



Original article

Age and the effect of exercise, nutrition and cognitive training on oxidative stress – The Vienna Active Aging Study (VAAS), a randomized controlled trial



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ABSTRACT

The purpose of this study was to investigate the effect of age – over or under life-expectancy (LE) – on six months resistance training alone or combined with a nutritional supplement, and cognitive training by analyzing markers for oxidative stress and antioxidant defense in institutionalized elderly, living in Vienna.

Three groups (n = 117, age = 83.1 ± 6.1 years) – resistance training (RT), RT combined with protein and vitamin supplementation (RTS) or cognitive training (CT) – performed two guided training sessions per week for six months. Oxidative stress, antioxidant defense and DNA strand breaks were analyzed and transformed into an “antioxidant factor” to compare the total effect of the intervention. Physical fitness was assessed by the 6-min-walking, the chair-rise and the handgrip strength tests.

We observed significant negative baseline correlations between 8-oxo-7,8-dihydroguanosine and handgrip strength (r = -0.350, p = 0.001), and between high sensitive troponin-T and the 6-min-walking test (r = -0.210, p = 0.035). RT and RTS groups, showed significant improvements in physical performance. Over LE, subjects of the RT group demonstrated a significant greater response in the “antioxidant factor” compared to RTS and CT (RT vs. RTS p = 0.033, RT vs. CT p = 0.028), whereas no difference was observed between the intervention groups under LE.

Six months of elastic band resistance training lead to improvements in antioxidant defense, DNA stability and oxidative damage, summarized in the “antioxidant factor”, however mainly in subjects over their statistical LE. Consuming a supplement containing antioxidants might inhibit optimal cellular response to exercise.

The study was approved by the ethics committee of the City of Vienna (EK-11-151-0811) and registered at ClinicalTrials.gov, NCT01775111.

1. Introduction

The steadily increasing life-expectancy (LE) of humans living in developed countries and consequently the incidence of age-related diseases is already a challenge for the health system. Sarcopenia, the loss of muscle mass and function with aging, as well as the aging process itself, are strongly correlated to increased oxidative damage, which in turn is linked to typical age-related diseases such as cardiovascular

disease, cancer, dementia and diabetes [1]. Especially elderly living in institutionalized facilities are experiencing a rapid decline of physical function often accompanied and partly caused by malnutrition and physical inactivity as soon as they change their living situation from “free-living” into a community-dwelling surrounding [2,3].

Resistance training, together with protein supplementation, seems to be most effective to increase muscle mass and strength in the elderly [4]. Especially the use of elastic bands has been shown to be an

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appropriate, versatile and effective tool to safely increase physical performance and muscle quality in older subjects [5–7]. Although there are official guidelines for conducting an effective and health-promoting resistance training program in the elderly [8], outcomes on parameters of oxidative stress and DNA damage after exercise are contradictory; either of improved but also of deteriorated mechanisms was reported [9,10]. Importantly, the acutely increased level of reactive-oxygen-species (ROS) caused by physical activity is an essential messenger to activate redox-sensitive pathways which ultimately improve antioxidant defense and DNA repair mechanisms [11,12]. Therefore the consumption of antioxidants could potentially influence optimal adaptation after exercise and should be considered carefully, especially in the elderly, where a low status of several micronutrients is common [2,13]. A sufficient availability of nutrients, especially antioxidant vitamins, such as vitamin C and E, is recommended to support antioxidant defense mechanisms. However, supplementing these nutrients, in too close proximity to an exercise stimulus, seems to restrain optimal adaptation of the redox-system [14,15]. Interestingly, the very oldest of our society, who are reaching an age beyond current statistical LE, demonstrate a lower incidence of age-related diseases, improved antioxidant defense, better DNA integrity and superior genome stability, although their physical fitness is still lower compared to those below LE [1,16]. This super-aging cohort urges deeper analyses to better understand their unique cellular resistance.

As exercise and nutrients intake seem to have an important effect on oxidative stress, antioxidant potential, DNA stability and physical fitness, it is of eager importance to investigate and optimize nutritional and exercise based strategies in the elderly to increase or at least keep strength and muscle mass until high age.

In the present study we hypothesized that after six months of strength training increased physical fitness improves resistance against oxidative stress as well as against oxidative damage in institutionalized elderly. Supplementing (antioxidant) vitamins and protein should further enhance this protective effect.

2. Methods

The presented data are part of the Vienna Active Ageing Study, which is a multidisciplinary project between the Centre for Sport Science and University Sports, the Faculty of Life Sciences, the Research Platform Active Ageing (all University of Vienna), the Karl Landsteiner Institute for Remobilization and Functional Health (Institute for Physical Medicine and Rehabilitation) and the Curatorship of Viennese Retirement Homes.

2.1. Subjects

Over a time period of 6 months (July 2011 - January 2012) 117 institutionalized elderly women and men (aged 65–98 years), were recruited from five different senior residencies in the area of Vienna (Curatorship of Viennese Retirement Homes) (Fig. 1). The subjects were mentally (Mini Mental State Examination \geq 23) and physically (Short Physical Performance Battery $>$ 4) able to participate in this training intervention study. They were sedentary (less than one hour of physical activity or exercise per week) and free of severe diseases that would contra-indicate medical training therapy or measurement of physical performance, including cardiovascular diseases, diabetic retinopathy and regular use of cortisone-containing drugs. Inclusion and exclusion criteria have been described in detail by Oesen et al. [6]. The health condition of all study subjects was assessed by specialists in internal medicine and gerontology. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. Subjects were not allowed to take part in any exhausting physical activity within 2 days before the blood sampling and fitness test. All participants followed their medication protocols as prescribed by their physicians. If supplements were consumed before

entering the study, details on further intake were discussed with their physicians.

2.2. Study design

The present study design was described previously in Franzke et al. [17]. Briefly, study participants were randomly assigned into three intervention groups – cognitive training (CT), resistance training (RT), RT + supplement (RTS) – and matched for gender in a randomized, controlled, observer-blind design. At baseline (T1), after three (T2) and after six months (T3) blood samples were taken as well as physical and functional tests were performed. The current study was conducted to investigate the effect of six months elastic band resistance training, either with or without consuming a supplement containing macro- and micro-nutrients on markers of oxidative stress (malondialdehyde), antioxidant potential (uric acid, ferric reducing ability potential, superoxide dismutase, catalase, glutathione peroxidase) and oxidized DNA/RNA (DNA strand breaks, 8-oxo-7,8-dihydro-2'-deoxyguanosine, 8-oxo-7,8-dihydroguanosine) in Austrian institutionalized elderly. With respect to the old age of our subjects (almost 60% were older than their statistical LE [18]) we further analyzed whether there was a different response between subjects over LE compared to subjects at or under LE.

