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

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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 \((P < 0.001)\). 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 com-
plaint 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 com-
ponent 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 lev-
els 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, mortal-
ity risk, and with negative outcomes, including inabil-
ity 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 et al. 2011). There is also evidence that stress, exhaustion and depression are associated with telo-
mere 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, Wolkwirz 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 glu-
cose and lipid profiles may impact energy levels. Liter-
ature on the impact of cardiovascular risk factors on energy levels, fatigue or quality of life is limited. Stud-
ies 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 supple-
mentation was found to be effective in alleviating che-
motherapy 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 (Lasa 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 associ-
ated 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 physi-
cal 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 pro-
files 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 dam-
age 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 addi-
tion, 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 capac-
ity. 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 litera-
ture, 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 measured in isolated PBMCs from members of this cohort, we sought to discover physical, physiological and cellular 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 techniques. 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 techniques (see discussion). A modified comet assay (comet FPG sensitive sites), utilizing the FPG (for mammidopyrimidine DNA glycosylase) enzyme that incises DNA at oxidative DNA lesions, facilitates estimation of oxidative DNA damage. The γH2AX staining method detects mainly DSBs. The telomere measurements were designed to record different aspects of telomere shortening: the mean telomere length and the load (relative quantity) of short telomeres. We included the load of short telomeres measure as evidence suggests that this parameter, not mean telomere length, is indicative of telomere dysfunction and cell viability (Hemann et al. 2001, Hao et al. 2005, Rajaraman et al. 2007, Armanios et al. 2009, Vera & Blasco 2012). It has been shown that the load of short telomeres increases with age in peripheral blood lymphocytes (Canela et al. 2007) and is associated with cellular 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 members 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 participants, used in this study, was not a completely random 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 Environment in 2010 and included blood tests, clinical examination (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 \times \left( \frac{(7 \text{ score of item 1}) + (7 \text{ score of item 2}) + \text{score of item 3} + \text{score of item 4})}{15} \right)\) to a scale ranging from 0 to 100, where higher value indicated a higher subjective vitality level. A total of 2434 out of the 2487 selected participants submitted their SF 36 vitality 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 participants that had valid measures for each pair of comparisons (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 damage test 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 participants that reported myocardial infarction or angina pectoris, or a stroke, or diabetes, or had taken antidepressant drugs or lipid modifying drugs, in the past 3 weeks, were excluded from this study. The current study was conducted according to the ethical principles of the Helsinki II declaration and good publication practice as stated in ‘Good publication practice in physiology 2013: revised author guidelines for Acta
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/C226 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 \( \gamma H2AX \) staining, 2 x 10^6 PBMCs were centrifuged onto microscope slides and immediately fixed and antibody stained for \( \gamma H2AX \) detection.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean (±SD)</th>
<th>P value</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA breaks by FADU (fluorescence')</td>
<td>77</td>
<td>33.28 (12.78)</td>
<td>0.624</td>
<td>-0.057</td>
</tr>
<tr>
<td>DNA breaks by comet (strand breaks/10^6 bp)</td>
<td>43</td>
<td>0.140 (0.069)</td>
<td>0.046</td>
<td>-0.306*</td>
</tr>
<tr>
<td>FPG sensitive sites by comet (FPG sensitive site/10^6 bp)</td>
<td>43</td>
<td>0.269 (0.158)</td>
<td>0.111</td>
<td>0.246</td>
</tr>
<tr>
<td>( \gamma H2AX ) sites (number of foci/cell)</td>
<td>20</td>
<td>9.91 (2.41)</td>
<td>0.55</td>
<td>-0.142</td>
</tr>
<tr>
<td>Mean telomere length (T/S ratio)</td>
<td>122</td>
<td>0.48 (0.120)</td>
<td>0.243</td>
<td>0.106</td>
</tr>
<tr>
<td>Load of short telomeres (number of telomeres below 750 bp)</td>
<td>29</td>
<td>5.79 (3.67)</td>
<td>0.439</td>
<td>-0.149</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>2073</td>
<td>5.56 (1.51)</td>
<td>0.088</td>
<td>-0.038</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>2058</td>
<td>3.09 (0.79)</td>
<td>0.201</td>
<td>-0.028</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>2058</td>
<td>1.42 (0.37)</td>
<td>0.164</td>
<td>0.031</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>2058</td>
<td>1.90 (1.09)</td>
<td>0.11</td>
<td>-0.035</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>2058</td>
<td>6.33 (1.07)</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>2081</td>
<td>26.13 (3.62)</td>
<td>0.002</td>
<td>-0.068**</td>
</tr>
<tr>
<td>Max jump force (cm)</td>
<td>1812</td>
<td>21.38 (4.66)</td>
<td>&lt;0.001</td>
<td>0.168**</td>
</tr>
<tr>
<td>Max handgrip strength (kg)</td>
<td>2077</td>
<td>49.27 (8.37)</td>
<td>&lt;0.001</td>
<td>0.104**</td>
</tr>
<tr>
<td>Number of chair rises/30 s</td>
<td>1827</td>
<td>21.74 (5.53)</td>
<td>&lt;0.001</td>
<td>0.188**</td>
</tr>
<tr>
<td>Per cent of predicted FEV1</td>
<td>2072</td>
<td>107.99 (17.41)</td>
<td>&lt;0.001</td>
<td>0.148**</td>
</tr>
</tbody>
</table>

