Biomedicine & Pharmacotherapy 193 (2025) 118726

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Inhibition of ATP citrate lyase does not affect muscle mitochondrial activity in apoE$^{-/-}$ mice

Chiara Macchi [a], Isabella Fichtner [a], Tasnim Mohamed [a], Elsa Franchi [a], Alessandra Stefania Rizzuto [b], Chiara Pavanello [a,c], Jurgen Gindlhuber [d], Simone Nerini [a], Cesare R. Sirtori [a], Elena Osto [d,e], Stefano Carugo [b,f], Marco Busnelli [a], Giulia Chiesa [a], Valerio Magnaghi [a], Alberto Corsini [a], Massimiliano Ruscica [a,b,*]

[a] Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", Università Degli Studi di Milano, Milan, Italy
[b] Department of Cardio-Thoracic-Vascular Diseases, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[c] Center of Dyslipidemias, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy
[d] Division of Physiology and Pathophysiology, Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Medical University of Graz, Graz, Austria
[e] Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[f] Department of Clinical Sciences and Community Health, Università Degli Studi di Milano, Milan, Italy

ARTICLE INFO

Keywords:
Atherosclerosis
Bempedoic acid
Mitochondrial activity
Skeletal muscles
Statins

ABSTRACT

Introduction: Lipid-lowering therapy is a cornerstone in preventing coronary disease. Bempedoic acid is recommended in patients who are unable to take statin therapy to achieve the low-density lipoprotein cholesterol goal. Based on the hypothesis that statins impact skeletal mitochondrial activity, this study aimed to evaluate the effect of bempedoic acid on this aspect in apoE$^{-/-}$ mice fed a high-fat high-cholesterol (HFHC) diet and compare it to the results obtained using atorvastatin.

Methods: Female apoE$^{-/-}$ mice. The following techniques were used: *En* face analysis of aorta, mitostress analysis of skeletal muscles mitochondria, and transmission electron microscopy analysis of muscle fibres.

Results: After 12 weeks, body weight, food intake, glycaemic profile, and liver enzymes were unaffected by bempedoic acid. However, the treatment significantly reduced the plaque in thoracic and abdominal segments, as well as the necrotic core area. Mitochondrial functionality of skeletal muscles (tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, quadriceps, biceps brachii) in mice receiving bempedoic acid was not reduced compared to mice fed only HFHC diet, whereas mice receiving HFHC plus atorvastatin showed a significant reduction in basal and maximal mitochondrial respiration. Cumulative energy expenditure and oxygen consumption were reduced in the atorvastatin group compared to control and bempedoic acid groups. Transmission electron microscopy analysis of muscle fibres of tibialis anterior showed that bempedoic acid did not affect the morphology and distribution of mitochondria. The same analysis in mice fed HFHC plus atorvastatin showed fragmented mitochondria.

Conclusions: In apoE$^{-/-}$ mice, bempedoic acid positively impacts plaque burden while preventing skeletal muscle mitochondrial dysfunction.

1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death and disability worldwide, accounting for over 18.5 million deaths despite advanced pharmacological treatments and revascularization [1]. Numerous epidemiologic and interventional studies, along with genetic evidence, strongly support that elevated levels of low-density lipoprotein cholesterol (LDLc) are a major causal factor for ASCVD [2]. Reducing cumulative exposure to LDLc decreases the number of atherogenic lipoproteins trapped within the artery wall,

* Correspondence to: Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", Università degli Studi di Milano, Via Balzaretti 9, Milan 20133, Italy.
E-mail address: massimiliano.ruscica@unimi.it (M. Ruscica).

https://doi.org/10.1016/j.biopha.2025.118726
Received 25 August 2025; Received in revised form 17 October 2025; Accepted 27 October 2025
Available online 4 November 2025

thereby slowing atherosclerosis progression and significantly lowering the lifetime risk of ASCVD [3]. Given their well-established excellent benefit-risk profile, statins are globally recommended as a first-line pharmacological approach for both primary and secondary cardiovascular prevention [4]. However, numerous real-world studies have shown that achieving treatment goals is sub-optimal for patients at very high cardiovascular risk [5]. Indeed, many patients who are prescribed statins do not adhere to therapy, with 30 % or more discontinuing within one year, which increases the risk of ASCVD [6]. Statin-associated adverse effects can be severe enough to lead to treatment discontinuation, with statin-associated muscle symptoms (SAMS) being the most common reason for stopping therapy, and the incidence of muscle symptoms increasing with treatment intensity [7]. Although still debated, evidence suggests that SAMS may result from impaired mitochondrial membrane potential, maximal oxygen uptake, and ATP levels [8].

Among the newly developed and approved cholesterol-lowering drugs, bempedoic acid lowers the risk of major adverse cardiovascular events among statin-intolerant patients [9]. Bempedoic acid inhibits an early step in the cholesterol biosynthetic pathway by modulating the activity of ATP citrate lyase (ACL), an enzyme upstream of 3-hydroxy-3--methyl-glutaryl-CoA reductase (HMGCR, the target of statins), leading to the upregulation of hepatic LDL receptor (LDLR) and reduced circulating LDLc levels [10]. Bempedoic acid is a pro-drug converted to its active form by very-long-chain acyl-CoA synthetase 1, which is mainly present in the liver and absent in skeletal muscles, thus limiting the risk of myalgia and myopathy [11]. Due to this peculiarity and the findings of the CLEAR Outcomes (Cholesterol Lowering via Bempedoic Acid, an ACL (adenosine triphosphate-citrate lyase)-Inhibiting Regimen) trial, which showed that bempedoic acid significantly reduced major adverse cardiovascular events among statin-intolerant patients [9], the drug received a class IB recommendation in the recent update of 2025 Focused Update on the European Guidelines for the management of dyslipidaemias. Bempedoic acid is recommended in patients who are unable to take statin therapy to achieve the LDL-C goal [12]. Despite this evidence, as well as preclinical data showing the mechanisms by which bempedoic acid lowers LDLc [13,14], there is a lack of characterization regarding its potential impact on skeletal muscle mitochondria. Thus, the primary endpoint of this study was to evaluate the impact of bempedoic acid on skeletal muscle mitochondrial dysfunction. Secondary outcomes included assessing the metabolic safety of bempedoic acid (e.g. effect on glycaemia and liver function) and its efficacy on atheroma burden. Based on our previous experimental paradigm on murine models [15], we fed apoE$^{-/-}$ mice a high-fat high-cholesterol diet (HFHC) for 12 weeks with bempedoic acid and compared the results with those obtained from a group receiving either a high dose atorvastatin or the HFHC diet alone.

2. Methods

2.1. Mouse model and experimental procedures

All animal procedures were performed in accordance with the guidelines from directive 2010/63/EU of the European Parliament on the protection of animals and were approved by the Ethical Committee (authorization n° 439/2023-PR to MR).

Forty-five female apoE$^{-/-}$ mice aged 5 weeks were bought from Charles River (Calco, Italy). Female mice have been chosen since in apoE-deficient mice, atherosclerotic lesions are larger and more advanced in young female (16 weeks) than in male [16]. After 10 days of acclimatization, all mice were fed HFHC for 15 days. Therefore, 15 mice were fed a HFHC (Mucedola, Italy), 15 were fed HFHC containing atorvastatin (333,3 mg of atorvastatin were added to each kg of diet to guarantee an intake of 40 mg/Kg/day) and 15 were fed HFHC containing bempedoic acid (250 mg of bempedoic acid were added to each kg of diet to guarantee an intake of 30 mg/Kg/day) (n = 15) for further 12 weeks (Fig. 1A). The dose regimens were chosen according to previous studies on this subject [13–15]. The mice were kept under controlled light/dark cycle (12 h of light/12 h of dark) and temperature-controlled conditions (21°C). They had free access to food and water.

Atorvastatin calcium salt trihydrate ≥ 98.0 % was purchased from VWR (Milan, Italy), code: TCIAA2476–5G. Bempedoic acid (ETC-1002) was purchased from Cayman Chemical (code: CA-26409; USA).

The following experimental procedures were conducted on live animals: intraperitoneal glucose tolerance test (ipGTT), indirect calorimetry, grip and locomotion tests.

2.2. Blood collection and tissue harvesting

Mice were euthanized at 21 weeks of age under general anesthesia with 3–4 % isoflurane. Blood was collected from cardiac puncture, placed into Eppendorf tubes and centrifuged for 15 min at 2000 rpm at 4°C. After overnight fasting, serum was collected and immediately stored at −80°C for the evaluation of total cholesterol (TC), high-density lipoprotein cholesterol (HDLc) and triglycerides (TG) (Cobas, Roche) and of murine proprotein convertase subtilisin/kexin type 9 (Pcsk9; by a commercial enzyme-linked immunosorbent assay (ELISA) kit - R&D Systems, Minneapolis, MN)). For this assay, serum samples were diluted 1:200 and then processed as per manufacturer instructions. The following organs and tissues were collected: spleen, liver, kidney, aorta, heart, white adipose tissue, skeletal muscles (tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, quadriceps, and biceps brachii).

