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RESEARCH ARTICLE

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Physical training promotes remodeling of the skeletal muscle extracellular matrix: An ultrastructural study in a murine model of Down syndrome

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Abstract

Down syndrome (DS) is a genetically based disease caused by triplication of chromosome 21. DS is characterized by multi-systemic premature aging associated with deficit in motor coordination, balance, and postural control. Using a morphological, morphometrical, and immunocytochemical ultrastructural approach, this study investigated in vastus lateralis muscle of Ts65Dn mouse, a murine model of DS, the effect of an adapted physical training on the extracellular matrix (ECM) characteristics and whether the forecasted exercise-induced ECM remodeling impacts on sarcomere organization. Morphometry demonstrated thicker basement membrane and larger collagen bundles with larger interfibrillar spacing as well as irregularly arrayed myofibrils and lower telethonin density on Z-lines in trisomic versus euploid sedentary mice. In agreement with the multi-systemic premature aging described in DS, these ECM alterations were similar to those previously observed in skeletal muscle of aged mice. Adapted physical training induced remodeling of ECM in both trisomic and euploid mice, that is, enlargement of the collagen bundles associated with hypertrophy of collagen fibrils and reduction of the interfibrillar spacing. A re-alignment of the myofibrils and a higher telethonin density on Z-line was found in trisomic mice. Altogether, our findings suggest that physical training is an effective tool in limiting/counteracting the trisomy-associated musculoskeletal structural anomalies. The current findings constitute a solid experimental background for further study investigating the possible positive effect of physical training on skeletal muscle performance.

Research Highlights

- Vastus lateralis muscle of trisomic mice shows aging-like alterations of extracellular matrix.
- Training promotes extracellular matrix remodeling.

- Training may be an effective tool to counteract trisomy-associated alterations of skeletal muscle.

KEYWORDS
aging, exercise, sarcomere, transmission electron microscopy, trisomy

1 | INTRODUCTION

Down syndrome (DS) is a genetically based disease caused by triplication of chromosome 21 and affecting 1 in 700 newborns (Parker et al., 2010). People with DS show a premature or accelerated aging of some body systems (Zigman, 2013), among which the musculoskeletal (Barnhart & Connolly, 2007). Deficit in motor coordination, balance, and postural control (Malak et al., 2013; Rigoldi et al., 2011) as well as severe muscle hypotonia and weakness are typical musculoskeletal abnormalities in DS (Davis & Kelso, 1982; Lagan et al., 2020; Morris et al., 1982). The early development of such physical dysfunctions critically impacts on the quality of daily life and functional capacity of affected people.

Understanding of mechanisms leading to the DS-associated musculoskeletal deficit is still limited. In this regard, animal models of DS represent a useful tool to investigate the DS-associated skeletal muscle structural and functional alterations. The Ts65Dn mouse is a well-characterized and widely studied mouse model of DS sharing many features with the human condition including locomotor deficit (Costa et al., 1999). A recent study from this laboratory (Cisterna et al., 2020) provided evidence of a "sarcoplasmic hypertrophy" associated with structural alterations of mitochondria and irregular arrangement of the myofibrils in muscle cells of the quadriceps muscle from trisomic Ts65Dn mice. The soleus muscle of the same model shows unbalance in glucose and fat metabolism as well as pathways involved into adenosine triphosphate biosynthesis (Cowley et al., 2012). These and other findings in both human and murine DS (Gomez et al., 2020; Mollo et al., 2020) suggest impaired mitochondrial biogenesis and bioenergetics in the DS skeletal muscle. Overall, structural and compositional trisomy-associated alterations of the myofiber is indicative of an early aging of the skeletal muscle cell in DS (Cisterna et al., 2013, 2020; Zigman, 2013).

The myofibers of skeletal muscle are structurally and functionally supported by the extracellular matrix (ECM), a complex network of collagens, glycosaminoglycans, proteoglycans, laminins, elastin, and fibronectins (Theocharis et al., 2014). ECM is involved in muscle development (Thorsteinsdóttir et al., 2011), growth, and repair (Calve et al., 2010) as well as transmission of contractile force (Street, 1983). As a dynamic compartment, the ECM is continuously remodeled. Therefore, alterations in the normal remodeling of the ECM can impact the functional properties of skeletal muscle (Kragstrup et al., 2011). In this regard, evidence has been provided that changes in the architecture and composition of ECM take place in the aging skeletal muscle (Csapo et al., 2020; Garg & Boppart, 2016; Kragstrup et al., 2011; Lofaro et al., 2021). An unbalanced ECM collagen turnover leads to increased collagen deposition and possible alterations of collagen composition/arrangement (Csapo et al., 2020; Kragstrup et al., 2011; Lofaro et al., 2021), likely causing enhancement of muscle stiffness (Csapo et al., 2020; Wood et al., 2014), a typical hallmark of muscle aging (Wood et al., 2014). Furthermore, the basement membrane (BM), which surrounds the myofibers acting as a bridge to the innermost layer of the ECM, thickens in aging in accordance with accumulation of its components (Kovanen et al., 1988; Lofaro et al., 2021); this suggests age-associated alterations of the anchoring system, that is, the structural and functional interconnection of the myofiber to the ECM (Lofaro et al., 2021). An important dysregulation of the expression of ECM components was demonstrated in DS (Conti et al., 2007; Galat et al., 2020), likely resulting in structural disorganization of the ECM (Mollo et al., 2022). Interestingly, DS is characterized by anomalies of stromal progenitor development strongly suggestive of a connective tissue impairment likely associated with musculoskeletal disorders (Galat et al., 2020).

A wealth of evidence indicates that physical training is effective in improving the skeletal muscle condition at all ages (Pedersen & Saltin, 2015). Physical training has been shown to improve muscle performance in both children and young adults with DS (Sugimoto et al., 2016). However, no quantitative changes in the tissue composition of skeletal muscle or fiber type were found in Ts65Dn trisomic mice upon training (Cisterna et al., 2022), strongly suggesting that other factors, for example, the ECM are involved in the locomotor deficit observed in DS.

