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Differential effects of endurance, interval, and resistance training on telomerase activity and telomere length in a randomized, controlled study

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Aims It is unknown whether different training modalities exert differential cellular effects. Telomeres and telomere-associated proteins play a major role in cellular aging with implications for global health. This prospective training study examines the effects of endurance training, interval training (IT), and resistance training (RT) on telomerase activity and telomere length (TL).

Methods and results One hundred and twenty-four healthy previously inactive individuals completed the 6 months study. Participants were randomized to three different interventions or the control condition (no change in lifestyle): aerobic endurance training (AET, continuous running), high-intensive IT (4 × 4 method), or RT (circle training on 8 devices), each intervention consisting of three 45 min training sessions per week. Maximum oxygen uptake (VO_{2max}) was increased by all three training modalities. Telomerase activity in blood mononuclear cells was up-regulated by two- to three-fold in both endurance exercise groups (AET, IT), but not with RT. In parallel, lymphocyte, granulocyte, and leucocyte TL increased in the endurance-trained groups but not in the RT group. Magnet-activated cell sorting with telomerase repeat-ampliflication protocol (MACS-TRAP) assays revealed that a single bout of endurance training—but not RT—acutely increased telomerase activity in CD14+ and in CD34+ leucocytes.

Conclusion This randomized controlled trial shows that endurance training, IT, and RT protocols induce specific cellular pathways in circulating leucocytes. Endurance training and IT, but not RT, increased telomerase activity and TL which are important for cellular senescence, regenerative capacity, and thus, healthy aging.

Keywords Exercise • Cellular senescence • Telomere length • Telomerase activity • Endurance training • Resistance training

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Translational perspective

Exercise is a cornerstone of cardiovascular disease prevention. This randomized, controlled, and prospective training study shows that specific training protocols lead to differential effects on cellular aging. Aerobic endurance and high-intensive interval training, but not resistance training, increases telomerase activity and telomere length in blood mononuclear cells. The study identifies telomerase activity and telomere length as sensitive cellular parameters to measure preventive effects of exercise interventions. Individual training recommendations guided by measurement of parameters associated with cellular senescence and training response may improve both adherence to and efficacy of exercise training programmes in cardiovascular prevention.

Introduction

Physical training is associated with improvements in many aspects of human health. These include exercise capacity, endothelial function, insulin sensitivity, autonomic function, and blood pressure as well as a reduction of abdominal fat, lipid metabolism, adverse psychosocial, and inflammatory parameters.[1,2] Endurance training and resistance training (RT) alone or in combination are recommended for cardiovascular disease prevention.[2–4] Compared with conventional aerobic endurance training (AET), high-intensity interval training (IT) is believed to be a more effective and time-efficient training method for improving cardiorespiratory fitness.[5] However, few randomized comparisons of different training modalities have been reported, and it is unknown whether they exert differential cellular effects.

Vascular aging is associated with endothelial dysfunction and atherogenesis.[6] A key cellular process of aging is the shortening of telomeres to a critical telomere length (TL) where cells enter replicative senescence or programmed cell death.[7] Telomere length in circulating leucocytes is associated with cardiovascular morbidity.[6,8–11] Telomere length is regulated by the reverse transcriptase telomerase that prevents successive shortening of telomeres.[7,12] The regulation of telomerase activity and TL in blood mononuclear cells (MNCs) parallels the regulation in the vessel wall and telomeres shorten at similar rates in somatic tissues of adults.[8,10,11,13,14]

Beneficial effects of physical activity on cellular regeneration and senescence have been observed.[10,12,13,15–19] For example, long-term endurance training is associated with higher telomerase activity and a reduced rate of telomere attrition in young- and middle-aged endurance athletes compared with inactive controls.[13] Twins with a higher level of physical activity exhibit longer telomeres in middle age compared with inactive siblings.[12] Moderate levels of physical activity may reduce telomere shortening by inducing a telomere-protective phenotype, indicating an important cellular adaptation that may prevent age-related diseases.[18] However, prospective studies are lacking, and the effect of different exercise modalities is unknown.[12]

Herein, we compare the long-term effects of three exercise modes, namely AET, high-intensity IT, and RT, with a sedentary control group on telomerase activity and TL in a prospective, randomized study in a primary prevention cohort. In addition, we measured the acute effects of endurance training and RT on telomerase activity in leucocytes subpopulations in a cross-over study.

Methods

Acute effects of aerobic endurance and resistance exercise

Exercise testing was performed by bicycle spiroergometry (Innocor 500; Innovision, Denmark; 30 W/min ramp protocol) in young healthy, moderately trained individuals. The acute exercise study consisted of two supervised exercise bouts of running the maximum distance for 45 min at separate days in a cross-over design, $n = 15$. Resistance exercise was circle training on eight strength training devices (details in Supplementary material online, *Methods*).

Prospective training study

The flowchart of the trial is illustrated in *Figure 2*. In total, 1534 volunteers were screened. Two hundred and sixty-six healthy, physically inactive non-smokers without permanent medication were randomized. The main inclusion criteria were selected to represent the typical beginner of a primary preventive training programme: age 30–60 years, a body mass index (BMI) below 30 kg/m^2, and a lack of regular physical exercise (<1 h/week in the last 12 months; VO_{2max} <45 mL/kg/min). The exclusion criteria were smoking, known cardiovascular disease, cardiovascular risk factors requiring medication (including fasting blood glucose concentration <6.1 mmol/L, resting blood pressure >160/100 mmHg, and presence of iron deficiency/anaemia), any condition or disease that may interfere with the training protocol. Subjects were recruited by advertisements in local media. Sex and VO_{2max} were used as criteria for stratified randomization with three strata for VO_{2max} (<30 mL/kg/min; 30–40 mL/kg/min and >40 mL/kg/min). During the study, 119 subjects dropped out, primarily because of lack of compliance with the training protocol. The rate of drop-outs and injuries was higher in the two endurance groups. Twenty-three individuals had to be excluded due to lack of exhaustion on the stress tests or missing samples.

Exercise testing

Exhaustive individual exercise tests were carried out using a combined step-wise and ramp-wise protocol as described in detail in the Supplementary material online, *Methods*.[20]

Training interventions

The details are described in the Supplementary material online, *Methods*. Subjects in the control group were advised to maintain their lifestyle and diet for the next 6 months. Subjects in the training groups exercised three times per week for 26 weeks. Aerobic endurance training consisted of 45 min of walking/running at 60% heart rate reserve. Interval training was performed according to the high-intensity 4 × 4 method. Resistance training was a circle training of eight machine-based exercises: back extension, crunch, pull-down, seated row, seated leg curl, seated leg extension, seated chest press, and lying leg press. Twenty repetition

maximum (RM) was determined every 6 weeks and training weights were adjusted.

Isolation of mononuclear cells

Mononuclear cells were isolated by Ficoll density gradient centrifugation before, after, and after 24 h in the acute exercise study.[13] In the prospective exercise study, MNCs were isolated at baseline and 48 h to 7 days after that last exercise bout after completion of the 6 months training intervention or control condition (see Supplementary material online, *Methods* for details).