2.3. Atherosclerosis evaluation - en face analysis of aorta and histology of aortic sinus

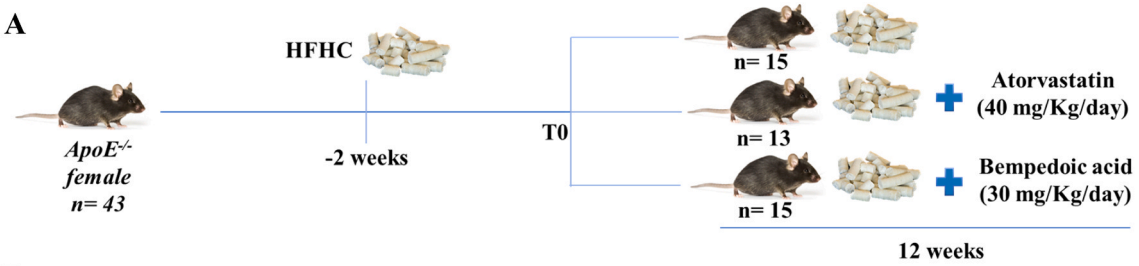
At sacrifice, the aorta was rapidly dissected from the aortic root down to the iliac bifurcation, and periadventitial fat was removed. Aortas were longitudinally opened, pinned flat on a black wax surface in ice-cold PBS, and photographed unstained for *en face* analysis [17]. For the histological evaluation of the aortic sinus, hearts were harvested, processed, and stained with haematoxylin and eosin to evaluate plaque area and with Masson's trichrome stain to define the necrotic core area [18,19]. Both aortic *en face* and aortic sinus histology were performed in accordance with American Heart Association recommendations [20].

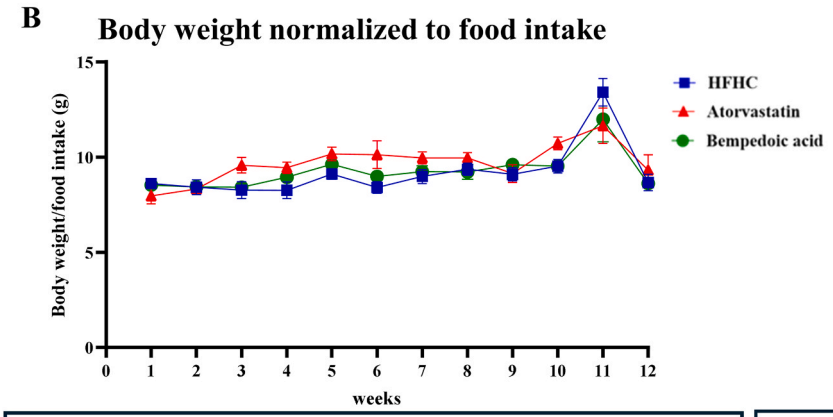
2.4. ipGTT

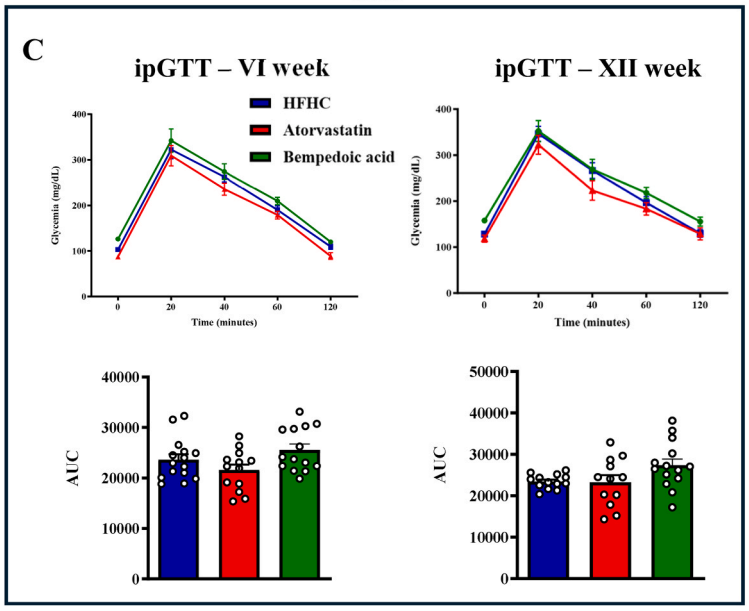
For the ipGTT, mice were fasted overnight (12 h), weighed and blood glucose measured by snipping the tail and using a glucometer (ONETOUCH Ultra) before and after intraperitoneal injection of 2 g/kg glucose solution. At the end of the test all the tail wounds were cauterized, and mice were provided with food. Glycaemia was measured at 0 and 20, 40, 60 and 120 min after the injection. The area under the curve (AUC) was calculated for glucose clearance following glucose tolerance test [21].

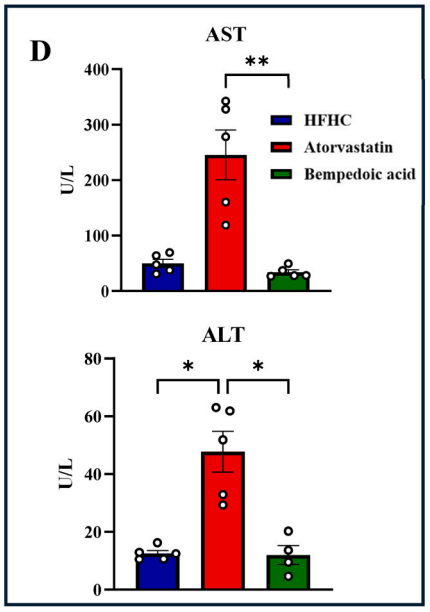
2.5. Metabolic cages and indirect calorimetric analysis

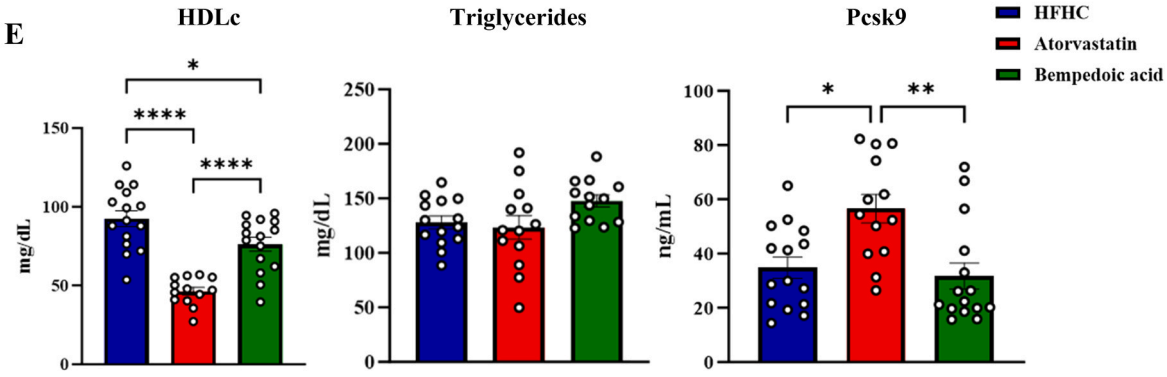
Metabolic analyses were performed by indirect calorimetry using a computer-controlled system (Promethion Metabolic Screening, Sable Systems International, USA). At the twelfth week of diet, all animals were housed individually in cages of the CAB-8 Environmental Control Cabinet (Promethion Core (Sable Systems International, USA). All mice had ad libitum access to food according to their dietary regimen and water during the study. Mice were let to acclimatize for 48 h before acquiring experimental data for a further 96 h. Respiratory gases were measured with an integrated fuel cell oxygen analyser, a CO_2 spectrophotometric analyser, and a capacitive partial pressure water vapour analyser (GA3m1, Sable Systems International, USA). Oxygen











(caption on next page)

Fig. 1. ApoE$^{-/-}$ mice were fed for 12 weeks with HFHC diet, HFHC plus atorvastatin or HFHC plus Bempedoic acid (A). (B) Body weight normalized to food-intake (C) Glucose tolerance test was performed after 6 weeks and 12 weeks and the relative AUC was calculated. (D) At the end of the treatment, circulating levels of AST and ALT were assessed. (E) HDLc, triglycerides and PCSK9 circulating levels were evaluated after 12 weeks of diet. In panel B between-group differences were assessed by two-way ANOVA. In panels C, D and E between-group differences were assessed by one-way ANOVA. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data are depicted as mean ± SEM. ALT, alanine transaminase; AST, aspartate transaminase; AUC, area under the curve; HDLc, high-density lipoprotein cholesterol; HFHC, high-fat high-cholesterol diet; ipGTT, intraperitoneal glucose tolerance test; PCSK9, proprotein convertase subtilisin/kexin type 9. Blue bars represent mice fed a HFHC diet (n = 15), red bars represent mice fed a HFHC diet plus atorvastatin (n = 13), and green bars represent mice fed a HFHC diet plus bempedoic acid (n = 15). ∘stands for individual values.

consumption and carbon dioxide (CO_2) production were measured for each mouse for 1 min at 7-min intervals. The analysis of total activity of mice was performed using Promethion Beam Break Activity Monitor (Sable Systems International, USA). Data were then analysed using the web tool Indirect Calorimetry Experiments CalR [22].

