Non-esterified fatty acids and telomere length in older adults: The Cardiovascular Health Study

Peter Ahiawodzi [a, *], Annette L. Fitzpatrick [b], Luc Djousse [c], Joachim H. Ix [d], Jorge R. Kizer [e], Kenneth J. Mukamal [f]

[a] Department of Public Health, Campbell University College of Pharmacy and Health Sciences, Buies Creek, NC, USA
[b] Departments of Family Medicine, Epidemiology, and Global Health, University of Washington, Seattle, WA, USA
[c] Division of Aging, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
[d] Divisions of Nephrology and Preventive Medicine, University of California, San Diego, CA, USA
[e] Cardiology Section, San Francisco Veterans Affairs Health Care System, And Departments of Medicine, Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA
[f] Division of General Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

ARTICLE INFO

ABSTRACT

Background: Telomeres shorten as organisms age, placing limits on cell proliferation and serving as a marker of biological aging. Non-esterified fatty acids (NEFAs) are a key mediator of age-related metabolic abnormalities. We aimed to determine if NEFAs are associated with telomere length in community-living older adults.

Material and methods: We cross-sectionally studied 1648 participants of the Cardiovascular Health Study (CHS) who underwent concomitant telomere length measurement from a sample of 4715 participants who underwent measurement of circulating total fasting NEFAs in stored specimens from their 1992-3 clinic visit. We used linear regression and inverse probability weighting to model telomere length as a function of NEFAs with adjustment for age, gender, race, clinic, BMI, marital status, smoking status, alcohol intake, diabetes status, years of education, hypertension status, prevalent cardiovascular disease, C-reactive protein, total adiponectin, albumin, fetuin-A, fasting insulin, eGFR, total cholesterol, HDL-cholesterol, triglycerides, and general health status.

Results: Higher NEFAs were significantly associated with shorter telomere length, after adjusting for age, gender, race, and clinic site ($\beta = -0.034$; SE $= 0.015$; $P = 0.02$). Estimates remained similar in fully adjusted models where each SD of NEFA increment was associated with 0.042 kilobase (kb) pairs shorter telomere length (standard error $= 0.016$; $P = 0.007$); for comparison the coefficient for a single year of age in the same model was -0.017. These results were similar in strata of sex, and waist circumference although they tended to be strongest among participants in the youngest tertile of age ($\beta = -0.079$; SE $= 0.029$; P $= 0.01$).

Conclusions: In this population-based cohort of community-living elders, we observed a significant inverse association between NEFAs and telomere length. If confirmed, NEFAs may represent a promising target for interventions to slow biological aging.

1. Introduction

Non-esterified fatty acids (NEFAs) are a biologically active class of carboxylic acids of various lengths and degrees of saturation. They arise in circulation predominantly from hydrolysis of triglycerides in adipose tissue but also, to a lesser degree, from food or phospholipids [1]. Circulating levels of NEFAs are modifiable in response to insulin and other metabolic regulators [1–4] and hence are attractive candidates as targetable risk factors for aging and age-related diseases.

Although NEFAs are best known as intermediates in age-related metabolic dysregulation, leading to diabetes, the biological effects of NEFAs may occur across a wider variety of organ systems and tissues and hence are plausibly related to biological aging. Indeed,

previous work in CHS demonstrated that higher NEFAs were associated not only with incident diabetes, but also dementia- and pulmonary-related mortality, atrial fibrillation, congestive heart failure, frailty and disability [5–8].

Telomere length is a biomarker of cumulative exposure to oxidative stress and an indicator of biological rather than chronological aging [9,10]. Telomeres are repetitive DNA sequences that cap the ends of chromosomes. In humans, the long stretches of the tandem repeats are made up of TTAGGG [11]. Telomeres regulate the proliferation of cells in organisms, for they shorten as the organism ages, and this shortening place limits on the number of replications that the cells of the organism can undergo.

Several genetic and environmental factors have been proposed to influence telomere length: many of these factors influence oxidative stress and inflammation, which are important drivers in telomere length dynamics and aging [12–20]. Because NEFAs have also been associated with oxidative stress and inflammation, we hypothesized that they may be associated with shorter telomere length and hence with biological aging. However, no previous studies have measured both factors simultaneously and investigated if the two are related to one another independent of chronological age or other comorbidities.

To address this question, we analyzed data from participants of the Cardiovascular Health Study (CHS), an ongoing population-based cohort study of older Americans that measured both total fasting NEFAs and telomere length in a subset of participants. *A priori*, we hypothesized that higher NEFA concentrations would be associated with shorter telomere length, independent of age and other comorbid diseases.

