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No support for a higher dystroglycan glycan-synthesis transcript score after structured training in older adults: a preregistered meta-analysis of public muscle transcriptomes, with human and rodent disuse

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Research Article

Keywords: skeletal muscle, disuse atrophy, exercise training, dystroglycan, transcriptome, meta-analysis, preregistration

Posted Date: November 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11876485/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

Background. Matriglycan on α-dystroglycan is the glycan through which the muscle fibre binds laminin, and eight enzymes build it. Whether the transcripts of these enzymes change when muscle is trained or unloaded is not known. This study tested whether a transcript score of the eight genes is higher after structured training in older adults, and whether it is lower during disuse in humans and in rodents. Transcript abundance of this set is not the amount of glycan.

Methods. A protocol registered before the final search and before any data access fixed an eight-gene transcript score (writer8; rank-based single-sample enrichment, scoring code locked), the hypotheses, the gates and the permitted wording. Public skeletal-muscle transcriptomes were screened in two stages. Of 482 records, 57 holding 67 independent cohorts were analysed. Within-cohort changes used the standardised mean change and between-group differences Hedges' g, pooled by restricted maximum likelihood with the modified Hartung–Knapp adjustment; humans and rodents were never pooled. Post hoc analyses, each registered before it was run, added compatibility bounds, co-measured programmes in the same biopsies and expression-matched random gene-set nulls.

Results. With structured training in older adults the registered hypothesis was not supported at the pooled level (k = 7; standardised mean change −0.190, 95% confidence interval [CI] −0.499 to 0.118), and the 90% CI reached 0.055. During human disuse the score did not show a decrease (k = 13; 0.172, −0.044 to 0.388). In the same biopsies, programmes that training and disuse are known to change did move: with training, collagen +0.910 (0.363 to 1.457) and capillary +0.920 (0.376 to 1.464) in every cohort; during disuse, respiratory-chain transcripts −1.994 (−2.822 to −1.166) and atrogenes +0.635 (0.369 to 0.900), each in all 13 cohorts. In rodent disuse the score was lower than in controls (k = 12; Hedges' g −2.315, −3.234 to −1.396, all 12 negative) and lay below every draw of an expression-matched null, but a mean z-score of the same eight genes gave −0.811 (−1.631 to 0.009), and the collagen and capillary programmes showed the same rodent–human split.

Conclusion. The registered hypothesis for training in older adults was not supported at the pooled level, and the score did not show a decrease during human disuse, in biopsies where programmes known to respond did move. The decrease in rodent disuse was larger than that of expression-matched random gene sets, but its size depended on how the same eight genes were scored, and the difference between rodents and humans was shared with unrelated co-measured programmes and is not specific to these transcripts. The glycan itself was not measured, and a scan of public repositories found no muscle dataset that pairs matriglycan with the transcriptome of the same samples.

Registration: https://doi.org/10.17605/OSF.IO/GDVJH

1. Introduction

Skeletal muscle fibres are anchored to their basement membrane through the dystrophin–glycoprotein complex, and the laminin-binding part of that link is a glycan rather than a protein: α-dystroglycan carries matriglycan, a polymer of repeating xylose and glucuronic acid units to which laminin binds directly [1]. Placed on an unrelated cell-surface protein, matriglycan alone reproduces dystroglycan-dependent ligand binding and function [2], and defects in the enzymes that build it cause muscular dystrophies in which the dystroglycan protein is made but its glycan is truncated [3–5]. The extracellular matrix of muscle responds to loading and unloading [6]; short periods of bed rest or immobilisation cause rapid loss of muscle, to which older adults are particularly vulnerable [7–9]; and structured exercise is recommended to preserve muscle function in older adults [10]. Whether the glycan link takes part in these adaptations is not known.

Transcripts of these enzymes are not the glycan, and this must be said before anything else. The glycan is built in a defined sequence on an O-mannose of the core M3 type: POMGNT2 and B3GALNT2 extend the mannose and POMK phosphorylates it [11]; FKTN and FKRP add two ribitol-phosphate units [3, 12]; RXYLT1 adds a xylose [4]; B4GAT1 adds the glucuronic acid that primes the polymer [13]; and LARGE1 synthesises the repeating unit [14]. These eight genes form the score tested here, the eight-gene dystroglycan glycan-synthesis transcript score (writer8). LARGE expression was reduced in many dystrophic biopsies without a corresponding loss of α-dystroglycan glycosylation [15], and matriglycan length depends on POMK and on the N-terminal domain of dystroglycan as well as on LARGE1 [5, 16]. In rodent hindlimb suspension, complex components changed in a direction that depended on muscle and duration [17], and they rose during long-term human bed rest [18]; dystrophin protein rose on reloading independently of its mRNA [19]; and in denervated rat muscle α-dystroglycan changed in molecular mass through post-translational modification while a single transcript was detected [20].

The module and its scoring code were fixed in an earlier reanalysis of muscle-wasting models [21], which is background and not evidence here. Genome-wide transcriptome syntheses of disuse and exercise exist [22–24]; this study instead tested one fixed module, with a protocol registered before the final search and before any expression value was accessed [25]. It asked whether the score is higher after structured training in older adults, and whether it is lower during disuse in humans and in rodents. A result that does not support a hypothesis is informative only when two further things are known: which sizes of change its interval makes implausible, and whether the same samples register changes that are expected to occur. A rank-based score of eight genes raises a third question, namely whether a large change reflects its own genes or a shift of the transcriptome around them. Post hoc analyses, each planned before it was run, addressed all three.

2. Materials and Methods

Full procedural detail (registration mechanics, amendment history, cohort identifiers, the screening procedure and its agreement statistics) is in the Supplementary Methods; only what is needed to read the results is kept here.

Registration and reporting. Protocol version 1.0 was frozen on 2026-09-30 and registered on Open Science Framework (OSF) Registries on 2026-10-01 [25], before the final search, the screening and any access to expression data, together with a disclosure of the author's earlier exposure to several candidate records in a separate project. Under the protocol's exposure rule, recovery became exploratory without a gate and three human disuse comparisons are labelled not blind. Two further amendments, covering the screening procedure and a sensitivity analysis excluding comparisons whose batch is completely confounded with condition, were registered before any data access. Every post hoc analysis reported here follows a dated amendment that was added to the registration, with the wording of each possible outcome fixed, before it was computed, or a plan frozen with a cryptographic digest before it was computed (Table S1). Reporting follows the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement [26] (Supplementary Document 1; Figure S1).

Eligibility, search and selection. Eligible records held skeletal muscle sampled in vivo from adult humans, mice or rats under disuse (bed rest, limb suspension or immobilisation, hindlimb unloading, intensive-care immobilisation, spaceflight), or under exercise training or electrical stimulation of at least 2 weeks. Older adults met the age criterion when inclusion criteria guaranteed at least 60 years or, under a screening reading made before any data access, when every per-sample age was at least 60 years. Control genotypes, at least 3 biological units per state and platform coverage of all eight genes were required. After a training period the primary analysis required the later biopsy at least 48 h after the last session. The Gene Expression Omnibus (GEO) [27] and the ArrayExpress collection of BioStudies [28, 29] were searched on 2026-10-01 with seven registered strings (Table S2); the dataset lists of two syntheses [22, 23] and an external review served as a recall check. Screening used metadata only, withholding Methods sentences that named a module gene or a glycosylation term, and one comparison per independent cohort was selected by a registered order of preference. Risk of bias was judged in seven registered domains.

Score. Seven records without per-sample counts were reprocessed from public reads with nf-core/rnaseq [30, 31], Salmon [32] and tximport [33]. The writer8 score was computed exactly as in the locked v1.0.0 analysis [21], pinned by code digests: a rank-based statistic derived from single-sample gene set enrichment analysis (ssGSEA) [34] (rank ssGSEA, $\alpha = 0.25$), computed within each sample over every mapped gene. Four five-gene composition proxies (myofibre, stromal, endothelial, immune) were scored the same way; used as outcomes rather than covariates they are called programmes.