Telomerase activity

Telomerase activity was quantified using the Telomerase Repeat Amplification Protocol[10,13,16,21] in protein extracts of 10 000 MNCs or MACS-isolated leucocyte subpopulations using a Lightcycler (Roche, Germany). Protein extracts from human embryonic kidney (HEK) cells were measured as positive controls in each assay, a 10-point standard titration curve was established from 5 to 2500 cells to ensure linearity (Supplementary material online, *Figure S2*, R^2 = 0.99). Details are described in the Supplementary material online, *Methods*.

Telomere length

Telomere length in MNCs and different leucocyte subpopulations was determined by three separate methods, namely flow cytometry and FISH (FlowFISH) was well as by real-time PCR as described. Details are given in the Supplementary material online, *Methods*. Southern blots were performed from the genomic DNA to standardize conversion of fluorescence values to base pairs.

PCR method

Briefly, 40 ng of genomic DNA were used for real-time PCR using 150 nmol/L primers for the single copy gene 36b4 (S) and telomere strands (T).[8,13,22] Experiments were run on a StepOnePlus Real-Time PCR System (Applied Biosystems). The comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$) was used to calculate telomere/single copy gene (T/S) ratios.

FlowFISH method

A telomere-specific green fluorescent peptide nucleic acid (PNA) probe (FITC-PNA, Panagene, Korea, via Cambridge Research Biochemicals, UK; sequence: FITC-OO-CCCTAA-CCCTAA-CCCTAA) was used to specifically bind telomeres.[13,23] Telomere fluorescence intensity was measured in different blood cell populations, namely lymphocytes and granulocytes as well as in bovine thymocytes from a single isolation, which were used as an internal standard in each sample.[23] Only MNCs with effective DNA counterstaining and effective PNA hybridization were gated. Lymphocytes and granulocytes were differentiated from thymocytes by scatter graphs, DNA signal, and gating in the FITC channel. From fluorescence intensities, molecules of soluble fluorescence (MESF) were calculated using calibration beads with defined fluorescence intensity. Additional validation experiments are listed in the Supplementary material online, *Methods*.

Southern blot determination of terminal restriction fragment length

The detailed procedure using the TeloTAGGG Telomere Length Assay Kit is described in the Supplementary material online, *Methods*. Terminal restriction fragment values of MNC samples of 20 participants of the chronic exercise study were plotted against their corresponding FlowFISH-based MESF values to generate a standard curve (Supplementary material online, *Figure S4*), which was used to calculate the TLs for the other samples.

Fluorescence-activated cell sorting (FACS) assay of leucocyte subpopulations in acute and chronic exercise

Characterization of leucocyte subpopulations was performed by multicolour flow cytometry (see Supplementary material online, *Methods*).

Telomerase activity in magnetic bead-isolated leucocyte subpopulations

CD34+ haematopoietic stem/progenitor cells as well as defined subpopulations of peripheral MNCs were isolated by magnetic-activated cell sorting (MACS) with magnetic bead-coated antibodies (Miltenyi Biotec MicroBeads; CD34, 130-046-703; CD4: 130-045-101; CD8: 130-045-201; CD14: 130-050-201; CD19: 130-050-301; CD56: 130-050-401) followed by magnetic enrichment and TRAP assay. A scheme of the isolation is provided in Supplementary material online, *Figure S6*. Tests of purity and viability are provided in Supplementary material online, *Figure S7*.

Quantification of cortisol and serum markers of exercise

Analysis of serum cortisol was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described. As control for extreme physical activity, samples from $n = 10$ participants of the 38th Berlin Marathon were included.[24] For more details see the Supplementary material online, *Methods*.

Statistical methods

Data are presented as individual data points with mean (bars) and standard deviation (SD, whiskers). For the two acute exercise cross-over studies, changes of telomerase activity over time were analysed by one-way analysis of variance (ANOVA) for repeated measurements within each training modality (*post hoc* test for linear trend). In the randomized controlled study, baseline characteristics between groups were compared by one-way ANOVA. Changes in blood composition, serum markers, chemistry, clinical variables, and physical fitness during the study period were analysed using *t*-tests for related samples and two-way ANOVA for repeated measurements (repeat factor: test; categorical predictor: group; *post hoc* test: Bonferroni's multiple comparison test) to compare groups. Changes of TL and telomerase activity within groups were analysed with *t*-tests and differences between groups were tested with one-way ANOVA and the Bonferroni *post hoc* tests for multiple comparisons. Generalized estimating equation (GEE) mixed models were employed to test the influence of study group (dummy coded), age, gender, BMI, and baseline VO_{2max} on the changes TL and telomerase activity from pre- to post-test. Missing samples were excluded. Kolmogorov–Smirnov tests were used to test the hypothesis of normal distribution and all variables used in GEE models were normally distributed. Associations between molecular regulators, clinical variables, and parameters of exercise capacity were tested by two-sided Pearson correlation. Differences in telomerase activity stratified by training response were analysed by *t*-tests. Analyses were performed with SPSS 20.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 5.01 (San Diego, CA, USA). *P*-values <0.05 were considered significant.

Study approval

The clinical study has been conducted according to the principles of Helsinki and was approved after institutional review by the ethics committee of the Ärztekammer des Saarlandes (No. 148/10) and registered at clinicaltrials.gov (NCT01263522). Written informed consent was received from all participants prior to any study-related procedure. Names of study subjects were blinded by pseudonymization.

Table 1 Baseline characteristics in the prospective 6 months training study

	Control group	Aerobic endurance training	Interval training	Resistance training	*P*-value
N = 124	35	26	29	34	
Gender (M/F)	12/23	9/17	10/19	14/20	
Age (years)	50.2 (7.4)	49.5 (7.0)	48.4 (6.5)	48.1 (7.5)	0.61
Clinical characteristics					
Body mass index (kg/m^2)	24.2 (3.1)	23.8 (3.3)	24.5 (3.0)	24.8 (3.4)	0.64
Systolic BP (mmHg)	131 (16)	132 (15)	125 (27)	132 (13)	0.35
Diastolic BP (mmHg)	84 (10)	86 (11)	82 (17)	86 (7)	0.63
Resting heart rate (b.p.m.)	64 (10)	65 (8)	68 (11)	67 (9)	0.32
Clinical chemistry					
Fasting glucose (mg/dL)	96 (9)	96 (6)	98 (8)	96 (8)	0.44
Fasting insulin (µIU/mL)	4.60 (2.2)	4.48 (2.3)	6.11 (2.6)	5.19 (2.6)	0.05
Total cholesterol (mg/dL)	212 (40)	224 (43)	212 (36)	206 (44)	0.41
HDL cholesterol (mg/dL)	55 (20)	58 (21)	53 (17)	56 (16)	0.77
LDL cholesterol (mg/dL)	105 (31)	114 (36)	106 (32)	102 (32)	0.59
Treadmill stress test					
Peak heart rate (b.p.m.)	178 (13)	183 (10)	178 (10)	182 (11)	0.19
VO_{2max} (mL/min/kg)	35.1 (5.3)	35.3 (6.3)	35.1 (5.0)	35.3 (5.3)	0.99
Maximum speed (km/h)	10.0 (1.5)	9.9 (1.6)	10.0 (1.2)	10.0 (1.5)	0.99
At 130 b.p.m.	5.5 (2.4)	4.5 (3.1)	4.5 (2.8)	5.6 (1.9)	0.14
At 150 b.p.m.	7.2 (1.3)	7.2 (0.9)	6.9 (0.6)	7.2 (1.1)	0.56
At lactate 2 mmol/L	4.9 (3.0)	4.6 (3.2)	4.7 (3.0)	5.3 (2.6)	0.82
Respiratory exchange rate	1.17 (0.10)	1.12 (0.09)	1.10 (0.13)	1.11 (0.22)	0.28

Data are represented as mean (SD).
BP, blood pressure; b.p.m., beats per minute; F, female; M, male; VO_{2max}, maximum oxygen uptake.