2. Material and Methods

CHS is a prospective cohort consisting of 5888 men and women aged ≥65 years who were randomly selected from Medicare-eligibility lists in four U.S. communities (Forsyth County, NC; Washington County, MD; Sacramento County, CA; and Pittsburgh, PA). A detailed description of methods and procedures in the CHS has previously been published [21]. Briefly, persons eligible to participate were not institutionalized or wheelchair dependent, did not require a proxy for consent, were not receiving treatment for cancer, and were expected to remain in their respective regions for 3 years. From 1989 to 1990, 5201 participants were recruited in the original cohort. Between 1992 and 1993, 687 subjects (predominantly African American) were additionally recruited. Baseline evaluation of study participants included standardized questionnaires, physical examination, anthropometric measurements, resting electrocardiography, and laboratory examinations. The institutional review board at each center approved the study, and each participant gave informed consent.

For this analysis, the 1992–93 clinic visit (i.e., the first visit that incorporated the additional 687 subjects who were recruited) was used for analysis. Of the 5265 participants present at the 1992-93 clinic visit, 4715 underwent NEFA measurement on stored fasting serum samples. During the same clinic visit, 1675 were randomly selected for leucocyte telomere length measurement if they had stored genetic samples available, had a signed consent for DNA use on file, and also attended the 1997–1998 CHS examination. Overall, 1648 participants underwent both NEFA and telomere length measurements.

2.1. Measurement of serum NEFA

Serum NEFA concentration was measured on fasting samples by the Wako enzymatic method at the CHS Central Laboratory at the University of Vermont. This technique relies on the acylation of CoA by the fatty acids in the presence of added acyl-CoA synthetase. Acyl-CoA produced is oxidized by added acyl-CoA oxidase with generation of hydrogen peroxide and in the presence of peroxidase permits the oxidative condensation of 3-methy-Nethyl- N(b-hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple-colored adduct. The latter is then measured colorimetrically at 550 nm. The inter-assay CV was 3.54–8.17% (detectable range 0.0156–1.50 mEq/L).

2.2. Measurement of telomere length

Telomere length was measured as the mean length of the terminal restriction fragments (TRFs) in peripheral leukocytes, using the Southern blot method. Briefly, DNA samples were digested overnight with the restriction enzymes HinfI (10 U) and *Rsa*I (10 U; Roche Applied Science, Indianapolis, Indiana). Eighteen DNA samples (~5 μg each) and four DNA ladders (one kilobase (kb) pair DNA ladder plus λ DNA/*Hin*dIII fragments; Invitrogen, Carlsbad, California) were resolved on 0.5% agarose gel (20 cm × 20 cm) at 50 V (GNA-200 electrophoresis unit; GE Healthcare, Piscataway, New Jersey). After 16 h, the DNA was depurinated for 30 min in 0.25 N hydrochloric acid, denatured for 30 min in 0.5 mol/L sodium hydroxide/1.5 mol/L sodium chloride, and neutralized for 30 min in 0.5 mol/L Tris (pH 8)/1.5 mol/L sodium chloride. The DNA was transferred for 1 h to a positively charged nylon membrane (Roche Applied Science) with a vacuum blotter (Boekel Scientific, Feasterville, Pennsylvania). Membranes were hybridized at 65 °C with the telomeric probe (digoxigenin 3′-end labeled 5′-(CCCTAA)₃) overnight in 5X sodium chloride/sodium citrate (SSC), 0.1% Sarkosyl, 0.02% sodium dodecyl sulfate, and 1% blocking reagent (Roche Applied Science). Membranes were washed three times at room temperature in 2X SSC, 0.1% sodium dodecyl sulfate each for 15 min and once in 2X SSC for 15 min. The digoxigenin-labeled probe was detected by means of the digoxigenin luminescent detection procedure (Roche Applied Science) and exposed on Hyperfilm (GE Healthcare).

Autoradiographs were scanned and the TRF signal between molecular weights of 3 kb and 20 kb was digitized; background was fixed as the signal in a non-DNA-loaded region of the film. The optical density values versus DNA migration distances were converted to optical density (adjusted for background)/molecular weight versus molecular weight. The mean TRF length was then calculated accordingly (between the nadir and 20 kb). Each sample was analyzed twice for telomere length measurement (on different gels on different occasions), and the mean was used for statistical analyses. The Pearson correlation coefficient for the duplicates of this sample was 0.97, with an average coefficient of variation for pair sets of 1.5%. The laboratory personnel conducting the TRF length and NEFA measurements were blinded to all characteristics of participants [22,23].

