Supplementary Information

Nonlinear age-related differences in adult knee extension strength reveal sex-specific early warning windows

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Supplementary Information

This supplementary file accompanies the Scientific Reports submission entitled *Nonlinear age-related differences in adult knee extension strength reveal sex-specific early warning windows*. The purpose of the supplement is to make the main analyses more transparent, reproducible, and reviewable by documenting the participant flow, age-distribution stability, nonlinear model selection process, breakpoint-search uncertainty, derivative-based interpretation, and the field testing protocol used for isometric knee extension strength.

Figure S1. Participant flowchart

Initial field-tested records
$n = 6{,}831$

↓

Records excluded before analysis
Missing age: $n = 0$
Invalid or failed isometric knee extension measurements (coded as -99 and treated as missing): $n = 70$

↓

Final analytic sample
$n = 6{,}761$

↓

Sex-stratified analytic samples
Men: $n = 3{,}020$
Women: $n = 3{,}741$

Figure 1: **Participant flowchart for the analytic sample.** The field-tested dataset contained 6,831 records. No participants were excluded because of missing age; 70 records with invalid or failed isometric knee extension measurements were excluded, yielding a final analytic sample of 6,761 adults.

Table S1. Sex- and age-specific sample distribution

Table 1: **Sex- and age-specific sample distribution and knee extension strength summary.**

Sex	Age band (years)	N	Age mean (SD)	Knee extension mean (SD), N	Knee extension median (IQR), N
Male	20–24	369	21.9 (1.5)	413.9 (132.6)	404.4 (319.5, 503.4)
Male	25–29	452	27.0 (1.5)	423.7 (139.1)	404.3 (332.9, 492.3)
Male	30–34	418	32.1 (1.4)	419.2 (131.1)	410.2 (328.0, 490.0)
Male	35–39	414	36.7 (1.4)	429.6 (144.0)	414.1 (335.7, 499.6)
Male	40–44	399	42.0 (1.4)	399.3 (135.0)	387.4 (315.5, 468.8)
Male	45–49	336	46.7 (1.4)	373.5 (124.2)	356.8 (295.3, 437.9)
Male	50–54	336	52.1 (1.4)	355.9 (134.7)	338.7 (267.5, 429.9)
Male	55–59	296	56.8 (1.5)	331.0 (105.9)	327.8 (261.5, 397.1)

Sex	Age band (years)	N	Age mean (SD)	Knee extension mean (SD), N	Knee extension median (IQR), N
Female	20–24	312	22.1 (1.5)	266.2 (84.2)	255.4 (210.0, 316.8)
Female	25–29	372	27.1 (1.4)	270.6 (93.5)	259.2 (207.0, 313.5)
Female	30–34	449	32.1 (1.4)	257.3 (89.9)	245.1 (200.9, 301.1)
Female	35–39	634	37.0 (1.4)	263.3 (84.0)	254.5 (208.3, 308.1)
Female	40–44	572	42.0 (1.3)	258.5 (83.6)	248.8 (208.7, 297.2)
Female	45–49	558	46.8 (1.5)	249.9 (80.6)	240.7 (199.5, 290.1)
Female	50–54	490	52.0 (1.3)	243.3 (75.2)	236.0 (191.6, 285.6)
Female	55–59	354	57.0 (1.4)	223.5 (69.5)	213.9 (172.8, 258.0)

Interpretation. The sample remained large across all 5-year age bands, but the female distribution was visibly more bounded near the upper age limit. This supports the boundary-sensitive interpretation of the female derivative minimum near 57 years.

Table S2. Full descriptive statistics by sex and age group

The full machine-readable version of Table S2 is supplied in `Supplementary_Tables.xlsx`. It contains sex- and age-group-specific n, mean, SD, median, and IQR for age, height, weight, BMI, grip strength, back strength, vertical jump, push-ups/knee push-ups, sit-ups, absolute knee extension strength, and relative knee extension strength.