2.3. Resistance training

The resistance training groups (RT and RTS) received two weekly sessions of resistance training, conducted on two non-consecutive days and were supervised by a sports scientist. Training attendance was recorded every session. Exercises were conducted using elastic bands, chairs and own body weight - for detailed training program see supplement of Oesen et al. [6]. The progressive resistance training protocol was designed based on the guidelines of the American College of Sports Medicine for resistance training with older subjects [8]. The about one hour lasting workout consisted of an initial 10 min warm up, a 30–40 min strength training for the main muscle groups (legs, back, abdomen, chest, shoulder and arms) and a 10 min cool down. The participants were motivated and controlled to adapt the resistance of the elastic band (shorter or stronger band) to keep exercise intensity within an effective range. After completing the initial phase of 4 weeks, where one set of 15 repetitions was performed, the intensity and volume has progressively been increased from two sets of light exercises to two sets of heavy resistance.

2.4. Resistance training and supplementation

The RTS group performed the same exercises together with the RT group and additionally received a liquid supplement every morning, as well as directly after each training session. Each drink supplied a total energy of 150 kcal and contained 20.7 g protein (56 energy (En)%, 19.7 g whey protein, 3.0 g leucine, $>$ 10 g essential amino acids), 9.3 g carbohydrates (25 En%, 0.8 BE), 3.0 g fat (18 En%), 1.2 g roughage (2 En%), 800IU (20 μ g) of vitamin D, 250 mg calcium, vitamins C, E, B6 and B12, folic acid and magnesium (FortiFit, NUTRICIA GmbH, Vienna, Austria). The intake of the nutritional supplement was controlled at breakfast as well as after the training sessions.

2.5. Cognitive training

The CT groups served as our control group and performed coordinative or cognitive tasks [19] two times per week, equally to the frequency of the RT and RTS groups. This was done to minimize the “bias” of being part of a social group activity.

Participants of all groups were instructed to maintain their regular food intake, which was controlled by food diaries.

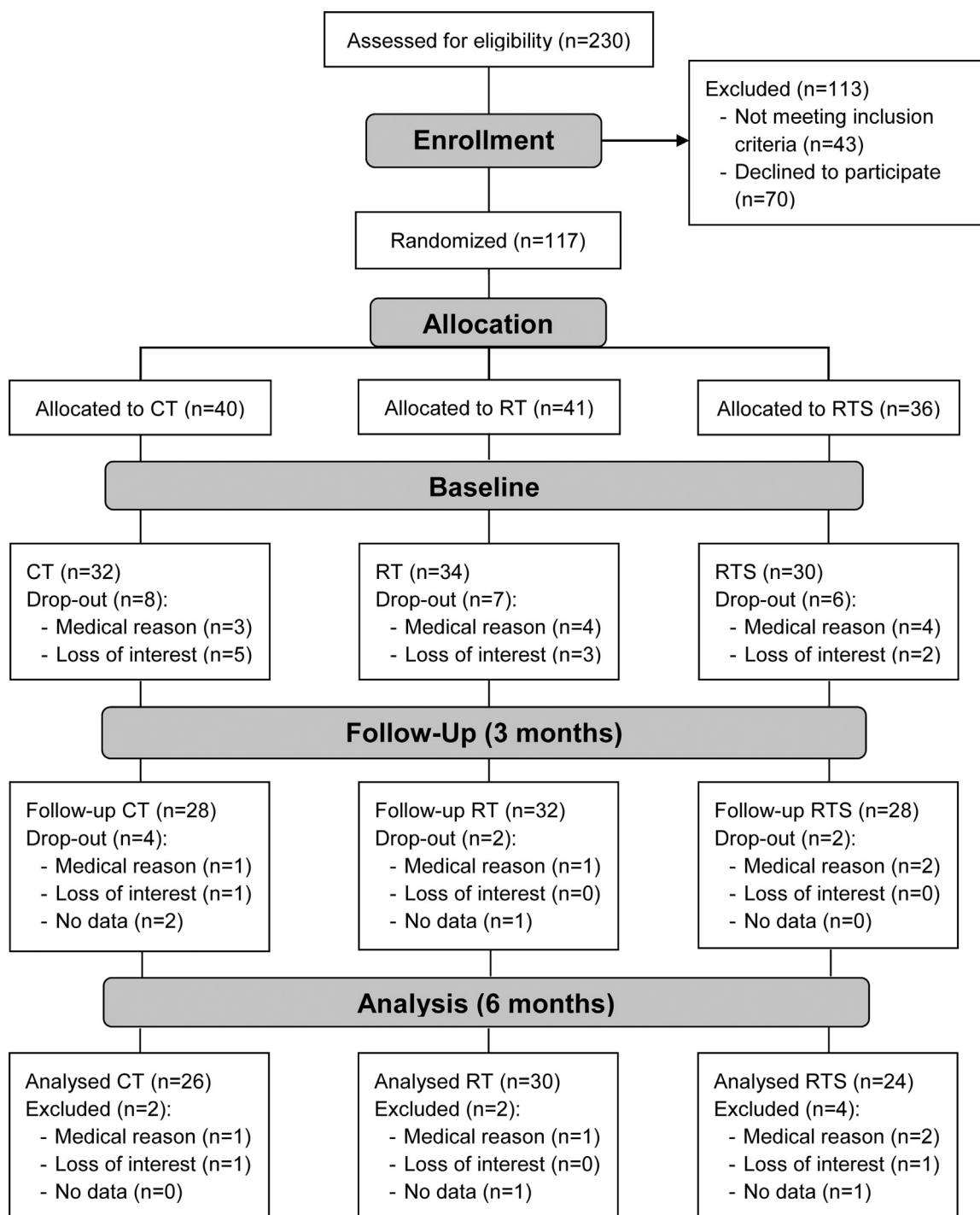


Fig. 1. Participants flow diagram, Vienna Active Ageing Study.

2.6. Comet assay

Blood samples were taken in the morning after an overnight fast using heparin, serum and EDTA tubes (Greiner Bio One, Austria). Peripheral blood lymphocytes were isolated using Ficoll separation tubes (Greiner Bio One, Austria). The samples were either freshly used for the comet assay or stored at -80°C for subsequent analyses.