Based on the above and considering the well-known ability of physical training to remodel ECM in normal skeletal muscle (Heinemeier et al., 2009; Hyldahl et al., 2015; Kjaer, 2004), the present study investigated the effect of adapted physical training on the ECM characteristics of vastus lateralis muscle of trisomic Ts65Dn mice. To assess whether the forecasted exercise-induced remodeling of the ECM impacts on sarcomere organization, the alignment as well as register of Z-lines (which guarantee the unison variation of myofibril length and thus the coordinated contraction of the myofiber) were evaluated. Moreover, the distribution/abundancy of telethonin was evaluated. Theletonin (t-cap titin), is a Z-line molecule binding two titin molecules to form a mechanically resistant and stable complex, which anchors titin (Lee et al., 2006) thereby maintaining the architecture of the sarcomere (Wadmore et al., 2021).

Using a morphological, morphometrical, and immunocytochemical ultrastructural approach, this study highlighted some interesting parallels between trisomy and aging, possibly opening the way to better understanding of the musculoskeletal deficit in DS. Moreover, findings indicated ECM remodeling in physically trained mice suggesting that

physical training may be an effective tool in limiting/counteracting the trisomy-associated musculoskeletal anomalies.

2 | MATERIALS AND METHODS

2.1 | Animals and adapted physical training

Ts65Dn [strain: B6EiC3Sn.BLia-Ts(17<16>)65Dn/DnJ] breeder mice were obtained from the Jackson Laboratory, Bar Harbor, ME, USA. Tissue for genotyping was obtained from tail clips (Reinholdt et al., 2011) in p11 male mice. The mice were housed in groups of 3–4 by genotype and maintained under standard conditions (24 ± 1°C ambient temperature, 60% ± 15% relative humidity, and 12 h light/dark cycle) and fed ad libitum with standard commercial chow. The trisomic mice presented deficits in balance and motor coordination by month 4 of age (Costa et al., 1999).

The study was approved by national committee board of the Italian Ministry of Health (ref.: 538/2015-PR), in compliance with the Italian Ministry of Health (DL March 4, 2014, n. 26) and the European Communities Council (Directive 63/2010/EU of the European Parliament and of the Council) guidelines for the care and use of animals. Eight (four trisomic and four euploid) male Ts65Dn mice aged 6 months were used for this study. For physical training, trisomic and euploid (control) mice were assigned to one of four groups: sedentary trisomic (ST; $n = 2$), sedentary euploid (SE; $n = 2$), trained trisomic (TT; $n = 2$), and trained euploid (TE; $n = 2$). Mice in the TT and TE group underwent training on a treadmill (Harvard Instruments, Crisel Instruments, Rome, Italy). Belt speed was 8 m/min (0% incline). Mice trained 45 min a day, 5 days a week for 1 month. In this work, physical training was adapted to optimize trisomic mice compliance to training (Fattoretti et al., 2018).

The Ts65Dn mouse is fragile and only a limited number of adult animals is made available for experiments at a time; accordingly, previously published data (Cisterna et al., 2022) of Z-line alignment in ST and SE mice were used for comparison with TT and TE.

2.2 | Tissue processing

Ts65Dn mice were deeply anesthetized using Tribromoethanol and perfused transcardially with phosphate buffer solution (PBS, containing 137 mM NaCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl) followed by 4% paraformaldehyde in PBS. During perfusion, both hindlimbs were secured on the surgical table by using surgical tape maintaining a 90° angle between thigh and leg. After perfusion, the vastus lateralis muscle was quickly removed, cut in fragments (about 1 mm^3), and then placed for 2 h at 4°C in either a 2.5% glutaraldehyde plus 2% paraformaldehyde in PBS (samples intended for ultrastructure morphology) or 4% paraformaldehyde and 0.2% glutaraldehyde in PBS (samples intended for immunocytochemical evaluation). After fixation, samples for ultrastructural morphology were rinsed with PBS, postfixed with 1% OsO4 and 1.5% $K_4Fe(CN)_6$ for 2 h at 4°C, dehydrated with acetone, and embedded in Epon 812 resin. For immunocytochemistry, samples were washed in PBS, treated with 0.5 M NH_4Cl solution in PBS for 45 min at 4°C to block free aldehyde groups, dehydrated in graded concentrations of ethanol at room temperature, and embedded in LRWhite resin. Due to the small size of the muscles, the vastus lateralis muscle from the right thigh was used for ultrastructural morphology and the muscle from the left thigh was processed for immunohistochemistry.

Muscles from all groups (ST, SE, TT, and TE), were processed in a single run to guarantee that all samples were prepared under the same conditions. Muscle samples were cut transversely at mid-length, and then appropriately placed in a mold to obtain longitudinal muscle sections upon cutting.

2.3 | Morphometrical evaluation of ECM components

Ultrathin (70–90 nm thick) sections of Epon-embedded muscles were stained with Reynolds' lead citrate for 2 min and observed with a Philips Morgagni transmission electron microscope operating at 80 kV and equipped with a Megaview III camera for digital image acquisition.

The thickness of the BM, that is, the electrodense sheath covering the myofiber, was measured on randomly selected areas (×44,000) of longitudinally sectioned muscles. Morphometric evaluation (200 measurements per ST, SE, TT, and TE) was carried out using the Radius software (EMSIS GmbH, Germany) implemented in the Philips Morgagni transmission electron microscope. Morphometry of the perimysium collagen bundles was carried out on a total of 50 longitudinally sectioned collagen bundles (×14,000) per mice group. Collagen bundles of a minimum of 4 μm in length were considered. Collagen bundle size (i.e., the width of the bundle) and the index of collagen bundle linearity (X/Y, i.e., the ratio between the real length [X] of the bundle measured following the profile of the bundle itself and its corresponding linear length [Y] measured between the two ends of the bundle) were assessed. Collagen fibril diameter and the distance between single collagen fibrils were measured on a total of 200 longitudinally sectioned collagen fibrils (×44,000) per mice group. The ImageJ software (NIH) was used for all measurements.