Table S3. Candidate RCS models and cross-validation results

Table 2: **Candidate restricted cubic spline (RCS) models and five-fold cross-validation results.**

Sex	Selection stage	Candidate	CV mean MSE	CV SD	Selected
Male	Degrees of freedom	df = 5	17542.025	1654.853	Yes
Male	Degrees of freedom	df = 6	17548.959	1651.926	No
Male	Degrees of freedom	df = 4	17550.129	1653.595	No
Male	Degrees of freedom	df = 3	17553.302	1668.031	No
Male	Prespecified knots	knots = [30, 40, 50]	17543.083	1654.999	Yes
Male	Prespecified knots	knots = [35]	17547.268	1664.276	No
Male	Prespecified knots	knots = [35, 45]	17548.035	1653.191	No
Male	Prespecified knots	knots = [40]	17554.322	1668.508	No
Male	Prespecified knots	knots = [45]	17566.621	1673.277	No
Female	Degrees of freedom	df = 4	6876.233	841.641	Yes
Female	Degrees of freedom	df = 3	6881.752	839.418	No
Female	Degrees of freedom	df = 6	6882.345	839.338	No
Female	Degrees of freedom	df = 5	6882.360	841.330	No

Sex	Selection stage	Candidate	CV mean MSE	CV SD	Selected
Female	Prespecified knots	knots = [40, 50]	6876.245	838.051	Yes
Female	Prespecified knots	knots = [55]	6876.375	837.065	No
Female	Prespecified knots	knots = [50]	6877.361	837.672	No
Female	Prespecified knots	knots = [45]	6879.354	838.667	No
Female	Prespecified knots	knots = [35, 45, 55]	6879.375	838.646	No

Interpretation. The selected models minimized cross-validated prediction error while remaining clinically interpretable. For women, the best two knot configurations were numerically close, which further supports cautious interpretation of high-age curvature.

Table S4. Multivariable RCS coefficients

The full machine-readable version of Table S4 is supplied in Additional file 2 (`Additional_file_2_Supplementary_tables.xlsx`). This file contains the regression coefficients, 95% confidence intervals, and P values for all spline basis terms and covariates in the sex-specific multivariable RCS models.

Table S5. Piecewise regression coefficients and breakpoint-search uncertainty

Table S5A. Full piecewise regression coefficients are supplied in Additional file 2 (`Additional_file_2_Supplementary_tables.xlsx`).

Table S5B. Breakpoint search profile.

Table 3: **Top-ranked candidate breakpoints from the one-breakpoint piecewise regression scan.**

Sex	Search range	Step	Bootstraps	Rank	Candidate breakpoint	RSS	95% CI of selected breakpoint
Male	22–57	1 y	100	1	38	52768471.669	29.0 to 40.5
Male	22–57	1 y	100	2	37	52777163.471	29.0 to 40.5
Male	22–57	1 y	100	3	39	52779341.064	29.0 to 40.5
Male	22–57	1 y	100	4	36	52784156.286	29.0 to 40.5
Male	22–57	1 y	100	5	35	52796379.857	29.0 to 40.5
Male	22–57	1 y	100	6	40	52823453.222	29.0 to 40.5
Female	22–57	1 y	100	1	50	25628364.162	36.9 to 53.0
Female	22–57	1 y	100	2	51	25630188.039	36.9 to 53.0
Female	22–57	1 y	100	3	49	25631420.789	36.9 to 53.0
Female	22–57	1 y	100	4	52	25638867.213	36.9 to 53.0
Female	22–57	1 y	100	5	48	25639949.674	36.9 to 53.0

Sex	Search range	Step	Bootstraps	Rank	Candidate breakpoint	RSS	95% CI of selected breakpoint
Female	22–57	1 y	100	6	44	25645643.107	36.9 to 53.0

Interpretation. In men, adjacent breakpoints around 37–39 years performed similarly, supporting a mid-to-late-30s transition rather than a perfectly discrete biological threshold. In women, the minimum RSS occurred at 50 years, but several neighboring breakpoints between 48 and 52 years performed similarly and the bootstrap interval remained comparatively wide.