2.6. Grip and rotarod tests and gait analysis

Grip test was performed by using the Grip Meter, which consists of a precision dynamometer connected to a grid, that allows measurement of the strength of limb grasping. Mice were handled by the tail, thus allowing to grasp the grid specifically with their forelimb and pulled out until the grip was left. The grip meter records the maximum weight that the mouse can pull; each mouse was tested for 5 series of pulls composed by 3 consecutives pulls and 1 min of resting in the cage. The average weight of the maximal performance of each series for each mouse was calculated. Strength (N) data were normalized on body weight (g), assessed immediately before the test was conducted. Walking analysis was performed in accordance with our previous publications [23]. The plantar surface of both hind paws was painted with ink and the animal was allowed to walk along a narrow corridor (100 cm long) with white paper on its base. Paper strips were scanned, and, among all parameters, we considered stride length (SL), and stride width (SW). We measured these parameters, averaging 8–10 footprints per mouse (5 measurements for SL and SW each). Analyses were performed using image J software. Rotarod analysis was performed to test sensory-motor functions and central coordination. Mice were placed on the rod of a rotarod apparatus, which turned on and progressively accelerated (0–40 rpm in 800 s), until the animals fell off. The average time spent on the turning rod, in a set of 3 repetitions, was measured. If the mouse completed the entire test (800 s), the test was not repeated, and maximum time was considered for analysis.

2.7. Mitochondria isolation, mitochondrial respiration analysis and transmission electron microscopy

To assess the impact of atorvastatin and bempedoic acid on skeletal muscle mitochondrial functionality, a Mito Stress Test (Agilent Technologies, Santa Clara, CA, USA) was performed. Fresh mitochondria were isolated from the following skeletal muscles: tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, quadriceps, and biceps brachii. They were weighed, placed in a cold basic medium, minced, and homogenized with a glass/Teflon Potter Elvehjem tissue grinder in a homogenization medium added with Proteinase K. The resulting solution was centrifuged to remove any undisrupted tissue. The supernatant was then centrifuged, and the pellet resuspended in a specific buffer and incubated on ice for myofibrillar repolymerization. Subsequently, it was centrifuged, the supernatant collected and further centrifuged to generate a mitochondrial pellet. This latter was resuspended in isolation medium. All the steps were performed on ice or at 4 °C. Mitochondrial protein content was then determined by bicinchoninic acid (BCA) assay. 7 μg of mitochondria were loaded in a 24 well Agilent Seahorse XF Cell Culture Microplate and the oxygen consumption rate (OCR) was recorded at the basal level and after the sequential injections of 0.7 mM ADP, 2.25 μg/mL Oligomycin (ATP synthase inhibitor), 10 μM FCCP (uncoupling agent) and a mixture of 2 μM Rotenone (complex I inhibitor) and 3.6 μM Antimycin A (complex III inhibitor) [15].

Transmission electron microscopy (TEM) was conducted on gastrocnemii. Tissues were dissected in small pieces of about 2 mm× 3 mm; they were immediately fixed in 2.5 % glutaraldehyde in sodium cacodylate buffer 0,1 M pH 7.2 and then put overnight at 4°C. The samples were then rinsed 3 times for 10 min with sodium cacodylate buffer 0.1 M, post-fixed in 1 % osmium tetroxide in 0.1 M cacodylate buffer for 2 h at room temperature. After 2 rinses with bidistilled water, samples were counter-stained with a 0.5 % uranyl acetate in bidistilled water overnight at 4°C in the dark.

After rinsing with bidistilled water, the tissues were dehydrated with a graded series of ethanol (30 %, 50 %, 70 %, 80 %, 90 %) for 20 min each and then dehydrated with 100 % ethanol for 20 min, 2 times.

The tissues were then permeated twice with 100 % propylene oxide for 20 min; Meanwhile, Epon-Araldite resin was prepared mixing properly Embed-812, Araldite 502, dodecenylsuccinic anhydride (DDSA) and Epon Accelerator DMP-30 according to manufacturer specifications. Samples were gradually infiltrated first with a mixture of Epon-Araldite and propylene-oxide 1:2 for 1 h, then with Epon-Araldite and propylene-oxide 1:1 for 1 h and left in a mixture of Epon-Araldite and propylene-oxide 2:1 overnight at room temperature. Finally, samples were put in pure resin before polymerization at 60°C for 48 h.

Ultra-thin sections preparation and sample observation at the transmission electron microscope: resin embedded samples were cut with the ultramicrotome (PowerTome XL, RMC) in semi-thin sections of 1 μm thickness with a glass knife, stained with 1 % toluidine blue in phosphate buffer and observed with a transmission light microscope to identify the region of the sample to investigate at the ultrastructural level. Ultra-thin sections of 80 nm were cut with a diamond knife (Ultra 45°, DIATOME) and collected on 200 mesh copper grids and observed under a transmission electron microscope Talos L120C (Thermo Fisher Scientific) operated at 120 kV. Images were acquired by a digital camera (Ceta CMOS Camera 4k x 4k, Thermo Fisher Scientific) using TIA software.

Section 2.8 (Liver proteomic analysis), **section 2.9** (RNA isolation and quantitative PCR), **section 2.10** (Western blot analysis), and **section 2.11** (Data representation and statistical analysis) are reported in supplemental material file.

3. Results

3.1. Bempedoic acid does not affect metabolic milieu in apoE$^{-/-}$ mice

The first step was to evaluate the effects of the diet and drug treatments on body weight. Fig. 1B which reports body weight normalized for food-intake showed no differences among the three groups throughout the experimental period (Fig. 1B). Weekly body weight (g) and food intake (g/mouse/day) are reported in Supplementary Fig. 1A and 1B. Mice receiving atorvastatin showed a reduction in non-normalized body weight after the first week of treatment (-16 %, $p < 0.01$), a trend that persisted throughout week 12 (Supplementary Figure 1A). These changes do not seem to be strictly related to food intake, but rather to a decrease in the weight of visceral adipose tissue of mice receiving atorvastatin (Supplementary Figure 1B-C).

Given the known association between statins, as a class, and the potential onset of diabetes, we assessed the glycaemic profile of the mice. Following intraperitoneal injection of 2 g/kg of pure glucose, the

AUC for the ipGTT showed no significant differences among groups at either mid-point (week 6)- or the end (week 12) of the treatment period (Fig. 1C). Since a dose–response relationship of atorvastatin and rise in serum concentration of aspartate transaminase (AST) or alanine transaminase (ALT) is reported in clinical trials [7], we measured these enzymes. After 12 weeks, elevated AST and ALT were observed only in apoE$^{-/-}$ mice receiving atorvastatin compared to those fed HFHC diet only or supplemented with bempedoic acid (Fig. 1D).

Compared to the HFHC-only group, mice given bempedoic acid showed a significant reduction in HDLc (-18 %; $p < 0.0238$), with no changes in triglycerides and Pcsk9 levels (Fig. 1E). The atorvastatin group exhibited a decrement in HDLc (-50 %; $p < 0.0001$) and an increase in Pcsk9 levels (+62 %; $p < 0.05$) compared to the HFHC-only group, while triglyceride levels remained unaffected (Fig. 1E). Regarding the hepatic cholesterol milieu, bempedoic acid significantly increased the gene expression of *Hmgcr* and *Ldlr,* decreasing that of *Sterol Regulatory Element Binding Transcription Factor 2 (Srebf2)* and *Pcsk9* compared to the HFHC-only group (Supplementary Figure 1D). However, bempedoic acid treatment led to increased hepatic intracellular protein levels of SREBP-2 compared to the other two groups (Supplementary Figure 1E), while circulating total cholesterol levels were reduced only in the atorvastatin group (Supplementary Figure 1F).

To further characterize our model, we performed an untargeted proteomic analysis of the liver. Functional enrichment analysis showed enhanced fatty acid catabolism in the bempedoic acid-treated group compared to the other two groups (Fig. 2A and Supplementary Fig. 2B). This finding aligns with increased hepatic gene expression of *Tfam* (Transcription Factor A, Mitochondrial; $p < 0.001$) and elevated protein levels of the electron transport chain's complexes II, III and V, with a tendency toward increased protein expression of complexes I and IV compared to the HFHC-only group (Fig. 2B-C).

3.2. Bempedoic acid improves atheroma burden in apoE$^{-/-}$ mice

En-face analysis of aortas harvested at the time of sacrifice showed that atherosclerotic lesions predominantly formed in the aortic arch across all experimental groups. As expected, apoE$^{-/-}$ mice on HFHC diet exhibited extensive atherosclerosis throughout all the three aortic segments (i.e. aortic arch, thoracic and abdominal segments). Compared to HFHC group, treatment with atorvastatin significantly reduced plaque area in all the segments by −49 % ($p < 0.01$), −79 % ($p < 0.0001$) and −97 % ($p < 0.001$), respectively (Fig. 3A).

As for bempedoic acid, although limited reduction in plaque was observed in the aortic arch compared to HFHC (-14 %), significant plaque reductions were found in the thoracic and abdominal segments by −61 % ($p < 0.01$) and −71 % ($p < 0.01$), respectively (Fig. 3A).

Consistent with the findings observed in the aortic arch, histological analysis of the aortic sinus showed significantly smaller plaques in mice receiving atorvastatin, whereas no significant differences were observed between HFHC and bempedoic acid. Notably, both atorvastatin and bempedoic acid led to a significant reduction in necrotic core area compared to HFHC group, with decreases of 38 % ($p < 0.01$) and 35 % ($p < 0.05$), respectively (Fig. 3B).

