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Elzen, W.P.J. den; Martin-Ruiz, C.; Zglinicki, T. von; Westendorp, R.G.J.; Kirkwood, T.B.L.; Gussekloo, J.

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Telomere length and anaemia in old age: results from the Newcastle 85-plus Study* and the Leiden 85-plus Study

Wendy P. J. Den Elzen[1,2,†], Carmen Martin-Ruiz[2,†], Thomas von Zglinicki[2], Rudi G. J. Westendorp[3,4], Thomas B. L. Kirkwood[2], Jacobijn Gussekloo[1]

[1]Department of Public Health and Primary Care, Leiden University Medical Centre, Leiden, The Netherlands
[2]Institute for Ageing and Health, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, UK
[3]Department of Gerontology and Geriatrics, Leiden University Medical Centre, Leiden, The Netherlands
[4]Netherlands Consortium for Healthy Ageing, Leiden, The Netherlands

Address correspondence to: W. P. J. den Elzen, Department of Public Health and Primary Care, Leiden University Medical Centre, Post zone V-0-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Tel: (+31) 71 526 8444; Fax. (+31) 71 526 8259. Email: w.p.j.den_elzen@lumc.nl

Abstract

Background: reduced telomere length in blood cells has been associated with increased risk of multiple age-related diseases and is widely regarded as a general biomarker of ageing. Therefore, it is important to know both the extent and limitations of this association. We investigated the relation between telomere length and anaemia in two independent cohorts, with the prior expectation of adding anaemia to the list of conditions for which telomere reduction is a risk factor.
Participants and methods: the present study is embedded in the Newcastle 85-plus Study and Leiden 85-plus Study, two population-based studies of inhabitants of Newcastle and North Tyneside, UK (n = 749) and Leiden, the Netherlands (n = 658) aged 85 and over. High-molecular-weight DNA was isolated from full fresh blood (Newcastle) and peripheral blood mononuclear cells samples (Leiden). Telomere length was measured as abundance of telomeric template versus a single gene by quantitative real-time polymerase chain reaction. Anaemia was defined according to World Health Organization criteria.
Results: in both studies, no differences in median telomere length were observed between participants with anaemia and participants without anaemia (Newcastle: 2,846 bp (interquartile range (IQR) 2,433–3,630) versus 2,920 bp (IQR 2,425–3,570), P = 0.63; Leiden: 4,136 bp (IQR 3,879–4,428) versus 4,167 bp (IQR 3,893–4,501), P = 0.41). Telomere length also did not correlate with any other haematological parameter in both men and women.
Conclusions: in contrast to other age-related diseases, telomere length is not associated with anaemia or any other haematological parameter in older individuals in the general population.

Keywords: *aging, anaemia, telomeres, elderly*

†Both authors contributed equally to this work.
*The Newcastle 85-plus Study Core Team comprises J. Collerton, K. Davies, C. Jagger, A. Kingston, J. Bond, M. P. Eccles, L. A. Robinson, C. Martin-Ruiz, T. von Zglinicki, O. F. W. James, T. B. L. Kirkwood.

Introduction

Reduced telomere length in blood cells has been associated with increased risk of multiple age-related diseases, such as dementia [1], myocardial infarction [2], heart failure [3], atherosclerosis [4] and solid tissue tumours [5] and is widely regarded as a general biomarker of aging [6]. Therefore, it is important to know both the extent and limitations of this association. In the present study, we investigated the relation between telomere length and anaemia, another highly common condition in older individuals [7], in two population-based studies of individuals aged 85 years and over, with the prior expectation of adding anaemia to the list of conditions for which telomere reduction is a risk factor.

Method

Study populations and procedures

Newcastle 85-plus Study

The Newcastle 85-plus Study is a population-based study of 85-year-old inhabitants of Newcastle and North Tyneside, UK. The study protocol and baseline findings have been described in detail elsewhere [8, 9]. In short, all individuals who turned 85 during the year 2006 (i.e. born in 1921) and who were registered with any Newcastle or North Tyneside Primary Care Trust general practice were eligible for study participation ($n = 1,453$). Of the 1,042 persons that agreed to participate (response rate 71.7%), 778 participants agreed to blood sampling. For the present analyses, data from 749 participants with complete haemoglobin and telomere data were included.