Table S6. First-derivative key estimates

Table 4: **Key first-derivative estimates from the covariate-adjusted RCS curves.**

Sex	Age (years)	Predicted strength, N	Derivative, N/year	Boundary-sensitive minimum	Interpretation
Male	20.0	421.80	-1.244	No	Observed age boundary
Male	30.0	414.28	0.239	No	Reference age point
Male	38.0	414.85	-1.410	No	Near piecewise breakpoint
Male	40.0	410.59	-3.025	No	Reference age point
Male	45.0	386.65	-5.631	No	Reference age point
Male	50.0	361.51	-3.789	No	Reference age point
Male	55.0	350.83	-0.888	No	Reference age point
Male	59.0	349.22	-0.155	No	Observed age boundary
Female	20.0	272.53	-0.965	No	Observed age boundary
Female	30.0	263.30	-0.830	No	Reference age point
Female	40.0	256.82	-0.433	No	Reference age point
Female	50.0	248.82	-1.798	No	Near piecewise breakpoint
Female	55.0	236.63	-2.972	No	Reference age point
Female	57.0	230.16	-3.199	Yes	Age of steepest derivative minimum
Female	59.0	223.81	-3.269	No	Observed age boundary

Interpretation. In men, the derivative became most negative around the mid-40s, consistent with a steeper cross-sectional age gradient after the late 30s transition. In women, the derivative continued to become more negative toward the upper end of the observed age range, supporting the interpretation of a broader post-50 pattern of more pronounced cross-sectional age-related differences rather than a fixed turning age at exactly 57 years.

Table S7. Quantile regression by sex and quantile

The full machine-readable version of Table S7 is supplied in Additional file 2 (`Additional_file_2_Supplementary_tables.xlsx`). This file contains sex-specific quantile regression coefficients for relative knee extension strength (Q10, Q25, Q50, Q75, and Q90), including the age slope at each quantile, pseudo-R^2, and the analytic sample size.

Table S8. Sex-specific normative grading

The full machine-readable version of Table S8 is supplied in Additional file 2 (`Additional_file_2_Supplementary_tables.xlsx`). This file contains the sex-specific cut-points used to define Excellent, Good, Average, Below Average, and Poor grades, together with their observed proportions in the analytic sample.

Machine-readable supplementary table package

For submission, the machine-readable supplementary tables are bundled in `Supplementary_Tables.xlsx` as an Excel workbook:

- `Additional_file_2_Supplementary_tables.xlsx`

The workbook contains separate sheets for Table S1, Table S2, Table S3, Table S4, Table S5A, Table S5B, Table S6, Table S7, and Table S8. The CSV exports used to generate the workbook were retained locally for version control and reproducibility.

Appendix S1. Isometric knee extension testing SOP and quality control

Purpose and setting. Isometric knee extension strength was used as the primary lower-limb strength outcome because it directly reflects quadriceps function and aligns with the functional demands of standing, walking, stair climbing, and other anti-gravity activities. The test was designed as a field-deployable procedure emphasizing safety, standardization, and feasibility for large-scale adult monitoring.

Equipment and limb selection. A portable isometric dynamometer was used to test the dominant leg. Before each field-testing session, the device and fixation system were checked and calibrated by trained examiners. The strap fixation point was standardized to a position 5 cm above the medial malleolus.

Participant positioning. Participants sat on a chair with a backrest, with the trunk against the support and both hands holding the sides of the chair for stabilization. The test-side thigh remained in contact with the chair, and the hip and knee were positioned in a standardized flexed posture, with the knee at approximately 90°.

Execution. Participants were instructed to keep the lower leg at the starting position and not to swing it backward before pushing forward. On a standardized verbal cue, they generated maximal forward force and maintained the contraction for 3 seconds. The highest force recorded during each attempt was stored by the device.

Repeated trials and scoring. Each participant completed three repeated trials with adequate recovery between attempts. The maximum value was used for the analytic dataset. This choice was intended to reduce random within-subject variability and better capture maximal voluntary effort in a field environment.

Quality-control rules. An attempt was judged unacceptable and was repeated when one or more of the following were observed: poor movement quality, obvious compensatory trunk or hip motion, loss of the standardized knee position, unstable strap fixation, or any interruption of force production before the required 3-second contraction was completed. Examiners monitored posture continuously and could terminate and repeat a trial immediately if the standardized execution was not maintained.

Methodological rationale. The broader project rationale for this test emphasized the need for a safe, convenient, and function-oriented lower-limb strength measure that could complement existing national physical fitness surveillance indicators. In the underlying project materials, development priorities included functional relevance, action optimization, safety evaluation, standardization, and eventual digitization for large-scale monitoring.