3.3. Bempedoic acid does not affect muscular performance of apoE$^{-/-}$ mice

To evaluate the impact of atorvastatin and bempedoic acid on muscular performance, apoE$^{-/-}$ mice underwent a series of behavioural tests assessing both aerobic and anaerobic capacity, including grip, gait analysis (walking test) and rotarod analysis. In the grip strength test (anaerobic), 12 weeks of treatment with either atorvastatin or bempedoic acid did not result in any significant differences in strength performance compared to the control group (HFHC-fed mice; Fig. 4A). This outcome remained consistent when each group was analysed individually, comparing results at week 12 to those at baseline (week 0), just before experimental diet intake. For gait analysis, there were no differences among groups in stride width, stride length, or distance to the opposite foot (TOF). The significant increase in TOF in both the control group and animals treated with bempedoic acid indicates that the drug unlikely affects gait [24].

Within-group comparisons showed a slight increase in stride width from baseline to week 12 in the HFHC group (Fig. 4B). Rotarod testing, which evaluates sensory-motor coordination and balance through latency to fall, remained unchanged after 12 weeks, with performance remaining stable across all groups (Fig. 4C).

3.4. Bempedoic acid does not affect energy expenditure in apoE$^{-/-}$ mice

To further explore the effects of pharmacological treatments on locomotor activity, metabolic cages analysis was conducted. No significant differences were observed among the three groups in terms of pedestrian locomotion and total distance travelled (Supplementary Figure 2A). However, night-time locomotor activity was significantly reduced in the atorvastatin-treated group compared to the bempedoic acid group, showing a −18 % reduction ($p < 0.05$; Fig. 5A). In agreement with these findings, energy expenditure measured over 24 h and during the dark phase was reduced in the atorvastatin group compared to control group (-15.7 %), and even more so when compared to the bempedoic acid group across all time intervals, i.e. full day (-15.6 %), light (-14.6 %), dark (-16.4 %) phases (Fig. 5B). Similarly, cumulative energy expenditure, representing total caloric output over the monitoring period, was lower in the atorvastatin group than in both the control and bempedoic acid groups during the full day (-25 %; $p < 0.02$ vs HFHC and −14 %; $p < 0.008$ vs bempedoic acid) and night-time periods (Fig. 5B). Supporting these observations, oxygen consumption was also reduced in the atorvastatin group compared to both the control group (full day: −16 %; $p = 0.037$) and the bempedoic acid group (Full day: −16 %; $p = 0.047$; Fig. 5B). In contrast, carbon dioxide production did not differ significantly among the groups (Supplementary Figure 2A).

3.5. Bempedoic acid does not affect either myofibril organization or mitochondrial morphology

Mitochondrial respiration was evaluated using freshly isolated mitochondria from skeletal muscles (including tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, quadriceps, and biceps brachii) immediately following euthanasia (Fig. 6A). ApoE$^{-/-}$ mice given atorvastatin showed a significant reduction in basal respiration compared to HFHC group (-35 %; $p = 0.002$), and this reduction remained persisted when maximal respiration was measured (-38 %; $p = 0.0459$). When compared to the bempedoic group, atorvastatin led to a significant drop in this parameter (-48 %; $p = 0.0198$). A comparable pattern was seen in spare respiratory capacity, which was 21 % lower in the atorvastatin group than in the bempedoic acid group ($p = 0.0002$). ATP production was also significantly reduced in atorvastatin-treated mice compared to both the HFHC group (- 34 %; $p < 0.0207$) and the bempedoic acid group (-56 %; $p = 0.0059$; Fig. 6A). Conversely, mice treated with bempedoic acid did not show significant differences in basal respiration, maximal respiration and ATP production relative to the HFHC group. Of note, bempedoic acid significantly enhanced spare respiratory capacity compared to HFHC-fed mice and atorvastatin-treated mice (+13 %, $p = 0.011$ and +26 %, $p = 0.0002$, respectively; Fig. 6A). In line with these findings, a significant change was found in the gene expression of *Tfam* in gastrocnemius of mice treated with bempedoic acid compared to controls (Fig. 6B).

To delve deeper into the effects of pharmacological treatments on skeletal muscle, TEM was conducted on gastrocnemius muscle samples from each group. The muscle fibres of animals fed with HFHC showed widened intermyofibrillar spaces and branched myofibrils with enlarged mitochondria. In the atorvastatin group, signs suggestive of

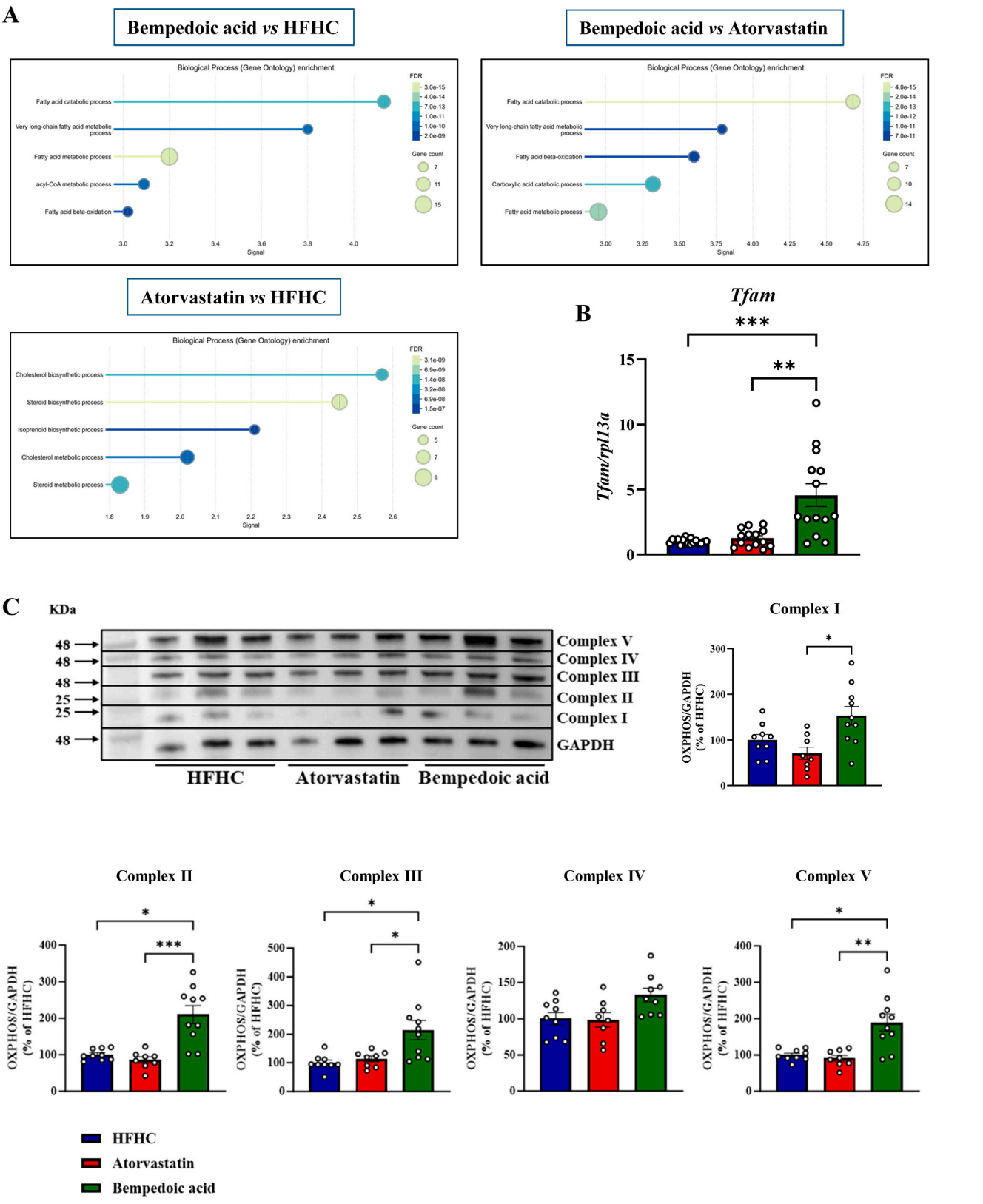


Fig. 2. Untargeted proteomic analysis and assessment of mitochondrial components expression in the liver. The top five biological processes that differ between bempedoic acid and HFHC, bempedoic acid and atorvastatin, atorvastatin and HFHC are shown (A). Panel B displays hepatic gene expression of Tfam, normalized to the expression of housekeeping gene rpl13a. Representative Western Blot depicting the expression of the five oxidative phosphorylation complexes, with GAPDH used as a housekeeping protein (C). Between-group differences were assessed by one-way ANOVA with Kruskal-Wallis test (B) or Brown-Forsythe and Welch ANOVA test (C). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Data are depicted as mean ± SEM. HFHC, high-fat high-cholesterol diet; tfam, mitochondrial transcription factor A. Blue bars represent mice fed a HFHC diet, red bars represent mice fed a HFHC diet plus atorvastatin, and green bars represent mice fed a HFHC plus bempedoic acid. n = 13–15 per group. ∘stands for individual values.