To measure oxidative DNA damage and resistance against H_2O_2 , the comet assay was conducted [20]. In short, a cell suspension of PBMCs (1×10^6 cells/mL in PBS) was mixed with 1% low melting agarose and applied on microscope slides (coated in 1% normal melting agarose). Slides for either “lysis”, “buffer”, “FPG” (formamidopyrimidine DNA

glycosylase) and “ H_2O_2 ” were prepared. All slides were placed into lysis solution (pH 10) for at least one hour. Only H_2O_2 slides were treated with a $100 \mu\text{M}$ H_2O_2 solution for 5 min at 4°C prior to lysis. After lysis and three washing steps with enzyme buffer, the buffer and FPG slides were either incubated with $50 \mu\text{L}$ of enzyme buffer or FPG solution at 37°C for 30 min in a moist box. All slides were put into an electrophoresis tank (CSL-COM40, Biozym, Austria), containing electrophoresis solution (pH > 13). After the unwinding process (20 min) and 30 min of electrophoresis (25 V, 300 mA, 4°C), the slides were washed with PBS and dried at room temperature. After staining the slides with $20 \mu\text{g}/\text{mL}$ of ethidium bromide, DNA damage was measured using a fluorescence microscope together with an image analysis system

Table 1
Baseline characteristics of participants over and under life-expectancy.

Parameter	Under LE	Over LE	p
Subjects [number]	43	62	
Age [years]	80 (65–83)	87 (84–98)	**
Lysis [%DNA in tail]	6.85 (3.21–10.96)	6.40 (1.51–9.87)	
H ₂ O ₂ [%DNA in tail]	25.33 (16.36–40.62)	26.63 (14.78–42.97)	
FPG [%DNA in tail]	6.77 (0.75–21.49)	8.57 (0.00–21.28)	
FRAP [μ mol/l]	1178 (737–2133)	1210 (771–2383)	
SOD [I.U./g Hb]	2116 (1611–2530)	1986 (1283–2834)	
CAT [I.U./g Hb]	123 (61–235)	123 (82–174)	
GSH-Px [I.U./g Hb]	34.5 (15.0–52.0)	34.0 (19.0–50.0)	
LDL-cholesterol [mg/dl]	121 (72–225)	103 (63–185)	*
Soxoguo [nM/mM]	3.70 (1.80–9.14)	3.90 (1.88–6.13)	
SoxodG [nM/mM]	1.70 (0.96–5.27)	1.70 (0.73–4.25)	
hs CRP [mg/l]	2.35 (0.80–14.30)	1.80 (0.50–10.60)	
Uric acid [mg/dl]	5.40 (3.20–8.10)	5.90 (2.60–8.80)	
hs TNT [ng/l]	10.00 (3.00–20.00)	11.00 (3.00–49.00)	*
MDA [μ M/l]	1.86 (1.04–3.22)	1.85 (1.32–2.87)	

Data are medians (minimum – maximum); p-values are calculated using Mann-Whitney-U-Test. *... < 0.05; **... < 0.001.

Life expectancy (LE), formamidopyrimidine DNA glycosylase (FPG), ferric reducing ability potential (FRAP), superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px), low-density-lipoprotein (LDL), 8-oxo-7.8-dihydroguanine (Soxoguo), 8-oxo-7.8-dihydro-2'-deoxyguanosine (SoxodG), high sensitive c-reactive protein (hs CRP), high sensitive Troponin-T (hs TNT), malondialdehyde (MDA).

(Komet 5.5, Kinetic Imaging, Liverpool, UK). The mean %DNA in tail of 50 comets per gel (2 gels per sample) was calculated. The results from buffer treatment were subtracted from those of FPG to calculate the net amount of FPG-sensitive sites.

2.7. Superoxide dismutase (SOD)

To assess SOD activity, the inhibition of autoxidation of pyrogallol was measured. One unit of SOD activity was defined as the required amount of SOD to inhibit autoxidation by 50% [21].

2.8. Catalase (CAT)

The activity of CAT was measured using photometrical analysis. Therefore the initial rate of H₂O₂ degradation was quantified. One unit of CAT activity was defined as the rate constant of the first-order reaction [21].

Table 2
The impact of 3 (T2) and 6 (T3) months of intervention on markers describing oxidative stress and antioxidant defense.

Intervention	RT				RTS				CT			
	T1	T2	T3	p	T1	T2	T3	p	T1	T2	T3	p
Subjects [number]	34	32	30		30	28	24		32	28	26	
BMI [kg/m ²]	29.0 ± 3.7	29.3 ± 3.8	29.0 ± 3.9		30.0 ± 6.3	30.1 ± 5.2	30.0 ± 5.4		28.6 ± 4.9	27.6 ± 5.0	27.9 ± 5.3	
FRAP [μ mol/l]	1161 ± 289	1261 ± 461	1271 ± 490		1312 ± 337	1436 ± 465	1393 ± 412		1221 ± 361	1272 ± 537	1263 ± 335	
LDL-cholesterol [mg/dl]	118 ± 37	119 ± 42	125 ± 39		119 ± 34	120 ± 37	126 ± 42		124 ± 37	125 ± 35	124 ± 38	
Soxoguo [nM/mM]	3.72 ± 1.27	3.91 ± 1.20	3.71 ± 1.18		3.95 ± 1.47	3.98 ± 1.49	3.56 ± 1.37		4.34 ± 1.99	3.87 ± 1.42	3.73 ± 1.14	
SoxodG [nM/mM]	1.91 ± 0.83	1.88 ± 0.78	1.58 ± 0.50		2.01 ± 0.90	1.69 ± 0.56	1.56 ± 0.71		1.98 ± 0.89	1.64 ± 0.64	1.66 ± 0.73	
hs CRP [mg/l]	3.03 ± 2.89	3.51 ± 3.07	3.60 ± 3.04		3.10 ± 2.80	3.72 ± 3.17	3.40 ± 3.29		3.79 ± 3.72	3.44 ± 3.21	3.91 ± 4.81	
Uric acid [mg/dl]	5.73 ± 1.35	5.83 ± 1.58	5.81 ± 1.51		5.99 ± 1.30	5.84 ± 1.86 ⁺	5.74 ± 1.17	**	5.73 ± 1.65	6.01 ± 1.34	5.79 ± 1.39	
hs TNT [ng/l]	10.5 ± 7.5	10.8 ± 7.4	12.2 ± 6.9		15.6 ± 13.2	14.9 ± 13.4	14.3 ± 12.5		16.7 ± 21.2	13.5 ± 9.1	14.5 ± 8.3	
MDA [μ M/l]	1.95 ± 0.41	1.95 ± 0.37	2.03 ± 0.41		1.89 ± 0.54	1.92 ± 0.63	2.13 ± 0.42 ⁺	*	1.95 ± 0.47	1.95 ± 0.36	1.97 ± 0.48	

Data are means ± SD; p-values are calculated using Friedman-Test for overall differences between time points, *... < 0.05; **... < 0.001; Wilcoxon-Test was performed for post-hoc analyses, significance level to T1: ⁺... < 0.05, ⁺⁺... < 0.001, significance level to T2: ^X... < 0.05.

