

Resistance training prevents the development of sarcopenia induced by chronic cholestatic liver disease

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Abstract

Sarcopenia secondary to chronic cholestatic liver disease (CCLD) is linked to activation of the ubiquitin-proteasome system (UPS) and autophagy-lysosome system (ALS), impaired mitochondrial dynamics, and diminished mTOR signaling. Resistance training (RT) has shown benefits against age-related sarcopenia, but its effects on CCLD-associated sarcopenia have not been explored. This study investigated the effects of RT in a mouse model of CCLD-induced sarcopenia. Male C57BL/6J mice developed CCLD-induced sarcopenia via a six-week diet containing the hepatotoxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine while performing RT. Assessments included muscle strength, mass, physical performance, fiber diameter, cross-sectional area (CSA), ultrastructure, mitochondrial morphology, dynamics, respiratory function, ALS and UPS markers at the transcriptional and protein levels, and mTOR phosphorylation. RT markedly enhanced muscle strength, mass, and performance in CCLD mice. It maintained muscle fiber diameter and CSA, prevented loss of sarcomere proteins, and improved ultrastructure. RT preserved mitochondrial density, dynamics (DRP-1, mitofusin-2, OPA-1), PGC-1 α levels, and oxygen consumption, normalized MuRF-1 and atrogin-1 expression, prevented transcriptional and protein induction of autophagy markers (LC3B, p62), and restored mTOR phosphorylation. These results demonstrate that RT prevents CCLD-related sarcopenia by inhibiting proteolytic systems and reactivating anabolic and mitochondrial pathways, offering a promising non-drug approach to protect skeletal muscle in this condition.

Keywords Liver disease · Resistance training · Sarcopenia · Skeletal muscle

Abbreviations

ALS Autophagy-lysosome system
ATP Adenosine triphosphate
ANOVA Analysis of variance

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Claudio Cabello-Verrugio, Mayalen Valero-Breton and Franco Tacchi contributed equally to this work.



BW	body weight
CCLD	Chronic cholestatic liver disease
CSA	Cross-Sectional Area
DDC	3,5-diethoxycarbonyl-1,4-dihydrocollidine
DRP-1	Dynamin-Related Protein 1
EDL	Extensor digitorum longus
GGT	gamma-glutamyl transferase
GOT	glutamate-oxaloacetate transaminase
GPT	glutamic pyruvic transaminase
mTOR	Mammalian target of rapamycin
OCR	Oxygen consumption rate
OPA-1	Optic atrophy 1
PGC-1 α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PBS	Phosphate-buffered saline
RIPA	Radioimmunoprecipitation assay
RT	Resistance training
SD	Standard deviation
SED	Sedentary
TA	Tibialis anterior
TEM	Transmission electron microscopy
UPS	Ubiquitin-proteasome system
WGA	Wheat germ agglutinin

Introduction

Skeletal muscle plays essential roles in movement, posture, and respiration, among other functions¹. Although specific properties, such as muscle shape, spatial organization, fascicle orientation, and attachment to tendons and bones, are established during development, skeletal muscle remains highly adaptable to physiological and pathological stimuli throughout life. This plasticity enables the tissue to modify its functional properties, including strength and mass, through structural and molecular adaptations in response to changing demands².

Resistance training (RT) is a well-established intervention that enhances muscle mass and strength through a process known as hypertrophy, characterized by increased muscle fiber diameter, organelle biogenesis, and elevated contractile protein content³. Furthermore, RT has been shown to trigger mitophagy, increase mitochondrial cristae density, and modify mitochondrial morphology, thereby improving muscle metabolism^{4,5}. This hypertrophic and metabolic adaptation is mainly driven by the Akt/mTOR pathway, which promotes protein synthesis and inhibits FoxO-dependent catabolic programs⁶. In parallel, mitochondrial adaptation to RT depends on a coordinated fission-fusion cycle, mediated by DRP-1 (fission) and mitofusin-2 and OPA-1 (fusion), together with PGC-1 α -driven biogenesis. Together, these processes maintain the mitochondrial network required to preserve muscle mass⁷.

In contrast, sarcopenia is defined as a progressive and generalized loss of muscle mass and strength, leading to impaired physical performance. It is associated with frailty, altered metabolic regulation, reduced quality of life, and increased mortality⁸. Sarcopenia is classified as primary when age-related and secondary when it arises in the context of chronic diseases, including chronic liver diseases. Its diagnosis is based on clinical assessments of muscle strength, mass, and physical performance⁹.

Chronic liver diseases represent a major global health burden and encompass a broad spectrum of hepatic disorders that can progress to cirrhosis and hepatocellular carcinoma¹⁰. These conditions are broadly categorized as

cholestatic and non-cholestatic etiologies¹¹. Chronic cholestatic liver disease (CCLD) results from impaired bile acid transport or bile duct obstruction and is characterized by ductular fibrosis, hepatocellular injury, and progressive liver dysfunction^{12,13}. A hallmark of CCLD is the accumulation of circulating bile acids, which occurs early in these diseases^{11–13}.

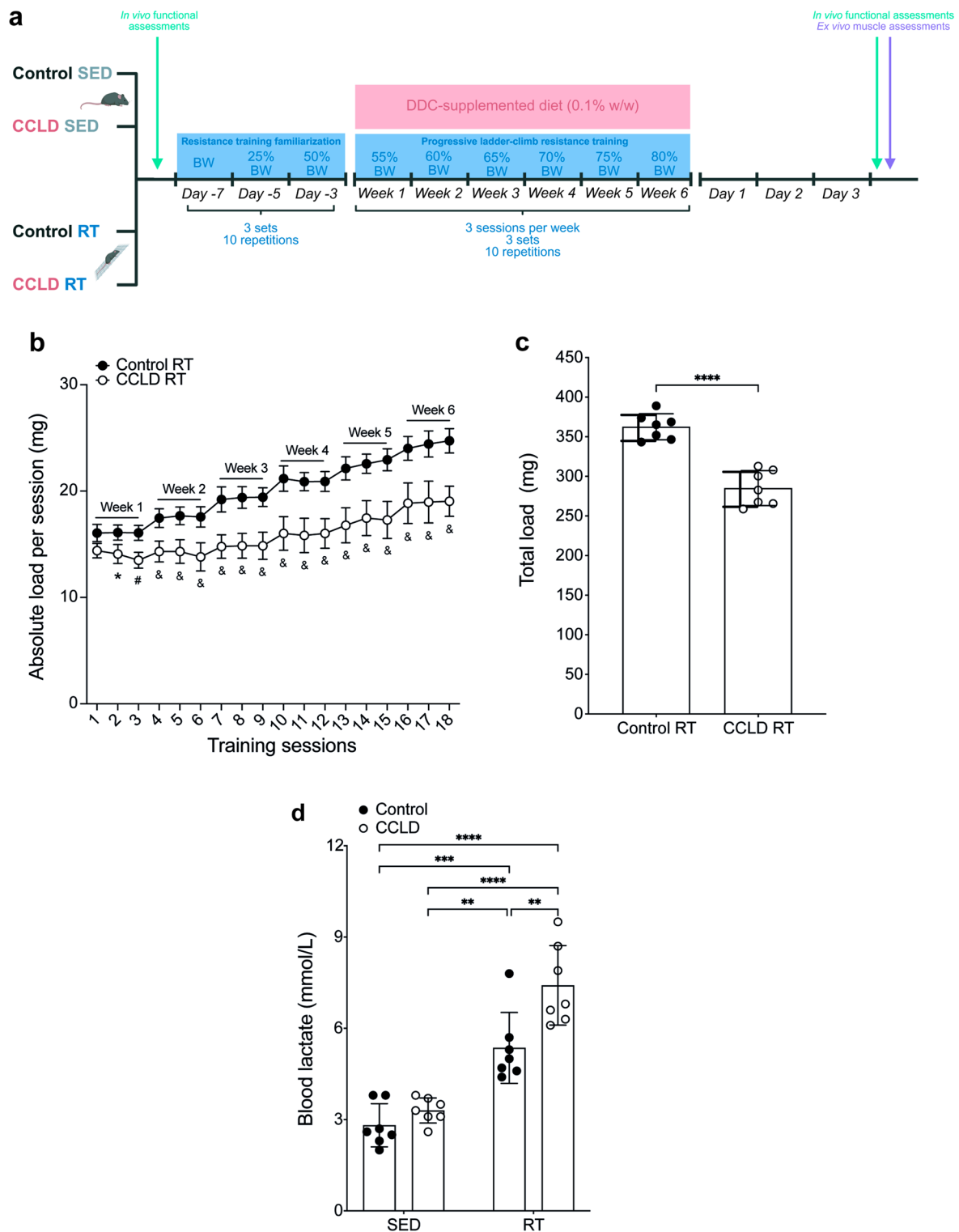
Among the complications of chronic liver diseases, sarcopenia is highly prevalent, affecting up to 60% of patients with advanced disease¹⁴. In this population, sarcopenia is associated with poor prognosis, increased hospitalization, higher risk of hepatic decompensation, and elevated mortality¹⁵. Notably, it is recognized as an independent predictor of mortality before and after liver transplantation, with sarcopenic patients exhibiting lower survival rates compared to non-sarcopenic individuals^{14,15}. In addition to their classical role in lipid digestion and absorption, bile acids also act as signaling molecules in extrahepatic tissues, including skeletal muscle^{16–18}. Our previous studies have demonstrated that elevated circulating bile acids in CCLD directly contribute to sarcopenia by activating multiple pathways that promote the loss of strength and muscle mass. This activation involves the ubiquitin-proteasome system (UPS), which is upregulated by typical atrogenes, such as E3-ubiquitin ligases MuRF-1 and atrogin-1^{16,19,20}. Muscle proteolysis also involves the autophagy-lysosome system (ALS), which is concurrently activated by the same FoxO1/FoxO3 transcriptional program that induces MuRF-1 and atrogin-1, providing a second, coordinated route for protein degradation^{21–23}. In murine models, the expression of bile acid receptors in skeletal muscle is required for the development of muscle dysfunction associated with CCLD, highlighting a mechanistic link between liver pathology and muscle wasting^{16,19,20}.