Results

Differential regulation of telomerase activity in mononuclear cell and MACS-isolated leucocyte subpopulations by acute exercise

The acute effects of aerobic endurance vs. resistance exercise were studied in *N* = 15 healthy volunteers in a cross-over design (Supplementary material online, *Table S1*). All individuals performed supervised cardiopulmonary exercise testing until exertion (mean respiratory coefficient 1.1), which showed no cardiac or pulmonary limitation and a moderately trained status (VO_{2max} 41.4 ± 6.3 mL/min/kg). Volunteers ran a mean distance of 7.3 ± 1.5 km and had a mean exercise heart rate of 155 ± 8 b.p.m. and a peak exercise heart rate of 178 ± 7 b.p.m., equalling a high-endurance intensity of 0.83 as calculated by the Karvonen formula. In the resistance exercise group, a mean weight of 10.3 ± 4.2 tons was moved.

Aerobic endurance training acutely increased telomerase activity (pre: 189 ± 90, post: 250 ± 140, and 24 h: 306 ± 182 HEK cell equivalents) (*Figure 1A*). This regulation was not observed when the same individuals performed intensive RT for the same duration. As depicted in Supplementary material online, *Table S2*, the endurance protocol increased absolute numbers of leucocytes, neutrophilic, and basophile granulocytes and monocytes. After 24 h, the blood counts returned to the baseline levels. Compared with these profound acute changes of blood composition after the endurance exercise bout, changes were much smaller after resistance exercise. Fluorescence-activated cell sorting (FACS) analyses showed that different T cell populations (T-helper, T-killer, and activated T cells) were increased and there was a numeric trend of increased B and NK cells. The strongest up-regulation was seen in CD34+ haematopoietic stem/progenitor cells (Supplementary material online, *Table S3*). However, this pattern was not observed after resistance exercise. CD14+ monocytes and CD34+ progenitor cells exhibited a potent increase of telomerase activity directly and 24 h after endurance exercise (four-fold in CD14+ and six-fold in CD34+ cells, *Figure 1B* and *C*), whereas RT was not associated with these changes. Telomerase activity was not detectable in CD4+, CD8+, CD19+, and CD56+ cells.

Prospective training study

The design of the randomized controlled training study is illustrated in *Figure 2*. The training groups were similar with respect to clinical characteristics, glucose and lipid parameters, and physical fitness. *Table 1* shows the baseline characteristics. The maximum oxygen uptake (VO_{2max}) was 35.2 ± 5.4 mL/min/kg. The 6 months training interventions improved physical fitness. All three exercise modalities led to a comparable increase of the ΔVO_{2max} (in mL/min/kg: control -1.0 ± 3.1; AET 2.7 ± 3.7; IT 2.8 ± 5.1; RT 3.0 ± 5.9). Maximum running speed as an indicator of maximum physical capacity was increased in

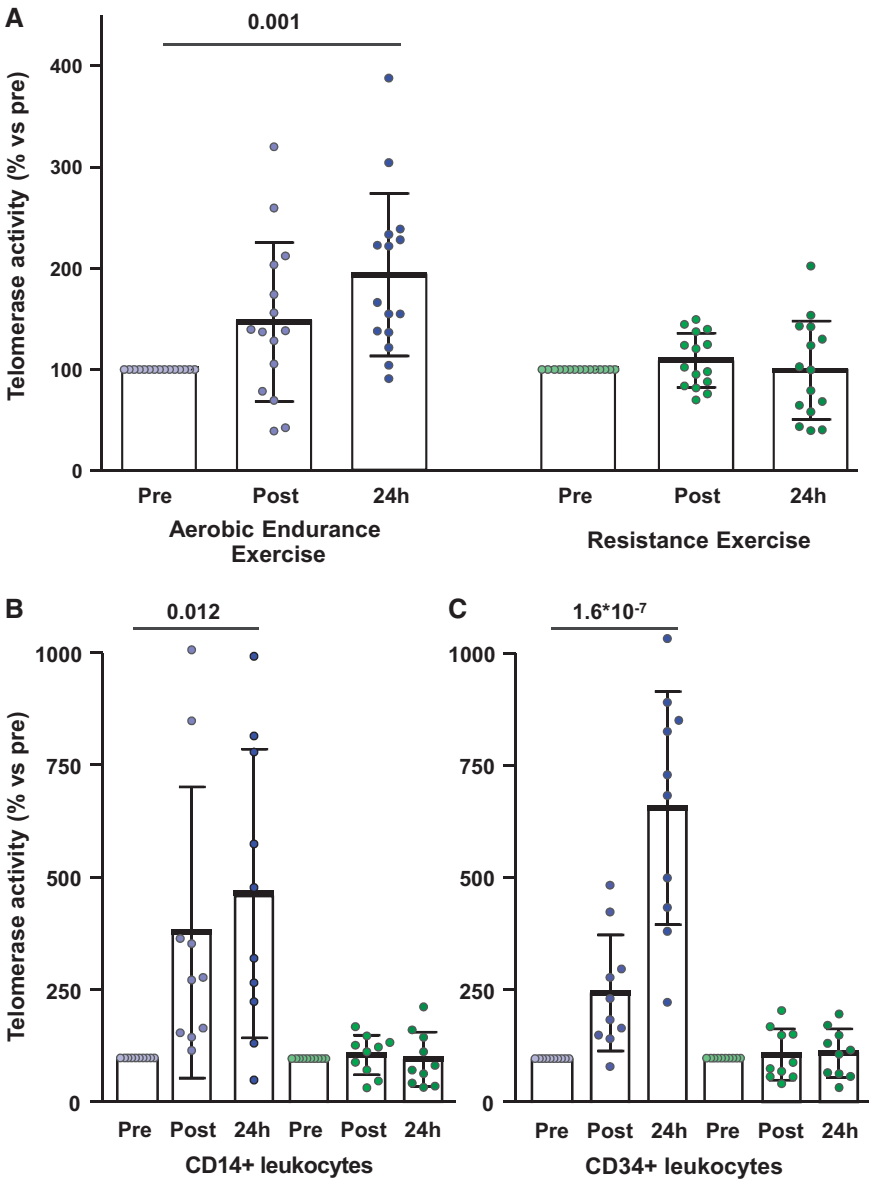


Figure 1 Acute effects of aerobic endurance vs. resistance exercise on telomerase activity. (*A*) Cross-over comparison of acute regulation of mononuclear cell telomerase activity pre, post, and 24 h after aerobic endurance training (45 min continuous running) and resistance exercise (45 min circle training on eight strength devices) in $n = 15$ healthy young individuals. Exercise was performed at least 48 h after any previous physical exercise. Telomerase activity of 10^4 mononuclear cell was compared with human embryonic kidney cells (telomere repeat amplification protocols, TRAP assay). Time course of individual % changes of telomerase activity in 10^4 magnetic-activated cell sorting-isolated (*B*) CD14+ and (*C*) CD34+ leucocyte subfractions compared with pre-exercise in $n = 10$ healthy young individuals (blue circles: endurance exercise; green circles: resistance exercise).