2.4 | Computational evaluation of the Z-lines alignment

The Z-lines alignment was evaluated in TT and TE using a semi-automatic routine written in MATLAB (2018b version, Mathworks; Cisterna et al., 2021) and results were compared with those obtained in sedentary mice (trisomic and euploid) of the same strain (Cisterna et al., 2021). Briefly, TEM images (×8900) of myofibers were randomly acquired avoiding the cell periphery, and the "ginput" MATLAB function was used to define the end points of the Z-lines of sarcomeres belonging to adjacent myofibrils. The following parameters

were measured: H step, representing the step between the Z-lines of two adjacent myofibrils and calculated as the distance between the point at end of one Z-line and the line passing through the adjacent Z-line; mean of the H step values along a single Z-staircase (where Z-staircase is used to indicate the sequence of adjacent Z-lines in adjacent myofibrils) calculated as $H_{mean,Z\text{-staircase}} = (H_1 + H_2 + \ldots H_n)/n$ where H_i ($i = 1, \ldots, n$) is the value of the step between the ($i + 1$)th Z-line and ith Z-line; standard deviation of the $H_{mean,Z\text{-staircase}}$ along a Z-staircase as assessment of the homogeneity/heterogeneity in sarcomere alignment. The distributions of the Z-line alignment parameters were plotted, and the frequency was the number of measurements in a bar width divided by the total number of measurements. Measurements were performed on 350 sarcomeres and 30 Z-staircase for each mice group.

2.5 | Ultrastructural immunocytochemistry

Ultrathin sections (70–90 nm thick) of LRWhite-embedded muscle collected on Formvar–carbon-coated nickel grids, were floated on normal donkey serum (NDS) diluted 1:100 in PBS for 3 min and then incubated at 4°C for 17 h with a polyclonal anti-telethonin primary antibody (sc-8725, Santa Cruz Biotechnology, Dallas, USA) diluted 1:25 in PBS containing 0.05% Tween and 0.1% BSA. After rinsing with PBS Tween and PBS, the grids were incubated with NDS as above, and then incubated for 30 min at room temperature with a goat anti-rabbit IgG (H + L) secondary antibody (Jackson Immuno Research Laboratories, West Grove, PA, USA) coupled with 12 nm colloidal gold, diluted 1:20 in PBS. The grids were rinsed with PBS and distilled water. As control, some grids were floated on the incubation mixture without the primary antibody, and then treated as above. Sections were stained for 30 min at room temperature with Uranyl Less EM stain (Electron Microscopy Sciences, Hatfield, PA, USA), followed by Reynolds' lead citrate for 1 min (Lacavalla & Cisterna, 2023). Sections were then observed with a Philips Morgagni transmission electron microscope operating at 80 kV.

Semiquantitative assessment of the anti-telethonin immunolabeling was carried out by estimating the gold grain density on 100 Z-lines per each mice group. The gold grains were counted, and the labeling density was expressed as number of gold grains/Z-line length (in nanometers).

2.6 | Statistical analysis

Quantitative and semiquantitative values for individual variable were pooled according to the experimental group (ST, SE, TT, and TE) and presented as mean ± standard error of the mean (SEM). The Shapiro–Wilk test showed that data for all measured variables were not normally distributed ($p < .001$). Consequently, statistical analysis was performed using the non-parametric Kruskal-Wallis test. In case of significance, post-hoc group–group comparison was performed with the Mann–Whitney test. The significance was set at $p \leq .05$. The IBM-SPSS (v.25, Armonk, NY, USA) statistical package was used for all analyses.

3 | RESULTS

3.1 | Morphometry of the ECM collagenous components

In all four groups of mice (SE, ST, TE, and TT) the BM covered the surface of the skeletal muscle cells as an electrodense sheath in connection with the network of collagen fibrils of the endomysium (Figure 1a–d).

The Kruskall-Wallis test showed a statistically significant difference in BM thickness in the four groups ($p < .0001$). Post-hoc analysis (Figure 1e) highlighted that BM was significantly thicker in ST versus SE. BM was significantly thinner in both TT and TE versus their sedentary counterpart. Significantly thicker BM was found in TT versus TE.

In all mice, the perimysium showed the typical organization of the ECM, rich in bundles of collagenous fibrils (Figure 2).

The Kruskall-Wallis test showed a statistically significant difference in the four groups for both collagen bundle size ($p = .0001$) and linearity ($p = .0002$). Post-hoc analysis (Figure 2e) highlighted that collagen bundles were significantly larger in ST versus SE. The bundles were significantly enlarged in TE and TT versus SE and ST, respectively.

The collagen bundles were spatially organized in various orientations and the X/Y parameter was calculated as an assessment of the linearity of the bundles in the perimysium (Figure 2f). Post-hoc analysis showed that collagen bundles were more linear in ST versus SE as well as TT versus TE. No significant difference was found in ST and SE versus their trained counterpart.

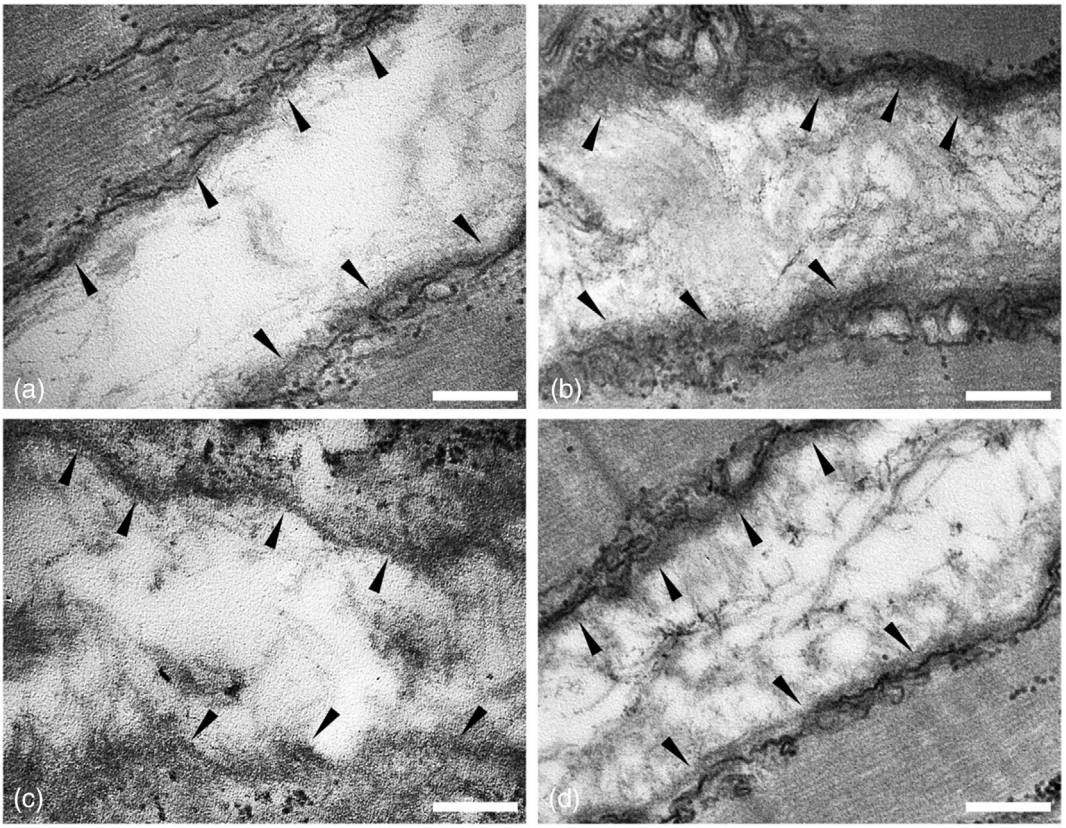
Each collagen bundle was made up of longitudinally arranged collagen fibrils running close to each other (Figure 3).

