Erschienen in: Acta Physiologica ; 213 (2015), 1. - S. 156-170
https://dx.doi.org/10.1111/apha.12296

Associations of subjective vitality with DNA damage, cardiovascular risk factors and physical performance

S. Maynard,[1,2] G. Keijzers,[1,2,3] Å.-M. Hansen,[4,5] M. Osler,[3,6] D. Molbo,[1,4] L. Bendix,[3,4] P. Møller,[4] S. Loft,[4] M. Moreno-Villanueva,[7] A. Bürkle,[7] C. P. Hvitby,[3,8] S. H. Schurman,[9] T. Stevnsner,[3,8] L. J. Rasmussen,[1,2] K. Avlund[1,3,4] and V. A. Bohr[1,10]

1 Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark
2 Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark
3 Danish Aging Research Center, University of Southern Denmark, Odense, Denmark
4 Department of Public Health, University of Copenhagen, Copenhagen, Denmark
5 National Research Centre for the Working Environment, Copenhagen, Denmark
6 Research Centre for prevention and Health, Glostrup University Hospital, Glostrup, Denmark
7 Molecular Toxicology Group, University of Konstanz, Konstanz, Germany
8 Department of Molecular Biology and Genetics, University of Aarhus, Aarhus, Denmark
9 Clinical Research Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA
10 Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Correspondence: S. Maynard, Department of Cellular and Molecular Medicine, University of Copenhagen, Panum Institute 18.1.26, Blegdamsvej 3b, 2200 Copenhagen N, Denmark.
E mail: scott@sund.ku.dk

Abstract

Aim: To examine associations of DNA damage, cardiovascular risk factors and physical performance with vitality, in middle-aged men. We also sought to elucidate underlying factors of physical performance by comparing physical performance parameters to DNA damage parameters and cardiovascular risk factors.

Methods: We studied 2487 participants from the Metropolit cohort of 11 532 men born in 1953 in the Copenhagen Metropolitan area. The vitality level was estimated using the SF-36 vitality scale. Cardiovascular risk factors were determined by body mass index (BMI), and haematological biochemistry tests obtained from non-fasting participants. DNA damage parameters were measured in peripheral blood mononuclear cells (PBMCs) from as many participants as possible from a representative subset of 207 participants.

Results: Vitality was inversely associated with spontaneous DNA breaks (measured by comet assay) ($P = 0.046$) and BMI ($P = 0.002$), and positively associated with all of the physical performance parameters (all $P < 0.001$). Also, we found several associations between physical performance parameters and cardiovascular risk factors. In addition, the load of short telomeres was inversely associated with maximum jump force ($P = 0.018$), with lowered significance after exclusion of either arthritis sufferers ($P = 0.035$) or smokers ($P = 0.031$).

Conclusion: Here, we show that self-reported vitality is associated with DNA breaks, BMI and objective (measured) physical performance in a cohort of middle-aged men. Several other associations in this study verify clinical observations in medical practice. In addition, the load of short telomeres may be linked to peak performance in certain musculoskeletal activities.

Keywords body mass index, DNA damage, vitality.

'Lack of energy' (i.e. low vitality) is a common complaint among older adults at physician visits (Bjorner *et al.* 1998, 2007, Avlund 2010, Brown *et al.* 2011). The SF 36 vitality scale is a subscale of the multi component Medical Outcomes Study Short Form 36 (SF 36) self reported fatigue scale that covers physical, social and mental aspects of fatigue. The SF 36 vitality scale was developed as a short questionnaire based subjective measure of vitality, to give a numerical value (out of 100) within a single continuum from energy to fatigue, to assess differences in subjective well being and disease burden, and is the best of the eight SF 36 components at discriminating between levels of health (Bjorner *et al.* 1998). Fatigue or low vitality may be indicative of underlying psychiatric or medical illness and is increasingly seen as an early indicator of frailty, caused by increased vulnerability in multiple biological and physiological systems (Avlund 2010). In fact, the SF 36 vitality level is associated with several clinical conditions, including anaemia, congestive heart failure, chronic obstructive pulmonary disease, chronic fatigue syndrome, mortality risk, and with negative outcomes, including inability to work, job loss and hospitalization (Buchwald *et al.* 1996, Bjorner *et al.* 2007).

There are several studies that link DNA strand breaks and oxidative DNA damage with psychological factors, including stress and depression (Irie *et al.* 2001, 2003, Dimitroglou *et al.* 2003, Gidron *et al.* 2006, Maes 2011). There is also evidence that stress, exhaustion and depression are associated with telomere length, in leucocytes, and glycan profiles in serum (Moreno Villanueva *et al.* 2013), suggesting that life stress may be linked to macromolecular changes, and contribute to acceleration of the rate of biological ageing (Epel *et al.* 2004, Kananen *et al.* 2010, Wolkowitz *et al.* 2011, Ahola *et al.* 2012). However, very little is known about the biological correlates to vitality, and particularly, potential DNA damage correlates. Therefore, a major aim of this study was to examine associations of DNA damage parameters (specifically DNA breaks and telomere length measures) with SF 36 vitality.

Clinical practice indicates that BMI as well as glucose and lipid profiles may impact energy levels. Literature on the impact of cardiovascular risk factors on energy levels, fatigue or quality of life is limited. Studies in this regard have focused on how to increase the patient's quality of life during illness or recovery from treatment, such as surgery or chemotherapy. For example, restoration of carnitine (a fundamental molecule for energy production and indispensable for glucose and lipid turnover) by levocarnitine supplementation was found to be effective in alleviating chemotherapy induced fatigue (Graziano *et al.* 2002). A study on diabetic men found that questionnaire based vigour activity correlated with total cholesterol level and inversely correlated with triglyceride levels (Lasaite *et al.* 2009). Therefore, another major aim in this study was to examine associations of cardiovascular risk factors (blood glucose, blood lipids, BMI) with vitality score.

SF 36 vitality had been shown to be strongly associated with other components of the SF 36 fatigue scale, such as SF 36 physical functioning, mental health, bodily pain scores and decreased sleep quality (all $P < 0.001$) (Bjorner *et al.* 1998, Jhamb *et al.* 2009). In addition, studies have shown that the SF 36 physical functioning is associated with objectively measured physical performance measures (such as grip strength and chair rises) (Syddall *et al.* 2009, Wanderley *et al.* 2011). However, no study has directly compared SF 36 vitality scores with objective measures of physical performance. So another major aim of this study was to examine associations of various common physical performance measures (jump force, handgrip strength, chair rises, lung function) with SF 36 vitality. Such analysis may help determine whether the subjective vitality level is linked to objective physical performance.

To further examine links to physical performance, we also compared physical performance measures to cardiovascular risk factors and to DNA damage parameters. The effect of BMI and glucose/lipid profiles on several physical performance activities is not obvious. Studies indicate an association of more favourable levels of some cardiovascular risk factors with better physical performance, such as habitual activities (Jimenez Pavon *et al.* 2010, Duvivier *et al.* 2013), speed strength and cardiorespiratory fitness (Vainionpaa *et al.* 2007). With respect to DNA damage as a link to physical performance, studies have found an association between DNA oxidative damage (known to induce DNA breaks) and muscle strength (Semba *et al.* 2007, Muzembo *et al.* 2014). In addition, Krauss *et al.* (2011) found that individuals with low exercise capacity (based on METs) had a greater likelihood of having short telomeres in their leucocytes compared to individuals with a greater exercise capacity. There is also evidence that physical training induces global molecular responses (Hagberg *et al.* 2012, Fernandez Gonzalo *et al.* 2013) and specifically exercise induced adaptive responses in telomere length and in the load of short telomeres (Puterman *et al.* 2010, Ludlow *et al.* 2013). Lastly, we compared the cardiovascular risk factors among themselves, as this has not been comprehensively tabulated in the literature, especially for non fasting participants.

To carry out this study, we compiled SF 36 vitality scores, as well as various measures of physical

performance, BMI and blood biochemistry from the Metropolit cohort of men born in 1953 (Osler *et al.* 2006). Using these values and the values from various DNA damage parameters that we have recently mea sured in isolated PBMCs from members of this cohort, we sought to discover physical, physiological and cel lular correlates to subjective vitality. Data from this study may give insight into early indicators of future frailty or fatiguing illnesses and also insight into underlying causes of low vitality.

