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Independent and Joint Effects of Sedentary Time and Cardiorespiratory Fitness on All-Cause Mortality: The Cooper Center Longitudinal Study

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Abstract

Objectives*:* Studies have linked prolonged sedentary time to higher mortality risk, yet previous research has not examined the sedentary behavior-mortality relationship while also considering the role of cardiorespiratory fitness (fitness), an objective and physiological consequence of physical activity and a strong predictor of morbidity and mortality. Thus, we examined the effects of sedentary time and fitness on all-cause mortality.

Design, Setting, Participants*:* A prospective study of 3,141 adult patients attending the Cooper Clinic (Dallas, TX) for preventive medical examinations. Participants provided information on sedentary behavior and completed a maximal exercise test (to determine fitness levels) at baseline and were followed until mortality (yes/no). Subsequently, we assessed the relationship between sedentary behavior and fitness (primary exposures) to all-cause mortality (outcome) utilizing multivariable analyses, while adjusting for confounders.

Results: Multivariable analyses revealed a significant linear relationship between increased fitness and lower mortality risk, even while adjusting for sedentary time, BMI, and clinical covariates (p for linear trend= 0.02). In comparison, the effects of prolonged sedentary time on increased mortality risk did not quite reach statistical significance once fitness and covariates were entered into the model (p for linear trend=0.05). When examining this relationship categorically, being sedentary for ≥23 hours weekly increased mortality risk by 29% without controlling for fitness (HR=1.29, 95%CI= 1.03-1.63); however, once fitness and covariates were taken into account this relationship did not reach statistical significance (HR=1.20, 95%CI= 0.95-1.51). In addition, high fitness levels significantly decreased mortality risk irrespective of sedentary time (HR=0.76, 95%CI= 0.59-0.97).

Conclusions: The relationship between increased sedentary time and higher mortality risk is less pronounced when fitness is taken into account.

Strengths and limitations of this study

- The first study to examine the effects of sedentary behavior on mortality, while taking cardiorespiratory fitness into account.
- Cardiorespiratory fitness was assessed objectively via maximal exercise testing; however, sedentary time was based on self-report.
- While we examined a sample of adults who attended a preventive medicine clinic with a multitude of information on patients' health, the sample was homogeneous with regards to socio-demographics.

BACKGROUND

Sedentary behavior and health has emerged as a new area of scientific investigation, based on accumulating studies linking prolonged sitting to morbidity and mortality. [1] In the US, adults spend close to 8 hours daily in sedentary behaviors, defined as low energy expenditure activities (1.0-1.5 Metabolic Equivalents) in a sitting or reclining posture.[2,3] These prolonged hours of sedentary time have been found to be related to cardiometabolic risk (primarily in cross-sectional studies),[4–7] and premature death from all-causes and from cardiovascular diseases in prospective studies.[8–10] For example, a review by Ford and Caspersen (2012) observed a 17% increased risk for cardiovascular events (fatal and non-fatal) per 2 hour/day increments of television (TV) viewing, and 5% more events per 2 hours increases in sitting time.[10] Additionally, recent meta-analyses by Chau et al. (2013) and Biswas et al. (2015) found a 34% and 24% higher risk (respectively) for all-cause mortality for prolonged sedentary time, even after adjusting for physical activity.[9,11]

These studies, however, have predominately taken into account self-reported physical activity (which is prone to recall bias), and have yet to control for cardiorespiratory fitness (fitness). Fitness, an objective and physiological consequence of habitual physical activity (also influenced by genetics) is an indicator of overall cardiovascular health.[12–14] Observational evidence has found that low fitness levels accounted for ~16% of deaths in a large cohort of over 40,000 individuals, [15] yet to date, studies have not accounted for fitness when examining the effects of sedentary behavior on mortality. Hence, we attempt to bridge this gap by examining whether sedentary behavior is associated with increased mortality risk, while considering the potential mitigating effects of fitness. Specifically, we examine the independent and joint effects of sedentary time and fitness on all-cause mortality among participants of the Cooper Center Longitudinal Study (CCLS).

METHODS

Participants and Design

The CCLS, described elsewhere,[16] is an observational study of primarily well-educated non-Hispanic white individuals who come to the Cooper Clinic (Dallas, Texas) for preventive medical examinations. In general, the CCLS aims to examine the effects of fitness on chronic disease morbidity and mortality.[17] The CCLS receives annual approval from the Cooper Institute Institutional Review Board and the present investigation received approval from the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston. In the current study, we prospectively assessed the effects of sedentary behavior and fitness on all-cause mortality among adults (≥20 years) who: (1) completed a 1982 survey including questions pertaining to sedentary behavior; and (2) came for a preventive medical visit which included a fitness test and a thorough medical history questionnaire at the Cooper Clinic within a 1 year time-frame.[12] Of 3,676 participants meeting these criteria with pertinent data on the study measures, 329 were excluded due to incomplete fitness testing, abnormal exercise ECG, less than one year of follow-up and underweight weight status. Additionally, 206 participants were excluded based on personal history of myocardial infarction, stroke, or cancer. These exclusion criteria resulted in an analytic sample of 2,716 men and 425 women (total n=3,141) with complete data on the primary exposures (sedentary behavior and fitness), and the outcome (mortality). Due to the small number of women in the sample, gender was adjusted for in multivariable analyses rather than performing stratified analysis (see statistical analysis section).

Measures

Exposures (Sedentary Behavior and Cardiorespiratory Fitness)

Sedentary behavior was assessed at baseline via reported time spent viewing TV and commuting in a car, as indicated in a 1982 survey.[12,18] For analysis, sedentary time (i.e. the sum of TV viewing and car commuting time) was categorized into sample-specific quartiles (i.e. quartile cut-points: 11, 16, 23 hours/ week). Fitness was assessed via maximal exercise testing on a treadmill adhering to the modified Balke protocol. In this protocol, described elsewhere,[16] the treadmill speed and incline are increased gradually up to 25 minutes or until volitional exhaustion.[19] From the final treadmill speed and grade maximal metabolic equivalents (METs; 1 MET = 3.5 ml O_2 uptake · kg body mass^{-1} · min^{-1}) were determined, which have been highly correlated (r>0.90) with maximal oxygen uptake. [20,21] Fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles, based on the distribution of the sample.

Outcome (all-cause mortality)

Participants were followed for mortality from all causes from baseline to either the date of death or through December 31, 2010 in order to determine vital status. The National Death Index (NDI) was the primary source of mortality information.[17] The NDI has been found to have 100% specificity and 96% sensitivity in ascertaining mortality among the general population.[22,23]

Statistical Analysis

Descriptive characteristics were computed for the entire sample and by vital status. The association between sedentary behavior and all-cause mortality was determined using Cox proportional hazard models to estimate the hazard ratio (HR) and 95% confidence intervals (CI). These models passed the proportional hazards assumption test adhering to the methodology suggested by Lin et al. (1993), which is based on cumulative sums of Martingale residuals.[24] A total of four regression models were computed adjusting for the following covariates: Model 1- age and gender; Model 2- age, gender, current smoking (dichotomous), alcohol intake (categorical), personal history of hypertension (dichotomous), personal history of diabetes (dichotomous), and family history of cardiovascular disease (dichotomous); Model 3- variables in model 2 along with fitness or sedentary time (both categorically); and Model 4- variables in model 3 as well as body mass index (BMI), glucose, systolic blood pressure, total cholesterol (all continuous), and self-reported physical activity (<500 MET-minutes per week; 500-1000 MET-minutes per week; >1000 MET-minutes per week; see footnote in Table 1).[17,25] Furthermore, we examined the joint effects of sedentary time and fitness on mortality risk, while adjusting for the other covariates in model 4. For the joint effects models, we collapsed fitness into two categories: low fitness and middle/high fitness. Multiplicative interactions were assessed by including their cross-product in the statistical model. For all analyses, p-values were two sided with an alpha of <0.05 considered statistically significant; SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) was utilized in analyses.

RESULTS

A total of 581 deaths occurred over a median follow-up period of 28.7 years (SD=4.4). At baseline, participants' mean age was 45.0 years (SD=9.6), 14.4% were current smokers, and participants consumed a median of 5 alcoholic beverages per week. In addition, participants were of normal weight (mean BMI=24.6, SD=3.0), had an average fitness level of 12.1 METs (SD=2.4), and spent 17.0 hours/week (SD=10.0) sedentary. Participants' baseline characteristics are described by vital status in Table 1.

The association between sedentary time and all-cause mortality is depicted in Table 2. Specifically, a significant linear relationship was found between higher sedentary time and increased mortality risk in three of the four multivariable models (linear trend p<0.05 for models 1-3), with the fully adjusted model (including fitness, physical activity, and clinical variables) not reaching statistical significance (linear trend p=0.05). When examining this relationship categorically, being sedentary for ≥23 hours weekly was significantly related to a 34% increase in mortality risk (HR=1.34, 95%CI= 1.06-1.68, p=0.01) without adjusting for fitness. However, once fitness was included in the model (model 3) the 22% higher mortality risk did not reach statistical significance (HR=1.22, 95%CI= 0.97-1.54, p-value=0.09). Moreover, in the fully adjusted model (model 4 which additionally controlled for fitness, physical activity, BMI, cholesterol, blood pressure and glucose) the 20% higher mortality risk similarly did not reach statistical significance (HR=1.20, 95%CI= 0.95-1.51, p-value=0.14).