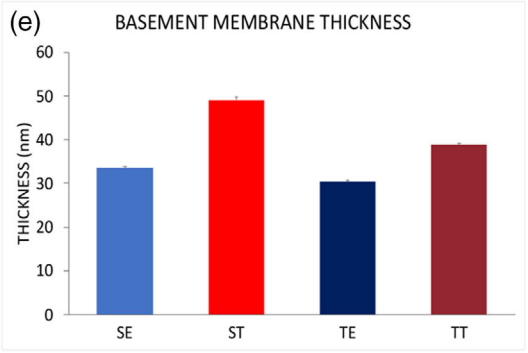
The Kruskall-Wallis test demonstrated a statistically significant difference in the four groups for both collagen fibril diameter ($p = .005$) and distance ($p < .0001$). Post-hoc analysis indicated no significant difference in collagen fibril diameter (Figure 3e) in SE versus ST as well as TE versus TT. The fibril thickness was significantly higher in trained mice (either euploid or trisomic) versus their respective sedentary counterpart.

The distance between collagen fibrils (Figure 3f) was statistically significantly higher in ST versus SE as well as TT versus TE. The interfibrillar distance was significantly reduced in trained mice (either euploid or trisomic) versus their respective sedentary counterpart.

3.2 | Ultrastructural morphology and computational evaluation of the Z-lines alignment

In all mice groups (ST, SE, TT, and TE), myofibers of vastus lateralis muscle were typically elongated, showing myonuclei in subsarcolemmal position containing a few heterochromatin clumps. Longitudinally





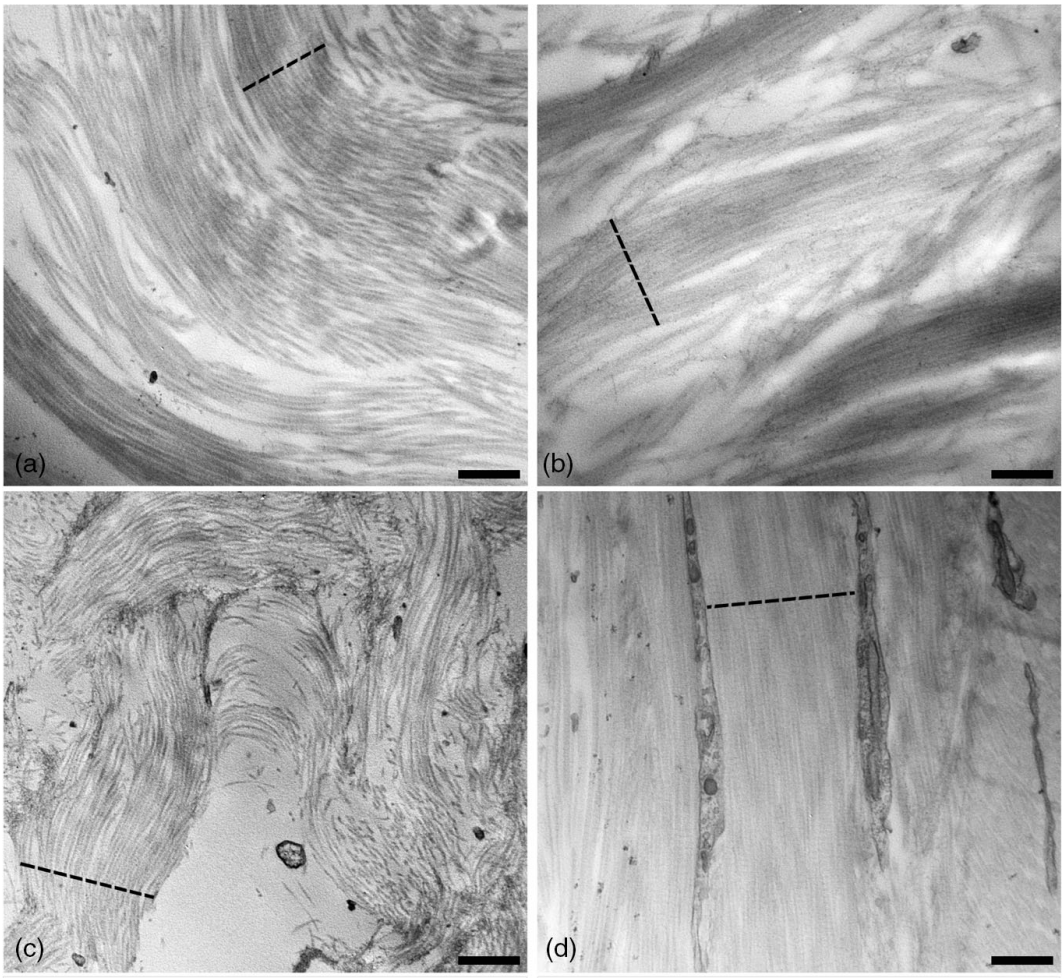
	ST	TE	TT
SE	< 0.0001	< 0.0001	< 0.0001
ST		< 0.0001	< 0.0001
TE			< 0.0001

FIGURE 1 Ultrastructural images of the basement membrane in sedentary euploid (SE, panel a), sedentary trisomic (ST, panel b), trained euploid (TE, panel c), and trained trisomic (TT, panel d) vastus lateralis muscle. Arrowhead, BM. Bars: 200 nm. The results of post-hoc morphometric analysis are reported in panel (e).

arrayed myofibrils occupied almost the entire cytoplasm, and ovoid mitochondria were lined in narrow sarcoplasm areas between myofibrils (Figure 4). However, in both ST (Figure 4b) and TT (Figure 4d) large mitochondria and dilated sarcoplasmic reticulum cisternae were aligned between irregularly arrayed myofibrils.