Ferric reducing ability potential (FRAP), low-density-lipoprotein (LDL), 8-oxo-7.8-dihydroguanine (Soxoguo), 8-oxo-7.8-dihydro-2'-deoxyguanosine (SoxodG), high sensitive c-reactive protein (hs CRP), high sensitive Troponin-T (hs TNT), malondialdehyde (MDA). Please see the data for Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) and the Comet assay (Formamidopyrimidine DNA glycosylase (FPG), H₂O₂, Lysis) in Franzke et al. [17].

2.9. Glutathione peroxidase (GSH-Px)

GSH-Px activity was analyzed with an indirect coupled assay. The activity that catalyses the oxidation of one nmol of nicotinamide adenine dinucleotide phosphate per minute was defined as one unit [21].

2.10. Ferric reducing ability potential (FRAP)

The antioxidant capacity of serum was measured by performing the ferric reducing ability potential (FRAP) assay as described by Benzie and Strain [22] in triplicates using trolox as standard. Absorbance was measured with BMG FLUOstar OPTIMA Microplate Reader (BMG LABTECH GmbH) at 593 nm and results are expressed as trolox equivalents in μ mol/l.

2.11. Biochemical parameters

Lipid profile, high sensitive c-reactive protein (hs CRP), uric acid and high sensitive Troponin-T (hs TNT) were analyzed immediately after blood sampling at a routine laboratory (study lab GmbH, Vienna).

2.12. Oxidative damage detected in urine

Urine samples were collected on the day of blood samplings, aliquoted and stored at –20 °C until analysis. 8-oxo-7.8-dihydro-2'-deoxyguanosine (SoxodG) and 8-oxo-7.8-dihydroguanine (Soxoguo) were measured at the Laboratory of Clinical Pharmacology, Rigshospitalet, in Copenhagen, using a validated method for ultra-performance LC and MS/MS [23]. SoxodG and Soxoguo were normalized against urinary creatinine concentration determined by the Jaffe reaction.

2.13. Malondialdehyde (MDA)

MDA levels were determined in duplicates in plasma as described earlier [24]. After heating (60 min, 100 °C) plasma samples were neutralized with methanol/NaOH, centrifuged (3 min, 3000 rpm) and MDA was measured with high-performance liquid chromatography (HPLC) (excitation: λ = 532 nm, emission: λ = 563 nm, LaChrom Merck Hitachi Chromatography System, Vienna, Austria; HPLC column 125 × 4 mm, 5 μ m; Merck, Vienna, Austria).

2.14. Chair rise test

In the chair rise test, the participants had to stand up and sit down

Table 3
The impact of 3 (T2) and 6 (T3) months of intervention on markers describing oxidative stress and antioxidant defense in subjects OVER life-expectancy.

Intervention	RT			RTS			CT		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Subjects [number]	18	16	15	18	16	12	20	18	17
Lysis [%DNA in tail]	5.77 ± 2.07	8.18 ± 2.30 ⁺	9.17 ± 2.06 ⁺	7.60 ± 2.08	8.72 ± 2.28	8.58 ± 2.69	6.49 ± 1.81	8.91 ± 3.59 ⁺	8.17 ± 1.61 ⁺
H ₂ O ₂ [%DNA in tail]	27.95 ± 7.50	20.63 ± 8.27 ⁺	21.31 ± 4.08 ⁺	26.70 ± 7.63	23.98 ± 8.05	24.66 ± 5.39	25.51 ± 5.51	20.74 ± 6.80 ⁺	23.77 ± 5.09
FFG [%DNA in tail]	9.81 ± 5.03	9.58 ± 7.73	7.10 ± 4.45	11.23 ± 10.58	11.31 ± 10.58	9.46 ± 6.79	10.33 ± 5.52	10.33 ± 5.52	8.54 ± 6.17
SOD [I.U./g Hb]	1881 ± 287	2017 ± 256	2106 ± 200	2152 ± 375	2047 ± 210	2238 ± 236 [×]	2056 ± 140	2097 ± 180	2160 ± 288
CAT [I.U./g Hb]	116 ± 28	140 ± 33	151 ± 35	132 ± 34	146 ± 26	144 ± 45	126 ± 28	142 ± 48	140 ± 27
GSH-Px [I.U./g Hb]	33.1 ± 7.8	38.1 ± 12.5	36.7 ± 6.6	37.2 ± 11.8	44.3 ± 13.1	36.7 ± 13.7	37.8 ± 9.9	34.5 ± 6.9	31.3 ± 9.6
FRAP [μmol/l]	1104 ± 306	1301 ± 589	1296 ± 654	1259 ± 253	1324 ± 352	1324 ± 352	1259 ± 394	1332 ± 623	1311 ± 362
LDL-cholesterol [mg/dl]	105 ± 33	96 ± 29	108 ± 32	113 ± 31	114 ± 34	117 ± 44	119 ± 41	121 ± 38	122 ± 45
8oxoGuo [nM/mM]	4.00 ± 1.27	4.09 ± 1.31	3.76 ± 0.89	4.51 ± 1.63	4.63 ± 1.56	4.15 ± 1.61	3.78 ± 1.65	3.40 ± 1.10	3.45 ± 0.81
8oxodG [nM/mM]	1.83 ± 0.57	1.84 ± 0.59	1.52 ± 0.42	2.07 ± 0.83	1.83 ± 0.55	1.75 ± 0.85	1.76 ± 0.65	1.45 ± 0.64	1.53 ± 0.68
hs CRP [mg/l]	2.04 ± 1.73	2.20 ± 1.33	2.52 ± 2.21	2.84 ± 2.38	4.11 ± 3.62	3.28 ± 3.56	4.11 ± 3.73	3.75 ± 3.51	3.31 ± 3.21
Uric acid [mg/dl]	5.80 ± 1.44	5.92 ± 1.69	5.86 ± 1.73	6.10 ± 1.37	6.24 ± 2.14 ⁺	5.87 ± 0.91	6.12 ± 1.77	6.24 ± 1.39	6.06 ± 1.39
hs TNT [ng/l]	12.3 ± 9.3	13.2 ± 9.1	15.1 ± 8.1	19.9 ± 15.0	17.4 ± 15.8	18.3 ± 14.6 [×]	20.6 ± 26.1	14.7 ± 10.1	15.1 ± 9.5
MDA [μM/l]	1.90 ± 0.30	1.95 ± 0.30	2.06 ± 0.39	1.87 ± 0.61	1.88 ± 0.76	2.10 ± 0.49	1.89 ± 0.39	1.98 ± 0.32	2.11 ± 0.39

Data are means ± SD; p-values are calculated using Friedman-Test for overall differences between time points; * ... < 0.05; ** ... < 0.001; Wilcoxon-Test was performed for post-hoc analyses, significance level to T1: + ... < 0.05, significance level to T2: x ... < 0.05.