We measured DNA damage using several tech niques. The comet assay and the fluorometric analysis of DNA unwinding (FADU) assay detect both single strand breaks (SSBs) and double strand breaks (DSBs) and thus are two alternative methods for measuring DNA strand breaks; it is prudent to use both tech niques (see discussion). A modified comet assay (comet FPG sensitive sites), utilizing the FPG (for mamidopyrimidine DNA glycosylase) enzyme that incises DNA at oxidative DNA lesions, facilitates esti mation of oxidative DNA damage. The γH2AX stain ing method detects mainly DSBs. The telomere measurements were designed to record different aspect of telomere shortening: the mean telomere length and the load (relative quantity) of short telomeres. We included the load of short telomeres measure as evi dence suggests that this parameter, not mean telomere length, is indicative of telomere dysfunction and cell viability (Hemann *et al.* 2001, Hao *et al.* 2005, Rajar aman *et al.* 2007, Armanios *et al.* 2009, Vera & Blasco 2012). It has been shown that the load of short telo meres increases with age in peripheral blood lympho cytes (Canela *et al.* 2007) and is associated with cell senescence (Bendix *et al.* 2010), and with depressive episodes in bipolar disorder (Elvsashagen *et al.* 2011).

Materials and methods

Cohort selection and vitality score evaluation

The study population consists of all of the 2487 mem bers of the Metropolit cohort who participated with relevant data in the Copenhagen Aging and Midlife Biobank (CAMB) data collection (Avlund *et al.* 2014). The Metropolit cohort originally consisted of 11 532 men born in 1953 in the Copenhagen Metropolitan area (Osler *et al.* 2006). The surviving 7750 cohort members, currently living in the study area, were invited, and 32 per cent of them chose to come to the test centre and participate in the full study (Avlund *et al.* 2014). This study population of 2487 partici pants, used in this study, was not a completely ran dom selection due to logistics (e.g. some locations were excluded) and typical social status bias; in fact, the CAMB study participants themselves represent a somewhat socially selected group (a larger proportion of CAMB participants tend to be employed compared with non participants) (Avlund *et al.* 2014). Although the participants represented a slightly socially selected group, Avlund *et al.* (2014) found that the number of contacts with general practitioners during the year 2009 was nearly the same for participants and non participants, suggesting that the two groups were likely to be comparable in overall health.

The CAMB data collection took place at the National Research Centre for the Working Environ ment in 2010 and included blood tests, clinical exam inations (height, weight, waist measurements, blood pressure and physical performance tests), cognitive tests and a postal questionnaire on health, health behaviour, depressive mood and social factors. Vitality was measured by the Medical Outcomes Study Short Form 36 vitality scale, which consists of four items scored from 1 to 6. The scores from the four items were summed together and transformed [100 x [mean ((7 score of item 1) + (7 score of item 2) + score of item 3 + score of item 4) 1]/5] to a scale ranging from 0 to 100, where higher value indicated a higher subjective energy level. A total of 2434 out of the 2487 selected participants submitted their SF 36 vital ity questionnaire. The average score was 65.66 with standard deviation of 19.10 and with no ceiling effect. Of the 2487 participants, the *N* for each comparison (except those involving DNA damage) ranged from 1812 to 2441 depending on the number of partici pants that had valid measures for each pair of com parisons (see Tables 1, 3, 4 and 5). A total of 207 participants for the DNA damage testing (DNA strand breaks and telomere length parameters measured in isolated PBMCs) were drawn from the ongoing data collection visits of the Metropolit cohort. Of the 207 participants, 205 submitted vitality scores (average vitality score 63.32; standard deviation 18.50; no ceiling effect). For practical reasons, each DNA dam age tests was performed on a different population size (reasons include: not enough PBMCs available from some participants for all tests, or some tests were too laborious to perform them on all available participant PBMC sample). These values were then compared with values obtained from other parameters that were measured on the same participants, resulting in *N* ranging from 17 to 122 (see Tables 1, and 2). All par ticipants that reported myocardial infarction or angina pectoris, or a stroke, or diabetes, or had taken antidia betic drugs or lipid modifying drugs, in the past 3 weeks, were excluded from this study. The current study was conducted according to the ethical princi ples of the Helsinki II declaration and good publica tion practice as stated in 'Good publication practice in physiology 2013: revised author guidelines for Acta

Table 1 Association of DNA damage parameters, cardiovascular risk factors and physical performance parameters with vitality score

Variable	*N*	Mean (±SD)	*P* value	*R*
DNA breaks by FADU (fluorescence†)	77	33.28 (12.78)	0.624	−0.057
DNA breaks by comet (strand breaks/10^6 bp)	43	0.140 (0.069)	0.046	−0.306*
FPG sensitive sites by comet (FPG sensitive site/10^6 bp)	43	0.269 (0.158)	0.111	0.246
γH2AX sites (number of foci/cell)	20	9.91 (2.41)	0.55	−0.142
Mean telomere length (T/S ratio)	122	0.48 (0.120)	0.243	0.106
Load of short telomeres (number of telomeres below 750 bp)	29	5.79 (3.67)	0.439	−0.149
Glucose (mM)	2073	5.56 (1.51)	0.088	−0.038
LDL (mM)	2058	3.09 (0.79)	0.201	−0.028
HDL (mM)	2058	1.42 (0.37)	0.164	0.031
Triglyceride (mM)	2058	1.90 (1.09)	0.11	−0.035
Cholesterol (mM)	2058	6.35 (1.07)	1	<0.001
BMI (kg/m^2)	2081	26.13 (3.62)	0.002	−0.068**
Max jump force (cm)	1812	21.38 (4.66)	<0.001	0.168**
Max handgrip strength (kg)	2077	49.27 (8.37)	<0.001	0.104**
Number of chair rises/30 s	1827	21.74 (5.53)	<0.001	0.188**
Per cent of predicted FEV1	2072	107.99 (17.41)	<0.001	0.148**

Pearson correlation analysis was performed to generate *P* values and Pearson *r* (*R*) coefficients.
N, population number.
†Fluorescence was determined by calculating 'T minus P' as described in Materials and Methods.
*Correlation is significant at $P < 0.05$, **Correlation is significant at $P < 0.01$.

Physiologica' (Persson 2014). We have recently published a parallel study (Maynard *et al.* 2013), in which the PBMCs from many of these 207 participants were used for association analysis of vitality with a different group of molecular measures (with a mitochondrial bioenergetics focus) than we have used in this current study.

PBMC isolation

peripheral blood mononuclear cells were isolated from 8 mL of blood using BD Vacutainer cell preparation tubes (CPT) containing sodium citrate (BD Biosciences, San Jose, CA, USA), according to the manufacturer's protocol and as described previously (Maynard *et al.* 2013). Cells were counted by a cell counter (CASY® cell counter, Roche Innovatis AG) and then diluted to 2 million cells per mL in PBS and aliquoted for the various tests. Aliquots of isolated PBMCs were either resuspended in freezing medium (75% fetal bovine serum, 20% DMEM, 5% DMSO), and frozen in liquid nitrogen for testing of DNA damage (0.25 million PBMCs for comet assay and 1 million PBMCs for FADU assay), or centrifuged to a pellet of 0.5 million PBMCs and frozen in liquid nitrogen for testing of telomere length, or, in the case of γH2AX staining, 2×10^6 PBMCs were centrifuged onto microscope slides and immediately fixed and antibody stained for γH2AX detection.

Comet assay

One method we used for measuring the level of DNA strand breaks was by way of the alkaline single cell gel electrophoresis (comet) assay, as previously described (Moller 2005). This assay measures both single and double strand breaks, but cannot distinguish between them. 50 100 images were scored per slide and two slides per subjects, in an Olympus fluorescence microscope at 400× magnification, using a five class scoring system. Besides DNA break measurement, we also performed a modified comet assay that makes use of the formamidopyrimidine DNA glycosylase (FPG) enzyme that specifically incises DNA at oxidative lesions. This provided us a means to estimate the extent of DNA oxidative lesions by way of determining the difference in score between cells treated with FPG enzyme and with buffer only. The number of lesions per 10^6 base pairs (bp) was calculated from a calibration curve assuming a yield of 0.29 strand breaks/Gy per 10^9 Daltons as described previously (Forchhammer *et al.* 2010).