2.3. Covariates

Baseline variables were used for statistical adjustment. These include clinic site, age, technician-measured body mass index, marital status (married or non-married), race (white or non-white) years of education; , hypertension status (normotensive, pre-hypertensive, or hypertensive), diabetes status (normal [fasting glucose <100 mg/dl], prediabetes [fasting glucose between 100 and 125 mg/dl], diabetic [fasting glucose >125 mg/dl or use of medication]), smoking status, alcohol intake, adjudicated prevalent cardiovascular disease (CHD, MI, stroke, CHF), and general health status. Other biomarkers measured at the CHS Central Laboratory included total adiponectin, albumin, fetuin-A, fasting insulin, cystatin-C (used to estimated glomerular filtration rate), C-reactive

protein (CRP), total and HDL-cholesterol, and triglycerides.

2.4. Statistical analyses

Because only 1648 participants (out of 4715 participants with NEFA measurements) underwent measurement of telomere length, we employed stabilized inverse probability weighting to calculate sample weights that estimated associations in the original sub-cohort of 4715. For Table 1, we made the *a priori* decision to describe study variables by NEFA levels in tertiles.

Linear regression models were used to model telomere length as a function of total NEFA (per SD; SD = 0.20) in a reduced model including age, gender, race, and clinic site, and in a fully adjusted model that added BMI, marital status, smoking status, alcohol intake, diabetes status, years of education, hypertension status, prevalent cardiovascular disease, C-reactive protein, total adiponectin, albumin, fetuin-A, fasting insulin, eGFR, total cholesterol, HDL-cholesterol, triglycerides, and general health status. In secondary analyses, we also performed stratified analyses with age, gender and waist circumference. For the stratified analyses, age was reclassified into tertiles; 64–71, 72–76, and >76, and waist circumference was dichotomized at the median as <97 cm and ≥97 cm. Restricted cubic splines were used to evaluate the shape of the relationship between total NEFAs and telomere length. All analyses were performed using SAS Version 9.4.

3. Results

Table- 1 shows the demographic characteristics of the 1648 included participants according to tertiles of NEFAs. As expected, NEFAs tended to be higher with older age, heavier BMI, and among women.

We observed a significant inverse association between NEFAs and telomere length in both reduced ($\beta = -0.034$; SE = 0.015; P = 0.02) and fully adjusted ($\beta = -0.042$; SE = 0.016; P = 0.01)

models. The association appeared stronger in the fully adjusted models relative to the reduced models. Fig. 1 demonstrates the dose-dependent, graded association of NEFAs with telomere length; we observed no evidence of non-linearity (p = 0.47), and a significant linear trend (p = 0.01). In a sensitivity analysis, we
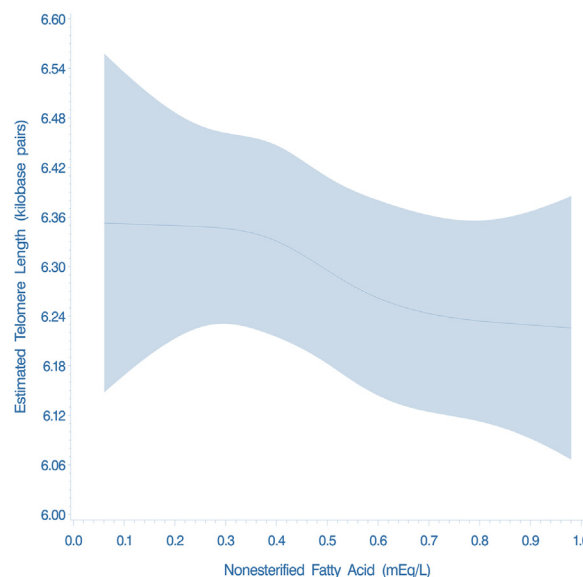
Fig. 1. Adjusted relationship between NEFAs and telomere length.
Results derived from a penalized cubic spline generalized linear model with 4 knots The model adjusted for age, sex, race, clinic site, body mass index, marital status, years of education, hypertension status, diabetes status, smoking status, alcohol intake, adjudicated prevalent cardiovascular disease (CHD, CHF, MI, and stroke), hypoglycemic and lipid-lowering medications, general health status, total adiponectin, albumin, fetuin-A, fasting insulin, glomerular filtration rate (cystatin-C), C-reactive protein (CRP), total and HDL-cholesterol, and triglycerides. Shaded area indicates 95% confidence interval. P values were 0.47 for non-linearity, 0.01 for a significant linear trend.