FRAP, ferric reducing ability potential (FRAP), superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px), low-density-lipoprotein (LDL), 8-oxo-7,8-dihydroguanine (8oxoGuo), 8-oxo-7,8-dihydro-2'-deoxyguanosine (8oxodG), high sensitive c-reactive protein (hs CRP), high sensitive Troponin-T (hs TNT), malondialdehyde (MDA).

from a chair (46 cm seat height) as often as possible within 30 s. To ensure a safe test-setting, the chair was placed against the wall. For one successful repetition, participants had to fully stand up (hip and knee fully extended) and sit back, with their arms crossed over their chest. A last-second-attempt was considered valid, if the person had covered more than 50% of the range of motion [6,25].

2.15. Handgrip strength

To assess handgrip strength, participants performed an isometric handgrip strength test (kg) using a dynamometer. The test was conducted in a sitting position and maximal isometric contraction within 4–5 s was measured (JAMAR compatible handgrip dynamometer adapted to handle different sizes). The highest score of maximum voluntary contraction was used for data analyses [6,26].

2.16. Six-minutes-walking test

The participants had to walk for six minutes as fast and as far as possible. The six-minute-walking test is a valid tool to evaluate aerobic endurance in the elderly. Participants were allowed to slow down and even take a short rest. Every subject performed the test separately without being disturbed by others. They had to walk back and forth on a 30 m shuttle track and the distance covered within six minutes was registered [6,27].

2.17. Statistics

Statistical analyses were performed with IBM SPSS Statistics 21. For all parameters included into the current analyses Shapiro-Wilk test was used to check for normal distribution which was violated for most of the blood-based parameters. Therefore, differences between age-groups were measured, using Kruskal-Wallis-h- and/or Mann-Whitney-U-Test. To assess the overall differences between the time points, Friedman-Test was conducted and if significant, Wilcoxon-Test was used to show the differences between each time point by considering Bonferroni correction. Linear correlations were calculated using the Spearman-Test. A p-value of less than 0.05 was considered an indication of significance. In order to have a broader picture an “antioxidant factor” was calculated as following: The 6 month percentage change of each oxidative parameter (antioxidant enzymes, FRAP, 8oxoGuo, 8oxodG, MDA, DNA strand breaks and uric acid) was classified, according to statistical quintiles, and transformed into a scale of a maximum of + or – 5 points (1 point always for the first quintile and 5 points for the highest/lowest quintile). The received points were summed up and the total of the points was analyzed. After transformation an increase was equal to an improved antioxidant status and a decrease was equal to a deteriorated status.

2.18. Statistical power and sample size

An a priori power analysis showed that at an α of 0.05/3 and a power of 0.85 a total number of 86 participants would be necessary to detect similar changes. With an estimated drop-out number of 40% over 6 months we aimed at starting the study with 120 subjects, see Fig. 1.

3. Results

3.1. Baseline characteristics

At baseline blood samples from 105 subjects were collected, however only 96 participants completed all tests (Fig. 1). The ratio of 12.4% male and 87.6% female presents a representative picture of the gender distribution in the houses of the Curatorship of Viennese Retirement Homes. The mean age of the participants was 82.9 ± 6.0 years for women and 84.9 ± 6.7 years for men. Neither BMI, physical fitness,

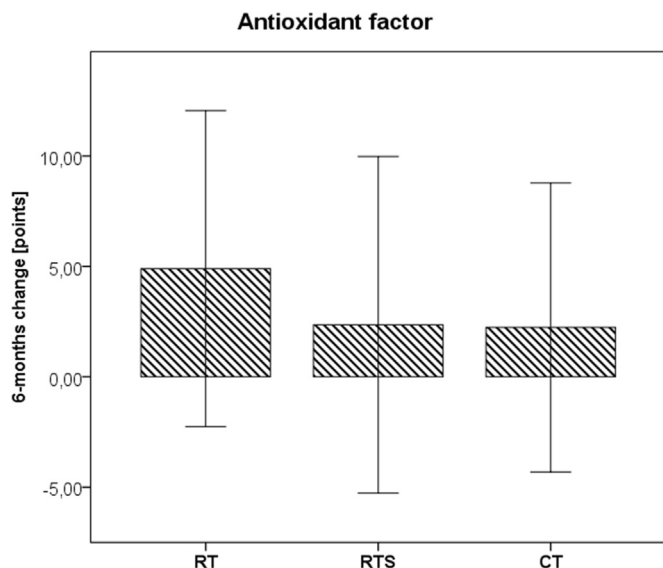
Table 4

The impact of 3 (T2) and 6 (T3) months of intervention on markers describing oxidative stress and antioxidant defense in subjects UNDER life-expectancy.