From every participant informed consent was obtained. If the individual was not able to give informed consent due to cognitive impairment, assent was sought from a caregiver, according to the UK Mental Capacity Act 2005. The study was approved by the Newcastle & North Tyneside Local Research Ethics Committee.

At baseline, all information was obtained during three home visits at the participant's place of residence. During these visits, several questionnaires on socio-economic status (income, level of education) and lifestyle were completed, and a number of measurements and function tests were performed (e.g. Mini Mental State Examination [10]). One additional visit was made to collect fasting blood samples. The participants' general practitioner records were reviewed to obtain information about medical history.

The Leiden 85-plus Study

The Leiden 85-plus Study is a population-based prospective study of inhabitants of Leiden, the Netherlands, aged 85 years and over. For the present analyses, we made use of the first cohort enrolled between December 1986 and March 1988. A detailed description of the study procedures can be found elsewhere [11]. In short, on 1 December 1986, the community of Leiden in the Netherlands had 105,000 inhabitants, of whom 1,258 (1%) were 85 years and over. During the enrolment period from 1 December 1986 to 1 March 1988, 221 participants died before they could be visited. Of the remaining 1,037 people who were eligible for the study, 977 (94%) agreed to participate. Blood was taken from 905 subjects. The present study includes data from 658 participants for whom complete data on telomere length and haematological parameters were available.

The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all subjects or their guardian for cognitively impaired subjects. During two home visits, participants were interviewed and blood samples were taken according to predefined protocols under non-fasting conditions. The medical history was obtained from the participants and in memory-impaired subjects from partners and carers.

Main laboratory measurements

Routine haematology was performed on anti-coagulated whole blood samples (ethylenediaminetetraacetic acid (EDTA)-coated tubes) using standard automated analysis systems. Anaemia was defined according to World Health Organization criteria (haemoglobin (Hb) <12 g/dl for women and Hb <13 g/dl for men) [12].

In the Newcastle 85-plus Study, DNA was extracted from full fresh blood (white blood cells, i.e. granulocytes and peripheral blood mononuclear cells (PBMC)) with the QiaAmp DNA Maxi kit (Qiagen Ltd, Crawley, UK). In the Leiden 85-plus Study, DNA was extracted from PBMC samples stored at −80°C using the QiaAmp DNA Mini kit (Qiagen Ltd, Crawley, UK).

DNA concentration and quality were monitored by agarose gel electrophoresis. Samples were discarded if DNA degradation (smear <20 kb) was visible. Telomere length was measured as the ratio of the starting quantity for telomeres versus the starting quantity for the single copy gene of glyceraldehyde-3-phosphate dehydrogenase (as control) by quantitative real-time polymerase chain reaction (PCR) [13] with modifications as described previously [14]. Measurements were performed in quadruplicates. Three DNA samples with known telomere lengths (3.0, 5.5 and 9.5 kb pairs) were run as internal standards together with each batch of 16 study samples to convert the ratios of starting quality into telomere lengths in base pairs. The intra-assay coefficient of variation for this PCR method in our lab is 2.65% and the inter-assay coefficient of variation is 5.12%.

Additional laboratory measurements

Additional laboratory measurements were performed in the Newcastle 85-plus Study. Ferritin levels were determined by

ADVIA Centaur chemiluminescence immunoassay in serum samples. A chemiluminescence microparticle immunoassay on an Abbott ARCHITECT analyser was used to determine vitamin B_{12} in plasma and red cell folate levels from EDTA anti-coagulated blood samples. Serum creatinine levels were measured as a proxy of renal function on an Olympus AU640 system. Serum high-sensitivity C-reactive protein (CRP) levels were determined as an indicator of inflammatory status by the Dade Behring CardioPhase hsCRP immunoassay.

Data analysis

In both studies, differences in telomere length between participants with and without anaemia were tested with Mann–Whitney *U* tests. The relation between telomere length and haemoglobin levels was displayed in scatter plots with superimposed regression lines, stratified by gender and analysed with linear regression analysis. Differences in haematological characteristics between quintiles of telomere length were tested with χ^2 tests (categorical data) or Jonkheere–Terpstra tests (continuous data), for men and women separately.

