

DISSERTATION

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„The effect of a lifestyle intervention on DNA damage
and genome stability in Austrian institutionalized elderly“

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Mag. Bernhard Franzke, Bakk.

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I. Abstract

Aging and its aligned loss of muscle mass are associated with higher levels of DNA damage and deteriorated antioxidant defense. In order to improve the body's overall resistance against DNA damage and genomic instability, maintaining a healthy and active lifestyle is desirable, especially in the elderly. Since people are getting older, many of the elderly have to change their residence from home living to an institution, which is often accompanied by malnutrition, depression and inactivity.

It has been shown that exercise training – depending on the individual training status, the training frequency and intensity, and the presence of chronically increased oxidative stress and/or inflammation – acutely and transiently increases the production of free radical oxygen species (ROS). However, the exercise-induced oxidative challenge is essential for triggering the adaptation processes after physical activity in order to improve antioxidant defense mechanisms.

The current study aimed at investigating the effect of a six month progressive resistance training (RT), with or without protein and vitamin supplementation (RTS), or a cognitive training (CT), on DNA strand breaks (Comet assay) and chromosomal damage (CBMN assay) in Austrian institutionalized women and men. One hundred seventeen subjects were recruited from five different houses of the Curatorship of Viennese Retirement Homes (KWP) and randomly assigned into the three intervention groups. Each group trained two times per week and the RTS group received the supplement together with their breakfast and directly after each workout.

At baseline, data from 97 women and men (13.4% male, 86.6% female) were included into the calculations. The participants had a mean-age of 83.0 ± 6.1 years. After six months of intervention, 80 participants completed all tests. DNA damage increased significantly in the RT group (T1-T2 +20%, $p=0.001$) and the RTS group (T1-T2 +17%, $p=0.002$) and showed a similar tendency in the CT group (T1-T2 +21%, $p=0.059$) after three months of intervention. However, %DNA in tail, in cells exposed to H_2O_2 (a well-known reactive oxygen species inducer), decreased significantly in the RT (T1-T2 -24%,

p= 0.030; T1-T3 -18%, p= 0.019) and CT (T1-T2 -21%, p= 0.004; T1-T3 -13%, p= 0.038) groups; only a tendency for an overall time-effect occurred in the RTS group (Friedman-Test p= 0.093). Antioxidant enzyme activity showed significant differences for time in the RT and RTS groups (RT +22% Catalase-activity T1-T3, p= 0.013; RTS +6% Superoxide Dismutase-activity T2-T3, p= 0.005). In this very heterogenic age group micronuclei (MN) frequency decreased in all intervention groups over six months (RT: -13%, RTS: -10%, CT: -10%), however not significantly. Only nucleoplasmic bridges (CT: p= 0.025) and apoptotic cells (RTS: p= 0.024; CT: p= 0.021) showed significant time effects. Vitamin status changed significantly only in the RTS group (plasma B12: +130%, p= 0.006; red blood cell folic acid: +43%, p= 0.018). We observed a significant negative correlation between the six months change of the B12 plasma level and the MN frequency in the RTS group (RTS: $r = -0.673$, p= 0.002; RT: $r = 0.087$, p= 0.692; CT: $r = -0.246$, p= 0.309).

Our results suggest that both, cognitive and resistance training improve resistance against H_2O_2 induced DNA damage and decrease chromosomal damage in institutionalized elderly. To improve the status of the vitamins B12 and folic acid, a supplementation could be useful. Furthermore, our data indicate that there might be a link between aerobic fitness and chromosomal damage, by means of lower MN frequency with an elevated fitness level, even in the very oldest of our population.

II. Zusammenfassung

Der mit dem Alterungsprozess einhergehende Verlust von Muskelmasse und Muskelkraft ist stark mit einer reduzierten Lebensqualität verbunden. Um diesem Abbau entgegen zu wirken und somit Mobilität und Unabhängigkeit im Alter zu erhalten oder wiederherzustellen, ist körperliche Aktivität, insbesondere in Form von Krafttraining, wichtig.

Krafttraining kombiniert mit einer optimalen Versorgung an Nährstoffen, allen voran Protein, scheint am effektivsten, um Muskelmasse und Kraft, auch bei SeniorInnen, zu erhöhen. Speziell um oxidative Schäden an der DNA und Zellmutationen zu reduzieren sind Antioxidantien sowie die Vitamine B12 und Folsäure wichtige Komponenten. Sowohl Altern, als auch der Abbau von Muskulatur sind mit Entzündungen, oxidativem Stress, DNA-Schäden und Genominstabilität verbunden.

Bekanntermaßen erhöht (Kraft-) Training akut und vorübergehend die Bildung reaktiver Sauerstoff-Spezies (ROS) und bewirkt, abhängig von Trainingsstatus, Häufigkeit und relativer Intensität des Trainingsreizes, erhöhten oxidativen Stress und erhöhte Entzündungswerte im menschlichen Organismus. Allerdings ist, durch akutes körperliches Training induzierter, oxidativer Stress essentiell, um Anpassungsprozesse im menschlichen Körper nach körperlicher Belastung auszulösen und antioxidative Abwehrmechanismen zu verbessern.

Das Forschungsziel der vorliegenden Arbeit war es den Einfluss von sechs Monaten Krafttraining (RT), alleine oder in Kombination mit einer Protein und Vitamin Supplementierung (RTS) und kognitivem Training (CT) auf DNA Schäden (Comet Assay) und Genomstabilität (CBMN Assay) bei institutionalisierten PensionistInnen zu untersuchen. Einhundertsiebzehn ProbandInnen wurden aus fünf Häusern des Kuratoriums Wiener Pensionisten-Wohnhäusern (KWP) rekrutiert und randomisiert den drei Interventionsgruppen zugeteilt. Es wurde in jeder Gruppe zweimal pro Woche trainiert, zusätzlich erhielt die RTS-Gruppe das Supplement täglich zum Frühstück, sowie direkt nach jedem Training.

Zu Studienbeginn konnten Daten von 97 ProbandInnen (13.4% Männer, 86.6% Frauen), mit einem Durchschnittsalter von 83.0 ± 6.1 Jahren, in die Berechnungen miteinbezogen werden. In der RT-Gruppe (T1-T2 +20%, $p= 0.001$), sowie in der RTS-Gruppe (T1-T2 +17%, $p= 0.002$), erhöhten sich die DNA Schäden nach dreimonatiger Intervention signifikant. Jedoch erhöhte sich die Widerstandsfähigkeit der Zellen gegenüber der Behandlung mit H_2O_2 nach drei und nach sechs Monaten signifikant in der RT-Gruppe (T1-T2 -24%, $p= 0.030$; T1-T3 -18%, $p= 0.019$) und der CT-Gruppe (T1-T2 -21%, $p= 0.004$; T1-T3 -13%, $p= 0.038$); lediglich eine Tendenz für einen Zeiteffekt konnte in der RTS-Gruppe beobachtet werden (Friedman-Test $p= 0.093$). Die Aktivität der antioxidativ wirkenden Enzyme zeigte signifikante Anstiege in den RT und RTS-Gruppen (RT +22% Catalase-activity T1-T3, $p= 0.013$; RTS +6% Superoxide Dismutase-activity T2-T3, $p= 0.005$).

In dieser sehr heterogenen Altersgruppe verringerte sich die Mikrokern-Frequenz, ein Marker für Genominstabilität, in allen drei Interventionsgruppen nach sechs Monaten (RT: -13%, RTS: -10%, CT: -10%), allerdings nicht signifikant. Zwei der Parameter des CBMN-Assays, nukleoplasmatische Brücken (CT: $p= 0.025$) und apoptotische Zellen (RTS: $p= 0.024$; CT: $p= 0.021$), zeigten signifikante Zeiteffekte. Die Nahrungsergänzung in der RTS-Gruppe hatte einen signifikanten Effekt auf den Status der Vitamine B12 und Folsäure nach sechs Monaten (Plasma B12: +130%, $p= 0.006$; Folsäure - rote Blutkörperchen: +43%, $p= 0.018$). Diese Erhöhung des B12 Plasma Spiegels korrelierte signifikant mit einer Reduktion der Mikrokern-Frequenz in der RTS-Gruppe (RTS: $r= -0.673$, $p= 0.002$; RT: $r= 0.087$, $p= 0.692$; CT: $r= -0.246$, $p= 0.309$).

Die Ergebnisse der vorliegenden Studie deuten darauf hin, dass sowohl regelmäßiges Gedächtnis-, als auch Krafttraining die Widerstandsfähigkeit gegenüber von H_2O_2 induzierten DNA Schäden erhöht und Chromosomenschäden bei institutionalisierten PensionistInnen reduziert. Die Daten zeigen weiters, dass bessere aerobe Fitness mit geringeren Chromosomenschäden korreliert. In dieser Altersgruppe scheint eine zusätzliche Supplementierung sinnvoll, um den Status von Vitamin B12 und Folsäure zu erhöhen.

III. Publications and Manuscripts

The present thesis is based on the following papers:

Paper 1

B. Franzke, B. Halper, M. Hofmann, S. Oesen, H. Peherstorfer, K. Krejci, B. Koller, K. Geider, A. Baierl, A. Tosevska, E.M. Strasser, B. Wessner, K.H. Wagner, V.A.A.S. Group, **The influence of age and aerobic fitness on chromosomal damage in Austrian institutionalised elderly**, *Mutagenesis*, 29 (2014) 441-445.

Paper 2

B. Franzke, B. Halper, M. Hofmann, S. Oesen, W. Jandrasits, A. Baierl, A. Tosevska, E.M. Strasser, B. Wessner, K.H. Wagner, V.A.A.S. Group, **The impact of six months strength training, nutritional supplementation or cognitive training on DNA damage in institutionalised elderly**, *Mutagenesis*, 30 (2015) 147-153.

Paper 3

B. Franzke, B. Halper, M. Hofmann, S. Oesen, B. Pierson, A. Cremer, E. Bacher, B. Fuchs, A. Baierl, A. Tosevska, E.M. Strasser, B. Wessner, K.H. Wagner, V.A.A.S.G. (VAAS), **The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly**, *Exp Gerontol*, 65 (2015) 16-22.

Paper 4

B. Franzke, O. Neubauer, K.-H. Wagner, **Successful DNAgging - New insights into DNA integrity, genome stability and telomeres in the oldest old**, *Mutat Res Rev* (2015) – **ACCEPTED for publication**

IV. Contents

I. Abstract	2
II. Zusammenfassung.....	4
III. Publications and Manuscripts	6
IV. Contents	7
V. Abbreviations	9
1. Aging and its consequences on DNA damage and genome stability	11
1.1 Integrative and network aging theories.....	12
1.2 Oxidative stress, DNA damage and mitochondria.....	14
1.3 Oxidative DNA damage & aging	17
1.4 Chromosomal damage & aging	18
1.5 Nutrition, DNA-, chromosomal damage & aging.....	19
1.6 Physical activity, DNA-, chromosomal damage & aging.....	22
1.7 Socialization, DNA damage and aging	23
2. Hypotheses and study aims.....	24
2.1 Hypotheses.....	24
3. Study Design	25
3.1 Subjects	25
3.2 Resistance training	26
3.3 Resistance training and supplementation.....	27
3.4 Cognitive training	27
4. Methods	28
4.1 Cytokinesis block micronucleus cytome assay (papers 1 & 3)	28
4.2 Comet – Assay (paper 2).....	28
4.3 Chair rise test (papers 2 & 3).....	29
4.4 Handgrip strength test (papers 2 & 3).....	30
4.5 Six minute walking test (papers 1, 2 & 3).....	30
4.6 Vitamin B12 and folate (paper 3)	30
4.7 Superoxide Dismutase (SOD) (paper 2).....	30
4.8 Catalase (CAT) (paper 2).....	31
4.9 Glutathione Peroxidase (GSH-Px) (paper 2)	31
4.10 Statistics.....	31
5. Summary of results and conclusions (papers 1-4)	32

5.1	Paper 1: Original Article. The influence of age and aerobic fitness on chromosomal damage in Austrian institutionalized elderly	35
5.2	Paper 2: Original Article. The impact of six months strength training, nutritional supplementation or cognitive training on DNA damage in institutionalized elderly.....	37
5.3	Paper 3: Original Article. The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly	38
5.4	Paper 4: Review Article. Super DNAGing - New insights into DNA integrity, genome stability and telomeres in the oldest old	40
6.	Further publications and scientific presentations.....	42
7.	Acknowledgement.....	43
8.	Literature	45
9.	Appendix.....	51

V. Abbreviations

ATP... adenosine triphosphate

BMI... Body Mass Index

CAT... catalase

CBMN assay... cytokinesis blocked micronucleus cytome assay

CT... cognitive Training

DNA... deoxyribonucleic acid

dTMP... deoxythymine monophosphates

dUMP... deoxyuracil monophosphates

EN... energy

FPG... formamidopyrimidine DNA glycosylase

GSH-Px... glutathione peroxidase

H₂O₂... hydrogen peroxide

KWP... Curatorship of Viennese Retirement Homes

MMSE... Mini Mental State Examination

MN... micronuclei

mt... mitochondrial

nBridges... nucleoplasmic bridges

nBuds... nuclear buds

O₂... Oxygen

pO₂... O₂ partial pressure

RONS... reactive oxygen and nitrogen species

ROS... reactive oxygen species

RT... resistance training

RTS... resistance training and supplementation

SOD... superoxide dismutase

SPPB... short physical performance battery

1. Aging and its consequences on DNA damage and genome stability

Aging is the inevitable accumulation of physical changes in organisms over time, which progressively increases the risk of disease and mortality [1, 2]. In developed countries the aging process constitutes the major risk factor for disease and mortality after the age of 28 years [3], with the rate of aging being low in early life, but, due to its exponential nature, rapidly increasing with age [4].

With the continuously increasing life-expectancy [5], predictions from the World Health Organization estimate that the global number of people aged 85 years and older is going to increase by 351% from 2010 to 2050. Particularly the sub-group of centenarians will increase 10-fold, which makes the oldest old group (aged 85 years or older) the fastest growing segment of the population in developed countries [6]. Importantly, many of the oldest old lose their ability for independent living, mostly caused by limited mobility, frailty or other signs of altered physical and/or cognitive function [6]. Especially the group of elderly aged around the statistical life-expectancy of humans (Austria: men around 78 years, women around 83 years) [5] is also most susceptible to disease and disability [7]. With this bio-demographic development the burden of chronic, age-related diseases such as cardiovascular diseases, type 2 diabetes, cancer, dementia and physical impairments associated with the age-related loss of skeletal muscle and function, is steadily increasing [7].

Aging is considered as a degenerative and multi-factorial process caused by accumulating molecular and cellular damage that leads to cell and tissue dysfunction [8-10]. Proposed mechanisms that contribute to the aging process and the development of chronic, age-associated diseases include increased levels of DNA damage, genotoxicity, oxidative stress, and shorter telomeres [11-15]. However, the underlying biological mechanisms of aging are still not fully discovered and understood, which is also displayed by the different theories trying to explain the aging process [2].

1.1 Integrative and network aging theories

Many theories have been proposed to explain the phenomenon of aging, yet none has been able to fully answer all aging related questions, and instead of a unifying theory of aging only the number of theories increased within the last decades (e.g. the somatic mutation theory, the telomere hypothesis, the free radical/ oxidative stress/ mitochondrial theory, altered protein accumulation theory) [1, 2, 16, 17]. However, in parallel to the different aging hypothesis, integrative approaches and “network” theories of aging were developed and gained more and more importance, claiming that for the process of aging the interactions between biological and molecular mechanisms may be at least as important as single actions [2, 17-20].

In 1997, Kirkwood and Kowald [18] were the first to describe a “network” approach of aging incorporating four aging theories – 1) accumulation of defective mitochondria; 2) the effect of aberrant proteins in protein synthesis; 3) the damaging actions of reactive oxygen species (ROS), and the protective role of antioxidant mechanisms; and 4) the turnover of proteins by proteolytic scavengers. Their model successfully predicted many observations, including:

- Age related increase in the fraction of inactive proteins, important for specific enzyme activity;
- Significant increase in protein half-life with age;
- Decreasing number of mitochondria with age;
- Increased frequency of defective mitochondria;
- Increased mitochondrial free radical production with age;
- Reduced level of ATP generation per mitochondrion.

In their integrative approach on aging Zglinicki et al. [20] emphasized the response to stress-induced damage to nuclear, telomeric and mtDNA as part of a synergistic network (**Fig. 1**). Further, they strengthened the importance of interacting networks, rather than individual actions, to explain the process of aging.

inter-cellular communication could trigger a wave of functional decline in the network and cause aging.

One of the newest network theories of aging is the one of Cohen et al. [21] suggesting complex higher-order physiological processes as a main component for the human aging process. They identified “integrated albumemia” as a valid predictor for mortality and frailty. Moreover, it’s behavior is more stable across populations and sub-populations (sex, race,...) than other aging-related biomarkers [21].

Within this PhD-theses we used markers for DNA damage (single- and double-strand breaks; measured by the Comet-assay), chromosomal damage (mutagenicity; measured by the CBMN-assay), and antioxidant defense (enzyme activity; SOD, CAT, GSH-Px) to investigate the effect of aging, exercise and nutrition in institutionalized elderly.

1.2 Oxidative stress, DNA damage and mitochondria

Mitochondria constitute the main source of ROS production in humans - up to 90% of total ROS are generated by mitochondria [22]. The total mitochondrial ROS emission is the net result from ROS generation at the electron transport chain and their reduction by the antioxidant defense (antioxidative enzymes) [23]. This process of redox-regulation is highly sensitive to the mitochondrial redox state; a dynamic state which can easily be altered by different physiological and pathological conditions [22, 23]. Under normal physiological conditions, ROS production is low and ROS scavenging is high, according to the redox-optimized ROS balance hypothesis [22-24]. However, if this balance shifts into either of both extremes (highly oxidized or highly reduced redox environment), oxidative stress occurs, either as a consequence of a depleted scavenger pool or exceeded scavenger capacity (Fig. 2) [22, 24]. In the long run (under conditions of chronic diseases, chronic inflammation...), a permanent state of oxidative stress could lead to sustained DNA damage, genome instability, cell death and further to mortality. Importantly, however, a low physiological

concentration of reactive oxygen and nitrogen species (RONS) essentially contributes to cellular signaling processes (e.g. exercise adaptation, immune response,...) [25].