γH2AX staining

γH2AX staining, as a measure of double strand breaks, was performed on 2×10^6 untreated PBMCs per microscope slide. Cytocentrifugation, fixation, staining, imaging and quantification were carried out

Table 2 Associations of DNA damage parameters with physical performance parameters

Variable		Max jump force (cm)	Max handgrip strength (kg)	Number of chair rises/30 s	Per cent of predicted FEV1
DNA breaks by FADU (abs†)	*R*	0.15	0.17	0.085	0.033
	P	0.22	0.14	0.489	0.775
	N	69	77	69	77
DNA breaks by comet (strand breaks/10^6 bp)	*R*	−0.102	0.193	−0.017	−0.151
	P	0.536	0.216	0.919	0.335
	N	39	43	38	43
FPG sensitive sites by comet (FPG sensitive site/10^6 bp)	*R*	−0.069	−0.06	−0.152	0.197
	P	0.675	0.701	0.361	0.206
	N	39	43	38	43
γH2AX sites (number of foci/cell)	*R*	−0.069	−0.127	−0.16	−0.093
	P	0.793	0.592	0.526	0.696
	N	17	20	18	20
Mean telomere length (T/S ratio)	*R*	0.039	0.04	0.026	0.146
	P	0.685	0.663	0.787	0.11
	N	110	122	109	121
Load of short telomeres (number of telomeres below 750 bp)	*R*	−0.477*	0.226	−0.277	−0.067
	P	0.018	0.239	0.181	0.729
	N	24	29	25	29

Pearson correlation analysis was performed to generate *P* values and Pearson r (*R*) coefficients. *N*, population number. †Fluorescence was determined by calculating 'T minus P' as described in Materials and Methods.
*Correlation is significant at $P < 0.05$.

as described previously (Schurman *et al.* 2012), with the following specific methodology: fixed and blocked slides were incubated overnight (4 °C, 12 h) with primary antibody anti γH2AX (Millipore, Billerica, MA, USA) diluted 1 : 1000 in 10% FBS/PBS, and 30 min at 37 °C with secondary antibody Alexa647 (Invitrogen). γH2AX foci were counted by eye for a minimum of 100 cells per participant at 100× magnification.

Fluorimetric detection of alkaline DNA unwinding

Levels of endogenous DNA strand breaks were measured using the automated and modified version of the fluorimetric detection of alkaline DNA unwinding (FADU) assay (Moreno Villanueva *et al.* 2009) as described previously (Garm *et al.* 2013). This assay measures both single and double strand breaks, but cannot distinguish between them. Three different parameters were determined in triplicate for each participant: *T* (total amount of double stranded DNA), *P* (amount of double stranded DNA at physiological conditions) and *B* (background fluorescence). *T* and *P* measurements were corrected for background fluorescence, and then the levels of endogenous strand breaks were calculated as the difference between T and P (so higher '*T* minus *P*' value reflects higher number of DNA strand breaks).

Telomere length

Telomere mean length quantification, expressed in relative length as the ratio of telomere repeat copy number (T) to RNase P single copy gene copy number (S) (i.e. T/S ratio), was carried out with an adaptation of the Q PCR method (Cawthon *et al.* 2003) as described previously (Bendix *et al.* 2014). For load of short telomere quantification, expressed as number of telomeres below 750 bp, Universal STELA was performed on genomic DNA as described previously (Bendix *et al.* 2010).

Physical and cardiovascular risk factor measurements

Jump height was measured by way of a two legged counter movement jump using a force platform (AMTI, model OR6 7 1000; Advanced Mechanical Technology, Watertown, MA, USA) and strain gauge amplifier (AMTI, model MiniAmp MSA 6). Handgrip strength was measured with a dynamometer (model G100; Biometrics Ltd, Newport, UK) wired to the computers signal conditioning interface enabling automatic recording of the grip strength force. Functional lower limb capacity was measured as maximal number of chair rises performed during a 30 s chair rise test, as described in more detail previously (Hansen *et al.* 2014). A skilled laboratory technician gave

instructions to each physical performance test, and the participants were encouraged to perform their best. Respiratory (lung) fitness was measured by forced expiratory volume in 1 s (FEV1) (Miller *et al.* 2005, Pellegrino *et al.* 2012). For BMI determination, body weight was measured in kilogram with light clothes and without socks and shoes, and percentage body fat was measured using a four compartment Tanita® MC 180 body composition analyser (Tanita Corporation, Tokyo, Japan). Height in metres was measured without shoes, heels together and buttock, shoulders and head touching the vertical surface on a standardized wall mounted height board. Body mass index (BMI) was calculated as kg/m^2.

For blood analysis, non fasting blood samples were collected, stored at −20 °C and then analysed within a maximum of 2 years for concentrations of high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol and triglycerides. Glucose was measured in ethylenediaminetetraacetic acid (EDTA) blood at the day of the test right after sampling with HemoCue® Glucose 201. Lyphochek Diabetes Control (Calibrator) from BioRad (Anaheim, CA, USA) for HbA_{1c} was used to follow the long term stability of the above methods. HDL, LDL and total cholesterol were carried out by use of a Cobas Mira Plus. The determination of HDL, LDL and total cholesterol and triglycerides were by way of ABX Pentra assays from Triolab (Sollentuna, Sweden). The analytical methods for measuring total cholesterol in serum have been evaluated by a method evaluation function design (Christensen *et al.* 1993) to estimate the random and systematic effects. This was based on a linear least squares regression analysis of the measured concentration vs. the conventional true concentration of a series of method evaluation samples containing the physiological response variable in the linear range of the method. The between assay variation (Hansen *et al.* 2007) was estimated to be 2.7% at 5.3 mmol/l cholesterol. Commercially available control samples for HDL, LDL and total cholesterol and triglycerides were analysed together with samples to show equivalence between different runs. Westgard control charts were used to document that the precision and the trueness of the analytical methods remained stable (Westgard *et al.* 1981).

Statistical analysis

Statistical analysis for Tables 1, 2, 3 and 5 was performed by Pearson correlation (two tailed), using either GraphPad Prism 5 software (La Jolla, California, USA) or SPSS statistical software, version 20.0 (IBM Corp. Released 2011; IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.). Statistical analysis for Table 4 was performed by multivariate linear regression analysis (using SAS version 9.2), to generate regression coefficients (specifically, the effect of one unit increase in the variable on the physical performance parameter), as an alternative to the Pearson correlation analysis performed on the same data set in Table 3. $P < 0.05$ was considered statistically significant.

Results

Correlates of vitality score

The analysis for potential links of DNA damage (strand breaks and telomere shortening), cardiovascular risk factors (blood biochemistry and BMI) and physical performance with human subjective vitality is shown in Table 1. DNA breaks by comet assay had an inverse association with vitality score (P 0.046; Table 1 and Figure S1); however, the DNA strand breaks measured by the FADU assay or by γH2AX staining did not show an association or obvious trend with vitality score (see discussion). BMI was inversely associated with vitality score (P 0.002). Interestingly, total cholesterol had no trend at all with vitality score (P 1), and this was reflected by the fact that the Pearson r directions of LDL (P 0.201, R −0.028) and HDL (P 0.164, R 0.031) with vitality score were opposite to each other. Strikingly, all of the physical performance parameters were strongly positively associated with vitality score (all $P < 0.001$). We also performed multivariate analysis, setting all the cardiovascular risk factors and physical performance parameters as covariates, and found that associations were not dramatically altered (data not shown. We could not do multivariate analysis on any analyses involving the DNA damage parameters (Tables 1 and 2), as the number of subjects became too small (this was not a problem for the physical performance and cardiovascular risk factor analyses, as the population numbers were all much higher (i.e. near 2000 participants)).

Relationships of DNA damage parameters with physical performance parameters

The data presented in Table 1 show that the only DNA damage parameter to show an association with vitality score was DNA breaks as detected by comet assay. We also tested whether the DNA damage parameters were associated with physical performance parameters (Table 2). The load of short telomeres was inversely associated with maximum jump force (P 0.018). Because smoking and arthritis can affect jump height (see Table 4), we looked more closely at

Table 3 Associations of cardiovascular risk factors with physical performance parameters

Variable		Max jump force (cm)	Max handgrip strength (kg)	Number of chair rises/30 s	Per cent of predicted FEV1
Glucose (mM)	*R*	−0.103**	−0.038	−0.02	−0.073**
	P	<0.001	0.084	0.391	0.001
	N	1848	2114	1864	2108
LDL (mM)	*R*	0.002	0.001	−0.015	−0.019
	P	0.941	0.967	0.533	0.383
	N	1837	2099	1848	2093
HDL (mM)	*R*	0.021	−0.060**	0.055*	0.053*
	P	0.375	0.006	0.017	0.015
	N	1837	2099	1848	2093
Triglyceride (mM)	*R*	−0.064**	0.014	−0.083**	−0.086**
	P	0.006	0.526	<0.001	<0.001
	N	1837	2099	1848	2093
Cholesterol (mM)	*R*	−0.01	0.004	0.014	0.024
	P	0.678	0.837	0.534	0.281
	N	1837	2099	1848	2093
BMI (kg/m^2)	*R*	−0.270**	0.135**	−0.208**	−0.079**
	P	<0.001	<0.001	<0.001	<0.001
	N	1855	2122	1870	2118

Pearson correlation analysis was performed to generate *P* values and Pearson r (*R*) coefficients. *N*, population number.
*Correlation is significant at $P < 0.05$, **Correlation is significant at $P < 0.01$.