In addition, Table 2 also presents the relationship between fitness and mortality while taking into account confounders. All multivariable models exhibited significant dose-response effects for increased fitness and reduced mortality risk, including models adjusting for sedentary behavior ($p<0.05$ for all). For example, in the fully adjusted model (model 4), while middle levels of fitness were associated with a 20% reduced mortality risk, high fitness levels were related to a 24% lower mortality risk in comparison to the reference group of low fitness (middle fitness: HR=0.80, 95%CI= 0.65-0.99, p=0.04; high fitness: HR=0.76, 95%CI=0.59-0.97, p=0.03). Further, when examining the joint effects of fitness and sedentary behavior on mortality, we found that in comparison to the 'high risk' reference group (low fitness/4th quartile of sedentary time) participants who were in the middle/high fitness category were primarily at reduced mortality risk irrespective of sedentary time (Table 3). For example, participants who were in the middle/high fitness strata and in the 4th quartile of sedentary time, had a 40% decreased risk for mortality (HR=0.60, 95%CI=0.43-0.86); whereas those in the middle/high fitness category and the lowest quartile of sedentary time were similarly at 40% reduced mortality risk (HR=0.60, 95%CI=0.44-0.82). Moreover, participants in the low fitness strata mostly did not have a reduced risk for mortality irrespective of sedentary time; with the exception of the 2nd quartile of sedentary behavior (see Table 3). These joint effect findings are consistent with the fact that there was no significant interaction effect between sedentary time and fitness in multivariable models (all- $p>0.10$).

DISCUSSION

The present study aimed to determine whether prolonged sedentary time is associated with increased mortality risk irrespective of and alongside fitness among a cohort of adults. Study findings reveal a significant relationship between prolonged sedentary time and increased mortality risk in models not controlling for fitness. However, once fitness was taken into account the sedentary behavior-mortality relationship was less pronounced. Specifically, being sedentary for 23 or more hours weekly significantly increased mortality risk by 29%, while accounting for confounders with the exception of fitness. Once fitness was added into the model, then increased mortality risk from prolonged sedentary time was 22% (yet without reaching significance). This 7% reduction in mortality risk stems from the protective health effects of fitness.[14] Interestingly, when accounting for additional clinical variables (e.g. BMI, blood pressure) that are on the causal pathway between sedentary time and mortality, then mortality risk was reduced by an additional 2%. This finding is understandable since studies have found that decreasing and breaking up sedentary time lowers obesity and cardiometabolic risk and these, in turn, are related to increased morality risk.[8,26,27] Thus including these intermediate variables into the model is likely to confound the relationship between the exposure and outcome.[11,28,29] However, when examining the relationship between fitness and mortality, higher fitness levels reduced mortality risk irrespective of the inclusion of sedentary time and the intermediate variables; this is indicative of the robust and causal relationship between fitness and mortality.[14]

Thus, current findings pertaining to the protective effects of fitness (e.g. 24% mortality reduction in the high fit strata fully adjusted model) are consistent with a large body of the literature that emphasizes the importance of achieving higher fitness levels to obtain health benefits.[14] Previous research has found 10-25% increased survival with a 1-MET increase in fitness. This represents a relatively small incremental change that is achievable for most individuals through increasing physical activity with the goal of reaching/ exceeding physical activity guidelines; i.e. 150 minutes of moderate or 75 minutes of vigorous intensity physical activity per week (or a combination of both). [14,25] Thus, while decades of research emphasize the health benefits of increasing fitness levels, particularly for individuals with low levels of fitness, [14] the evidence pertaining to sedentary behavior and health outcomes (independent of physical activity) is accumulating but not as well established.

The most recent systematic review/meta-analysis on the topic conducted by Biswas et al. (2015), found a 24% increased all-cause mortality risk for prolonged sedentary behavior, when adjusting for physical activity; however, virtually all studies adjusted for self-reported physical activity, and none considered the protective impact of fitness. They additionally observed that high sedentary time coupled with low levels of physical activity resulted in an even higher risk (46%) for all-cause mortality.[11] In the current study, we examined the interaction between objectively measured fitness and sedentary time in relation to mortality; we did not observe a significant interaction effect. Thus, for example, individuals who were highly fit were at a reduced risk for mortality irrespective of their sedentary time. An earlier study by Warren et al. (2010) found that prolonged sedentary behavior increased cardiovascular disease mortality risk in a larger sample of men from the Cooper Clinic;[18] however, they relied on self-reported physical activity, did not take fitness into account, and therefore did not comparatively examine its impact on mortality. Thus, in the current study, we demonstrate that sedentary behavior is related to mortality risk; however fitness 'buffers' some of the adverse health effects of sedentary behavior. The underlying mechanism as to why increased sedentary time leads to higher mortality risk warrants further investigation. The hypothesized biological mechanism of the unique impact of sedentary time, described elsewhere,[30] includes the suppression of lipoprotein lipase activity, which results in the reduction of HDL cholesterol and increased insulin resistance.[12,30,31] In a previous study of the CCLS cohort, we observed that sedentary time was cross-sectionally related to a proxy of insulin resistance even after adjusting for fitness. [12]

Current study findings should be tempered by the study's limitations. While we examined a sample of adults who attended a preventive medicine clinic with objectively measure fitness and a multitude of information on patients' health, the sample was homogeneous with regards to socio-demographics. Thus, examination of the study question among more diverse samples is warranted to generalize findings. Further, while fitness was measured via maximal exercise testing, sedentary time was based on self-reported data on TV viewing and time spent in a car, which are proxies of sedentary behavior and do not include all domains of sitting (e.g. occupational sitting). The inclusion of an objective measure of sitting (e.g. activPal accelerometer) would have been preferable, but was not available in the CCLS database. In addition, values of both sedentary time and fitness were measured at baseline and might have changed during the follow-up period. [17] Finally, dietary information was not available in the dataset and therefore was not adjusted for in the multivariable analysis. [12]

In summary, this is the first study to account for fitness when examining the sedentary behavior-mortality relationship. Findings reveal that prolonged sedentary time is related to higher mortality risk from all-causes when fitness is not accounted for; however, once controlling for fitness the sedentary behavior-mortality relationship is reduced. Thus higher levels of fitness appear to have protective effects from prolonged sedentary time by lowering mortality risk. In addition, higher levels of fitness are protective against mortality risk irrespective of sedentary time. Therefore, increasing fitness levels through meeting or exceeding physical activity guidelines is of paramount public health importance. Nonetheless, additional research is needed to explore the relationship between sedentary behavior and morbidity and mortality while taking the protective effects of fitness into account.

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Conflict of Interest: The authors declare that no competing interests exist.

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References

1 Owen N, Sparling PB, Healy GN, *et al.* Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin Proc* 2010;**85**:1138–41.

2 Matthews CE, Chen KY, Freedson PS, *et al.* Amount of time spent in sedentary behaviors in the United States, 2003-2004. *Am J Epidemiol* 2008;**167**:875–81.

3 Sedentary research network. Standardized use of the terms ‘ sedentary ’ and ‘ sedentary behaviours ’. *Appl Physiol Nutr Metlab* 2012;**37**:540–2.

4 Shuval K, Gabriel KP, Leonard T. TV viewing and BMI by race/ethnicity and socio-economic status. *PLoS One* 2013;**8**:e63579.

5 Healy GN, Matthews CE, Dunstan DW, *et al.* Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J* 2011;**32**:590–7.

6 Gardiner PA, Healy GN, Eakin EG, *et al.* Associations between television viewing time and overall sitting time with the metabolic syndrome in older men and women: the Australian Diabetes, Obesity and Lifestyle study. *J Am Geriatr Soc* 2011;**59**:788–96.

7 Thorp AA, Healy GN, Owen N, *et al.* Deleterious Associations of Sitting Time and Television Viewing Time with Cardiometabolic Risk Biomarkers. *Diabetes Care* 2010;**33**:327–34.

8 Veerman JL, Healy GN, Cobiac LJ, *et al.* Television viewing time and reduced life expectancy: a life table analysis. *Br J Sports Med* 2012;**46**:927–30.

9 Chau JY, Grunseit AC, Chey T, *et al.* Daily sitting time and all-cause mortality: a meta-analysis. *PLoS One* 2013;**8**:e80000.

10 Ford ES, Caspersen CJ. Sedentary behaviour and cardiovascular disease: a review of prospective studies. *Int J Epidemiol* 2012;**41**:1338–53.

11 Biswas A, Oh PI, Faulkner GE, *et al.* Sedentary Time and Its Association With Risk for Disease Incidence, Mortality, and Hospitalization in Adults. *Ann Intern Med* 2015;**162**:123.

12 Shuval K, Finley CE, Barlow CE, *et al.* Sedentary Behavior, Cardiorespiratory Fitness, Physical Activity, and Cardiometabolic Risk in Men: The Cooper Center Longitudinal Study. *Mayo Clin Proc* 2014;**88**:1052–62.

