb the cellular integrity and as a consequence affect cardiac function. Notably, the life span of CGI-58KOM mice was significantly prolonged compared with that of ATGL-deficient mice despite impaired mitochondrial FAO and comparable TG levels in CM of both animal models. This observation suggests that the severity of cardiac dysfunction in CGI-58KOM and ATGL-deficient mice is not exclu-

FIGURE 8. Analysis of TG homeostasis, ATGL protein levels, and mRNA expression of FA metabolizing genes in exercised CGI-58KOM mice. a, shown are tissue TG levels in m. tibialis of resting (non-fasted), exercised, and 14 h fasted female 12–14-week-old CGI-58KOM mice compared with controls (n = 4–5). ON, overnight. b, TG hydrolytic activities in SM homogenates (m. soleus) prepared from resting, exercised, and ON (14 h) fasted female mice, respectively (samples from at least 2 pools per genotype, each containing tissues of 3 mice), were analyzed. c, shown are ATGL protein levels in m. soleus prepared from resting and exercised mice; GAPDH served as the loading control. d, shown are mRNA levels of selected PPARα and β/δ target genes, LPL, and CD36 in m. tibialis prepared from resting and exercised mice, respectively (n = 4–5). AOX, acyl-CoA oxidase; MCAD, medium-chain acyl-CoA oxidase; LCAD, long-chain acyl-CoA oxidase; CPT1b, carnitine palmitoyltransferase-1b. Data are shown as the mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus flox/flox mice. #, p < 0.05, and ##, p < 0.01, refer to resting flox/flox mice versus exercised and fasted flox/flox mice, respectively.
sively determined by defective cardiac lipolysis and steatosis but additionally involves systemic changes in energy catabolism. Global ATGL deficiency is associated with a marked drop in circulating FA levels due to defective TG mobilization adipose tissue, whereas plasma FA levels were unchanged in CGI-58KOM mice. It is conceivable that the early lethal phenotype of ATGL-deficient mice is a consequence of both cardiometabolic derangements and CM starvation due to systemic energy depletion. Whether the divergent clinical picture of humans carrying mutated CGI-58 or ATGL alleles involves systemic changes in energy catabolism is not known.

Purified recombinant human or mouse CGI-58 has been reported to exhibit LPA acyltransferase activity (31, 37), leading to increased formation of phosphatidic acid, which is an intermediate of phospholipid and TG synthesis. Yet in our study LPA acyltransferase activity was mildly increased in CGI-58-deficient CM compared with flox/flox preparations, suggesting that CGI-58 does not play a significant role in LPA acylation in CM.

The lack of muscle CGI-58 also substantially increased TG levels in SM, indicating that the lack of CGI-58 similarly impairs TG catabolism in SM as in CM. However, and in contrast to CM, the lack of CGI-58 in SM neither affected TG hydrolytic activities nor PPARα-activated gene expression and subsequently mitochondrial FAO. Notably, the addition of recombinant CGI-58 markedly stimulated in vitro TG hydrolytic activities in SM preparations of both flox/flox and CGI-58KOM mice, demonstrating that the TG hydrolytic capacity of SM depends on the availability of CGI-58. Further-

FIGURE 9. Increased glucose tolerance and glucose uptake in CM of CGI-58KOM mice. Blood glucose (a) and plasma insulin (b) concentrations were determined in re-fed 16–18-week-old female flox/flox and CGI-58KOM mice, respectively (n = 6–8). Glucose (c) and insulin (d) tolerance tests of 18-week-old female mice were fasted for 6 and 4 h, respectively (n = 5–6; AUC, normalized area under the curve). e, tissue-specific glucose uptake was determined in 14–16-week-old female mice (n = 6) using [2-3H]-deoxyglucose as radioactive tracer. f, shown are RQs in 15–16-week-old male flox/flox and CGI-58KOM mice during the light and dark period. The rise in RQ values of CGI-58KOM mice is an indication for augmented glucose oxidation. Data are shown as the mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus flox/flox mice.