Synthesis. The unit was the participant or animal. Within-cohort changes were expressed as the standardised mean change with raw-score standardisation, in units of the standard deviation at the earlier time point [35]; between-group differences as Hedges' g [36]; controlled changes as Morris's d_ppc2 [37]. Pooling used restricted maximum likelihood (REML) with the modified Hartung–Knapp adjustment [38] and t with $k - 1$ degrees of freedom. Humans and rodents were never pooled, nor were the three contrast types. Inference required $k \geq 3$, and heterogeneity statistics and prediction intervals are not interpreted below $k = 5$. No sample size was set in advance: every eligible cohort returned by the registered search was analysed, and the smallest change that a pool could detect with 80% power is reported with the results (post hoc; Table 3). Training in older adults and recovery formed a Holm family [39]; countermeasures and training in younger adults, and the five rodent comparisons, formed Benjamini–Hochberg families [40]. Each gate required $k \geq 3$, a pooled estimate above 0 and a family-adjusted P of at most 0.05; otherwise the registered wording is "not supported at the pooled level", never "no effect". For each comparison and proxy a regression adjusted the score for the proxy before pooling; a result was classified sensitive to measured proxies if any proxy reversed the sign or attenuated the estimate by more than 50%.

Table 3

Post hoc estimates (Amendment A4, Part 1; not registered, not blind): resolution, subsets, reliability, Bayesian description and co-measured programmes.

row	status	k	estimate (95% CI)	post hoc values
Training in older adults (H-b); SMCR, after minus before training				
writer8, post hoc subset: all sampling times, patients	post hoc (A4 Part 1, P8); description, no test; includes 1 comparison(s) labelled not blind	5	−0.449 (− 0.970 to 0.072)	stromal 0.729 (− 0.744 to 2.201); endothelial 1.338 (− 0.092 to 2.769)
writer8, post hoc subset: all sampling times, other adults	post hoc (A4 Part 1, P8); description, no test	10	−0.235 (− 0.479 to 0.010)	stromal 0.835 (0.340 to 1.330); endothelial 0.958 (0.494 to 1.422)
writer8, post hoc subset: all sampling times, sampled < 48 h or at an unknown time	post hoc (A4 Part 1, P8); description, no test; includes 1 comparison(s) labelled not blind	8	−0.396 (− 0.740 to − 0.052)	stromal 0.671 (− 0.172 to 1.514); endothelial 1.214 (0.380 to 2.049)
writer8, post hoc subset: cohorts that met the wording of protocol decision D04 (K013, K027 and K079)	post hoc (A5, X2); description, no gate	3	−0.371 (− 1.302 to 0.560)	P 0.229 (two-sided, unadjusted); without GSE157585 (A1 item 6) k = 2, not evaluable
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	7		90% CI − 0.435 to 0.055; minimal detectable effect (80% power) 0.423 at two-sided α 0.05 and 0.489 at α 0.025, the α the gate in effect required; one-sided P against μ ≥ 0.1, 0.2, 0.3 and 0.5: 0.031, 0.011, 0.004 and 0.000778
writer8, reliability (P6): pre–post correlation and the pool with a common r	post hoc (A4 Part 1, P6)	7	−0.182 (− 0.469 to 0.105)	median pre–post r 0.13 on arrays (k = 5) and 0.54 on RNA-seq (k = 2); estimate with every cohort's r set to the pool median r of 0.333 (estimate column)
writer8, Bayesian random-effects description (P9)	post hoc (A4 Part 1, P9)			over 6 prior settings: P(μ > 0.2 | data) 0.004 to 0.012 and BF_{+0} 0.058 to 0.145 with all cohorts (k = 7); 0.006 to 0.022 and 0.071 to 0.189 without GSE157585 (k = 6)
stromal (collagen) programme, stromal_fap5	post hoc (A4 Part 1, P2)	7	0.910 (0.363 to 1.457)	negative in 0 of 7 cohorts
endothelial (capillary) programme, endothelial5	post hoc (A4 Part 1, P2)	7	0.920 (0.376 to 1.464)	negative in 0 of 7 cohorts
myofibre programme, myofiber5	post hoc (A4 Part 1, P2)	7	−0.357 (− 0.974 to 0.260)	negative in 5 of 7 cohorts
immune programme, immune5	post hoc (A4 Part 1, P2)	7	0.435 (0.022 to 0.848)	negative in 0 of 7 cohorts
Training in younger adults (H-b'); SMCR, after minus before training				
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	5		90% CI − 0.658 to 0.222; minimal detectable effect (80% power) 0.768 at two-sided α 0.05; one-sided P against μ ≥ 0.1, 0.2, 0.3 and 0.5: 0.099, 0.056, 0.033 and 0.013
Human disuse (H-a0); SMCR, end of disuse minus before				
writer8, post hoc subset: without the comparisons labelled not blind	post hoc (A4 Part 1, P5); description, no test	10	0.125 (− 0.112 to 0.363)	

The rows come from Amendment A4, dated and approved 2026-10-27 (Part 1), written after every registered analysis had been reported and after the editorial decision; they are not registered and not blind, carry no gate and change no registered result. P1: the 90% CI is the pair of one-sided 95% bounds; the minimal detectable effect is (t(1 − α/2, k − 1) + t(0.80, k − 1)) × SE_HK; the one-sided P values are compatibility statements against the fixed grid 0.1, 0.2, 0.3 and 0.5 in the pool's own standardised units, not equivalence tests, and no value of the grid is a smallest effect of interest. P2: the four registered composition proxies used as outcomes in exactly the primary comparisons, with the same effect sizes and pooling as writer8; post hoc P values are not shown (no gate; Table S9). P4, P5 and P8: subsets and strata pooled the same way, described without tests; the status column gives the number of comparisons labelled not blind that each contains. P6: the median pre–post correlation of the score by platform, and the pool with every cohort's correlation replaced by the pool median. P9: normal–normal hierarchical model with μ ~ N(0, σ²), σ in {0.5, 1}, and τ ~ half-normal with scale in {0.25, 0.5, 1}; BF_{+0} is the directional Bayes factor for μ > 0 against μ = 0.

Rows marked A5 come from Amendment A5 (approved 2026-11-01 and added to the registration as version 6 of the form before it was run): X2 pools the registered estimates of the 3 training cohorts that met the wording of protocol decision D04 (age counted only when the inclusion criteria guarantee it), and X3 the rodent cohorts without GSE273092, whose v1.0.0 value was known before registration. Both are descriptions without a gate; with k = 3, τ², I^2 and the prediction interval of X2 are not interpreted.

Gene lists of the programmes and modules: Table 2 notes. Metrics differ between estimands (SMCR, Morris d_ppc2, Hedges' g); humans and rodents are never pooled. The score describes the within-sample rank of eight transcripts and does not measure matriglycan, laminin binding or muscle function. Every post hoc Part 1 table is in Table S9, and the Part 2 tables are in Tables S10 and S15.