Pearson correlation analysis was performed to generate P values and Pearson r (R) coefficients. N, population number.

\( \gamma H2AX \) staining

\( \gamma H2AX \) staining, as a measure of double strand breaks, was performed on 2 x 10^6 untreated PBMCs per microscope slide. Cytocentrifugation, fixation, staining, imaging and quantification were carried out
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 \( \gamma H2AX \) (Millipore, Billerica, MA, USA) diluted 1 : 1000 in 10% FBS/PBS, and 30 min at 37 °C with secondary antibody Alexa647 (Invitrogen). \( \gamma H2AX \) 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

### Table 2 Associations of DNA damage parameters with physical performance parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Max jump force (cm)</th>
<th>Max handgrip strength (kg)</th>
<th>Number of chair rises/30 s</th>
<th>Per cent of predicted FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA breaks by FADU (abs¹)</td>
<td>( R ) 0.15</td>
<td>0.17</td>
<td>0.085</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.22</td>
<td>0.14</td>
<td>0.489</td>
<td>0.775</td>
</tr>
<tr>
<td></td>
<td>( N ) 69</td>
<td>77</td>
<td>69</td>
<td>77</td>
</tr>
<tr>
<td>DNA breaks by comet (strand breaks/10⁶ bp)</td>
<td>( R ) -0.102</td>
<td>0.193</td>
<td>-0.017</td>
<td>-0.151</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.536</td>
<td>0.216</td>
<td>0.919</td>
<td>0.335</td>
</tr>
<tr>
<td></td>
<td>( N ) 39</td>
<td>43</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>FPG sensitive sites by comet (FPG sensitive site/10⁶ bp)</td>
<td>( R ) -0.069</td>
<td>-0.06</td>
<td>-0.152</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.675</td>
<td>0.701</td>
<td>0.361</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>( N ) 39</td>
<td>43</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>( \gamma H2AX ) sites (number of foci/cell)</td>
<td>( R ) -0.069</td>
<td>-0.127</td>
<td>-0.16</td>
<td>-0.093</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.793</td>
<td>0.592</td>
<td>0.326</td>
<td>0.696</td>
</tr>
<tr>
<td></td>
<td>( N ) 17</td>
<td>20</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Mean telomere length (T/S ratio)</td>
<td>( R ) 0.039</td>
<td>0.04</td>
<td>0.026</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.685</td>
<td>0.663</td>
<td>0.787</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>( N ) 110</td>
<td>122</td>
<td>109</td>
<td>121</td>
</tr>
<tr>
<td>Load of short telomeres (number of telomeres below 750 bp)</td>
<td>( R ) -0.477*</td>
<td>0.226</td>
<td>-0.277</td>
<td>-0.067</td>
</tr>
<tr>
<td></td>
<td>( P ) 0.018</td>
<td>0.239</td>
<td>0.181</td>
<td>0.729</td>
</tr>
<tr>
<td></td>
<td>( N ) 24</td>
<td>29</td>
<td>25</td>
<td>29</td>
</tr>
</tbody>
</table>

Pearson correlation analysis was performed to generate \( P \) values and Pearson \( r \) (\( R \)) coefficients. \( N \), population number. ¹Fluorescence was determined by calculating ‘\( T \) minus \( P \)’ as described in Materials and Methods.  *Correlation is significant at \( P < 0.05 \).
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 kilograms 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 with out 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/m².