While RT is widely recognized as an effective strategy to counteract age-related sarcopenia, most exercise-based interventions in patients with chronic liver disease have focused on endurance training²⁴. Nevertheless, emerging evidence from a limited number of clinical studies suggests that progressive RT may improve muscle strength and mass in patients with chronic liver disease, particularly those with cirrhosis, potentially leading to enhanced quality of life^{25,26}. However, no study to date has specifically investigated the impact of RT on sarcopenia secondary to CCLD, leaving a critical gap in the understanding of therapeutic strategies for this condition. Therefore, this study aimed to evaluate the effects of 6 weeks of RT on muscle strength, muscle mass, and physical performance in a murine model of CCLD-induced sarcopenia. We also assessed sarcomere protein content, muscle fiber diameter, CSA, ultrastructural features, and mitochondrial morphology parameters to provide a comprehensive analysis of RT-induced adaptations.

Results

Mice with CCLD-induced sarcopenia perform resistance training with lower absolute loads but exhibit higher physiological effort

To characterize training load across groups, absolute per-session load and cumulative training volume were monitored throughout the 6-week protocol (Fig. 1a). As shown in Fig. 1b, both Control RT and CCLD RT animals progressively increased the load they carried across sessions. However, due to lower body weight, CCLD animals lifted significantly less absolute load than controls across multiple sessions (interaction effect, $p < 0.0001$). This was also reflected in the total cumulative load, which was significantly lower in the CCLD RT group than in the Control RT group (Fig. 1c, $p < 0.0001$, 362.7 ± 16.37 vs. 285.1 ± 22.04). Notably, despite carrying lower loads, CCLD RT animals exhibited significantly higher blood lactate concentrations than Control RT (Fig. 1d), indicating a greater relative physiological effort ($p = 0.0029$; 5.357 ± 1.165 vs. 7.414 ± 1.307). Both RT groups showed higher lactate levels than their sedentary counterparts (exercise effect, $p < 0.0001$). Additionally, RT partially prevented liver alterations in CCLD, as indicated by bile acid and GOT/GGT enzyme levels (Supplemental Material 1).



These results confirm that the training stimulus required substantial effort across groups, thereby validating the effectiveness of the protocol even under CCLD conditions.

Fig. 1 Load progression and physiological effort during the resistance training protocol. **(a)** Experimental timeline showing the 6-week protocol combining a DDC-supplemented diet (0.1% w/w) to induce chronic cholestatic liver disease and a progressive ladder-climb resistance training program. Before the intervention, all mice underwent a 1-week familiarization period (0–50% of body weight). Training sessions were conducted three times a week, consisting of three sets of ten repetitions with progressively increasing loads (55–80% of body weight). **(b)** Absolute load per session was recorded for each of the 18 training sessions, and **(c)** total training load. **(d)** Blood lactate concentration was measured immediately after the last exercise session. Data represent independent biological samples (one animal per datapoint) and are presented as mean $\pm$ SD ($n=7$ animals per group). Two-way ANOVA with Tukey's post hoc test (**b**, **d**) and unpaired t-test (**c**) were performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Resistance training in mice with CCLD-induced sarcopenia prevents losses in muscle strength, fat-free mass, and physical performance

Resistance training prevented sarcopenia by preserving muscle strength (Fig. 2). The loss of grip strength in the CCLD SED group compared to Control SED in the forelimb (Fig. 2a; $p<0.0001$, 6.10 ± 0.19 vs. 3.96 ± 0.43) and hindlimb (Fig. 2b; $p<0.0046$, 7.37 ± 0.80 vs. 5.83 ± 0.35) was mitigated in the CCLD RT group (Fig. 2a; forelimb, $p<0.0001$, 3.96 ± 0.43 vs. 5.80 ± 0.35 ; Fig. 2b; hindlimb, $p<0.001$, 5.83 ± 0.35 vs. 7.91 ± 0.57). Ex vivo contractile force of the tibialis anterior muscle was also preserved in CCLD RT mice (Fig. 2c), with significantly higher force generation than CCLD SED at 10 Hz ($p=0.013$), 20 Hz ($p<0.0001$), 40 Hz ($p<0.0001$), 60 Hz ($p<0.0001$), 80 Hz ($p<0.0001$), 100 Hz ($p<0.0001$), and 150 Hz ($p<0.0001$) (Fig. 2c). Additionally, maximal specific force was significantly higher in CCLD RT than in CCLD SED (Fig. 2d; $p<0.0001$, 150.11 ± 9.86 vs. 104.87 ± 10.45) and was higher than Control SED (Fig. 2d; $p=0.0388$, 150.11 ± 9.86 vs. 134.09 ± 9.71).

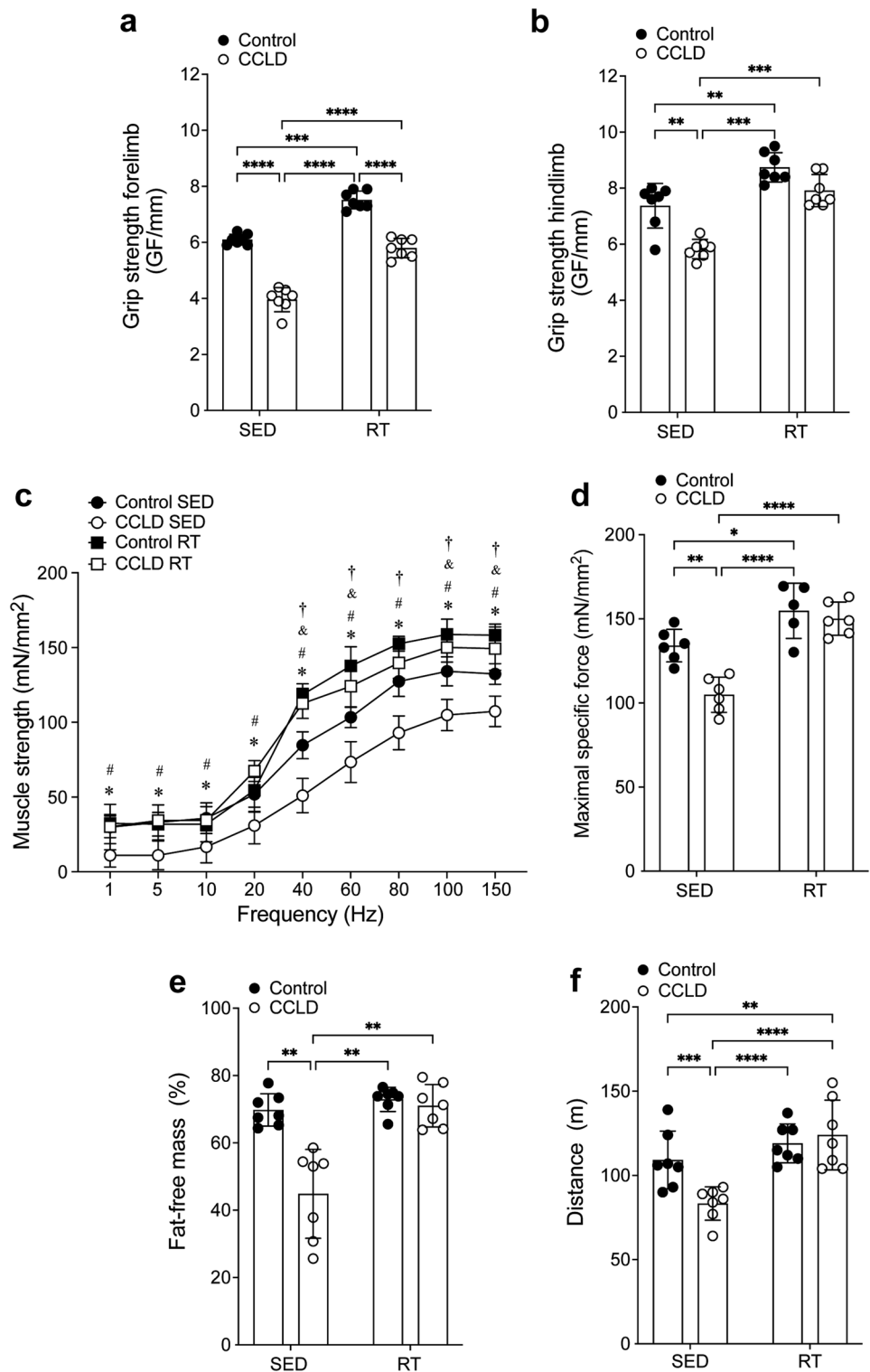
Muscle mass, assessed by fat-free mass through bioimpedance, was reduced in CCLD SED animals (Fig. 2e; $p=0.0020$, vs. 69.77 ± 4.81 vs. 44.85 ± 13.19), whereas it was preserved in CCLD RT animals (Fig. 2e; $p=0.9847$, 69.77 ± 4.81 vs. 71.03 ± 6.31) compared to Control SED. Moreover, physical performance, assessed through an incremental treadmill test, CCLD SED animals showed a significant decline in running distance compared to Control SED (Fig. 2f; $p=0.0004$, 109.14 ± 17.18 vs. 83.29 ± 9.93), whereas was maintained in CCLD RT animals (Fig. 2f; $p<0.0001$, 109.14 ± 17.18 vs. 124.00 ± 20.72).

These findings demonstrate that RT effectively prevents loss of muscle strength, muscle mass, and function in CCLD-induced sarcopenia, reinforcing its role as a therapeutic strategy.