all training groups. All training modalities reduced resting heart rate. Data depicted in *Table 2* and Supplementary material online, *Table S4*.

Differential regulation of leucocyte telomere length by endurance and resistance training

Telomere length was measured by FACS-FlowFISH and real-time PCR. After 6 months, a small decline of TL was observed in the control group and in the RT group. In contrast, the two endurance-based training groups, the AET, and the IT, showed a significant increase of both lymphocyte and granulocyte TL (AET: Δ lymphocyte TL 218 ± 211 bp, Δ granulocyte TL 248 ± 349 bp; IT: Δ lymphocyte TL 214 ± 307 bp, Δ granulocyte TL 261 ± 332 bp; *Figure 3A* and *B*). Comparisons of the relative intra-individual changes of TL yielded a similar result with a non-significant decline of TL in the control and RT groups, while lymphocytes and granulocytes in the AET and IT groups were characterized by 3.3–3.5% longer telomeres (*Figure 3D*

Table 2 Effects of 6 months training on clinical parameters and physical fitness

	Co	Aerobic endurance training		Interval training		Resistance training	
	Δ post vs. pre	Δ post vs. pre	*P* vs. Co	Δ post vs. pre	*P* vs. Co	Δ post vs. pre	*P* vs. Co
Clinical parameters							
BMI (kg/m^2)	0.0 (0.8)	-0.5 (1.0)	**0.041**	-0.5 (0.9)	**0.015**	-0.2 (0.8)	0.18
Systolic BP (mmHg)	2.7 (11.1)	-1.3 (12.2)	0.20	1.8 (9.7)	0.75	-3.2 (9.4)	**0.022**
Diastolic BP (mmHg)	0.0 (7.3)	-2.2 (8.5)	0.28	-1.8 (5.3)	0.29	-2.8 (8.3)	0.14
Resting HR (b.p.m.)	-2.7 (6.0)	-5.4 (6.2)	0.11	-6.8 (7.3)	**0.020**	-6.0 (8.1)	0.07
Fasting glucose (mg/dL)	-0.7 (9.3)	-3.4 (6.2)	0.21	0.2 (7.1)	0.67	-0.7 (7.3)	0.97
Fasting insulin (μIU/mL)	0.06 (2.23)	-0.31 (1.59)	0.47	-0.55 (2.28)	0.29	-0.37 (1.91)	0.39
Total cholesterol	3.6 (25.5)	-10.62 (19.6)	**0.023**	1.21 (21.5)	0.69	3.09 (28.8)	0.94
Treadmill stress test							
Peak heart rate (b.p.m.)	1.1 (6.0)	-2.5 (7.8)	0.06	-4.8 (5.2)	**<0.001**	0.2 (6.1)	0.56
VO_{2max} (mL/min/kg)	-1.0 (3.1)	2.7 (3.7)	**<0.001**	2.8 (5.1)	**0.001**	3.0 (5.9)	**0.001**
Maximum speed (km/h)	-0.1 (0.6)	1.1 (0.6)	**<0.001**	1.4 (0.6)	**<0.001**	0.4 (0.7)	**0.002**
At 130 b.p.m.	0.2 (0.4)	0.4 (0.6)	**0.032**	0.6 (0.5)	**0.001**	0.2 (0.4)	0.82
At 150 b.p.m.	0.2 (0.4)	0.7 (0.7)	**<0.001**	1.0 (0.5)	**<0.001**	0.2 (0.4)	0.98
At lactate 2 mmol/L	-0.1 (0.6)	0.8 (0.6)	**<0.001**	0.5 (0.7)	**0.019**	-1.0 (0.5)	0.94
RER	-0.04 (0.10)	-0.02 (0.12)	0.45	0.00 (0.16)	0.24	-0.10 (0.13)	**0.032**

Data are represented as mean (SD).
Significant differences to pre time-point (2-way ANOVA) are in bold face.
b.p.m., beats per minute; BMI, body mass index; BP, blood pressure; Co, control group; HR, heart rate; RER, respiratory exchange ratio.

and *E*). GEE mixed models confirmed significantly longer telomeres at the post-time point in the AET and IT groups, but not in the control and RT groups. Inclusion of baseline variables such as age, gender, BMI, and baseline VO_{2max} did not alleviate this association (Supplementary material online, *Table S6*). In the second assay, leucocyte telomere length (LTL) was measured in DNA isolates of MNCs by real-time PCR. While the control and RT groups exhibited no significant change after 6 months, LTL significantly increased in both the AET and IT groups (*Figure 4A*).

Differential regulation of telomerase activity by endurance and resistance training

The trial revealed no significant change of telomerase activity in the control group and in the resistance exercise group. In contrast, 6 months of AET or high-intensity IT increased telomerase activity by two-fold. The mean relative changes of telomerase activity were control 124 ± 74%, AET 298 ± 334%, IT 264 ± 231%, and RT 169 ± 114% ($P < 0.05$ for AET and IT vs. control and RT, *Figure 4B*). Changes of telomerase activity correlated with changes in lymphocyte TL (Δ telomerase vs. Δ lymphocyte TL: $R = 0.26$, $P = 0.009$).

Effects of training on cell numbers and telomerase activity in leucocytes subpopulations

In the chronic exercise study, cell numbers of leucocytes, monocytes, lymphocytes, CD3+, CD4+, CD8+, CD19+, CD19+, CD16+/56+, and CD3+HLA-DR+ did not differ between the four study groups and did not differ between the start and the end of the training period. CD34+ haematopoietic progenitor cells were markedly up-regulated by endurance training and IT, but not in the control or the resistance groups (Supplementary material online, *Table S5*).