Table 1
Descriptive characteristics of 1648 CHS participants with NEFA measurement in 1992−1993.

	Non-Esterified Fatty Acids (mEq/L)		
VARIABLE	<0.40 (N = 534)<	0.40−0.55 (N = 526)	>0.55 (N = 588)
Age, Years	74.1 (5.0)	74.9 (5.2)	75.3 (5.4)
BMI, kg/m^2	26.5 (4.2)	27.0 (5.0)	27.2 (5.0)
Male, n (%)	327 (48.4)	206 (30.5)	143 (21.2)
White, n (%)	466 (32.4)	460 (32.0)	512 (35.6)
Married, n (%)	414 (36.6)	360 (31.9)	356 (33.0)
Education (≥H.S), n (%)	428 (34.7)	381 (30.9)	424 (34.4)
Current Smoker, n (%)	80 (35.1)	75 (32.9)	73 (32.0)
Alcohol, #/wk	2.3 (5.1)	1.9 (4.4)	2.1 (5.1)
Diabetes, n (%)	62 (24.0)	86 (33.3)	110 (42.6)
Diabetes Medication Use, n (%)	41 (27.0)	47 (30.9)	64 (42.1)
Hypertension, n (%)	195 (28.3)	200 (29.0)	294 (42.7)
Hypertension Medication Use, n (%)	272 (31.2)	260 (29.9)	339 (38.9)
Coronary Heart Disease, n (%)	141 (36.9)	113 (29.6)	128 (33.5)
Congestive Heart Failure, n (%)	41 (33.3)	43 (35.0)	39 (31.7)
Stroke, n (%)	27 (30.0)	35 (38.9)	28 (31.1)
Myocardial Infarction, n (%)	78 (39.6)	54 (27.4)	65 (33.0)
General Health (Good-Excellent), n(%)	439 (33.1)	430 (32.5)	456 (34.4)
Fetuin A, g/L	0.46 (0.09)	0.48 (0.09)	0.49 (0.1)
Albumin, gm/dl	3.89 (0.27)	3.91 (0.27)	3.95 (0.27)
Adiponectin, ng/mL	12072.6 (7102.0)	13681.4 (7205.1)	15130.6 (8331.3)
Total Cholesterol, mg/dL	203.3 (36.6)	209.8 (37.4)	215.5 (39.4)
Triglyceride, mg/dL	142.5 (81.3)	152.0 (97.9)	157.1 (86.4)
HDL, mg/dL	48.2 (12.0)	52.1 (13.5)	56.4 (15.3)
Insulin, IU/ml	16.7 (34.6)	13.8 (14.8)	14.9 (25.1)
C-Reactive Protein, mg/L	5.4 (11.2)	6.2 (11.1)	6.8 (11.7)
Glomerular Filtration Rate (Cystatin-C)	70.8 (18.4)	70.5 (19.9)	72.0 (18.8)
Hypoglycemic Medication, n (%)	32 (27.1)	32 (27.1)	54 (45.8)
Lipid-Lowering Medication, n (%)	43 (34.9)	36 (29.3)	44 (35.8)

added diabetes medication use, hypertension medication use, hypoglycemic medication use, and lipid-lowering medication use to the fully adjusted model, but including these in the model did not alter our findings significantly ($\beta = -0.047$; SE $= 0.018$; P $= 0.01$).

When we examined covariates individually, we found that the stronger association in the fully adjusted model was attributable to albumin and hypertension status. For comparison, the only other variables significantly associated with telomere length in these models were age, sex, race, smoking, and hypertension. The regression coefficient for 1 SD increment in NEFAs was approximately equivalent to that for 2.5 years of age ($\beta = -0.017$ for a year of age) in multivariable models.

In stratified analyses (Fig. 2), the association of NEFAs with telomeres was nearly identical among men ($\beta = -0.047$; SE $= 0.027$; P $= 0.07$) and women ($\beta = -0.041$; SE $= 0.020$; P $= 0.03$), and was generally similar by waist circumference as well ($\beta = -0.023$; SE $= 0.023$; P $= 0.30$, for waist circumference <97 cm; and $\beta = -0.057$; SE $= 0.022$; P $= 0.01$, for waist circumference ≥97 cm). Participants in the 64−71 age group had the largest telomere length decrease ($\beta = -0.079$; SE $= 0.029$; P $= 0.01$), compared to the older two tertiles, which were similar to each other ($\beta = -0.022$; SE $= 0.026$; P $= 0.40$, for age group 72−76 years; and $\beta = -0.024$; SE $= 0.027$; P $= 0.37$, for age >76 years). The interactions were not significant for age (p $= 0.30$), gender (p $= 0.90$) or waist circumference (p $= 0.64$).