To evaluate the effect of RT on muscle fiber morphology, muscle cross-sections were stained with WGA to delineate fibers and determine their diameters and CSA by fluorescence microscopy (Fig. 3a). In CCLD SED animals, a significant reduction in muscle fiber diameter was observed in three of the four hindlimb muscles analyzed compared to Control SED. Specifically, reduced mean fiber diameter was evident in the tibialis anterior (Fig. 3b, $p=0.0064$, 40.68 ± 1.03 vs. 34.71 ± 1.21), and CSA was also diminished (Fig. 3c, $p=0.0045$, $1,775.09\pm44,81$ vs. $1,514.75\pm52.60$). In the gastrocnemius, the CCLD SED group showed a reduced fiber diameter (Fig. 3d, $p=0.0193$, 33.56 ± 1.01 vs. 28.87 ± 2.68), and CSA (Fig. 3e, $p=0.0476$, $2,148.07\pm64,64$ vs. $1,919.88\pm69.42$) compared to Control SED. In the EDL, fiber diameter (Fig. 3f, $p=0.0025$, 29.64 ± 1.86 vs. 25.43 ± 1.03) and CSA (Fig. 3g, $p=0.0008$, $1,199.56\pm75,35$ vs. $1,029.33\pm41.54$) were decreased in the CCLD SED compared to Control SED. No changes were observed in soleus muscle fiber diameter (Fig. 3h, $p=0.5211$, 32.34 ± 3.07 vs. 31.14 ± 3.59) or CSA (Fig. 3i, $p=0.5563$, $875.95\pm83,18$ vs. 843.30 ± 97.19). This effect was also reflected in the fiber size distribution curves, with a clear leftward shift in CCLD SED compared to Control SED in tibialis anterior (Supplemental Material 2a, group $\times$ size interaction, $p<0.0001$), gastrocnemius (Supplemental Material 2c, group $\times$ size interaction, $p<0.0001$) and EDL muscles (Supplemental Material 2e, group $\times$ size interaction, $p<0.0001$). In contrast, the soleus muscle showed no significant differences in either mean fiber diameter (Fig. 3e, $p=0.8855$, 32.34 ± 3.07 vs. 31.14 ± 3.59) or fiber size distribution (Supplemental Material 2 g, group $\times$ size interaction, $p=0.5255$).

Regarding the effects of RT, significant increases in mean fiber diameter (Figs. 3b, $p=0.0018$, 34.71 ± 1.21 vs. 39.22 ± 1.44) and CSA (Fig. 3c, $p=0.0019$, $1,514.75\pm52.60$ vs. $1,711.45\pm62.95$) were observed in CCLD

Fig. 2 RT prevents the decline in strength, fat-free mass, and performance in CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Forelimb and **(b)** hindlimb grip strength were measured. **(c)** The force-frequency curve was obtained from electrical stimulation of the tibialis anterior muscle across a range of frequencies (1–150 Hz), and **(d)** maximal specific force was extracted from the frequency response curve. **(e)** Fat-free mass percentage, and **(f)** running distance covered in a treadmill exhaustion test. Data represent independent biological samples (one animal per datapoint) and are presented as mean $\pm$ SD ($n=5-7$ animals per group). Mixed-effects models with Tukey's post hoc test were performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



RT animals when compared to CCLD SED in both the tibialis anterior and gastrocnemius (Fig. 3d, $p=0.0068$, 28.87 ± 2.68 vs. 34.02 ± 3.43), (Fig. 3e, $p=0.0287$, $1,919.88 \pm 69.42$ vs. $2,177.40 \pm 219.79$). Additionally, similar changes were detected in the EDL for fiber diameter (Fig. 3f, $p=0.0030$, 25.43 ± 1.03 vs. 29.32 ± 1.15) and CSA (Fig. 3g, $p=0.0023$, $1,029.33 \pm 41.54$ vs. $1,182.73 \pm 46.72$). In contrast, no significant changes were detected

in soleus muscle fiber diameter (Fig. 3h, $p=0.8391$, 31.14 ± 3.59 vs. 31.54 ± 2.08) or CSA (Fig. 3i, $p=0.8470$, 843.30 ± 97.19 vs. 854.08 ± 56.27). Distribution analysis further confirmed the effect of RT on the tibialis anterior (Supplemental Material 2b, group $\times$ size interaction, $p<0.0001$) and EDL (Supplemental Material 2f, group $\times$ size interaction, $p<0.0001$), with a rightward shift in fiber size within specific diameter ranges. In the gastrocnemius, however, although the mean fiber diameter was increased, the distribution curve did not show an apparent displacement (Supplemental Material 2d, group $\times$ size interaction, $p=0.2656$). No changes were observed in the soleus distribution (Supplemental Material 2h, group $\times$ size interaction, $p=0.5255$).

These results indicate that RT mitigates muscle fiber atrophy in CCLD-induced sarcopenia by preserving fiber diameter and preventing the shift toward smaller diameters in specific hindlimb muscles.

Resistance training prevents alterations in sarcomere ultrastructure and reductions in sarcomere protein levels in mice with CCLD-induced sarcopenia

Transmission electron microscopy analysis revealed alterations in sarcomere structure of tibialis anterior muscles (Fig. 4a), including reduced Z-line width in CCLD SED compared to Control SED (Fig. 4b; $p=0.0162$, 76.0 ± 5.13 vs. 57.97 ± 6.32), an effect that was prevented by RT ($p=0.0059$, 57.97 ± 6.32 vs. 83.58 ± 2.37). Similarly, sarcomere length was significantly shorter in CCLD SED than in Control SED (Fig. 4c; $p=0.0219$, 2190.41 ± 217.59 vs. 1795.10 ± 66.96), whereas RT preserved this parameter ($p=0.0072$, 1795.10 ± 66.96 vs. 2378.97 ± 38.21), with values similar to the Control SED group.

Sarcomere proteins are essential for skeletal muscle contraction and function. Tropomyosin and troponin I protein levels, assessed by western blot (Fig. 4d), were significantly reduced in tibialis anterior from CCLD SED compared to Control SED (Fig. 4e; tropomyosin: $p=0.0002$; 1.00 ± 0.16 vs. 0.44 ± 0.17 ; Fig. 4f; troponin I: $p=0.0160$; 1.00 ± 0.16 vs. 0.44 ± 0.15). RT prevented this reduction, with higher protein abundance in CCLD RT than CCLD SED for tropomyosin ($p<0.0001$; 1.72 ± 0.23 vs. 0.44 ± 0.17) and troponin I ($p<0.0001$; 1.49 ± 0.17 vs. 0.44 ± 0.15).

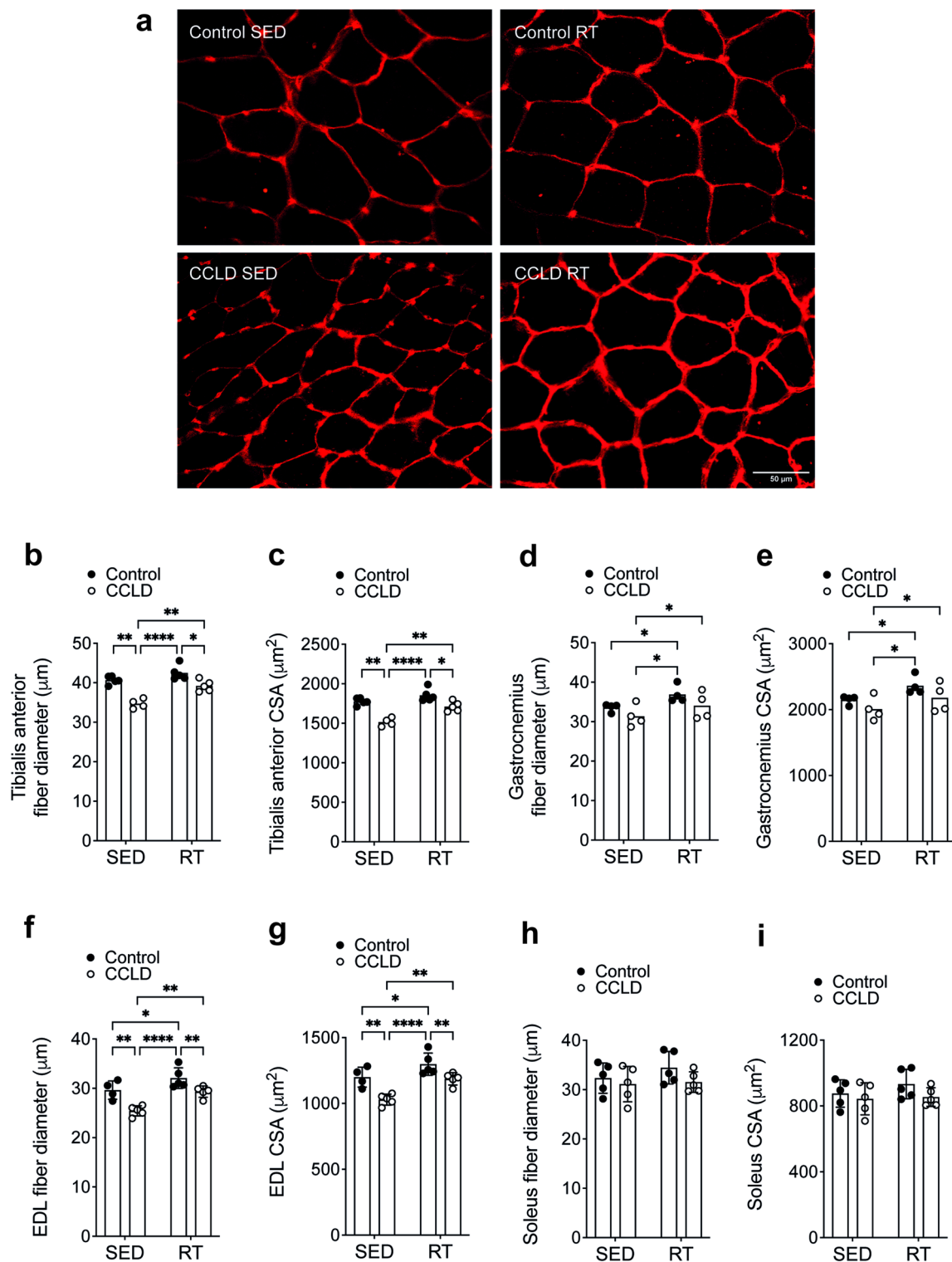
These findings highlight RT as an effective intervention to counteract sarcopenia-induced structural deterioration and preserve key components of the contractile and functional unit of skeletal muscle.

Resistance training suppresses atrogenes and prevents alterations in autophagy and mTOR phosphorylation in mice with CCLD-induced sarcopenia

Atrogenes (MuRF-1 and atrogin-1) are key mediators and markers of skeletal muscle atrophy, driving UPS proteolysis under catabolic stress and declining in response to anabolic stimuli. Atrogin-1 and MuRF-1 protein levels were assessed in tibialis anterior muscles by western blot (Fig. 5a). MuRF-1 was higher in CCLD SED than in Control SED (Fig. 5b; $p=0.032$; 1.00 ± 0.41 vs. 1.86 ± 0.34), while atrogin-1 showed a similar trend (Fig. 5c; $p=0.0136$; 1.00 ± 0.29 vs. 1.92 ± 0.49). RT prevented these increases: CCLD RT was lower than CCLD SED (MuRF-1: $p=0.0002$; 1.86 ± 0.34 vs. 0.861 ± 0.19 ; atrogin-1: $p=0.0034$; 1.92 ± 0.49 vs. 1.04 ± 0.43).