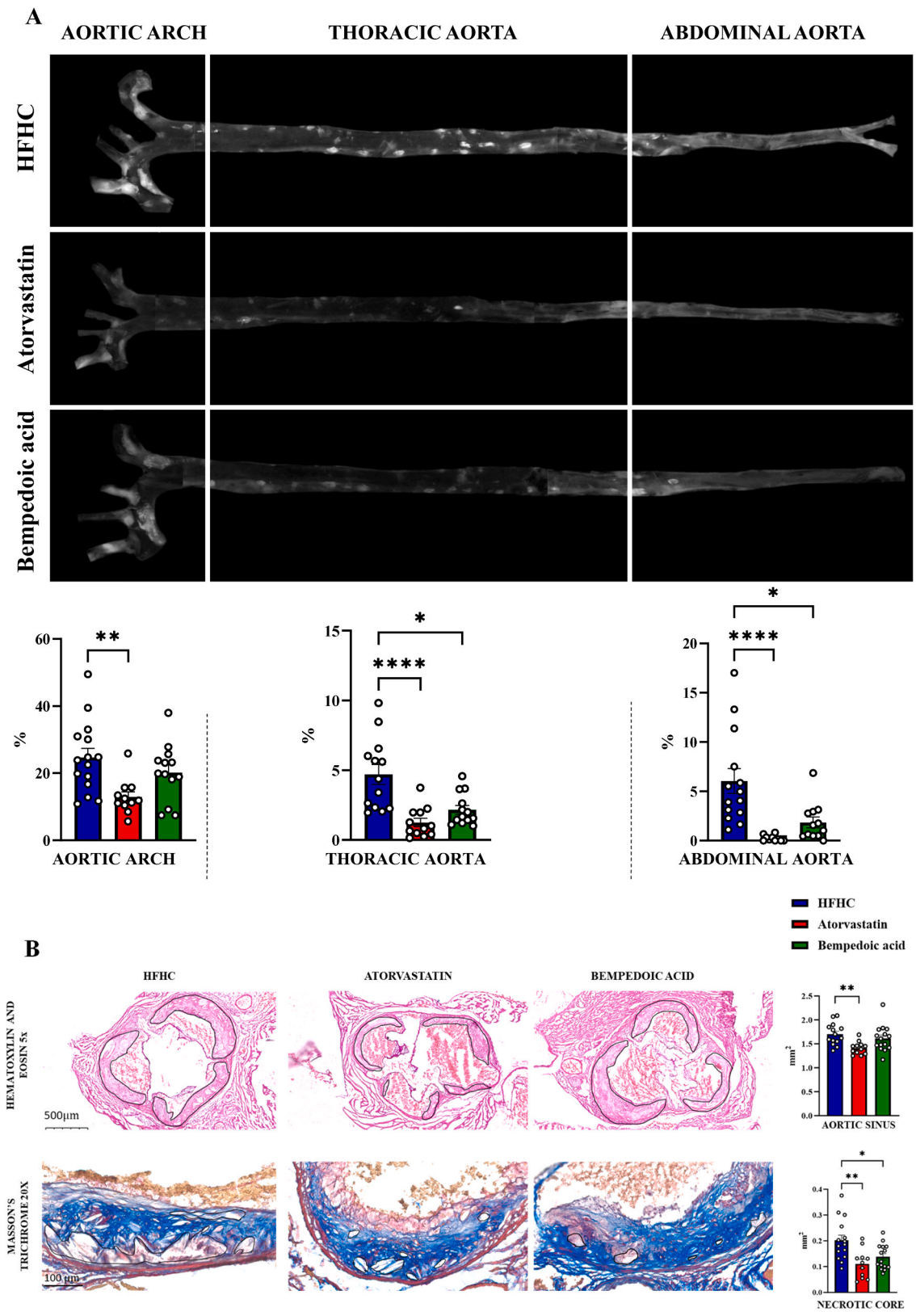


Fig. 3. Atherosclerosis development in the whole aorta and at the aortic sinus. Representative en-face images of atherosclerosis development in the whole aorta are shown (A); the exposed plaques (white areas) were quantified as a percentage of the whole area (gray surface). Representative hematoxylin and eosin stained pictures of atherosclerosis development at the aortic sinus; bar length: 500 µm (B). Representative Masson's trichrome stained pictures of plaques to highlight necrotic core area; bar length: 100 µm (B). Between-group differences were assessed by one-way ANOVA with Kruskal-Wallis test. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data are depicted as mean ± SEM. HFHC, high-fat high-cholesterol diet. Blue bars represent mice fed a HFHC diet, red bars represent mice fed a HFHC diet plus atorvastatin, and green bars represent mice fed a HFHC diet plus bempedoic acid. n = 13–15 per group. ∘stands for individual values.

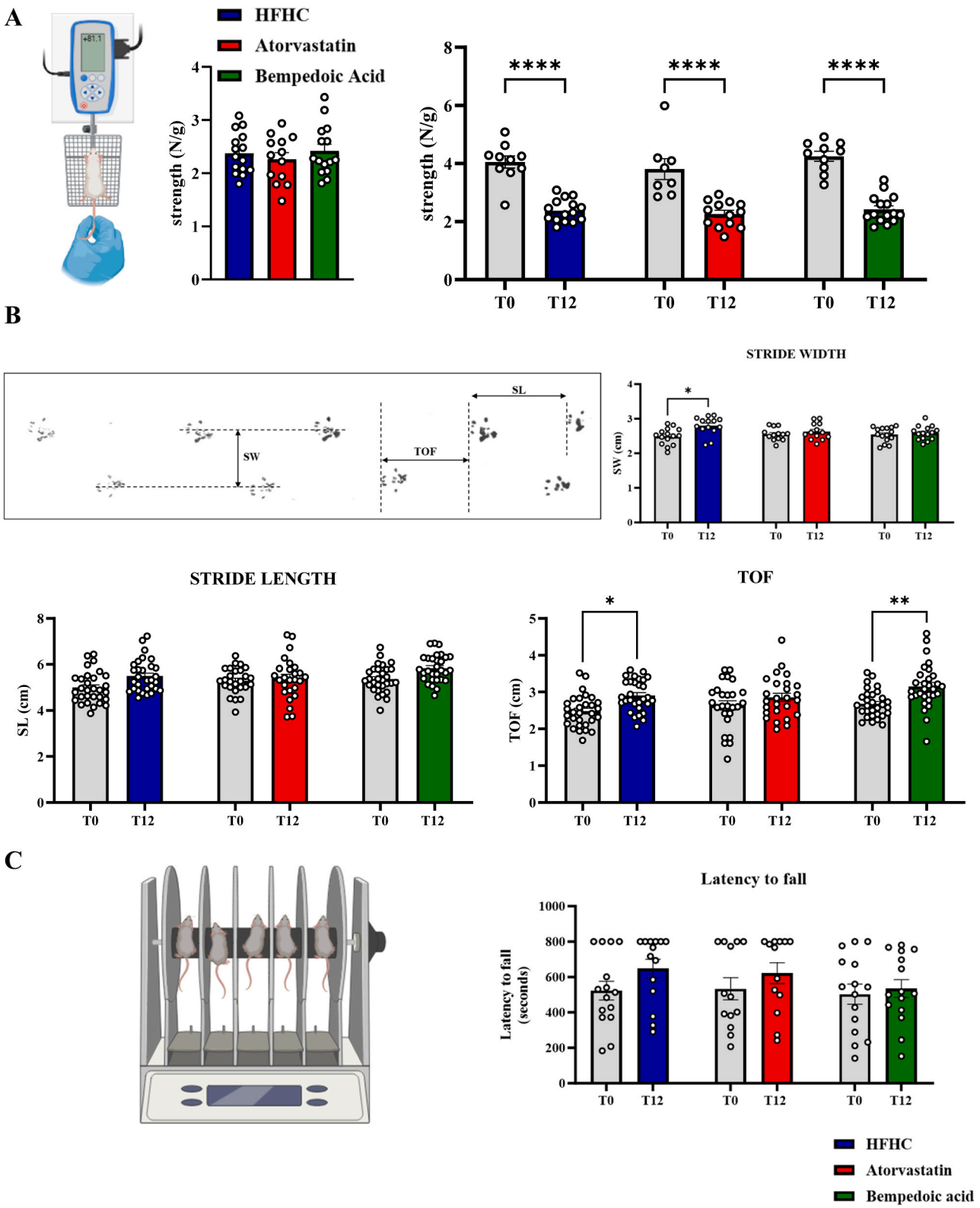


Fig. 4. Muscle strength and locomotion. Grip test was used to assess muscle strength and data were expressed as strength (N) per animal weight (g) (A). Locomotion was assessed by walking analysis behavioral test. Stride width, length and the distance to the opposite foot (TOF) were obtained averaging 5 consequent steps for each animal; both the right and left sides are represented for stride length and TOF (B). Coordination was assessed by rotarod test (C). Differences between groups were assessed by one-way ANOVA with Kruskal-Wallis test (panels A, B, stride width, stride length and panel C) or Brown-Forsythe and Welch ANOVA test (panel B, TOF). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data are depicted as mean ± SEM. HFHC, high-fat high-cholesterol diet; SL, stride length; SW, stride width. Blue bars represent mice fed a HFHC diet, red bars represent mice fed a HFHC diet plus atorvastatin, and green bars represent mice fed a HFHC diet plus bempedoic acid. Grey bars represent the animals at time 0, before starting the treatment. ○ stands for individual values.