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 HbA₁c 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 physio logical 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
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

Table 3 Associations of cardiovascular risk factors with physical performance parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Max jump force (cm)</th>
<th>Max handgrip strength (kg)</th>
<th>Number of chair rises/30 s</th>
<th>Per cent of predicted FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mm)</td>
<td>R = −0.103**</td>
<td>−0.038</td>
<td>−0.02</td>
<td>−0.073**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>0.084</td>
<td>0.391</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>N 1848</td>
<td>2114</td>
<td>1864</td>
<td>2108</td>
</tr>
<tr>
<td>LDL (mm)</td>
<td>R 0.002</td>
<td>0.001</td>
<td>−0.015</td>
<td>−0.019</td>
</tr>
<tr>
<td></td>
<td>P 0.941</td>
<td>0.967</td>
<td>0.533</td>
<td>0.383</td>
</tr>
<tr>
<td></td>
<td>N 1837</td>
<td>2099</td>
<td>1848</td>
<td>2093</td>
</tr>
<tr>
<td>HDL (mm)</td>
<td>R 0.021</td>
<td>−0.060**</td>
<td>0.055*</td>
<td>0.053*</td>
</tr>
<tr>
<td></td>
<td>P 0.375</td>
<td>0.006</td>
<td>0.017</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>N 1837</td>
<td>2099</td>
<td>1848</td>
<td>2093</td>
</tr>
<tr>
<td>Triglyceride (mm)</td>
<td>R = −0.064**</td>
<td>0.014</td>
<td>−0.083**</td>
<td>−0.086**</td>
</tr>
<tr>
<td></td>
<td>P 0.060</td>
<td>0.526</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>N 1837</td>
<td>2099</td>
<td>1848</td>
<td>2093</td>
</tr>
<tr>
<td>Cholesterol (mm)</td>
<td>R −0.01</td>
<td>0.004</td>
<td>0.014</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>P 0.678</td>
<td>0.837</td>
<td>0.534</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>N 1837</td>
<td>2099</td>
<td>1848</td>
<td>2093</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>R = −0.270**</td>
<td>0.135**</td>
<td>−0.208**</td>
<td>−0.079**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>N 1855</td>
<td>2122</td>
<td>1870</td>
<td>2118</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Max jump force (cm)</th>
<th>Max handgrip strength (kg)</th>
<th>Number of chair rises/30 s</th>
<th>Per cent of predicted FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mm)</td>
<td>b = −0.207**</td>
<td>−0.274**</td>
<td>0.018</td>
<td>−0.499</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>0.007</td>
<td>0.810</td>
<td>0.015</td>
</tr>
<tr>
<td>LDL (mm)</td>
<td>b 0.207</td>
<td>−0.438</td>
<td>−0.487</td>
<td>−2.308**</td>
</tr>
<tr>
<td></td>
<td>P 0.355</td>
<td>0.269</td>
<td>0.070</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL (mm)</td>
<td>b = −1.207**</td>
<td>−1.160</td>
<td>−0.884</td>
<td>−2.609</td>
</tr>
<tr>
<td></td>
<td>P 0.001</td>
<td>0.055</td>
<td>0.032</td>
<td>0.033</td>
</tr>
<tr>
<td>Triglyceride (mm)</td>
<td>b = −0.071</td>
<td>−0.160</td>
<td>−0.278</td>
<td>−1.397**</td>
</tr>
<tr>
<td></td>
<td>P 0.507</td>
<td>0.390</td>
<td>0.029</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mm)</td>
<td>b 0.024</td>
<td>0.493</td>
<td>0.577**</td>
<td>2.533**</td>
</tr>
<tr>
<td></td>
<td>P 0.891</td>
<td>0.106</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>b = −0.397**</td>
<td>0.239**</td>
<td>−0.332**</td>
<td>−0.466**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>b = −1.309**</td>
<td>−1.387**</td>
<td>−1.868**</td>
<td>−8.119**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arthritis</td>
<td>b = −0.871**</td>
<td>−0.558**</td>
<td>−1.136**</td>
<td>−1.135**</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>0.155</td>
<td>&lt;0.001</td>
<td>0.153</td>
</tr>
</tbody>
</table>

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.
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>
<thead>
<tr>
<th>Variable</th>
<th>LDL</th>
<th>HDL</th>
<th>Triglycerides</th>
<th>Total cholesterol</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>$R$ -0.109**</td>
<td>-0.016</td>
<td>0.034</td>
<td>-0.103**</td>
<td>0.078**</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.001$</td>
<td>0.477</td>
<td>0.12</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>$N$ 2104</td>
<td>2104</td>
<td>2104</td>
<td>2117</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>$R$ -0.094**</td>
<td>0.143**</td>
<td>0.790**</td>
<td>0.082**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.001$</td>
<td>0.001</td>
<td>$&lt; 0.001$</td>
<td>$&lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N$ 2105</td>
<td>2105</td>
<td>2105</td>
<td>2102</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>$R$ -0.457**</td>
<td>0.126**</td>
<td>-0.311**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.001$</td>
<td>0.001</td>
<td>$&lt; 0.001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N$ 2105</td>
<td>2105</td>
<td>2102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>$R$ 0.236**</td>
<td></td>
<td>0.239**</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.001$</td>
<td></td>
<td>$&lt; 0.001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N$ 2105</td>
<td>2102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>$R$</td>
<td></td>
<td></td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td></td>
<td></td>
<td>0.114</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N$</td>
<td></td>
<td></td>
<td>2102</td>
<td></td>
</tr>
</tbody>
</table>