Table 4 Multivariate linear regression: Associations of cardiovascular risk factor measures, smoking and arthritis with physical performance parameters

Variable		Max jump force (cm)	Max handgrip strength (kg)	Number of chair rises/30 s	Per cent of predicted FEV1
Glucose (mM)	*b*	−0.207**	−0.274**	0.018	−0.499
	P	<0.001	0.007	0.810	0.015
LDL (mM)	*b*	0.207	−0.438	−0.487	−2.308**
	P	0.355	0.269	0.070	0.004
HDL (mM)	*b*	−1.207**	−1.160	−0.884	−2.609
	P	0.001	0.055	0.032	0.033
Triglyceride (mM)	*b*	−0.071	−0.160	−0.278	−1.397**
	P	0.507	0.390	0.029	<0.001
Cholesterol (mM)	*b*	0.024	0.493	0.577**	2.553**
	P	0.891	0.106	0.006	<0.001
BMI (kg/m^2)	*b*	−0.397**	0.239**	−0.332**	−0.466**
	P	<0.001	<0.001	<0.001	<0.001
Smoking	*b*	−1.309**	−1.387**	−1.868**	−8.119**
	P	<0.001	0.001	<0.001	<0.001
Arthritis	*b*	−0.871**	−0.558**	−1.136**	−1.135**
	P	<0.001	0.155	<0.001	0.153

Multivariate linear regression analysis was performed to generate *P* values and regression coefficients *b* (the effect of one unit increase in the variable on the physical performance parameter; refer to Table 1 for the mean values to give perspective on the extent of this effect). *N* 2441.
**Correlation is significant at $P < 0.01$.

this correlation by excluding either or both of these covariates (Figure S2). If both of these covariates were excluded, the statistical significance was lost. We retained significant association when only one of either covariate was excluded. None of the other DNA damage parameters, including average length of

telomeres, showed significant associations with the physical performance measures.

Relationships of cardiovascular risk factors with physical performance parameters

We tested for associations of the cardiovascular risk factors with the physical performance parameters, using Pearson correlation analysis (Table 3) and also by multivariate analysis (Table 4) [since the cardiovascular risk factors are linked (see Table 5)]. In Table 4, we also included values on smoking and arthritis, as they are likely to affect physical performance. Indeed, smoking had a significant inverse association with all four physical performance parameters (all $P \leq 0.001$), while arthritis had a significant inverse association with maximum jump force ($P < 0.001$) and number of chair rises ($P < 0.001$) (Table 4). The Pearson correlation analysis of Table 3 displays information on links of each variable to the physical performance parameters, irrespective of the other variables, to assess potential biomarkers and for comparison to the multivariate analysis of Table 4. In Table 3, it can be seen that a measurement of only cholesterol will not be predictive of any physical performance parameters. However, if the other cardiovascular risk factors, along with smoking and arthritis, are taken into account (Table 4), it becomes predictive of chair rises (P 0.006) and per cent of predicted FEV1 ($P < 0.001$). As can be seen by comparing Tables 3 and 4, changes occur in the outcome of several other paired association analyses. BMI appears to be the most independent predictor of the physical performance parameters, as it retains significance (including direction) when the multivariate analysis is performed.

Associations among the cardiovascular risk factor variables

We also analysed the cardiovascular risk factors parameters for associations among themselves (Table 5). There were several associations, including positive association of BMI with triglyceride, glucose and LDL (all $P < 0.001$) and inverse association of BMI with HDL ($P < 0.001$), which would be expected under non fasting conditions (Zhu *et al.* 2002, Ford *et al.* 2009a). The intercardiovascular risk factor associations that we have demonstrated in this study could be informative for researchers or clinicians who are interested in evaluating the extent of these correlations or trends independent of age effects (all our participants were the same age) and when the blood is from persons in their typical daily non fasting state.

Discussion

We measured a number of DNA damage parameters, cardiovascular risk factors and physical performance parameters in PBMCs of the Metropolit cohort of middle aged men. We analysed if these variables were associated with self reported SF 36 vitality levels. We also tested whether the DNA damage parameters and the cardiovascular risk factors were associated with the physical performance measures.

Table 5 Associations among the cardiovascular risk factors

Variable		LDL	HDL	Triglycerides	Total cholesterol	BMI
Glucose	*R*	−0.109**	−0.016	0.034	−0.103**	0.078**
	P	<0.001	0.477	0.12	<0.001	<0.001
	N	2104	2104	2104	2104	2117
LDL	*R*		−0.094**	0.143**	0.790**	0.082**
	P		<0.001	<0.001	<0.001	<0.001
	N		2105	2105	2105	2102
HDL	*R*			−0.457**	0.126**	−0.311**
	P			<0.001	<0.001	<0.001
	N			2105	2105	2102
Triglycerides	*R*				0.236**	0.239**
	P				<0.001	<0.001
	N				2105	2102
Total cholesterol	*R*					0.034
	P					0.114
	N					2102

Pearson correlation analysis was performed to generate *P* values and Pearson r (*R*) coefficients. Units: glucose, LDL, HDL, triglycerides and cholesterol, mM; BMI, kg/m^2.
**Correlation is significant at $P < 0.01$.

There was a weak but significant association (P 0.046) of vitality score with DNA breaks by comet assay (Table 1 and Figure S1). However, it was not associated (or showing any obvious trend) with DNA breaks by FADU assay. As noted in the Intro duction, these two techniques use different strategies to detect DNA strand breaks. The comet assay is based on fluorescent detection of damaged DNA as a 'comet' visualized on single cells; the FADU assay is based on fluorescent signal from damaged DNA from a group of cells. There was also no association of vitality with DSBs as detected by γH2AX signal. Thus, we speculate that in our hands, the FADU assay may have been more sensitive to DNA DSBs (relative to SSBs) and/or DNA breaks due to apoptosis (Moreno Villanueva *et al.* 2009, Korwek *et al.* 2012), and so the signal from these sources may mask out associa tions due to DNA single strand breaks. Moreover, in our comet assay, we used pH >13, whereas the FADU assay called for pH of 12.5. Higher pH may promote formation of SSBs from alkali labile sites such as AP sites (intermediates of excision repair) (Collins 2004, Wen *et al.* 2011), so the association of vitality with DNA breaks comet that we see, may in fact, to some degree, represent these sites. There is debate as to whether the pH affects the degree to which SSBs relative to DSBs are detected. To be sure of these aspects with respect to our study, more work would have to be car ried out comparing these two techniques for detection of basal levels of DNA damage from PBMCs in participant cohorts. Therefore, based on our collective association data with DNA damage assays and vitality score, we propose that vitality is associated with SSBs and/or alkali labile sites. However, more work would need to be carried out to confirm this, especially because the low numbers of subjects in each analysis of DNA damage leave room for both type 1 (false positive finding) and type 2 (false negative finding) errors.

In addition, BMI was inversely associated with vitality score (Table 1). The other blood measures were not significantly associated with vitality, but there were apparent trends, such as glucose (P 0.088) with negative Pearson r values (same direction as BMI; Table 1). In the case of total choles terol, we find that there is no correlative trend with vitality (P 1). In Table 5, we show that several blood measures are correlated with BMI and with each other, and in the expected direction. As BMI was the only cardiovascular risk factor that was signifi cantly associated with vitality, it is perhaps a central risk factor, that is, it is affected by the blood chemis try and in turn affects vitality. There have been several cohort association based studies indicating links between BMI and SF 36 or its subset scales such as physical functioning, general health and, as in this current study, vitality (Vasiljevic *et al.* 2008, Castres *et al.* 2010, Wang *et al.* 2012, Delgado *et al.* 2013, Oldenburg *et al.* 2013). Studies have also shown that chronic fatigue syndrome and fibromyalgia (both of which have fatigue and low vitality as core symptoms) are associated with larger waist circumference and higher serum triglycerides (Loevinger *et al.* 2007, Maloney *et al.* 2010). Moreover, studies show an association between mitochondrial dysfunction and BMI or fat mass (Lanouette *et al.* 2001, Wang *et al.* 2007, Wortmann *et al.* 2009). Thus, our current study may promote research into the link of mitochondrial dysfunction (source of energy as ATP) and BMI with SF 36 vitality. In fact, we have recently reported that SF 36 vitality is associated with some biochemical activities (reactive oxygen species production and dNTP balance), in PBMCs, that are known to be impacted negatively by mitochondrial dysfunction (Maynard *et al.* 2013).