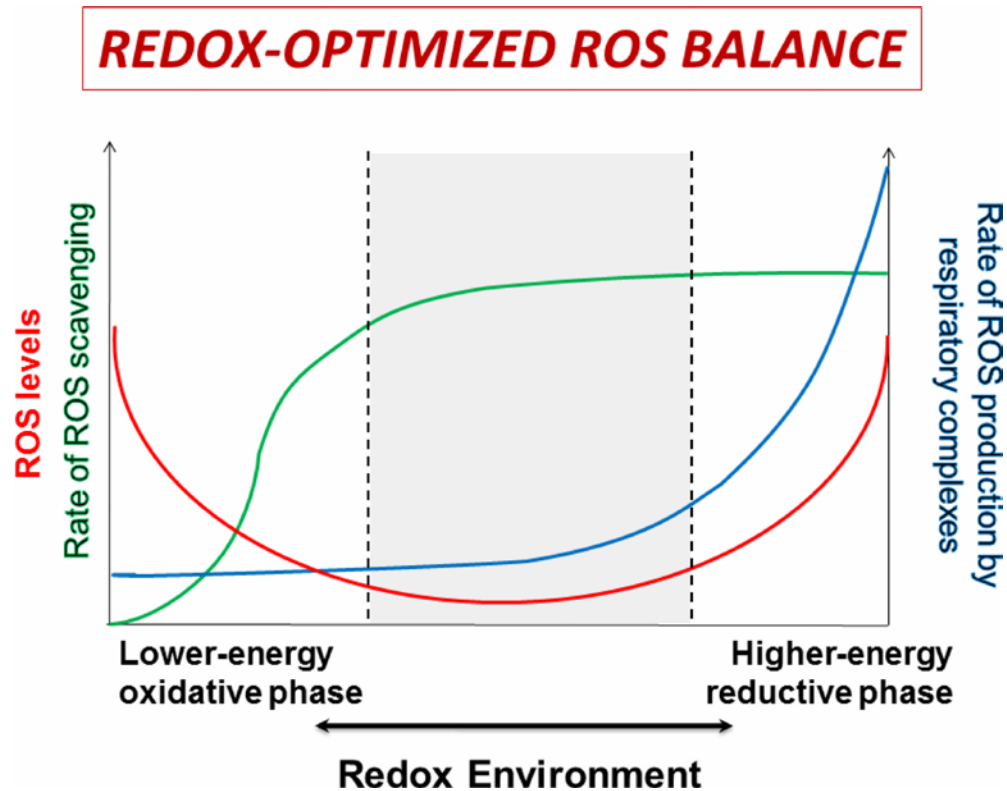


Fig. 2: The Redox-Optimized ROS Balance hypothesis [22].

Importantly, mitochondria are set-up for maximizing energy output while at the same time keeping ROS production at a minimum and therefore optimized for exercise-like conditions [22, 24]. According to these observations, in the absence of chronically elevated oxidative stress (diseases, chronic inflammation, hypoxia...), relative ROS production, inside the mitochondrion is decreased under conditions of exercise, compared to the resting state (Fig. 3). When mitochondria are shifting from resting respiration (state 4) to active respiration (state 3) (physical activity), despite increased energy demand and increased mitochondrial O_2 consumption, intra-mitochondrial ROS production is relatively low due to fast electron flow and decreased O_2 partial pressure. However, the increased mitochondrial flow-rate under exercise-like conditions also increases the over-all net ROS production

per unit of time, which constitutes the product of mitochondrial ROS production minus antioxidant scavenging (Fig. 2). Notably, at physical inactivity and sedentary conditions (state 4 of mitochondrial energy transition), when electron flow and energy production is low, intra-mitochondrial ROS production is relatively high, but in total kept at an physiological lower level through scavenging processes (Fig. 3) [26].

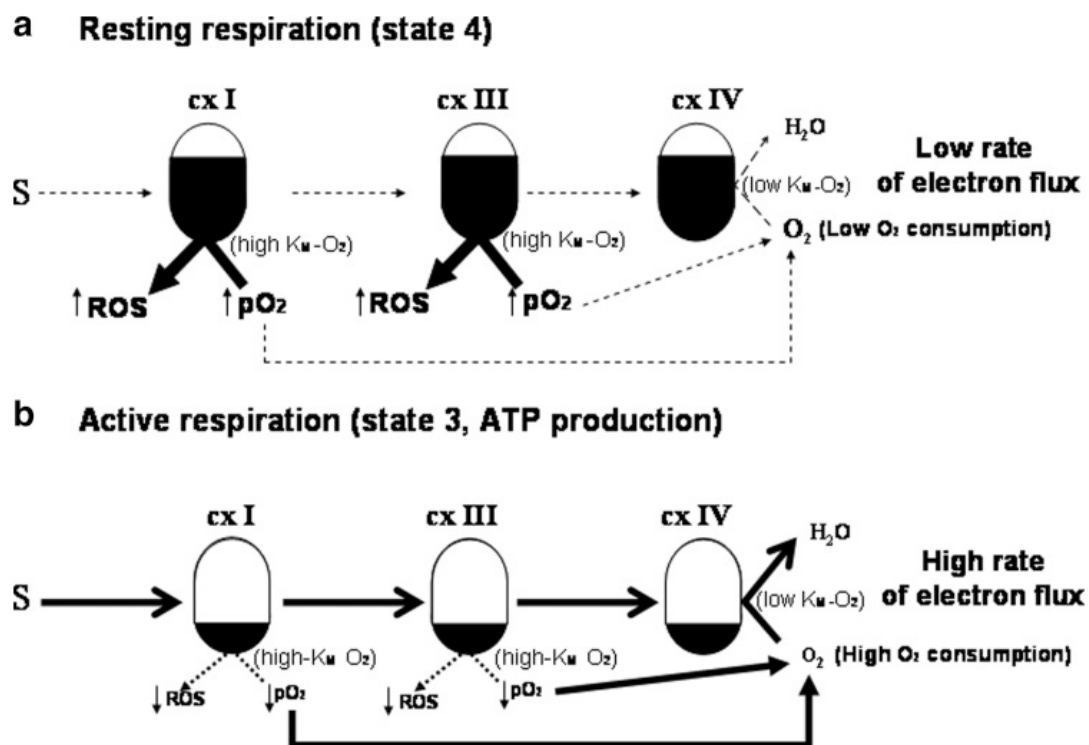


Fig. 3: Mitochondrial ROS and energy production in rest (state 4) and under activity (state 3) [26].

The above described mechanisms support the fact that regularly performed exercise has rather protective effects and does not contribute to increased mitochondrial ROS production (under healthy conditions) [26]. It has been shown, that regular exercise increases the number of mitochondria and decreases the mitochondrial ROS production in animals [26] and also in elderly humans [27, 28] and might therefore successfully contribute to lower rates of DNA damage and “healthy aging” [29, 30].

1.3 Oxidative DNA damage & aging

The process of aging has shown to be linked to increased DNA damage [31]. These findings are supported by the free radical theory of aging, suggesting that the production of ROS by mitochondria accumulates over the lifespan and, therefore, lead to a state of chronic oxidative stress at old age [3]. As antioxidant defense mechanisms and DNA repair capacity seem to be impaired in the elderly, DNA damage has been proposed to be a consequence of aging [32]. However, the free radical theory of aging has not conclusively been proven; and whether ROS are the primary cause of aging or a consequence remains a matter of debate [33]. Furthermore, evidence has been increasing in the recent years that ROS are not only damaging agents, but also act as signaling molecules for initiating adaptive responses to exercise [34-36]. In support of these findings, DNA damage has been shown to be lower in “successful agers” (reaching an age of 85+ years), compared to people below their statistical lifespan [37-39], indicating that for these individuals the radical theory of aging is not applicable. Previous studies suggested that age-related increased DNA damage is accompanied by enhanced DNA repair mechanisms, which might, at least to a certain extent, explain that there is no further increase of DNA damage after the age of 70 to 80 years [40, 41].

In a study performed by Humphreys et al. [41] the subjects in the oldest age-group (75-82 years) demonstrated higher basal DNA damage but also better repair and protective, antioxidant mechanisms, compared to the younger age-groups (aged 20-35 years and 63-70 years).

Studies in nonagenarians (90-99 years) and centenarians (100-109 years) concerning DNA damage are still rare but revealed interesting results. Human individuals in this age group represent an excellent model for healthy aging [42]. Importantly, Hyland et al. [37] showed that in their group of nonagenarians plasma antioxidant capacity was significantly higher compared to a middle-aged (40-60 years) control population. Moreover, the level of DNA damage was comparable to the younger cohort, which indicates that there is no further age-dependent increase of DNA damage in the “survivors”, whose age is beyond the statistical life-expectancy. Accordingly to the previous findings,

no age-related link could be observed in the Newcastle 85+ study [43]. Wiley et al. [43] concluded that ROS production and mitochondrial dysfunction in white blood cells do not serve as valid biomarkers for subjects aged 85 years or older.

Investigating fibroblasts from centenarians, Chevanne et al. [38] observed similar DNA repair activity and antioxidant enzyme activity compared to younger age groups, but at the same time less susceptibility to H₂O₂ induced DNA damage. In agreement with these findings [38], cells from centenarians demonstrated significantly improved repair capacity after H₂O₂ exposure than cells from elderly (69-75 years) subjects. Notably, in cells of the oldest, DNA repair activity was comparable to cells of the youngest group (19-26 years) [39].

1.4 Chromosomal damage & aging

Cytogenetic aberrations, such as acentric fragments, nondisjunction, aneuploidy, chromosomal loss, and the formation of micronuclei are elevated in the elderly and in subjects with age-related diseases such as cancer, cardio-vascular diseases and diabetes [44, 45]. It is well reported that chromosomal damage increases over the human lifespan and shows a linear relationship until the age of 60-70 years [46-48].

Only a few investigations on subjects within the oldest age-groups of nonagenarians or centenarians have been performed so far. These findings indicate that there might be a threshold of genome instability that limits the upper rate of MN formation (a marker for chromosomal damage). If the “survivors” are able to reach that high age of 85 years or above, they seem to be either more resistant to MN formation or cells with MN are more likely to undergo apoptosis [47, 49, 50].

However, available data indicate that this particular group of the oldest old might be more resistant to mutagenicity than elderly below or at their statistical life-expectancy. Franceschi et al. [51] reported a significant lower frequency of sister chromatid exchange in cells from centenarians compared to younger age-groups (23-79 years) which constitutes a high genomic stability in the

oldest old. In support of these data, Erceg et al. [52] observed similar chromosomal aberrations in subjects between 80 and 100 years compared to the younger middle-aged control group. In subjects between 65 and 80 years of age about twice as high frequencies of cytogenetic aberrations were observed versus younger control groups [52].

Bonassi et al. [53] were the first to report rather a reduction of the micronucleus frequency in the latest age classes. By analyzing data of 181 subjects (20-98 years), we recently demonstrated a leveling-off of the micronucleus frequency beyond the age of 60 to 70 years [54]. Our findings are supported by the results of Wojda et al. [50], which also indicate no further increase in the micronucleus frequency of centenarians and a plateau-like effect of chromosomal aberrations after the age of 60 years [49].

Genomic instability of human cells increases with age but, comparable to the data on DNA damage (previous chapter), in subjects beyond their statistical life-expectancy no further increase can be observed, rather a trend for improved chromosomal stability in this age-group is reported.

1.5 Nutrition, DNA-, chromosomal damage & aging

The living situation in many elderly individuals is changing towards surroundings such as homes for the elderly, which is often accompanied by conditions of malnutrition, loss of appetite, multiple medications or increased depression and loss of life quality [55, 56]. Especially the deficiency of several micronutrients has been reported to highly affect DNA stability and to increase the risk of chronic, age-related diseases [57]. There is sufficient evidence that deficiencies of specific vitamins and minerals correlate with DNA damage, DNA repair mechanisms, cellular mutation, and telomere shortening [58-60]. Therefore, diet quality, in addition to physical activity and psychological stress, plays a key role in preventing age-related diseases by minimizing DNA damage and enhancing repair capacity [57].

Mounting evidence supports the importance of an optimal status of vitamins, minerals and other bioactive dietary components to lower DNA damage, improve cellular repair mechanisms, enhance genome stability, and to improve telomerase activity [57, 59-61]. Figure 4 shows the nutrient network, which is discussed to enhance genome stability, telomere maintenance and antioxidant defense [60].

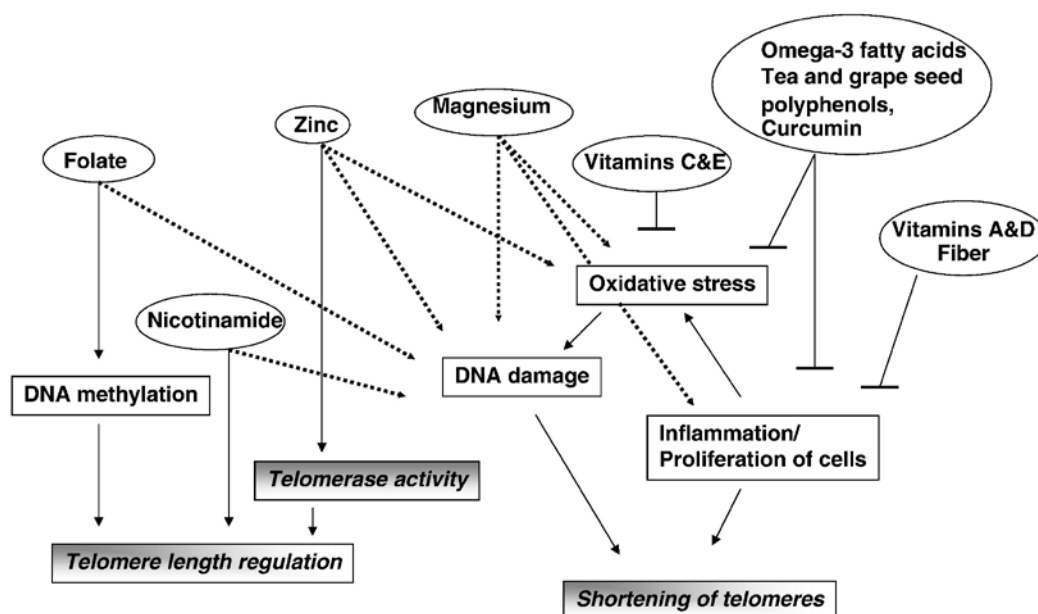


Fig. 4: Potential mechanisms behind the influence of nutrients on telomere length and DNA damage. Dashed line indicates effects by deficiency of a nutrient [60].

Within this thesis a main research focus was genome stability, as measured by the cytokinesis-block micronucleus cytome (CBMN) assay. To prevent from mutation and keep chromosomal damage low, the vitamins folate and B12 are mainly discussed [48, 61] and were also part of our biomarker toolbox [47, 54].

Folate and vitamin B12 play a crucial role in converting uracil (dUMP) to thymidine (dTMP) during DNA methylation (Fig. 5). In absence or deficiency of vitamin B12 this process is diminished which makes folate unavailable and consequently increases the uracil incorporation into DNA, which in turn makes DNA very susceptible for strand breaks and increases genome instability [62, 63].

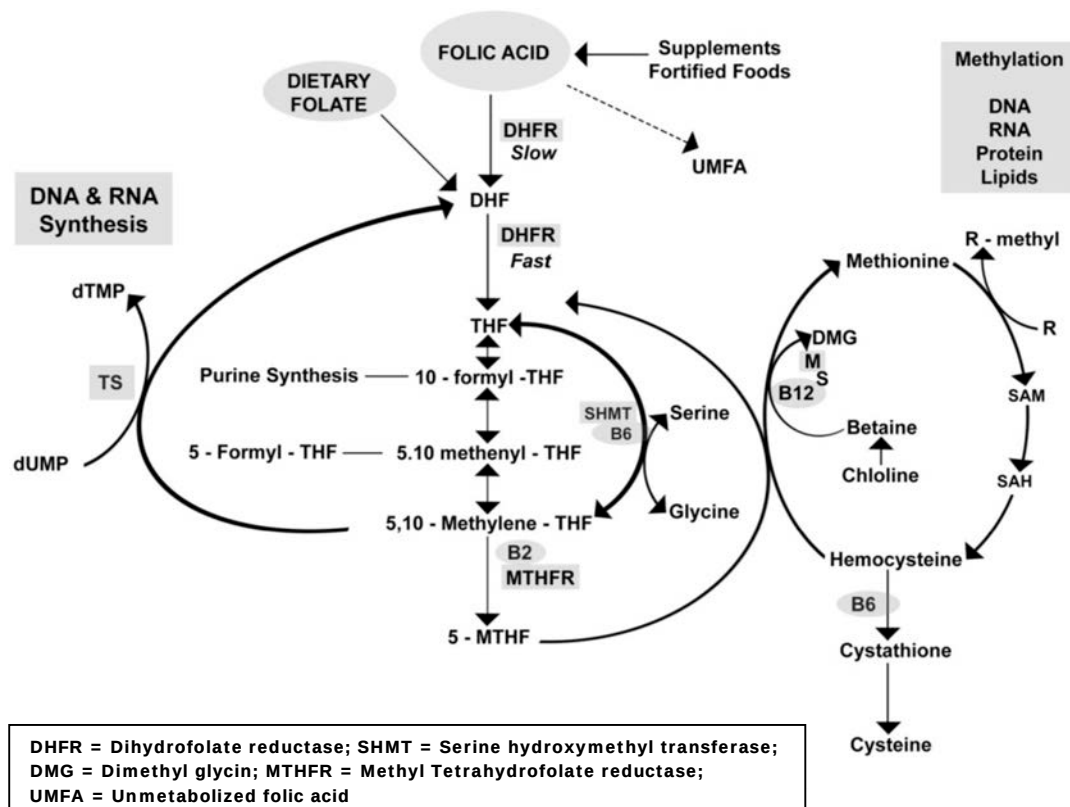


Fig. 5: Folate and vitamin B12 metabolism [64].

Although a sufficient micronutrient intake is crucial for improved antioxidant defense, a lower grade of DNA damage and therefore reduced incidence of various (age-related) diseases, this approach fails in prolonging the maximum lifespan [65]. Interestingly, in mammals, a successful method to increase mean, as well as maximum lifespan is the dietary strategy of caloric restriction, which has also been shown to reduce DNA damage and improve DNA repair [66].

Recent findings emphasize the importance of mitochondrial DNA as a main target for aging, because of its close proximity to the free radical generation. Inside the mitochondrion, it is the mitochondrial ROS production that mainly determines the concentration of ROS, which seems to be the main factor for longevity, rather than antioxidant defense [65]. Caloric restriction seems to have the potential to lower the mitochondrial ROS production rate and therefore increase the lifespan among animals [65]. Possible links between mitochondrial ROS production, oxidative damage, DNA mutations,

caloric restriction and aging are shown in Figure 6. However, whether this approach is applicable to humans or not, demands for more research.