In the Newcastle 85-plus Study, telomere length was also compared between participants with red blood cell count (RBC), platelets and white blood cell count (WBC) in the lowest (gender-dependent) quartile and participants with RBC, platelets and WBC in the highest (gender-dependent) quartile. In addition, we used Mann–Whitney *U* tests to analyse differences in telomere length between participants with explained and unexplained anaemia. Explained anaemia was defined as the presence of anaemia and one or more of the following: ferritin concentration <15 μg/l, vitamin B_{12} concentration <170 pg/ml, red cell folate concentration <160 μg/l, CRP concentration >5 mg/l and/or creatinine concentration >155 μmol/l. In the Newcastle 85-plus study, unexplained anaemia was defined as the presence of anaemia with normal ferritin, vitamin B_{12}, red cell folate, CRP and creatinine concentrations. Furthermore, we compared telomere length between participants with unexplained anaemia and participants without anaemia and normal ferritin, vitamin B_{12}, red cell folate, CRP and creatinine concentrations.

Data analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL. USA).

Results

Table 1 describes the socio-demographic and haematological characteristics of both study populations. All participants in the Newcastle 85-plus Study were aged 85 years. The median age of the cohort from Leiden was 89 years (interquartile range (IQR) 88–92). Compared with participants in the Newcastle 85-plus Study, the prevalence of institutionalisation and smoking was higher among participants in the Leiden 85-plus Study. The prevalence of stroke, myocardial infarction and malignancy was lower in the Leiden 85-plus Study (Table 1).

Table 1. Socio-demographic, haematological and clinical characteristics of the study populations

	Newcastle 85-plus Study (*N* = 749)	Leiden 85-plus Study (*N* = 658)
Socio-demographics		
Age	85	89 (88–92)
Men	295 (39.4%)	176 (26.7%)
Institutionalisation	62 (8.3%)	354 (53.8%)
Current smoking	42 (5.6%)	102 (15.5%)
Haematological characteristics		
Haemoglobin (g/dl)	13.1 (12.1–14.1)	13.4 (12.3–14.2)
Haematocrit (%)	0.40 (0.37–0.43)	0.41 (0.38–0.43)
MCV (fl)	94 (90–97)	91 (88–94)
Anaemia[a]	222 (29.6%)	127 (19.3%)
Microcytic anaemia (MCV < 80 fl)	9 (4.1%)	13 (10.2%)
Normocytic anaemia (MCV 80–100 fl)	190 (85.6%)	104 (81.9%)
Macrocytic anaemia (MCV > 100 fl)	23 (10.4%)	10 (7.9%)
RBC ($\times 10^9$/l)	4.3 (3.9–4.6)	4.5 (4.2–4.8)
Platelets ($\times 10^9$/l)	249 (209–297)	NA
WBC ($\times 10^9$/l)	6.4 (5.4–7.6)	6.1 (5.2–7.3)
Telomere length (bp)	2,889 (2,427–3,589)	4,163 (3,885–4,495)
Men	3,005 (2,517–3,832)	4,277 (3,972–4,684)
Women	2,797 (2,356–3,418)	4,113 (3,849–4,425)
Other clinical characteristics		
MMSE (points)	28 (25–29)	26 (20–29)
Ferritin (μg/l)	59 (28–121)	NA
Vitamin B_{12} (pg/ml)	313 (230–437)	NA
Red cell folate (μg/l)	383 (271–565)	NA
Creatinine (μmol/l)	99 (86–119)	NA
C-reactive protein (mg/l)	2.6 (1.2–5.9)	NA
Comorbidity		
Stroke	100 (13.4%)	20 (3.0%)
Myocardial infarction	110 (14.7%)	49 (7.4%)
Dementia	52 (7.0%)	50 (7.6%)
Diabetes mellitus	101 (13.5%)	82 (12.5%)
Malignancy	184 (24.6%)	106 (16.1%)

Categorical data are presented as *N* (%). Continuous data are presented as median (interquartile range).
MCV, mean cell volume; RBC, red blood cell count; WBC, white blood cell count; MMSE, Mini Mental State Examination.
[a]Haemoglobin <12 g/dl for women and <13 g/dl for men.

The prevalence of anaemia was 29.6% ($n = 222/749$) in the Newcastle 85-plus Study and 19.3% ($n = 127/658$) in the Leiden 85-plus Study. In the Newcastle 85-plus Study, the prevalence of anaemia in men was 33.2% (98/295) and 27.3% in women (124/454), χ^2 test $P = 0.08$. In the Leiden 85-plus Study, the prevalence of anaemia was higher in men than in women: men 26.7% (47/176) versus women 16.6% (80/482), $P < 0.01$). Participants in the Newcastle 85-plus Study had shorter telomeres than participants in the Leiden 85-plus Study. In both studies, women had shorter telomeres than men (Mann–Whitney *U* test $P < 0.01$).