13 Sui X, LaMonte MJ, Laditka JN, *et al.* Cardiorespiratory fitness and adiposity as mortality predictors in older adults. *J Am Med Assoc* 2007;**298**:2507–16.

14 Kaminsky L a, Arena R, Beckie TM, *et al.* The importance of cardiorespiratory fitness in the United States: the need for a national registry: a policy statement from the American Heart Association. *Circulation* 2013;**127**:652–62.

15 Blair SN. Physical inactivity: the biggest public health problem of the 21st century. *Br J Sports Med* 2009;**43**:1–2.

16 Willis BL, Gao A, Leonard D, *et al.* Midlife fitness and the development of chronic conditions in later life. *Arch Intern Med* 2012;**172**:1333–40.

17 Shuval K, Barlow CE, Chartier KG, *et al.* Cardiorespiratory fitness, alcohol, and mortality in men: the Cooper Center longitudinal study. *Am J Prev Med* 2012;**42**:460–7.

18 Warren TY, Barry V, Hooker SP, *et al.* Sedentary behaviors increase risk of cardiovascular disease mortality in men. *Med Sci Sports Exerc* 2010;**42**:879–85.

19 Shuval K, Finley CE, Chartier KG, *et al.* Cardiorespiratory Fitness, Alcohol Intake, and Metabolic Syndrome Incidence in Men. *Med Sci Sport Exerc* 2013;**44**:2125–31.

20 Pollock ML, Bohannon RL, Cooper KH, *et al.* A comparative analysis of four protocols for maximal treadmill stress testing. *Am Heart J* 1976;**92**:39–46.

21 Pollock M, C F, Schmidt D, *et al.* Comparative analysis of physiologic responses to three different maximal graded exercise test protocols in healthy women. *Am Heart J* 1982;**103**:363–73.

22 Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B HC. Test of the National Death Index. *Am J Epidemiol* 1984;**119**:837–9.

23 Farrell SW, Fitzgerald SJ, McAuley P a, *et al.* Cardiorespiratory fitness, adiposity, and all-cause mortality in women. *Med Sci Sports Exerc* 2010;**42**:2006–12.

24 Lin DY, Wei LJ, Ying Z. Checking the Cox Model with Cumulative Sums of Martingale-Based Residuals. *Biometrika* 1993;**80**:557.

25 U.S. Department of Health and Human Services. Physical Activity Guidelines for Americans. 2008. http://www.health.gov/paguidelines/pdf/paguide.pdf

26 Healy GN, Dunstan DW, Salmon J, *et al.* Breaks in Sedentary Time: Beneficial associations with metabolic risk. *Diabetes Care* 2008;**31**. doi:10.2337/dc07-2046.Abbreviations

27 Dunstan DW, Kingwell BA, Larsen R, *et al.* Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 2012;**35**:976–83.

28 Matthews CE, George SM, Moore SC, *et al.* Amount of time spent in sedentary behaviors and cause-specific mortality in US adults. *Am J Clin Nutr* 2012;**95**:437–45.

29 Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. Third Edit. Philadelphia, PA: : Lippincott Williams & Wilkins 2008.

30 Bey L, Hamilton MT. Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity: a molecular reason to maintain daily low-intensity activity. *J Physiol* 2003;**551**:673–82.

31 Hamilton MT, Hamilton DG, Zderic TW. Role of Low Energy Expenditure and Sitting in Obesity, Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Disease. *Diabetes* 2007;**56**:2655–67.

Table 1. Baseline characteristics of participants by vital status, the Cooper Center Longitudinal Study.

	All	Survivors	Decedents	P-value[a]
N	3141	2560	581	
Men, n (%)	2716 (86.5)	2206 (86.2)	510 (87.8)	0.306
Women, n (%)	425 (13.5)	354 (13.8)	71 (12.2)	
Follow up (years)	27.3 (4.4)	28.9 (0.7)	20.4 (6.4)	<0.001
Age (years)	45.0 (9.6)	43.1 (8.6)	53.1 (9.7)	<0.001
Resting systolic blood pressure (mmHg)	116.9 (12.7)	115.8 (12.1)	121.7 (14.1)	<0.001
Resting diastolic blood pressure (mmHg)	78.9 (8.7)	78.3 (8.4)	81.4 (9.5)	<0.001
Total cholesterol (mg/dL)	204.8 (34.4)	203.4 (34.1)	211.1 (35.2)	<0.001
LDL-C (mg/dL)	134.3 (31.3)	133.3 (31.1)	138.8 (31.8)	<0.001
HDL-C (mg/dL)	48.0 (11.8)	48.0 (11.6)	47.8 (12.4)	0.683
Triglycerides (mg/dL)	112.8 (61.6)	110.6 (61.0)	122.6 (63.7)	<0.001
Glucose (mg/dL)	96.2 (13.3)	95.3 (11.7)	100.3 (18.5)	<0.001
Body Mass Index (kg/cm^2)	24.6 (3.0)	24.5 (3.0)	25.2 (3.3)	<0.001
Physical Activity Guidelines[b]				
Not meeting Guidelines	1798 (57.2)	1460 (57.0)	338 (58.2)	0.609
Meeting Guidelines	571 (18.2)	467 (18.2)	104 (17.9)	
Exceeding Guidelines	772 (24.6)	633 (24.7)	139 (23.9)	
Cardiorespiratory Fitness[c], n (%)				
Low	1105 (35.2)	843 (32.9)	262 (45.1)	<0.001
Middle	1025 (32.6)	854 (33.4)	171 (29.4)	
High	1011 (32.2)	863 (33.7)	148 (25.5)	
Quartiles of Sedentary time[d], n (%)				
Q1	895 (28.5)	749 (29.3)	146 (25.1)	
Q2	687 (21.9)	571 (22.3)	116 (20.0)	
Q3	845 (26.9)	673 (26.3)	172 (29.6)	
Q4	714 (22.7)	567 (22.2)	147 (25.3)	0.008

Alcoholic drinks per week[e] (median, 25th, 75th percentile)	5 (1, 10)	4 (1, 10)	5 (1, 11)	0.371
Current Smoker, n (%)	452 (14.4)	350 (13.7)	102 (17.6)	0.003
Personal history of hypertension, n (%)	520 (16.6)	368 (14.4)	152 (26.2)	<0.001
Personal history of diabetes, n (%)	60 (1.9)	39 (1.5)	21 (3.6)	<0.001
Family History of CVD n (%)	451 (14.4)	384 (15.0)	67 (11.5)	0.031

Values are Mean (SD) unless otherwise indicated

Abbreviations: HDL-C : high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MET: metabolic equivalent; CRF: cardiorespiratory fitness Q: quartile; hrs/wk: hours per week; CVD: cardiovascular disease; SD: standard deviation.

[a] Wald trend test p-values for continuous variables; Jonckeheere-Terpstra trend test p-values for categorical variables

[b] Physical activity was based on self-reported type, time, and intensity of activity which were converted into MET minutes per week. METs were then categorized into: (1) not meeting physical activity guidelines (<500 MET minutes per week); meeting physical activity guidelines (500-1000 MET minutes per week); and (3) exceeding physical activity guidelines (>1000 MET minutes per week).

[c] Cardiorespiratory fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample.

[d] Sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk), Q3 (16-22 hrs/wk), and Q4 (>23 hrs/wk).

[e] A total of 29 participants had missing values for alcohol intake and thus a 'missing' category was utilized in multivariable analysis.

Table 2. Association between sedentary time [a], cardiorespiratory fitness [b] and all-cause mortality: multivariable models[c]

All-cause Mortality		n	Cases	Model 1 HR (95% CI) [d]	Model 2 HR (95% CI)[e]	Model 3 HR (95% CI)[f]	Model 4 HR (95% CI)[g]
Sedentary Time							
	Q1	895	146	1.0	1.0	1.0	1.0
	Q2	687	116	0.99 (0.78-1.26)	0.95 (0.74-1.22)	0.92 (0.72-1.18)	0.92 (0.72-1.18)
	Q3	845	172	1.25 (1.00-1.56)	1.20 (0.96-1.50)	1.15 (0.92-1.43)	1.12 (0.89-1.40)
	Q4	714	147	1.34 (1.06-1.68)	1.29 (1.03-1.63)	1.22 (0.97-1.54)	1.20 (0.95-1.51)
	Linear trend, p-value			0.003	0.007	0.03	0.05
Cardiorespiratory Fitness	Low	1105	262	1.0	1.0	1.0	1.0
	Middle	1025	171	0.68 (0.56-0.82)	0.72 (0.59-0.88)	0.73 (0.60-0.89)	0.80 (0.65-0.99)
	High	1011	148	0.60 (0.49-0.73)	0.66 (0.54-0.81)	0.67 (0.55-0.83)	0.76 (0.59-0.97)
	Linear trend, p-value			<0.0001	<0.0001	0.0002	0.03

Abbreviations: Hazards Ratio; CI- confidence interval; PY, person years; Q: quartile; CVD: cardiovascular disease;

[a] Sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk), Q3 (16-22 hrs/wk), and Q4 (>23 hrs/wk).

[b] Cardiorespiratory Fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample.