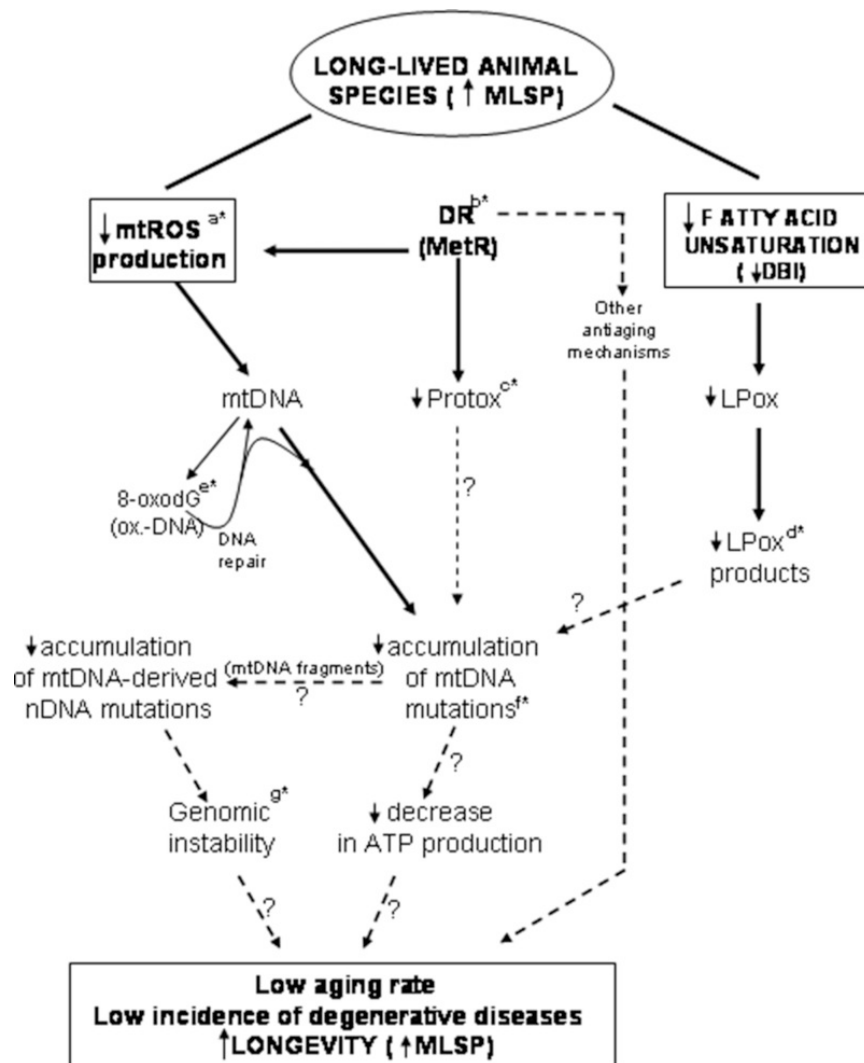


Fig. 6: Mitochondrial ROS production, oxidative damage, DNA mutations, aging and the possible influence or dietary restriction (DR) [65].

1.6 Physical activity, DNA-, chromosomal damage & aging

Existing data about the effects of physical activity on DNA damage are inconsistent, which, to a certain extent, is due to differences in the kind of acute exercise or the exercise training program and the training status of included individuals [67, 68]. The most conclusive picture that emerges from data about acute exercise and exercise training on DNA damage is that very strenuous and – in

relation to the training status – unaccustomed, acute exercise can lead to (mostly transient) DNA damage, whereas regular exercise training likely results in the adaptation of protective mechanisms including improved endogenous antioxidant defense and enhanced DNA repair mechanisms [67, 69]. Especially in the elderly, where DNA repair capacity might have already been reduced [68, 70], very intensive and unaccustomed exercise might lead to increased oxidative DNA damage [35]. However, evidence has emerged that a transient generation of ROS and moderate levels of oxidative stress are essential for inducing redox-sensitive cellular pathways that lead to adaptive responses to exercise training, particularly to adaptation in endogenous antioxidant defense and other cell-protective mechanisms [35, 36].

After chronic exercise, enzyme activity and DNA repair mechanisms have been shown to be up-regulated [71]. Investigating groups of young sedentary (26.0 ± 4.5 years), young physically active (30.2 ± 7.9 years), old sedentary (63.4 ± 4.7 years), and old physically active (62.4 ± 2.9 years) men, Radak et al. [73] observed improved DNA repair mechanisms in skeletal muscle after an acute exercise bout in young and old physically active subjects only, but not in the sedentary. Parise et al. [74] also reported that resistance exercise training decreased oxidative damage to DNA in older adults.

Moreover, elderly subjects with higher fitness levels demonstrated no difference in telomere length compared to a younger cohort [75], as well as shorter telomere length [76], and less chromosomal damage [54] compared to untrained controls of the same age.

1.7 Socialization, DNA damage and aging

Similar to physical activity, socialization and psychological stress seem to have strong impacts on DNA damage [77-80]. In institutionalized elderly oxidative stress was higher when compared to free-living subjects [81]. Social isolation and the psychosocial environment can have significant effects on oxidative stress markers and the endocrinal system, which in turn might affect DNA stability [82, 83].

2. Hypotheses and study aims

The present study was performed to measure the effect of six months elastic band resistance training, with or without protein and vitamin supplementation, or cognitive training on DNA damage and genome stability in institutionalized women and men, living in Vienna.

2.1 Hypotheses

- Regular elastic band resistance training improves genome stability.
- Regular elastic band resistance training improves resistance against DNA damage.
- An additional supplementation of vitamins and protein enhances the exercise induced effects.
- Cognitive training has no preventive effects against mutagenicity and DNA damage.
- Regular elastic band resistance training improves antioxidative defense.
- Vitamin supplementation improves nutritional status, particular regarding nutrients which are linked to genomic instability.

3. Study Design

The present study was conducted in a randomized, controlled, observer-blind design. The participants were randomly divided into three parallel groups – cognitive training (CT), resistance training (RT), RT + supplement (RTS) – and matched for gender. Blood samples were taken and physical performance tests were executed before (T1), after three months (T2) and after six months (T3) of intervention. The goal was to assess and compare the effects of either a resistance training intervention, a resistance training and nutritional supplementation intervention or a cognitive training intervention on institutionalized elderly.

3.1 Subjects

One hundred seventeen institutionalized elderly women and men (aged 65-98 years), recruited from five different senior residences in the area of Vienna (Curatorship of Viennese Retirement Homes (KWP)), volunteered for the study (Fig. 7 – Flow Chart). The subjects were mentally (Mini Mental State Examination (MMSE) ≥ 23) and physically (Short Physical Performance Battery (SPPB) > 4) able to participate in the study. They were sedentary (less than one hour of physical activity or exercise per week) and free of severe diseases (cardiovascular diseases, diabetic retinopathy and regular use of cortisone-containing drugs) that would contra-indicate medical training therapy or measurement of physical performance. The health condition of all study subjects was assessed by specialists in internal medicine and gerontology. Furthermore, regular strength training (more than once per week within the last six months before inclusion), was an exclusion criterion. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. This study was approved by the ethics committee of the City of Vienna (EK-11-151-0811) and registered at ClinicalTrials.gov, NCT01775111.

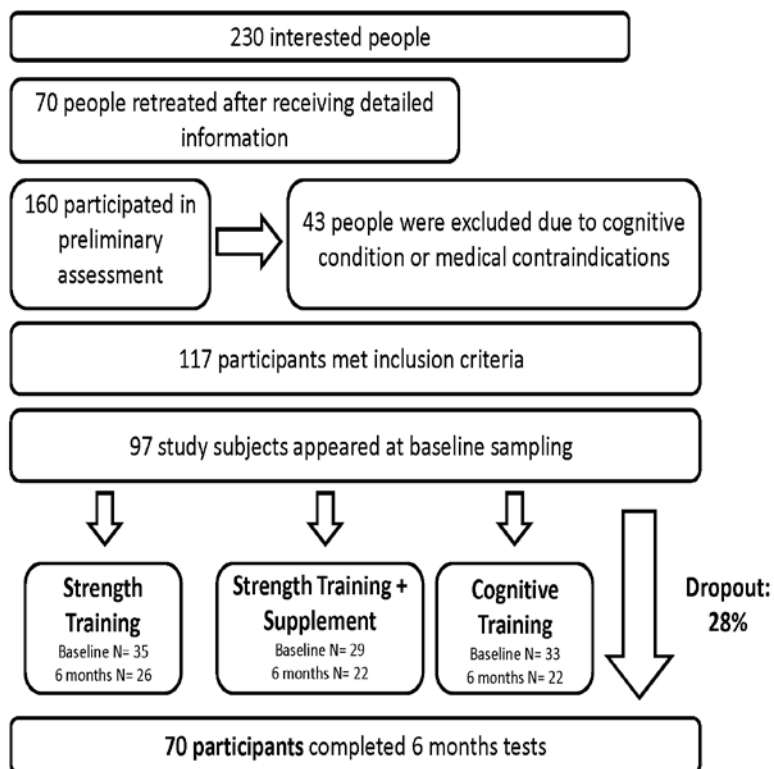


Figure 7: Flow chart: Number of study participants from first information to study end.

3.2 Resistance training

The resistance training groups (RT and RTS) received two weekly sessions of resistance training, conducted on two non-consecutive days and supervised by a sport scientist. Training attendance was recorded every session. The only equipment used were elastic bands and a chair. The progressive resistance training protocol was designed based on the guidelines of the American College of Sports Medicine for resistance training with older subjects [84]. The main part consisted of ten exercises for the main muscle groups (legs, back, abdomen, chest, shoulder and arms). One training session started with 10 minutes of warm-up, continued with 30-40 minutes of strength training and finished with a 10 minute cool-down. To keep the training stimulus high enough, the exercise program was adjusted to the participants' individual needs, by either adapting the resistance of the elastic band

(shorter or stronger band) or by modifying the exercise, by means of performing a more difficult version. In the initial phase (4 weeks) one set of 15 repetitions was performed in order to learn the correct form of each exercise. From the fifth week on the intensity and volume has progressively been increased from two sets of light exercises to two sets of heavy resistance. If the participants could easily perform two sets of 15 repetitions they were told to either take more resistance or to perform a more difficult version of the exercise.

3.3 Resistance training and supplementation

The RTS group followed the same training protocol as the RT group. Additionally they received a liquid supplement every morning, as well as directly after each training session. It consisted of 20.7g protein (56 energy (En)%, 19.7 g whey protein, 3 g leucine, > 10 g essential amino acids), 9.3g carbohydrates (25 En%, 0.8 BE), 3.0g fat (18 En%), 1.2g roughage (2 En%), 800 IU (20µg) of vitamin D, 250mg calcium, vitamins C, E, B6 and B12, folic acid and magnesium (1 portion FortiFit, Nutricia). Total energy per drink was 150 kcal. The intake of the nutritional supplement was controlled at breakfast as well as after the training sessions.

3.4 Cognitive training

The CT groups performed coordinative or cognitive tasks two times per week, equally to the RT and RTS groups. In contrary to a classical control group, which would not get any treatment, the training frequency of this group was the same as in the other groups. Therefore the “bias” of regularly being part in group activities was minimized (socialization factor). The participants of the CT group mainly performed memory training and finger dexterity exercises in a sitting position [85].

4. Methods

4.1 Cytokinesis block micronucleus cytome assay (papers 1 & 3)

Blood samples were taken early morning after an overnight fast using heparin, serum and EDTA tubes (Greiner Bio-One, Kremsmunster, Austria). Peripheral blood lymphocytes were isolated using Ficoll separation tubes (Greiner Bio- One). The cytokinesis block micronucleus cytome (CBMN) assay was conducted according to the protocol of Fenech [86] and Wallner et al. [46]. Cells were stimulated to perform mitotic division with phytohemagglutinin (PAA, Pasching, Austria) using a concentration of 1×10^6 cells/ml in culture medium. Samples were incubated at 37°C and 5% CO₂, and after 44 h, cytochalasin B (Sigma Aldrich, Vienna, Austria) was added to stop further cell division. Cells were spotted onto microscope slides, stained (Diff-Quick; Medion Diagnostics, Duding, Switzerland) and counted using a bright field microscope (1000- fold magnification; Olympus, Wien, Austria). For each sample, duplicates were performed and two slides of each duplicate were produced. From the four resulting slides, 500 cells per slide (2000 per subject) were counted equally to minimize experimental variation and eliminate scorer bias.

To assess chromosomal damage of blood lymphocytes, the frequency of MN, nucleoplasmic bridges and nuclear buds in 2000 binucleate (BN) cells was counted, as well as the number of apoptotic and necrotic cells. Furthermore, the nuclear division index (NDI) was calculated to measure cytostatic effects and the mitogenic response of lymphocytes [86].

4.2 Comet – Assay (paper 2)

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 H₂O₂, the comet assay was conducted [87, 88]. In short, a cell suspension of PBMCs (1x10⁶ 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 “H₂O₂” were prepared. All slides were placed into lysis solution (pH 10) for at least one hour. Only H₂O₂ slides were treated with a 100 µM H₂O₂ solution for 5 minutes 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 µL of enzyme buffer or FPG solution at 37°C for 30 minutes 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 minutes) and 30 minutes 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 µg/mL of ethidium bromide, DNA damage was measured using a fluorescence microscope together with an image analysis system (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.

4.3 Chair rise test (papers 2 & 3)

To perform well in the chair rise test, the participants had to stand up from a chair (46cm seat height) as often as possible within 30 seconds. 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 [89].

4.4 Handgrip strength test (papers 2 & 3)

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 seconds was measured (JAMAR compatible handgrip dynamometer adapted to handle different sizes). The better result of two trials (one minute break in between) for each hand was noted [90].

4.5 Six minute walking test (papers 1, 2 & 3)

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 [91]. 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 meter shuttle track and the distance covered within six minutes was registered [91].

4.6 Vitamin B12 and folate (paper 3)

Plasma concentrations of vitamin B12 and folate in erythrocytes were measured according to Müllner et al. [45] by using radioimmunoassay. Standard curves were drawn and sample values calculated according to the protocol published by the kit producer (MP Biomedicals, Germany).

4.7 Superoxide Dismutase (SOD) (paper 2)

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% [92, 93].

4.8 Catalase (CAT) (paper 2)

The activity of CAT was measured using photometrical analysis. Therefore the initial rate of H_2O_2 degradation was quantified. One unit of CAT activity was defined as the rate constant of the first-order reaction [94].

4.9 Glutathione Peroxidase (GSH-Px) (paper 2)

GSH-Px activity was analysed 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 [95].

4.10 Statistics

Statistical analyses were performed with IBM SPSS Statistics 21. Gender differences were measured, using Mann-Whitney-U- and Chi-Squared-Test. To assess the overall differences between the time points, Friedman-Test was conducted and if significant, Wilcoxon-Test was used to calculate the differences between each time point by considering Bonferroni correction. Linear and curve fitting regressions were calculated for MN-frequency and 6-minute-walking-performance as well as for MN-frequency and age. Spearman test was performed to assess the link between B12 plasma levels and MN frequency. Except for descriptive statistics, MN frequency was adjusted for sex as described in Bonassi et al. [96]. To analyze the age effect, we used additional data from our working group, as previously published by Wallner et al. [46]. Both, the present study and the one of Wallner et al. [46], performed the CBMN-assay according to the protocol of Fenech [86]. To minimize scoring variation, all scorers have been trained on the same slides by the same persons and regularly reassessed their scoring, to keep quality on a highest level. A p-value of less than 0.05 was considered significant.

5. Summary of results and conclusions (papers 1-4)

DNA damage, decreased antioxidant defense, and chromosomal instability are highly associated with the ageing-process, which is further accompanied by decreased physical activity, loss of muscle mass, and in many individuals by an institutionalized living situation. Especially in the elderly an active and healthy lifestyle is suggested to improve the individual resistance against unphysiological, oxidative stress and so to possibly decrease the incidence of age-related diseases.

Within this PhD-thesis and the papers it is based on, we investigated the effect of a six months progressive resistance training (RT), with or without protein and vitamin supplementation (RTS), or a cognitive training (CT), on DNA strand breaks and chromosomal damage in Austrian institutionalized women and men.

At baseline, data from 97 women and men were included into the calculations. Twenty of the former 117 participants quit the study before obtaining the first blood samples. From the 97 study subjects, 13.4% were male and 86.6% female, which constitutes a representative distribution in the houses of the Curatorship of Viennese Retirement Homes (KWP). The participants had a mean-age of 83.0 ± 6.1 years and a BMI of 29.27 kg/m^2 ($\pm 5.00 \text{ kg/m}^2$).

After six months of intervention, 80 participants completed all tests. However, for the CBMN-assay, ten samples demonstrated too low cell numbers to be included into the analyses. The drop-outs were due to health problems and lack of motivation. Still, the six month drop-out rate of about 28% was lower than expected (a 35-40% drop-out was predicted before starting the study) for this high-age group.

This study on a very old, institutionalized, still insufficiently investigated cohort revealed new and interesting results:

- DNA damage increased significantly in the RT group (T1-T2 +20%, $p = 0.001$) and the RTS group (T1-T2 +17%, $p = 0.002$) and showed a similar tendency in the CT group (T1-T2 +21%,

p= 0.059) after three months of intervention. %DNA in tail, in cells exposed to H₂O₂, decreased significantly in the RT (T1-T2 -24%, p= 0.030; T1-T3 -18%, p= 0.019) and CT (T1-T2 -21%, p= 0.004; T1-T3 -13%, p= 0.038) groups; only a tendency for an overall time-effect occurred in the RTS group (Friedman-Test p= 0.093).

- Antioxidant enzyme activity showed significant differences for time in the RT and RTS groups (RT +22% CAT-activity T1-T3, p= 0.013; RTS +6% SOD-activity T2-T3, p= 0.005).
- In this very heterogenic age group MN frequency decreased in all intervention groups over six months (RT: -13%, RTS: -10%, CT: -10%), however not significantly. Only nucleoplasmic bridges (CT: p= 0.025) and apoptotic cells (RTS: p= 0.024; CT: p= 0.021) showed significant time effects.
- Vitamin status changed significantly only in the RTS group (plasma B12: +130%, p= 0.006; red blood cell folic acid: +43%, p= 0.018). All data are shown in Table 1.
- We observed a significant negative correlation between the six months change of the B12 plasma level and the MN frequency in the RTS group (RTS: r= -0.673, p= 0.002; RT: r= 0.087, p= 0.692; CT: r= -0.246, p= 0.309).
- Subjects over their statistical life-expectancy demonstrate consistent genome stability; even tendencies for decreased chromosomal damage in this high age group were observed. Their antioxidant defense and DNA repair capacity was shown to be superior to subjects at or below life-expectancy and comparable to much younger cohorts, which might, at least to a certain extent, be influenced by hereditary factors.