Insulin-like growth factor-1, alpha-Klotho, irisin, and cortisol concentrations do not correlate with the long-term training effects on telomeres

Insulin-like growth factor-1, alpha-Klotho, and irisin have been proposed to correlate with exercise-induced effects.[16,20,25,26] Alpha-Klotho was discretely up-regulated after 6 months endurance exercise (Supplementary material online, *Table S4*). No differences were observed for IGF-1 and irisin in the long-term training groups. In the acute exercise study, IGF-1 showed a biphasic response after both running and RT (Supplementary material online, *Table S2*) as described.[27]

As positive control, cortisol was quantitated in $n = 10$ participants of the Berlin Marathon.[24] As expected, the physical stress of a marathon markedly increased serum cortisol by 5.5-fold. A 24 h after the marathon, cortisol levels had returned to baseline levels (*Figure 5A*). In the acute training study, we observed a minor increase of cortisol by 17.8% immediately after running and no change after the resistance protocol. In the prospective long-term exercise study, cortisol serum concentrations were similar between all groups and did not differ before and after the training period (*Figure 5B*). In the situation of chronic endurance training, the observed chronic changes of telomerase activity appear to be independent of stress hormones.

Nitric oxide (NO) synthases are closely involved in the cellular mechanisms of exercise.[15] Protective signalling effects of running in the vessel wall depend on both endothelial NO synthase and telomerase activity.[13] In blood cells, the inducible isoform of NOS

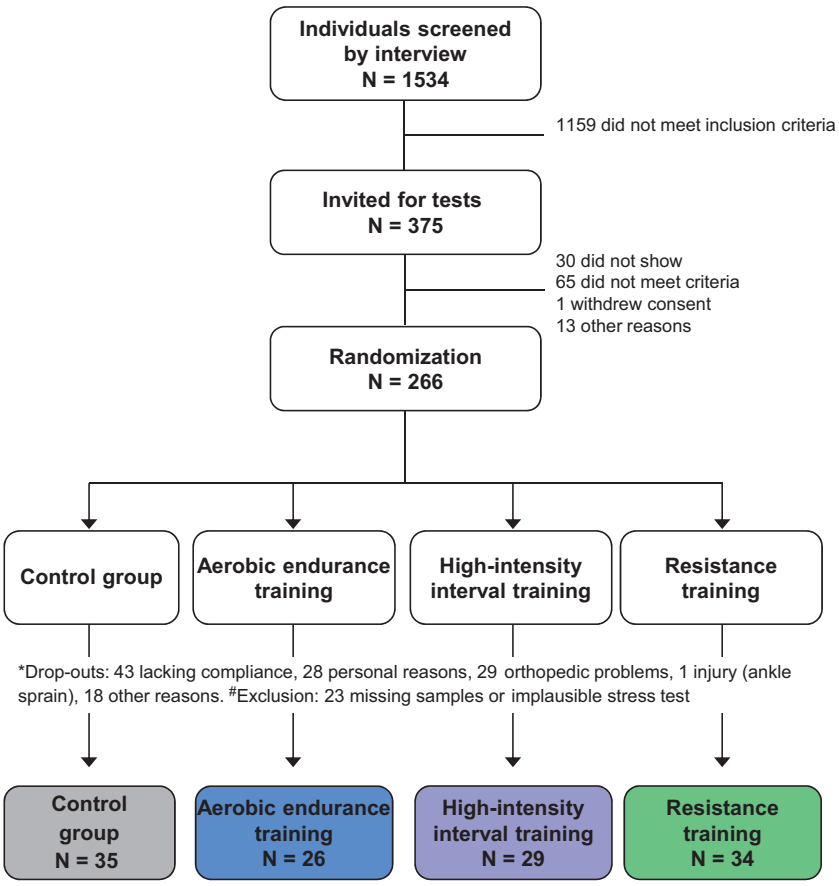


Figure 2 Design of the prospective, randomized, and controlled 6 months training study. Individuals enrolled in the study were randomized to the control group (no change of inactive life style) or one of three training groups (three sessions per week, 45 min each): (i) aerobic endurance training (continuous running); (ii) high-intensity interval training (4 × 4 method); (iii) resistance training: circle training on eight strength–endurance training devices. Blood samples were drawn at baseline (pre) and after 6 months (post). *Discontinuation for orthopaedic reasons was not training-associated. #Plausible stress test was defined as a respiratory exchange ratio of >1.00 in both stress tests.

(iNOS) is predominant. Recent evidence revealed that iNOS is crucial for arterial collateral formation in the context of running.[17] Here, iNOS mRNA expression was up-regulated in both endurance exercise groups, but not with RT (*Figure 5C*).

Association of training response with cellular response

We hypothesized that the cellular response could be predicted by changes in physical performance. The mean VO_{2max} increase between training groups was comparable (Table 2, Supplementary material online, *Table S4*), however, in individuals with a training effect above the mean of ΔVO_{2max} increases, the activity of the telomerase after 6 months training was higher compared with weaker responders (*Figure 5D*). Changes in TL were not associated with changes of VO_{2max}.

Discussion

This is the first prospective, randomized controlled training trial assessing the effects of different training protocols on telomerase activity and LTL in a primary prevention cohort. The main novel finding of the study is that the activity of the telomerase and TL in circulating MNCs is increased by endurance training and by high-intensive IT but not after RT. These results characterize the cellular 'anti-aging' effects of exercise, imply that telomeres adapt to physiological stress and identify differential cellular effects of established training modalities (*Take home figure*). In middle-aged subjects without a relevant training history, completing a training programme of moderate or intensive endurance exercise led to potent 'anti-aging effects' in circulating blood cells, consistent with beneficial effects on cardiovascular health in the long term.

Telomeres cap the ends of all eukaryotic chromosomes and are a prerequisite of genomic stability.[6] During the process of natural aging of somatic cells, TL is one of the major determinants of the cells' capability to divide and function. The process of telomere attrition plays both a causal as well as a potentiating role in human disease processes and is susceptible to life style factors.[7,10,28] Telomere length in circulating leucocytes has been linked to cardiovascular risk factors and diseases.[6,8–11,29] Physical exercise is a regulator of cellular senescence and TL.[10,12,13,16,18,19] However, previous training studies in