4. Discussion

In this cohort of community-living older adults, higher serum NEFA levels were significantly associated with shorter telomere length and indeed among the few measured variables to do so. To our knowledge, this the first epidemiological study to examine this association and adds to the growing list of adverse health measures associated with circulating NEFAs in elders.

The mechanisms by which NEFAs might lead to accelerated biological aging are speculative, but our results suggest that they may be independent (or at least superimposed upon) the effects of NEFAs on inflammation and glucose metabolism (for which we controlled).

One potential pathway linking NEFAs and telomere length is oxidative stress. Higher circulating levels of NEFAs have been shown to promote de novo lipogenesis, causing abnormal hepatic lipid accumulation and oxidative stress [24]. Studies have also shown that reduction of NEFAs lowers oxidative stress [25]. In a systematic review of oxidative stress and telomere length, Von Zglinicki [9] noted that in most cases the replicative senescence of

telomeres is a stress response arising from external oxidative stress or diminished internal antioxidant defenses. His review of experiments on stress dependency of telomere shortening revealed that 19 out of 22 independent experiments from seven different laboratories showed increased telomere shortening rate under conditions of increased oxidative stress.

Inflammation and oxidative stress increase the demand for cell replication and hence accelerated shortening of telomeres [10,26,27]. In fact, inflammation and oxidative stress increase the number of telomere repeats lost per cell replication [28−30]. The association of inflammation and oxidative stress with diseases of aging [31,32] may explain the increased incidence and prevalence of late-life diseases, particularly cardiovascular disease among individuals with shorter telomeres [27,33−35].

Telomere shortening has been associated with neurodegenerative diseases, infections, diabetes, malignancies, autoimmune diseases, hypertension, atherosclerosis, cardiovascular disease, and mortality especially from cardiovascular disease [26,27,33,36−39]. Many of these conditions have also been associated with higher serum NEFAs, and future research is warranted to determine if NEFAs are associated with that subset of conditions that have not yet been tested. Because NEFA levels are modifiable − by improving adipocyte insulin sensitivity, for example − they may reflect one potential pathway by which telomere length can be preserved.

Our stratified analyses by age group did not show a formally significant interaction between age in tertiles and NEFA levels. Although we studied a cohort of older adults, a closer look at age differences demonstrates that participants 64−71 years had a larger telomere length decrease compared to older participants, although we observed no dose-response relationship above this age. This could reflect the loss of telomere length that occurs with age [9,10,40], such that modifiable factors like NEFAs may be most important in individuals of less advanced age. This hypothesis will require testing in younger cohorts.

The stratified analyses by gender did not show major differences between males and females for the association between NEFAs and telomere lengths. Nonetheless, sex itself was a determinant of telomere length, consistent with previous work by Bekaert at al. [41] who showed that females have significantly longer telomeres compared to males. Although we did not find significant gender differences in the associations of NEFAs with telomere length, the antioxidant properties of estrogen may serve to preserve telomeres of females or at least slow the rate of attrition. Estrogen acts to scavenge free radicals, inhibit their formation, and stimulate enzymes to detoxify free radicals. [42]

Our study has several strengths. First, this is the first study to our knowledge that has investigated the association between NEFAs and telomere lengths. CHS provides high-quality data and is one of few cohorts to have both NEFAs and telomere lengths measured. Second, we had a good representation of both gender in our study sample, and risk estimates were nearly identical by gender. Third, we controlled for potential confounding as CHS enjoys a comprehensive list of covariates.

Our study is also not without limitations. First, we found associations that require further confirmation given the novelty of our findings. Nonetheless, our findings are consistent with the biology of telomere attrition and possible role of NEFA-induced oxidative stress. Second, our findings are correlative, and we cannot say with certainty that NEFAs caused telomere shortening. Moreover, the cross-sectional study design does not allow evaluation of directionality, and it remains possible that shorter telomere length precedes increases in plasma NEFA concentrations. Third, we did not assess oxidative stress or other potential mechanistic links in this study and as such cannot directly ascertain the pathways involved. Finally, our results apply directly only to older adults, as
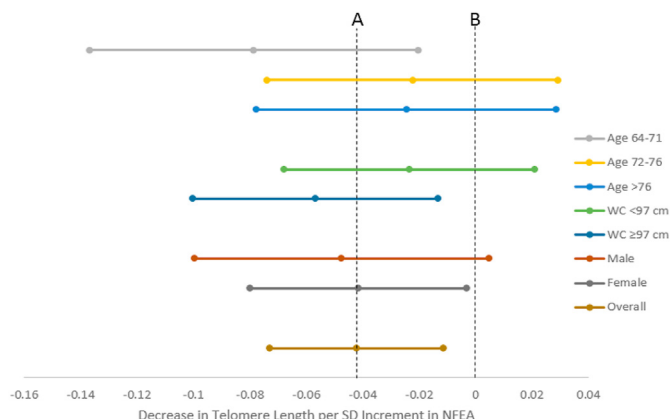