Our data suggest that both, cognitive and resistance training improve resilience against H₂O₂ induced DNA damage and decrease chromosomal damage in institutionalized elderly. To prevent from an increase in chromosomal damage by a low status of the vitamins B12 and folic acid, a supplementation could be useful particularly for this old age group. Furthermore, our data indicate that there might be a link between aerobic fitness and chromosomal damage, by means of lower MN frequency with an elevated fitness level, even in the very oldest of our population.

Table 1: The impact of 3 (T2) and 6 (T3) months of intervention [resistance training (RT), resistance training and supplementation (RTS) and cognitive training (CT)] on chromosomal and DNA damage, physical performance, vitamin status, and antioxidant enzymes. P-values were calculated performing Friedmann-Test for differences over all time points.

	RT				RTS				CT			
Time-point	T1	T2	T3	P	T1	T2	T3	P	T1	T2	T3	P
Subjects [number]	35	23	26		29	27	22		33	22	22	
Cells with MN [per 1000 BN cells]	24.6±12.8	18.9±10.6	19.9±11.0	0.561	22.9±13.4	19.4±11.1	18.9±12.0	0.411	21.9±9.0	16.7±6.1	19.2±9.5	0.252
MN total [per 1000 BN cells]	27.6±13.9	22.8±13.2	24.1±14.9	0.943	25.0±10.8	23.9±15.7	22.4±14.4	0.522	26.0±10.9	19.7±7.4	23.5±12.5	0.278
nBridges [per 1000 BN cells]	1.13±1.14	0.53±0.72	0.50±0.49	0.491	0.88±0.89	0.52±0.62	0.59±1.16	0.129	1.23±1.05	0.57±0.63	0.64±0.85	0.025
nBuds [per 1000 BN cells]	3.56±2.75	3.24±2.34	3.93±3.96	0.940	3.34±2.62	2.63±2.45	2.98±2.35	0.422	4.23±4.57	3.53±3.83	3.03±3.08	0.759
Apoptotic cells [per 1000 BN cells]	0.83±0.64	0.58±0.28	0.51±0.32	0.257	0.69±0.37	0.51±0.34	0.48±0.23	0.024	0.73±0.58	0.62±0.35	0.43±0.23	0.021
Necrotic cells [per 1000 BN cells]	0.73±0.47	0.55±0.37	0.47±0.25	0.113	0.65±0.35	0.59±0.54	0.41±0.26	0.084	0.59±0.41	0.54±0.38	0.46±0.27	0.838
H ₂ O ₂ [%DNA tail]	27.0±7.1	20.9±8.6	22.5±4.3	0.050	28.1±7.8	23.1±6.2	24.9±4.4	0.093	27.4±5.8	21.7±6.9	23.8±4.8	0.008
Lysis [%DNA tail]	6.9±2.7	8.3±2.2	8.1±2.6	0.012	7.5±2.1	8.7±2.4	8.8±2.3	0.023	6.9±2.5	8.4±2.7	7.7±1.8	0.112
FPG [%DNA tail]	8.8±8.9	12.5±8.5	9.5±4.6	0.296	10.8±9.8	10.5±6.4	8.4±4.8	0.229	10.5±5.9	11.4±5.7	9.5±6.1	0.834
Chair Rise [repetitions]	11.9±3.3	13.6±3.7	14.6±3.7	0.002	13.0±4.4	14.3±4.6	16.1±5.4	0.001	11.9±4.2	11.8±3.5	11.3±3.3	0.829
6 Minutes Walking [m]	373±87	394±89	422±91	0.021	357±101	399±158	396±143	0.015	373±102	374±117	383±111	0.650
Handgrip [kg]	19.8±6.6	20.4±6.2	20.2±6.1	0.610	19.1±6.4	19.4±6.2	19.4±5.9	0.629	16.7±6.0	17.2±7.0	16.3±6.8	0.316
B12 [pmol/l]	346±268	388±349	384±352	0.738	452±432	799±802	1047±934	0.006	388±433	607±925	439±629	0.956
Folic acid [nmol/l]	204±116	186±111	228±123	0.678	154±33	231±150	216±83	0.018	192±142	207±129	206±70	0.204
SOD [IU/g Hb]	1981±280	2056±234	2142±224	0.174	2183±322	2124±219	2258±196	0.011	2092±177	2147±217	2182±273	0.096
CAT [IU/g Hb]	126±33	149±37	153±38	0.011	132±39	144±23	149±39	0.727	129±31	138±41	149±32	0.280
GSH-Px [IU/g Hb]	33.9±8.7	35.9±11.6	35.0±8.7	0.733	35.6±10.1	41.5±11.1	36.8±11.6	0.272	37.7±9.3	35.0±6.7	32.3±9.8	0.269

5.1 Paper 1: Original Article. The influence of age and aerobic fitness on chromosomal damage in Austrian institutionalized elderly

Within this first manuscript we analyzed baseline data of our study subjects concerning chromosomal damage and its link to aerobic fitness.

Chromosomal damage was measured by conducting the Cytokinesis Block Micronucleus Cytome (CBMN) assay. Aerobic fitness of the participants was assessed using the six minute walking test. To investigate the effect of age on micronuclei (MN) frequency and evaluate the particular age group, our data were merged with data from a recent study [46].

Age and MN frequency correlated significantly for squared regression ($r = 0.577$; $p = 0.000$) and showed, a levelling-off at about 60 years of age (Fig. 8). Furthermore we observed a significant negative linear correlation ($r = -0.222$; $p = 0.03$) between MN frequency and six min walking performance. There was a plateau-like effect of the MN frequency above the age of 60-70 years, indicating a higher resistance against chromosomal damage of the “survivors” of the regular life span. This study suggests that aerobic fitness “protects” against chromosomal damage at high age.

These data are the first to show that there is no further incline of the MN frequency after the age of 60 to 70 years in institutionalized elderly. Importantly, we observed a negative correlation between aerobic fitness and chromosomal damage, concluding that regular physical activity enhances resistance against genotoxicity in the elderly. Investigations in the age-group of the oldest people are generally rare, therefore this study adds important insights to this rapidly growing sub-population.

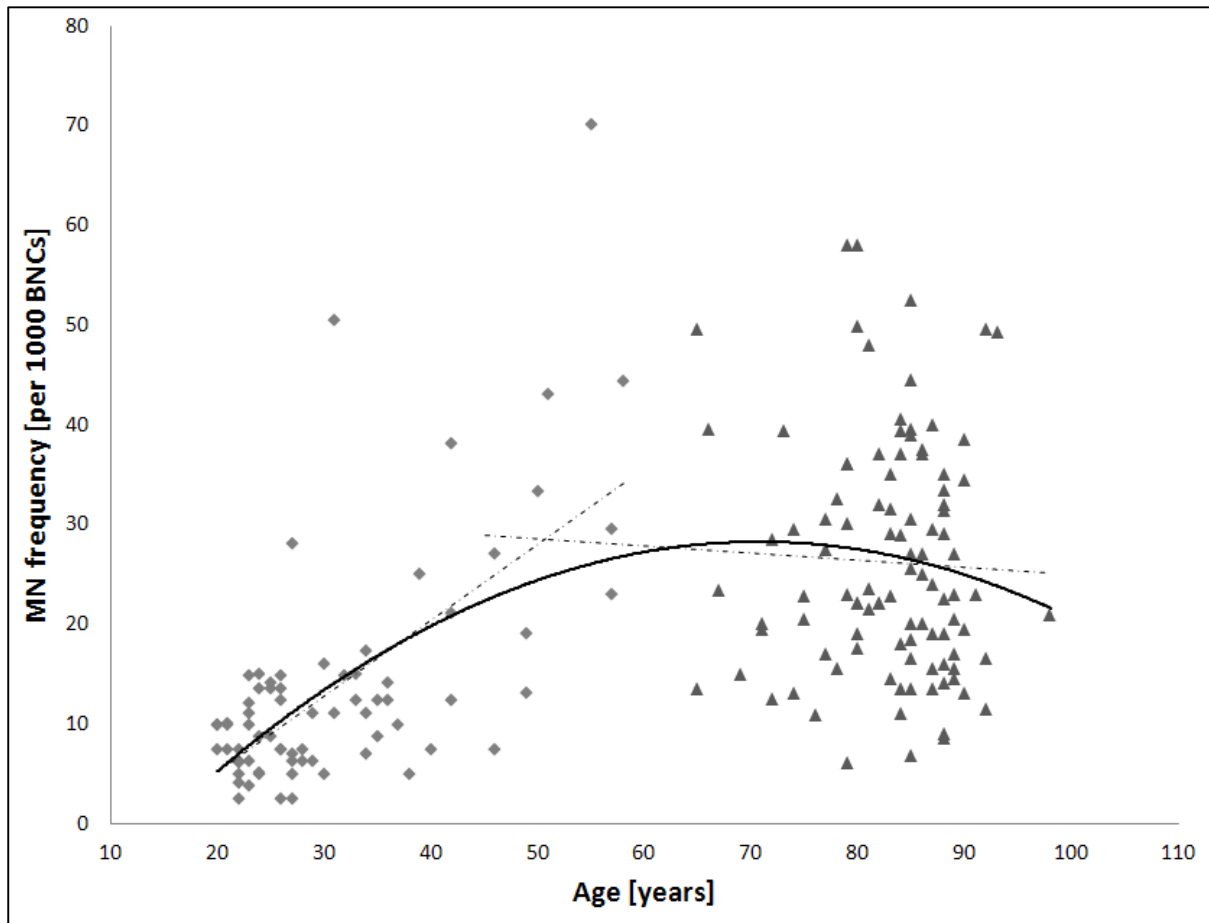


Fig. 8: Squared regression between MN frequency [per 1000 BNCs] and age [years] (—), $r = 0.577$, $p = 0.000$; linear regression under and over 60 years (— • — •).

5.2 Paper 2: Original Article. The impact of six months strength training, nutritional supplementation or cognitive training on DNA damage in institutionalized elderly

In paper two we examined the effect of six months of resistance training (RT), with or without protein and vitamin supplementation (RTS), or cognitive training (CT), on DNA strand breaks.

DNA damage was detected by performing the single cell gel electrophoresis (comet) assay. Physical fitness was assessed using the chair rise, the six minute walking and the handgrip strength test. In addition, antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were analyzed.

Basal DNA damage (lysis) increased significantly after three months of intervention in the RT group (T1-T2 +20%, $p = 0.001$) and the RTS group (T1-T2 +17%, $p = 0.002$), and showed a similar tendency in the CT group (T1-T2 +21%, $p = 0.059$). %DNA in tail decreased in cells exposed to H_2O_2 significantly in the RT (T1-T2 -24%, $p = 0.030$; T1-T3 -18%, $p = 0.019$) and CT (T1-T2 -21%, $p = 0.004$; T1-T3 -13%, $p = 0.038$) groups. Only RT and RTS groups showed significant differences over time in enzyme activity (RT +22% CAT-activity T1-T3, $p = 0.013$; RTS +6% SOD-activity T2-T3, $p = 0.005$). Contrary to the time effects, no difference between groups was detected for any parameter at any time point.

Our results suggest that both cognitive and resistance training improve resistance against H_2O_2 induced DNA damage and that a nutritional supplement has no further protective effect to prevent from single- or double-strand breaks in institutionalized elderly.

5.3 Paper 3: Original Article. The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly

The third paper continues with the six months observations now considering the parameters of genome stability.

All three intervention groups demonstrated a tendency of a reduced frequency of cells with MN (-15%) as well as for the total number of MN (-20%), however no significant time-effect was observed. Besides a significant increase in plasma B12 and red blood cell folate status, the six month change of B12 was negatively correlated with the six month change of the MN frequency in the RTS group ($r = -0.584$, $p = 0.009$) (Fig. 9). Our results suggest that in this age group either physical or cognitive training may result in similar biochemical changes and therefore enhance resistance against genomic instability. A supplementation with the vitamins B12 and folic acid could contribute to reduced chromosomal damage in institutionalized elderly. Importantly, subjects with higher chromosomal damage and lower physical performance at baseline were more likely to quit the study before the last samplings.

To our knowledge, we were the first to investigate the effect of a six-month lifestyle intervention of cognitive training, resistance training alone or combined with a nutritional supplement on resistance against chromosomal damage in highly aged institutionalized elderly. Our results from studying such a cohort of very old institutionalized elderly lead us to several conclusions:

- 1) Higher B12 plasma levels, due to an oral supplementation containing vitamin B12, are negatively linked to chromosomal damage.
- 2) Compliance to a human intervention study seems to be influenced by the subjects' baseline physical condition.

3) In this age group either physical or cognitive training could achieve similar biochemical changes which tend to enhance resistance against genomic instability.

4) Social events and social well-being might significantly contribute to institutionalized elderly's health.

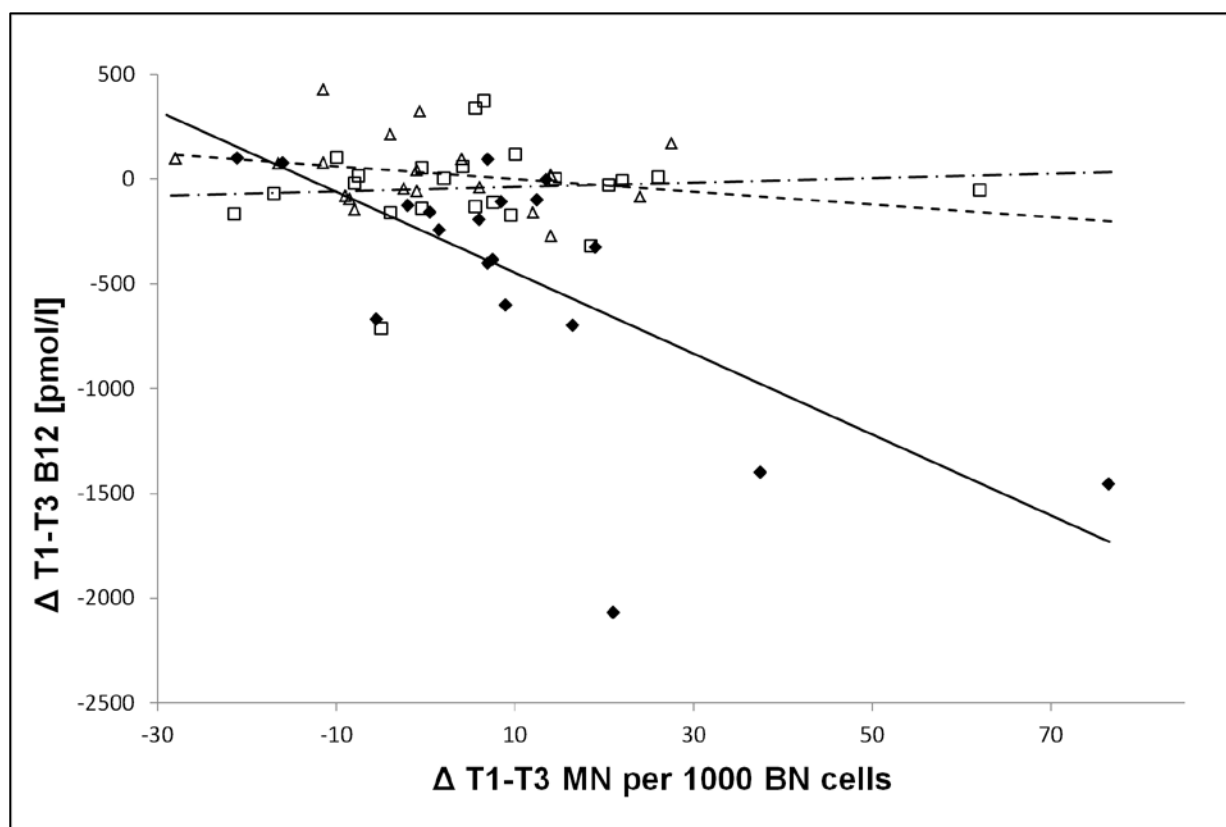


Figure 9: Correlation between six month change (T1-T3) of plasma B12 status and MN frequency. Filled square – RTS group (full line), empty square – RT group (dotted line), triangle – CT group (broken line); RTS: $r = -0.584$, $p = 0.009$; RT: $r = 0.081$, $p = 0.715$; CT: $r = -0.259$, $p = 0.284$.

5.4 Paper 4: Review Article. Super DNAgging - New insights into DNA integrity, genome stability and telomeres in the oldest old

Aging is considered as a degenerative and multi-factorial process caused by accumulating molecular and cellular damage that leads to cell and tissue dysfunction. Proposed mechanisms that contribute to the aging process and the development of chronic, age-associated diseases include increased levels of DNA damage, genotoxicity, oxidative stress, and incidence of shorter telomeres. Importantly, however, people exceeding the statistical life-expectancy, and especially the very oldest age-groups – nonagenarians (90-99 years), centenarians (100-109 years) and super-centenarians (110 years and older) – demonstrate a different picture of age-related diseases compared to study cohorts at or below life-expectancy. This phenomenon of “successful aging” contradicts the theories of aging where age linearly correlates with the accumulation of reactive oxygen species, DNA damage, mitochondrial dysfunction, and shortening of the telomeres.

In this review we therefore investigated the impact of DNA damage for successfully and healthy aging in the oldest old of our society and tried to explore the crucial difference between elderly individuals at or below life-expectancy and the “super old” – nonagenarians, centenarians and super-centenarians. We focused on reports about DNA damage (double/single strand breaks, DNA repair, antioxidant defense, mitochondrial DNA), mutagenicity (sister chromatid exchange, acentric fragments, nondisjunction, aneuploidy, chromosomal loss, formation of micronuclei) and telomeres (telomere length, telomerase activity) within the oldest age-groups. Furthermore, recognizing the importance of mitochondria and, especially, mitochondrial (mt)DNA stability and integrity for aging as well as the functionality of skeletal muscle in elderly individuals we discussed findings on mtDNA damage in skeletal muscle of aging humans. Finally we examine potential life-style based strategies for successful “DNAgging” and reaching the individually predetermined lifespan.

This review gives important and novel insights into what distinguishes individuals that are exceeding the statistical life-expectancy from those prior to death, on a cellular level. Conclusively, longevity

seems to be, at least to a certain part, determined by an individual genetical predisposition rather than an extraordinary health behavior at old age. However, independent of the achievable age, to fully exploit one's maximum lifespan, it is of great need to follow a healthy lifestyle (Fig. 10). Although the available data about DNA damage in the oldest old point into the same direction (towards a genetical predisposition), the relatively small number of studies within this age group urges for further investigations on cohorts beyond life-expectancy and their health behavior particularly in their early life periods.