[c] Cox proportional hazard models were utilized to estimate the hazard ratio (HR) and 95% confidence intervals (CI).

[d] Adjusted for age and gender.

[e] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, and family history of CVD.

[f] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, and cardiorespiratory fitness or sedentary time.

[g] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, cardiorespiratory fitness or sedentary time, physical activity, BMI, total cholesterol, systolic blood pressure, and glucose.

Table 3. Joint effects of sedentary time and cardiorespiratory fitness on all-cause mortality

		Cardiorespiratory Fitness[a]			
		Low		Middle/High	
		Cases	HR[c] (95%CI)	Cases	HR[c] (95%CI)
Sedentary Time[b]	Q1	48	0.71 (0.50-1.02)	98	0.60 (0.44-0.82)
	Q2	47	0.63 (0.44-0.90)	69	0.58 (0.41-0.81)
	Q3	80	0.80 (0.59-1.09)	92	0.68 (0.49-0.93)
	Q4	87	1.0	60	0.60 (0.43-0.86)

[c] Cox proportional hazard regression was utilized to estimate the hazard ratio (HR) and 95% confidence intervals. The model was adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, BMI, total cholesterol, systolic blood pressure, and glucose.

Abbreviations: HR: hazards ratio; CI: confidence interval; CVD: cardiovascular disease; Q: quartile.

[a] Cardiorespiratory fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample. Cardiorespiratory fitness was then dichotomized into low and middle/high for the joint effects analysis.

[b] Sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk), Q3 (16-22 hrs/wk), and Q4 (>23 hrs/wk).

[c] Cox proportional hazard regression was utilized to estimate the hazard ratio (HR) and 95% confidence intervals. The model was adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, physical activity, BMI, total cholesterol, systolic blood pressure, and glucose.

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Independent and Joint Effects of Sedentary Time and Cardiorespiratory Fitness on All-Cause Mortality: The Cooper Center Longitudinal Study

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Abstract

Objectives: To examine the independent and joint effects of sedentary time and cardiorespiratory fitness (fitness) on all-cause mortality.

Design, Setting, Participants: A prospective study of 3,141 Cooper Center Longitudinal Study participants. Participants provided information on TV viewing and car time in 1982 and completed a maximal exercise test during a 1-year time frame; they were then followed until mortality or through 2010. TV viewing, car time, total sedentary time, and fitness were the primary exposures and all-cause mortality was the outcome. The relationship between the exposures and outcome was examined utilizing Cox proportional hazard models.

Results: A total of 581 deaths occurred over a median follow-up period of 28.7 years (SD=4.4). At baseline participants' mean age was 45.0 years (SD=9.6), 86.5% were men, and their mean BMI was 24.6 (SD=3.0). Multivariable analyses revealed a significant linear relationship between increased fitness and lower mortality risk, even while adjusting for total sedentary time and covariates (p=0.02). The effects of total sedentary time on increased mortality risk did not quite reach statistical significance once fitness and covariates were adjusted for (p=0.05). When examining this relationship categorically, in comparison to the reference category (≤10 hours/week), being sedentary for ≥23 hours weekly increased mortality risk by 29% without controlling for fitness (HR=1.29, 95%CI= 1.03-1.63); however, once fitness and covariates were taken into account this relationship did not reach statistical significance (HR=1.20, 95%CI= 0.95-1.51). Moreover, spending >10 hours in the car weekly significantly increased mortality risk by 27% in the fully adjusted model. The association between TV viewing and mortality was not significant.

Conclusions: The relationship between total sedentary time and higher mortality risk is less pronounced when fitness is taken into account. Increased car time, but not TV viewing, is significantly related to higher mortality risk, even when taking fitness into account, in this cohort.

Strengths and limitations of this study

- The first study, to our knowledge, to examine the effects of sedentary behavior on mortality, while taking cardiorespiratory fitness into account.
- Cardiorespiratory fitness was assessed objectively via maximal exercise testing; however, sedentary behavior was based on self-report.
- While the study sample consists of participants with extensive clinical and behavioral information with a long duration of follow-up, the sample was drawn from a single preventive medicine clinic.

BACKGROUND

Sedentary behavior and health has emerged as a new area of scientific investigation, based on accumulating studies linking prolonged sitting to morbidity and mortality. [1] In the US, adults spend close to 8 hours daily in sedentary behaviors, defined as low energy expenditure activities (1.0-1.5 Metabolic Equivalents) in a sitting or reclining posture.[2,3] These prolonged hours of sedentary time have been found to be related to cardiometabolic risk (primarily in cross-sectional studies),[4–7] and premature death from all-causes and from cardiovascular diseases in prospective studies.[8–10] For example, a review by Ford and Caspersen (2012) observed a 17% increased risk for cardiovascular events (fatal and non-fatal) per 2 hour/day increments of television (TV) viewing, and 5% more cardiovascular events per 2 hours increases in sitting time.[10] Additionally, recent meta-analyses by Chau et al. (2013) and Biswas et al. (2015) found a 34% and 24% higher risk (respectively) for all-cause mortality for prolonged sedentary time, even after adjusting for physical activity.[9,11]

These studies, however, have predominately taken into account self-reported physical activity (which is prone to recall bias), and have yet to control for cardiorespiratory fitness (fitness). Fitness, an objective and physiological consequence of habitual physical activity (also influenced by genetics) is an indicator of overall cardiovascular health.[12–14] Observational evidence has found that low fitness levels accounted for ~16% of deaths in a large cohort of over 40,000 individuals, [15] yet to date, studies have not accounted for fitness when examining the effects of sedentary behavior on mortality. Hence, we attempt to bridge this gap by examining whether sedentary behavior is associated with increased mortality risk, while considering the potential mitigating effects of fitness. Specifically, we examine the independent and joint effects of sedentary time and fitness on all-cause mortality among participants of the Cooper Center Longitudinal Study (CCLS).

METHODS

Participants and Design

The CCLS, described elsewhere,[16] is an observational study of patients who self-referred or were referred by their employer or physician to the Cooper Clinic (Dallas, Texas) for preventive medical examinations.[17] In general, the CCLS aims to examine the effects of fitness on chronic disease morbidity and mortality.[18] The CCLS receives annual approval from the Cooper Institute Institutional Review Board and the present investigation received approval from the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston. In the current study, we prospectively assessed the effects of sedentary behavior and fitness on all-cause mortality among adults (≥20 years) who: (1) completed a 1982 survey including questions pertaining to sedentary behavior; and (2) came for a preventive medical visit which included a fitness test and a thorough medical history questionnaire at the Cooper Clinic within a 1 year time-frame.[12] Of 3,676 participants meeting these criteria with pertinent data on the study measures, 329 were excluded due to incomplete fitness testing, abnormal exercise ECG, less than one year of follow-up and underweight weight status. Additionally, 206 participants were excluded based on personal history of myocardial infarction, stroke, or cancer. These exclusion criteria resulted in an analytic sample of 2,716 men and 425 women (total n=3,141) with complete data on the primary exposures (sedentary behavior and fitness), and the outcome (all-cause mortality). Due to the small number of women in the sample and the lack of a significant interaction effect between gender and the exposures (p-value>0.10) in relation to mortality, gender was adjusted for in multivariable analyses rather than performing stratified analysis.

Measures

Exposures (Sedentary Behavior and Cardiorespiratory Fitness)

Sedentary behavior was assessed at baseline via reported time spent viewing TV and commuting in a car, as indicated in a 1982 survey.[12,19] Specifically, participants were asked the following two questions pertaining to their sedentary behavior: (1) "How much time do you spend riding in a car each week? __hours per week"; and (2) "How much time do you spend watching TV each week? __hours per week". For analysis, the hours of car driving and TV viewing per week were considered separate exposure variables. The combined amount (hours per week) of sedentary time (i.e. the sum of TV viewing and car commuting time) was regarded as an additional exposure variable. These exposure variables were each categorized into sample-specific quartiles. Quartile cut-points for the combined sedentary time are: 11, 16, 23 hours/ week; the quartile cut-points of TV viewing and car time appear in Table 1. Fitness was assessed via maximal exercise testing on a treadmill adhering to the modified Balke protocol. In this protocol, described elsewhere,[16] the treadmill speed and incline are increased gradually up to 25 minutes or until volitional exhaustion.[20] From the final treadmill speed and grade maximal metabolic equivalents (METs; 1 MET = 3.5 ml O_2 uptake · kg body mass^{-1} · min^{-1}) were determined, which have been highly correlated (r>0.90) with maximal oxygen uptake. [21,22] Fitness METs of the analytic sample were categorized into age (20-39, 40-49, 50-59, and ≥60 years) specific tertiles (low, medium, high) for each gender separately. [23]

Outcome (all-cause mortality)

Participants were followed for mortality from all causes from baseline to either the date of death or through December 31, 2010 in order to determine vital status. The National Death Index (NDI) was the primary source of mortality information.[18] The NDI has been found to have 100% specificity and 96% sensitivity in ascertaining mortality among the general population.[24,25]