The Kruskall-Wallis test showed statistically significant difference in the four groups of mice for all Z-line alignment parameters (H step, $p < .0001$; mean value of $H_{mean,Z\text{-staircase}}$, $p < .0001$; standard deviation of $H_{mean,Z\text{-staircase}}$ along a Z-staircase, $p < .0001$).

Post-hoc analysis showed in both SE and TE similar values for H step (Figure 4e), $H_{mean,Z\text{-staircase}}$ (Figure 4f), and standard deviation of $H_{mean,Z\text{-staircase}}$ along a Z-staircase (Figure 4g), in agreement with morphological findings showed in Figure 4a,c. The Z-lines were not perfectly aligned in ST (Figure 4b) versus SE, as demonstrated by



	ST	TE	TT
SE	*0.0078*	*0.0003*	*< 0.0001*
ST		0.13	*0.05*
TE			0.63

	ST	TE	TT
SE	*0.0002*	0.95	*0.01*
ST		*0.001*	0.34
TE			*0.04*

FIGURE 2 Ultrastructural images of the collagen bundles of sedentary euploid (SE, panel a), sedentary trisomic (ST, panel b), trained euploid (TE, panel c), and trained trisomic (TT, panel d) perymisium. Dashed line shows the size of collagen bundle. Bars: 500 nm. The results of post-hoc analysis are reported in panels (e) and (f).

significantly higher values of H step (Figure 4e), $H_{\text{mean,Z-staircase}}$ (Figure 4f), and standard deviation of $H_{\text{mean,Z-staircase}}$ along a Z-staircase in the former. Significantly lower values for Z-line parameters were found in TT versus ST (Figure 4e–g), in agreement with the Z-line alignment observed in TT (Figure 4d).

The distributions of H step, mean value of $H_{\text{mean,Z-staircase}}$, and standard deviation of $H_{\text{mean,Z-staircase}}$ along a Z-staircase (Figure 5) demonstrated a misalignment of the Z-lines together with high heterogeneity in such a misalignment in ST versus SE. The distributions of all Z-line parameters were similar in TE versus TT.

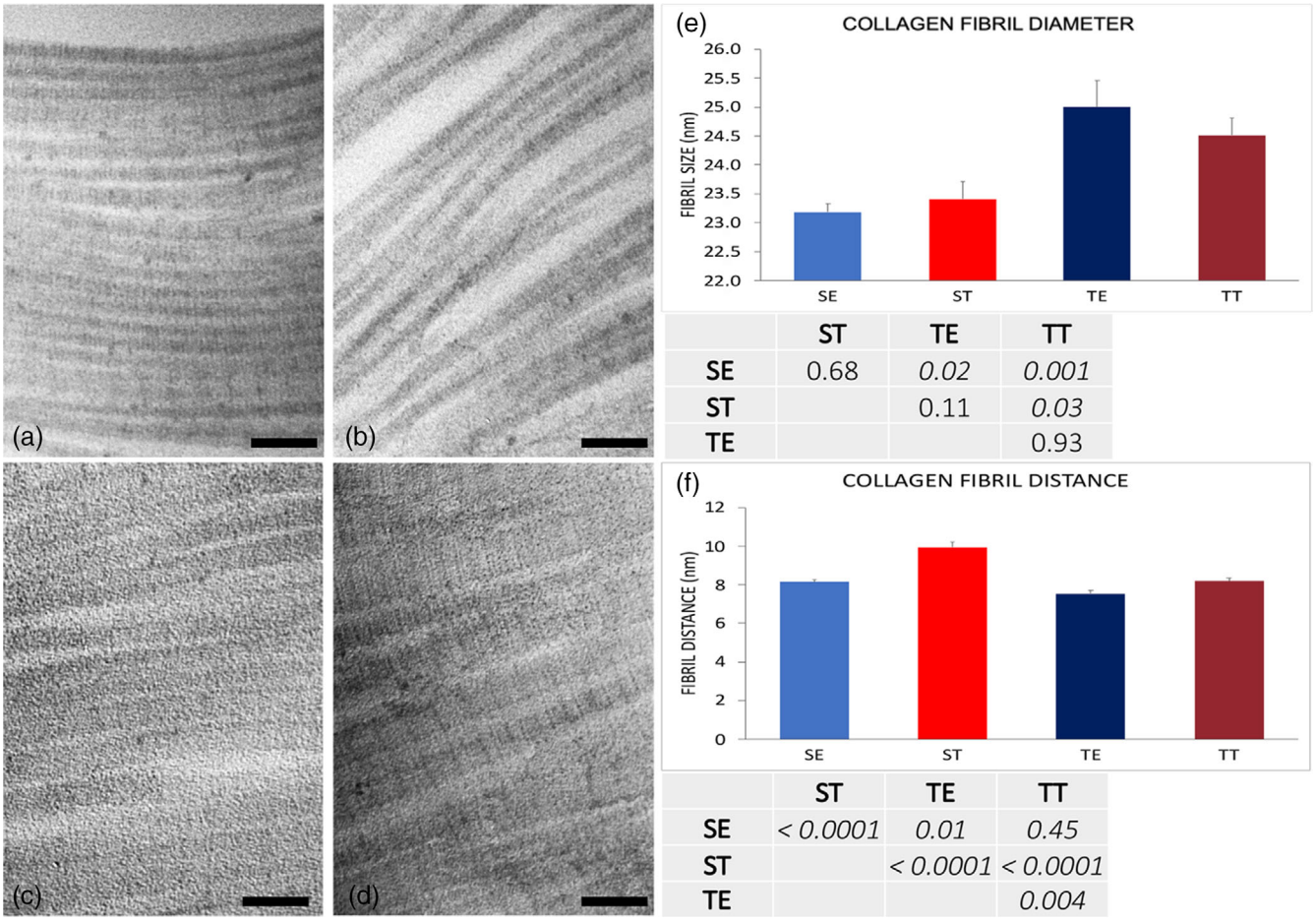


FIGURE 3 Ultrastructural images of the collagen fibrils of sedentary euploid (SE, panel a), sedentary trisomic (ST, panel b), trained euploid (TE, panel c), and trained trisomic (TE, panel d) perymisium. Bars: 100 nm. The results of post-hoc analysis are reported in panels (e) and (f).