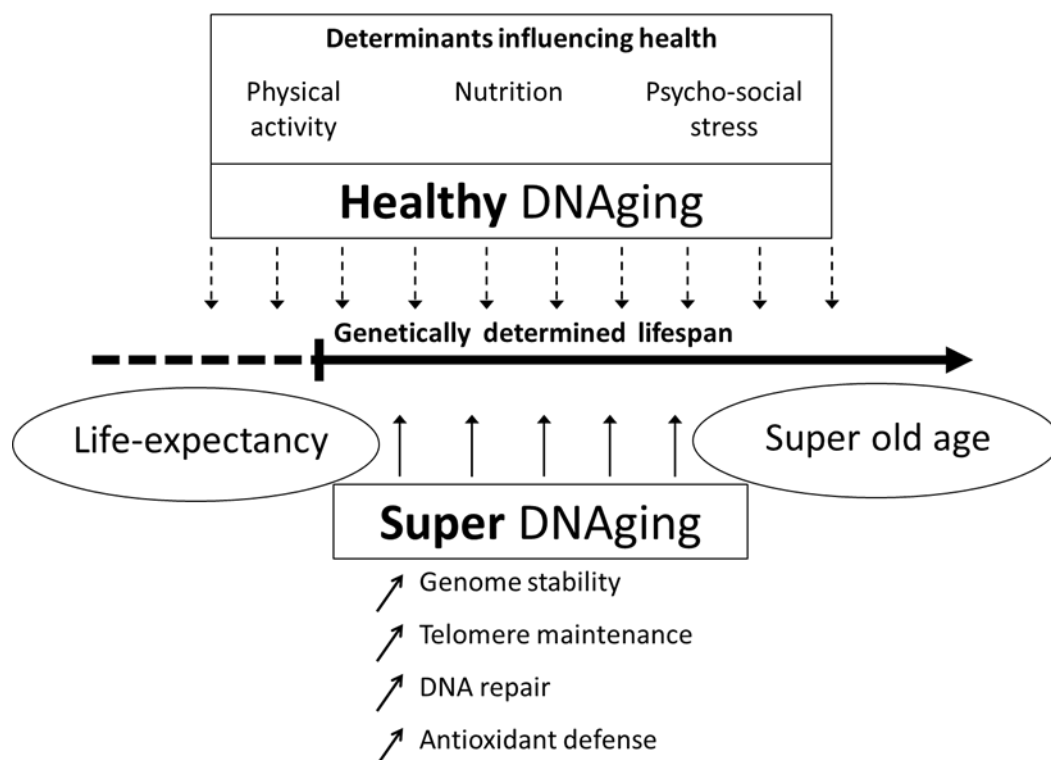


Fig. 10: Hypothesized model of factors that may explain why super old agers surpass life-expectancy from a DNA point of view. Dashed line arrows indicate influence of health behavior to possibly reach one's "genetically determined" age. Full line arrows indicate potential factors for reaching super old age.

6. Further publications and scientific presentations

M. Hofmann , B. Halper, S. Oesen, **B. Franzke**, P. Stuparits, H. Tschan, N. Bachl, E.M. Strasser, M. Quittan, M. Ploder, K.H. Wagner, B. Wessner, **Serum concentrations of insulin-like growth factor-1, members of the TGF-beta superfamily and follistatin do not reflect different stages of dynapenia and sarcopenia in elderly women**, Exp Gerontol, 64 (2015) 35-45.

B. Halper, M. Hofmann , M., S. Oesen, **B. Franzke**, Vidotto, C., H. Tschan, N. Bachl, E.M. Strasser, M. Quittan, M. Ploder, K.H. Wagner, B. Wessner, **Influence of age and physical fitness on miRNA-21, TGF- β and its receptors in leukocytes of healthy women**, Exerc Immunol Rev, 21 (2015) 154-63.

S. Oesen, B. Halper, M. Hofmann, W. Jandrasits, **B. Franzke**, E.M. Strasser, A. Graf, H. Tschan, N. Bachl, M. Quittan, M. Ploder, K.H. Wagner, B. Wessner, **Effects of elastic-band resistance training and nutritional supplementation on physical performance of institutionalised elderly - a randomized controlled trial**, Exp Gerontol – accepted for publication.

B. Franzke, M. Hofmann, **Workshop Funktionelle Tests**, 1. Symposium Active Aging, April 18th, 2015, Vienna, Austria.

B. Franzke, B. Wessner, K.H. Wagner, **Krafttraining, Gedächtnistraining und Ernährung bei institutionalisierten Hochbetagten – Forschungsplattform „Active Ageing“**, Ernährung Aktuell, 4 (2014) 1-5.

B. Franzke, B. Halper, M. Hofmann, S. Oesen, A. Baierl, A. Tosevska, E.M. Strasser, B. Wessner, K.H. Wagner, **The effect of a six months resistance exercise, nutritional or cognitive intervention on chromosomal and DNA damage in Austrian institutionalised elderly**, poster presentation, ÖGE Jahrestagung 2014, November 26th-27th, 2014, Vienna, Austria.

7. Acknowledgement

"We should be taught not to wait for inspiration to start a thing. Action always generates inspiration. Inspiration seldom generates action". (Frank Tibolt)

Action is what drives me, what motivates me, what inspires me.

"A complex system that works is invariably found to have evolved from a simple system that works."
(John Gaule)

A big thank you goes to my supervisor, Karl-Heinz Wagner, for incorporating me into the system he had built and giving me the support and trust to do my research.

"My life has no purpose, no direction, no aim, no meaning, and yet I'm happy. I can't figure it out. What am I doing right?" (Charles M. Schulz)

For me, life has no value without happiness... people "blame" me for always being happy... and I "blame" my mother for making me who I am today – happy without a purpose... because I can.

"A wise man will make more opportunities than he finds." (Sir Francis Bacon)

I want to thank my father for not just teaching, but living his bounce-back-ability, as well as for his trust into me and my activities.

"Silly is you in a natural state, and serious is something you have to do until you can get silly again."
(Mike Myers)

My silly/natural state is definitely a challenge to deal with and I want to thank my girlfriend, my love, Resi, for accepting this challenge, and for loving and joining me to enjoy the silliness together.

"It is our responsibilities, not ourselves that we should take seriously." (Peter Ustinov)

"Not everything that can be counted counts, and not everything that counts can be counted." (Albert Einstein)

A big thank you goes to my colleagues and my master students for making the last years possible and first and foremost impossibly entertaining and full of laughter.

"What's another word for Thesaurus?" (Steven Wright)

Last but definitely not least, a big thank you goes to my colleague and friend Oliver for being a perfect counterpart in countless conversations about work, sports, nutrition and life.

"Well, you know, I'm the forever optimist."

(Arnold Schwarzenegger)

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9. Appendix

Curriculum Vitae

Personal Information:

Name: Bernhard "Billy" Franzke
Telephone number: +43 1 4277 – 54932
Email: bernhard.franzke@univie.ac.at
Date of Birth: June 6th, 1981
Citizenship: Austria

Current position:

Scientific assistant at the Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna. Co-coordinator and PhD-student at the research platform "Active Ageing" – an intervention study on the effects of exercise and nutrition in the elderly. PhD-student of Nutritional & Sports Sciences.

Education:

1999 Graduation Highschool, Wr. Neustadt, Austria
2000-2005 Bachelor Sports Science, University of Vienna, Austria
2005-2008 Master Sports Science, University of Vienna, Austria
(Master Thesis "Proprioceptive training of the upper body")
from 2010 Doctoral Studies Sports Science & Nutritional Science,
University of Vienna, Austria
(PhD Thesis "The effect of a lifestyle intervention on DNA
damage and genome stability in Austrian institutionalized
elderly")

Career:

from 2011 Employee at the research platform "Active Ageing",
Department of Nutritional Sciences, University of Vienna,
Austria
from 2010 Vienna City Boot Camp, Founder & Head Coach, Vienna, Austria
2008-2012 INSPOSO (Individual Sports Solution), Freelancer, Vienna,
Austria
2006-2011 University Sports Institute (USI), Lecturer & Instructor, Vienna,
Austria
2009-2011 Life, Institut for Health Promotion, Head Coach, Baden, Austria

Research Interests:

- (1) The effects of training and nutrition on ageing.
 - a. DNA damage

- b. Cellular mutation
 - c. Mechanistic effects
- (2) Mechanistic reactions and adaptations on training and nutrition.
 - a. DNA modification
 - b. Growth factors
 - c. Hormones
 - d. Physiological adaptation limitations

Professional Qualifications:

- PC: Word, Excel, Power Point, SPSS, Inkscape
- Languages: German, English, French, Spanish
- NLP Practitioner
- Training & exercise: Specialist in body forming, weight management & performance improvement

Paper 1

Original Research Article

The influence of age and aerobic fitness on chromosomal damage in Austrian institutionalised elderly

Bernhard Franzke¹, Barbara Halper¹, Marlene Hofmann², Stefan Oesen¹, Heidemarie Peherstorfer³, Klemens Krejci³, Birgit Koller³, Karin Geider³, Andreas Baier⁴, Anela Tosevska¹, Eva-Maria Strasser⁵, Barbara Wessner^{1,2}, Karl-Heinz Wagner^{1,3,*} and Vienna Active Ageing Study Group

¹Research Platform Active Ageing, Faculty of Life Sciences and Centre for Sport Science and University Sports, University of Vienna, Althanstraße 14, 1090 Vienna, Austria, ²Department of Sport and Exercise Physiology, Centre for Sport Science and University Sports, University of Vienna, Auf der Schmelz 6, 1150 Vienna, Austria, ³Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria, ⁴Department of Statistics and Decision Support Systems, Institute for Statistics and Operations Research, University of Vienna, Grillparzerstraße 7, 1010 Vienna, Austria and ⁵Karl Landsteiner Institute for Remobilization and Functional Health, Institute for Physical Medicine and Rehabilitation, Kaiser Franz Joseph Hospital, SMZ-Süd, Kundratstrasse 3, 1100 Vienna, Austria

*To whom correspondence should be addressed. Tel: +43 1427 754930; Fax: +43 1427 79549; Email: karl-heinz.wagner@univie.ac.at

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Ageing goes hand in hand with altered DNA repair and defence mechanisms against DNA damage. To improve the body's overall resistance against chromosomal damage, maintaining a healthy and active lifestyle is of great concern, especially in the elderly. As more and more people are getting older, they change from home living to an institutionalised situation, which is often accompanied by malnutrition, depression and inactivity. So far, there is a lack of data on chromosomal damage in relation to age and fitness status. The aim of this study was to examine the effect of age and aerobic fitness on end-points of DNA damage in 105 institutionalised women and men (65–98 years) living in Vienna. Chromosomal damage was measured by conducting the cytokinesis block micronucleus cytome assay. Aerobic fitness of the participants was assessed using the 6-min walking test. To investigate the effect of age on micronuclei (MN) frequency and evaluate the particular age group, our data were merged with data from a recent study by Wallner *et al.* (Effects of unconjugated bilirubin on chromosomal damage in individuals with Gilbert's syndrome measured with the micronucleus cytome assay. *Mutagenesis* 2012; 27: 731–735). Age and MN frequency correlated significantly for squared regression ($r = 0.577$; $P = 0.000$) and showed a levelling-off at ~60 years of age. Furthermore, we observed a significant negative linear correlation ($r = -0.222$; $P = 0.03$) between MN frequency and 6-min walking performance. There was a plateau-like effect of the MN frequency above the age of 60–70 years, indicating a higher resistance against chromosomal damage of the 'survivors' of the regular lifespan. This study suggests that aerobic fitness 'protects' against chromosomal damage at high age.

Introduction

Ageing is a physiological process influenced by endogenous and exogenous factors, such as diet, exercise, exposure to microorganisms, pollutants, ionising radiation and production of reactive oxygen species (ROS), mostly accompanied by decreased physical activity and an increase of chronic diseases (1–3). Furthermore, with ageing, the mechanisms to counteract DNA modification and cellular damage are impaired, and as a consequence, cellular mutation rate is elevated (4–6).

The formation frequency of micronuclei (MN) is a well-established marker for chromosomal damage in various post-mitotic cell types (7,8). Micronucleus frequency is influenced by age, sex and lifestyle factors such as smoking, diet and physical activity (9) but also in a chronic disease state, like diabetes, cardiovascular disease or cancer, higher frequencies of MN are found (10,11). Most studies on the incidence of MN and ageing have reported a linear relationship between these parameters until the age of 60 years (9,12–14). However, only a very few have investigated people in their 80s or 90s, thereby finding hints for opposite observations (15–18). Almost two decades ago, Bonassi *et al.* (17) mentioned a plateauing effect of chromosomal damage with ageing. Surprisingly, ever since, only Wojda *et al.* (16) reported on the same effect in elderly people being over 100 years of age. Importantly, both studies did not really report on subjects between 75 and 95 years of age.

Since people are growing older, a deeper look on chromosomal damage of the very oldest of our population (8th, 9th and 10th decade) is required. Currently, there is a lack of data for this age group. This aspect is of interest because the living situation in many elderly people is changing in this period towards a transfer to new surroundings such as elderly homes, which is often accompanied by conditions of malnutrition, multiple medications or increased depression and loss of life quality (19,20).

Besides the ageing process, aerobic fitness seems to play a key role in the formation of MN in human peripheral blood mononucleated cells (21). Although acute physical stress induced by exercise increases the incidence of MN, moderate regular exercise seems to enhance the defence mechanisms against oxidative stress and as a result of which lower the frequency of MN (22–24). As no data on aerobic fitness and MN formation in institutionalised elderly people are available, we conducted this study to examine the impact of ageing and aerobic fitness on the formation of MN in institutionalised women and men in Vienna, Austria. With our study cohort subjects, being at or above life expectancy in Austria (study/Austria: women 82.86/83.25 years; men 84.85/77.95 years) (25) and 75% of our population being 80 years old or older, we were able to generate novel data on MN frequency in this particular age group of institutionalised elderly and link them with aerobic fitness in the very oldest of the population.

Materials and methods

Subjects

A total of 105 institutionalised elderly women and men (aged 65–98 years), recruited from five different senior residencies in the area of Vienna

[Curatorship of Viennese retirement homes (KWP)] volunteered for the study. For the current data set, we used baseline characteristics of study participants intended to take part in a prospective training study. The subjects were mentally (mini-mental state examination ≥ 23) and physically (short physical performance battery > 4) able to participate in the study. They were sedentary (< 1 h of physical activity or exercise per week) and free of severe diseases that would contraindicate medical training therapy or measurement of physical performance, including cardiovascular diseases, diabetic retinopathy and regular use of cortisone-containing drugs. Furthermore, regular strength training ($> 1 \times$ week) in the last 6 months before inclusion was an exclusion criterion. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. This study was approved by the ethics committee of the City of Vienna (EK-11-151-0811).

Cytokinesis block micronucleus cytome assay

Blood samples were taken early morning after an overnight fast using heparin, serum and EDTA tubes (Greiner Bio-One, Kremsmünster, Austria). Peripheral blood lymphocytes were isolated using Ficoll separation tubes (Greiner Bio-One). The cytokinesis block micronucleus cytome (CBMN) assay was conducted according to the protocol of Fenech (8) and Wallner *et al.* (14). Cells were stimulated to perform mitotic division with phytohaemagglutinin (PAA, Pasching, Austria) using a concentration of 1×10^6 cells/ml in culture medium. Samples were incubated at 37°C and 5% CO_2 , and after 44 h, cytochalasin B (Sigma Aldrich, Vienna, Austria) was added to stop further cell division. Cells were spotted onto microscope slides, stained (Diff-Quick; Medion Diagnostics, Dudinggen, Switzerland) and counted using a bright field microscope (1000-fold magnification; Olympus, Wien, Austria).

For each sample, duplicates were performed and two slides of each duplicate were produced. From the four resulting slides, 500 cells per slide (2000 per subject) were counted equally to minimise experimental variation and eliminate scorer bias.

To assess chromosomal damage of blood lymphocytes, the frequency of MN, nucleoplasmic bridges and nuclear buds in 2000 binucleate (BN) cells was counted, as well as the number of apoptotic and necrotic cells. Furthermore, the nuclear division index (NDI) was calculated to measure cytostatic effects and the mitogenic response of lymphocytes (8).

Six-min walking test

The participants had to walk for 6 min as fast and as far as possible. The 6-min walking test is a valid tool to evaluate the aerobic endurance in the elderly people. Participants were allowed to slow down and even take a short rest, if they had to. Every person 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 6 min was registered (26).

Statistics

Statistical analyses were performed with IBM SPSS Statistics 21. Gender differences were measured, using Mann–Whitney *U* test and chi-squared test. Linear and curve fitting regressions were calculated for MN frequency and 6-min walking performance as well as for MN frequency and age. Except for descriptive statistics, MN frequency was adjusted for sex as described in Bonassi *et al.* (13). To analyse the age effect, we used additional data from our working group, as previously published by Wallner *et al.* (14). Both, this study and the one of Wallner *et al.* (14), performed the CBMN assay according to the protocol of Fenech (8). To minimise scoring variation, all scorers have been trained on the same slides by the same persons and regularly reassessed their scoring, to keep quality on a highest level. A *P*-value of < 0.05 was considered significant.

Results

Subject characteristics

From the 105 subjects included, 12.4% were male and 87.6% female, which constitutes a representative distribution of the houses of the Curatorship of Viennese retirement homes. The participants had a mean age ($\pm$ standard deviation) of 83.10 years (± 6.08 years) and a body mass index (BMI) of 29.27 kg/m^2 ($\pm 5.00 \text{ kg/m}^2$).

No higher frequency of MN was found in women than in men (cells with MN, $P = 0.816$; total number of MN, $P = 0.867$). The same non-significant differences were observed for nucleoplasmic bridges, nuclear buds and the NDI—the other parameters of the CBMN assay (Table I).

The mean walking distance at the 6-min walking test was 365 m (± 101 m) with no difference between men and women. Rikli and Jones (27) defined ‘Criterion-Referenced Fitness Standards for Maintaining Physical Independence in Older Adults’ to assess the relative fitness level of people between 60 and 94 years of age. Compared with their data of the 6-min walking test, the subjects of this study only covered 67.7% ($\pm 18.6\%$) of the reference distance for maintaining physically independent.

MN and age

The frequency of MN with 26.84 ± 14.19 MN/1000 BN cells and the number of cells with MN with 22.64 ± 11.34 MN/1000 BN cells reflect the high age of the population. To investigate the effect of age on MN frequency, data from this study (Table I) and from that of Wallner *et al.* (14) (Table II), who investigated younger subjects in the same lab, were statistically merged. Age and the frequency of MN correlated significantly for squared regression ($r = 0.577$; $P = 0.000$; Figure 1). We found a levelling-off at ~ 60 years of age. There was a significant increase up to the age of 60 years ($r = 0.646$; $P = 0.000$), but over 60 years, no further change in the MN frequency was observed ($r = -0.039$; $P = 0.700$).