Covariates

Covariates include age, gender, current smoking, alcohol intake, personal history of hypertension and diabetes, family history of cardiovascular disease, leisure-time physical activity, body mass index (BMI), blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, and fasting glucose. Participants' age, gender, current smoking status, and alcohol intake (drinks per week) were based on responses to a medical history questionnaire. Alcohol intake was categorized for analyses into: non-drinkers, (2) light drinkers (≤3 drinks a week for women and men), moderate drinkers (>3-7 drinks a week for women or >3- 14 drinks a week for men), and heavy drinkers (>7 drinks per week for women or >14 drinks/week for men).[20,26] Leisure time physical activity was based on survey questions pertaining to the frequency and the amount of time spent in the following activities: running, treadmill, swimming, stationary cycling, bicycling, elliptical, aerobic dance, racket sports, vigorous sports, and other activity.[27] MET values for each activity were based on the physical activity compendium and multiplied by the frequency and intensity of activity performed resulting in MET min/week.[28] The sum of the MET values from all activities was subsequently grouped into the following three categories based on the Health and Human Services Physical Activity Guidelines: (1) not meeting guidelines (<500 MET min/week); (2) meeting guidelines (500-1000 MET min/week); and (3) exceeding guidelines (>1000 MET min/week). [29] In this study, meeting physical activity guidelines was significantly associated with cardiorespiratory fitness levels (Spearman rho=0.46, p-value<0.001). In addition, personal and family history of disease was based on self-report on the medical history questionnaire. [30] BMI and clinical indicators were determined during the clinical examination. Specifically, BMI was computed from height and weight using the standard formula (kg/m^2), resting blood pressure was measured with a calibrated sphygmomanometer, and serum samples were analyzed for glucose and lipids adhering to standard procedures after a 12 hour fast. [18]

Statistical Analysis

Descriptive characteristics were computed for the entire sample and by vital status. The association between sedentary behavior and all-cause mortality was determined using Cox proportional hazard models to estimate the hazard ratio (HR) and 95% confidence intervals (CI). These models passed the proportional hazards assumption test adhering to the methodology suggested by Lin et al. (1993), which is based on cumulative sums of Martingale residuals.[31] A total of four regression models were computed adjusting for the following covariates: Model 1- age and gender; Model 2- age, gender, current smoking (dichotomous), alcohol intake (categorical), personal history of hypertension (dichotomous), personal history of diabetes (dichotomous), and family history of cardiovascular disease (dichotomous); Model 3- variables in model 2 along with fitness or total sedentary time (both categorical); and Model 4- variables in model 3 as well as body mass index (BMI), glucose, systolic blood pressure, total cholesterol (all continuous), and self-reported physical activity (categorical).[18,29] We then reanalyzed models one through four, replacing total sedentary time with either car time (categorical) or TV viewing (categorical) as the exposure of interest. Furthermore, we examined the joint effects of total sedentary time, car time, and TV viewing coupled with fitness on mortality risk, while adjusting for the other covariates in model 4. For the joint effects models, we collapsed fitness into two categories: low fitness and middle/high fitness. Multiplicative interactions were assessed by including their cross-product in the statistical model. For all analyses, p-values were two sided with an alpha of <0.05 considered statistically significant; SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) was utilized in analyses.

RESULTS

A total of 581 deaths occurred over a median follow-up period of 28.7 years (SD=4.4). At baseline, participants' mean age was 45.0 years (SD=9.6), 14.4% were current smokers, and participants consumed a median of 5 alcoholic beverages per week. In addition, participants were of normal weight (mean BMI=24.6, SD=3.0), had an average fitness level of 12.1 METs (SD=2.4), and spent 17.0 hours/week (SD=10.1) in total sedentary time (i.e. time spent in the car and watching TV). Participants' baseline characteristics are described by vital status in Table 1.

The association between sedentary behaviors and all-cause mortality is depicted in Table 2. Specifically, a significant linear relationship was found between higher total sedentary time and increased mortality risk in three of the four multivariable models (linear trend $p<0.05$ for models 1-3), with the fully adjusted model (including fitness, physical activity, and clinical variables) not quite reaching statistical significance (linear trend $p=0.05$). When examining this relationship categorically, being sedentary for ≥23 hours weekly was significantly related to a 34% increase in mortality risk (HR=1.34, 95%CI= 1.06-1.68) without adjusting for fitness, in comparison to the reference group (≤10 hours of sedentary time weekly). However, once fitness was included in the model (model 3) the 22% higher mortality risk did not reach statistical significance (HR=1.22, 95%CI= 0.97-1.54). Moreover, in the fully adjusted model (model 4 which additionally controlled for fitness, physical activity, BMI, cholesterol, blood pressure and glucose) the 20% higher mortality risk similarly did not reach statistical significance (HR=1.20, 95%CI= 0.95-1.51). When examining the relationship between each sedentary behavior (car time or TV viewing) and mortality, the associations differed markedly (Table 2). Specifically, more time spent in a car per week was significantly associated with a higher risk for all-cause mortality in all multivariable models (linear trend $p<0.05$ in all models). While the addition of fitness into the models reduced the risk for mortality, the associations still remained significant in the models. Thus, spending more than 10 hours in the car per week increased the risk for all-cause mortality by 27% in the fully adjusted model (HR=1.27, 95%CI=1.01-1.59). In comparison, the association between TV viewing and mortality was not significant in all the models (Table 2). In addition, Table 2 also presents the relationship between fitness and mortality while taking into account confounders. All multivariable models exhibited significant dose-response effects for increased fitness and reduced mortality risk, including models adjusting for sedentary behavior (linear trend $p<0.05$ for all). For example, in the fully adjusted model (model 4), while middle levels of fitness were associated with a 20% reduced mortality risk, high fitness levels were related to a 24% lower mortality risk in comparison to the reference group of low fitness (middle fitness: HR=0.80, 95%CI= 0.65-0.99; high fitness: HR=0.76, 95%CI=0.59-0.97).

When examining the joint effects of fitness and combined sedentary behavior on mortality, we found that in comparison to the 'high risk' reference group (low fitness/4th quartile of combined sedentary time) participants who were in the middle/high fitness category were at reduced mortality risk irrespective of sedentary time (Table 3). For example, participants who were in the middle/high fitness strata and in the 4th quartile of sedentary time, had a 40% decreased risk for mortality (HR=0.60, 95%CI=0.43-0.86); whereas those in the middle/high fitness category and the lowest quartile of sedentary time were similarly at 40%

reduced mortality risk (HR=0.60, 95%CI=0.44-0.82). In comparison, participants in the low fitness strata with lower levels of total sedentary time had a reduced risk for mortality; however, the association was statistically significant only among those classified in the second quartile of sedentary time (second quartile: HR=0.63, 95%CI=0.44-0.90). A similar pattern was observed when examining the joint effects of car time and fitness on mortality. Specifically, participants in the low fitness strata who had lower levels of car time had reduced mortality risk (first quartile: HR=0.63, 95%CI= 0.46- 0.88; second quartile: HR=0.61, 95%CI=0.44-0.85); whereas those in the middle/high fitness group were at a similarly lower risk for mortality both in the lowest and highest levels of car time (first quartile: HR=0.58, 95%CI=0.42, 0.80; fourth quartile: HR=0.60, 95%CI=0.42, 0.86). With regard to the joint effects of TV viewing and fitness on mortality risk, few statistically significant findings were observed (see Table 3).

DISCUSSION

The present study aimed to determine whether sedentary behavior is associated with increased mortality risk irrespective of and alongside fitness among a cohort of adults. Study findings reveal a significant relationship between prolonged sedentary time and increased mortality risk in models not controlling for fitness. However, once fitness was taken into account the sedentary behavior-mortality relationship was less pronounced. Specifically, being sedentary for 23 or more hours weekly significantly increased mortality risk by 29%, while accounting for confounders with the exception of fitness. Once fitness was added into the model, then increased mortality risk from prolonged sedentary time was 22%. This 7% reduction in mortality risk likely stems from the protective health effects of fitness.[14] Notably, when accounting for additional clinical variables (e.g. BMI, blood pressure) that could be on the causal pathway between sedentary time and mortality,[32] then mortality risk was reduced by an additional 2%. This finding is understandable since studies have found that lower levels of sedentary behavior have been linked to lower obesity and cardiometabolic risk which, in turn, could potentially lower morality risk.[8,33,34] Thus including these intermediate variables into the model is likely to confound the relationship between the exposure and outcome.[11,32,35]

In addition to examining the effects of total sedentary time on mortality, we also examined the relationship between time spent in a car and TV viewing in relation to mortality risk. Study results reveal the more time spent in a car significantly increased mortality risk even while taking fitness into account. This finding is consistent with a previous CCLS study observing that longer commute distances are associated with elevated blood pressure even while considering the protective effects on both physical activity and fitness. [17] Our null findings pertaining to TV viewing and mortality could potentially stem from: (1) the fact that while car time exclusively involves sitting, individuals watching TV could be multi-tasking (e.g. moving about while watching TV viewing); or (2) the amount of time participants spent watching TV among this cohort is markedly lower than present day TV viewing habits. The later explanation might be more likely since most of the literature has observed higher mortality risk for those watching excessive amounts of TV. [36]

We additionally examined the relationship between fitness and mortality, finding that higher fitness levels reduced mortality risk irrespective of controlling for sedentary behavior and the intermediate variables. This is indicative of the robust and causal relationship between fitness and mortality.[14] Current findings pertaining to the protective effects of fitness (e.g. 24% mortality reduction in the high fit strata fully adjusted model) are consistent with a large body of the literature that emphasizes the importance of achieving higher fitness levels to obtain health benefits.[14] Previous research has found 10-25% increased survival with a 1-MET increase in fitness.[14] This represents a relatively small incremental change that is achievable for most individuals through increasing physical activity with the goal of reaching/ exceeding physical activity guidelines; i.e. 150 minutes of moderate or 75 minutes of vigorous intensity physical activity per week (or a combination of both). [14,29] Thus, while decades of research emphasize the health benefits of increasing fitness levels, particularly for individuals with low levels of fitness, [14] the evidence pertaining to sedentary behavior and health outcomes (independent of physical activity) is accumulating but not as well established.