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TABLE 3

Body mass composition, tissue weights, hepatic TG levels, and TG hydrolytic activities in non-muscle tissue of control and CGI-58KOM mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>flox/flox</th>
<th>CGI-58KOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean body mass (%)</td>
<td>71.4 ± 1.6</td>
<td>74.1 ± 3.1</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>22.0 ± 1.7</td>
<td>19.4 ± 3.5</td>
</tr>
<tr>
<td>WAT weight (%)</td>
<td>0.64 ± 0.17</td>
<td>0.47 ± 0.12</td>
</tr>
<tr>
<td>BAT weight (%)</td>
<td>0.13 ± 0.03</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>Liver weight (%)</td>
<td>4.33 ± 0.32</td>
<td>4.46 ± 0.93</td>
</tr>
<tr>
<td>Liver TG non-fasted (μg TG/mg protein)</td>
<td>12.97 ± 3.49</td>
<td>9.84 ± 5.49</td>
</tr>
<tr>
<td>Liver TG 14 h fasted (μg TG/mg protein)</td>
<td>77.60 ± 13.92</td>
<td>58.93 ± 7.95</td>
</tr>
<tr>
<td>WAT TG hydrolyase activity (nmol FFA/h/mg protein)</td>
<td>687.3 ± 90.8</td>
<td>648.7 ± 118.5</td>
</tr>
<tr>
<td>BAT TG hydrolyase activity (nmol FFA/h/mg protein)</td>
<td>362.4 ± 38.9</td>
<td>382.5 ± 66.6</td>
</tr>
<tr>
<td>Liver TG hydrolyase activity (nmol FFA/h/mg protein)</td>
<td>15.2 ± 3.3</td>
<td>13.4 ± 1.5</td>
</tr>
</tbody>
</table>

*p < 0.05.

more, ATGL protein levels were markedly increased in oxidative m. soleus, probably as an adaptation to impaired TG catabolism in the absence of CGI-58. Notably, mRNA levels of LPL and CD36, two major players that mediate FA influx to myocytes, were markedly increased in the SM of exercised CGI-58KOM mice compared to controls. We hypothesize that an enhanced FA influx may compensate for the defect in endogenous TG catabolism and the reduced generation of FA as oxidative fuel. Taken together, these data suggest that the substantial increase in SM TG levels is caused by impaired TG catabolism, which is compensated by increased FA influx (increased LPL and CD36 mRNA) and glucose utilization (increased RQ). Such a conclusion is in line with a recent study reporting that the knockdown of CGI-58 in differentiated human myotubes markedly reduced TG catabolism and increased glucose oxidation (40).

Defective lipolysis in ATGL-deficient mice markedly reduced mRNA expression levels of established PPARα target genes in the heart and liver, suggesting that PPARα-activated gene expression critically depends on endogenous TG catabolism (17). This view is also consistent with our study, showing that defective cardiac lipolysis in the absence of CGI-58 impairs PPARα-activated gene expression. In contrast to the heart, expression of genes involved in FAO was not impaired in SM of CGI-58KOM mice, although SM showed marked TG accumulation. Because LPL mRNA levels were markedly reduced in SM of CGI-58KOM mice, whereas LPL mRNA levels were increased in SM of fasted or exercised CGI-58KOM mice, this may suggest that in contrast to CM, PPARα-activated gene expression in SM does not critically depend on endogenous TG catabolism. In line with this notion, mice globally lacking ATGL similarly showed no obvious defect in mitochondrial FAO of SM (41) despite massive TG accumulation.

Increased SM TG deposition often correlates with the development of insulin resistance (4). However, this view was challenged by the phenotype of ATGL-deficient mice, which showed increased glucose and insulin tolerance despite massive TG accumulation in SM. The severe decline of circulating insulin levels of ATGL-deficient mice is believed to be the consequence of both defective pancreatic insulin secretion and increased tissue insulin sensitivity (36, 42). Defective adipose tissue lipolysis in ATGL-deficient mice provoked a decreased availability of FA as oxidative fuel that could favor organ glucose utilization in line with the Randle (34) hypothesis. Similarly, CGI-58KOM mice showed increased glucose tolerance, elevated RQ levels, and significantly reduced plasma insulin levels, whereas glucose uptake was substantially increased in CM. Yet circulating FA levels were unchanged in CGI-58KOM mice, strongly suggesting that changes in glucose homeostasis and insulin levels of CGI-58KOM mice are a consequence of impaired muscle TG catabolism.

In summary, our study demonstrates that functional muscle lipolysis critically depends on both proteins, CGI-58 and ATGL. Defective muscle lipolysis interfered with whole body energy homeostasis of CGI-58KOM mice. The more moderate phenotype of CGI-58KOM mice compared with the early lethality of ATGL-deficient mice may indicate that the severe cardiomyopathy of NLSD with myopathy patients is not the exclusive consequence of defective cardiac lipolysis but additionally involves changes in whole body energy catabolism. Furthermore, it appears that CGI-58 is not limiting in cardiac lipolysis in humans in contrast to mice. This would explain the divergent cardiac phenotype between NLSD with myopathy and with ichthyosis patients.

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