Fig. 2. Relation of NEFA with telomere length by stratified covariates.

CHS is a cohort of older adults. Future studies are needed to determine if results generalize to younger persons.

5. Conclusion

In a population of older community-living adults, higher serum NEFA levels were independently associated with shorter telomere length. NEFAs are known to promote oxidative stress and may be an important target for intervention against telomere shortening. This finding adds to the available literature on NEFA and telomere biology and suggests that NEFAs may be associated with more rapid biological aging.

Authors contributions

All authors were involved in the conception and design of the study. Peter Ahiawodzi and Kenneth Mukamal performed the statistical analyses. All authors were involved in the interpretation of the data. Peter Ahiawodzi wrote the manuscript, and all authors critically revised it. The final version of the manuscript was approved by all authors.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***.

a Give information separately for exposed and unexposed groups.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2—3
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	4—5
Objectives	3	State specific objectives, including any prespecified hypotheses	4—5
Methods			
Study design	4	Present key elements of study design early in the paper	5—9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5—9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	5—9
Variable	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5—9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5—9
Bias	9	Describe any efforts to address potential sources of bias	8—9
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8—9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8—9
		(*b*) Describe any methods used to examine subgroups and interactions	8—9
		(*c*) Explain how missing data were addressed	8—9
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	6
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8—9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9 —13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9 —13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Declaration of competing interest

None of the authors have any conflict of interest to disclose.

References

[1] Campbell PJ, Carlson MG, Hill JO, Nurjhan N. Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. Am J Physiol 1992;263(6):1063. https://doi.org/10.1152/ajpendo.2006.263.6.E1063. Accessed Oct 16, 2019.

[2] Iozzo P, Lautamaki R, Geisler F, et al. Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. Diabetologia 2004;47(7):1149–56. https://doi.org/10.1007/s00125-004-1443-2. Accessed Oct 16, 2019.

[3] Ferrannini E, Camastra S, Coppack SW, Fliser D, Golay A, Mitrakou A. Insulin action and non-esterified fatty acids. the european group for the study of insulin resistance (EGIR). Proc Nutr Soc 1997;56(2):753–61. S0029665197000293 [pii].

[4] Lv Z, Ma P, Luo W, et al. Association between serum free fatty acid levels and possible related factors in patients with type 2 diabetes mellitus and acute myocardial infarction. BMC Cardiovasc Disord 2014;14(1):159. https://doi.org/10.1186/1471-2261-14-159. https://www.ncbi.nlm.nih.gov/pubmed/25399059.

[5] Mukamal KJ. Nonesterified fatty acids, cognitive decline, and dementia. Curr Opin Lipidol 2020;31(1):1–7. https://doi.org/10.1097/MOL.0000000000000656. Accessed Feb 12, 2020.

[6] Miedema MD, Maziarz M, Biggs ML, et al. Plasma-free fatty acids, fatty acid-binding protein 4, and mortality in older adults (from the cardiovascular health study). Am J Cardiol 2014;114(6):843–8. https://doi.org/10.1016/j.amjcard.2014.06.012. Accessed Feb 12, 2020.

[7] Djoussé L, Benkeser D, Arnold A, et al. Plasma free fatty acids and risk of heart failure: the cardiovascular health study. Circulation. Heart failure. 2013;6(5):964–9. https://doi.org/10.1161/CIRCHEARTFAILURE.113.000521. https://www.ncbi.nlm.nih.gov/pubmed/23926204.

[8] Khawaja O, Bartz TM, Ix JH, et al. Plasma free fatty acids and risk of atrial fibrillation (from the cardiovascular health study). Am J Cardiol 2012;110(2):212–6. https://doi.org/10.1016/j.amjcard.2012.03.010. Accessed Oct 16, 2019.

[9] Tv Zglinicki. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27(7):339–44. https://doi.org/10.1016/S0968-0004(02)02110-2. Accessed Jan 30, 2020, https://www.cell.com/trends/biochemical-sciences/abstract/S0968-0004(02)02110-2.