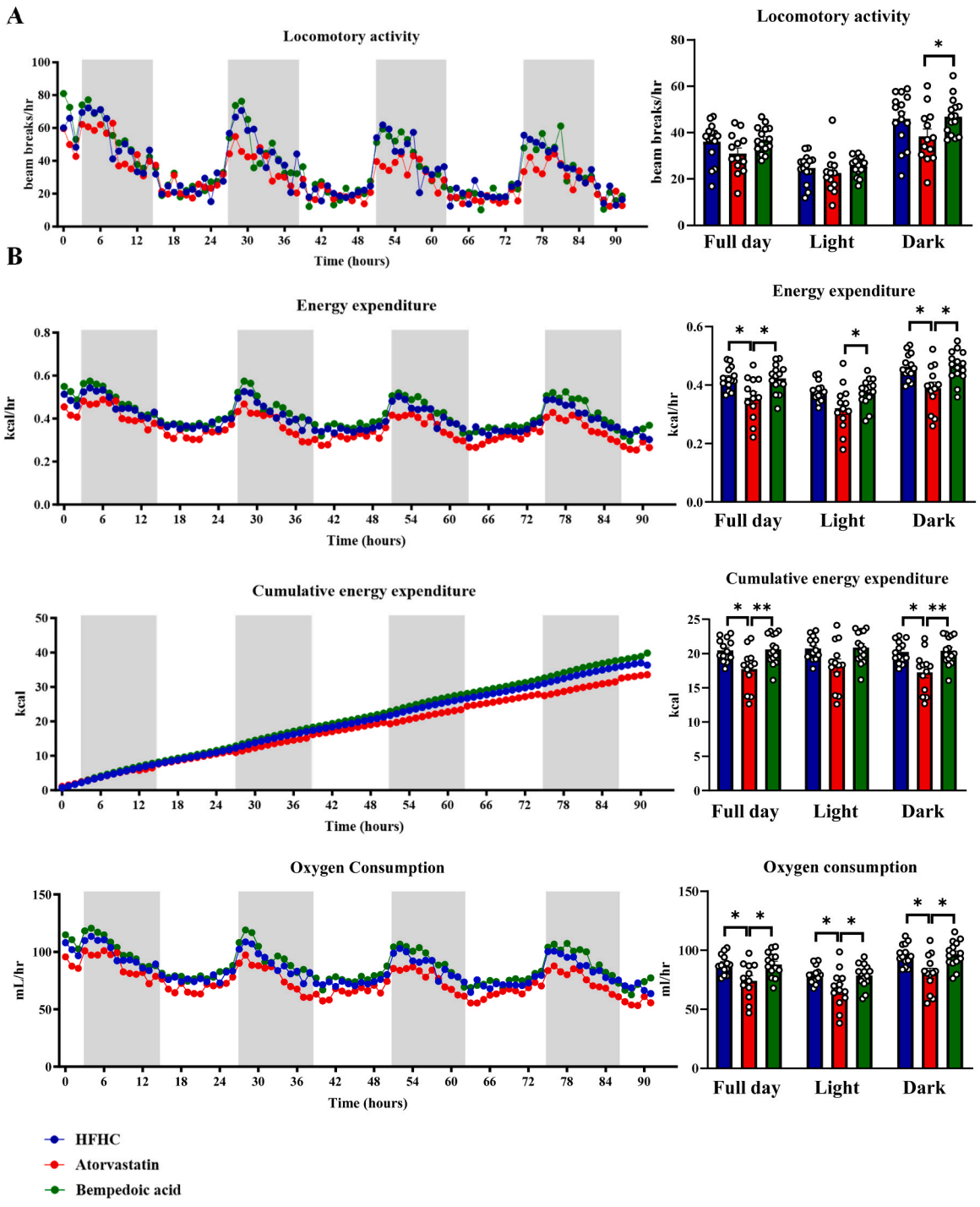


Fig. 5. Behavioral and metabolic phenotyping. Metabolic cages were used to assess hourly locomotory activity (A), energy expenditure, cumulative energy expenditure and oxygen consumption (B). Between-group differences were assessed by one-way ANOVA with Kruskal-Wallis test (panels A, B) * $p < 0.05$; ** $p < 0.01$. Data are depicted as mean ± SEM. HFHC, high-fat high-cholesterol diet; Blue bars represent mice fed a HFHC diet, red bars represent mice fed a HFHC diet plus atorvastatin, and green bars represent mice fed a HFHC diet plus bempedoic acid. n = 15 in HFHC and bempedoic acid group; n = 13 in atorvastatin group. ◦stands for individual values.

mitochondria degeneration were observed, whereas bempedoic acid treatment did not appear to effect myofibril organization or mitochondrial morphology (Fig. 6C).

4. Discussion

The overall key findings of this study highlight that bempedoic acid, in contrast to atorvastatin, does not impact on mitochondrial functionality and structural organization within skeletal muscle fibres in apoE$^{-/-}$ mice. Conversely, the subtle mitochondrial alterations observed in mice receiving atorvastatin could represent early indicators of statin-induced myalgia, especially considering that no differences in aerobic or anaerobic exercises were detected across treatment groups (i.e. mice fed only HFHC, HFHC plus atorvastatin and HFHC plus bempedoic acid). This supports previous observations suggesting that statins impair mitochondrial respiration in skeletal muscle without necessarily