Similar results were observed in the mRNA levels of *murf-1* and *atrogin-1* (Supplemental Material 3). *murf-1* and *atrogin-1* mRNA levels were significantly increased in tibialis anterior from CCLD SED compared to Control SED (Supplemental Material 3a; *murf-1*: $p<0.0001$; 1.00 ± 0.17 vs. 7.06 ± 0.37 ; Supplemental Material 3b; *atrogin-1*: $p<0.0001$; 1.00 ± 0.11 vs. 5.11 ± 0.39). RT prevented this increase, with lower mRNA abundance in CCLD RT than CCLD SED for *murf-1* (Supplemental Material 3a; $p<0.0001$; 7.06 ± 0.37 vs. 1.64 ± 0.43) and *atrogin-1* (Supplemental Material 3b; $p=0.0003$; 5.11 ± 0.39 vs. 1.70 ± 0.34).

In addition, we evaluated the LC3II/LC3I ratio and p62 levels as ALS parameters (Fig. 5d). LC3-II/LC3-I ratio, a marker of autophagosome formation, was increased in CCLD SED compared to Control SED (Fig. 5e; $p=0.0185$; 1.00 ± 0.14 vs. 1.83 ± 0.37), whereas p62 protein levels did not change across groups (Fig. 5f; $p=0.4696$; 1.00 ± 0.10 vs. 1.07 ± 0.16). RT normalized LC3II/LC3I ratio (Fig. 5e; $p=0.0439$; 1.83 ± 0.37 vs. 1.10 ± 0.22) and maintained unaltered the p62 levels (Fig. 5f; $p=0.3882$; 1.07 ± 0.16 vs. 0.83 ± 0.38).



In the mRNA level, *lc3b* and *p62* were significantly increased in tibialis anterior from CCLD SED compared to Control SED (Supplemental Material 3c; *lc3b*: $p < 0.0004$; 1.00 ± 0.26 vs. 2.49 ± 0.37 ; Supplemental Material 3d; *p62*: $p = 0.0006$; 1.00 ± 0.23 vs. 3.26 ± 0.51). RT prevented this increase, with lower mRNA abundance in CCLD RT than CCLD SED for *lc3b* (Supplemental Material 3c; $p = 0.0024$; 2.49 ± 0.37 vs. 1.18 ± 0.11) and *p62* (Supplemental Material 3d; $p = 0.0053$; 3.26 ± 0.51 vs. 1.54 ± 0.22).

Fig. 3 RT prevents fiber atrophy in specific hindlimb muscles in CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Representative images of WGA-stained cross-sections. Quantification of muscle fiber diameter in **(b)** tibialis anterior, **(d)** gastrocnemius, **(f)** EDL, and **(h)** soleus. Quantification of cross-sectional area (CSA) of **(c)** tibialis anterior, **(e)** gastrocnemius, **(g)** EDL, and **(i)** soleus. Data represent independent biological samples (one animal per data point) and are presented as mean $\pm$ SD ($n=4-5$ animals per group). For morphometric analysis, approximately 400–1,000 fibers were quantified per EDL and soleus muscles, 2,000–3,500 fibers per tibialis anterior, and 5,000–8,500 fibers per gastrocnemius. Mixed-effects models with Sidak's post hoc test were performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Scale bar: 50 μ m.

These findings indicate that the ALS is activated in parallel to the UPS in CCLD-induced sarcopenia.

We next assessed the anabolic pathway of muscle protein turnover by measuring mTOR phosphorylation, a key readout of mTORC1 activity and protein synthesis (Fig. 5g). Phospho-mTOR (p-mTOR) levels were reduced in CCLD SED compared to Control SED (Fig. 5h; $p=0.0031$; 1.00 ± 0.05 vs. 0.26 ± 0.12) and were fully prevented by RT (Fig. 5h; $p=0.0096$; 0.26 ± 0.12 vs. 1.23 ± 0.25). Notably, RT further increased phospho-mTOR in the Control group above sedentary levels (Fig. 5h; $p=0.0066$; 1.00 ± 0.05 vs. 1.40 ± 0.14), confirming that the training protocol used here effectively engages the anabolic mTORC1 pathway^{6,27}.

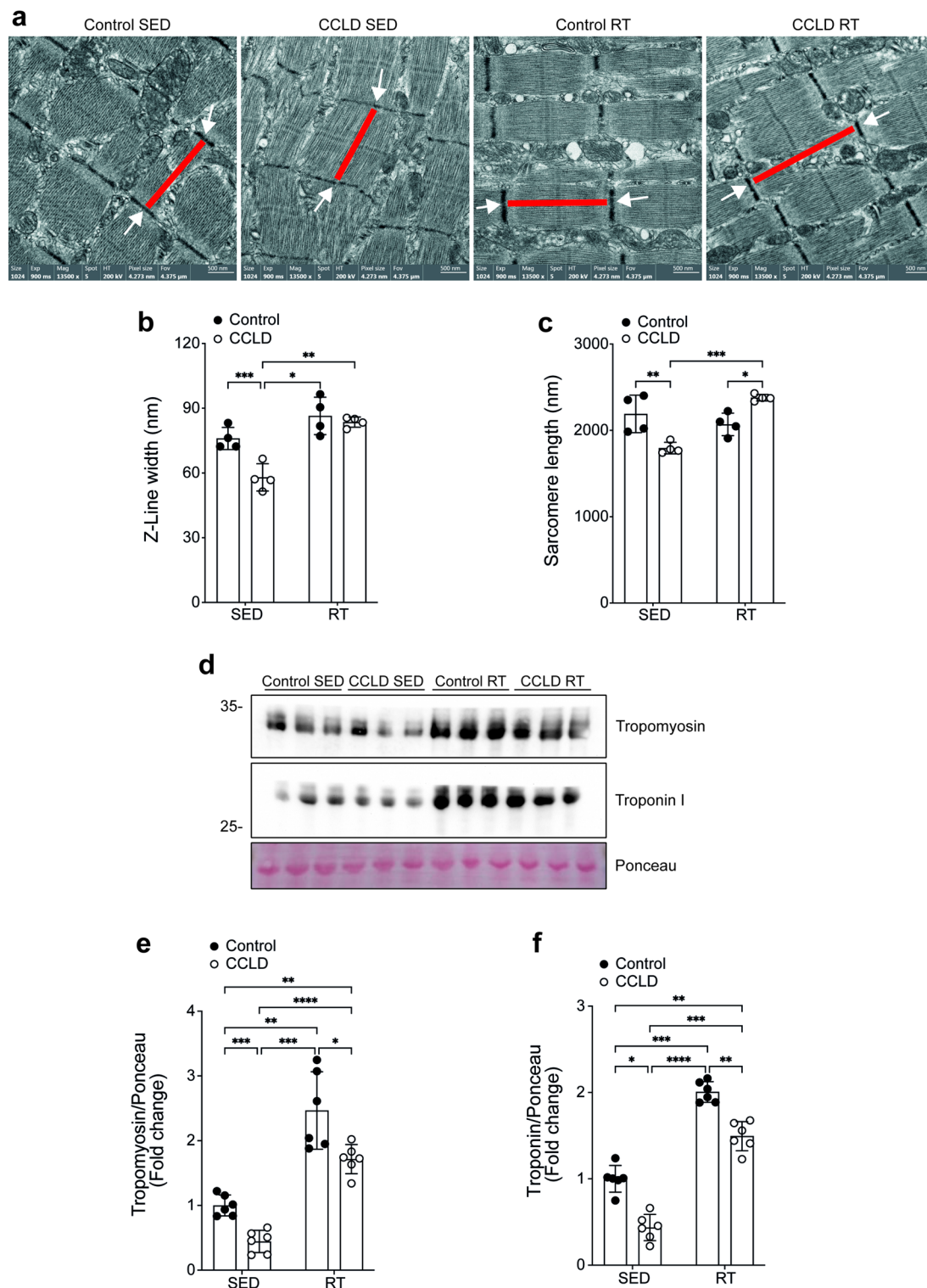
These findings suggest that RT prevents increases in proteins involved in the UPS and ALS catabolic pathways and also prevents decreases in anabolic pathway proteins in CCLD-induced sarcopenia.

Resistance training prevents alterations in morphology and mitochondrial dynamics in mice with CCLD-induced sarcopenia

Mitochondrial alterations have been reported in muscle from CCLD. Thus, we evaluated mitochondrial morphology parameters by TEM (Fig. 6a). Mitochondrial density was significantly reduced in TA CCLD SED compared to Control SED (Fig. 6b; $p=0.009$, 4.9 ± 1.1 vs. 2.2 ± 0.7), an alteration that was prevented by RT ($p=0.0017$, 2.2 ± 0.7 vs. 9.2 ± 1.4). Mitochondrial elongation was also lower in CCLD SED (Fig. 6c; $p=0.0378$, 0.64 ± 0.05 vs. 0.51 ± 0.09), but RT did not mitigate this alteration ($p=0.2606$, 0.51 ± 0.09 vs. 0.58 ± 0.03). However, CCLD RT did not differ from Control SED, indicating a partial protective effect ($p=0.1975$, 0.64 ± 0.05 vs. 0.58 ± 0.03). Mitochondrial size was not significantly altered in CCLD SED (Fig. 6d; $p=0.4247$, $157,821\pm41,852$ vs. $121,271\pm27,091$), but RT increased mitochondrial size in the CCLD group ($p=0.0013$, $121,271\pm27,091$ vs. $239,660\pm33,8281$). Mitochondrial cristae density showed no reduction in CCLD SED (Fig. 6e; $p=0.1582$, 0.000035 ± 0.000007 vs. 0.000026 ± 0.00001), and RT likewise did not induce significant changes ($p=0.0689$, 0.000026 ± 0.00001 vs. 0.000039 ± 0.00001).