Intervention	RT			RTS				CT				
	T1	T2	T3	p	T1	T2	T3	p	T1	T2	T3	p
Subjects [number]	16	16	15		12	12	12		12	10	9	
Lysis [%DNA in tail]	7.95 ± 2.63	8.51 ± 2.01	7.04 ± 2.59		7.17 ± 2.40	9.21 ± 2.60 ⁺	8.98 ± 2.03	*	7.51 ± 2.93	8.20 ± 1.51	6.72 ± 1.72	
H ₂ O ₂ [%DNA in tail]	23.97 ± 5.83	22.14 ± 8.75	24.43 ± 4.68		28.80 ± 8.32	24.29 ± 5.27	25.08 ± 3.28		29.49 ± 6.01	24.96 ± 6.93	23.94 ± 4.36	
FPG [%DNA in tail]	8.70 ± 10.50	12.07 ± 9.15	6.94 ± 3.96		9.40 ± 5.66	12.95 ± 7.01	6.04 ± 4.76		12.77 ± 6.35	11.97 ± 7.69	6.45 ± 6.17	
SOD [I.U./g Hb]	2068 ± 250	2090 ± 214	2174 ± 246		2215 ± 275	2200 ± 210	2277 ± 157		2162 ± 229	2247 ± 263	2225 ± 253	
CAT [I.U./g Hb]	134 ± 35	156 ± 39	154 ± 41		132 ± 44	142 ± 22	154 ± 34		134 ± 37	130 ± 22	167 ± 36	
GSH-Px [I.U./g Hb]	34.6 ± 9.7	33.9 ± 10.8	33.5 ± 10.2		33.9 ± 8.3	38.7 ± 8.4	36.8 ± 9.6		37.5 ± 8.8	36.0 ± 6.7	34.5 ± 10.5	
FRAP [μmol/l]	1225 ± 264	1222 ± 300	1246 ± 263		1396 ± 443	1465 ± 651	1462 ± 472		1157 ± 300	1180 ± 379	1177 ± 279	
LDL-cholesterol [mg/dl]	134 ± 37	140 ± 41	142 ± 39		129 ± 37	130 ± 40	136 ± 40		131 ± 31	133 ± 29	126 ± 19	
8oxoGuo [nM/mM]	3.28 ± 1.18	3.70 ± 1.08	3.66 ± 1.45		3.20 ± 0.80	3.03 ± 0.68	2.98 ± 0.75		5.37 ± 2.21	4.54 ± 1.58	4.18 ± 1.48	
8oxodG [nM/mM]	2.02 ± 1.14	1.94 ± 0.99	1.64 ± 0.59		1.93 ± 1.03	1.50 ± 0.53	1.38 ± 0.50		2.37 ± 1.15	1.96 ± 0.55	1.89 ± 0.81	
hs CRP [mg/l]	4.37 ± 3.60	4.82 ± 3.76	4.68 ± 3.43		3.58 ± 3.53	3.21 ± 2.52	3.52 ± 3.16		3.26 ± 3.79	2.92 ± 2.71	5.19 ± 7.28	
Uric acid [mg/dl]	5.65 ± 1.27	5.75 ± 1.53	5.75 ± 1.30		5.83 ± 1.22	5.32 ± 1.30	5.62 ± 1.42		5.09 ± 1.27	5.59 ± 1.19	5.21 ± 1.30	
hs TNT [ng/l]	8.4 ± 4.2	8.6 ± 4.8	9.4 ± 4.2		9.2 ± 6.1	11.6 ± 8.9	10.3 ± 9.0		10.4 ± 4.6	11.3 ± 7.0	13.3 ± 5.0	
MDA [μM/l]	2.02 ± 0.53	1.94 ± 0.45	2.01 ± 0.43		1.91 ± 0.42	1.98 ± 0.41	2.16 ± 0.37 ⁺	*	2.03 ± 0.59	1.90 ± 0.44	1.72 ± 0.55	

Data are means ± SD; p-values are calculated using Friedman-Test for overall differences between time points; *... < 0.05; **... < 0.001; Wilcoxon-Test was performed for post-hoc analyses, significance level to T1: ⁺... < 0.05.

Formamidopyrimidine DNA glycosylase (FPG), ferric reducing ability potential (FRAP), superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px), low-density-lipoprotein (LDL), 8-oxo-7,8-dihydroguanine (8oxoGuo), 8-oxo-7,8-dihydro-2'-deoxyguanosine (8oxodG), high sensitive c-reactive protein (hs CRP), high sensitive Troponin-T (hs TNT), malondialdehyde (MDA).

**Fig. 2.** Six-months change in the “antioxidant factor”.

nor blood based markers did differ between RT, RTS or CT groups (RT vs. RTS vs. CT: BMI $p = 0.960$, SOD $p = 0.060$, CAT $p = 0.911$, GSH-Px $p = 0.378$, FRAP $p = 0.491$, LDL $p = 0.881$, 8oxoGuo $p = 0.921$, 8oxodG $p = 0.830$, hsCRP $p = 0.964$, uric acid $p = 0.843$, hsTNT $p = 0.309$, MDA $p = 0.365$).

We observed not powerful, however significant negative correlations between 8oxoGuo and handgrip strength ($r = -0.350$, $p = 0.001$) as well as between hs TNT and the 6-min-walking test ($r = -0.210$, $p = 0.035$) at baseline. Baseline data for physical fitness, enzymes and the comet assay have been published previously in Franzke et al. [17] and Oesen et al. [6], however they were not analyzed and discussed in the context of aging (over and under LE) or oxidative stress and therefore constitute essential co-parameters. Antioxidant enzyme activity did not differ between age groups at baseline (over vs. under LE: SOD $p = 0.061$, CAT $p = 0.280$, GSH-Px $p = 0.765$), however, as described previously [17], a weak negative correlation of age with SOD ($r = -0.236$, $p = 0.043$) and CAT ($r = -0.312$, $p = 0.007$) but not with GSH-Px ($r = -0.031$,

$p = 0.610$) activity was observed.

3.1.1. Baseline characteristics over and under life-expectancy

As almost 60% of our subjects hold an age older than the statistical LE (Austria: women 83.25 years; men 77.95 years), we performed further analyses dividing our sample population in a group over and a group under LE, respecting the sex-specific difference in LE [18]. We observed significantly lower LDL-cholesterol ($p = 0.022$) and higher hs TNT ($p = 0.010$) levels in subjects over LE and a trend for reduced SOD activity ($p = 0.061$) in the older group (Table 1). If divided by age groups, subjects of the youngest sub group presented the highest hsCRP values ($p < 0.05$) (65–74 years = 6.42 ± 5.25 mg/l, 75–84 years = 2.65 ± 1.96 mg/l, 85+ years = 3.20 ± 2.98).

Significant, however weak, negative correlations between 8oxoGuo and handgrip strength were observed in both, under ($r = -0.403$, $p = 0.018$) and over LE ($r = -0.317$, $p = 0.026$). Further, in subjects under LE chair rise test correlated negatively with hs TNT ($r = -0.345$, $p = 0.027$).

Subjects over LE showed a significant positive correlation between CAT-activity and chair rise test ($r = 0.401$, $p = 0.014$).

3.2. Intervention

Attendance at the training sessions was 71% ($\pm 26.5\%$) with no significant differences between groups ($p \geq 0.05$). After six months of intervention, we observed significant improvements in both exercise groups in chair rise (RT: $p = 0.006$, RTS: $p = 0.002$) and six minutes walking test (RT: $p = 0.004$, RTS: $p = 0.029$). For further details about training attendancy and the effects on physical function parameters please see Oesen et al. [6]. BMI did not change in any intervention group.

Only the RTS group demonstrated a significant increase in uric acid ($p = 0.010$) and MDA ($p = 0.036$) (Table 2).

Interestingly, after six months of intervention RT and RTS groups demonstrated significantly higher basal DNA damage; RT and CT groups showed improved resistance against H₂O₂ exposure, and RT and RTS groups revealed significantly increased activity in CAT and SOD, respectively. Six months data of the comet assay and antioxidant enzymes are not shown as they have previously been published in Franzke et al. [17].