row	status	k	estimate (95% CI)	post hoc values
Training in older adults (H-b); SMCR, after minus before training				
writer8, post hoc subset: bed rest	post hoc (A4 Part 1, P5); description, no test; includes 2 comparison(s) labelled not blind	8	0.273 (− 0.033 to 0.579)	
writer8, post hoc subset: unilateral suspension or immobilisation	post hoc (A4 Part 1, P5); description, no test; includes 1 comparison(s) labelled not blind	5	−0.041 (− 0.461 to 0.379)	
writer8, post hoc subset: 5 to 14 days of disuse	post hoc (A4 Part 1, P5); description, no test; includes 3 comparison(s) labelled not blind	7	0.146 (− 0.134 to 0.426)	
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	13		90% CI − 0.005 to 0.348; minimal detectable effect (80% power) 0.302 at two-sided α 0.05; one-sided P against μ ≤ −0.1, − 0.2, − 0.3 and − 0.5: 0.009, 0.001, 0.000232 and 0.00000981
stromal (collagen) programme, stromal_fap5	post hoc (A4 Part 1, P2)	13	0.214 (− 0.334 to 0.763)	negative in 4 of 13 cohorts
endothelial (capillary) programme, endothelial5	post hoc (A4 Part 1, P2)	13	0.001 (− 0.173 to 0.175)	negative in 7 of 13 cohorts
myofibre programme, myofiber5	post hoc (A4 Part 1, P2)	13	0.334 (0.056 to 0.612)	negative in 2 of 13 cohorts
immune programme, immune5	post hoc (A4 Part 1, P2)	13	0.457 (0.173 to 0.740)	negative in 3 of 13 cohorts
Recovery (H-a1); SMCR, recovery minus end of disuse				
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	3		90% CI − 1.698 to 0.600; minimal detectable effect (80% power) 2.110 at two-sided α 0.05
Exercise or electrical stimulation during disuse (H-a2); Morris d_ppc2, disuse with countermeasure minus disuse alone				
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	4		90% CI − 1.401 to 0.934; minimal detectable effect (80% power) 2.064 at two-sided α 0.05 and 2.557 at α 0.025, the α the gate in effect required; one-sided P against μ ≥ 0.1, 0.2, 0.3 and 0.5: 0.275, 0.223, 0.181 and 0.118
Rodent disuse (R-a0); Hedges' g, disuse minus control				
writer8, post hoc subset: hindlimb unloading without co-intervention	post hoc (A4 Part 1, P4); description, no test	8	−1.956 (− 2.928 to − 0.984)	
writer8, post hoc subset: hindlimb unloading for 5 to 14 days	post hoc (A4 Part 1, P4); description, no test	7	−1.907 (− 3.031 to − 0.783)	stromal − 2.739 (− 4.499 to − 0.979); endothelial − 1.836 (− 3.031 to − 0.640)

The rows come from Amendment A4, dated and approved 2026-10-27 (Part 1), written after every registered analysis had been reported and after the editorial decision; they are not registered and not blind, carry no gate and change no registered result. P1: the 90% CI is the pair of one-sided 95% bounds; the minimal detectable effect is (t(1 − α/2, k − 1) + t(0.80, k − 1)) × SE_HK; the one-sided P values are compatibility statements against the fixed grid 0.1, 0.2, 0.3 and 0.5 in the pool's own standardised units, not equivalence tests, and no value of the grid is a smallest effect of interest. P2: the four registered composition proxies used as outcomes in exactly the primary comparisons, with the same effect sizes and pooling as writer8; post hoc P values are not shown (no gate; Table S9). P4, P5 and P8: subsets and strata pooled the same way, described without tests; the status column gives the number of comparisons labelled not blind that each contains. P6: the median pre–post correlation of the score by platform, and the pool with every cohort's correlation replaced by the pool median. P9: normal–normal hierarchical model with μ ~ N(0, σ²), σ in {0.5, 1}, and τ ~ half-normal with scale in {0.25, 0.5, 1}; BF_{+0} is the directional Bayes factor for μ > 0 against μ = 0.

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Gene lists of the programmes and modules: Table 2 notes. Metrics differ between estimands (SMCR, Morris d_ppc2, Hedges' g); humans and rodents are never pooled. The score describes the within-sample rank of eight transcripts and does not measure matriglycan, laminin binding or muscle function. Every post hoc Part 1 table is in Table S9, and the Part 2 tables are in Tables S10 and S15.

row	status	k	estimate (95% CI)	post hoc values
Training in older adults (H-b); SMCR, after minus before training				
writer8, post hoc subset: cohorts with at least four animals per group	post hoc (A4 Part 1, P4); description, no test	8	−2.507 (−3.720 to −1.293)	
writer8, post hoc subset: without GSE273092 (value known before registration)	post hoc (A5, X3); description, no gate	11	−2.293 (−3.301 to −1.284)	P 0.000487; τ² 1.346; I² 68.3%; prediction interval −5.067 to 0.482
writer8, resolution and compatibility bounds (P1)	post hoc (A4 Part 1, P1)	12		90% CI − 3.065 to − 1.566; minimal detectable effect (80% power) 1.284 at two-sided α 0.05
stromal (collagen) programme, stromal_fap5	post hoc (A4 Part 1, P2)	12	−2.105 (−3.514 to −0.697)	negative in 10 of 12 cohorts
endothelial (capillary) programme, endothelial5	post hoc (A4 Part 1, P2)	12	−1.507 (−2.093 to −0.922)	negative in 12 of 12 cohorts
myofibre programme, myofiber5	post hoc (A4 Part 1, P2)	12	0.143 (−0.750 to 1.037)	negative in 6 of 12 cohorts
immune programme, immune5	post hoc (A4 Part 1, P2)	12	−0.310 (−0.706 to 0.087)	negative in 7 of 12 cohorts
The rows come from Amendment A4, dated and approved 2026-10-27 (Part 1), written after every registered analysis had been reported and after the editorial decision; they are not registered and not blind, carry no gate and change no registered result. P1: the 90% CI is the pair of one-sided 95% bounds; the minimal detectable effect is (t(1 − α/2, k − 1) + t(0.80, k − 1)) × SE_HK; the one-sided P values are compatibility statements against the fixed grid 0.1, 0.2, 0.3 and 0.5 in the pool's own standardised units, not equivalence tests, and no value of the grid is a smallest effect of interest. P2: the four registered composition proxies used as outcomes in exactly the primary comparisons, with the same effect sizes and pooling as writer8; post hoc P values are not shown (no gate; Table S9). P4, P5 and P8: subsets and strata pooled the same way, described without tests; the status column gives the number of comparisons labelled not blind that each contains. P6: the median pre–post correlation of the score by platform, and the pool with every cohort's correlation replaced by the pool median. P9: normal–normal hierarchical model with μ ~ N(0, σ²), σ in {0.5, 1}, and τ ~ half-normal with scale in {0.25, 0.5, 1}; BF_{+0} is the directional Bayes factor for μ > 0 against μ = 0.				
Rows marked A5 come from Amendment A5 (approved 2026-11-01 and added to the registration as version 6 of the form before it was run): X2 pools the registered estimates of the 3 training cohorts that met the wording of protocol decision D04 (age counted only when the inclusion criteria guarantee it), and X3 the rodent cohorts without GSE273092, whose v1.0.0 value was known before registration. Both are descriptions without a gate; with k = 3, τ², I² and the prediction interval of X2 are not interpreted.				
Gene lists of the programmes and modules: Table 2 notes. Metrics differ between estimands (SMCR, Morris d_ppc2, Hedges' g); humans and rodents are never pooled. The score describes the within-sample rank of eight transcripts and does not measure matriglycan, laminin binding or muscle function. Every post hoc Part 1 table is in Table S9, and the Part 2 tables are in Tables S10 and S15.				

Post hoc analyses. These are not registered, not blind, carry no gate, change no registered result and are never pooled with a registered estimate. They comprise compatibility bounds and minimal detectable effects; the four composition proxies and four externally defined programmes (nuclear-encoded respiratory chain, fibrillar collagens, type IV collagens, and the atrogenes FBXO32 and TRIM63) scored in the same comparisons; 1,000 expression-matched random eight-gene sets per pool, drawn both independently in each cohort and identically across cohorts; two alternative scorings of the same eight genes; and a restricted ranking universe. Four further checks were added after the registered and amended analyses had been reported, and are reported here for the first time: a comparison of the per-gene directions with two published genome-wide syntheses; an extraction of the muscle loss each source publication reports for the sampled muscle; a scan of public proteomics repositories for the eight enzymes; and a repeat of the registered composition rule with the four five-gene proxies replaced by seven cell-type marker sets (myofibre, satellite, fibroblast, endothelial, pericyte, immune, adipocyte) taken from an ontology-grounded marker resource. Their plans, inputs and the wording of every possible outcome were frozen with cryptographic digests before each was computed (Supplementary Methods, section 17).

Generative AI. Large language models were used for the two screening passes under the registered procedure (Supplementary Methods), for writing and testing the analysis code, and for drafting the text. The tools, models and uses are listed under "Use of generative artificial intelligence"; the author checked and approved all output.