Then, we measured the oxygen consumption rate (OCR) using the XF24-3 Seahorse Flux Analyzer in primary muscle culture cells (Supplemental Material 4), a key indicator of mitochondrial oxygen consumption. Compared to the Control SED, the CCLD SED group showed decreased basal (Supplemental Material 4a; $p=0.030$, 2.94 ± 0.42 vs. 2.47 ± 0.33) and maximal OCR (Supplemental Material 4b; $p=0.028$, 6.34 ± 0.95 vs. 5.24 ± 0.76), as well as diminished ATP-linked (Supplemental Material 4c; $p=0.028$, 3.4 ± 0.95 vs. 2.77 ± 0.76). CCLD RT cells prevented all these mitochondrial alterations.

Then, we evaluated parameters of mitochondrial fission (phospho-DRP-1) and fusion (OPA-1 and mitofusin-2), through western blot analysis (Fig. 7a), which have been shown to be altered in several sarcopenia models⁷. A clear decrease in phospho-DRP1 (Fig. 7b; $p=0.0040$, 1.00 ± 0.12 vs. 0.31 ± 0.07), OPA-1 (Fig. 7c; $p=0.017$, 1.00 ± 0.37 vs. 0.15 ± 0.06), and mitofusin-2 (Fig. 7d; $p=0.0007$, 1.00 ± 0.09 vs. 0.25 ± 0.15) protein levels was observed in CCLD mice compared to Control SED. These alterations in CCLD SED were fully prevented in the CCLD-RT mice (Fig. 7b, p-DRP-1, $p=0.0036$, 0.31 ± 0.07 vs. 0.83 ± 0.09 ; Fig. 7c, OPA-1, $p=0.013$, 0.15 ± 0.06 vs. 1.40 ± 0.20 ; Fig. 7d, mitofusin-2, $p=0.0027$, 0.25 ± 0.15 vs. 1.48 ± 0.19). In the Control group, RT further increased the levels of phospho-DRP1 (Fig. 7b; $p=0.082$, 1.00 ± 0.12 vs. 1.40 ± 0.41), OPA-1 (Fig. 7c; $p=0.0058$, 1.00 ± 0.37 vs. 1.98 ± 0.36) and mitofusin-2 (Fig. 7d; $p=0.049$, 1.00 ± 0.09 vs. 1.59 ± 0.33) above sedentary levels. In addition, and consistent with impaired mitochondrial biogenesis, PGC-1 α mRNA expression was reduced in



CCLD mice compared to Control SED (Supplemental Material 3e; $p < 0.0001$; 1.00 ± 0.37 vs. 0.15 ± 0.06) and normalized by RT in CCLD conditions (Supplemental Material 3e; $p < 0.0001$; 0.15 ± 0.06 vs. 1.40 ± 0.20).

Collectively, these results indicate that CCLD induces a broad disruption of skeletal muscle mitochondrial homeostasis, while RT preserves mitochondrial dynamics and bioenergetic function in CCLD-induced sarcopenia.

Fig. 4 RT preserves sarcomere structure and sarcomere protein levels in the tibialis anterior of CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Representative transmission electron microscopy (TEM) images of tibialis anterior muscles. Quantification of **(b)** Z-line width, and **(c)** sarcomere length. **(d)** Representative western blot showing tropomyosin and troponin protein levels, and **(e, f)** densitometric quantification normalized to Ponceau. Data represent independent biological samples (one animal per data point) and are presented as mean $\pm$ SD ($n=4-6$ animals per group). For TEM analysis, three micrographs per muscle were quantified and averaged to obtain a single value per animal. Two-way ANOVA with Tukey's post hoc test was performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Scale bar: 500 nm.

Discussion

Sarcopenia is a major complication of chronic liver disease with profound negative impacts on patient outcomes, including increased morbidity, mortality, and reduced transplant survival rates^{14,28}. Despite its clinical significance, effective therapeutic strategies remain limited. Building on the established benefits of resistance training (RT) in primary sarcopenia²⁹, this study evaluated whether RT could counteract muscle loss and functional decline in a murine model of CCLD-induced secondary sarcopenia. Our findings demonstrate that RT effectively prevents sarcopenia by preserving muscle strength, mass, and physical performance, which are primary diagnostic criteria according to current consensus guidelines⁹.

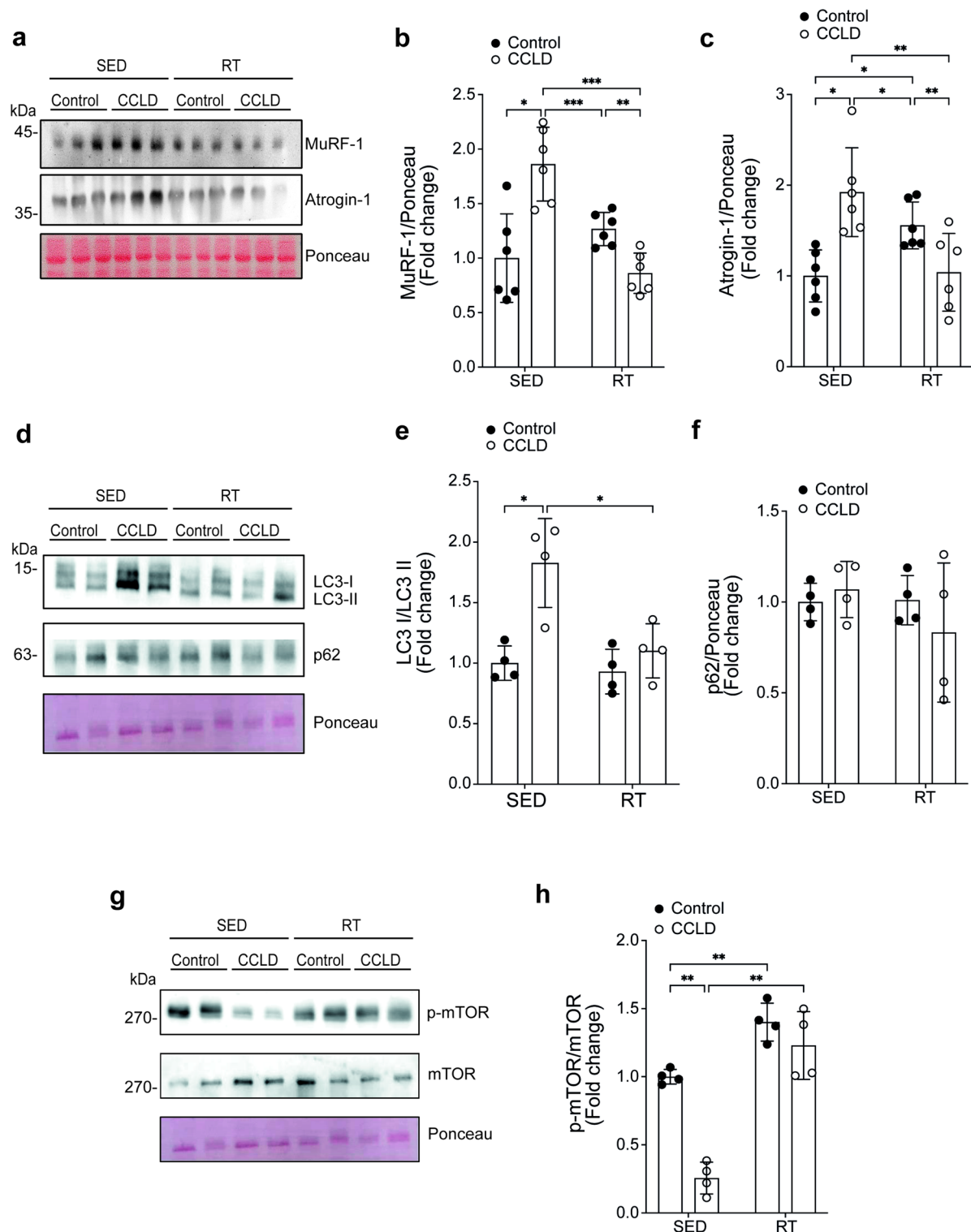
Despite lower absolute training loads due to reduced body weight, CCLD animals exhibited elevated blood lactate levels, indicating greater relative physiological effort, consistent with evidence that blood lactate is a robust marker of exercise intensity³⁰. The capacity of CCLD mice to sustain training despite their compromised state validates both the model's relevance and the feasibility of RT as a therapeutic intervention.

RT preserved forelimb and hindlimb grip strength and ex vivo muscle force, demonstrating maintained functional capacity in trained CCLD animals. These findings align with studies showing that RT enhances muscle strength in primary sarcopenia^{3,31}. The increase in fat-free mass further supports the anabolic effect of RT^{3,31}. Trained CCLD mice also exhibited greater mobility than sedentary counterparts, indicating RT not only preserves muscle integrity but also enhances functional capacity. This is particularly relevant given that sarcopenia-related mobility impairments are associated with increased fall risk and reduced quality of life^{32,33}. Considering the severe clinical consequences of sarcopenia, including higher hospitalization and mortality rates³⁴, these findings underscore the need for interventions that preserve muscle integrity in chronic cholestatic liver disease.

Morphologically, RT prevented fiber atrophy in fast-twitch glycolytic muscles (tibialis anterior and EDL) and the mixed-fiber gastrocnemius, with no effects observed in the oxidative soleus. This muscle fiber type-specific response is consistent with findings that RT preferentially stimulates hypertrophy in fast-twitch fibers³⁵, which are more susceptible to atrophy under pathological conditions. Since fiber diameter correlates directly with force-generating capacity, this morphological preservation provides a mechanistic basis for the observed improvements in muscle strength³⁶. These findings underscore the importance of tailoring exercise interventions to muscle fiber composition³⁷. However, a limitation of this study is the absence of fiber-type-specific analyses, which would have enabled evaluation of qualitative changes and fiber-type transitions.

RT also prevented structural deterioration at the level of sarcomere proteins. CCLD SED animals showed reductions in troponin I and tropomyosin protein levels, Z-line width, and sarcomere length, essential elements for force transmission^{38,39}. RT preserved these sarcomere proteins, maintaining myofibrillar architecture and contractile function, consistent with evidence that physical exercise sustains structural proteins in skeletal muscle^{40,41}.