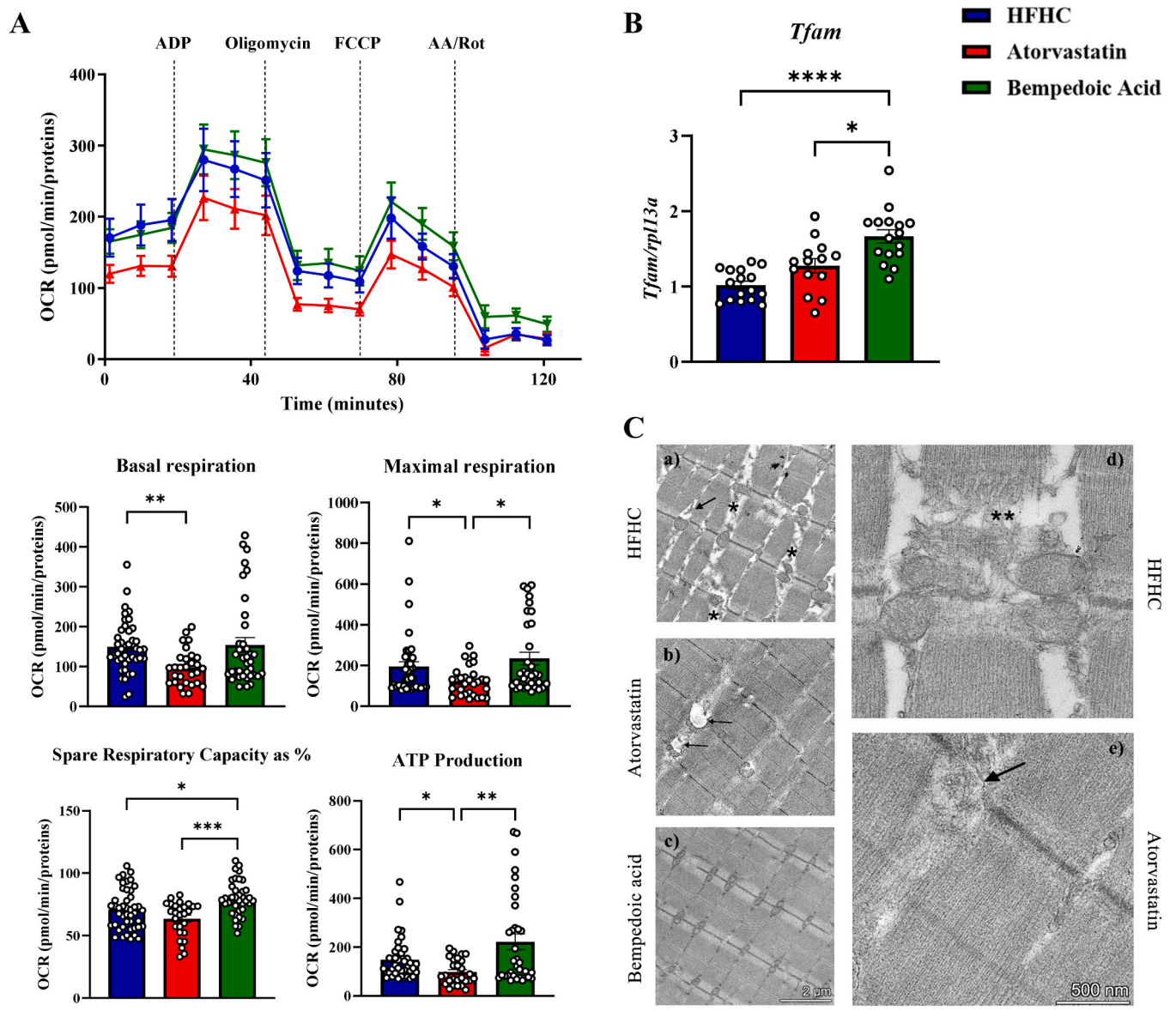


Fig. 6. Evaluation of mitochondria functionality and morphology in skeletal muscle. Oxygen consumption rate was assessed in mitochondria of skeletal muscles of apoE$^{-/-}$ mice fed a HFHC diet, a HFHC diet plus atorvastatin, and a HFHC diet plus bempedoic acid (A). Skeletal muscles were a pool of tibialis anterior, extensor digitorum longus, soleus, gastrocnemius, quadriceps, and biceps brachii. Panel B displays gene expression of Tfam in gastrocnemius of mice fed a HFHC diet, a HFHC diet plus atorvastatin, and a HFHC diet plus bempedoic acid. *Rpl13a* (Ribosomal Protein L13a) was used as housekeeping gene (B). Representative images TEM showing muscle fibers from the gastrocnemius of mice fed a HFHC diet (a), a HFHC diet plus atorvastatin (b), and a HFHC diet plus bempedoic acid (c). In image a), asterisks indicate enlargements in intermyofibrillar spaces and the black arrow points to branched myofibrilla. In image b), black arrows indicate potential mitochondrial degeneration. Image d) is a magnification of image a), where asterisks mark sarcomere ruptures. Image e) is a magnification of image b), with the black arrow indicating mitochondrial degenerations (C). Scale bar = 500 nm. Differences between groups were assessed by one-way ANOVA with Kruskal-Wallis test (panel A) or Brown-Forsythe and Welch ANOVA test (panel B). * $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$; **** $p < 0.0001$. Data are depicted as mean ± SEM. ADP, adenosine triphosphate; AA, antimycin A; FCCP, carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazone; HFHC, high-fat high-cholesterol diet; OCR, Oxygen consumption rate; Tfam, mitochondrial transcription factor A. Blue bars represent mice fed a HFHC diet, red bars represent mice fed a HFHC diet plus atorvastatin, and green bars represent mice fed a HFHC plus bempedoic acid. n = 15 in HFHC and bempedoic acid group; n = 13 in atorvastatin group. ∘stands for individual values; all three replicates are shown individually in Panel A.

compromising contractile function [25]. On this matter, muscle biopsies from patients with statin-induced myopathies revealed a significant decrease in mitochondrial complex III enzyme activity and mitochondrial ATP production capacity [26]. A significant reduction in maximal ADP-stimulated respiration was also found in permeabilized fibre bundles from muscle biopsies from patients treated with 40 and 80 mg/day atorvastatin [27]. In general, several lines of evidence suggest statin therapy is associated with impaired mitochondrial plasticity [28], impairment of several mitochondrial functions, including, reduced mitochondrial biogenesis [29], increased mitochondrial ROS production, and reduced antioxidant capacity and calcium buffering [30]. This perspective underscores the relevance of our findings. It is well-established that LDLc-lowering pharmacotherapy is an effective approach for preventing both primary and secondary ASCVD events [31]. However, it is equally important to recognize that enhancing long-term adherence to lipid lowering therapies is critical [32]. Given that the effectiveness of lipid lowering therapies (e.g. statins) depends more on the degree, timing, and duration of LDLc reduction than on the specific agent used, employing treatments with fewer muscle-related side effects could significantly improve therapeutic outcomes [33]. In

line with this, the 2025 update of the ESC/EAS guidelines for the management of dyslipidaemia reported that the use of bempedoic acid for statin-intolerant patients represents a significant development for this challenging population. The drug's unique activation pathway, which circumvents skeletal muscle expression, offers a solution to the longstanding clinical challenge of statin intolerance while maintaining cardiovascular efficacy [12]. Our results should be viewed from these perspectives: bempedoic acid, compared to atorvastatin, did not alter mitochondrial ATP production, basal or maximal mitochondrial respiration in skeletal muscle of apoE$^{-/-}$ mice, while reducing the atherosclerotic plaque burden, as evidenced by decreased plaque extent in the aorta and the presence of necrotic core areas. This evidence aligns with the observed reduction in HDLc, increased *Ldlr* expression and unchanged circulating PCSK9 levels compared to HFHC-only group. Additionally, it is worth noting that the decrease in HDLc aligns with the cholesterol metabolism typical of apoE$^{-/-}$ mice, where cholesterol is predominantly carried as HDL [34].

Considering that mitochondrial morphology directly reflects the mitochondrial function, TEM was used to examine gastrocnemius muscle fibres. The absence of changes in mitochondrial morphology or distribution in the bempedoic acid group supports clinical findings showing an incidence of muscular and serious adverse events comparable to placebo [35]. To our knowledge, this represents the first direct evidence of such effect with bempedoic acid. Conversely, mice given atorvastatin exhibited signs of possible mitochondrial degeneration. This observation aligns with evidence showing that cerivastatin triggered mitochondria swelling, lysosomal activation, the formation inclusion bodies within mitochondria, and the presence of autophagic vacuoles in the soleus muscles of rats [36].

To explore these aspects further, we assessed a range of behavioural changes, including neuromuscular function (gait analysis), motor coordination (rotarod test), muscular strength (grip test) and overall locomotor activity (using metabolic cages). These parameters were selected based on the understanding that the limb pain experienced by patients on statins is sometimes attributed by some to polyneuropathy. Some studies have proposed a connection between statin use and axonal neuropathy [37]. Despite this, our gait and rotarod test results showed no significant differences among groups. This outcome was expected, as these tests are primarily used to assess nerve damage and regeneration efficacy in peripheral nerve injuries or to detect motor skill impairments. We then employed a comprehensive lab animal metabolic activity monitoring system to measure spontaneous activity and physiological indicators (e.g. respiration). Compared to the bempedoic acid group, mice given atorvastatin showed reduction in spontaneous locomotor activity, lower energy expenditure, and decreased oxygen consumption, mainly during the dark phase, which aligns with nocturnal activity at night-time [38]. Locomotor activity is the parameter that would most accurately reflect energy expenditure because it encompasses both the smaller activities and larger activities that make up a mouse's total activities.

Regarding overall safety, bempedoic acid did not affect food intake, weight gain, glycaemia and hepatic enzymes. According to recent data showing that hepatic ATP-citrate lyase plays an unexpected role in restraining diet-dependent lipid accumulation and that bempedoic acid exerts substantial effects on hepatic lipid metabolism independently of ATP-citrate lyase possibly through activation of PPARα [39,40], also our enrichment analysis showed that mice receiving bempedoic acid had an enhanced fatty acid catabolism. In line with this, we found an increase in the expression of the mitochondrial electron transport chain's complexes (OXPHOS) in the group treated with bempedoic acid compared to the two other groups. This increase in the protein expression of complexes could reflect an enhanced mitochondrial biogenesis and respiratory capacity [41], as well as an increase in the catabolic activity of energetic substrates, like lipids.

5. Limitations

These results should be interpreted in light of certain limitations, such as the absence of a chow diet control group, and a group receiving a lower dose of atorvastatin. Specifically, including a group fed exclusively a chow diet would have provided a reference baseline for assessing skeletal muscle mitochondria activity (e.g. seahorse analysis) and mitochondrial morphology within muscle fibres. However, the robustness of our findings is supported by the use of a control group matched for sex, age and diet. Second, we acknowledge that evaluating the effects of a low dose atorvastatin on skeletal muscle mitochondria activity would have been informative. In this regard, the observed morphological alterations in skeletal muscles may be dose-dependent, as such changes have not been reported in literature when mice were given a very low dose of atorvastatin (3 mg/kg/day) [42]. However, it is important to underline that our preclinical model reflects a high-risk cardiovascular scenario, which typically requires a pharmacological treatment with a high-intensity statin regimen.

Third, regarding the evaluation of physical performance, a treadmill test could also have been included. However, the lack of significant differences observed in the rotarod, walking, and grip tests suggests that the inclusion of the treadmill would likely not have yielded substantial additional information. Furthermore, despite the choice of the murine model should not have influenced our findings on skeletal muscle mitochondrial activity, the *Ldlr*$^{-/-}$ mouse model could have been also used to replicate the data [43]. It would be worthwhile to consider replicating our study in apoE$^{-/-}$ male mice considering that in the CLEAR Outcomes study, bempedoic acid similarly reduced major adverse cardiovascular events in both females and males compared with placebo [44].

6. Conclusions

Considering that the reduction in cardiovascular risk achieved with bempedoic acid is comparable to that obtained with statins for the same absolute decrease in LDL-C levels [45], our data in apoE$^{-/-}$ mice shows that bempedoic acid positively impacts plaque burden while preventing skeletal muscle mitochondrial dysfunction. Finally, since adherence to lipid-lowering treatments is influenced by a multifaceted interaction of real and nocebo side effects, concerns about medication safety, and poor health literacy [46], to provide evidence describing a specific mechanism (e.g. the absence of impact on skeletal muscle mitochondrial function) might enhance the success to adherence.

CRediT authorship contribution statement

Simone Nerini: Methodology, Formal analysis. **Cesare R Sirtori:** Writing – review & editing. **Chiara Pavanello:** Formal analysis. **Jurgen Gindlhuber:** Formal analysis. **Alberto Corsini:** Writing – review & editing, Funding acquisition. **Massimiliano Ruscica:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Alessandra Stefania Rizzuto:** Data curation. **Valerio Magnaghi:** Writing – review & editing. **Tasnim Mohamed:** Formal analysis. **Elsa Franchi:** Formal analysis. **Marco Busnelli:** Writing – review & editing, Data curation. **Chiara Macchi:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Giulia Chiesa:** Writing – review & editing. **Isabella Fichtner:** Data curation. **Elena Osto:** Writing – review & editing. **Stefano Carugo:** Writing – review & editing.

Funding

This research was funded by Daiichi Sankyo Italy to Università degli Studi di Milano.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alberto Corsini reports financial support was provided by Daiichi Sankyo Italy for lectures. If there are other authors, they declare that they have no known competing financial interests or personal relationshipsthat could have appeared to influence the work reported in this paper.