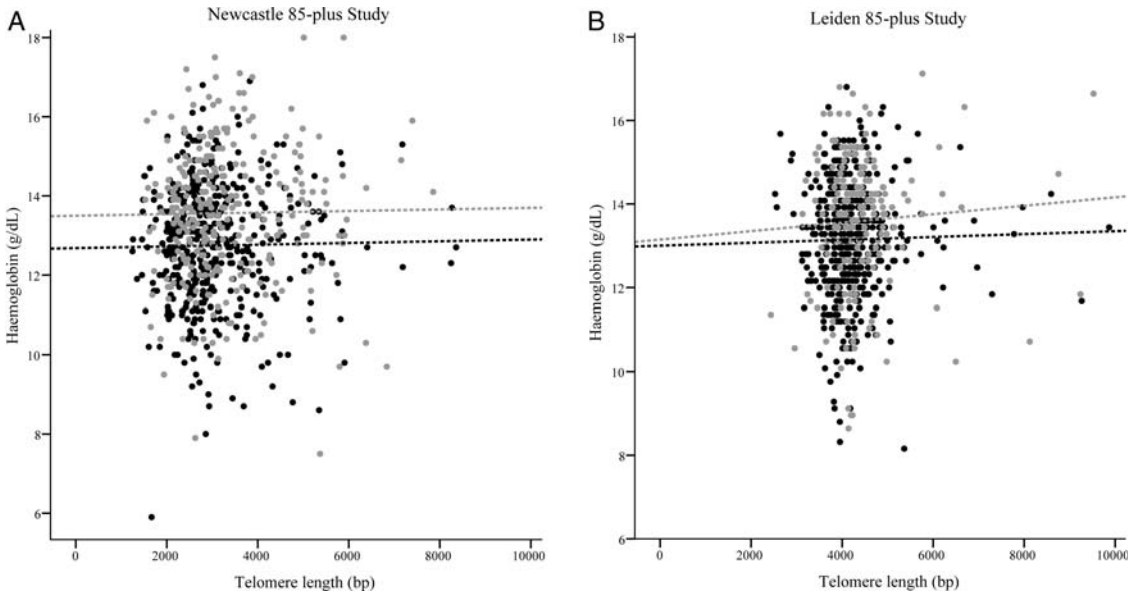

Figure 1. Scatter plots with superimposed regression lines of haemoglobin levels (*y*-axis) against telomere length (*x*-axis) in the Newcastle 85-plus Study (A, $n = 749$) and Leiden 85-plus Study (B, $n = 658$). Legend: grey circle, men; black circle, women. No significant associations were observed (all $P > 0.40$).

Figures 1A and B are scatter plots with superimposed regression lines of haemoglobin levels (*y*-axis) against telomere length (*x*-axis) for men and women separately. There was no relation between telomere length and haemoglobin levels in either men or women (linear regression analyses, $P > 0.40$). No associations were found in both populations when we adjusted the regression analyses in the total study populations for gender (Hb 0.021 g/dl ($P = 0.69$) per 1,000 bp increase in telomere length in the Newcastle 85-plus Study and Hb 0.055 g/dl ($P = 0.40$) in the Leiden 85-plus Study). Similar results were found when we also adjusted for stroke, myocardial infarction, dementia, diabetes mellitus and malignancy (data not shown).

In addition, no association between telomere length and haemoglobin was found when we performed one regression analysis combining both study populations (Hb 0.034 g/dl ($P = 0.40$) per 1,000 bp increase in telomere length, adjusted for gender and study population).

No differences in median telomere length were observed between participants with anaemia and participants without anaemia (Table 2, Newcastle 85-plus Study: 2,846 bp (IQR 2,433–3,630) versus 2,920 bp (IQR 2,425–3,570), $P = 0.63$; Leiden 85-plus Study: 4,136 bp (IQR 3,879–4,428) versus 4,167 bp (IQR 3,893–4,501), $P = 0.41$). Similar results were found when we stratified on gender or when we restricted the analysis in the Leiden 85-plus Study to participants aged 85–89 years ($n = 384$).