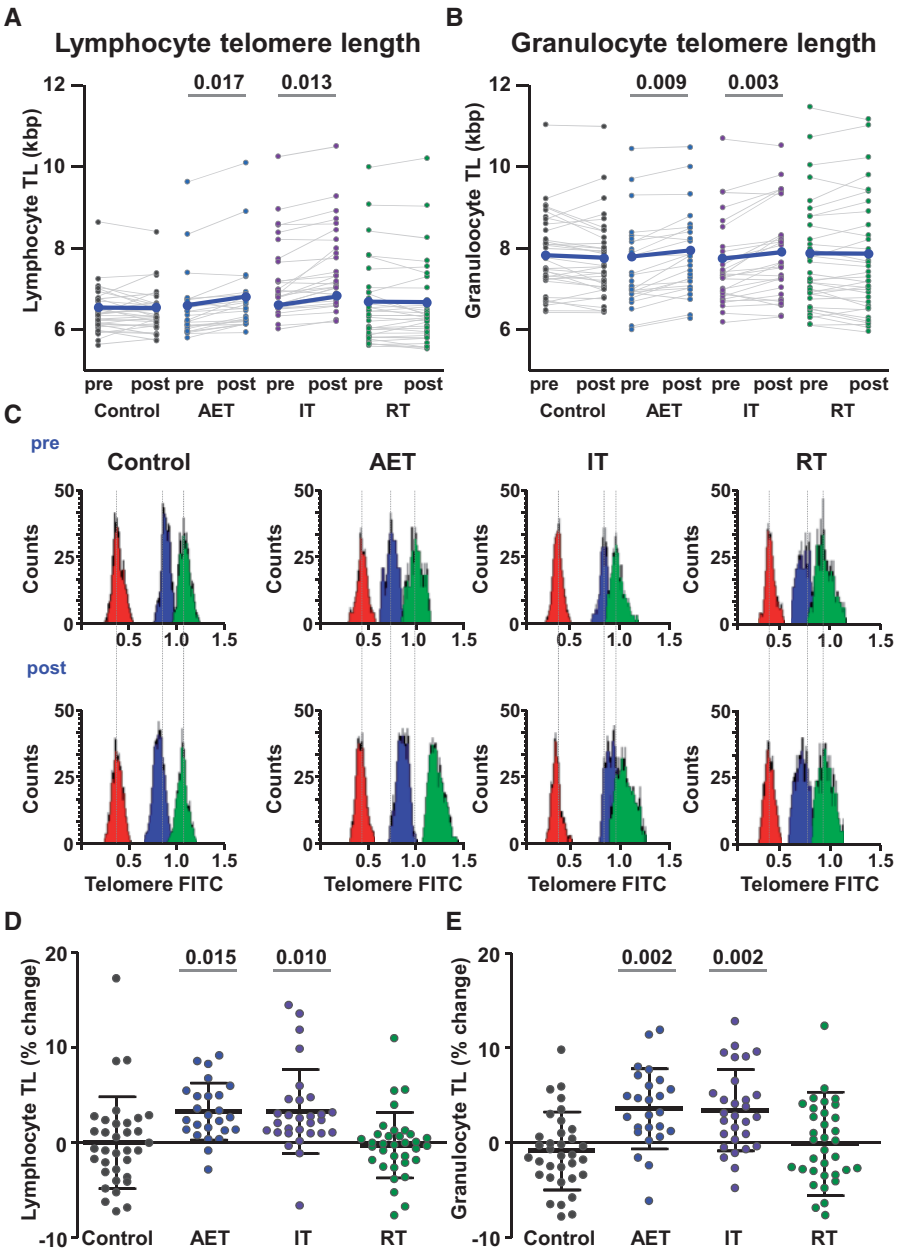


Figure 3 Endurance but not resistance training induces telomere elongation in lymphocytes and granulocytes. Telomere length was measured in mononuclear cells using the FlowFISH method. Absolute values of telomere length (kbp) were calculated using Southern blot standardization. Individual absolute values of (*A*) lymphocyte and (*B*) granulocyte telomere length pre- and post-study time points for each group and means of each group are shown as bold blue bars. AET, aerobic endurance training; IT, interval training; RT, resistance training. (*C*) FlowFISH FACS blots showing telomere fluorescence intensity peaks for a representative subject of each study group (columns) and pre- vs. post-time points (upper and lower row, respectively). Red peaks are the internal standard (bovine thymocytes) in each sample, blue peaks are lymphocytes, and green peaks are granulocyte measurements. Dotted lines are for visualization of rightward or leftward shifts in the peaks during the study. (*D*) Mean and SD of relative individual changes of lymphocyte (left panel) and (*E*) granulocyte (right panel) telomere length for each group.

humans did not provide a conclusive answer with regard to telomere biology because of lack of prospective randomization, a control group, methodological homogeneity, and sufficient duration.[12] To our knowledge, no randomized controlled prospective studies comparing different exercise modalities with respect to cellular senescence have been performed to date.

Data on the acute effects of exercise on TL and telomere-associated proteins after a single or a few bouts of exercise are scarce, especially with regard to RT.[30,31] Here, we present the first data of a cross-over study in healthy young volunteers directly comparing endurance and resistance exercise. A single bout of AET (running) acutely up-regulated MNC telomerase activity. The novel and

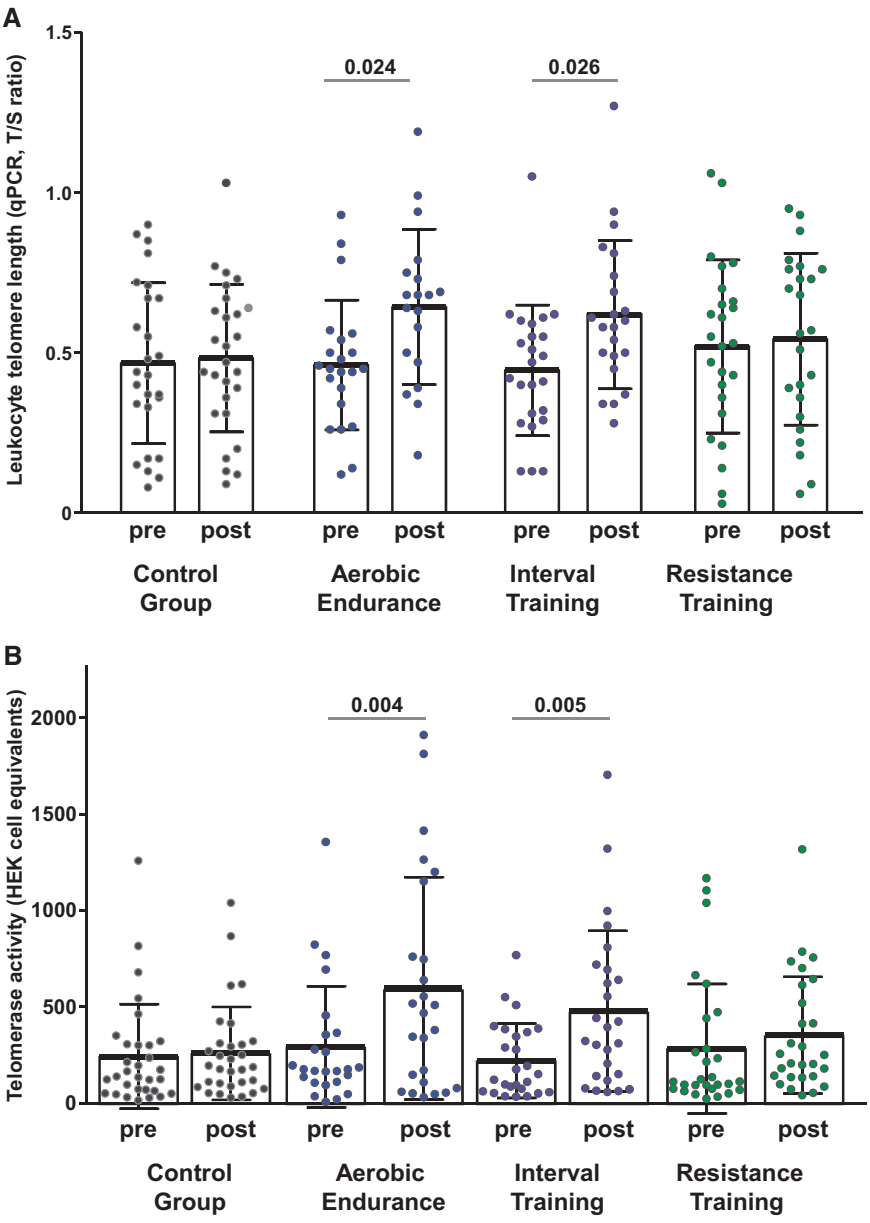


Figure 4 Differential effects of training on telomerase activity and telomere length in circulating mononuclear cells. (*A*) Leucocyte telomere length was determined in genomic DNA isolates by real-time PCR. Beside telomere (T) DNA, the single (S) copy gene 36b4 was amplified to allow calculation of T/S ratio for each subject at both time points. Data are represented as mean ± SD of T/S ratio per group. (*B*) Telomerase activity was determined by TRAP assay. Data are represented as mean ± SD of telomerase activity expressed as human embryonic kidney cell equivalents.