Ethics. This study reanalysed public, de-identified expression data and their metadata. No new human participants, animals or samples were involved, and no new procedure was performed, so no approval was sought for the reanalysis itself. The ethical approvals and consents for the source studies are held by their original investigators and are reported in the source publications listed in Table S12.

3. Results

3.1 Records and cohorts

Of 482 records screened, 57 records holding 67 independent cohorts were analysed; one record was not analysable. Table 1 lists the cohorts of the three main pools, which contribute 32 comparisons, and Figure S1 gives the PRISMA flow. Risk of bias was judged in the seven registered domains. Across those

32 comparisons, batch (21 unknown) and attrition (19 unknown, 3 high) were the domains most often unknown; batch was completely confounded with condition in 2 comparisons, and in rodent disuse allocation was unknown in 8 of 12 comparisons and the comparator was high risk in 3 (Table S3).

Table 1
Cohorts of the primary pools for training in older adults, human disuse and rodent disuse.

cohort	record	species	population	design	duration	muscle	n	platform	post hoc subset (Fig. 3)
Training in older adults (H-b); SMCR, after minus before training; k = 7 cohorts									
K013	GSE117525	human	frail or pre-frail older adults (Fried criteria); men and women; 66–91 y	progressive full-body RT, 2×/wk; training + placebo drink arm	24 wk	VL	14	array	
K014	GSE117525	human	healthy older adults, not frail (Fried criteria); men and women; 65–86 y	progressive full-body RT, 3×/wk; training + placebo drink arm	24 wk	VL	18	array	
K026	GSE53598	human	healthy men; subgroup with per-sample age ≥ 60 y (60–66 y)	combined endurance and resistance training; single arm	12 wk	VL	10	array	
K027	GSE157585	human	healthy men and women ≥ 65 y (MASTERS trial)	progressive RT + placebo (plaPRT arm)	14 wk	VL	24	RNA-seq	
K072	GSE47881	human	healthy adults; subgroup with per-sample age ≥ 60 y (64–75 y); sex not in sample attributes	supervised resistance exercise training; single arm	20 wk	VL	16	array	
K078	GSE8479	human	healthy older men and women; 65–79 y	supervised whole-body RT, twice weekly	26 wk	VL	14	array	
K079	GSE97084	human	older men and women recruited at 65–80 y; no structured exercise; cardiovascular, metabolic and renal disease excluded	supervised HIIT, RT or combined training (randomised arms, one comparison)	12 wk	VL	23	RNA-seq	
Human disuse (H-a0); SMCR, end of disuse minus before; k = 13 cohorts									
K001	GSE21496	human	healthy sedentary young men; mean 22.1 y	ULLS	48 h	VL (unloaded leg)	7	array	–
K009	GSE104999	human	healthy men; mean 27 y	normoxic bed rest (PlanHab)	21 d (day 21)	VL	12	array	–
K011†	GSE113165	human	healthy young adults, 18–28 y; men and women	supervised bed rest	5 d	VL	9	RNA-seq	5–14 d
K012†	GSE113165	human	healthy older adults, 60–79 y; men and women	supervised bed rest	5 d	VL	19	RNA-seq	5–14 d
K019	GSE148152	human	healthy men, 26–41 y	6° HDT bed rest; bed-rest-only arm	day 84 of 90 d	VL	11	array	–
K020	GSE14901	human	healthy young men and women; mean 20.5 and 21.1 y	unilateral knee-brace immobilisation	14 d	VL (immobilised leg)	24	array	5–14 d
K032	GSE186045	human	healthy adults, 20–54 y (5 men, 1 woman sequenced)	−6° HDT bed rest; bed-rest-only arm	35 d	VL	6	RNA-seq	–

cohort	record	species	population	design	duration	muscle	n	platform	post hoc subset (Fig. 3)
Training in older adults (H-b); SMCR, after minus before training; k = 7 cohorts									
K037†	GSE211204	human	recreationally active young men; inclusion 18–35 y	ULLS; no countermeasure	10 d	VL	11	RNA-seq	5–14 d
K041	GSE224900	human	healthy older adults > 60 y; men and women	bed rest; placebo arm	5 d	VL	9	RNA-seq	5–14 d
K042	GSE226973	human	healthy men, 25–45 y	6° HDT bed rest; control arm without exercise	day 64 of 70 d	VL	7	array	–
K046	GSE24215	human	healthy young men, 24–27 y	bed rest	day 9 (end of bed rest)	VL	10	array	5–14 d
K061	GSE310344	human	young healthy adults; mean 26.5 y; 7 men, 3 women	unilateral knee-brace immobilisation (first period)	14 d	VL (immobilised leg)	9	RNA-seq	5–14 d
K067	GSE33886	human	healthy men; 21 y	ULLS (right leg)	3 wk	VL	3	array	–
Rodent disuse (R-a0); Hedges' g, disuse minus control; k = 12 cohorts (8 mouse, 4 rat)									
K003	E-MTAB-4228	rat	Sprague Dawley; sex and age not stated	mechanical ventilation with neuromuscular blockade vs no ventilation	4.5 to 9 d	plantaris	5 + 4	RNA-seq (reads requantified)	–
K007	GSE102284	mouse	male C57BL/6J; adult, 6 mo	HU vs control	10 d	soleus	4 + 9	RNA-seq (reads requantified)	HU, 5–14 d
K008	GSE102284	mouse	male C57BL/6J; aged, 22–24 mo	HU vs control	10 d	soleus	6 + 9	RNA-seq (reads requantified)	HU, 5–14 d
K017	GSE141827	rat	mature Wistar; sex not stated	HU vs control	7 d	soleus	3 + 3	RNA-seq (reads requantified)	HU, 5–14 d
K028	GSE167462	mouse	aged male C57BL/6; 22–24 mo	HU (water vehicle arm) vs ambulatory control	14 d	gastrocnemius	4 + 4	RNA-seq	HU, 5–14 d
K045	GSE240909	mouse	C57BL/6J AMPKα1/α2 floxed no-Cre controls; 3–4 mo; males and females	HU vs ambulatory control	14 d	soleus	7 + 8	RNA-seq	HU, 5–14 d
K048	GSE244259	rat	adult male Wistar; 14–16 wk	controlled mechanical ventilation vs non-ventilated control	12 h	diaphragm	4 + 4	array	–
K050	GSE273092	mouse	female Zfp697 flox/flox (control genotype); 8 wk at start	HU vs cage control	10 d	gastrocnemius	3 + 3	RNA-seq	HU, 5–14 d

cohort	record	species	population	design	duration	muscle	n	platform	post hoc subset (Fig. 3)
Training in older adults (H-b); SMCR, after minus before training; k = 7 cohorts									
K053	GSE278016	mouse	male C57BL/6J; 16 wk at sampling	immobilisation with 60% caloric restriction and PBS pump vs ad-libitum reference	14 d	quadriceps	15 + 15	RNA-seq	–
K062	GSE310849	rat	male Fischer 344; 23 mo	TTX nerve block vs sham operation	6–7 d	tibialis anterior	3 + 3	RNA-seq	–
K069	GSE40578	mouse	female C57BL/6J; 7 wk	HU vs weight bearing	5 d	gastrocnemius and plantaris	3 + 3	array	HU, 5–14 d
K081	GSE9802	mouse	male ICR; 6 mo	hindlimb suspension vs ambulating controls	24 h	gastrocnemius	5 + 5	array	HU
Cohorts of the registered primary analysis sets of three estimands (one comparison per cohort per estimand, protocol section 7.1). Records are GEO series except E-MTAB-4228 (ArrayExpress). Population, design and duration are shortened from the strings recorded at screening; the full strings, the later-sample timing and every alternative arm are in Tables S11 and S16.									
n: complete before-and-after pairs for the human within-person changes; disuse + control animals for the rodent between-group differences.									
† Comparison labelled not blind under Amendment A1 (item 3): K011, K012, K037. The human disuse pool is a supportive estimate without a gate.									
Training in older adults: under the screening reading of 2026-10-05 (ages from sample attributes), 4 of the 7 cohorts entered because every participant's per-sample age was at least 60 years: K014, K026, K072 and K078. K026 and K072 are the older subgroups of mixed-age cohorts.									
Post hoc subset: membership of the subsets plotted in Fig. 3 (Amendment A4, Part 1, items P5(c) and P4(a, b); not registered). Human disuse: 5–14 d of disuse. Rodent disuse: HU, hindlimb unloading without co-intervention; HU, 5–14 d, the same restricted to 5 to 14 days of unloading. A dash marks a cohort outside every plotted subset.									
Humans and rodents are never pooled, and within-person and between-group changes are never pooled together. Mouse and rat cohorts are pooled within rodent disuse (reading of 2026-10-07).									
Abbreviations: CR, caloric restriction; HDT, head-down tilt; HIIT, high-intensity interval training; HU, hindlimb unloading or suspension; mo, months; PBS, phosphate-buffered saline; plaPRT, the source's label of the progressive resistance training + placebo arm; RT, resistance training; SMCR, standardised mean change (raw-score standardisation); TTX, tetrodotoxin; ULLS, unilateral lower-limb suspension; VL, vastus lateralis; wk, weeks; y, years.									