3.3 | Ultrastructural immunocytochemistry

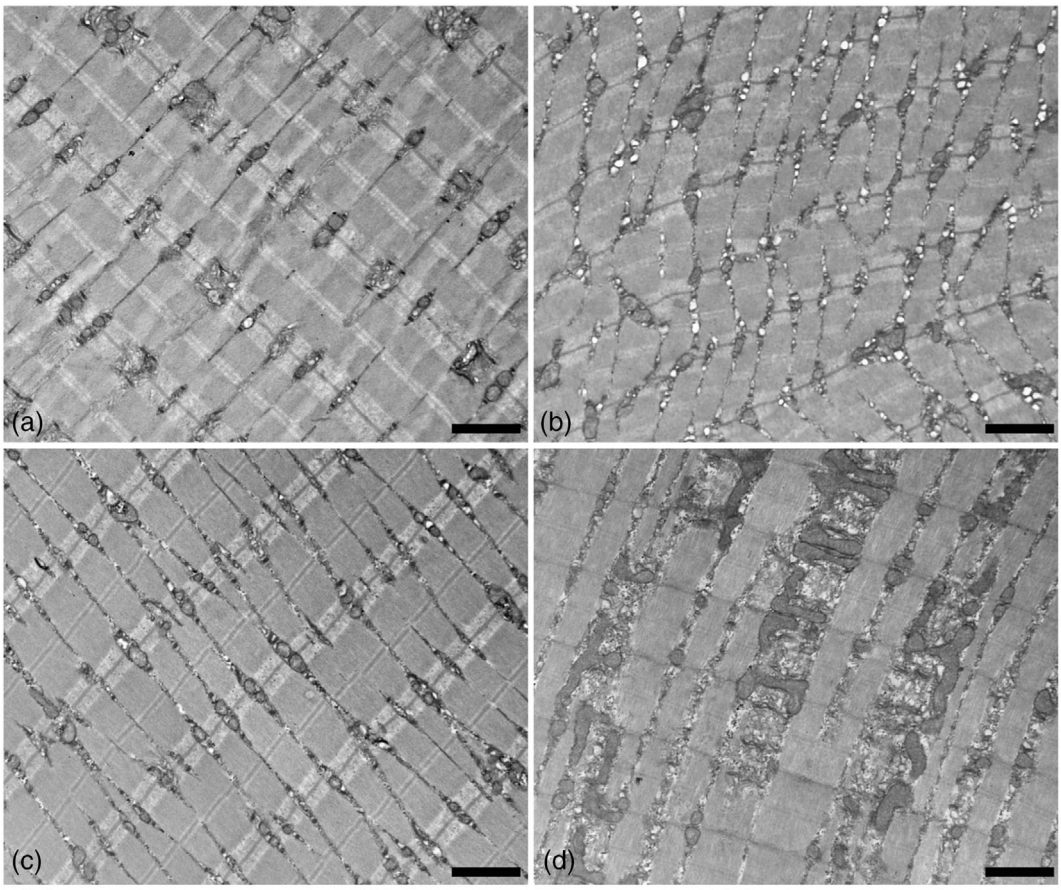
The telethonin immunolabeling on the Z-line (Figure 6a) was considered for semiquantitative evaluation of the linear density of the telethonin molecule.

The Kruskall-Wallis test showed a statistically significant difference in the four groups of mice ($p = .01$) for anti-telethonin immunolabeling linear density. Post-hoc analysis highlighted that telethonin immunolabeling was significantly less dense in ST versus SE. The telethonin density was significantly higher in TT versus ST, but not TE. No significant difference was found in SE versus TE.

4 | DISCUSSION

DS is characterized by a multi-systemic early aging (Zigman, 2013) which also affects the musculoskeletal apparatus (Barnhart & Connolly, 2007). The Ts65Dn mouse shares with DS human condition several phenotypes including locomotor deficit (Costa et al., 1999) and multi-systemic premature aging (Cisterna et al., 2013, 2020; Vacano et al., 2012). In this work, the Ts65Dn mouse was used to expand our knowledge of the trisomy-associated alterations of the myofiber by exploring the organization of the skeletal muscle ECM, its involvement in the Z-line misalignment observed in ST mice (Cisterna et al., 2021), and the effect of adapted physical exercise on both ECM and sarcomere.

A thicker BM was found in vastus lateralis muscle of trisomic versus euploid mice of both sedentary and physically trained groups, suggesting a trisomy-associated accumulation of BM components, for example, laminin and type IV collagen. This is supported by the finding of high amount of laminin in the soleus muscle of Ts65Dn mice (Cowley et al., 2012), up-regulation of type IV collagen in DS cells and tissues (Vilardell et al., 2011), and high amount of type IV collagen in trisomic fibroblast lysate (Mollo et al., 2022). Interestingly, thickening of the BM was found in aged muscle (Lofaro et al., 2021) along with accumulation of both laminin and type IV collagen (Kovanen et al., 1988; Lofaro et al., 2021). The increased thickness of the BM, common to both trisomic and aged mice, may induce an impairment of the anchoring system, which structurally and functionally interconnects the myofiber to the ECM (Lofaro et al., 2021). Type VI collagen is a key player in the anchoring system that is crucially involved in the function and stability of skeletal muscle (Cescon et al., 2015; Sabatelli



(e)	ST	TE	TT
SE	< 0.0001	0.32	0.004
ST		< 0.0001	< 0.0001
TE			< 0.0001

(f)	ST	TE	TT
SE	< 0.0001	0.75	0.008
ST		< 0.0001	< 0.0001
TE			0.005

(g)	ST	TE	TT
SE	< 0.0001	0.23	0.065
ST		< 0.0001	< 0.0001
TE			0.006

FIGURE 4 Ultrastructural images of vastus lateralis myofibers from sedentary euploid (SE, panel a), sedentary trisomic (ST, panel b), trained euploid (TE, panel c), and trained trisomic (TT, panel d). (a, c) The cytoplasm is occupied by regularly arrayed myofibrils with well-preserved triad areas. (b) Less regularly arrayed myofibrils characterize the myofiber. (d) Less regularly arrayed but aligned myofibrils occupied the cytoplasm. (b, d) Note the large-size mitochondria and dilated sarcoplasmic reticulum. Bars: 1 mm. Results of post-hoc analysis of H step, $H_{mean,Z\text{-staircase}}$ value along the Z-line of the adjacent sarcomere, and standard deviation of the $H_{mean,Z\text{-staircase}}$ along a Z-staircase are reported in panels (e–g).