unexpected finding is that circle training on eight strength devices did not induce these cellular effects in the same individuals. Magnet-activated cell sorting with telomerase repeat-amplification protocol (MACS-TRAP) assays revealed increased telomerase activity in CD34+ and in CD14+ cells that was observed immediately after endurance exercise and persisted after 24 h. Similarly, up-regulation of telomerase activity was specific for the endurance protocol.

The participants in the training groups exercised three times per week for 26 weeks. The blood draw was performed between 48 h and 7 days after the last training bout. In addition to the comparison of traditional endurance training and RT, high-intensity IT was included. Low-volume high-intensity interval exercise may induce more potent effects on fitness and metabolism, both in healthy individuals as well as patients with heart failure.[5] The IT led to comparable changes in physical fitness compared with AET. These data confirm the recent SAINTEX-CAD study.[32] While acute endurance exercise-induced up-regulation of leucocytes,[33] the cell numbers of leucocytes, monocytes, lymphocytes, CD3+, CD4+, CD8+, CD19+, CD19+, CD16+/56+, CD3+, and

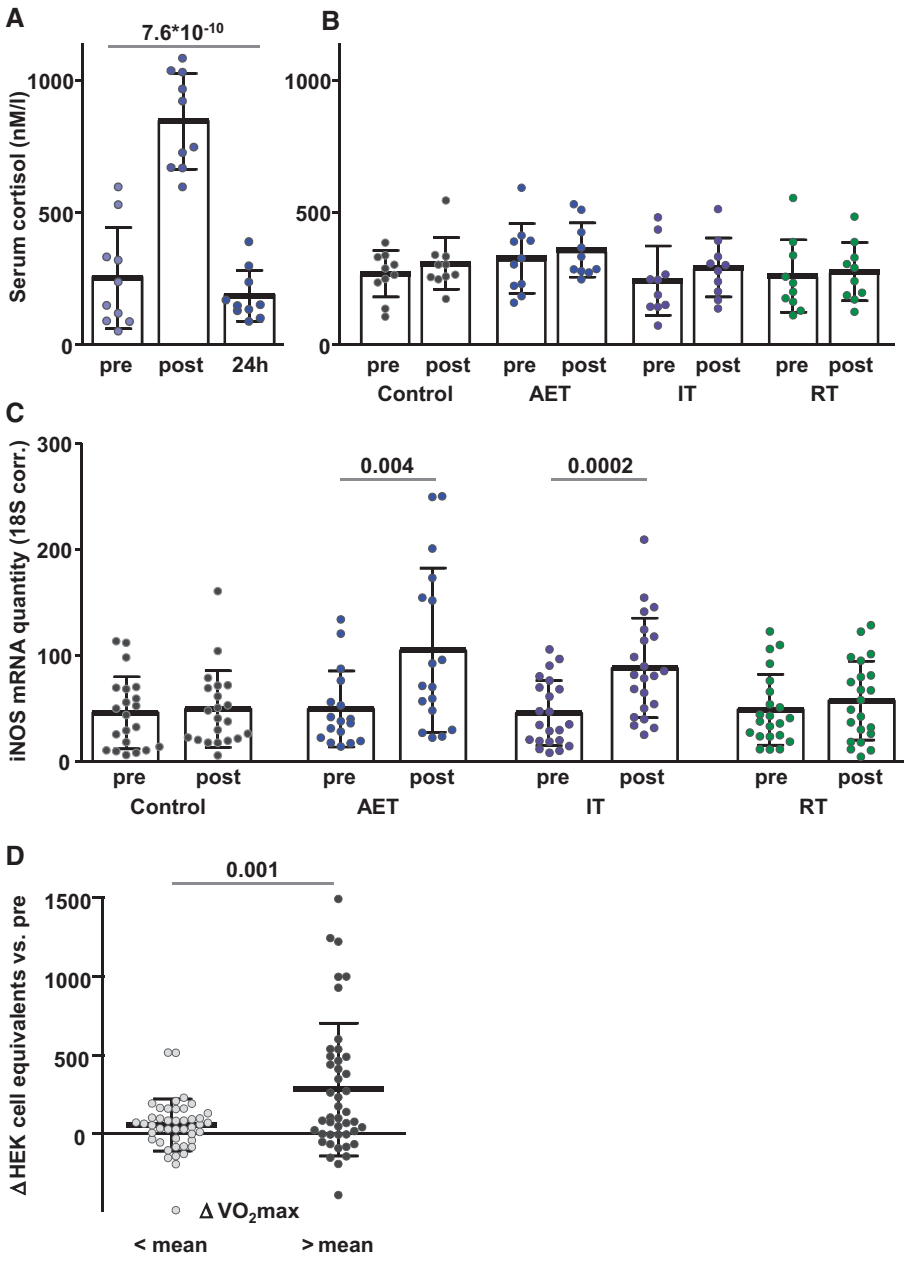
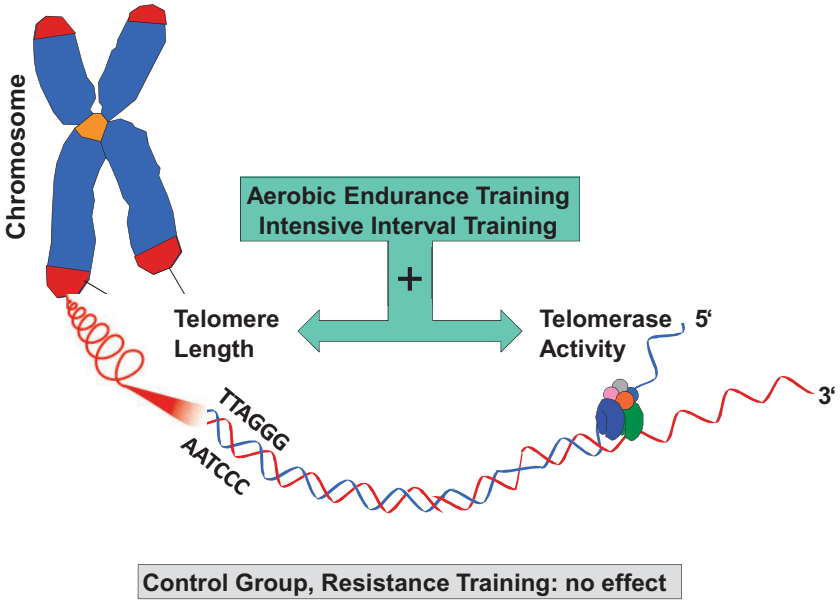