3.2 Registered results: the human side

The registered hypothesis that the score is higher after structured training in older adults was not supported at the pooled level (Fig. 1A; Table 2). Over 7 cohorts the pooled standardised mean change was − 0.190 (95% confidence interval [CI] − 0.499 to 0.118; Holm-adjusted P 0.364). Four of the 7 estimates were negative. Every registered sensitivity analysis kept the negative sign, including the one that removes the cohort affected by the exposure disclosure (− 0.229). Training in younger adults, the registered reference, gave − 0.218 (− 0.792 to 0.355; k = 5). The registered composition analysis classified training in older adults as sensitive to measured proxies, because adjustment for the stromal or the endothelial proxy reversed the sign of the pooled estimate; this limits a myofibre-intrinsic reading of the result.

Table 2
Registered gates and pooled writer8 estimates, with the registered sensitivity analyses and supplementary modules.

row	status	k	estimate (95% CI)	P	family-adjusted P; gate	τ^2; I^2	prediction interval	leave one cohort out	composition (registered)
Training in older adults (H-b); SMCR, after minus before training									
writer8, primary analysis	registered: primary (gate GH-b)	7	−0.190 (− 0.499 to 0.118)	0.182	0.364 (Holm, family of 2); GH-b not supported at the pooled level: direction not positive	τ^2 0.028; I^2 15.5%	−0.702 to 0.321	−0.229 (omitting K027) to − 0.069 (omitting K079); k = 6 each	sensitive to measured composition proxies (sign reversed by adjustment for stromal_fap5 and endothelial5)[X]; unadjusted change − 0.0017 (− 0.0041 to 0.0008) score units, k = 7
writer8, without GSE157585 (A1 item 6)	registered: sensitivity analysis, unadjusted, no gate	6	−0.229 (− 0.630 to 0.172)	0.202	unadjusted; no gate				
writer8, without batch-confounded GSE8479 (A3)	registered: sensitivity analysis, unadjusted, no gate	6	−0.193 (− 0.552 to 0.165)	0.224	unadjusted; no gate				
writer8, all sampling times (adds cohorts sampled < 48 h or at an unknown time)	registered: sensitivity analysis, unadjusted, no gate; not blind (GSE27536, A1 item 3)	15	−0.298 (− 0.499 to − 0.096)	0.007	unadjusted; no gate				
core3 module	registered: supplementary module, no gate	7	−0.100 (− 0.457 to 0.257)	0.518	unadjusted (supplementary module)				
pathway13 module	registered: supplementary module, no gate	7	−0.002 (− 0.221 to 0.217)	0.980	unadjusted (supplementary module)				
Training in younger adults (H-b'); SMCR, after minus before training									
writer8, primary analysis	registered: secondary (no gate)	5	−0.218 (− 0.792 to 0.355)	0.350	0.670 (Benjamini–Hochberg, family of 2); no gate	τ^2 0.148; I^2 76.1%	−1.429 to 0.993		not evaluable; unadjusted change − 0.0020 (− 0.0071 to 0.0032) score units, k = 5
Human disuse (H-a0); SMCR, end of disuse minus before									
writer8, primary analysis	registered: supportive (no gate); 3 comparisons labelled not blind (Amendment A1)	13	0.172 (− 0.044 to 0.388)	0.109	not in a multiplicity family; no gate	τ^2 0.014; I^2 17.4%	−0.166 to 0.509		not evaluable; unadjusted change 0.0020 (− 0.0003 to 0.0043) score units, k = 12
core3 module	registered: supplementary module, no gate	13	0.518 (0.182 to 0.855)	0.006	unadjusted (supplementary module)				

row	status	k	estimate (95% CI)	P	family-adjusted P; gate	τ²; I²	prediction interval	leave one cohort out	composition (registered)
Training in older adults (H-b); SMCR, after minus before training									
pathway13 module	registered: supplementary module, no gate	13	0.367 (0.051 to 0.683)	0.026	unadjusted (supplementary module)				
Recovery (H-a1); SMCR, recovery minus end of disuse									
writer8, primary analysis	registered: exploratory, not blind (A1 item 2)	3	−0.549 (− 2.242 to 1.144)	0.297	exploratory under Amendment A1 item 2; enters the Holm family as P = 1 (family of 2); GH-a1 withdrawn	τ² 0.320; I² 69.4%; not interpreted at this k	not interpreted at this k		sensitive to measured composition proxies (sign reversed by adjustment for endothelial5)[2]; unadjusted change − 0.0054 (− 0.0200 to 0.0091) score units, k = 3
Exercise or electrical stimulation during disuse (H-a2); Morris d_ppc2, disuse with countermeasure minus disuse alone									
writer8, primary analysis	registered: secondary (gate GH-a2)	4	−0.233 (− 1.812 to 1.345)	0.670	0.670 (Benjamini–Hochberg, family of 2); GH-a2 not supported at the pooled level: direction not positive	τ² 0.427; I² 48.9%; not interpreted at this k	not interpreted at this k		no composition model (controlled change)
Rodent disuse (R-a0); Hedges' g, disuse minus control									
writer8, primary analysis	registered: secondary (rodent family, no gate)	12	−2.315 (− 3.234 to − 1.396)	0.000174	0.000868 (Benjamini–Hochberg, family of 5); no gate	τ² 1.215; I² 65.6%	−4.909 to 0.279	−2.517 (omitting K045) to − 2.126 (omitting K003); k = 11 each	robust to measured composition proxies (attenuation within the limit for all four proxies: myofiber5 0.04, stromal_fap5 − 0.24, endothelial5 0.06, immune5 0.05); unadjusted change − 0.0174 (− 0.0266 to − 0.0082) score units, k = 12
writer8, without batch-confounded GSE141827 (A3)	registered: sensitivity analysis, unadjusted, no gate	11	−2.411 (− 3.414 to − 1.407)	0.000323	unadjusted; no gate				
core3 module	registered: supplementary module, no gate	12	−1.579 (− 2.709 to − 0.449)	0.011	unadjusted (supplementary module)				
pathway13 module	registered: supplementary	12	−1.359 (− 2.343	0.011	unadjusted (supplementary				