et al., 2001). By forming a microfibrillar network surrounding the BM, type VI collagen interacts with type IV collagen and fibronectin (Kovanen et al., 1988) as well as fibrillar type I collagen (Bonaldo et al., 1990). Several evidence showed in DS a dysregulation of type VI collagen (Conti et al., 2007; Dey et al., 2013; Galat et al., 2020), type I and IV collagens, and fibronectin (Conti et al., 2007; Galat et al., 2020), thus supporting the hypothesis of a possible altered organization of anchoring system in trisomy.

BM thickness decreased in both trisomic and euploid physically trained mice in comparison with their sedentary counterpart. Physical exercise is known to induce a remodeling of ECM (Heinemeier et al., 2009; Hyldahl et al., 2015; Kjaer, 2004), characterized by an increase in the metalloproteinases that target collagen type IV and laminin in the BM (Ogasawara et al., 2014; Rullman et al., 2009). Findings reported herein thus suggest that a more efficient turnover of BM components may be a positive response of skeletal muscle to

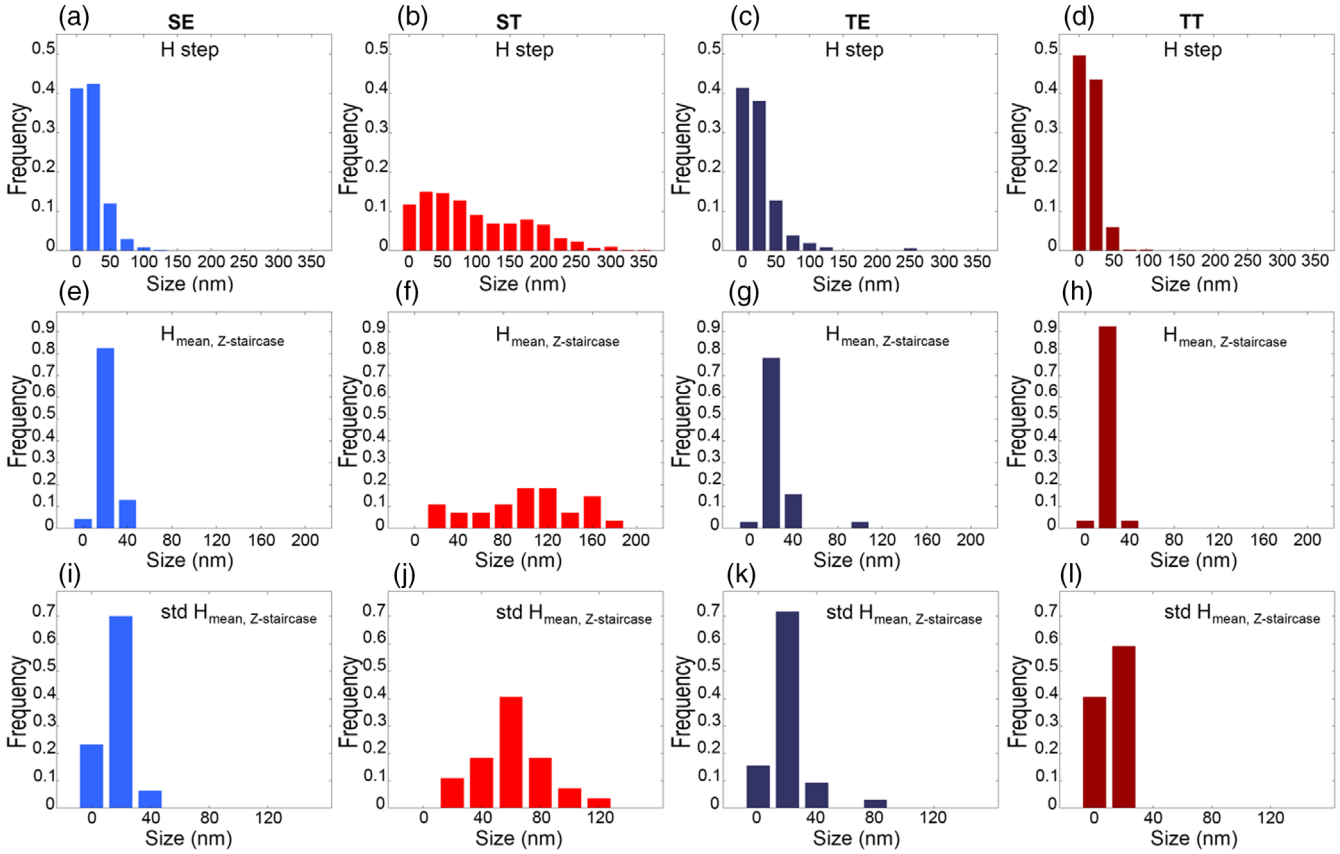
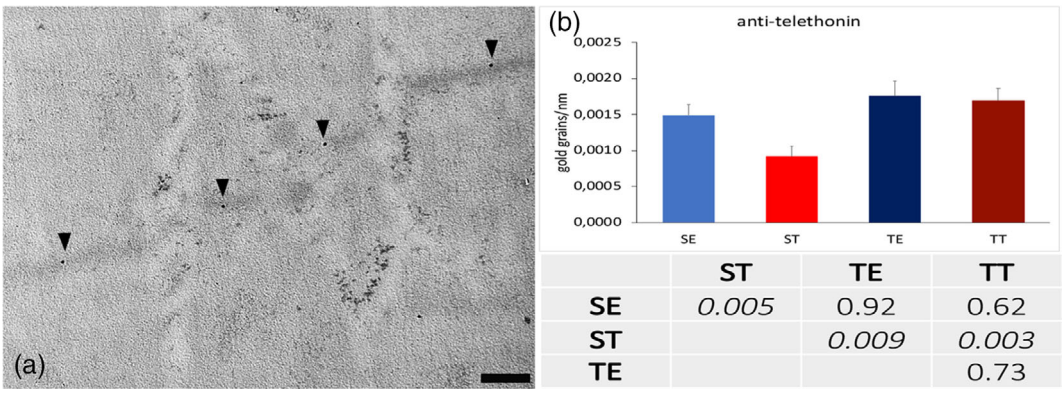


