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Last updated by author(s):	YYYY-MM-DD

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For a	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	\square The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	A description of all covariates tested
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	igstyle Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Sof	tware and code

Policy information about <u>availability of computer code</u>

Data collection

LabVIEW (Version) Matlab (Version) Wattbike Hub (app) Polar Beat (app) LabChart 7 Powerlab 16/35

Data analysis

RStudio(version 2022.07.2)

R packages Ime4, LmerTest, mixedpower

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data is available upon request

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity</u> and <u>racism</u>.

Reporting on sex and gender

Sex was included as a fixed factor in the data analysis model. Sex was not included as an interaction effect in the model as this did not improve overall model fit.

Reporting on race, ethnicity, or other socially relevant groupings

Race and ethnicity were not included as variables in the current study. The sample was recruited from the eastern suburbs of Melbourne, Australia. The sample size was small and predominantly caucasian, therefore meaningful conclusions regarding the impact of race or ethnicity were unlikely to be identified based on the current data.

Population characteristics

Participants included in the final dataset were 23 healthy adults aged 55 - 75 years (mean age = 66.68 years, SD = 5.32) with no major medical or psychiatric diagnoses. The sample comprised 15 women and 8 men.

Recruitment

Participants were recruited via local community groups including educational services and fitness groups targeted at older age. Due to its nature as an exercise research study, there was likely a self-selection bias toward individuals with higher cardiorespiratory fitness and a greater level of comfort with physical exercise.

Ethics oversight

Ethical approval was provided by Monash University Human Research Ethics Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Behavioural & social sciences

Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This study involved quantitative data and a mixed experimental design.

Research sample

Participants were healthy older adults aged 55 - 75 recruited via targeted advertisements in the local area. Participants were predominantly recruited via advertisements to local educational institutions for older adults and gyms with older-age specific classes. The sample was therefore unlikely to be representative, as it comprised predominantly higher fit and more active older adults.

Sampling strategy

Participants were recruited via convenience sampling. A target sample size of 28 participants was determined based on an effect size of d = 0.96 as reported in Stavrinos & Coxon, (2017) for a Time \times Exercise Condition interaction to assess motor learning retention (alpha = .05, power = 80%).

Data collection

Exercise data were collected using Polar Beat app and heart rate monitor, with cycling data collected using Wattbike Hub. Additional measures were taken manually with pen and paper, including heart rate, cadence, power output and percieved exertion. Measures were taken at 1 minute intervals during exercise or 5-minute intervals during active rest.

Behavioural data were collected using LabVIEW 2015 and collated using Matlab 2018a.

During graded fitness test, data was collected using LabChart, with manual measures taken each minute as described above. The researchers were not blind to group allocation or the hypothesis of the research.

Timing

Data were collected across two time periods, one between 01/07/2019 - 18/12/2019, and another period between 29/06/2022 - 28/11/2022.

Data exclusions

Three participants were excluded prior to data analysis due to performance below a pre-established cutoff on a cognitive screening measure. One additional participant was excluded due to technical problems during testing, which resulted in incomplete data.

	44 participants commenced participation in the research study, however due to extended COVID-19 lockdowns and university closures between 2020 and 2022, 20 participants withdrew, were no longer able to participate, or ceased responding and did not complete all aspects of the study.
Randomization	Participants were randomly allocated to an active rest or exercise group via random number generation.

Reporting for specific materials, systems and methods

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