The data from Table 1 also showed that all of the physical performance parameters were strongly posi tively associated ($P < 0.001$) with vitality (Table 1). Wanderley *et al.* (2011) examined SF 36 vitality in relation to physical performance parameters and reported that SF 36 vitality was associated with hand grip strength (in agreement with our data) and with a measure of aerobic fitness (6 min walk test) that we did not include in our study. Our data also agree with a recent study by Manty *et al.* (2012) in which self reported fatigue in both men and women aged 75 was strongly associated ($P < 0.001$) with slower walking speed. They give further data suggesting that muscle strength (as measured by hand grip strength) is one of the underlying factors explaining this association. However, unlike our study, their study used a measure of mobility related fatigue to assess tiredness in rela tion to specific mobility activities, and so does not have vitality sore as a component. From these data, we speculate that SF 36 subjective vitality may be linked to low ATP levels (although no direct evidence yet, as discussed in (Maynard *et al.* 2013)), a disease state, or physical impairment (such as arthritis; Table 4) that negatively impacts measured physical performance.

When we looked for DNA damage correlates with physical performance parameters, we found that the load of short telomeres was significantly inversely associated with maximum jump force (P 0.018, R −0.477; Table 2 and Figure S2A). When we excluded smokers (Figure S2D) or arthritis suffers (Figure S2C), or both of these covariates (Figure S2B), the P values were smaller. This is at least in part due to the lower population size as the covariates are removed; in fact, the r values did not dramatically differ. These data suggest that the load of short

telomeres may be important in terms of negative impact on some aspects of physical function; a larger population size would be necessary to determine con clusively whether smoking and arthritis modify this apparent link. However, a recent study suggests that telomere attrition is linked to the cumulative oxidative stress and inflammation induced by smoking (Bab izhayev *et al.* 2011). Other research has shown that load of short telomeres is associated with bone dysfunction, such as arthritis in the knee and hip (O'Donovan *et al.* 2011, Harbo *et al.* 2012, 2013, Le *et al.* 2013). This could obviously impact jump force, as shown in a cohort of physically active woman (Hakkinen *et al.* 2002) and also a cohort of children, in which the arthritis was accompanied by measured underlying biomechanical deviations that affected jump height (Ford *et al.* 2009b).

In Table 3, we showed that several of the cardio vascular risk factors were also associated with several physical performance parameters. BMI was inversely associated with maximum jump force, number of chair rises and per cent of predicted FEV1; however, it is positively associated with maximum grip strength. Certainly, other studies besides ours have found higher BMI to be associated with poorer physical per formance measures, such as slower walking speed and poorer chair rises and standing balance performance (Ferrucci *et al.* 2000, Brach *et al.* 2004, Forrest *et al.* 2006, Kuh *et al.* 2006, Houston *et al.* 2007, Sergi *et al.* 2007, Stenholm *et al.* 2008). The relationship between BMI and handgrip strength is less consistent (Kuh *et al.* 2006, Woo *et al.* 2007), and it appears that sarcopenic obesity (a combination of weak mus cle strength and high adiposity) is particularly detri mental to physical performance (Houston *et al.* 2007, Stenholm *et al.* 2008). A recent study (Hardy *et al.* 2013) has found similar results to ours, specifically in their case for both men and women, that higher BMI is associated with poorer performance on chair rise (as we have found in our male cohort), walking speed and standing balance. They also found that higher BMI was associated with stronger grip strength in men only (as we have found in our male cohort). Handgrip strength is of course heavily influenced by muscle mass, and studies suggest that BMI is highly correlated with muscle mass (corrected for height) in older adults (Micozzi & Harris 1990, Iannuzzi Sucich *et al.* 2002). Thus, our data and the above cited stud ies suggest that generally middle aged and older peo ple with higher BMI have a stronger grip strength and that this is due to higher muscle mass.

When the analysis of Table 3 was reanalysed using multivariate linear regression (Table 4), several of these associations were altered dramatically, as would be expected as they are interrelated (e.g. Table 5). The addition of smoking and arthritis, as covariates, now also revealed strong associations between these two variables and physical functioning that would be expected from clinicians and from literature (Fukuba *et al.* 1993, Forrest *et al.* 2006). BMI and smoking were the only two variables in the table that showed association with all the physical performance parame ters (all $P \leq 0.001$). The above data reaffirm that a complete blood workup, BMI, smoking and arthritis should be measured for optimal prediction of the four measured physical performance parameters and by extension other force, strength and cardiorespiratory fitness activities. However, the associations with other specific activities would have to be determined in an appropriate cohort.

Strengths and limitations

One strength in the current study is the large popula tion size for the comparison among any of the vitality, physical performance or cardiovascular risk factors (N of 1812 2441 depending on the number overlapping participants for each comparison), and thus the chance of type 1 (false positive finding) and type 2 (false negative finding) errors is minimal. Conversely, a weakness is the smaller population size for the com parison involving the DNA damage parameters. The low and variable numbers of subjects in each analysis of DNA damage leaves room for both type 1 and 2 errors. Another potential limitation with respect to the smaller cohort of 207 participants is that, due to over lapping selection protocol from a separate study that was in place to examine social classes, there was some degree of inadvertent selection for social class. In fact, we tested by chi square and found that the higher and lower social class were significantly overrepresented ($P < 0.0001$) in the smaller set of 207 participants used for blood collection/DNA damage tests. This may have influenced comparisons, because social class had been found to be correlated with some physical performance parameters (Hansen *et al.* 2014) and thus calls for cautious interpretation of results involving the DNA damage parameters.

Another strength is that our study involved only men, all of the same age. This design gives more power, as one does not have to stratify for age or gen der. However, a weakness is that it does not allow us to assess sex or age differences, and thus is not indica tive of the whole population. We must be careful when comparing to other studies, which typically deal with cohort participants of both sexes and with a range of ages. For example, at least two studies link ing DNA oxidative damage (in peripheral leucocytes) to fatigue related measures (depression and stress) were only valid in women (Irie *et al.* 2001, 2003).

The association of DNA damage comet with vitality is not strong (P 0.046). In addition, DNA damage FADU did not show an association with vitality, as discussed. Certainly, more work is needed to delineate how DNA damage could be linked to vitality. We also note that the DNA damage assays we have used in this study detect only nuclear DNA damage; mito chondrial DNA cannot be detected by the techniques we have used in this study. Potential links of mito chondrial DNA damage to perception of energy would be interesting to examine because mitochondrial DNA damage is closely linked to defects in ATP generation (mitochondrial oxidative phosphorylation), partly due to the close proximity of mitochondrial DNA to the electron transport chain.

Summary

The results of this current study suggest that subjec tive vitality in healthy men is linked to BMI (but not glucose/lipid levels), physical performance and DNA breaks, and thus suggests that these measures could serve as biomarker warning signals for imminent frailty or fatiguing illnesses. These associations give us new insight into the underlying physiological and cel lular parameters that impact subjective vitality.

Our study also provides a uniquely comprehensive tabulation of the complex association of various common physical performance measures with car diovascular risk factors (Tables 3 and 4) and the associations among the cardiovascular risk factors (Table 5). With respect to mechanisms and sugges tions generated from data of this study, our data from Table 5 suggest that higher levels of glucose, triglyce rides and LDL and lower HDL are partial determi nants of higher BMI, which we have shown can then negatively impact vitality and physical performance, except handgrip strength, which it positively impacts (Tables 1, 3 and 4); that is, BMI appears to be 'con vergent destination' of defects in blood chemistry, and a marker for vitality and physical performance. Our data reiterate that proper blood chemistry levels, nutrition and exercise habits are important in main taining vitality during daily activities and also to maintain vitality and quality of life for patients undergoing treatment for various medical reasons, such as cancer.

With respect to the DNA damage associations, our data suggest that vitality may in fact have links to sev eral underlying molecular events because DNA dam age is a 'convergent destination' for many cellular dysfunctions. Our data also reveal an association of load of short telomeres with jump height. This may involve arthritis and its link to short telomeres. Fur ther research into the mechanisms by which DNA damage leads to physical impairment or lower vitality deserves further study.

Conflict of interest

The authors have no competing interests to declare.

This study was supported by grants from Nordea fonden and the VELUX Foundation. The Copenhagen Aging and Midlife Biobank (CAMB) has been supported by a generous grant from the VELUX FOUNDATION. Authors thank the staff at Institute of Public Health and the National Research Center for the Working Environment who undertook the data collec tion. Further thanks to Helle Bruunsgaard, Nils Erik Fiehn, Poul Holm Pedersen, Rikke Lund and Erik Lykke Mortensen who initiated and established the Copenhagen Aging and Midlife Biobank from 2009 to 2011 together with Kirsten Avlund, Åse Marie Hansen and Merete Osler. The authors acknowledge the crucial role of the initiators and steering groups of the Metropolit Cohort, The Copenhagen Perinatal Cohort and The Danish Longitudinal Study on Work Unem ployment and Health. The FPG enzyme was a gift from Andrew R Collins, University of Oslo, Norway.