We previously demonstrated that the ubiquitin-proteasome system (UPS) is critical for sarcopenia in CCLD mice⁴², with increased expression of E3 ubiquitin ligases MuRF-1 and atrogin-1¹⁹. RT attenuated increases in these protein levels, suggesting prevention of UPS activation associated with CCLD-induced sarcopenia. This could explain how RT attenuates the loss of troponin I and tropomyosin. Previous studies confirm that RT decreases UPS-related catabolic gene expression, thereby preserving muscle protein content under metabolic or inflammatory stress⁴³. Although the UPS is a major proteolytic pathway, the ALS is concurrently activated in this model, as reflected by an increased LC3-II/LC3-I ratio in CCLD SED muscle, which was normalized by RT,



while p62 levels remained unchanged. At the transcriptional level, both *lc3b* and *p62* mRNA were increased in CCLD SED muscle and normalized by RT. These data allow us to speculate that the FoxO-dependent autophagy program may be engaged in this model. Because p62 protein levels remained unchanged despite transcriptional activation, it is likely that autophagic degradation balanced the increased synthesis. Nevertheless, this pattern should be interpreted cautiously, since bile acids have also been reported to increase the LC3-II/LC3-I ratio while

Fig. 5 RT normalizes alterations in pathways involved in protein metabolism in the tibialis anterior of CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Representative western blot of tibialis anterior muscles showing MuRF-1 and atrogin-1 protein levels, and densitometric quantification normalized to Ponceau for **(b)** MuRF-1 and **(c)** atrogin-1. **(d)** Representative western blot of tibialis anterior muscles showing LC3-I, LC3-II, and p62 protein levels. Densitometric quantification, normalized to Ponceau, of **(e)** the LC3II/LC3I ratio and **(f)** p62. **(g)** Representative western blot of tibialis anterior muscles showing phospho-mTOR (p-mTOR) and mTOR protein levels. Densitometric quantification, normalized to mTOR, of **(h)** p-mTOR. Data represent independent biological samples (one animal per data point) and are presented as mean $\pm$ SD ($n=4-6$ animals per group). Two-way ANOVA with Tukey's post hoc test was performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

reducing autophagic flux in skeletal muscle cells⁵⁰, suggesting that the terminal, degradative step of autophagy may be only partially efficient in this model.

These results indicate that RT restrains CCLD-induced muscle proteolysis through coordinated suppression of both the UPS and the ALS, which is consistent with a possible shared regulation of atrogenes and autophagy genes by the FoxO1/FoxO3 transcriptional network²¹⁻²³. Although speculative, this link could be part of our model, given that results in the CCLD RT group show normalization of *murf-1/atrogin-1* and *lc3b/p62* transcript levels. In contrast, we found that phospho-mTOR, a central regulator of translation initiation and muscle protein synthesis, was reduced in CCLD SED mice and fully restored by RT. This could be due to increased CCL5 levels observed in CCLD mice⁴⁴, a myokine that has been previously reported to decrease protein synthesis in skeletal muscle⁴⁵. Furthermore, RT increased phospho-mTOR levels beyond sedentary control levels, indicating activation of the Akt/mTOR anabolic pathway⁶. Further experiments evaluating target proteins downstream of mTOR (p70S6K, rpS6, or 4E-BP1) to confirm mTORC1 pathway activation. Moreover, Akt-driven mTORC1 activation and Akt-driven FoxO inhibition are interconnected pathways, which could explain the molecular basis for the anabolic and anti-catabolic effects of RT in this model.

Mitochondrial dysfunction is a hallmark of sarcopenia, characterized by impaired ATP production and defective mitochondrial quality control⁴⁶. CCLD SED mice showed reduced mitochondrial density, suggesting impaired biogenesis or increased degradation. Mitochondrial morphology was altered with changes in elongation indicating disrupted dynamics; however, RT did not fully restore this parameter⁴⁷. Despite this, RT increased mitochondrial density and size, consistent with studies demonstrating RT enhances mitochondrial biogenesis^{48,49}. Given that prior research identified elevated oxidative stress in this model²⁴, the partial restoration of mitochondrial features by RT suggests a potential role in reducing oxidative injury⁵. Previous findings show that in muscle cells treated with bile acids, mitochondrial ROS generation increases and mitochondrial membrane potential decreases^{17,50,51}. Mitochondrial dysfunction has also been described in other sarcopenia models³⁹. RT is established as a means of inducing beneficial mitochondrial adaptations in sarcopenic conditions, and our results confirm this role. The CCLD-induced reduction in mitochondrial density and elongation observed by TEM was accompanied by concurrent decreases in the fission regulator phospho-DRP1 and the fusion regulators mitofusin-2 and OPA-1, along with reduced PGC-1 α mRNA expression, indicating a global suppression of mitochondrial dynamics and biogenesis rather than a shift toward fission-driven fragmentation^{7,52}. RT restored phospho-DRP1, mitofusin-2, OPA-1, and PGC-1 α expression, consistent with the RT-induced increase in mitochondrial density and size and with the partial recovery of elongation, since OPA-1 levels in CCLD RT remained below those in Control RT, indicating incomplete restoration of the fusion machinery in the CCLD-sarcopenia context⁵³⁻⁵⁵. This coordinated prevention of mitochondrial dynamics and biogenesis-associated parameters parallels the normalization of oxygen consumption rate (basal, maximal, and ATP-linked OCR) observed by Seahorse analysis, providing a mechanistic bridge between the molecular, ultrastructural, and functional mitochondrial outcomes of RT in CCLD-induced sarcopenia.

These findings provide evidence that RT effectively prevents CCLD-induced sarcopenia. The beneficial effects may be partially attributed to ameliorated hepatic dysfunction, particularly the partial restoration of serum bile acid levels, which are directly involved in muscle wasting¹⁶. The observed improvements in force generation,

fiber diameter, and mitochondrial adaptations suggest RT acts through multiple mechanisms to counteract muscle deterioration.

In conclusion, RT effectively prevents the development of sarcopenia in a murine model of CCLD by mitigating muscle strength loss, preserving muscle mass, and maintaining physical performance through preservation of muscle fiber integrity, sarcomere structure, and mitochondrial morphology and dynamics (DRP1, mitofusin-2, OPA-1), parameters associated with biogenesis (PGC-1 α), and respiratory function. It concurrently inhibits UPS- and ALS-mediated proteolysis at both the transcriptional and protein levels and reactivates mTOR anabolic phosphorylation. These findings support RT as a promising non-pharmacological strategy for preventing sarcopenia in chronic cholestatic liver disease.

Materials and methods

Animals

C57BL/6J mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and further, the colony was amplified in the animal housing from the Center for Research on Pandemic Resilience at Universidad Andres Bello. Male mice (16–18 weeks old) were randomly assigned to either a sedentary (SED) group or a resistance training (RT) group. Only males were used to avoid potential sex-related confounding effects on muscle phenotype. Chronic cholestatic liver disease (CCLD) was induced in mice by administering a diet supplemented with 0.1% (w/w) 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) for 6 weeks. During this period, the mice in the RT groups were also subjected to a resistance training protocol. Therefore, the experimental groups were the following: (1) Control SED, (2) CCLD SED, (3) Control RT, and (4) CCLD RT.

A total of 4–7 animals per group were used, depending on the specific endpoint analyzed (functional, histological, or molecular). The exact sample size for each experiment is indicated in the corresponding figure legends. Each data point represents an independent biological sample obtained from a different animal. No technical replicates were pooled for statistical analyses. Animals were randomized and housed in polycarbonate cages, maintained at a controlled temperature and relative humidity, and fed a standard (Control) or DDC (CCLD) diet and water ad libitum. Animals were euthanized by isoflurane overdose (5% induction followed by sustained exposure at 5%) and subsequent cervical dislocation.

All animal procedures complied with international, national, and institutional guidelines for animal care and were approved by the Animal Ethics Committee at the Universidad Andrés Bello (approval number 004/2020).

Resistance training protocol

Mice performed a progressive ladder-climb resistance training protocol adapted from previously validated models^{17,56,57}. The training apparatus consisted of an inclined ladder with a porous surface that allowed animals to grip securely during climbing. A dark resting chamber was placed at the top of the ladder to motivate the mice to complete each climb and to allow them to rest between sets. Before starting the experimental intervention, animals underwent a three-day familiarization period. On day 1, mice climbed the ladder carrying only their body weight. On day 2, a load equivalent to 25% of their body weight was attached to the base of the tail, and on day 3, a 50% load was used. Each familiarization session consisted of three sets of 10 climbs, with approximately 60 s of rest between sets. After familiarization, mice performed six weeks of progressive resistance training concurrently with DDC exposure. Training sessions were conducted three times per week, each consisting of three sets of 10 repetitions. Training began with 55% of body weight in the first week, and the load was increased by 5% each subsequent week until it reached 80% in the final week. Three days after the final training session, animals were euthanized, and skeletal muscles were collected, frozen in isopentane, and stored at -80 °C until processing (Fig. 1a).