Acknowledgments

Unitech Nolimits, an advanced imaging facility established by the Università degli Studi di Milano. The authors acknowledge support from the University of Milan through the APC initiative.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118726.

Data availability

Data will be made available on request.

References

[1] G.B.D. Diseases, C. Injuries, Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019, Lancet 396 (10258) (2020) 1204–1222.
[2] A. Pirillo, et al., Global epidemiology of dyslipidaemias, Nat. Rev. Cardiol. 18 (10) (2021) 689–700.
[3] B.A. Ference, E. Braunwald, A.L. Catapano, The LDL cumulative exposure hypothesis: evidence and practical applications, Nat. Rev. Cardiol. 21 (10) (2024) 701–716.
[4] F. Mach, et al., 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Eur. Heart J. 41 (1) (2020) 111–188.
[5] J. Brandts, et al., Optimal implementation of the 2019 ESC/EAS dyslipidaemia guidelines in patients with and without atherosclerotic cardiovascular disease across Europe: a simulation based on the DA VINCI study, Lancet Reg. Health Eur. 31 (2023) 100665.
[6] S.F. Nielsen, B.G. Nordestgaard, Negative statin-related news stories decrease statin persistence and increase myocardial infarction and cardiovascular mortality: a nationwide prospective cohort study, Eur. Heart J. 37 (11) (2016) 908–916.
[7] M. Ruscica, et al., Side effects of statins: from pathophysiology and epidemiology to diagnostic and therapeutic implications, Cardiovasc Res 118 (17) (2023) 3288–3304.
[8] E.S. Stroes, et al., Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus Panel Statement on Assessment, Aetiology and Management, Eur. Heart J. 36 (17) (2015) 1012–1022.
[9] S.E. Nissen, et al., Bempedoic Acid and Cardiovascular Outcomes in Statin-Intolerant Patients, N. Engl. J. Med 388 (15) (2023) 1353–1364.
[10] M. Ruscica, et al., ETC-1002 (Bempedoic acid) for the management of hyperlipidemia: from preclinical studies to phase 3 trials, Expert Opin. Pharmacother. 20 (7) (2019) 791–803.
[11] M. Ruscica, et al., Bempedoic Acid: for Whom and When, Curr. Atheroscler. Rep. 24 (10) (2022) 791–801.
[12] F. Mach, et al., 2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias, Eur. Heart J. (2025).
[13] S.L. Pinkosky, et al., Liver-specific ATP-citrate lyase inhibition by bempedoic acid decreases LDL-C and attenuates atherosclerosis, Nat. Commun. 7 (2016) 13457.
[14] J.P. Samsoondar, et al., Prevention of Diet-Induced Metabolic Dysregulation, Inflammation, and Atherosclerosis in Ldlr(-/-) Mice by Treatment With the ATP-Citrate Lyase Inhibitor Bempedoic Acid, Arterioscler. Thromb. Vasc. Biol. 37 (4) (2017) 647–656.
[15] C. Macchi, et al., Impact of Atorvastatin on Skeletal Muscle Mitochondrial Activity, Locomotion and Axonal Excitability-Evidence from ApoE(-/-) Mice, Int J. Mol. Sci. 23 (10) (2022).
[16] G. Caligiuri, et al., Effects of sex and age on atherosclerosis and autoimmunity in apoE-deficient mice, Atherosclerosis 145 (2) (1999) 301–308.
[17] M. Busnelli, et al., Effect of Diets on Plasma and Aorta Lipidome: A Study in the apoE Knockout Mouse Model, Mol. Nutr. Food Res 67 (2) (2023) e2200367.
[18] M. Busnelli, et al., Lack of ApoA-I in ApoEKO Mice Causes Skin Xanthomas, Worsening of Inflammation, and Increased Coronary Atherosclerosis in the Absence of Hyperlipidemia, Arterioscler. Thromb. Vasc. Biol. 42 (7) (2022) 839–856.
[19] M. Busnelli, et al., Rupatadine Treatment Is Associated with Atherosclerosis Worsening and Altered T Lymphocyte Recruitment, Thromb. Haemost. 122 (5) (2022) 853–856.
[20] A. Daugherty, et al., Recommendation on Design, Execution, and Reporting of Animal Atherosclerosis Studies: A Scientific Statement From the American Heart Association, Arterioscler. Thromb. Vasc. Biol. 37 (9) (2017) e131–e157.
[21] C. Macchi, et al., Monocarboxylate transporter 1 deficiency impacts CD8(+) T lymphocytes proliferation and recruitment to adipose tissue during obesity, iScience 25 (6) (2022) 104435.
[22] L. Da Dalt, et al., The inhibition of inner mitochondrial fusion in hepatocytes reduces non-alcoholic fatty liver and improves metabolic profile during obesity by modulating bile acid conjugation, Cardiovasc Res 119 (18) (2024) 2917–2929.
[23] V. Magnaghi, et al., Altered peripheral myelination in mice lacking GABAB receptors, Mol. Cell Neurosci. 37 (3) (2008) 599–609.
[24] S. Girirajan, et al., How much is too much? Phenotypic consequences of Rai1 overexpression in mice, Eur. J. Hum. Genet 16 (8) (2008) 941–954.
[25] T. Somers, et al., Effect of statins on mitochondrial function and contractile force in human skeletal and cardiac muscle, Biomed. Pharmacother. 180 (2024) 117492.
[26] T.J. Schirris, et al., Statin-Induced Myopathy Is Associated with Mitochondrial Complex III Inhibition, Cell Metab. 22 (3) (2015) 399–407.
[27] T.E. Ryan, et al., High-dose atorvastatin therapy progressively decreases skeletal muscle mitochondrial respiratory capacity in humans, JCI Insight 9 (4) (2024).
[28] C.R. Mikus, et al., Simvastatin impairs exercise training adaptations, J. Am. Coll. Cardiol. 62 (8) (2013) 709–714.
[29] J. Bouitbir, et al., Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a 'mitohormesis' mechanism involving reactive oxygen species and PGC-1, Eur. Heart J. 33 (11) (2012) 1397–1407.
[30] A.J. Dirks, K.M. Jones, Statin-induced apoptosis and skeletal myopathy, Am. J. Physiol. Cell Physiol. 291 (6) (2006) C1208–C1212.
[31] B.A. Ference, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (32) (2017) 2459–2472.
[32] A. Shroufi, J.W. Powles, Adherence and chemoprevention in major cardiovascular disease: a simulation study of the benefits of additional use of statins, J. Epidemiol. Community Health 64 (2) (2010) 109–113.
[33] K.K. Ray, et al., Combination lipid-lowering therapy as first-line strategy in very high-risk patients, Eur. Heart J. 43 (8) (2022) 830–833.
[34] K.S. Meir, E. Leitersdorf, Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress, Arterioscler. Thromb. Vasc. Biol. 24 (6) (2004) 1006–1014.
[35] A. Sayed, et al., The Clinical Efficacy and Safety of Bempedoic Acid in Patients at Elevated Risk of Cardiovascular Disease: A Meta-Analysis of Randomized Clinical Trials, Cardiovasc Drugs Ther. 38 (6) (2024) 1415–1420.
[36] H. Obayashi, et al., Cerivastatin induces type-I fiber-, not type-II fiber-, predominant muscular toxicity in the young male F344 rats, J. Toxicol. Sci. 36 (4) (2011) 445–452.
[37] U. Jeppesen, et al., Statins and peripheral neuropathy, Eur. J. Clin. Pharmacol. 54 (11) (1999) 835–838.
[38] P. Hawkins, H.D.R. Golledge, The 9–5 Rodent - Time for Change? Scientific and animal welfare implications of circadian and light effects on laboratory mice and rats, J. Neurosci. Methods 300 (2018) 20–25.
[39] J.Y. Liu, et al., Bempedoic acid suppresses diet-induced hepatic steatosis independently of ATP-citrate lyase, Cell Metab. 37 (1) (2025) 239–254, e7.
[40] J.G. Supplee, R. Marmorstein, K.E. Wellen, Molecular targets of bempedoic acid and related decoy fatty acids, Trends Endocrinol. Metab. (2025).
[41] C.F. Bennett, P. Latorre-Muro, P. Puigserver, Mechanisms of mitochondrial respiratory adaptation, Nat. Rev. Mol. Cell Biol. 23 (12) (2022) 817–835.
[42] P. Zheng, et al., Low-dose atorvastatin protects skeletal muscle mitochondria in high-fat diet-fed mice with mitochondrial autophagy inhibition and fusion enhancement, Eur. J. Pharmacol. 959 (2023) 176085.
[43] G.S. Getz, C.A. Reardon, Do the Apoe-/- and Ldlr-/- Mice Yield the Same Insight on Atherogenesis? Arterioscler. Thromb. Vasc. Biol. 36 (9) (2016) 1734–1741.
[44] L. Cho, et al., Impact of Bempedoic Acid on Cardiovascular Outcomes by Sex, Circulation 149 (22) (2024) 1775–1777.
[45] A.M. Lincoff, et al., Comparative Cardiovascular Benefits of Bempedoic Acid and Statin Drugs, J. Am. Coll. Cardiol. 84 (2) (2024) 152–162.
[46] K. Whitmore, et al., Review of strategies to improve adherence to lipid-lowering therapy in the primary prevention of cardiovascular disease, Eur. J. Prev. Cardiol. 32 (13) (2025) 1204–1215.