Author contributions

S.M., G.K., S.L., A.B., T.S., L.J.R., K.A. and V.A.B designed the study; G.K., S.M., L.B., P.M., M.M.V and C.P.H performed the laboratory research; S.M., G.K., Å.M.H, D.M., L.B. and S.H.S analysed and interpreted the data; Å.M.H., M.O., D.M. and K.A collected the patient samples and clinical data. S.M drafted the manuscript; V.B. and S.H.S helped develop the manuscript, and all authors critically reviewed the manuscript and gave their final approval.

References

Ahola, K., Siren, I., Kivimaki, M., Ripatti, S., Aromaa, A., Lonnqvist, J. & Hovatta, I. 2012. Work related exhaustion and telomere length: a population based study. *PLoS ONE* **7**, e40186.

Armanios, M., Alder, J.K., Parry, E.M., Karim, B., Strong, M.A. & Greider, C.W. 2009. Short telomeres are sufficient to cause the degenerative defects associated with aging. *Am J Hum Genet* **85**, 823 832.

Avlund, K. 2010. Fatigue in older adults: an early indicator of the aging process? *Aging Clin Exp Res* **22**, 100 115.

Avlund, K., Osler, M., Mortensen, E.L., Christensen, U., Bruunsgaard, H., Holm Pedersen, P., Fiehn, N.E., Han sen, A.M., Bachkati, S.H., Meincke, R.H., Jepsen, E., Molbo, D. & Lund, R. 2014. Copenhagen aging and midlife biobank (CAMB): an introduction. *J Aging Health* **26**, 5 20.

Babizhayev, M.A., Savel'yeva, E.L., Moskvina, S.N. & Yego rov, Y.E. 2011. Telomere length is a biomarker of cumula tive oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement

associated with smoking behavior. *Am J Ther* **18**, e209 e226.

Bendix, L., Horn, P.B., Jensen, U.B., Rubelj, I. & Kolvraa, S. 2010. The load of short telomeres, estimated by a new method, Universal STELA, correlates with number of senescent cells. *Aging Cell* **9**, 383 397.

Bendix, L., Thinggaard, M., Fenger, M., Kolvraa, S., Avlund, K., Linneberg, A. & Osler, M. 2014. Longitudinal changes in leukocyte telomere length and mortality in humans. *J Gerontol A Biol Sci Med Sci* **69**, 231 239.

Bjorner, J.B., Damsgaard, M.T., Watt, T. & Groenvold, M. 1998. Tests of data quality, scaling assumptions, and reliabil ity of the Danish SF 36. *J Clin Epidemiol* **51**, 1001 1011.

Bjorner, J.B., Wallenstein, G.V., Martin, M.C., Lin, P., Blais dell Gross, B., Tak, P.C. & Mody, S.H. 2007. Interpreting score differences in the SF 36 Vitality scale: using clinical conditions and functional outcomes to define the minimally important difference. *Curr Med Res Opin* **23**, 731 739.

Brach, J.S., VanSwearingen, J.M., FitzGerald, S.J., Storti, K.L. & Kriska, A.M. 2004. The relationship among physi cal activity, obesity, and physical function in community dwelling older women. *Prev Med* **39**, 74 80.

Brown, L.F., Kroenke, K., Theobald, D.E. & Wu, J. 2011. Comparison of SF 36 vitality scale and Fatigue Symptom Inventory in assessing cancer related fatigue. *Support Care Cancer* **19**, 1255 1259.

Buchwald, D., Pearlman, T., Umali, J., Schmaling, K. & Katon, W. 1996. Functional status in patients with chronic fatigue syndrome, other fatiguing illnesses, and healthy individuals. *Am J Med* **101**, 364 370.

Canela, A., Vera, E., Klatt, P. & Blasco, M.A. 2007. High throughput telomere length quantification by FISH and its application to human population studies. *Proc Natl Acad Sci U S A* **104**, 5300 5305.

Castres, I., Folope, V., Dechelotte, P., Tourny Chollet, C. & Lemaitre, F. 2010. Quality of life and obesity class rela tionships. *Int J Sports Med* **31**, 773 778.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A. & Kerber, R.A. 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lan cet* **361**, 393 395.

Christensen, S.L., Anglov, J.T.B., Christensen, J.M., Olsen, E. & Poulsen, O.M. 1993. Application of a new AMIQAS computer program for integrated quality control, method evaluation and proficiency testing. *Fresenius J Anal Chem* **345**, 343 350.

Collins, A.R. 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol Bio technol* **26**, 249 261.

Delgado, C., Doyle, J.W. & Johansen, K.L. 2013. Associa tion of frailty with body composition among patients on hemodialysis. *J Ren Nutr* **23**, 356 362.

Dimitroglou, E., Zafiropoulou, M., Messini Nikolaki, N., Doudounakis, S., Tsilimigaki, S. & Piperakis, S.M. 2003. DNA damage in a human population affected by chronic psychogenic stress. *Int J Hyg Environ Health* **206**, 39 44.

Duvivier, B.M., Schaper, N.C., Bremers, M.A., van Crom brugge, G., Menheere, P.P., Kars, M. & Savelberg, H.H. 2013. Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. *PLoS ONE* **8**, e55542.

Elvsashagen, T., Vera, E., Boen, E., Bratlie, J., Andreassen, O.A., Josefsen, D., Malt, U.F., Blasco, M.A. & Boye, B. 2011. The load of short telomeres is increased and associ ated with lifetime number of depressive episodes in bipolar II disorder. *J Affect Disord* **135**, 43 50.

Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D. & Cawthon, R.M. 2004. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A* **101**, 17312 17315.

Fernandez Gonzalo, R., Lundberg, T.R. & Tesch, P.A. 2013. Acute molecular responses in untrained and trained muscle subjected to aerobic and resistance exercise training versus resistance training alone. *Acta Physiol (Oxf)* **209**, 283 294.

Ferrucci, L., Penninx, B.W., Leveille, S.G., Corti, M.C., Pahor, M., Wallace, R., Harris, T.B., Havlik, R.J. & Gu ralnik, J.M. 2000. Characteristics of nondisabled older per sons who perform poorly in objective tests of lower extremity function. *J Am Geriatr Soc* **48**, 1102 1110.

Forchhammer, L., Johansson, C., Loft, S., Moller, L., Gods chalk, R.W., Langie, S.A., Jones, G.D., Kwok, R.W., Col lins, A.R., Azqueta, A. *et al.* 2010. Variation in the measurement of DNA damage by comet assay measured by the ECVAG inter laboratory validation trial. *Mutagene sis* **25**, 113 123.

Ford, K.R., Myer, G.D., Melson, P.G., Darnell, S.C., Brun ner, H.I. & Hewett, T.E. 2009a. Land jump performance in patients with juvenile idiopathic arthritis (JIA): a com parison to matched controls. *Int J Rheumatol* **2009**, 478526.

Ford, E.S., Li, C., Zhao, G., Pearson, W.S. & Mokdad, A.H. 2009b. Hypertriglyceridemia and its pharmacologic treatment among US adults. *Arch Intern Med* **169**, 572 578.

Forrest, K.Y., Zmuda, J.M. & Cauley, J.A. 2006. Correlates of decline in lower extremity performance in older women: a 10 year follow up study. *J Gerontol A Biol Sci Med Sci* **61**, 1194 1200.

Fukuba, Y., Takamoto, N., Kushima, K., Ohtaki, M., Kiha ra, H., Tanaka, T., Une, S. & Munaka, M. 1993. [Ciga rette smoking and physical fitness]. *Ann Physiol Anthropol* **12**, 195 212.

Garm, C., Moreno Villanueva, M., Burkle, A., Petersen, I., Bohr, V.A., Christensen, K. & Stevnsner, T. 2013. Age and gender effects on DNA strand break repair in periph eral blood mononuclear cells. *Aging Cell* **12**, 58 66.

Gidron, Y., Russ, K., Tissarchondou, H. & Warner, J. 2006. The relation between psychological factors and DNA dam age: a critical review. *Biol Psychol* **72**, 291 304.