row	status	k	estimate (95% CI)	P	family-adjusted P; gate	τ²; I²	prediction interval	leave one cohort out	composition (registered)
Training in older adults (H-b); SMCR, after minus before training									
	module, no gate		to − 0.376)		module)				
Registered rows keep the registered numbers and wording. Pooling: REML with the modified Hartung–Knapp 95% CI and P from t with k − 1 df. P is two-sided and unadjusted; the family-adjusted P is the one the registered gates use. "Not supported" means not supported at the pooled level; it is never a statement of no effect or of equivalence.									
τ² is the REML between-cohort variance and I² the share of variation attributed to heterogeneity. Prediction interval: v1.0.0 form, μ ± t(0.975, k − 1) × √(τ² + SE²) with the Hartung–Knapp SE. With 3 or 4 cohorts, τ², I² and the prediction interval are not interpreted (A4 rules). Leave one cohort out: range of the pooled estimate over the fits omitting each cohort in turn.									
Composition (protocol section 12.2) is reported beside the gates and never changes them. ▯ The association was sensitive to adjustment for measured composition proxies, which limits a myofibre-intrinsic interpretation (the sentence protocol section 13.2 attaches to this classification; it applies to each row so marked). For training in older adults the classification came from a sign change of adjusted estimates whose unadjusted interval includes 0, and the stromal and endothelial proxies used for adjustment themselves rise with training (post hoc, Table 3), so adjusting for them conditions on part of the training response. The unadjusted change is the pooled estimate of the composition model on the raw score scale (protocol section 12.1); it pools array and RNA-seq inputs, whose raw scales differ, and k counts the cohorts with an estimable model.									
Programmes: writer8, POMGNT2, POMK, B3GALNT2, FKTN, FKRP, RXYLT1, B4GAT1, LARGE1; stromal_fap5, PDGFRA, COL1A1, COL1A2, COL3A1, DCN; endothelial5, PECAM1, EMCN, KDR, VWF, RAMP2; myofiber5, ACTA1, MYH1, MYH2, MYH4, CKM; immune5, PTPRC, LST1, TYROBP, CD68, AIF1 (protocol section 12.1). core3 (POMGNT2, POMK, B3GALNT2) and pathway13 (writer8 with POMT1, POMT2, CRPPA, LARGE2 and DAG1) overlap writer8 and are not independent replications. Small rank-based gene sets scored in the same samples can move together.									
Metrics differ between estimands (SMCR, Morris d_ppc2, Hedges' g); humans and rodents are never pooled, and no rodent result passes a human gate. The score describes the within-sample rank of eight transcripts and does not measure matriglycan, laminin binding or muscle function. Every pooled estimate of every registered analysis is in Table S14; the post hoc estimates are in Table 3.									

During human disuse the score did not show a decrease. Over 13 cohorts, of which 3 are labelled not blind, the pooled standardised mean change was 0.172 (− 0.044 to 0.388; P 0.109), a supportive result without a gate (Fig. 1B; Table 2). The two registered supplementary modules, which overlap the eight genes, were higher with intervals above 0 (0.518, 0.182 to 0.855; and 0.367, 0.051 to 0.683).

In the same biopsies, programmes that training and disuse are known to change did move (post hoc, not registered, not blind; Fig. 2; Table 3 and Table S10). With training in older adults the collagen programme rose by 0.910 (0.363 to 1.457) and the capillary programme by 0.920 (0.376 to 1.464), with every one of the 7 cohort estimates positive; fibrillar collagens rose by 1.140 (0.512 to 1.768). During human disuse, nuclear-encoded respiratory-chain transcripts fell by − 1.994 (− 2.822 to − 1.166), negative in all 13 cohorts, and the atrogenes rose by 0.635 (0.369 to 0.900), positive in all 13; type IV collagens rose by 1.030 (0.593 to 1.466), also in all 13. Because the protocol had defined the proxies as covariates and because rank scores of small sets in the same samples can move together, this is a partial positive control, not a complete one.

Public transcriptomes could not answer two of the registered questions. Recovery of at least 7 days pooled 3 cohorts to − 0.549 (− 2.242 to 1.144), and exercise or electrical stimulation during disuse pooled 4 cohorts to − 0.233 (− 1.812 to 1.345), not supported at the pooled level; the smallest change those 4 cohorts could have detected with 80% power was about 2.1 standard-deviation units (Table 3).

3.3 Registered result: the rodent side

The score was lower with rodent disuse than in controls. Over 12 cohorts (8 mouse, 4 rat) the pooled Hedges' g was − 2.315 (− 3.234 to − 1.396; Benjamini–Hochberg q 0.000868) and all 12 estimates were negative (Fig. 1C; Table 2). Heterogeneity was substantial and the prediction interval included 0. By the registered rule the result was robust to the measured composition proxies, which does not establish a myofibre-intrinsic effect; adjustment for the stromal proxy enlarged the decrease. No single cohort carried it: in the registered leave-one-cohort-out analysis the pooled estimate stayed between − 2.517 and − 2.126, and the upper limit of its interval stayed at or below − 1.209, including when the discordant cohort E-MTAB-4228 was omitted.

3.4 How much of the rodent decrease belongs to these eight genes (post hoc; not registered, not blind, no gate)

Three checks, planned before they were run, bound the reading of that number (Figs. 3 and 4; Tables S10 and S15).

First, the change was more negative than for expression-matched random gene sets in the same comparisons. The pooled estimate lay below every one of 1,000 draws of the pooled null, whose 2.5th and 97.5th percentiles were − 0.991 and 0.410. Drawing the null from glycosyltransferases alone left it in the same position, and using the same random genes in every cohort gave percentile 0.2 with 2.5th and 97.5th percentiles of − 1.504 and 1.227. This is a diagnostic of a generic transcriptomic shift, not a test that the module is coherent or causal. In training in older adults and in human disuse, by contrast, the change was not distinguishable from that of expression-matched random gene sets (percentiles 21.9 and 92.5; 50.3 and 58.9 with the same genes in every cohort).

Second, the size depended on how the same eight genes were scored. A mean within-sample percentile rank gave − 2.163 (− 3.069 to − 1.256), but a mean z-score gave − 0.811 (− 1.631 to 0.009), an interval that includes 0. During human disuse the mean z-score of the same genes rose by 0.335 (0.041 to 0.629). Restricting the ranking universe to genes reaching 1 count per million in half the samples left the rodent RNA-seq subset at − 2.444, against − 2.242

unrestricted, so the decrease did not depend on ranking against barely expressed genes; within those inputs, however, the score correlated with library size (Pearson r − 0.842 to 0.540) and with the number of detected genes (− 0.750 to 0.657).

Third, the rodent–human difference was not specific to these transcripts. In the band of 5 to 14 days, human writer8 changed by 0.146 (− 0.134 to 0.426) with the collagen programme at − 0.002 and the capillary programme at 0.007; with rodent hindlimb unloading over the same band, writer8 changed by − 1.907 (− 3.031 to − 0.783), the collagen programme by − 2.739 (− 4.499 to − 0.979) and the capillary programme by − 1.836 (− 3.031 to − 0.640) (Fig. 3).

Two further descriptions bound that reading (Table S21). Ranked by the size of the pooled change among the nine scores measured in the same comparisons, writer8 was second of nine in rodent disuse, behind the fibrillar collagens at − 2.654 (− 3.925 to − 1.383); in human disuse and with training in older adults it was eighth of nine (Fig. 4C). Between cohorts, the scores did not track each other: across the 12 rodent cohorts the writer8 change was not distinguishable from no co-variation with the collagen programme (Pearson r 0.469, 95% CI − 0.144 to 0.822), and the same held for the capillary programme and for both programmes in the two human pools. The pooled estimates of these scores therefore move together while their cohort-level estimates do not.

3.5 Checks added after the registered analyses (post hoc; not registered, not blind, no gate)

Per-gene direction against published syntheses. The dataset lists of two genome-wide syntheses [22, 23] share 18 of the accessions analysed here. At the sources' own thresholds none of the eight genes was reported lower with inactivity and none higher with training; two training entries passed a threshold and both were in the lower direction. Directions agreed with the per-gene pooled estimates here for 37 of 48 gene-condition pairs (Table S17). Because cohorts are shared, this is a cross-check of scoring, not a replication.

Reported muscle loss. The source publication of each disuse cohort was read for the change of the sampled muscle at the sampled time. A usable value exists for 7 of the 12 rodent cohorts (reported losses 4.4% to 36.1%) and for 5 of the 13 human cohorts (0.3% to 9.0%); for training in older adults, 2 of 7 (Table S18; Figure S2). Protocol section 10.3 requires at least 10 cohorts with a known value before a meta-regression is fitted, so none was fitted and no slope is reported. The reported losses barely overlap between species, so in these data the species difference in the score is not separable from the magnitude of atrophy.