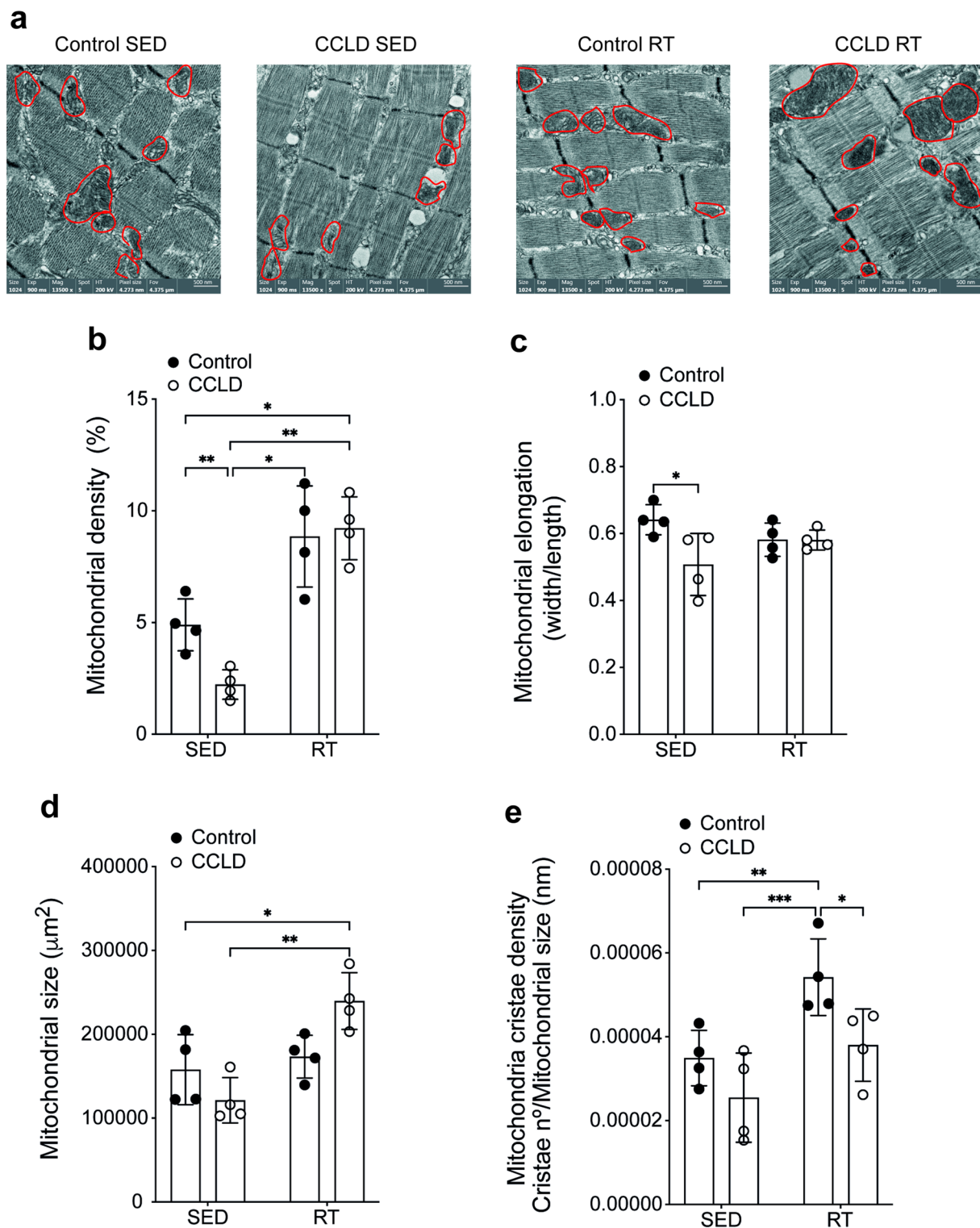


Fig. 6 RT preserves mitochondrial ultrastructure in the tibialis anterior of CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Representative transmission electron microscopy (TEM) images from tibialis anterior muscles, quantification of **(b)** mitochondrial density, **(c)** mitochondrial elongation, **(d)** mitochondrial size, and **(e)** mitochondria cristae density. For TEM analysis, three micrographs per muscle were quantified and averaged to obtain a single value per animal ($n=4$ animals per group). Two-way ANOVA with Tukey's post hoc test was performed. $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$. Scale bar: 500 nm.

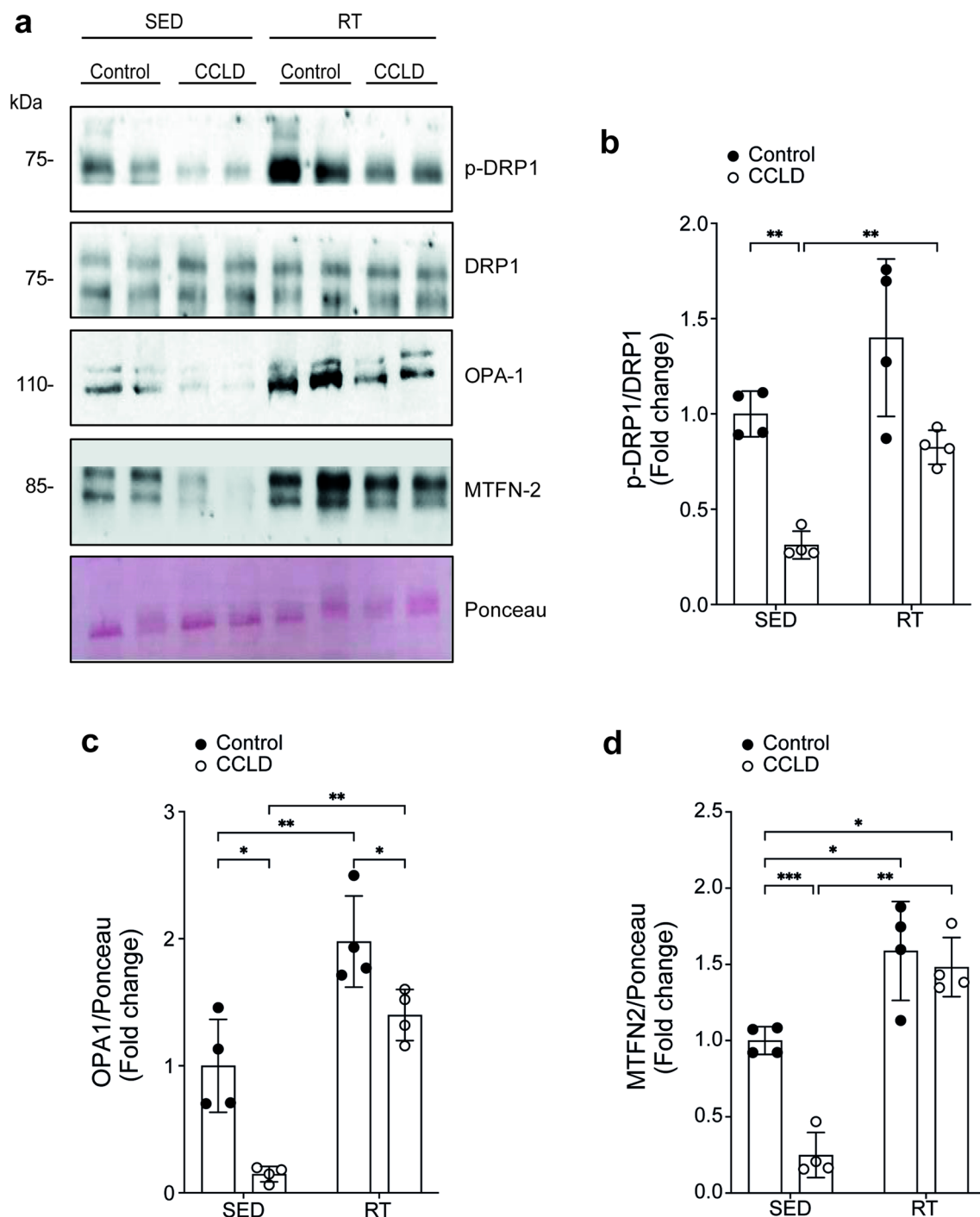


Fig. 7 RT prevents the decrease of DRP-1 phosphorylation, OPA-1, and Mitofusin-2 protein levels in the tibialis anterior of CCLD mice. C57BL/6 male mice were fed a control (Control) or DDC-supplemented diet (CCLD) concomitantly without exercise (SED) or with resistance training (RT) for 6 weeks. **(a)** Representative western blot of tibialis anterior muscles showing phosphor-DRP-1 (p-DRP-1), DRP-1, OPA-1, and mitofusin-2 protein levels. Densitometric quantification of **(b)** p-DRP-1 normalized to DRP-1, **(c)** OPA-1 normalized to Ponceau, **(d)** mitofusin-2 normalized to Ponceau. Data represent independent biological samples (one animal per data point) and are presented as mean $\pm$ SD ($n=4$ animals per group). Two-way ANOVA with Tukey's post hoc test was performed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Strength test

Muscle strength was assessed using a blinded grip-strength test (Grip Strength meter 47200, Ugo Basile, Gemonio, Italy). Briefly, mice performed 15 force repetitions for both forelimb and hindlimb, and the top 3 values from each group were recorded. Each value was then normalized by the tibia length of the mouse^{50,51}.

Contractile measurements of isolated skeletal muscle

The contractile properties of isolated skeletal muscle were determined by electrostimulation. After euthanasia, TA muscles were quickly removed by cutting the tendon at one extremity and leaving the joint to the patella at the other extremity. They were then attached to a force transducer (PowerLab 4/35, ADInstruments, Colorado Springs, CO, USA). The muscle was fixed with non-muscular tissue and maintained in an oxygenated (bubbled with 95% O₂ and 5% CO₂) Krebs-Ringer solution at 25 °C during evaluation. The optimal muscle length (Lo) was determined by micromanipulation to obtain maximal isometric twitch force. The voltage was empirically adjusted during preliminary tests to achieve the maximal twitch response and was then applied consistently across all recordings. The muscles were stimulated at frequencies between 1 and 100 Hz (450 ms duration), with a 2-minute rest interval between stimuli, using a Grass S48 stimulator (Grass Instruments, Astro-Med, Inc., West Warwick, RI, USA). A frequency-force curve was then obtained. The specific net force was calculated and expressed in mN/mm²^{45,51,58–61}.

Bioimpedance measurement

Mice were anesthetized with 3% isoflurane in air, and body composition was determined using the ImpediVET single-channel, tetrapolar bioimpedance spectroscopy (BIS) device (Impedimed, Carlsbad, CA, USA). Three consecutive measurements were performed with a single placement of the 4-needle electrodes to assess fat-free mass⁵¹. The data was analyzed using the provided software (ImpediVet Vet BIS1 v.1.0.0.4).

Running test

Physical performance was evaluated on a treadmill device (LE8610MTS, Panlab Harvard Apparatus, Barcelona, Spain). The test used an incremental speed protocol: the animals began with a 2-minute warm-up at 5 cm/s, followed by a 2 cm/s increase every minute until exhaustion. The total distance traveled by the animals on the treadmill was recorded.

Liver parameters assay

Mice were anesthetized using a face mask delivering 5% isoflurane in air. Blood samples were collected from the retro-orbital sinus using Na-heparinized capillary tubes (Hirschmann Laboratory, Eberstadt, Alemania). The collected blood was centrifuged at 4,000 rpm for 10 min at 4 °C (Centurion Scientific, UK. K3 series) and the resulting serum was used for biochemical analyses. Bile acid concentrations were measured using a commercial assay kit (Abcam, Cambridge, MA, USA, cat# ab239702) according to the manufacturer's instructions. In addition, serum levels of hepatic enzymes gamma-glutamyl transferase (GGT), glutamate-oxaloacetate transaminase (GOT), and glutamic pyruvic transaminase (GPT) were determined using the SPOTCHEM™ EZ analyzer (SP-4430) II KENSHIN-2 (77188) in accordance with the manufacturer's protocol (Arkay Global Business, Inc., Nakagyo-ku, Kyoto, Japan). Data were expressed as fold change relative to the Control SED group.