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 Introduction, 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 associations 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 carried 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 cholesterol, we find that there is no correlate 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 significantly associated with vitality, it is perhaps a central risk factor, that is, it is affected by the blood chemistry 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 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 positively 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 relation 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 conclusively 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 (Babizhayev 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 cardiovascular 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 performance 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 muscle strength and high adiposity) is particularly detrimental 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 studies suggest that generally middle aged and older people 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 parameters (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 population 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 comparison 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 overlapping 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 gender. However, a weakness is that it does not allow us to assess sex or age differences, and thus is not indicative 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 linking 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; mitochondrial DNA cannot be detected by the techniques we have used in this study. Potential links of mitochondrial 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 subjective 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 fatigue. These associations give us new insight into the underlying physiological and cellular parameters that impact subjective vitality.

Our study also provides a uniquely comprehensive tabulation of the complex association of various common physical performance measures with cardiovascular risk factors (Tables 3 and 4) and the associations among the cardiovascular risk factors (Table 5). With respect to mechanisms and suggestions generated from data of this study, our data from Table 5 suggest that higher levels of glucose, triglycerides and LDL and lower HDL are partial determinants 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 a ‘convergent 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 maintaining 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 several underlying molecular events because DNA damage 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. Further 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.

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**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., A.M.H, D.M., L.B and S.H.S analysed and interpreted the data; A.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**


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


cise training, genetics and type 2 diabetes related pheno
types. *Acta Physiol (Oxf)* 205, 456 471.

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

of individual reference intervals in small sample sizes. *Int J
Hyg Environ Health* 210, 471 478.

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 criti
cally short telomeres in human osteoarthritic knees. *Artbri
tis Res Ther* 14, R12.

Harbo, M., Delaisse, J.M., Kjaersgaard Andersen, P., Soeren
between ultra short telomeres, aging of articular cartilage
and the development of human hip osteoarthritis. *Mech Ageng
Dev* 134, 367 372.

Hardy, R., Cooper, R., Aihie, S.A., Ben Shlomo, Y., Cooper,
C., Deary, I.J., Demakakos, P., Gallacher, J., Martin,
Kuh, D. 2013. Body mass index, muscle strength and phys
ical performance in older adults from eight cohort studies:
the HALCyon programme. *PLoS ONE* 8, e56843.

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 phys
ical performance in the Health, Aging and Body Com
position study. *Int J Obes (Lond)* 31, 1680 1687.

Prevalence of sarcopenia and predictors of skeletal muscle
mass in healthy, older men and women. *J Gerontol A Biol

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.

state relates to female oxidative DNA damage via neutro
phil activation. *Biochem Biophys Res Commun* 311, 1014
1018.

Jham, 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
1786.

between objectively measured habitual physical activity
and adiposity in children and adolescents: Systematic
review. *Int J Pediatr Obes* 3, 3 18.

Kanane, L., Purnekka, I., Pirkola, S., Suvisaari, J., Lonnqvist,
adversities are associated with shorter telomere length at
adult age both in individuals with an anxiety disorder and

Korwek, Z., Sewastianuk, T., Bielak Zmijewsk, A., Mosie
niak, 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., Farzanb, F., 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.

Klamer, C.M., Giacobini, J.P., Perusse, L., Lacaille, M.,
Yvon, C., Chagnon, M., Kuhn, F., Bouchard, C., Muzzin,
pling protein 3 gene and obesity related phenotypes in the

Kajajevic, G. & Golestas, 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 (Kazan)* 45, 85 94.

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

Metabolic syndrome in women with chronic pain. *Metabo
lism* 56, 87 93.

Ludlow, A.T., Ludlow, L.W. & Roth, S.M. 2013. Do telo
meres adapt to physiological stress? exploring the effect of
exercise on telomere length and telomere related proteins.

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

Maloney, E.M., Boneva, R.S., Lin, J.M. & Rees, W.C.
2010. Chronic fatigue syndrome is associated with meta
bolic syndrome: results from a case control study in Geor
gia. *Metabolism* 59, 1351 1357.

Mantz, M., de Leon, C.F., Rantanen, T., Era, P., Pedersen,


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.
A multi rule Shewhart chart for quality control in clinical
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
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

**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.