MN and aerobic fitness

To assess the effect of aerobic fitness on chromosomal damage, the calculated percentage of the 6-min walking reference distance was linked to the MN frequency. A significant negative linear correlation was observed ($r = -0.222$; $P = 0.03$), which predicts a lower rate of MN at a higher aerobic fitness level (Figure 2).

An even higher correlation was found using the third polynomial ($r = 0.379$; $P = 0.003$; Figure 3). Considering the subjects who performed lower than 50% of the walking reference, there is a strong negative linear correlation ($r = -0.766$; $P = 0.006$) with MN. Between 50–80% of reference, no link was found ($r = -0.172$; $P = 0.174$), but in subjects performing over 80% of the walking reference distance, again a stronger, negative, non-significant correlation between aerobic fitness and MN frequency ($r = -0.332$; $P = 0.141$) was observed.

Discussion

The aim of this study was to investigate the formation of MN in a cohort of elderly institutionalised, Viennese women and men and to examine the influence of age and aerobic fitness, measured with the 6-min walking test, on chromosomal damage.

Although more women participated in this study (87.6%), the gender distribution was representative for the group of people living in retirement homes in Vienna (28).

The mean age of the study population (women 82.9 ± 6.0 years; men 84.9 ± 6.7 years) reflects the high age of subjects in institutionalised facilities and was for men almost 7 years higher than their life expectancy in Austria, which is at the moment the age of 78 (29). Linking body weight to DNA damage is in general inconsistent and gives no clear answer as to whether or not a higher BMI is connected to higher DNA damage in young and old adults (30–32). Only in obese and overweight children, Scarpato *et al.* (33) observed higher MN frequencies compared with normal weight children. In this study, no correlation between CBMN parameters and BMI was observed, and therefore, it is not further discussed.

Table I. Subject characteristics, parameters of the CBMN assay and aerobic performance

Parameter	All	Men	Women	<i>P</i> -value
Subjects, <i>n</i>	105	13	92	0.000
Age, years	83.10 (±6.08)	84.85 (±6.72)	82.86 (±5.98)	0.474
BMI, kg/m ²	29.27 (±5.00)	27.80 (±4.47)	29.47 (±5.05)	0.216
Parameters of the CBMN assay				
Cells with MN (per 1000 BN cells)	22.64 (±11.34)	20.77 (±9.00)	22.92 (±11.67)	0.816
Total number of MN (per 1000 BN cells)	26.84 (±14.19)	24.46 (±10.04)	27.19 (±14.71)	0.867
Nucleoplasmic bridges (per 1000 BN cells)	1.08 (±1.05)	1.38 (±0.82)	1.03 (±1.07)	0.103
Nuclear buds (per 1000 BN cells)	3.77 (±3.39)	4.58 (±2.94)	3.66 (±3.45)	0.188
Apoptotic cells (% per 1000 BN cells)	0.76 (±0.54)	1.08 (±0.79)	0.71 (±0.48)	0.157
Necrotic cells (% per 1000 BN cells)	0.65 (±0.42)	0.62 (±0.41)	0.65 (±0.42)	0.776
NDI	1.98 (±0.07)	2.01 (±0.07)	1.97 (±0.07)	0.116
Aerobic performance				
Six-minute walking, m	365.1 (±100.9)	387.9 (±150.1)	361.8 (±95.2)	0.487
Percentage of walking reference, %	67.74 (±18.57)	73.61 (±30.83)	66.87 (±16.12)	0.663

Data are means ± standard deviation; *P*-values are calculated using Mann–Whitney *U* test and chi-square test for gender differences; percentage of walking reference was calculated using the reference values of Rikli and Jones (27); significant differences are indicated in bold.

Table II. Subject characteristics of young adults (Wallner *et al.* (14))

Parameter	All	Men	Women	<i>P</i> -value
Subjects, <i>N</i>	76	56	20	0.000
Age, years	32.09 (±11.42)	31.46 (±11.36)	33.85 (±11.70)	0.453
Cells with MN (per 1000 BN cells)	10.78 (±8.97)	8.82 (±5.98)	16.47 (±13.16)	0.002
Total number of MN (per 1000 BN cells)	12.24 (±11.02)	10.18 (±8.32)	18.21 (±15.30)	0.003

Data are means ± standard deviation. *P*-values are calculated using Mann–Whitney *U* test and chi-square test for gender differences; significant differences are indicated in bold.

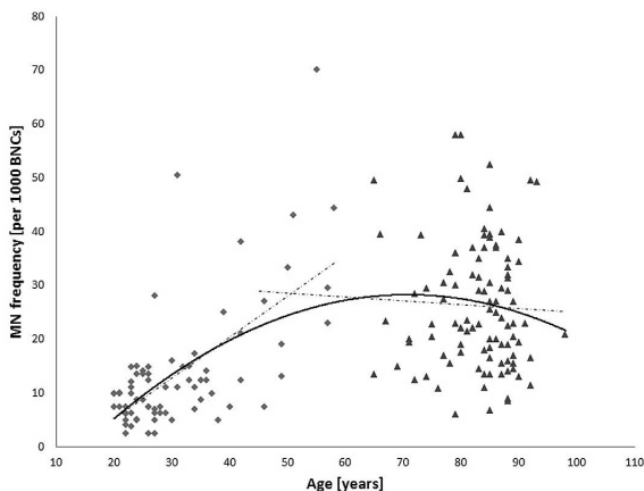


Fig. 1. Squared regression between MN frequency (per 1000 BN cells) and age (years) (line), $r = 0.577$, $P = 0.000$; linear regression under and over 60 years (dotted line).

Gender aspects of the CBMN assay in the elderly group

Although only very limited data are available for this age group (8th, 9th and 10th decade), our results (Table I) seem to be within the given range for the CBMN assay (8). Notably, our data on MN formation in the elderly people clearly demonstrate that there is a great heterogeneity in this age group, with the mean value (26.84 ± 14.19 MN/1000 BN cells) being at the upper limit of the expected range for the CBMN assay (0–30 MN/1000 BN cells) (8). Considering the individual range, we observed MN frequencies from 5.5 MN/1000 BN cells up to 98 MN/1000 BN cells. The same has been detected for all other parameters of the

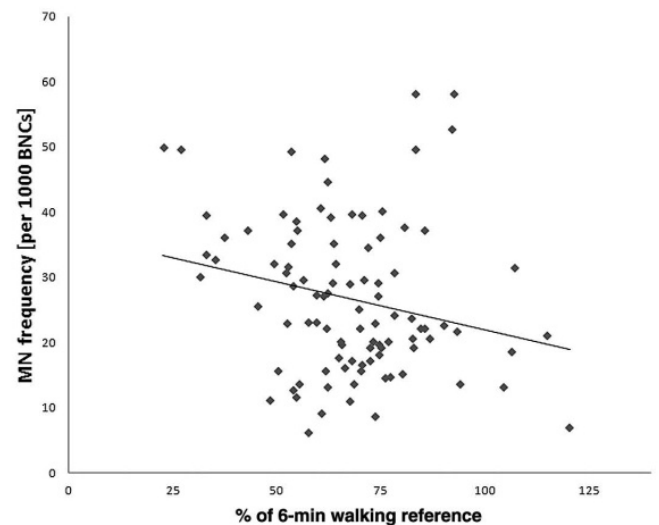


Fig. 2. Linear regression between MN frequency (per 1000 BN cells) and percentage of 6-min walking reference distance (%); $r = -0.222$, $P = 0.03$.

CBMN assay, which showed a broad range around the mean (Table I). Deviating from what has been reported on younger age groups, no significant difference between men and women was found. However, as described in the literature (34,35), the total number of MN and cells with MN was ~10% higher in women than in men, but, probably due to the smaller sample size of only 13 men in this age cohort, the difference was not statistically significant. Equally, for the other parameters of the CBMN assay, nucleoplasmic bridges, nuclear buds and the NDI, no gender differences have been observed (Table I), which also reflects the broad range of the single values in this age group.

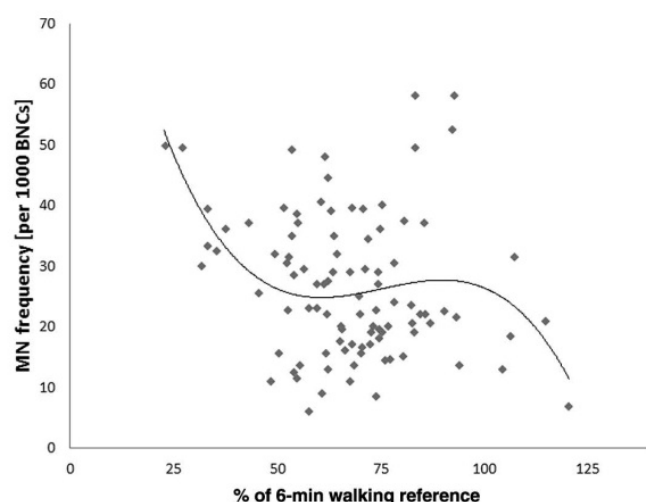


Fig. 3. Third polynomial regression between MN frequency (per 1000 BNCs) and percentage of 6-min walking reference distance (%); $r = 0.379$, $P = 0.003$.

MN and age

It is well known that the number of MN increases with age (12–14), with limited observations of a ‘saturation’ effect at ~60 to 70 years of age (15–18). This effect was perfectly seen in our elderly cohort; there was no age effect regarding increased MN formation, even a tendency to decreased MN with higher age occurred. To examine the effect of age, we merged our data with data previously produced by our working group (15). Our results go in line with the findings of Wojda *et al.* (16) and indicate a levelling-off of the MN frequency at ~60 years of age. Up to 60 years, we observed a significant increase ($r = 0.646$; $P = 0.000$) and from there on, no further change of the MN frequency was found ($r = -0.039$; $P = 0.700$; Figure 1).

Based on those findings, it seems that there is a threshold of genome instability that limits the upper rate of MN formation. If the ‘survivors’ are able to reach that high age of 85 years or older, they seem to be either more resistant to MN formation or MN are more likely to undergo apoptosis (16).

MN and aerobic fitness

Besides the ageing process, several lifestyle factors, including the aerobic fitness level, play a key role in the incidence of chromosomal damage (21). Although acute physical exercise causes an increase of ROS and therefore a higher oxidation rate of the DNA (24,36), the adaptation process to exercise has shown to improve the mechanisms to handle elevated ROS levels and as a consequence also to lower age-related DNA damage (37). Our results (Figure 2) show a significant negative correlation between the MN frequency and the percentage of the 6-min walking test reference distance ($r = -0.222$; $P = 0.03$). In detail, a negative correlation with the formation of MN was observed in subjects performing <50% of reference distance or >80%, whereas for those between 50–80%, MN frequency plateaued. This indicates that, at a very low aerobic fitness level in the elderly people (<50% relative to a reference population of the same age), improved endurance levels have a significant effect on the frequency of MN. Therefore, it is very likely that those with the highest fitness level (close to 100% of reference or higher) also have the lowest chromosomal damage (Figure 3). Mota *et al.* (22) observed, that in the ageing process, people

with a lower fitness level had significantly more damaged DNA and that a loss of mitochondrial activity only occurred in the elderly, when fitness was low.

In summary, our results from investigating a very old institutionalised cohort clearly show that there is a levelling-off of the MN frequency with age, starting at ~60 years of age. It seems that either the ‘survivors’ developed resistance against chromosomal damage as ageing progresses, or that only those with naturally given better defence mechanisms attain a great age. Further research is needed to identify the mechanisms behind this age phenomenon.

Furthermore, our data indicate that there might be a link between aerobic fitness and chromosomal damage, by means of lower MN frequency with an elevated fitness level, even in the very oldest of our population. Especially in this age group, improving resistance against free radical species and lowering the mutation rate by improving one’s fitness could be of great concern to keep resilience against age-related diseases, even at the end of the lifespan. Respecting the importance of the ageing society, more needs to be done to investigate, whether or not improving one’s aerobic fitness actually reduces the formation of MN in the elderly.

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Conflict of interest statement: None declared.

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Paper 2

Original Research Article

Original Manuscript

The impact of six months strength training, nutritional supplementation or cognitive training on DNA damage in institutionalised elderly

Bernhard Franzke¹, Barbara Halper¹, Marlene Hofmann², Stefan Oesen¹, Waltraud Jandrasits³, Andreas Baierl⁴, Anela Tosevska¹, Eva-Maria Strasser⁵, Barbara Wessner^{1,2}, Karl-Heinz Wagner^{1,3,*} and Vienna Active Ageing Study Group

¹ Research Platform Active Ageing, University of Vienna, Althanstraße 14, 1090 Vienna, Austria, ² Department of Sport and Exercise Physiology, Centre for Sport Science and University Sports, University of Vienna, Auf der Schmelz 6, 1150 Vienna, Austria, ³ Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria, ⁴ Institute for Statistics and Operations Research, University of Vienna, Grillparzerstraße 7, 1010 Vienna, Austria and ⁵ Karl Landsteiner Institute for Remobilization and Functional Health/Institute for Physical Medicine and Rehabilitation, Kaiser Franz Joseph Spital, SMZ-Süd, Kundratstraße 3, 1100 Vienna, Austria

*To whom correspondence should be addressed. Tel: +43 1 4277 54930; Fax: +43 1 4277 9549; Email: karl-heinz.wagner@univie.ac.at

Abstract

Aging and its aligned loss of muscle mass are associated with higher levels of DNA damage and deteriorated antioxidant defence. To improve the body's overall resistance against DNA damage, maintaining a healthy and active lifestyle is desirable, especially in the elderly. As people age, many have to change their residence from home living to an institution, which is often accompanied by malnutrition, depression and inactivity. The current study aimed at investigating the effect of a 6-month progressive resistance training (RT), with or without protein and vitamin supplementation (RTS), or cognitive training (CT), on DNA strand breaks in 105 Austrian institutionalised women and men (65–98 years). DNA damage was detected by performing the single cell gel electrophoresis (comet) assay. Physical fitness was assessed using the chair rise, the 6-min-walking and the handgrip strength test. In addition, antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were analysed. Basal DNA damage (lysis) increased significantly after 3 months of intervention in the RT group (T1 – T2 + 20%, $P = 0.001$) and the RTS group (T1 – T2 + 17%, $P = 0.002$) and showed a similar tendency in the CT group (T1 – T2 + 21%, $P = 0.059$). %DNA in tail decreased in cells exposed to H_2O_2 significantly in the RT (T1 – T2 – 24%, $P = 0.030$; T1 – T3 – 18%, $P = 0.019$) and CT (T1 – T2 – 21%, $P = 0.004$; T1 – T3 – 13%, $P = 0.038$) groups. Only RT and RTS groups showed significant differences overtime in enzyme activity (RT + 22% CAT-activity T1 – T3, $P = 0.013$; RTS + 6% SOD-activity T2 – T3, $P = 0.005$). Contrary to the time effects, no difference between groups was detected for any parameter at any time point. Our results suggest that both CT and RT improve resistance against H_2O_2 induced DNA damage and that a nutritional supplement has no further protective effect in institutionalised elderly.

Introduction

Aging is highly associated with a loss of muscle mass, strength, function and therefore a reduction in life quality (1,2). Regular

exercise, especially strength training, is important to prevent severe muscle atrophy, to enhance function and to maintain physical independence in the elderly (3,4). Both the process of aging and the loss of muscle mass are associated with higher levels of DNA

damage and deteriorated antioxidant defence (5). It is well known that subjects with chronic diseases such as cardiovascular diseases (CVD), type 2 diabetes and cancer show increased levels of DNA damage (6,7).

A combination of resistance training (RT) and protein supplementation seems to be the most effective for increasing muscle mass and strength in the elderly (8,9). In terms of improving DNA defence and repair mechanisms, the intake of antioxidants is considered to be beneficial (10–12).

Studies on regularly performed training, either aerobic training or resistance exercise, showed inconsistent results regarding DNA damage (13). Reports are published of decreased (14–16) but also of increased (17–19) levels of DNA damage. There seems to be a dose-dependent effect of exercise on DNA damage—too much and too intense physical activity could induce damage to DNA (20) but reactive oxygen species (ROS) production caused by moderate exercise is also necessary for different adaptation processes and therefore important for enhanced antioxidant defence mechanisms (21).

As the population is aging, there is a great need to investigate the health and fitness in the very oldest of the population (8th, 9th and 10th decade). Currently there is a general lack of data for this age group. This aspect is of particular interest since the living situation in many elderly individuals is changing in this period with a transfer to new surroundings such as homes for the elderly, which is often accompanied by conditions of malnutrition, multiple medications or increased depression and loss of life quality (22,23).

We conducted the current study to investigate the effect of a 6-month progressive RT, with or without protein and vitamin supplementation, on DNA damage measured by the single cell gel electrophoresis (comet) assay in Austrian institutionalised elderly. With our study cohort subjects, being on average at or above life expectancy in Austria (study/Austria: women 82.86/83.25 years; men 84.85/77.95 years) (24) and 75% of our study population being 80 years old or older, we were able to generate novel data on DNA damage and physical performance in this particular age group of institutionalised elderly.

Material and Methods

Subjects

One hundred and five institutionalised elderly women and men (aged 65–98 years), recruited from five different senior residences in the area of Vienna [Curatorship of Viennese Retirement Homes (KWP)], volunteered for the study. The subjects were mentally [Mini Mental State Examination (MMSE) ≥ 23] and physically [Short Physical Performance Battery (SPPB) > 4] able to participate in the study. They were sedentary (< 1 h physical activity or exercise per week) and free of severe diseases that would contra-indicate medical training therapy or measurement of physical performance, including CVD, diabetic retinopathy and regular use of cortisone-containing drugs. Furthermore, regular strength training (more than once per week in the last 6 months before inclusion), was an exclusion criterion. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. This study was approved by the ethics committee of the City of Vienna (EK-11-151-0811) and registered at ClinicalTrials.gov, NCT01775111.

Study design

The present study was conducted in a randomized, controlled, observer-blind design. The participants were randomly divided into three parallel groups—cognitive training (CT), resistance training (RT), RT + supplement (RTS)—and matched for sex. Blood samples

were taken and physical performance tests were executed before (T1), after 3 months (T2) and after 6 months (T3) of intervention. The goal was to assess and compare the effects of either a RT intervention, a RT and nutritional supplementation intervention or a CT intervention on institutionalised elderly.