Appendix S2. Analytical robustness and sensitivity considerations

Covariate strategy. The primary multivariable nonlinear models adjusted for height, weight, and grip strength. BMI was retained for descriptive and correlation analyses but excluded from the final primary RCS models because of collinearity with height and body weight.

Reason for treating quantile regression as supplementary. Quantile regression was used to evaluate heterogeneity in the cross-sectional age gradient across different relative-strength strata. It improves practical interpretability, but identification of key age windows remained anchored to the triangulated evidence from covariate- adjusted

RCS curves, first-derivative analysis, and piecewise regression.

Boundary sensitivity. The female derivative minimum occurred near the upper boundary of the observed age range. For this reason, the female value at 57.0 years was explicitly labeled boundary-sensitive and was not interpreted as a stable physiological threshold. The preferred interpretation is that women showed progressively more pronounced cross-sectional age differences after approximately 50 years.

Model-selection robustness. The candidate-model table shows that the chosen RCS specifications had the lowest cross-validated prediction error within the prespecified model space, but neighboring candidates for women performed similarly. Likewise, the piecewise breakpoint profile indicates that several adjacent candidate breakpoints produced very similar residual sums of squares. These features support emphasis on age windows and structural regions rather than overly literal interpretation of any single age as a hard biological cut-point.

Interpretive implication. Taken together, the supplementary analyses support the stability of the main narrative: in men, the late-30s to mid-40s period represents a key window of more marked cross-sectional age differences, whereas in women, stronger age-related differences emerge later and are best interpreted as a broader post-50 pattern with residual boundary uncertainty near the upper age limit. These findings were not derived from a nationally representative sample. Instead, they are most directly applicable to a field-measured Chinese adult cohort assessed under standardized community or field-testing conditions. Their applicability to adults aged $\geq$60 years, clinical populations, and frail older adults remains to be established in dedicated validation studies.

Appendix S3. STROBE checklist

Table 5: **STROBE checklist for this cross-sectional study.**

Item	Recommendation summary	Location in manuscript
1	State the design in the title or abstract.	Title; Abstract
2	Explain the scientific background and rationale.	Background
3	State specific objectives and hypotheses.	Background, final paragraph
4	Present key elements of study design early.	Methods: Study Design and Participants

Item	Recommendation summary	Location in manuscript
5	Describe the setting, locations, and dates.	Methods: Study Design and Participants; Data Collection and Measurements
6	Give eligibility criteria and sources/methods of participant selection.	Methods: Study Design and Participants
7	Clearly define outcomes, exposures, predictors, and confounders.	Methods: Data Collection and Measurements; Statistical Analysis
8	Describe data sources and methods of assessment.	Methods: Data Collection and Measurements; Appendix S1
9	Describe efforts to address potential bias.	Methods: Data Collection and Measurements; Appendix S1; Discussion: Strengths and Limitations
10	Explain how study size was arrived at.	Results: final analytic sample size; Figure S1
11	Explain handling of quantitative variables.	Methods: Statistical Analysis
12	Describe all statistical methods, subgroup analyses, missing data handling, and sensitivity considerations.	Methods: Statistical Analysis; Appendix S2
13	Report numbers of individuals at each stage.	Methods: Study Design and Participants; Figure S1
14	Give characteristics of study participants and missing-data profile.	Results: Basic Characteristics; Table 1; Table S1; Table S2
15	Report numbers of outcome events or summary measures.	Results: Tables 1–3; Tables S1–S8
16	Give unadjusted and adjusted estimates with precision.	Results: Tables 2A, 2B, 3; Tables S3–S8
17	Report other analyses such as subgroup and supplementary analyses.	Results: quantile regression description; Tables S7–S8; Appendix S2
18	Summarize key results with reference to objectives.	Discussion: Principal Findings and Overall Significance

Item	Recommendation summary	Location in manuscript
19	Discuss limitations, including potential bias or imprecision.	Discussion: Strengths and Limitations; Appendix S2
20	Provide an overall interpretation considering objectives, limitations, and external evidence.	Discussion, all subsections
21	Discuss generalizability.	Discussion: Strengths and Limitations
22	Report funding source and role of funders.	Declarations: Funding