Lactate measurements

Blood lactate concentration was measured immediately after the last exercise session, six weeks after the start of training. A drop of blood was obtained via tail vein puncture and analyzed for lactate concentration using disposable strips with the Lactate Scout 4 device (SensLab, Bautzner, Leipzig, Germany).

Fluorescence microscopy

Cryosections (10 μ m) of the gastrocnemius, tibialis anterior, soleus, and Extensor digitorum longus (EDL) muscles were stained with wheat germ agglutinin (WGA) conjugated to Alexa Fluor 594 (Thermo Scientific, Waltham, MA, USA) according to standard procedures. Fiber sizes were determined by the minimum Feret diameter and cross-sectional area (CSA) of each fiber. To represent the minimum Feret diameter, the values were grouped into 5- μ m diameter intervals. Images were acquired using a Motic BA310 epifluorescence microscope (Motic, Hong Kong), and quantification was performed using ImageJ software (National Institutes of Health [NIH], Bethesda, MD, USA).

Western blot analysis

For the protein extracts, tibialis anterior muscles were homogenized in a radioimmunoprecipitation assay (RIPA) buffer. Then, samples (20 μ g) were subjected to SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Thermo Fisher Scientific, Waltham, MA, USA) as previously described¹⁹. Immunoblotting was performed using the following primary antibodies: mouse anti-Troponin I (1:1,000; Santa Cruz Biotechnology, Dallas, TX, USA, cat # SC-374549), rabbit anti-Tropomyosin (1:1,000; Cell Signaling, Danvers, MA, USA, cat # 3910 S), mouse anti-MuRF-1/TRIM63 (1:500; Abcam, Cambridge, MA, USA, cat # A30694), rabbit anti-atrogin-1/FBXO32 (1:500; Abcam, Cambridge, MA, USA, cat # A14404), rabbit anti-phospho-mTOR (1:500; Cell Signaling, Danvers, MA, USA, cat # 2971s), rabbit anti-mTOR (1:500; Cell Signaling, Danvers, MA, USA, cat # 2983s), rabbit anti-LC3B (1:500; Cell Signaling, Danvers, MA, USA, cat # 2775s), mouse anti-SQSTM1(p62) (1:500; Santa Cruz Biotechnology, Dallas, TX, USA, cat # SC-28359), mouse anti-OPA-1 (1:500; BD Biosciences, San Jose, CA, USA, cat # 612606), rabbit anti-mitofusin-2 (1:500; Abcam, Cambridge, MA, USA, cat # ab56889), mouse anti-DRP1 (1:500; Santa Cruz Biotechnology, Dallas, TX, USA, cat # SC-101270), and rabbit anti-phospho-DRP1 (Ser⁶¹⁶) (1:500; Cell Signaling Technology, Danvers, MA, USA, cat # 3455 S). Furthermore, the membranes were incubated with the secondary antibodies, goat anti-mouse IgG-HRP (1:5,000; Santa Cruz Biotechnology, Dallas, TX, USA, cat # Bsc-2005) and anti-rabbit IgG-HRP (1:5,000; Santa Cruz Biotechnology, Dallas, TX, USA, cat # SC-2357). All immunoreactions were visualized by enhanced chemiluminescence (Thermo Fisher Scientific, Waltham, MA, USA). Images were acquired using the FOTO/Analyst Luminary Workstation Systems (Fotodyne Incorporated, Hartland, WI, USA). Band quantification was performed using densitometric analysis with ImageJ software [National Institutes of Health (NIH), Bethesda, MD, USA]. Normalization of the phosphorylated proteins was made using the non-phosphorylated form of these proteins. All other proteins were normalized by Ponceau Red. The original images for Western blots and Ponceau red are shown at Supplemental Material 5, 6, and 7.

RT-qPCR

Total RNA was extracted from TA muscles using Chomczynski's phenol-chloroform method, followed by precipitation with isopropanol. Purified RNA was resuspended in nuclease-free water and converted into cDNA using standard reverse transcription protocols as previously described. The resulting cDNA was analyzed to evaluate the expression of *murf-1* (F: GCTGGTGGAAAACATCATTGACAT, R: CATCGGGTGGCTGCCTTT), *atrogin-1* (F: CACATTCTCTCCTGGAAGGCG, R: TTGATAAAGTCTTGAGGGGAAAGTG), *Sqstm1/p62* (F: T

GTGGAACATGGAGGGAAGAG, R: TGTGCCTGTGCTGGAACCTTTC), *Map1lc3b* (*LC3B*) (F: GACGGCTTCCTGTACATGGTTT, R: TGGAGTCTTACACAGCCATTGC), *Ppargc1a* (*PGC-1α*) (F: TCAAGCCAAACCAACAACCTTTATCT, R: GGTTCGCTCAATAGTCTTGTTCTCA), and *β-actin* (F: GTGACGTTGACATCCGTAAAGA, R: GCCGGACTCATCGTACTCC). Target gene mRNA expression was calculated using the $\Delta\Delta C_t$ method, normalized to β -actin expression.

Primary skeletal muscle cell culture

Hindlimb muscles from C57BL/6 mice underwent mechanical and enzymatic dissociation. Briefly, muscles were finely minced with a scalpel into $\sim 1 \times 1$ mm fragments and incubated in Ham's F-12 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing type II collagenase (285 U/mg; Worthington, Lakewood, NJ, USA, cat # LS004176) at 37 °C for 40 min in a thermostatically controlled water bath. Following digestion, 15% horse serum was added to stop enzymatic activity, and the homogenate was centrifuged at $1,000 \times g$ for 5 min. The supernatant was removed, and the pellet was resuspended in fresh digestion solution containing Dispase II (1.82 U/mg; Sigma-Aldrich, St. Louis, MO, USA, cat # D4692) and type II collagenase (285 U/mg; Worthington, Lakewood, NJ, USA, cat # LS004176) for a second incubation at 37 °C for 30 min. The resulting suspension was sequentially filtered through 100 μ m, 70 μ m, and 40 μ m strainers and centrifuged at $1,000 \times g$ for 10 min. The final cell pellet was resuspended and subjected to pre-plating steps to reduce fibroblast contamination.

Mitochondrial oxygen consumption

Primary myotubes were obtained from the skeletal muscles of healthy control mice and mice with chronic cholestatic liver disease (CCLD), either sedentary or subjected to RT, as described above. Mitochondrial respiration was assessed using the Seahorse XF24 Extracellular Flux Analyzer (Agilent Technologies, Seahorse Bioscience, North Billerica, MA, USA). Before the assay, the culture medium was replaced with Seahorse XF assay medium supplemented with 5 mM glucose and 10 mM sodium pyruvate (pH 7.4). Oligomycin, FCCP, and rotenone/antimycin A were sequentially injected during the assay according to the manufacturer's instructions. Basal oxygen consumption rate (OCR), maximal OCR, and ATP-linked OCR were determined from the corresponding phases of the mitochondrial stress test. OCR values were normalized to the total protein content of each well.

Transmission electron microscopy

The tibialis anterior muscles were fixed in 4% glutaraldehyde after exercise training. After fixation, the samples were rinsed in 0.1 M sodium cacodylate buffer and post-fixed in 2% osmium tetroxide prepared in the same buffer for 2 h. Following additional washes with cacodylate buffer, the samples were incubated in 1% uranyl acetate for 2 h. The tissues were then dehydrated through a graded series of acetone and embedded in Epon resin. Finally, 80-nm sections were cut and mounted on electron microscopy grids for examination using a transmission electron microscope (Philips, Tecnai 12 at 80 kV). Quantitative analyses included measurement of Z-line width and sarcomere length (nm), mitochondrial cross-sectional area (μ m²), mitochondrial elongation (width-to-length ratio), mitochondrial density (expressed as a percentage of total area), and mitochondrial cristae density (defined as the number of cristae per mitochondrial area in nm²).

Statistics

The Shapiro-Wilk test was used to assess the data distribution, and the analysis showed that all dependent variables were normally distributed. To evaluate the main effects of liver condition (Control vs. CCLD), exercise intervention (Sedentary vs. Resistance Training), and their interaction, either a two-way analysis of variance (ANOVA) or a mixed-effects model was used. An unpaired t-test was also used in Fig. 1 to compare total training

loads. Data are presented as the mean $\pm$ standard deviation (SD). All statistical analyses were performed using GraphPad Prism 11.02 (GraphPad Software, San Diego, CA, USA). The p -values < 0.05 were considered statistically significant.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-026-75478-0>.

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Author contributions Conceptualization, C.C-V., M.V-B., F.T. Methodology, C.C-V., M.V-B., F.T., M.P-R., J.S-V., E.C-S., M.M-P., C.G.F., J.A.S., M.C-S., F.S. Validation, F.T., M.P-R., J.S-V., E.C-S., M.M-P., C.C-V. Investigation, C.C-V., M.V-B., M.P-R., J.S-V., E.C-S., M.M-P., C.G.F., J.A.S., A.A.E., A.M., M.C-S. Visualization, C.C-V., M.V-B., J.S-V. Supervision, C.C-V., C.G.F., J.A.S., M.C-S., F.S. Project administration, C.C-V., F.S. Formal Analysis, C.C-V., F.T., M.V-B., M.P-R., J.S-V., E.C-S., M.M-P. Writing-Original Draft Preparation, C.C-V., M.V-B., C.G.F., J.A.S., M.C-S., F.S. Writing-Review & Editing, F.T., M.P-R., E.C-S., M.M-P., M.V-B., C.G.F., J.A.S., M.C-S., F.S., C.C-V.

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Data availability The datasets generated and/or analyzed during the current study are available in the Zenodo repository, <https://doi.org/10.5281/zenodo.23101636>.

Declarations

Competing interests The authors declare no competing interests.

Approval for animal experiments All animal procedures complied with international, national, and institutional guidelines for animal care and were approved by the Animal Ethics Committee at the Universidad Andrés Bello (approval number 004/2020).

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