Resistance training

The RT groups (RT and RTS) performed two sessions of RT per week, supervised by a sport scientist, conducted on two non-consecutive days. Training attendance was recorded every session. The only equipment used was exercise bands and a chair. The progressive RT protocol was designed based on the guidelines of the American College of Sports Medicine for RT with older subjects (25). The main part consisted of 10 exercises for the main muscle groups (legs, back, abdomen, chest, shoulder and arms). One training session started with 10 min of warm-up, continued with 30–40 min of strength training and ended with a 10-min cool-down. To keep the training stimulus high enough, the exercise program was adjusted to the participants' individual needs, by either adapting the resistance of the elastic band (shorter or stronger band) or by modifying the exercise, by means of performing a more difficult version. In the initial phase (4 weeks) one set of 15 repetitions was performed in order to learn the correct form of each exercise. From the fifth week on, the intensity and volume were progressively increased from two sets of light exercises to two sets of heavy resistance. If the participants could easily perform two sets of 15 repetitions they were told either to take more resistance or to perform a more difficult version of the exercise.

RT and supplementation

The RTS group followed the same training protocol as the RT group. Additionally they received a supplement every morning, as well as directly after each training session. It consisted of 20.7 g protein [56 energy (En) %, 19.7 g whey protein, 3 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 %), 800 IU (20 μ g) of vitamin D, 250 mg calcium, vitamins C, E, B6 and B12, folic acid and magnesium (one portion FortiFit, Nutricia). Total energy per drink was 150 kcal. The intake of the nutritional supplement was controlled at breakfast as well as after the training sessions.

Cognitive training

The CT groups performed coordinative or cognitive tasks two times per week, similarly to the RT and RTS groups. In contrast to a classical control group, which would not get any treatment, the training frequency of this group was the same as in the other groups. Therefore the “bias” of regularly being part in group activities were minimized (socialization factor). The participants of the CT group mainly performed memory training and finger dexterity exercises in sitting position (26).

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). 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 (27,28). In short, a cell suspension of peripheral blood mononuclear cells (PBMCs) (1×10^6 cells/ml in phosphate buffered saline) was mixed with 1% low melting agarose

and applied on microscope slides (coated in 1% normal melting agarose). Slides for either 'lysis', 'buffer', 'formamidopyrimidine DNA glycosylase (FPG)' and ' H_2O_2 ' were prepared. All slides were placed into lysis solution (pH 10) for at least 1 h. Only H_2O_2 slides were treated with a 100 μM 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 μl 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 phosphate buffered saline and dried at room temperature. After staining the slides with 20 $\mu g/ml$ of ethidium bromide, DNA damage was measured using a fluorescence microscope together with an image analysis system (Komet 5.5, Kinetic Imaging, Liverpool, UK). The mean %DNA in tail of 50 comets per gel (two 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.

Superoxide dismutase

To assess superoxide dismutase (SOD) activity, the inhibition of autooxidation of pyrogallol was measured. One unit of SOD activity was defined as the required amount of SOD to inhibit autooxidation by 50% (29,30).

Catalase

The activity of catalase (CAT) was measured using photometrical analysis. Therefore, the initial rate of H_2O_2 degradation was quantified. One unit of CAT activity was defined as the rate constant of the first-order reaction (31).

Glutathione peroxidase

Glutathione peroxidase (GSH-Px) activity was analysed with an indirect coupled assay. The activity that catalyses the oxidation of 1 nmol of nicotinamide adenine dinucleotide phosphate per minute was defined as one unit (32).

Chair rise test

To perform well in the chair rise test, the participants had to stand up 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 >50% of the range of motion (33).

Handgrip test

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 better result of two trials (1 min break in between) for each hand was noted (34).

Six-minute-walking test

The participants had to walk for 6 min as fast and as far as possible. The 6-min-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 6 min was registered (35).

Statistics

Statistical analyses were performed with IBM SPSS Statistics 21. Sex differences were measured, using 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 <0.05 was considered an indication of significance.

Results

Baseline characteristics

From the 105 subjects included, 12.4% were male and 87.6% female, which constitutes a representative distribution in the houses of the Curatorship of Viennese Retirement Homes. The participants had a mean age of 83.10 ± 6.08 years (Table I).

No sex differences were observed in the parameters measured by the comet assay (lysis *P* = 0.932, H_2O_2 *P* = 0.351, FPG *P* = 0.401). Equally, no significant difference occurred for SOD (*P* = 0.821), CAT (*P* = 0.958) and GSH-Px (*P* = 0.243) activities. At baseline, age correlated negatively with SOD (*r* = -0.236, *P* = 0.043) and CAT (*r* = -0.312, *P* = 0.007) but not with GSH-Px (*P* = 0.610) activity.

The parameters for physical fitness showed a tendency for better performances in men, but only the handgrip test revealed a significant difference between women and men (men: 28 ± 5.4 kg, women: 17 ± 5.3 kg, *P* = 0.000).

RT, RTS and CT group did not differ significantly in any parameter at baseline.

Intervention effects

At baseline, data from 96 women and men were included into the calculations. Nine participants quit the study before obtaining the first blood samples. After 6 months of intervention, 80 participants completed all tests. The drop outs were due to health problems and lack of motivation. The 6 months drop-out rate of about 24% was lower than expected (35–40% predicted before the study) for this high-age group. All data are presented in Table II.

There was a significant increase in %DNA in tail, for cells that had only been treated with lysis, in the RT ($T1 - T2 + 20\%$, *P* = 0.001; $T1 - T3 + 18\%$, *P* = 0.082) and the RTS ($T1 - T2 + 17\%$, *P* = 0.002; $T1 - T3 + 18\%$, *P* = 0.081) groups, but not significantly in the CT group although there was a tendency at $T2$ ($T1 - T2 + 21\%$, *P* = 0.059).

%DNA in tail decreased in cells exposed to H_2O_2 significantly in the RT ($T1 - T2 - 24\%$, *P* = 0.030; $T1 - T3 - 18\%$, *P* = 0.019) and CT ($T1 - T2 - 21\%$, *P* = 0.004; $T1 - T3 - 13\%$, *P* = 0.038) groups. A tendency for an overall effect occurred in the RTS group (Friedman test *P* = 0.093); however, the Wilcoxon test showed a significant difference between $T1$ and $T2$ in this group ($T1 - T2 - 18\%$, *P* = 0.024). No difference between the groups was observed for any time point.

No intervention effects were detected for FPG-sensitive sites (Table II).

Both physical intervention groups improved significantly in the chair rise test and the 6-min-walking test, but not in the handgrip (RT: chair rise: $T1 - T3 + 17\%$, *P* = 0.006; 6-min-walking: $T1 - T3$

Table I. Baseline characteristics for comet assay, physical performance and enzymes activities

Parameter	All	Men	Women	P value
Subjects (number)	105	13	92	0.000
Age (years)	83.10 ± 6.08	84.85 ± 6.72	82.86 ± 5.98	0.474
SCGE-parameters				
Lysis (%DNA in tail)	7.05 ± 2.52	7.38 ± 3.64	6.97 ± 2.14	0.932
H ₂ O ₂ (%DNA in tail)	27.09 ± 6.97	25.56 ± 7.59	27.06 ± 6.82	0.351
FPG (%DNA in tail)	10.30 ± 7.17	13.72 ± 12.54	9.93 ± 6.29	0.401
Performance parameters				
Chair rise (repetitions)	12.0 ± 4.0	13.5 ± 5.5	11.9 ± 3.8	0.385
6-min walking (m)	365 ± 101	388 ± 150	365 ± 87	0.487
Handgrip (kg)	18.4 ± 6.5	28.0 ± 5.4	17.0 ± 5.3	0.000
Enzymes				
SOD (IU/g Hb)	2077 ± 275	2079 ± 273	2077 ± 277	0.821
CAT (IU/g Hb)	129 ± 34	125 ± 23	129 ± 35	0.958
GSH-Px (IU/g Hb)	35.6 ± 9.4	40.8 ± 12.6	35.0 ± 8.8	0.243

Data are means ± SD; P values are calculated using chi-squared and Mann–Whitney *U* test for gender differences; significant differences are highlighted using bold formatting.

+ 6%, $P = 0.004$; RTS: chair rise: T1 – T3 + 19%, $P = 0.002$; 6-min-walking: T1 – T3 + 8%, $P = 0.029$).

Only RT and RTS group showed significant differences over time in enzyme activities (Table II). In the RT group there was a 22% increase in CAT-activity (T1 – T3, $P = 0.013$) and in the RTS group a 6% increase in SOD activity (T2 – T3, $P = 0.005$).

No difference at any time point between groups was detected for any parameter.

Discussion

The aim of the present study was to investigate the effect of either a strength training, a strength training and nutrition or a CT intervention on DNA strand breaks in PBMCs in a cohort of elderly institutionalised, Viennese women and men.

Although more women participated in this study (87.6%), the sex distribution was representative for the group of people living in retirement homes in Vienna (36).

The mean age of the study population (women 82.9 ± 6.0 years; men 84.9 ± 6.7 years) reflects the high age of subjects in institutionalised facilities and was for men almost 7 years higher than the present life expectancy in Austria (24,37).

In our cohort of institutionalised elderly, we did not observe sex differences in DNA damage in any parameter of the comet assay, probably also due to the uneven sex distribution in this age group. However, it seems that men tend to have a higher rate of DNA damage than women (38–40). Besides a better lifestyle of females this could also be explained by the higher level of estrogen in women, which provides them with a better defence system against free radical species (41,42).

Similarly to the results of DNA damage, but contrary to some literature data (43), no difference between women and men was found for SOD, CAT and GSH-Px activities. Nevertheless, negative correlations were observed between age and SOD activity ($r = -0.236$, $P = 0.043$) and between age and CAT activity ($r = -0.312$, $P = 0.007$) within our group of highly aged institutionalised elderly. An age-associated decrease in enzyme activity (44–46) was not present for GSH-Px activity.

Besides sex, the aging process also has an impact on the formation of DNA strand breaks in PBMCs. Some, but not all previous studies have indicated that DNA damage increases with age (47–49).

No such effect could be detected in our cohort of elderly people. This can be explained by the high average age of 83 years and the general narrow age range of the study group. We were also able to show that the formation of micronuclei, which is well known to increase over lifetime, reached a plateau and even showed a slight trend to decrease between 7th and 9th decade of life (50).

At baseline, the subjects of the present study demonstrated in average a low physical performance when compared to reference values for maintaining physical independence at this age (51,52). Relatively, they performed best at the chair rise test where they reached 104 ± 37% of the reference for their age. However, handgrip strength, with 90 ± 30%, and the 6-min-walking test, with only 68 ± 18% compared to normative values, demonstrated the relatively weak physical condition of the institutionalised participants. With advanced aging, especially the aerobic fitness, as measured with the 6-min-walking test, seems to have an impact on DNA stability and repair mechanisms. Mota *et al.* (53) clearly demonstrated that, even in the aging process, subjects with higher $\text{VO}_{2\text{max}}$ showed less DNA damage and higher mitochondrial activity.

The existing data on the effect of physical training on DNA damage seem to be inconsistent (54,55). It was reported that regular training (resistance or aerobic training) could lead either to a decrease (14–16) or an increase (17–19) in DNA damage. However, there seems to be a dose-dependent effect of exercise on DNA damage since too long, too intense and too frequently performed exercise could impair the balance between ROS production and the antioxidant defence system and therefore could lead to chronic oxidative stress. As Radak *et al.* (21) pointed out in their review, moderate levels of oxidative damage are essential for the adaptive response after exercise.

In the present study, after 3 and 6 months of RT, RTS and CT intervention, higher levels of DNA damage (lysis) were observed in institutionalised elderly (Table II). RT and RTS groups showed significant changes in %DNA in tail (3 months changes: RT +20.4%, RTS +17%; 6 months changes: RT +17.9%, RTS +17.5%). Our results indicate that, for this age group, progressive RT with elastic bands causes elevated rates of DNA damage, measured with the comet assay. However, even the increased level of DNA damage was within the normal range for healthy individuals (56,57). With respect to the high age of our study cohort, DNA repair capacity might have already been reduced and therefore exercise-induced oxidative

Table II. The impact of 3 (T2) and 6 (T3) months of intervention on DNA damage and enzymes activities

Intervention	RT			P	RTS			P	CT			P
	T1	T2	T3		T1	T2	T3		T1	T2	T3	
Subjects (number)	34	32	30		30	28	24		32	28	26	
SCGE-parameter												
Lysis (%DNA in tail)	6.86 ± 2.73	8.26 ± 2.18	8.09 ± 2.59	0.012	7.47 ± 2.08	8.74 ± 2.38	8.78 ± 2.34	0.023	6.93 ± 2.50	8.36 ± 2.71	7.67 ± 1.76	0.112
H ₂ O ₂ (%DNA in tail)	27.00 ± 7.06	20.92 ± 8.57	22.54 ± 4.27	0.050	28.12 ± 7.80	23.08 ± 6.18	24.87 ± 4.37	0.093	27.43 ± 5.83	21.65 ± 6.90	23.83 ± 4.76	0.008
FPG (%DNA in tail)	8.82 ± 8.90	12.46 ± 8.49	9.54 ± 4.63	0.296	10.80 ± 9.80	10.50 ± 6.44	8.37 ± 4.75	0.229	10.49 ± 5.88	11.35 ± 5.72	9.47 ± 6.08	0.834
Enzymes												
SOD (IU/g Hb)	1981 ± 280	2056 ± 234	2142 ± 224	0.174	2183 ± 322	2124 ± 219	2258 ± 196	0.011	2092 ± 177	2147 ± 217	2182 ± 273	0.096
CAT (IU/g Hb)	125.8 ± 33.0	148.5 ± 36.6	153.0 ± 37.9	0.011	132.0 ± 38.6	144.3 ± 23.4	148.6 ± 39.1	0.727	129.0 ± 30.8	137.8 ± 41.0	148.8 ± 32.3	0.280
GSH-Px (IU/g Hb)	33.9 ± 8.7	35.9 ± 11.6	35.0 ± 8.7	0.773	35.6 ± 10.1	41.5 ± 11.1	36.8 ± 11.6	0.272	37.7 ± 9.3	35.0 ± 6.7	32.3 ± 9.8	0.269

Data are means ± SD; P values are calculated using Friedman test for differences between time points; significant differences are highlighted using bold formatting.

damage accumulated (13,58). Shahar *et al.* (9) conducted a similar study where they investigated the effect of a 12-week exercise intervention with elastic bands and protein supplementation either alone or in combination on physical fitness and oxidative stress; this study however, was in younger elderly between 60 and 75 years of age. In contrast to our findings, they did not see any changes in oxidation products but a reduction in SOD activity for the RT-only group. Although their subjects were diagnosed as sarcopenic, the mean age of the participants was almost 20 years younger than our subjects. For the younger subjects the training stimulus with elastic bands might have been lower than for the very old ones. Ghandi *et al.* (17) reported on higher %DNA in tails of younger adults' PBMCs when they perform regular strenuous exercise.

Although DNA damage increased significantly in the RT and RTS intervention group, a decrease in %DNA in tail of cells treated with hydrogen peroxide was measured (Table II). Exercise but also oxidative stress is known to up-regulate antioxidant enzyme activity and DNA repair mechanisms (59–61). Therefore, the intervention might have provided our subjects with a better defence system against H₂O₂-induced oxidative stress. This is also confirmed by a general increase in antioxidant enzyme activity which was significant in the RT and RTS group after 6 months (RT: CAT +21%; RTS: SOD +3.4%; $P < 0.05$). FPG-sensitive sites showed no significant changes.

No effects of the nutritional intervention on DNA damage could be observed. In the houses of the KWP residents are provided with food for breakfast, lunch and dinner, and even healthy snacks are served. At baseline, nutrients intake did not differ between the groups. The protein intake of ~0.8–1 g/kg did not significantly change after 3 and 6 months ($P = 0.460$). We observed significantly higher intake values for vitamin D and folic acid in the RTS group (T1 vs. T3: vitamin D 5.9 ± 4.3 µg/day vs. 20.6 ± 4.4 µg/day, $P = 0.006$; folic acid 199 ± 66 µg/day vs. 400 ± 100 µg/day, $P < 0.001$) compared to baseline and compared to the RT and the CT group.

Against our expectations, the subjects of the CT group demonstrated similar results to those of the training intervention groups in the parameters of the comet assay and showed similar tendencies in antioxidant enzymes activities. Besides physical activity, social activation and psychological stress also seem to have an impact on DNA damage (62–66). Notably, in elderly institutionalised people oxidative stress is higher when compared to free-living people (67). Social isolation and the psychosocial environment have severe impacts on the subject's wellbeing, influencing stress parameters and the endocrinal system (68,69) and therefore affecting DNA stability. As the subjects of the current study met twice a week for 60 min and performed either strength training or cognitive exercises, these social events might have improved resistance against H₂O₂-induced DNA damage in the CT group to a similar extent as in the RT and RTS groups. Still, DNA damage (strand breaks) tended to increase in this group however, not significantly. We hypothesise that taking part in this study motivated the subjects of the CT group to become more active on their own, which induced similar biochemical challenges for their body as the physical training groups.

After 6 months of intervention, the RT and the RTS group had significantly improved their performances at the chair rise test and at the 6-min-walking test but did not improve at handgrip strength. As expected, no change in physical performance was observed in the CT group. Importantly, the improvement of aerobic fitness (6-min-walking test) is of great interest for this age group to lower DNA damage and reduce the risk of age related diseases (53). In the same cohort, we were able to demonstrate that higher aerobic fitness significantly correlates with a lower rate of chromosomal damage (50).

To our knowledge, we were the first to investigate the effect of a 6-month lifestyle intervention of CT, RT alone or combined with a supplement on resistance against DNA damage in highly aged (83 ± 6 years) institutionalised elderly. We observed significant lower rates of H_2O_2 -induced DNA strand breaks. Interestingly, the participants of the CT group demonstrated similar effects as the RT and RTS groups. In already well-nourished institutionalised elderly, a supplementation of protein and vitamins shows no further effect on DNA damage, physical performance and antioxidant enzyme activity. Our results indicate that in this age group either physical training or CT could produce similar biochemical changes and therefore enhance the antioxidant defence. As the improvements of training with elastic bands are limited, further studies are warranted to investigate the effect of RT using strength machines on DNA damage and oxidative stress in institutionalised elderly.