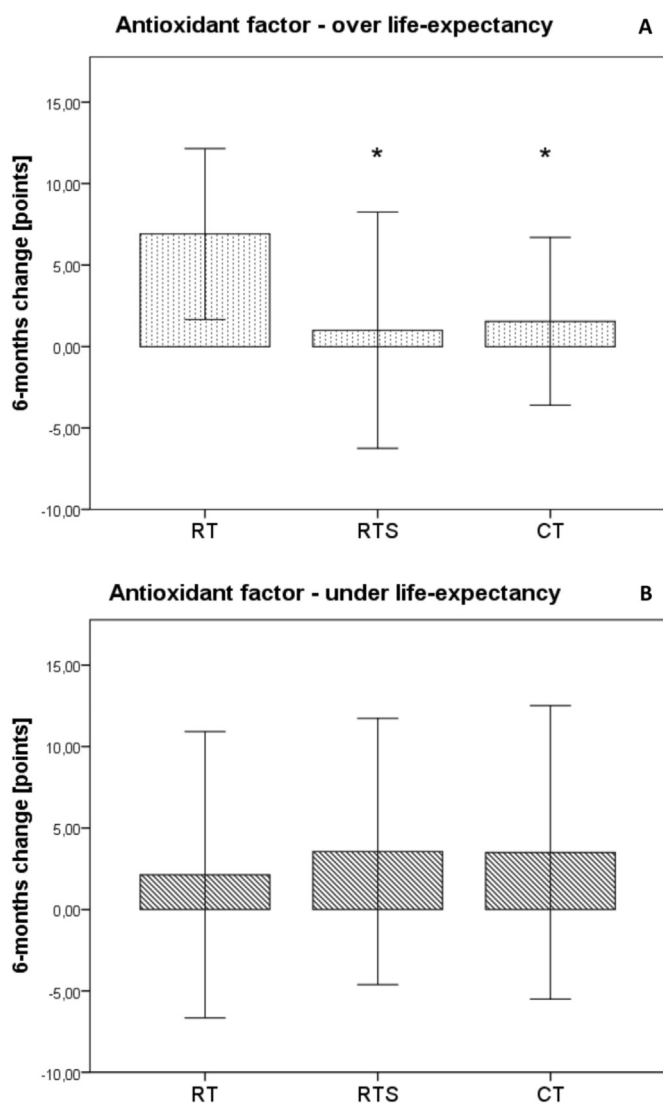


Fig. 3. Six-months change in the “antioxidant factor” for subjects over (A) and under life-expectancy (B). * indicates significant difference ($p < 0.05$) to RT group.

3.2.1. Correlations

After six months a decrease in DNA strand breaks significantly correlated with an increase in handgrip strength in RT group ($r = -0.480$, $p = 0.010$) and an increase in chair rise performance in the RTS group ($r = -0.494$, $p = 0.019$). RTS groups further showed a significant correlation between an increase in chair rise performance and increased MDA ($r = 0.438$, $p = 0.047$). Both, decreased GSH-Px activity ($r = -0.510$, $p = 0.015$) and decreased hs CRP ($r = -0.469$, $p = 0.028$) significantly correlated with increased six-minutes-walking performance in RTS groups.

The six-months deltas in the CT group revealed a significant negative correlation between six-minutes-walking test and DNA damage induced by H_2O_2 exposure ($r = -0.470$, $p = 0.024$), as well as a significant positive correlation between MDA and lysis ($r = 0.438$, $p = 0.028$).

3.2.2. The age effect

Regarding the age effect, in subjects over their statistical LE we observed a significant reduction in 8oxodG in the RT group ($p = 0.039$) but not in the other groups. Under LE a trend for reduced 8oxodG in the RTS group ($p = 0.060$) occurred.

A significant increase in %DNA in tail in cells treated with lysis in

the RT ($p = 0.009$) and CT ($p = 0.028$) groups over LE was observed. At the same time a significantly improved resistance against H_2O_2 exposure was measured (RT: $p = 0.013$; CT: $p = 0.019$). Subjects of the RTS group over LE showed no significant effect in the parameters of the comet assay (Table 3).

Under LE only the RTS group showed a significant effect of the perceived intervention with an increase in lysis ($p = 0.046$) (Table 4).

Over LE SOD activity increased significantly in the RTS group ($p = 0.006$) (Table 3). Younger subjects showed no significant effects for enzyme activity; a trend for increased CAT activity under LE was observed ($p = 0.057$) in the RT group.

Uric acid significantly decreased in the RTS group over LE ($p = 0.046$).

Under LE MDA significantly increased in the RTS group ($p = 0.013$).

3.2.3. The “antioxidant factor”

As described above, we calculated an “antioxidant factor” (the sum of the categorized six-months percentage change of all parameters related to oxidative damage, stress and antioxidant defense) to obtain a clearer picture about the total effect of the interventions on our subjects. The “antioxidant factor” revealed an about twice as high six-months change in the RT group (4.9 ± 7.2 points) compared to RTS (2.4 ± 7.6 points) and CT (2.2 ± 6.5 points) group, however, there was no statistical difference between the groups (Fig. 2). Over LE, we observed significantly higher changes in the RT subjects (6.9 ± 5.2 points) compared to RTS (1.0 ± 7.3 points, $p = 0.033$) and CT subjects (1.6 ± 5.1 points, $p = 0.028$). Under LE no statistical difference between the groups was observed (RT = 2.1 ± 8.8 points, RTS = 3.6 ± 8.2 points, CT = 3.5 ± 9.0 points) (Fig. 3).

4. Discussion

The current study was conducted to investigate the effect of either a strength training, a strength training and protein-vitamin supplement or a cognitive training intervention on markers of oxidative stress, oxidative damage and antioxidant defense in a cohort of elderly institutionalized, Viennese women and men. Our previous work guided our attention towards the phenomenon of longevity, in this perspective towards characteristics of DNA integrity and genome stability and the different responses between people reaching an age above their statistical life-expectancy and those dying earlier [1].

Despite only 12.4% of our study participants being male the sex ratio was representative for the group of people living in retirement homes in Vienna [28]. The high age of our study population (women 82.9 ± 6.0 years; men 84.9 ± 6.7 years) is comparable to the mean age of people living in institutionalized facilities and was for men almost seven years higher than the present life expectancy in Austria [18,29].