The most recent systematic review/meta-analysis on the topic conducted by Biswas et al. (2015), found a 24% increased all-cause mortality risk for prolonged sedentary behavior, when adjusting for physical activity; however, virtually all studies adjusted for self-reported physical activity, and none considered the protective impact of fitness. They additionally observed that high sedentary time coupled with low levels of physical activity resulted in an even higher risk (46%) for all-cause mortality.[11] An earlier study by Warren et al. (2010) found that prolonged sedentary behavior increased cardiovascular disease mortality risk in a larger sample of men from the Cooper Clinic;[19] however, they relied on self-reported physical activity, did not take fitness into account, and therefore did not comparatively examine its impact on mortality. Thus, in the current study, we demonstrate that sedentary behavior is related to mortality risk, yet fitness 'buffers' some of the adverse health effects of total sedentary behavior. The underlying mechanism as to why increased sedentary time leads to higher mortality risk warrants further investigation. The hypothesized biological mechanism of the unique impact of sedentary time, described elsewhere,[37] includes the suppression of lipoprotein lipase activity, which results in the reduction of HDL cholesterol and increased insulin resistance.[12,37,38] In a previous study of the CCLS cohort, we observed that sedentary time was cross-sectionally related to a proxy of insulin resistance even after adjusting for fitness. [12]

Current study findings should be tempered by the study's limitations. We examined a sample of adults who attended one preventive medicine clinic with objectively measure fitness and a multitude of information on patients' health.Thus, examination of the study question among a more representative sample is warranted to generalize findings. Further, while fitness was measured via maximal exercise testing, sedentary time was based on self-reported data on TV viewing and time spent in a car at baseline, which are proxies of sedentary behavior and do not include all domains of sitting (e.g. occupational sitting). Furthermore, participants' TV viewing habits and the car time measured at baseline (1982) are likely lower than present day sedentary behaviors.[2] In addition, sedentary behaviors and physical activity were based on self-report, which might be subject to under or over reporting. Moreover, the exposure measures (i.e. sedentary behavior, physical activity, and fitness), assessed at baselined, might have changed during the follow up period. [18] Finally, dietary information was not available in the dataset and therefore was not adjusted for in the multivariable analysis. [12]

In summary, this is the first study to account for fitness when examining the sedentary behavior-mortality relationship. Findings reveal that increased total sedentary time is related to higher mortality risk from all-causes when fitness is not accounted for; however, once controlling for fitness the sedentary behavior-mortality relationship is reduced. Fitness may also buffer some of the negative effects of time spent in a car, however, the inverse relationship between car time and mortality risk remained significant despite the inclusion of fitness into the models. Thus higher levels of fitness appear to have some protective effects from prolonged sedentary time by lowering mortality risk. In addition, higher levels of fitness are protective against mortality risk irrespective of sedentary time. Therefore, increasing fitness levels through meeting or exceeding physical activity guidelines is of paramount public health importance. Nonetheless, additional research is needed to explore the relationship between sedentary behavior and morbidity and mortality while taking the protective effects of fitness into account.

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References

1 Owen N, Sparling PB, Healy GN, *et al.* Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin Proc* 2010;**85**:1138–41.

2 Matthews CE, Chen KY, Freedson PS, *et al.* Amount of time spent in sedentary behaviors in the United States, 2003-2004. *Am J Epidemiol* 2008;**167**:875–81.

3 Sedentary research network. Standardized use of the terms ‘ sedentary ’ and ‘ sedentary behaviours ’. *Appl Physiol Nutr Metlab* 2012;**37**:540–2.

4 Shuval K, Gabriel KP, Leonard T. TV viewing and BMI by race/ethnicity and socio-economic status. *PLoS One* 2013;**8**:e63579.

5 Healy GN, Matthews CE, Dunstan DW, *et al.* Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur Heart J* 2011;**32**:590–7.

6 Gardiner PA, Healy GN, Eakin EG, *et al.* Associations between television viewing time and overall sitting time with the metabolic syndrome in older men and women: the Australian Diabetes, Obesity and Lifestyle study. *J Am Geriatr Soc* 2011;**59**:788–96.

7 Thorp AA, Healy GN, Owen N, *et al.* Deleterious Associations of Sitting Time and Television Viewing Time with Cardiometabolic Risk Biomarkers. *Diabetes Care* 2010;**33**:327–34.

8 Veerman JL, Healy GN, Cobiac LJ, *et al.* Television viewing time and reduced life expectancy: a life table analysis. *Br J Sports Med* 2012;**46**:927–30.

9 Chau JY, Grunseit AC, Chey T, *et al.* Daily sitting time and all-cause mortality: a meta-analysis. *PLoS One* 2013;**8**:e80000.

10 Ford ES, Caspersen CJ. Sedentary behaviour and cardiovascular disease: a review of prospective studies. *Int J Epidemiol* 2012;**41**:1338–53.

11 Biswas A, Oh PI, Faulkner GE, *et al.* Sedentary Time and Its Association With Risk for Disease Incidence, Mortality, and Hospitalization in Adults. *Ann Intern Med* 2015;**162**:123.

12 Shuval K, Finley CE, Barlow CE, *et al.* Sedentary Behavior, Cardiorespiratory Fitness, Physical Activity, and Cardiometabolic Risk in Men: The Cooper Center Longitudinal Study. *Mayo Clin Proc* 2014;**88**:1052–62.

13 Sui X, LaMonte MJ, Laditka JN, *et al.* Cardiorespiratory fitness and adiposity as mortality predictors in older adults. *J Am Med Assoc* 2007;**298**:2507–16.

14 Kaminsky L a, Arena R, Beckie TM, *et al.* The importance of cardiorespiratory fitness in the United States: the need for a national registry: a policy statement from the American Heart Association. *Circulation* 2013;**127**:652–62.

15 Blair SN. Physical inactivity: the biggest public health problem of the 21st century. *Br J Sports Med* 2009;**43**:1–2.

16 Willis BL, Gao A, Leonard D, *et al.* Midlife fitness and the development of chronic conditions in later life. *Arch Intern Med* 2012;**172**:1333–40.

17 Hoehner CM, Barlow CE, Allen P, *et al.* Commuting distance, cardiorespiratory fitness, and metabolic risk. *Am J Prev Med* 2012;**42**:571–8.

18 Shuval K, Barlow CE, Chartier KG, *et al.* Cardiorespiratory fitness, alcohol, and mortality in men: the Cooper Center longitudinal study. *Am J Prev Med* 2012;**42**:460–7.

19 Warren TY, Barry V, Hooker SP, *et al.* Sedentary behaviors increase risk of cardiovascular disease mortality in men. *Med Sci Sports Exerc* 2010;**42**:879–85.

20 Shuval K, Finley CE, Chartier KG, *et al.* Cardiorespiratory Fitness, Alcohol Intake, and Metabolic Syndrome Incidence in Men. *Med Sci Sport Exerc* 2013;**44**:2125–31.

21 Pollock ML, Bohannon RL, Cooper KH, *et al.* A comparative analysis of four protocols for maximal treadmill stress testing. *Am Heart J* 1976;**92**:39–46.

22 Pollock M, C F, Schmidt D, *et al.* Comparative analysis of physiologic responses to three different maximal graded exercise test protocols in healthy women. *Am Heart J* 1982;**103**:363–73.

23 Kendzor DE, Finley CE, Barlow CE, *et al.* The Association of Fitness With Reduced Cardiometabolic Risk Among Smokers. *Am J Prev Med* 2015;**48**:561–9. doi:10.1016/j.amepre.2014.12.004

24 Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B HC. Test of the National Death Index. *Am J Epidemiol* 1984;**119**:837–9.

25 Farrell SW, Fitzgerald SJ, McAuley P a, *et al.* Cardiorespiratory fitness, adiposity, and all-cause mortality in women. *Med Sci Sports Exerc* 2010;**42**:2006–12.

26 Mukamal KJ, Chen CM, Rao SR, *et al.* Alcohol Consumption and Cardiovascular Mortality Among U.S. Adults, 1987 to 2002. *J Am Coll Cardiol* 2010;**55**:1328–35.