Public proteomics. A scan of ProteomeXchange/PRIDE, MassIVE and jPOST on 2026-11-07 found 8 skeletal-muscle datasets of disuse or training with quantification tables that could be opened; 3 quantified at least one of the eight enzymes, and none quantified matriglycan (Table S19). The dystroglycan core protein was quantified in seven of the eight. In the two deepest human datasets, a 21-day bed-rest proteome of 1,567 protein groups and a 14-day immobilisation proteome of 3,209 identified proteins, none of the eight enzymes was quantified at all.

4. Discussion

The registered hypothesis, that a transcript score of the eight enzymes that build matriglycan is higher after structured training in older adults, was not supported at the pooled level, and the score did not show a decrease during human disuse. In rodent disuse the score was more than two standard-deviation units lower than in controls. The post hoc analyses bound how each of these three results can be read, and none of them is a statement about matriglycan.

What was excluded on the human side. The registered training result was not supported at the pooled level, and the post hoc bounds say which increases the data make less compatible: the 90% CI reached 0.055 standard-deviation units, the one-sided P against an increase of at least 0.1 was 0.031, and the smallest change detectable with 80% power at the gate's effective threshold was 0.489. Small increases are not excluded. During human disuse the score did not show a decrease, with a 90% CI of − 0.005 to 0.348 and a one-sided P against a decrease of at least 0.1 of 0.009. Neither of these is a statement that nothing happened; the glycan was not measured, and a bulk-tissue rank score is a coarse instrument.

What did move in the same samples. The obvious objection is that the design could not detect anything. It did detect other things in the same biopsies: two programmes rose in every training cohort, and the respiratory chain fell and the atrogenes rose in every one of the 13 human disuse cohorts. This weakens, without excluding, an insensitive design as the explanation, because detection depends on each programme's own effect size and expression, and the atrogene programme has only two genes.

How far the rodent decrease is specific. Two observations hold at once and must be read together. The pooled rodent change lay below every draw of an expression-matched null, including a null that uses the same random genes in every cohort, while the two human pools sat inside theirs. And a mean z-score of those same eight genes gave an interval that includes 0. The first indicates that the eight transcripts moved together more than matched sets do; the second indicates that most of the magnitude comes from ranking each sample's genes against the rest of its transcriptome. Taken together, both are properties of the score rather than of the glycan, and the first is a diagnostic of a generic transcriptomic shift, not a test that the module is coherent or causal.

Why the species difference is not a species difference in glycan synthesis. In the matched duration band the collagen and capillary programmes split between rodents and humans as the writer8 score did, and the writer8 change was not even the largest of the nine scores measured in those rodent comparisons. Design differs too (within-person changes in human vastus lateralis against between-group differences in six rodent muscles under hindlimb unloading, ventilation, nerve block or immobilisation), and the reported muscle loss differs: the five human cohorts with a published value lost 0.3% to 9.0%, the seven rodent cohorts 4.4% to 36.1%. With fewer than 10 cohorts per species carrying a value, no moderator analysis is permitted, so atrophy magnitude and species cannot be separated here. What can be said is that the difference is not specific to the glycan-synthesis transcripts.

The glycan itself. In every comparison the glycan was not measured. The proteins of the complex, α-dystroglycan glycosylation and matriglycan length are regulated at levels other than the transcripts of these enzymes [5, 15, 16, 19, 20]. The proteomics scan found no public muscle dataset pairing transcriptome and matriglycan in the same samples, and in the two deepest human disuse proteomes none of the eight enzymes was quantified. The measurement that would settle the question therefore does not exist in public data, and generating it would mean assaying matriglycan or laminin-binding activity in biopsies whose transcripts are profiled across disuse, recovery and training.

What public data cannot answer. Recovery and exercise during disuse were estimated with intervals wider than 3 standard-deviation units; for countermeasures the smallest detectable change was about 2.1. These two questions are open, and public transcriptomes as they stand cannot close them.

4.1 Limitations

First, k is small (7 cohorts for training in older adults, 3 for recovery, 4 for countermeasures), so heterogeneity and prediction intervals are unstable, and the certainty of the evidence was not graded. Second, a within-cohort change also carries time, repeated biopsy and regression to the mean, while the rodent comparison is between independent groups. Third, the post hoc analyses are neither registered nor blind; the registered proxies are only a partial positive control, and the externally defined programmes used as one were chosen after the registered results were known. Fourth, bulk tissue cannot separate the cell sources of the eight transcripts, nor a rank score the movement of its genes from that of the genes around them, and some genes were barely detected in some inputs. Replacing the four proxies by seven cell-type marker sets left all three composition classifications unchanged, but on 5 of 7, 8 of 13 and 10 of 12 cohorts, because the remaining cohorts could not be re-derived from the public files to the tolerance set in advance (Table S20); marker-set scores are not cell fractions, and a cell-type score built from ranks shares the behaviour that limits the score itself, so this narrows neither limitation (post hoc, not registered, not blind). Fifth, the pools combine resistance, interval and combined training and, in rodents, exposures from 12 h to 14 days. Sixth, every registered search string combined disuse with recovery, exercise, countermeasures or stimulation, so the disuse pools are the disuse arms of the records retrieved rather than a systematic sample of disuse studies; a human 3-week bed-rest series public before the search was not retrieved [41]. A disuse-only search has since been registered as Amendment A6 (Supplementary Document 4) and, run on metadata alone, returns 210 records of which 153 were not among the 482 screened here, including that series; no eligibility decision has been made for any of them and no extended pool has been computed. Seventh, parts of the human pools are not blind, training in older adults is confirmatory only under the registered reading of the exposure rule, and four of its seven cohorts qualified only under a screening reading that counted per-sample ages; the stricter reading leaves 3 cohorts (− 0.371, − 1.302 to 0.560, post hoc). One rodent cohort had been scored before registration; without it the pooled rodent change was − 2.293 (− 3.301 to − 1.284, post hoc). Eighth, 11 of the 15 rodent cohorts of the primary analysis sets were single-sex (9 male, 2 female); 2 were mixed-sex and sex was not stated for the remaining 2. There was no second human screener.

5. Conclusion

In conclusion, in this preregistered meta-analysis the hypothesis that a bulk-tissue dystroglycan glycan-synthesis transcript score is higher after structured training in older adults was not supported at the pooled level, and the score did not show a decrease during human disuse, in samples where collagen, capillary, respiratory-chain and atrogene programmes did move. In rodent disuse the score was more than two standard-deviation units lower than in controls, but that difference was shared with the collagen and capillary programmes and its size depended on how the same eight genes were scored. These findings concern the relative ranks of eight transcripts, not matriglycan. Stated bounds, same-sample positive controls and an expression-matched null changed how both a result that was not supported and a large difference could be read.

Declarations

Acknowledgments

No person is acknowledged other than the author.

Use of generative artificial intelligence

During this work the author used Anthropic Claude (for the first three versions, Claude Code with the models claude-opus-5, claude-opus-5-5 and claude-fable-5-1, and Claude Science with the model claude-opus-5) for four kinds of task: (1) the first screening pass under the registered procedure of Amendment A2 (reported in Methods); (2) writing, testing and running the analysis code, the data readers and the scripts that build the tables and figures, under the author's direction and against the registered protocol and its amendments; (3) drafting the plans of the post hoc analyses (Amendments A4 to A6 and the plans frozen before the checks of the third version), each of which the author revised or approved before it was run, running the search of Amendment A6 on repository metadata only, extracting the reported muscle loss from the source publications and scanning public proteomics repositories; and (4) drafting, reviewing and formatting the text of every version of this article, checking its numbers against the result files and verifying the references against PubMed and Crossref records. For this fourth version Claude Code was used to compare publication routes and to restructure the abstract, introduction, discussion and conclusion around the registered question without changing any number. OpenAI Codex (gpt-5.6-sol) ran the blind second screening pass under Amendment A2 and reviewed drafts of the first version; it was not used for the later versions.