Appendices 1 and 2 available as Supplementary data in *Age and Ageing* online show the haematological characteristics depending on quintiles of telomere length for men and women, respectively. In both men and women, telomere length was not associated with any of the haematological parameters, either in the Newcastle 85-plus Study or in the Leiden 85-plus Study. We did find a *P* value <0.05 for the association between telomere length and platelets for men in the Newcastle 85-plus Study, but this may be considered a chance finding as there was not a clear dose-dependent relation between telomere length and platelet count.

In the Newcastle 85-plus Study, no differences in telomere length were found between participants with RBC, platelets and WBC in the lowest gender-dependent quartile ($n = 30$) and participants with RBC, platelets and WBC in the highest gender-dependent quartile ($n = 14$) (2,771 (IQR 2,127–3,099) versus 3,024 (2,654–3,724), $P = 0.18$). In addition, we did not find any differences in median telomere length between participants with unexplained anaemia ($n = 79$) and participants with explained anaemia ($n = 143$): 2,809 bp (IQR 2,278–3,491) versus 2,880 bp (IQR 2,494–3,759), $P = 0.40$. Furthermore, no differences in median telomere length were found between participants with unexplained anaemia ($n = 79$) and participants without anaemia and normal ferritin, vitamin B_{12}, red cell folate, CRP and creatinine concentrations ($n = 318$) (2,809 bp (IQR 2,278–3,491) versus 2,932 bp (2,405–3,574) respectively, $P = 0.35$).

Discussion

We examined the relation between telomere length and anaemia, with the prior expectation of adding anaemia to the list of conditions for which telomere reduction is a risk factor. A surprise negative finding in one population led us to verify this finding in a second independent cohort. In both cohorts of older individuals, we found no association

Table 2. Telomere length in base pairs for participants with and without anaemia

	Anaemia[a]		No anaemia		P
	n	Median (IQR)	*n*	Median (IQR)	
Newcastle 85-plus Study					
Total population	222	2,846 (2,433–3,630)	527	2,920 (2,425–3,570)	0.63
Men	98	2,994 (2,518–3,789)	197	3,025 (2,513–3,899)	0.71
Women	124	2,782 (2,341–3,536)	330	2,816 (2,362–3,399)	0.54
Leiden 85-plus Study					
Total population	127	4,136 (3,879–4,428)	531	4,167 (3,893–4,501)	0.41
Men	47	4,194 (3,955–4,585)	129	4,329 (3,982–4,701)	0.26
Women	80	4,085 (3,877–4,360)	402	4,121 (3,844–4,443)	0.51

Data are presented as median (interquartile range). *P* values were obtained by Mann–Whitney *U* tests.[a]Haemoglobin <12 g/dl for women and <13 g/dl for men.

between telomere length and haemoglobin concentration or any other haematological parameter.

The lack of association between telomere length and anaemia was unexpected. First, telomere length has been correlated with many other major age-related diseases [1–5]. Second, myelodysplastic syndromes or other types of bone marrow failure are thought to explain the increased frequency of (unexplained) anaemia in older individuals [7, 15, 16]. Adult haematopoietic stem cells show a severe loss of telomeric DNA compared with cells from fetal liver or umbilical cord blood [17] and aged mice have a decreased capacity to replace blood cells during haematopoietic stress compared with younger mice [15, 18], indicating a loss of replicative potential for bone marrow stem cells with age [17, 19] and a possible incapacity to react to the physiologic demand for blood cell replenishment with age [15, 16, 18]. Since the results of earlier studies indicate that patients with myelodysplastic syndromes or other types of bone marrow failure syndromes have shortened telomeres [20–22], shorter telomere length has been associated with an increased risk of anaemia in chronic heart failure patients [23] and shorter telomere length was an independent predictor of lower red blood cell counts in a study of middle-aged subjects (aged 35–55 years) [24], we hypothesised that telomere length is a marker of haematopoietic ageing and bone marrow failure, and as a result would be associated with anaemia in older individuals in the general population. The results of this study, however, do not support this hypothesis.