27 Barlow CE, Shuval K, Balasubramanian B, *et al.* Sitting Time, Physical Activity and Cardiorespiratory Fitness- CCLS Cohort. *J Phys Act Health*

28 Ainsworth BE, Haskell WL, Herrmann SD, *et al.* 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011;**43**:1575–81.

29 U.S. Department of Health and Human Services. Physical Activity Guidelines for Americans. 2008. http://www.health.gov/paguidelines/pdf/paguide.pdf

30 Shuval K, Chiu C-Y, Barlow CE, *et al.* Family history of chronic disease and meeting public health guidelines for physical activity: the cooper center longitudinal study. *Mayo Clin Proc* 2013;**88**:588–92.

31 Lin DY, Wei LJ, Ying Z. Checking the Cox Model with Cumulative Sums of Martingale-Based Residuals. *Biometrika* 1993;**80**:557.

32 Matthews CE, George SM, Moore SC, *et al.* Amount of time spent in sedentary behaviors and cause-specific mortality in US adults. *Am J Clin Nutr* 2012;**95**:437–45.

33 Healy GN, Dunstan DW, Salmon J, *et al.* Breaks in Sedentary Time: Beneficial associations with metabolic risk. *Diabetes Care* 2008;**31**. doi:10.2337/dc07-2046.Abbreviations

34 Dunstan DW, Kingwell BA, Larsen R, *et al.* Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 2012;**35**:976–83.

35 Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. Third Edit. Philadelphia, PA: : Lippincott Williams & Wilkins 2008.

36 Grøntved A, Hu FB. Television Viewing and Risk of Type 2 Diabetes, Cardiovascular Disease, and All-Cause Mortality A Meta-analysis. *J Am Med Assoc* 2011;**305**:2448–55.

37 Bey L, Hamilton MT. Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity: a molecular reason to maintain daily low-intensity activity. *J Physiol* 2003;**551**:673–82.

38 Hamilton MT, Hamilton DG, Zderic TW. Role of Low Energy Expenditure and Sitting in Obesity, Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Disease. *Diabetes* 2007;**56**:2655–67.

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411 **Table 1.** Baseline characteristics of participants by vital status, the Cooper Center Longitudinal Study.

	All	Survivors	Decedents	*p*-value[a]
N	3141	2560	581	
Men, n (%)	2716 (86.5)	2206 (86.2)	510 (87.8)	0.306
Women, n (%)	425 (13.5)	354 (13.8)	71 (12.2)	
Follow up (years)	27.3 (4.4)	28.9 (0.7)	20.4 (6.4)	<0.001
Age (years)	45.0 (9.6)	43.1 (8.6)	53.1 (9.7)	<0.001
Resting systolic blood pressure (mmHg)	116.9 (12.7)	115.8 (12.1)	121.7 (14.1)	<0.001
Resting diastolic blood pressure (mmHg)	78.9 (8.7)	78.3 (8.4)	81.4 (9.5)	<0.001
Total cholesterol (mg/dL)	204.8 (34.4)	203.4 (34.1)	211.1 (35.2)	<0.001
LDL-C (mg/dL)	134.3 (31.3)	133.3 (31.1)	138.8 (31.8)	<0.001
HDL-C (mg/dL)	48.0 (11.8)	48.0 (11.6)	47.8 (12.4)	0.683
Triglycerides (mg/dL)	112.8 (61.6)	110.6 (61.0)	122.6 (63.7)	<0.001
Glucose (mg/dL)	96.2 (13.3)	95.3 (11.7)	100.3 (18.5)	<0.001
Body Mass Index (kg/cm^2)	24.6 (3.0)	24.5 (3.0)	25.2 (3.3)	<0.001
Physical Activity Guidelines[b]				
Not Meeting Guidelines	1798 (57.2)	1460 (57.0)	338 (58.2)	
Meeting Guidelines	571 (18.2)	467 (18.2)	104 (17.9)	0.609
Exceeding Guidelines	772 (24.6)	633 (24.7)	139 (23.9)	
Cardiorespiratory Fitness[c], n (%)				
Low	1105 (35.2)	843 (32.9)	262 (45.1)	<0.001
Middle	1025 (32.6)	854 (33.4)	171 (29.4)	
High	1011 (32.2)	863 (33.7)	148 (25.5)	
Car Time[d], n (%)				

Q1	925 (29.4)	746 (29.1)	179 (30.8)	
Q2	848 (27.0)	695 (27.1)	153 (26.3)	0.8904
Q3	637 (20.3)	534 (20.9)	103 (17.4)	
Q4	731 (23.3)	585 (22.9)	146 (25.1)	
TV Viewing[d], n (%)				
Q1	793 (25.2)	668 (26.1)	125 (21.5)	
Q2	837 (26.6)	697 (27.2)	140 (24.1)	0.0034
Q3	812 (25.9)	636 (24.8)	176 (30.3)	
Q4	699 (22.3)	559 (21.8)	140 (24.1)	
Total Sedentary Time[d], n (%)				
Q1	895 (28.5)	749 (29.3)	146 (25.1)	0.0081
Q2	687 (21.9)	571 (22.3)	116 (20.0)	
Q3	845 (26.9)	673 (26.3)	172 (29.6)	
Q4	714 (22.7)	567 (22.2)	147 (25.3)	
Alcohol Intake [e]				
Non Drinkers	722 (23.0)	588 (23.0)	134 (23.1)	0.3064
Light drinkers	426 (13.6)	360 (14.1)	66 (11.4)	
Moderate drinkers	1424 (45.3)	1162 (45.4)	262 (45.1)	
Heavy drinkers	540 (17.2)	429 (16.8)	111 (19.1)	
Current Smoker, n (%)	452 (14.4)	350 (13.7)	102 (17.6)	0.003
Personal history of hypertension, n (%)	520 (16.6)	368 (14.4)	152 (26.2)	<0.001
Personal history of diabetes, n (%)	60 (1.9)	39 (1.5)	21 (3.6)	<0.001
Family History of CVD n (%)	451 (14.4)	384 (15.0)	67 (11.5)	0.031

Values are Mean (SD) unless otherwise indicated

Abbreviations: HDL-C : high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MET: metabolic equivalent, Q: quartile; hrs/wk: hours per week; CVD: cardiovascular disease; SD: standard deviation.

[a] Wald trend test p-values for continuous variables; Jonckeheere-Terpstra trend test p-values for categorical variables

[b] Physical activity was based on self-reported type, time, and intensity of activity which were converted into MET minutes per week. METs were then categorized into: (1) not meeting physical activity guidelines (<500 MET minutes per week); meeting physical activity guidelines (500-1000 MET minutes per week); and (3) exceeding physical activity guidelines (>1000 MET minutes per week).

420 [c] Cardiorespiratory fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample.

421 [d] Total sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk),
422 Q3 (16-22 hrs/wk), and Q4 (≥23 hrs/wk). Quartiles of car time: (Q): Q1 (0-4 hrs/wk), Q2 (5-7 hrs/wk), Q3 (8-10 hrs/wk), and Q4 (≥11 hrs/wk). Quartiles of TV
423 viewing: (Q): Q1 (0-3 hrs/wk), Q2 (4-7 hrs/wk), Q3 (8-12 hrs/wk), and Q4 (≥13 hrs/wk).

424 [e] Non Drinker : 0 drinks per week; Light drinker ≤3 drinks per week; Moderate drinker: >3-7 drinks a week for women and >3-14 drinks per week for men; Heavy
425 drinker >7 drinks per week for women and >14 drinks/week for men. A total of 29 participants had missing values for alcohol intake and thus a 'missing' category
426 was utilized in multivariable analysis.

427

428 **Table 2.** Association between sedentary time [a], cardiorespiratory fitness [b] and all-cause mortality:
429 multivariable models[c]

All-cause Mortality		n	Cases	Model 1 HR (95% CI) [d]	Model 2 HR (95% CI)[e]	Model 3 HR (95% CI)[f]	Model 4 HR (95% CI)[g]
Car Time[a]							
	Q1	925	179	1.0	1.0	1.0	1.0
	Q2	848	153	0.96 (0.77, 1.19)	0.94 (0.76, 1.17)	0.93 (0.74, 1.16)	0.92 (0.74, 1.15)
	Q3	637	103	1.00 (0.79, 1.28)	1.02 (0.80, 1.30)	1.00 (0.78, 1.28)	0.96 (0.75, 1.23)
	Q4	731	146	1.37 (1.10, 1.71)	1.36 (1.09, 1.70)	1.31 (1.05, 1.64)	1.27 (1.01, 1.59)
	Linear trend, p-value			0.006	0.006	0.016	0.040
TV Viewing[a]							
	Q1	793	125	1.0	1.0	1.0	1.0
	Q2	837	140	1.01 (0.80, 1.29)	0.98 (0.77, 1.25)	0.94 (0.74, 1.20)	0.93 (0.73, 1.19)
	Q3	812	179	1.28 (1.02, 1.61)	1.21 (0.96, 1.53)	1.15 (0.91, 1.45)	1.13 (0.90, 1.43)
	Q4	699	140	1.12 (0.88, 1.43)	1.07 (0.84, 1.37)	1.02 (0.80, 1.30)	0.99 (0.77, 1.27)
	Linear trend, p-value			0.134	0.272	0.538	0.671
Total Sedentary Time[a]							
	Q1	895	146	1.0	1.0	1.0	1.0
	Q2	687	116	0.99 (0.78-1.26)	0.95 (0.75-1.22)	0.92 (0.72-1.18)	0.92 (0.72-1.18)
	Q3	845	172	1.25 (1.00-1.56)	1.20 (0.96-1.50)	1.14 (0.92-1.43)	1.12 (0.89-1.40)
	Q4	714	147	1.34 (1.06-1.68)	1.30 (1.03-1.63)	1.22 (0.97-1.55)	1.20 (0.95-1.51)