Graziano, F., Bisonni, R., Catalano, V., Silva, R., Rovidati, S., Mencarini, E., Ferraro, B., Canestrari, F., Baldelli, A.M., De, G.A., Giordani, P., Testa, E. & Lai, V. 2002. Potential role of levocarnitine supplementation for the treatment of chemotherapy induced fatigue in non anaemic cancer patients. *Br J Cancer* **86**, 1854 1857.

Hagberg, J.M., Jenkins, N.T. & Spangenburg, E. 2012. Exercise training, genetics and type 2 diabetes related phenotypes. *Acta Physiol (Oxf)* **205**, 456–471.

Hakkinen, A., Haanonan, P., Nyman, K. & Hakkinen, K. 2002. Aerobic and neuromuscular performance capacity of physically active females with early or long term rheumatoid arthritis compared to matched healthy women. *Scand J Rheumatol* **31**, 345–350.

Hansen, A.M., Garde, A.H. & Eller, N.H. 2007. Estimation of individual reference intervals in small sample sizes. *Int J Hyg Environ Health* **210**, 471–478.

Hansen, A.M., Andersen, L.L., Skotte, J., Christensen, U., Mortensen, O.S., Molbo, D., Lund, R., Nilsson, C.J. & Avlund, K. 2014. Social class differences in physical functions in middle aged men and women. *J Aging Health* **26**, 88–105.

Hao, L.Y., Armanios, M., Strong, M.A., Karim, B., Feldser, D.M., Huso, D. & Greider, C.W. 2005. Short telomeres, even in the presence of telomerase, limit tissue renewal capacity. *Cell* **123**, 1121–1131.

Harbo, M., Bendix, L., Bay Jensen, A.C., Graakjaer, J., Soe, K., Andersen, T.L., Kjaersgaard Andersen, P., Koelvraa, S. & Delaisse, J.M. 2012. The distribution pattern of critically short telomeres in human osteoarthritic knees. *Arthritis Res Ther* **14**, R12.

Harbo, M., Delaisse, J.M., Kjaersgaard Andersen, P., Soerensen, F.B., Koelvraa, S. & Bendix, L. 2013. The relationship between ultra short telomeres, aging of articular cartilage and the development of human hip osteoarthritis. *Mech Ageing Dev* **134**, 367–372.

Hardy, R., Cooper, R., Aihie, S.A., Ben Shlomo, Y., Cooper, C., Deary, I.J., Demakakos, P., Gallacher, J., Martin, R.M., McNeill, G., Starr, J.M., Steptoe, A., Syddall, H. & Kuh, D. 2013. Body mass index, muscle strength and physical performance in older adults from eight cohort studies: the HALCyon programme. *PLoS ONE* **8**, e56483.

Hemann, M.T., Strong, M.A., Hao, L.Y. & Greider, C.W. 2001. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* **107**, 67–77.

Houston, D.K., Ding, J., Nicklas, B.J., Harris, T.B., Lee, J.S., Nevitt, M.C., Rubin, S.M., Tylavsky, F.A. & Kritchevsky, S.B. 2007. The association between weight history and physical performance in the Health, Aging and Body Composition study. *Int J Obes (Lond)* **31**, 1680–1687.

Iannuzzi Sucich, M., Prestwood, K.M. & Kenny, A.M. 2002. Prevalence of sarcopenia and predictors of skeletal muscle mass in healthy, older men and women. *J Gerontol A Biol Sci Med Sci* **57**, M772–M777.

Irie, M., Asami, S., Nagata, S., Miyata, M. & Kasai, H. 2001. Relationships between perceived workload, stress and oxidative DNA damage. *Int Arch Occup Environ Health* **74**, 153–157.

Irie, M., Asami, S., Ikeda, M. & Kasai, H. 2003. Depressive state relates to female oxidative DNA damage via neutrophil activation. *Biochem Biophys Res Commun* **311**, 1014–1018.

Jhamb, M., Argyropoulos, C., Steel, J.L., Plantinga, L., Wu, A.W., Fink, N.E., Powe, N.R., Meyer, K.B. & Unruh, M.L. 2009. Correlates and outcomes of fatigue among incident dialysis patients. *Clin J Am Soc Nephrol* **4**, 1779–1786.

Jimenez Pavon, D., Kelly, J. & Reilly, J.J. 2010. Associations between objectively measured habitual physical activity and adiposity in children and adolescents: Systematic review. *Int J Pediatr Obes* **5**, 3–18.

Kananen, L., Surakka, I., Pirkola, S., Suvisaari, J., Lonnqvist, J., Peltonen, L., Ripatti, S. & Hovatta, I. 2010. Childhood adversities are associated with shorter telomere length at adult age both in individuals with an anxiety disorder and controls. *PLoS ONE* **5**, e10826.

Korwek, Z., Sewastianik, T., Bielak Zmijewska, A., Mosieniak, G., Alster, O., Moreno Villanueva, M., Burkle, A. & Sikora, E. 2012. Inhibition of ATM blocks the etoposide induced DNA damage response and apoptosis of resting human T cells. *DNA Repair (Amst)* **11**, 864–873.

Krauss, J., Farzaneh Far, R., Puterman, E., Na, B., Lin, J., Epel, E., Blackburn, E. & Whooley, M.A. 2011. Physical fitness and telomere length in patients with coronary heart disease: findings from the Heart and Soul Study. *PLoS ONE* **6**, e26983.

Kuh, D., Hardy, R., Butterworth, S., Okell, L., Wadsworth, M., Cooper, C. & Aihie, S.A. 2006. Developmental origins of midlife grip strength: findings from a birth cohort study. *J Gerontol A Biol Sci Med Sci* **61**, 702–706.

Lanouette, C.M., Giacobino, J.P., Perusse, L., Lacaille, M., Yvon, C., Chagnon, M., Kuhne, F., Bouchard, C., Muzzin, P. & Chagnon, Y.C. 2001. Association between uncoupling protein 3 gene and obesity related phenotypes in the Quebec Family Study. *Mol Med* **7**, 433–441.

Lasaite, L., Lasiene, J., Kazanavicius, G. & Gostautas, A. 2009. [Associations of emotional state and quality of life with lipid concentration, duration of the disease, and the way of treating the disease in persons with type 2 diabetes mellitus]. *Medicina (Kaunas)* **45**, 85–94.

Le, G.T., Jullien, L., Touzot, F., Schertzer, M., Gaillard, L., Perderiset, M., Carpentier, W., Nitschke, P., Picard, C., Couillault, G. *et al.* 2013. Human RTEL1 deficiency causes Hoyeraal Hreidarsson syndrome with short telomeres and genome instability. *Hum Mol Genet* **22**, 3239–3249.

Loevinger, B.L., Muller, D., Alonso, C. & Coe, C.L. 2007. Metabolic syndrome in women with chronic pain. *Metabolism* **56**, 87–93.

Ludlow, A.T., Ludlow, L.W. & Roth, S.M. 2013. Do telomeres adapt to physiological stress? exploring the effect of exercise on telomere length and telomere related proteins. *Biomed Res Int* **2013**, 601368.

Maes, M. 2011. An intriguing and hitherto unexplained co-occurrence: depression and chronic fatigue syndrome are manifestations of shared inflammatory, oxidative and nitrosative (IO&NS) pathways. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 784–794.

Maloney, E.M., Boneva, R.S., Lin, J.M. & Reeves, W.C. 2010. Chronic fatigue syndrome is associated with metabolic syndrome: results from a case control study in Georgia. *Metabolism* **59**, 1351–1357.

Manty, M., de Leon, C.F., Rantanen, T., Era, P., Pedersen, A.N., Ekmann, A., Schroll, M. & Avlund, K. 2012.

Mobility related fatigue, walking speed, and muscle strength in older people. *J Gerontol A Biol Sci Med Sci* **67**, 523 529.

Maynard, S., Keijzers, G., Gram, M., Desler, C., Bendix, L., Budtz Jorgensen, E., Molbo, D., Croteau, D.L., Osler, M., Stevnsner, T., Rasmussen, L.J., Dela, F., Avlund, K. & Bohr, V.A. 2013. Relationships between human vitality and mitochondrial respiratory parameters, reactive oxygen species production and dNTP levels in peripheral blood mononuclear cells. *Aging (Albany NY)* **5**, 850 864.

Micozzi, M.S. & Harris, T.M. 1990. Age variations in the relation of body mass indices to estimates of body fat and muscle mass. *Am J Phys Anthropol* **81**, 375 379.

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casa buri, R., coates, A., Crapo, R., van den Grinten, C.P.M., Gustafsson, P., Jensen, R. *et al.* 2005. Standardisation of spirometry. *Eur Respir J* **26**, 319 338.