One possible explanation for the lack of association between telomere length and anaemia in our study may be that telomere length in WBC in old age is very unstable and does not have enough predictive power for age-related morbidity and mortality at ages over 85 years [25]. This explanation, however, seems unlikely, because we recently found a strong and significant relation between telomere length and left ventricular function in the pilot study of the Newcastle 85-plus Study ($n = 89$, aged 85 years) [3]. Another explanation for the lack of association between telomere length and anaemia may be that, despite the results from earlier studies suggesting otherwise [23, 24], there, in fact, is no association between telomere length and bone marrow failure, or bone marrow failure and anaemia, in the general population of older individuals. This explanation is supported by the results from a study by Mollica *et al.* [26] in which no correlation was found between telomere length and blood counts in a population-based sample of 717 women aged 38–100 years (median 72 years).

Our findings do not preclude that short telomeres may drive aplastic syndromes. However, the repetitive negative findings presented here suggest that in the population at large, a myelodysplastic syndrome per se is an unlikely explanation for the presence of anaemia in older people. Similar discrepancies between studies in selected populations and population-based studies have been reported previously. For instance, Cherkas *et al.* [27] found an association between socio-economic status and telomere length in female twins aged 18–75 years, but no such association was found by Adams et al. [28] in an unselected cohort of 50-year-olds.

We observed a large difference in median telomere length between the two cohorts. Participants in the Newcastle 85-plus Study had shorter telomeres than participants in the Leiden 85-plus Study. This difference could not be explained by differences in laboratory techniques, since both collections of samples were measured using the same equipment and the same reagents and assays, and subsequent real-time PCR data analysis was performed by the same person (C.M.-R.). The fact that DNA samples were derived from WBC in the Newcastle 85-plus Study and from PBMC in the Leiden 85-plus Study is unlikely to explain this difference in telomere length. Granulocytes tend to have longer telomeres than lymphocytes [29, 30]. The difference in telomere length between these two cell populations increases with age [29, 30], which would have led us to expect longer median telomere length in the cohort from Newcastle. The difference in telomere length between the populations is as yet unexplained; possible factors include differences in disease incidence, dietary patterns or socio-economical status. In addition, in both study

populations we found shorter telomeres in women compared with men, the opposite of what is generally expected. This finding illustrates the rather strange telomere biology in the older populations, and may indicate that telomere length cannot be used unconditionally as a biomarker of the human aging process [25].

A limitation of the present study is its cross-sectional design, which restricts our capacity to draw conclusions about causal relations. Given that a proportion of older persons with unexplained anaemia may be due to myelodysplastic syndrome [7, 15, 16] and that earlier studies have linked myelodysplasia with shorter telomeres [20–22], it is a limitation of our study that the information on the causes of anaemia was only present in the Newcastle 85-plus Study. Another limitation is that in neither of the studies bone marrow biopsies were available to confirm the diagnosis of myelodysplasia or bone marrow failure [15].

A strength of our analysis is that we made use of data from two large studies of individuals aged 85 years and over, giving us a unique opportunity to cross-validate our negative findings, especially since in both studies telomere length was measured in the same laboratory using the same validated technique [14, 25]. Another strong point of the present study is the population-based nature of both cohorts, allowing us to generalise our results to the population of older people at large.

In conclusion, in contrast to other age-related diseases, telomere length is not associated with anaemia or any other haematological parameter in older individuals in the general population, despite the plausible biological mechanism underlying this association. To further investigate this intriguing matter, studies incorporating bone marrow biopsies will be needed.

Key points

- Reduced telomere length in blood cells has been associated with increased risk of multiple age-related diseases.
- Telomere length is not associated with anaemia or any other haematological parameter in old age.
- To further investigate this matter, studies incorporating bone marrow biopsies will be needed.

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Conflicts of interest

None declared.

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Author contributions

J.G. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: W.P.J.d.E., C.M.-R., T.v.Z., R.G.J.W., T.B.L.K., J.G.

Acquisition of data: C.M.-R., J.G.

Analysis and interpretation of the data: W.P.J.d.E., C.M.-R., T.v.Z., R.G.J.W., T.B.L.K., J.G.

Drafting of the manuscript: W.P.J.d.E., C.M.-R., J.G.

Critical revision of the manuscript for important intellectual content: T.v.Z., R.G.J.W., T.B.L.K.

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The sponsors did not have any role in the design, methods, subject recruitment, data collections, analysis and preparation of the manuscript.

Supplementary data

Supplementary data mentioned in the text is available to subscribers in *Age and Ageing* online.

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