While observing no difference between intervention groups at baseline, our analyses partly confirmed what has been shown in the literature. Better physical performance was linked to reduced oxidative DNA damage [3] and decreased levels of cardiac risk markers [30]. hs CRP values were comparable to other study cohorts of the same age and within the normal range for old humans [31–33]. Interestingly, hs CRP, which is commonly known to increase with age [34], was observed to be highest ($p < 0.05$) in our youngest age-group, which could partly be explained by a natural “aging-advantage” of people achieving high age. Successful agers seem to be genetically predisposed and better equipped to more efficiently cope with the accumulation of deleterious endo- and exogenous processes leading to cellular senescence and finally to mortality [1,35]. Latest observations are pointing towards a superior cellular resilience of the oldest old humans, who are demonstrating rarely a link to traditional cardiovascular disease risk factors [1,34]. However, hs TNT, a cardiac risk marker, was significantly higher in the oldest participants over statistical LE (Table 1),

which might validate hs TNT as a potential biomarker for aging. Martin-Ruiz et al. [36] identified 10 valid markers for aging, after testing 74 candidate biomarkers, however they did not measure hs TNT, but BNP (N-terminal pro-B-type natriuretic peptide) which represents a cardiac marker which is strongly linked to hs TNT [37,38].

Comparing our baseline characteristics of subjects over their statistical LE with those under LE, it seems that in the oldest subjects physical and functional strength play an important role in preventing from oxidative damage as well as in keeping the antioxidant defense systems active. These observations are supported by the work of Martin-Ruiz et al. [36], who identified hand grip strength and timed up and go test, which are all strength related markers, as strong predictors for aging. Weak results in those tests are highly correlated to multi-morbidity, disability, cognitive impairment and mortality [36], which in turn are linked to increased oxidative stress, DNA damage and impaired antioxidant defense [39,40]. Furthermore, as strength, muscle mass and physical function are steadily declining with age, the expected loss of strength and function in the oldest subjects of our cohort endorses the importance of physical fitness in this high-age-group [41,42].

The effect of exercise training on markers of oxidative stress and DNA damage in elderly are controversially discussed in literature. There are reports about an exercise-induced decline in oxidative damage [10], but also an increase in oxidation products is considered [9]. The most conclusive picture is that strenuous and unaccustomed acute exercise can lead to (mostly transient) DNA damage, whereas regular exercise training likely results in protective adaptations including endogenous antioxidant defense and DNA repair mechanisms [12,43]. Especially in the elderly, where DNA repair capacity might have already been reduced [44], very intensive and non-individualized exercise programs might lead to chronically elevated oxidative stress and unphysiologically increased DNA damage [42,45]. However, evidence has emerged that a transient generation of ROS is essential for inducing redox-sensitive cellular pathways that lead to adaptive responses to exercise training [11].

Besides increasing functional fitness parameters in both resistance training groups [6], six months of either performing elastic band resistance training, alone or combined with the provided supplement, or cognitive training revealed interesting results regarding parameters of oxidative stress and DNA strand breaks (see Tables 2–4).

Since our analyses of mainly single oxidation parameters did not create clear insights and did not allow to draw precise conclusions, we consequently calculated an “antioxidant factor” (the sum of the categorized six-months percentage change of all parameters related to oxidative damage, stress and antioxidant defense) to better compare the total effect of the interventions on our subjects (Fig. 2).

Following the pattern of our baseline analyses we compared subjects over LE with the younger ones. Here our data showed a significant higher six-month change in the “antioxidant factor” in RT subjects, compared to CT and RTS, only in the group over LE but not in the younger group (Fig. 3). Interestingly, over LE the RTS group demonstrated only marginal adaptations, similar to the results from subjects of the CT group, indicating that the consumed antioxidants from the supplement might have inhibited optimal responses to resistance training. As exercise acutely elevates the production of ROS, the consumption of antioxidant supplements close to exercise training is intensively discussed [13,15,46]. Meanwhile however, the important role of ROS as messenger molecules in exercise adaptation seems to be well established and the (over-) consumption of antioxidant supplements has been shown to suppress optimal response of the redox system after a training stimulus [14]. As a consequence, in our already well-nourished institutionalized elderly subjects [6,47], especially regarding antioxidants (determined by the mini-nutritional-assessment-questionnaire, nutritional protocols, and plasma status – data not shown), the antioxidants which were part of the supplement could have buffered the physiologically increased ROS level inhibiting optimal exercise responses in the oldest old, by “killing the messenger”.

Humans aged over their statistical LE show improved antioxidant defense, more efficient DNA repair mechanisms, longer telomeres and a better genome stability, compared to humans below LE [1,48], which might, at least to a certain extent, be affected by a genetic predisposition [16]. This superior resilience of exceptionally old people against oxidative damage might also be accompanied by a more sensitive redox-system, which possibly leads to faster and more effective adaptations to exercise induced ROS exposure. Despite observing no significant difference in the six-months deltas of physical performance between age-groups, the “antioxidant factor” revealed an obvious age-influence in our study population.

It is well known, that muscle strength, mass and function decline with age [49]. With about 10 years of difference between the mean age of our study groups over and under LE respectively (Table 1), the further progressed loss of muscle mass and strength could have established a baseline condition, which made the oldest subjects more susceptible to this type of “low-intensity” resistance training, regarding redox-regulation. Elastic band resistance training has been shown to effectively increase muscle strength, function and muscle mass [4,5], however it was, comparable to our data, not able to improve oxidation markers in elderly below LE, aged between 60 and 74 years [4].

Noteworthy, there are some limitations in our study. Although all training sessions were guided and supervised by sport scientists, determining exercise intensity and keeping it at an effective level for all subjects is a concern using elastic bands. The individually perceived exertion oftentimes does not correlate with the one repetition maximum and therefore the actual training stimulus might have been lower, compared to using weight machines or free weights [50]. Further, our interpretations of the results should be considered carefully as the number of subjects within each subgroup was notably lower when analyzing the three intervention groups above and below LE after 6 months (under LE: $N_{RT} = 15$, $N_{RTS} = 12$, $N_{CT} = 9$; over LE: $N_{RT} = 15$, $N_{RTS} = 12$, $N_{CT} = 17$). Although our in- and exclusion criteria aimed at producing an as homogenous sample of elderly as possible, the very advanced age of our participants (around their statistical LE) produced a broad standard deviation in all of our parameters by nature.

5. Conclusion

Collectively, in our study cohort of very old institutionalized elderly we could show that six months of elastic band resistance training improved physical function, as well as over-all redox status. Further, a better fitness level was linked to reduced oxidative DNA damage and decreased levels of cardiac risk markers, especially in the oldest subjects. Interestingly, older subjects seemed to be more susceptible to elastic band resistance training regarding the general oxidative state described as the “antioxidant factor”. Noteworthy, the antioxidants of the provided supplement might have contributed to a suppression of the adaptation to exercise of the redox-system in the RTS group.

The phenomenon of successful aging and the superior DNA integrity, antioxidant defense and DNA repair capacity of the super-aging cohort urges for deeper investigations to better understand their unique cellular resilience.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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