Moller, P. 2005. Genotoxicity of environmental agents assessed by the alkaline comet assay. *Basic Clin Pharmacol Toxicol* **96**(Suppl 1), 1 42.

Moreno Villanueva, M., Pfeiffer, R., Sindlinger, T., Leake, A., Muller, M., Kirkwood, T.B. & Burkle, A. 2009. A modified and automated version of the 'Fluorimetric Detec tion of Alkaline DNA Unwinding' method to quantify for mation and repair of DNA strand breaks. *BMC Biotechnol* **9**, 39.

Moreno Villanueva, M., Morath, J., Vanhooren, V., Elbert, T., Kolassa, S., Libert, C., Burkle, A. & Kolassa, I.T. 2013. N glycosylation profiling of plasma provides evi dence for accelerated physiological aging in post traumatic stress disorder. *Transl Psychiatry* **3**, e320.

Muzembo, B.A., Nagano, Y., Eitoku, M., Ngatu, N.R., Mat sui, T., Bhatti, S.A., Hirota, R., Ishida, K. & Suganuma, N. 2014. A cross sectional assessment of oxidative DNA damage and muscle strength among elderly people living in the community. *Environ Health Prev Med* **19**, 21 29.

O'Donovan, A., Pantell, M.S., Puterman, E., Dhabhar, F.S., Blackburn, E.H., Yaffe, K., Cawthon, R.M., Opresko, P.L., Hsueh, W.C., Satterfield, S., Newman, A.B., Ayona yon, H.N., Rubin, S.M., Harris, T.B. & Epel, E.S. 2011. Cumulative inflammatory load is associated with short leu kocyte telomere length in the Health, Aging and Body Composition Study. *PLoS ONE* **6**, e19687.

Oldenburg, C.S., Boll, D., Nicolaije, K.A., Vos, M.C., Pijnen borg, J.M., Coebergh, J.W., Beijer, S., van de Poll Franse, L.V. & Ezendam, N.P. 2013. The relationship of body mass index with quality of life among endometrial cancer survivors: a study from the population based PROFILES registry. *Gynecol Oncol* **129**, 216 221.

Osler, M., Lund, R., Kriegbaum, M., Christensen, U. & Andersen, A.M. 2006. Cohort profile: the Metropolit 1953 Danish male birth cohort. *Int J Epidemiol* **35**, 541 545.

Pellegrino, R., Viegi, G., Brusasco, V., Crapo, R., Burgos, F., Casaburi, R., Coates, A., van den Grinten, C.P.M., Gustafsson, P., Hankinson, J. *et al.* 2012. Interpretive strat egies for lung function tests. *Eur Respir J* **26**, 948 968.

Persson, P.B. 2014. Good publication practice in physiology 2013: revised author guidelines for Acta Physiologica. *Acta Physiol* **209**, 250 253.

Puterman, E., Lin, J., Blackburn, E., O'Donovan, A., Adler, N. & Epel, E. 2010. The power of exercise: buffering the effect of chronic stress on telomere length. *PLoS ONE* **5**, e10837.

Rajaraman, S., Choi, J., Cheung, P., Beaudry, V., Moore, H. & Artandi, S.E. 2007. Telomere uncapping in progenitor cells with critical telomere shortening is coupled to S phase progression in vivo. *Proc Natl Acad Sci U S A* **104**, 17747 17752.

Schurman, S.H., Dunn, C.A., Greaves, R., Yu, B., Ferrucci, L., Croteau, D.L., Seidman, M.M. & Bohr, V.A. 2012. Age related disease association of endogenous gamma H2AX Foci in mononuclear cells derived from leukaphere sis. *PLoS ONE* **7**, e45728.

Semba, R.D., Lauretani, F. & Ferrucci, L. 2007. Carotenoids as protection against sarcopenia in older adults. *Arch Bio chem Biophys* **458**, 141 145.

Sergi, G., Perissinotto, E., Toffanello, E.D., Maggi, S., Manz ato, E., Buja, A., Coin, A., Frigo, A.C., Inelmen, E.M. & Enzi, G. 2007. Lower extremity motor performance and body mass index in elderly people: the Italian Longitudinal Study on Aging. *J Am Geriatr Soc* **55**, 2023 2029.

Stenholm, S., Harris, T.B., Rantanen, T., Visser, M., Krit chevsky, S.B. & Ferrucci, L. 2008. Sarcopenic obesity: defi nition, cause and consequences. *Curr Opin Clin Nutr Metab Care* **11**, 693 700.

Syddall, H.E., Martin, H.J., Harwood, R.H., Cooper, C. & Aihie, S.A. 2009. The SF 36: a simple, effective measure of mobility disability for epidemiological studies. *J Nutr Health Aging* **13**, 57 62.

Vainionpaa, A., Korpelainen, R., Kaikkonen, H., Knip, M., Leppaluoto, J. & Jamsa, T. 2007. Effect of impact exercise on physical performance and cardiovascular risk factors. *Med Sci Sports Exerc* **39**, 756 763.

Vasiljevic, N., Ralevic, S., Marinkovic, J., Kocev, N., Maksi movic, M., Milosevic, G.S. & Tomic, J. 2008. The assess ment of health related quality of life in relation to the body mass index value in the urban population of Bel grade. *Health Qual Life Outcomes* **6**, 106.

Vera, E. & Blasco, M.A. 2012. Beyond average: potential for measurement of short telomeres. *Aging (Albany NY)* **4**, 379 392.

Wanderley, F.A., Silva, G., Marques, E., Oliveira, J., Mota, J. & Carvalho, J. 2011. Associations between objectively assessed physical activity levels and fitness and self reported health related quality of life in community dwell ing older adults. *Qual Life Res* **20**, 1371 1378.

Wang, X., Axelsson, J., Nordfors, L., Qureshi, A.R., Avesani, C., Barany, P., Schalling, M., Heimburger, O., Lindholm, B. & Stenvinkel, P. 2007. Changes in fat mass after initiation of maintenance dialysis is influenced by the uncoupling protein 2 exon 8 insertion/deletion polymorphism. *Nephrol Dial Transplant* **22**, 196 202.

Wang, R., Wu, M.J., Ma, X.Q., Zhao, Y.F., Yan, X.Y., Gao, Q.B. & He, J. 2012. Body mass index and health related quality of life in adults: a population based study in five cities of China. *Eur J Public Health* **22**, 497 502.

Wen, Y., Zhang, P.P., An, J., Yu, Y.X., Wu, M.H., Sheng, G.Y., Fu, J.M. & Zhang, X.Y. 2011. Diepoxybutane

induces the formation of DNA DNA rather than DNA pro tein cross links, and single strand breaks and alkali labile sites in human hepatocyte L02 cells. *Mutat Res* **716**, 84 91.
Westgard, J.O., Barry, P.L., Hunt, M.R. & Groth, T. 1981. A multi rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* **27**, 493 501.
Wolkowitz, O.M., Mellon, S.H., Epel, E.S., Lin, J., Dhabhar, F.S., Su, Y., Reus, V.I., Rosser, R., Burke, H.M., Kupfer man, E., Compagnone, M., Nelson, J.C. & Blackburn, E.H. 2011. Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress preliminary findings. *PLoS ONE* **6**, e17837.
Woo, J., Leung, J. & Kwok, T. 2007. BMI, body composi tion, and physical functioning in older adults. *Obesity (Sil ver Spring)* **15**, 1886 1894.
Wortmann, S.B., Zweers van, E.H., Rodenburg, R.J., van den Heuvel, L.P., de Vries, M.C., Rasmussen Conrad, E., Smeitink, J.A. & Morava, E. 2009. Mitochondrial energy production correlates with the age related BMI. *Pediatr Res* **65**, 103 108.
Zhu, S., Wang, Z., Heshka, S., Heo, M., Faith, M.S. & Hey msfield, S.B. 2002. Waist circumference and obesity associ ated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin Nutr* **76**, 743 749.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Correlation between DNA breaks comet assay and vitality score. Pearson correlation analysis between the DNA breaks by comet assay and vitality score (see also Table 1), using GraphPad Prism 5.0 software, with a cut off of $P < 0.05$ considered as sig nificant. Vitality score scale ranges from 0 to 100.

Figure S2. Correlation between load of short telo meres and maximum jump force. Pearson correlation analysis between number of short telomeres (below 750 bp) (i.e. load of short telomeres) and maximum jump force (measured by jump height in cm, on a force platform), using GraphPad Prism 5.0 software, with a cut off of $P < 0.05$ considered as significant. Including (A) (also see Table 2), or excluding (B) par ticipants that suffer from arthritis and also partici pants who smoke. (C) Excluding only participants that suffer from arthritis. (D) Excluding only partici pants that smoke.