Figure 5 Cortisol production in acute extreme exercise vs. chronic exercise and training response. (*A*) Serum concentrations of the stress hormone cortisol were measured in $N = 10$ male samples of the Berlin Beat of Running marathon study and compared with (*B*) cortisol concentrations in 40 samples (10 per experimental group) of the chronic exercise study. (*C*) mRNA expression of the inducible nitric oxide synthase (real-time PCR). (*D*) Training response is associated with markers of cellular senescence: responders in the training groups with respect to the change of maximum oxygen uptake (ΔVO_{2max}) after the study compared with baseline were defined as the individuals above the mean of ΔVO_{2max} as opposed to 'weak' responders with a ΔVO_{2max} below the mean. Comparison of the change in telomerase activity shown as Δ human embryonic kidney cell equivalents between subjects below vs. above the mean of the ΔVO_{2max}.

HLA-DR+CD3+ did not differ in the chronic exercise study between the four study groups and did not differ between the start and the end of the training period. CD34+ haematopoietic progenitor cells were up-regulated by endurance training and IT as expected,[15,34] but not in the control or the endurance groups. Assessment of stress hormones confirmed marked up-regulation of cortisol after the extreme physical stress of marathon running.[35] However, the chronic levels of cortisol did not differ between the four groups before and after the training period in the chronic exercise study.

To test whether the increased telomerase activity will persist long term with endurance training, a prospective randomized training study including a control group was conducted. The main finding of



Take home figure In a primary prevention cohort of untrained healthy middle-aged subjects, aerobic endurance training, or intensive interval training for 6 months increased telomerase activity and telomere length, indicating vascular anti-aging effects. No changes were observed in the control and resistance training groups.

our training study is the differential effects of the three training modalities on TL. In order to account for the methodological challenges of TL measurements in humans, especially with regard to PCR,[36] two established independent methods were used.[8,12,13,22,23] Both assays were carefully controlled (details described in Supplementary material online, *Methods*). In addition, the findings are supported by the measurements of telomerase activity. While the two endurance-based training protocols markedly increased telomerase activity and TL after 6 months, the strength-based protocol did not.[37] So far, only one prospective study addressed the effects of training on TL.[38] Melk *et al.* trained $n = 59$ men for 210 minutes per week for 6 months. Consistent with our findings they found an increase in TL and telomerase activity. However, that study was not controlled or randomized, did not test IT or RT and did not report which methodology was used for TL measurement. We would like to highlight the importance of a prospective randomized design, a control group and a sufficient follow-up for the interpretation of a training study.

Endurance training and RT induce a number of differential haemodynamic, metabolic, and/or neurohumoral responses, both acute and chronic.[4,39,40] Precise comparisons between endurance and a resistance exercise protocols are scarce. Our intra-individual comparisons of heart rate during acute AET, intensive IT, and resistance exercise (Supplementary material online, *Figure S8*) showed that the mean and the maximum heart rate are higher in the endurance training modalities. This may suggest that, compared with resistance exercise, endurance training may induce a higher rate of (laminar) vascular shear stress, which may, e.g. via NO, potentially contribute to the observed cellular effects.[17] Endothelial NO synthase and telomerase activity have shown to be linked in a signalling pathway mediating exercise-induced vascular protection.[13] In agreement with this hypothesis, we observed a differential regulation of iNOS which was only up-regulated by the endurance training protocols. From an evolutionary perspective, endurance training and high-intensity IT may mimic advantageous travelling and fight and fly behaviour better than strength training.[19] Our data set the stage for future studies to elucidate the details of these regulatory processes. While acute physical stress leads to up-regulation of stress hormones and leucocytes, these alterations are not observed chronically in the basal state after long-term regular exercising. However, we believe that it is likely that the repeated bouts of stress-induced changes induced by the endurance protocols three times a week lead to a shift towards increased baseline telomerase activity when applied long term. This hypothesis represents a possible explanation for the increase of TL with endurance, but not resistance exercise.

Telomere length has become a widely accepted molecular/cellular biomarker of aging.[6] We and others have demonstrated that changes in blood cell telomeres and their associated factors reflect changes in the vessel wall and the myocardium.[13,14] This may be important for the mechanism of cardiovascular prevention by exercise, because increased telomere erosion is associated with disease incidence and severity.[8,11,41] Importantly, telomere dysfunction by chronic inflammation, as in cardiovascular disease, can induce cellular senescence without telomere shortening.[42] Training responses are characterized by an inter-individual variability. Our study shows that changes in telomerase activity correlate not only with the training modality but also with the changes of the individual performance. Measurements of TL could therefore be a useful indicator of 'biological age' in future intervention studies.

Limitations

The sample size is smaller than other cardiovascular studies, however, this trial is the largest prospective randomized controlled training study performed to date regarding the cellular effects of exercise that includes randomization to well-defined and supervised 6 months training protocols. The study included a sedentary control group. Adherence is the primary challenge of all randomized interventions that address lifestyle changes, especially exercise. This is reflected by drop-out rates in the literature which are comparable to our study.[43,44] The protocol involved fresh isolation of MNC from peripheral blood after each blood draw. Therefore, incomplete samples in very few patients are expected. Per design, the increase of aerobic capacity was similar between the three training groups. Minor differences of the training intensity are possible but inherent to the respective modalities. The every-day activities of the participants outside the supervised training sessions may have contained elements of the other modalities, but this most likely was also the case in the control group during the 6 months observation period.

Conclusion

In summary, this randomized controlled training study shows that specific modalities of physical exercise mediate differential effects on regulators of cellular senescence. The activity of telomerase and TL is increased by endurance training and high-intensive IT but not after strength training. The data improve the molecular understanding of the protective effects of exercise in primary prevention and underline the potency of physical training in reducing the impact of age-related diseases. Telomerase activity is a sensitive parameter to measure preventive effects of exercise on the cellular level, both acute and chronic. Therefore, cellular senescence markers could be useful parameters to guide the efficacy of preventive exercise programmes. The effects on clinical outcomes need to be scrutinized in a large prospective trial, taking potential differences of training modalities into consideration.

With regard to training recommendations for the prevention of cellular aging, our data support the ESC's current guideline recommendations that resistance exercise should be complimentary to endurance training rather than a substitute.[2]

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

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Corrigendum

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Corrigendum to: Innovations in Medicine and Cost Savings: An Impossible Equation? [*Eur Heart J* (2018); 39 (11): 911–912]

The authors of the above paper wish to inform readers that the author, Eloi Marijon's name was given incorrectly in the paper as first published. This has now been corrected online.