FIGURE 5 Distributions of H step (a–d), $H_{mean,Z\text{-}staircase}$ value along the Z-line of the adjacent sarcomere (e–h), and standard deviation of the $H_{mean,Z\text{-}staircase}$ along a Z-staircase (i–l) in SE (a, e, i), ST (b, f, j), TE (c, g, k), TT (d, h, l). Frequency is the number of measurements in a bar width divided by the total number of measurements. The distributions of all Z-line parameters were similar in SE (a, e, i), TE (c, g, k), and TT (d, h, l). High heterogeneity was present in ST (b, f, j) versus SE, TE, and TT.



	ST	TE	TT
SE	*0.005*	0.92	0.62
ST		*0.009*	*0.003*
TE			0.73

FIGURE 6 Representative TEM micrograph after telethonin immunolabeling (a). Immunogold labeling (arrowheads) occurs on Z-lines. Bar: 200 nm. (b) Quantitative evaluation of anti-telethonin density on Z-line in sedentary euploid (SE, sedentary trisomic (ST), trained euploid (TE), and trained trisomic (TE) is reported in panel (b). Results of post-hoc analysis are reported in panel (b).

physical exercise in both euploid and trisomic mice. In trisomy, this may likely counteract the accumulation/disorganization of BM compounds.

Significantly larger collagen bundles were found in perimysium of ST versus SE. Such an enlargement may be justified by the greater distance between collagen fibrils in ST rather than fibril diameter (which are similar in ST vs. SE). The larger interfibrillar space may facilitate the interposition of other ECM constituents, as already suggested for old versus adult mice (Lofaro et al., 2021). The interaction of collagen with proteoglycans has been described as a key supporter of the structure/organization of the skeletal muscle matrisome (Lofaro et al., 2021) by intervening in the regulation of collagen fibrillogenesis,

fibrillar thickness, and interfibrillar spacing (Ameye & Young, 2002). Interestingly, down-regulation of ECM components, for example, heparan sulfate proteoglycans, which are involved in both ECM remodeling and maintaining of the physical connection between its components, has been demonstrated in DS (Galat et al., 2020).

In both TE and TT, the collagen bundles enlarged concomitantly with an increase in the collagen fibrils diameter and a decrease in the interfibrillar spacing. Similarly, resistance training has been demonstrated to induce increased levels of type I and type III collagen transcripts in both young and old rats (Guzzoni et al., 2018) and jumping training was associated with increased amount of type I collagen in hindlimb muscle of rabbit (Ducomps et al., 2003). Such an increased concentration of fibrillar collagen was associated with hypertrophy of collagen fibrils in mouse tendon after several weeks of physical loading on treadmill (reviewed in Mavropalias et al., 2022), thus suggesting a similar training-associated effect in both trisomic and euploid mice.

Collagen bundles were more linear in both sedentary and TT mice versus their euploid counterpart. Interestingly, a similar condition was observed in old versus adult mice (Lofaro et al., 2021), thus suggesting a similar impairment of the mechanical properties of ECM in trisomy and aging (Stearns-Reider et al., 2017). In fact, loosely packed and linear collagen bundles, as well as a disorganized BM, may negatively impact on the transmission of contractile force in ST, thus affecting the mechanical properties of skeletal muscle as proposed in aging (Kragstrup et al., 2011). During contraction, the regular lateral Z-line registration ensures the *in unison* change of the myofibril length. Therefore, the Z-line misalignment in ST may impair an efficiently coordinated contraction of the myofiber thus compromising the muscle contractile strength. In TT, Z-lines realigned and telethonin density increased. Telethonin binds two titin molecules at Z-line, thus mediating much of the structural integrity of the Z-disk hinges (Bertz et al., 2009) and sarcomere (Sadikot et al., 2010). In cardiomyocytes, evidence suggested that telethonin may be critically involved in sarcomere–membrane interactions as mechanical stretch sensor (Knöll et al., 2011). Interestingly, telethonin knockdown resulted in an irregular alignment of Z-lines in zebrafish muscle (Zhang et al., 2009), thus suggesting that a trisomy-associated dysregulation of telethonin may be involved in the misalignment of sarcomeres. Physical exercise may thus counteract such an alteration in trisomy, thereby promoting a re-alignment of the Z-lines without, however, limiting the structural alterations of the "sarcoplasmic hypertrophy" suggested for trisomic myofiber (Cisterna et al., 2020). In fact, large mitochondria and dilated cisternae of sarcoplasmic reticulum were also observed in TT.

In conclusion, the results of this study indicated an impairment in composition and assembly of skeletal muscle ECM in trisomic mice. Altered expression and/or organization of ECM components have been already proposed in DS as contributor to organ malformations (Danopoulos et al., 2021; Garcia et al., 2010), such as the congenital heart defects (Gittenberger-De Groot et al., 2003; Mollo et al., 2022).

In agreement with the multi-systemic premature aging described in DS (Zigman, 2013), we found ECM structural alterations similar to those observed in skeletal muscle of aged mice (Lofaro et al., 2021). Interestingly, our protocol of adapted physical training partially remodels ECM in both trisomic and euploid mice. This remodeling, along with the re-alignment of the myofibrils, may thus counteract the structural alterations of skeletal muscle in trisomy.

Altogether, our findings constitute a solid experimental background for further study investigating the possible positive effect of physical training on skeletal muscle performance.

AUTHOR CONTRIBUTIONS

Barbara Cisterna: Investigation; methodology; writing – review and editing; writing – original draft; data curation. **Federico Boschi:** Data curation; methodology; investigation; writing – original draft. **Maria Assunta Lacavalla:** Investigation. **Gaetano Nicola Alfio Vattemi:** Data curation; methodology. **Carlo Zancanaro:** Funding acquisition; methodology; writing – original draft; writing – review and editing. **Manuela Malatesta:** Conceptualization; funding acquisition; methodology; project administration; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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