	Linear trend, p-value			0.003	0.007	0.028	0.053
Cardiorespiratory Fitness[b]	Low	1105	262	1.0	1.0	1.0	1.0
	Middle	1025	171	0.68 (0.56-0.82)	0.72 (0.59-0.87)	0.73 (0.60-0.88)	0.80 (0.65-0.99)
	High	1011	148	0.60 (0.49-0.73)	0.66 (0.54-0.80)	0.67 (0.55-0.82)	0.76 (0.59-0.97)
	Linear trend, p-value			<0.001	<0.001	0.030	0.030

Abbreviations: Hazards Ratio; CI- confidence interval; PY, person years; Q: quartile; CVD: cardiovascular disease;

[a] Total sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk), Q3 (16-22 hrs/wk), and Q4 (≥23 hrs/wk). Quartiles of car time: (Q): Q1 (0-4 hrs/wk), Q2 (5-7 hrs/wk), Q3 (8-10 hrs/wk), and Q4 (≥11 hrs/wk). Quartiles of TV viewing: (Q): Q1 (0-3 hrs/wk), Q2 (4-7 hrs/wk), Q3 (8-12 hrs/wk), and Q4 (≥13 hrs/wk).

[b] Cardiorespiratory Fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample.

[c] Cox proportional hazard models were utilized to estimate the hazard ratio (HR) and 95% confidence intervals (CI).

[d] Adjusted for age and gender.

[e] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, and family history of CVD.

[f] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, and cardiorespiratory fitness or sedentary time.

[g] Adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, cardiorespiratory fitness or sedentary time, physical activity, BMI, total cholesterol, systolic blood pressure, and glucose.

Table 3. Joint effects of sedentary time and cardiorespiratory fitness on all-cause mortality

		Cardiorespiratory Fitness[a]			
		Low		Middle/High	
		Cases	HR[c] (95%CI)	Cases	HR[c] (95%CI)
Car Time[b]	Q1	67	0.63 (0.46, 0.88)	112	0.58 (0.42, 0.80)
	Q2	62	0.61 (0.44, 0.85)	91	0.52 (0.38, 0.72)
	Q3	49	0.71 (0.49, 1.01)	54	0.51 (0.35, 0.73)
	Q4	84	1.0	62	0.60 (0.42, 0.86)
TV Viewing[b]	Q1	39	0.93 (0.63, 1.38)	86	0.74 (0.53, 1.04)
	Q2	66	0.93 (0.67, 1.30)	74	0.65 (0.46, 0.92)
	Q3	81	0.97 (0.71, 1.34)	95	0.91 (0.66, 1.27)
	Q4	76	1.0	64	0.68 (0.48, 0.97)
Total Sedentary Time[b]	Q1	48	0.71 (0.50-1.02)	98	0.60 (0.44-0.82)
	Q2	47	0.63 (0.44-0.90)	69	0.58 (0.41-0.81)
	Q3	80	0.80 (0.59-1.09)	92	0.68 (0.49-0.93)
	Q4	87	1.0	60	0.60 (0.43-0.86)

Abbreviations: HR: hazards ratio; CI: confidence interval; CVD: cardiovascular disease; Q: quartile.

[a] Cardiorespiratory fitness was categorized into age (20-39, 40-49, 50-59, and ≥60 years) and gender specific tertiles based on the distribution of the sample. Cardiorespiratory fitness was then dichotomized into low and middle/high for the joint effects analysis.

[b] Total sedentary time (i.e. the sum of reported TV viewing and car time) was categorized into sample-specific quartiles (Q): Q1 (0-10 hrs/wk), Q2 (11-15 hrs/wk), Q3 (16-22 hrs/wk), and Q4 (≥23 hrs/wk). Quartiles of car time: (Q): Q1 (0-4 hrs/wk), Q2 (5-7 hrs/wk), Q3 (8-10 hrs/wk), and Q4 (≥11 hrs/wk). Quartiles of TV viewing: (Q): Q1 (0-3 hrs/wk), Q2 (4-7 hrs/wk), Q3 (8-12 hrs/wk), and Q4 (≥13 hrs/wk).

[c] Cox proportional hazard regression was utilized to estimate the hazard ratio (HR) and 95% confidence intervals. The model was adjusted for age, gender, current smoking, alcohol, personal history of hypertension, personal history of diabetes, family history of CVD, physical activity, BMI, total cholesterol, systolic blood pressure, and glucose.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract This is a prospective study- this is stated in the abstract- page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported This is clearly stated in the Introduction (page 3)
Objectives	3	State specific objectives, including any prespecified hypotheses The aim of the study is clearly stated (page 3): "we aim to examine the independent and joint effects of sedentary time and fitness on all-cause mortality among participants of the Cooper Center Longitudinal Study (CCLS)."
Methods		
Study design	4	Present key elements of study design early in the paper This appears in the first sentence of the Methods (line 78- page 4): "an observational study…"
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection: This appears in the Participants and design section of the Methods as well as the Outcome section (pages 4-5). For example- baseline data collection (1982) and follow up until 2010.
Participants	6	(*a*) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up This inclusion criteria in elaborately described (beginning line 84): : (1) completed a 1982 survey including questions pertaining to sedentary behavior; and (2) came for a preventive medical visit which included a fitness test and a thorough medical history questionnaire at the Cooper Clinic within a 1 year time-frame… Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable There are specific sections of exposure and outcome variables where they are clearly defined within the Methods. For example: "Outcome (all-cause mortality): Participants were followed for mortality from all causes from baseline to either the date of death or through December 31, 2010 in order to determine vital status. The National Death Index (NDI) was the primary source of mortality information.[18] The NDI has been found to have 100% specificity and 96% sensitivity in ascertaining mortality among the general population.[23,24]"

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. This is provided as well- such as in the example above where NDI was used to determine mortality.
Bias	9	Describe any efforts to address potential sources of bias All biases are clearly described in the limitation section. In addition, we adjust for a multitude of covariates to control for confounding.
Study size	10	Explain how the study size was arrived at This is clearly explained in the first paragraph of the Methods: we prospectively assessed the effects of sedentary behavior and fitness on all-cause mortality among adults (≥20 years) who: (1) completed a 1982 survey including questions pertaining to sedentary behavior; and (2) came for a preventive medical visit which included a fitness test and a thorough medical history questionnaire at the Cooper Clinic within a 1 year time-frame.[12] Of 3,676 participants meeting these criteria with pertinent data on the study measures, 329 were excluded due to incomplete fitness testing, abnormal exercise ECG, less than one year of follow-up and underweight weight status. Additionally, 206 participants were excluded based on personal history of myocardial infarction, stroke, or cancer. These exclusion criteria resulted in an analytic sample of 2,716 men and 425 women (total n=3,141) with complete data on the primary exposures (sedentary behavior and fitness), and the outcome (mortality).
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why This is depicted in the Stats section. For example: "…Model 3- variables in model 2 along with fitness or sedentary time (both categorically)…"
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding All below is clearly described in the Stats section (see page 5-6)
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

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 analysed This is clearly described in the Methods
		(b) Give reasons for non-participation at each stage This is clearly described in the Methods
		(c) Consider use of a flow diagram No need since we elaborate in the Methods
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders This is provided: e.g., " At baseline, participants' mean age was 45.0 years (SD=9.6), 14.4% were current smokers, and participants consumed a median of 5 alcoholic beverages per week."
		(b) Indicate number of participants with missing data for each variable of interest The analytic cohort was without missing variables (excluding alcohol). A total of 29 participants had missing alcohol intake which is indicated in table 1).
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) This appears in the first sentence of the Results (page 6)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time Yes- number of deaths appear in the table and text
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
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 This is provided (see Table 2 and the Results)
		(*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 See Table 3 for joint effects analysis
Discussion		
Key results	18	Summarise key results with reference to study objectives: The study objective and key findings appear in the first paragraph of the Discussion: The present study aimed to determine whether prolonged sedentary time is associated with increased mortality risk irrespective of and alongside fitness among a cohort of adults. Study findings reveal….
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 This is elaborately discussed (Page 9): "We examined a sample of adults who attended one preventive medicine clinic with objectively measure fitness and a multitude of information on patients' health.Thus, examination of the study question among a more representative sample is warranted to generalize findings. …."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence See limitation section

Generalisability	21	Discuss the generalisability (external validity) of the study results: Mentioned in limitation section: (e.g. Thus, examination of the study question among a more representative sample is warranted to generalize findings.)
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. Sources of funding are provided (NIH)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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.