[10] Aviv A. Telomeres and human aging: facts and fibs. Sci Aging Knowl Environ 2004;2004(51):pe43. https://doi.org/10.1126/sageke.2004.51.pe43. Accessed Jan 30, 2020.

[11] Meyne J, Ratliff RL, Moyzis RK. Conservation of the human telomere sequence (TTAGGG)n among vertebrates. Proc Natl Acad Sci U S A 1989;86(18):7049–53. https://doi.org/10.1073/pnas.86.18.7049. Accessed Jan 30, 2020.

[12] Cherkas LF, Hunkin JL, Kato BS, et al. The association between physical activity in leisure time and leukocyte telomere length. Arch Intern Med 2008;168(2):154–8. https://doi.org/10.1001/archinternmed.2007.39. Accessed Jan 30, 2020.

[13] Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. Lancet 2005;366(9486):662–4. https://doi.org/10.1016/S0140-6736(05)66630-5. Accessed Jan 30, 2020.

[14] Woo J, Suen EWC, Leung JCS, Tang NLS, Ebrahim S. Older men with higher self-rated socioeconomic status have shorter telomeres. Age Ageing 2009;38(5):553–8. https://doi.org/10.1093/ageing/afp098. Accessed Jan 30, 2020.

[15] Njajou OT, Cawthon RM, Blackburn EH, et al. Shorter telomeres are associated with obesity and weight gain in the elderly. Int J Obes 2012;36(9):1176–9. https://doi.org/10.1038/ijo.2011.196. Accessed Jan 30, 2020.

[16] Sun Q, Shi L, Prescott J, et al. Healthy lifestyle and leukocyte telomere length in U.S. women. PloS One 2012;7(5):e38374. https://doi.org/10.1371/journal.pone.0038374. Accessed Jan 30, 2020.

[17] Xu Q, Parks CG, DeRoo LA, Cawthon RM, Sandler DP, Chen H. Multivitamin use and telomere length in women. Am J Clin Nutr 2009;89(6):1857–63. https://doi.org/10.3945/ajcn.2008.26986. Accessed Jan 30, 2020.

[18] Marcon F, Siniscalchi E, Crebelli R, et al. Diet-related telomere shortening and chromosome stability. Mutagenesis 2012;27(1):49–57. https://doi.org/10.1093/mutage/ger056. Accessed Jan 30, 2020.

[19] De Meyer T, Rietzschel ER, De Buyzere ML, et al. Paternal age at birth is an important determinant of offspring telomere length. Hum Mol Genet 2007;16(24):3097–102. https://doi.org/10.1093/hmg/ddm271. Accessed Jan 30, 2020.

[20] Lin J, Epel E, Blackburn E. Telomeres and lifestyle factors: roles in cellular aging. Mutat Res 2012;730(1-2):85–9. https://doi.org/10.1016/j.mrfmmm.2011.08.003. Accessed Jan 30, 2020.

[21] Fried LP, Borhani NO, Enright P, et al. The cardiovascular health study: design and rationale. Ann Epidemiol 1991;1(3):263–76. https://doi.org/10.1016/1047-2797(91)90005-w. Accessed Jan 30, 2020.

[22] Okuda K, Khan MY, Skurnick J, Kimura M, Aviv H, Aviv A. Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. Atherosclerosis 2000;152(2):391–8. https://doi.org/10.1016/s0021-9150(99)00482-7. Accessed Apr 17, 2020.

[23] Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. Hypertension 2001;37(2 Pt 2):381–5. https://doi.org/10.1161/01.hyp.37.2.381. Accessed Apr 17, 2020.

[24] Zhang B, Li M, Zou Y, et al. NFκB/Orai1 facilitates endoplasmic reticulum stress by oxidative stress in the pathogenesis of non-alcoholic fatty liver disease. Front Cell Dev Biol 2019;7. https://doi.org/10.3389/fcell.2019.00202. Accessed Jan 29, 2020, https://www.frontiersin.org/articles/10.3389/fcell.2019.00202/full.

[25] Phielix E, Jelenik T, Nowotny P, Szendroedi J, Roden M. Reduction of non-esterified fatty acids improves insulin sensitivity and lowers oxidative stress, but fails to restore oxidative capacity in type 2 diabetes: a randomised clinical trial. Diabetologia 2014;57(3):572–81. https://doi.org/10.1007/s00125-013-3127-2. Accessed Jan 29, 2020.