No AI tool selected samples on the basis of outcomes, approved an amendment or is an author. The post hoc plans and their results are used in this article only with the author's approval. No figure contains AI-created images; all figures are plots of the result tables drawn by code. The author reviewed and edited all output of these tools and takes full responsibility for the content of this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics statement

This study reanalysed publicly available, de-identified expression data and their metadata. No new human participants, animals or samples were involved, and no new procedure was performed, so no approval by an institutional review board or ethics committee was required for the reanalysis itself. The ethical approvals and consents for the source studies are held by their original investigators and are reported in the source publications listed in Table S12. Patient consent: not applicable, because no participant was recruited and no identifiable information is reported.

Preregistration

The protocol (version 1.0) was frozen on 2026-09-30 and registered, together with Amendment A1, on Open Science Framework (OSF) Registries on 2026-10-01 (https://doi.org/10.17605/OSF.IO/GDVJH) [25], before the final search, the screening and any access to expression data for this study; Amendment A1 discloses the author's earlier exposure to several candidate records in a separate project. Amendments A2 (screening procedure, dated 2026-10-01) and A3 (sensitivity analysis without comparisons whose batch is completely confounded with condition, dated 2026-10-07) were added to the registration form in version 3 on 2026-10-07, before the first access to expression data on the same day; version 4 named the public update packet on Zenodo [42]. The post hoc analyses follow Amendment A4 (Supplementary Document 2), written after every registered analysis had been reported and after an editorial decision on an earlier version of this article, and approved on 2026-10-27; it was added to the registration form in version 5 on 2026-11-01, before its Part 2 was run, and archived on Zenodo [43]. Three further post hoc checks follow Amendment A5 (Supplementary Document 3), written and approved on 2026-11-01 after an independent re-read of the revised article; it was added to the registration form in version 6 the same day, before any of the checks was computed, and archived on Zenodo [44]. They are not part of the registered analysis and are not blind. After a second editorial decision, the registered pooled estimates were reproduced from the archived per-comparison tables before any further analysis, and the checks reported in Results section 3.5, the marker-set repeat of the composition rule, and the ranking and cohort-level correlations of the co-measured scores were run on 2026-11-07 under plans frozen with cryptographic digests before each was computed (Supplementary Methods, section 17); they are not registered and not blind. A disuse-only search, Amendment A6 (Supplementary Document 4), was drafted on 2026-11-07, run on repository metadata only, and added to the registration form in version 7 on 2026-11-07 and archived on Zenodo [45]; its screening and extended pools have not been run, and no expression value of any record it finds has been opened. Two scans of public proteomics repositories and PubMed for muscle matriglycan data (2026-10-26 and 2026-11-07) are also post hoc and not registered. Every amendment, dated reading, deviation and frozen plan is listed in Table S1.

Conflict of interest

The author declares compliance with the Peer Community In rule of having no financial conflicts of interest in relation to the content of the article, and has no other conflict of interest to declare.

Data availability statement

All expression data analysed in this study are public. The repository, accession, species, platform and role of every record are listed in Table S12 (GEO series, ArrayExpress studies, and the MoTrPAC rat endurance-training study read from its GEO deposit, GEO: GSE242354 with the sample groups of GEO: GSE242358). The registered protocol, Amendment A1, the frozen analysis code and the synthetic-test receipt are in the OSF registration [25]; Amendments A2 and A3, the dated readings and the screening records are in the registration update packet on Zenodo [42], Amendment A4 with its Part 2 code and gene lists is in the version 5 packet [43], and Amendment A5 with its code and synthetic tests in the version 6 packet [44]; Amendment A6 with the record of its metadata-only dry run is in the version 7 packet [45]. The version 1.0.0 analysis code and module definition that this study reuses are on Zenodo [21]. The study's own result tables (pooled estimates, gates, families, per-comparison results, leave-one-cohort-out, composition, alternative arms, gene-level description, descriptive comparisons), the exposure log, the screening and extraction records, and the scripts that built the tables and figures of this article are in a public Zenodo record [46], together with the post hoc material: the drafts of Amendments A4 and A5, the Part 1 code and its output tables (items P1 to P10 and the rebuilt per-comparison effects), the Part 2 code with its gene lists and output tables (items Q1 to Q4, the acceptance check and the manifest), the A5 code and output tables (items X1 to X3, the acceptance check and the manifest), the run records and the record of the post hoc repository scans. Version 3.0 of that record adds the material of the third version of this article: the reproduction gate (R0), the frozen plans with their digests, the per-gene direction table (R2), the extraction of reported muscle loss (R3), the proteomics scan (R4), the marker-set composition repeat with its acceptance check (R5), the cohort-level correlations and ranking (R6), the draft of Amendment A6 with its metadata-only search record, the data-access log of that round, and the figure and verification code of the third version.

Code availability statement

The registered analysis code is rehab_core.py (SHA-256 26d5f777548f328e60c654f044c0cd9a63508d6b8d2abbd08c41b8361cb83d95), frozen with the protocol on 2026-09-30 and included in the registration [25]. It imports the version 1.0.0 scoring code unmodified and only after the digests of module_definition.tsv (5eae7b09c8aa5e84355a9f22e911cfa469dff88782e9b40ab19fc48a4a724fe0), analysis_core.py (5d13001e0fb85d389c91b637c93d and reanalyze.py (d6cf83c9723f8f05e133994da9dca0173115e6a4f536193300689d3bd04930c8) match the archived version 1.0.0 release [21]. The 40 synthetic tests passed before registration (Python 3.12.11, numpy 2.3.1, scipy 1.16.0, pandas 2.3.1, R 4.0.2, metafor 2.4.0). The main analysis was run with Python 3.12.12, numpy 2.3.5, scipy 1.18.0 and pandas 3.0.5; metafor 2.4.0 under R 4.0.2 [47] was used for reference checks and for the platform meta-regression. Reads deposited without per-sample counts were quantified with nf-core/rnaseq 3.27.0 [30] (Nextflow [31]; Trim Galore v2.3.0 [48]; Salmon [32] with gene-level summarisation by tximport [33]) against Ensembl release 116 [49]. The post hoc Part 1 code, part1_posthoc.py (SHA-256 32d5551f2ee6092b2b936bfd35497b06a9d7d666e77dfc1a4a41e4f975726d2d), reads only the registered result files and code, whose digests its manifest records, and was run on 2026-10-27 with Python 3.13.17, numpy 2.5.4, scipy 1.16.0 and pandas 3.1.0. The post hoc Part 2 code, part2_matrix.py (SHA-256

2c978f7a26327f3921ac7c5153060f2c98e2a9fb825e380e67eaeb2fa04f6d05), reads the expression inputs again through the registered loading code, whose data loader file differs in digest from the registered run's copy, and was run on 2026-11-01 with Python 3.12.12, numpy 2.3.5, scipy 1.18.0 and pandas 3.0.5, the versions of the registered run; before producing any result it reproduced the registered per-unit scores to within 5.0×10^{-7}. Its gene lists were built from the HGNC release of 2026-10-16 [50], and each list's membership hash is pinned in the script and recorded in its manifest. The code of Amendment A5, a5_checks.py (SHA-256 179ad7ba8a3295c04515031316d06a4c5fe75afc61026aaa046a83cc2991feb6), imports the Part 2 code unmodified, was tested on synthetic data before registration and was run on 2026-11-01 in the same environment; before producing any result it reproduced the registered per-unit scores to within 5.0×10^{-7}. The checks added in the third version (r0_reproduce.py, r0_phase2_rescore.py, r2_build.py with r2_metamex.R, r3_description.py, r4_scan.py, r5_composition.py, r6_specificity.py) read only the archived result tables, the archived expression inputs of already included records and public summary tables; each was run on 2026-11-07 with Python 3.11.15, numpy 2.4.6, scipy 1.17.1 and pandas 2.3.3 under a plan frozen with a SHA-256 digest before it was computed (Table S1), and the reproduction gate was repeated by the author in the environment of the registered run. The driver scripts, the scripts that build the tables, figures and reference table of this article from the result files (make_tables.py, make_figures.py, make_references_table.py, make_tables_v2.py, make_figures_v2.py, make_table_s12.py, make_table_s1_v2.py, check_part2_ and the post hoc code are in the same Zenodo record [46].

Author contributions

Kazuya Izumi: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration.

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