[26] Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the framingham heart study. Aging Cell 2006;5(4):325–30. https://doi.org/10.1111/j.1474-9726.2006.00224.x. Accessed Jan 30, 2020.

[27] Fitzpatrick AL, Kronmal RA, Gardner JP, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am J Epidemiol 2007;165(1):14–21. https://doi.org/10.1093/aje/kwj346. Accessed Jan 23, 2020, https://academic.oup.com/aje/article/165/1/14/232589.

[28] Tchirkov A, Lansdorp PM. Role of oxidative stress in telomere shortening in cultured fibroblasts from normal individuals and patients with ataxia-telangiectasia. Hum Mol Genet 2003;12(3):227–32. https://doi.org/10.1093/hmg/ddg023. Accessed Jan 30, 2020.

[29] Sitte N, Saretzki G, von Zglinicki T. Accelerated telomere shortening in fibroblasts after extended periods of confluency. Free Radic Biol Med 1998;24(6):885–93. https://doi.org/10.1016/s0891-5849(97)00363-8. Accessed Jan 30, 2020.

[30] Saretzki G, Sitte N, Merkel U, Wurm RE, von Zglinicki T. Telomere shortening triggers a p53-dependent cell cycle arrest via accumulation of G-rich single stranded DNA fragments. Oncogene 1999;18(37):5148–58. https://doi.org/10.1038/sj.onc.1202898. Accessed Jan 30, 2020.

[31] Finch CE, Crimmins EM. Inflammatory exposure and historical changes in human life-spans. Science 2004;305(5691):1736–9. https://doi.org/10.1126/science.1092556. Accessed Jan 30, 2020.

[32] Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell 2005;120(4):483–95. https://doi.org/10.1016/j.cell.2005.02.001. Accessed Jan 30, 2020.

[33] Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003;361(9355):393–5. https://doi.org/10.1016/S0140-6736(03)12384-7. Accessed Jan 30, 2020.

[34] Benetos A, Gardner JP, Zureik M, et al. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. Hypertension 2004;43(2):182–5. https://doi.org/10.1161/01.HYP.0000113081.42868.f4. Accessed Jan 30, 2020.

[35] Fitzpatrick AL, Kronmal RA, Kimura M, et al. Leukocyte telomere length and mortality in the cardiovascular health study. J Gerontol A Biol Sci Med Sci 2011;66(4):421–9. https://doi.org/10.1093/gerona/glq224. Accessed Jan 30, 2020.

[36] Weng NP, Levine BL, June CH, Hodes RJ. Human naive and memory T lymphocytes differ in telomeric length and replicative potential. Proc Natl Acad Sci U S A 1995;92(24):11091–4. https://doi.org/10.1073/pnas.92.24.11091. Accessed Jan 30, 2020.

[37] Son NH, Murray S, Yanovski J, Hodes RJ, Weng N. Lineage-specific telomere shortening and unaltered capacity for telomerase expression in human T and B lymphocytes with age. J Immunol 2000;165(3):1191–6. https://doi.org/10.4049/jimmunol.165.3.1191. Accessed Jan 30, 2020.

[38] Weischer M, Bojesen SE, Cawthon RM, Freiberg JJ, Tybjærg-Hansen A, Nordestgaard BG. Short telomere length, myocardial infarction, ischemic heart disease, and early death. Arterioscler Thromb Vasc Biol 2012;32(3):822–9. https://doi.org/10.1161/ATVBAHA.111.237271. Accessed Jan 30, 2020.

[39] Farzaneh-Far R, Cawthon RM, Na B, Browner WS, Schiller NB, Whooley MA. Prognostic value of leukocyte telomere length in patients with stable coronary artery disease: data from the heart and soul study. Arterioscler Thromb Vasc Biol 2008;28(7):1379–84. https://doi.org/10.1161/ATVBAHA.108.167049. Accessed Jan 30, 2020.

[40] Vaziri H, Schächter F, Uchida I, et al. Loss of telomeric DNA during aging of normal and trisomy 21 human lymphocytes. Am J Hum Genet 1993;52(4):661–7. Accessed Aug 16, 2020.

[41] Bekaert S, De Meyer T, Rietzschel ER, et al. Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. Aging Cell 2007;6(5):639—47. https://doi.org/10.1111/j.1474-9726.2007.00321.x. Accessed Jan 30, 2020.

[42] Aviv A. Telomeres, sex, reactive oxygen species, and human cardiovascular aging. J Mol Med 2002;80(11):689—95. https://doi.org/10.1007/s00109-002-0377-8. Accessed Feb 27, 2020.