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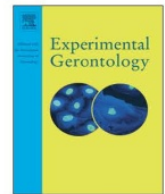
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Paper 3

Original Research Article



The effect of six months of elastic band resistance training, nutritional supplementation or cognitive training on chromosomal damage in institutionalized elderly

Bernhard Franzke^a, Barbara Halper^a, Marlene Hofmann^b, Stefan Oesen^a, Béatrice Pierson^c, Ariane Cremer^c, Evelyn Bacher^c, Birgit Fuchs^c, Andreas Baierl^d, Anela Tosevska^a, Eva-Maria Strasser^e, Barbara Wessner^{a,b}, Karl-Heinz Wagner^{a,c,*}, Vienna Active Ageing Study Group (VAAS)

^a University of Vienna, Research Platform Active Ageing, Althanstraße 14, 1090 Vienna, Austria

^b University of Vienna, Centre for Sport Science and University Sports, Department of Sport and Exercise Physiology, Auf der Schmelz 6, 1150 Vienna, Austria

^c University of Vienna, Faculty of Life Sciences, Department of Nutritional Sciences, Althanstraße 14, 1090 Vienna, Austria

^d University of Vienna, Institute for Statistics and Operations Research, Grillparzerstraße 7, 1010 Vienna, Austria

^e Karl Landsteiner Institute for Remobilization and Functional Health/Institute for Physical Medicine and Rehabilitation, Kaiser Franz Joseph Spital, SMZ-Süd, Kundratstraße 3, 1100 Vienna, Austria

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ABSTRACT

Increased DNA and chromosomal damage are linked to aging and age-related diseases like cardiovascular diseases, diabetes or cancer. Physical activity and an optimal status of micro- and macronutrients are known to reduce the incidence of MN, a marker for chromosomal instability and mutagenicity. Once older people reach a certain age they change from a home-living situation to an institutionalized situation, which is often accompanied by malnutrition, depression and inactivity. We conducted the current study to investigate the effect of a six month progressive resistance training (RT), with or without protein and vitamin supplementation (RTS) or cognitive training (CT) only, on chromosomal damage measured by the cytokinesis block micronucleus cytome assay in 97 Austrian institutionalized women and men (65–98 years). All three intervention groups demonstrated a tendency of a reduced frequency of cells with MN (–15%) as well as for the total number of MN (–20%), however no significant time-effect was observed. Besides a significant increase in plasma B12 and red blood cell folate status, the six month change of B12 was negatively correlated with the six month change of the MN frequency in the RTS group ($r = -0.584$, $p = 0.009$). Our results suggest that in this age group either physical or cognitive training may result in similar biochemical changes and therefore enhance resistance against genomic instability. Supplementation with the vitamins B12 and folic acid could contribute to reduced chromosomal damage in institutionalized elderly.

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1. Introduction

The loss of muscle mass, strength, function and the associated reduction of life quality is linked to the process of aging (Johnston et al., 2008; Seene et al., 2012). Physical activity, regular exercise, and especially resistance training are important in preventing severe muscle atrophy, enhancing function and maintaining physical independence in the elderly (Demontis et al., 2013; Arnold and Bautmans, 2014). The process of aging and the loss of muscle mass are both associated with higher levels of DNA damage and deteriorated antioxidant defense (Fulle et al., 2004; Gianni et al., 2004). It is well known that there are increased levels of chromosomal and DNA damage in people with chronic diseases

such as cardiovascular diseases, type 2 diabetes and cancer (Battershill et al., 2008; Müllner et al., 2014). Resistance training, together with protein supplementation, seems to be most effective for increasing muscle mass and strength in the elderly (Shahar et al., 2013; Lustgarten et al., 2014). To lower the frequency of micronuclei (MN), which is a well-established marker for chromosomal damage and highly correlated with cellular mutation, an adequate status of the vitamins B12 and folate is recommended (Fenech and Bonassi, 2011; Ni et al., 2012).

Studies concerning the effect of exercise and training on MN frequency are rare. However, examinations on that topic show varying results. Regular physical activity seems to lower chromosomal damage (Goon et al., 2008; Huang et al., 2009), whereas acute exhausting exercise tends to increase the MN frequency (Schiffi et al., 1997). Training status also influences the body's ability to deal with exercise-induced oxidative stress. After an ironman triathlon, trained individuals showed no increase in chromosomal damage or even a reduced MN frequency

* Corresponding author at: Department of Nutritional Sciences, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria.

E-mail address: karl-heinz.wagner@univie.ac.at (K.-H. Wagner).

(Reichhold et al., 2008), whereas only untrained subjects demonstrated an increased mutation rate even after 30 min of intense running (Umegaki et al., 1998).

As life expectancy is still growing, it is of great need to investigate health- and fitness-related markers in the oldest of the population (8th, 9th and 10th decade). Currently there is a general lack of data for this age group. This aspect is of particular interest since the living situation of many elderly individuals is changing in this period towards a transfer to new surroundings such as elderly homes, which is often accompanied by conditions of malnutrition, multiple medications or increased depression and loss of life quality (Smoliner et al., 2009).

We conducted the current study to investigate the effect of a six month progressive resistance training (using elastic bands), with or without protein and vitamin supplementation, on chromosomal damage in Austrian institutionalized elderly. The cytokinesis block micronucleus cytome assay (CBMN-assay) was performed to measure genome integrity. In the present study we hypothesized that increased physical fitness improves resistance against chromosomal instability in institutionalized elderly. Protein and vitamin supplementation should enhance this protective effect. With our study cohort subjects, being at or above life expectancy in Austria (study/Austria: women 82.86/83.25 years; men 84.85/77.95 years) (Statistics Austria, 2014) and 75% of our study population being 80 years old or older, we were able to generate novel data on chromosomal damage and physical performance after a six month training period in this particular age group of institutionalized elderly.

2. Methods

2.1. Subjects

Ninety-seven institutionalized elderly women and men (aged 65–98 years), recruited from five different senior residencies in the area of Vienna (Curatorship of Viennese Retirement Homes), volunteered for the study. The subjects were mentally (Mini Mental State Examination ≥ 23) and physically (Short Physical Performance Battery > 4) able to participate in the study. They were sedentary (less than 1 h 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. The health condition of all study subjects was assessed by specialists in internal medicine and gerontology. Furthermore, regular strength training (more than once per week) in the last six months before inclusion was an exclusion criterion. Written informed consent was obtained from all participants before entry into the study in accordance with the Declaration of Helsinki. This study was approved by the ethics committee of the City of Vienna (EK-11-151-0811) and registered at ClinicalTrials.gov, NCT01775111.

2.2. Study design

The present study was conducted in a randomized, controlled, observer-blind design. The participants were randomly divided into three parallel groups – cognitive training (CT), resistance training (RT), RT + supplement (RTS) – and matched for gender. Blood samples were taken and physical performance tests were executed before (T1), after three months (T2) and after six months (T3) of intervention. The goal was to assess and compare the effects of either a resistance training intervention, a resistance training and nutritional supplementation intervention or a cognitive training intervention on institutionalized elderly.

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 supervised by a sport scientist. Training attendance was recorded every

session. The only equipment used were elastic bands and a chair. The progressive resistance training protocol was designed based on the guidelines of the American College of Sports Medicine for resistance training with older subjects (Nelson et al., 2007). The main part consisted of ten exercises for the main muscle groups (legs, back, abdomen, chest, shoulder and arms). One training session started with 10 min of warm-up, continued with 30–40 min of strength training and ended with a 10 minute cool-down. To keep the training stimulus high enough, the exercise program was adjusted to the participants' individual needs, by either adapting the resistance of the elastic band (shorter or stronger band) or by modifying the exercise, by means of performing a more difficult version. In the initial phase (4 weeks) one set of 15 repetitions was performed in order to learn the correct form of each exercise. From the fifth week on the intensity and volume has progressively been increased from two sets of light exercises to two sets of heavy resistance. If the participants could easily perform two sets of 15 repetitions they were told to either take more resistance or to perform a more difficult version of the exercise.

2.4. Resistance training and supplementation

The RTS group followed the same training protocol as the RT group. Additionally they received a liquid supplement every morning, as well as directly after each training session. It consisted of 20.7 g protein (56 energy (En)%, 19.7 g whey protein, 3 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%), 800 IU (20 μ g) of vitamin D, 250 mg calcium, vitamins C, E, B6 and B12, folic acid and magnesium (1 portion FortiFit, Nutricia). Total energy per drink was 150 kcal. The intake of the nutritional supplement was controlled at breakfast as well as after the training sessions.

2.5. Cognitive training

The CT groups performed coordinative or cognitive tasks two times per week, equally to the RT and RTS groups. In contrast to a classic control group, which would not get any treatment, the training frequency of this group was the same as in the other groups. Therefore the “bias” of regularly being part in group activities was minimized (socialization factor). The participants of the CT group mainly performed memory training and finger dexterity exercises in sitting position (Gatterer and Croy, 2004).

2.6. Cytokinesis block micronucleus cytome assay

Blood samples were taken early morning after an overnight fast using heparin, serum and EDTA tubes (Greiner Bio-One, Kremsmünster, Austria). Peripheral blood lymphocytes were isolated using Ficoll separation tubes (Greiner Bio-One). The cytokinesis block micronucleus cytome (CBMN) assay was conducted according to the protocol of Fenech (Fenech, 2007) and Wallner et al. (Wallner et al., 2012). Cells were stimulated to perform mitotic division with phytohemagglutinin (PAA, Pasching, Austria) using a concentration of 1×10^6 cells/ml in culture medium. Samples were incubated at 37 °C and 5% CO₂, and after 44 h, cytochalasin B (Sigma Aldrich, Vienna, Austria) was added to stop further cell division. Cells were spotted onto microscope slides, stained (Diff-Quick; Medion Diagnostics, Dudingen, Switzerland) and counted using a bright field microscope (1000-fold magnification; Olympus, Wien, Austria). For each sample, duplicates were performed and two slides of each duplicate were produced. From the four resulting slides, 500 cells per slide (2000 per subject) were counted equally to minimize experimental variation and eliminate scorer bias.

To assess chromosomal damage of blood lymphocytes, the frequency of MN, nucleoplasmic bridges and nuclear buds in 2000 binucleate (BN) cells was counted, as well as the number of apoptotic and necrotic cells. Furthermore, the nuclear division index (NDI) was calculated to

measure cytostatic effects and the mitogenic response of lymphocytes (Fenech, 2007).

2.7. Chair rise test

To perform well in the chair rise test, the participants had to stand up 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 (Jones et al., 1999).

2.8. Handgrip strength test

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 better result of two trials (one minute break in between) for each hand was noted (Mijnarends et al., 2013).

2.9. Six minute walking test

The participants had to walk for 6 min as fast and as far as possible. The 6 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 meter shuttle track and the distance covered within 6 min was registered (Steffen et al., 2002).

2.10. Vitamin B12 and folate

Plasma concentrations of vitamin B12 and folate in erythrocytes were measured according to Müllner et al. (Müllner et al., 2013) by using radio-immunoassay. Standard curves were drawn and sample values calculated according to the protocol published by the kit producer (MP Biomedicals, Germany).

2.11. Statistics

Statistical analyses were performed using IBM SPSS Statistics 21. Baseline group differences were measured, using the Kruskal–Wallis-H and chi-squared tests. MN frequency was adjusted for sex as described in Bonassi et al. (Bonassi et al., 2001). To assess the overall differences between the time points, the Friedman test was conducted and if significant, the Wilcoxon test was used to calculate the differences between each time point by considering Bonferroni correction. The CBMN-assay was performed according to the protocol of Fenech (Fenech, 2007). To minimize scoring variation, all scorers have been trained on the same slides by the same persons and regularly reassessed their scoring, to keep quality on the highest level. The Spearman test was performed to assess the link between B12 plasma levels and MN frequency. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Baseline characteristics

At baseline, data from 97 women and men were included into the calculations. Twenty of the former 117 participants quit the study before obtaining the first blood samples (Fig. 3). From the 97 study subjects, 13.4% were male and 86.6% female, which constitutes a representative distribution in the houses of the Curatorship of Viennese Retirement Homes. The participants had a mean-age of 83.0 ± 6.1 years (Table 1).

No significant baseline differences between the intervention groups were observed, except for the Nuclear Division Index (NDI) ($p = 0.017$). Baseline data of the parameters of the CBMN-assay, of physical performance, and of vitamin status (B12 and folic acid) are presented in Table 1.

3.2. Intervention effects

After six months of intervention, 70 participants completed all tests. The drop-outs were due to health problems and lack of motivation. The six month drop-out rate of about 28% was lower than expected (35–40% predicted before starting the study) for this high-age group. Data of the CBMN-assay are presented in Table 2. Table 3 shows the results for performance and vitamin status.

For the parameters of the CBMN-assay, no significant difference between the intervention groups occurred, however not only the RT

Table 1

Baseline characteristics for CBMN assay, physical performance and vitamin status of the intervention group resistance training (RT), resistance training and supplementation (RTS) and cognitive training (CT).

Parameter	All	RT	RTS	CT	p-Value
Subjects [number]	97	35	29	33	0.756
Age [years]	83.0 ± 6.1	82.8 ± 5.7	82.5 ± 7.5	83.5 ± 5.4	0.769
<i>CBMN-parameter</i>					
Cells with MN [per 1000 BNCs]	23.2 ± 11.8	24.6 ± 12.8	22.9 ± 13.4	21.9 ± 9.0	0.785
Total number of MN [per 1000 BNCs]	26.3 ± 12.0	27.6 ± 13.9	25.0 ± 10.8	26.0 ± 10.9	0.880
Nucleoplasmic bridges [per 1000 BNCs]	1.09 ± 1.04	1.13 ± 1.14	0.88 ± 0.89	1.23 ± 1.05	0.411
Nuclear buds [per 1000 BNCs]	3.72 ± 3.43	3.56 ± 2.75	3.34 ± 2.62	4.23 ± 4.57	0.972
Apoptotic cells [% per 1000 BNCs]	0.76 ± 0.55	0.83 ± 0.64	0.69 ± 0.37	0.73 ± 0.58	0.769
Necrotic cells [% per 1000 BNCs]	0.66 ± 0.42	0.73 ± 0.47	0.65 ± 0.35	0.59 ± 0.41	0.270
Nuclear division index	1.98 ± 0.07	1.96 ± 0.08	1.99 ± 0.06	1.99 ± 0.07	0.017
<i>Performance parameters</i>					
Chair rise [repetitions]	12.1 ± 4.1	11.9 ± 3.3	12.6 ± 4.8	11.9 ± 4.1	0.674
6 minute walking [m]	368 ± 97	373 ± 87	353 ± 102	377 ± 103	0.600
Handgrip [kg]	18.4 ± 6.5	19.8 ± 6.6	18.7 ± 6.6	16.7 ± 5.9	0.095
<i>Vitamin status</i>					
B12 [pmol/l]	393 ± 379	346 ± 268	452 ± 432	388 ± 433	0.721
Folic acid [nmol/l]	186 ± 110	204 ± 116	154 ± 33	192 ± 142	0.441

Data are means $\pm$ standard deviation; p-values are calculated using Kruskal–Wallis-H test and chi-square test for group differences; significant differences are highlighted using bold formatting. Data for MN have been sex-adjusted according to Bonassi et al. (Steffen et al., 2002).

Table 2

The effect of 3 (T2) and 6 (T3) months of intervention [resistance training (RT), resistance training and supplementation (RTS) and cognitive training (CT)] on the parameters of the CBMN-assay.

Intervention	RT				RTS				CT			
	T1	T2	T3	p	T1	T2	T3	p	T1	T2	T3	p
Subjects [number]	35	23	26		29	27	22		33	22	22	
CBMN-parameter												
Cells with MN [per 1000 BNCs]	24.6 ± 12.8	18.9 ± 10.6	19.9 ± 11.0	0.561	22.9 ± 13.4	19.4 ± 11.1	18.9 ± 12.0	0.411	21.9 ± 9.0	16.7 ± 6.1	19.2 ± 9.5	0.252
Total number of MN [per 1000 BNCs]	27.6 ± 13.9	22.8 ± 13.2	24.1 ± 14.9	0.943	25.0 ± 10.8	23.9 ± 15.7	22.4 ± 14.4	0.522	26.0 ± 10.9	19.7 ± 7.4	23.5 ± 12.5	0.278
Nucleoplasmic bridges [per 1000 BNCs]	1.13 ± 1.14	0.53 ± 0.72	0.50 ± 0.49	0.491	0.88 ± 0.89	0.52 ± 0.62	0.59 ± 1.16	0.129	1.23 ± 1.05	0.57 ± 0.63	0.64 ± 0.85	0.025
Nuclear buds [per 1000 BNCs]	3.56 ± 2.75	3.24 ± 2.34	3.93 ± 3.96	0.940	3.34 ± 2.62	2.63 ± 2.45	2.98 ± 2.35	0.442	4.23 ± 4.57	3.53 ± 3.83	3.03 ± 3.08	0.759
Apoptotic cells [% per 1000 BNCs]	0.83 ± 0.64	0.58 ± 0.28	0.51 ± 0.32	0.257	0.69 ± 0.37	0.51 ± 0.34	0.48 ± 0.23	0.024	0.73 ± 0.58	0.62 ± 0.35	0.43 ± 0.23	0.021
Necrotic cells [% per 1000 BNCs]	0.73 ± 0.47	0.55 ± 0.37	0.47 ± 0.25	0.113	0.65 ± 0.35	0.59 ± 0.54	0.41 ± 0.26	0.084	0.59 ± 0.41	0.54 ± 0.38	0.46 ± 0.27	0.838
Nuclear division index	1.96 ± 0.08	2.00 ± 0.06	1.97 ± 0.08	0.186	1.99 ± 0.06	1.97 ± 0.08	1.96 ± 0.08	0.285	1.99 ± 0.07	1.96 ± 0.07	1.97 ± 0.06	0.471

Data are means ± standard deviation; p-values are calculated using Friedman test for differences between time points; significant differences are highlighted using bold formatting.

and RTS but also the CT group showed similar tendencies for reduction of chromosomal damage. Possibly due to the large standard deviation, only nucleoplasmic bridges (CT: $p = 0.025$) and apoptotic cells (RTS: $p = 0.024$; CT: $p = 0.021$) showed significant time effects. After six months of intervention, we observed a non-significant reduction of cells with MN (RT: -19% , RTS: -18% , CT: -12%) as well as the total number of MN (RT: -13% , RTS: -10% , CT: -10%) in our study cohort of institutionalized elderly (Table 2).

The participants of the RT and the RTS groups demonstrated significant improvements in the chair rise and the 6 minute walking tests (chair rise: RT: $+23\%$, $p = 0.002$, RTS: $+24\%$, $p = 0.001$; 6 minute walking: RT: $+13\%$, $p = 0.021$, RTS: $+11\%$, $p = 0.015$). The CT group did not improve in functional parameters and none of the three groups improved significantly at the handgrip strength test.

Vitamin status changed significantly only in the RTS group (plasma B12: $+130\%$, $p = 0.006$; red blood cell folate: $+43\%$, $p = 0.018$) after six months. Furthermore, only in the RTS group, a significant negative correlation between the six month change of the B12 plasma level and the MN frequency was observed (RTS: $r = -0.584$, $p = 0.009$; RT: $r = 0.081$, $p = 0.715$; CT: $r = -0.259$, $p = 0.284$), which indicates decreased chromosomal damage with increased B12 plasma status (Fig. 1).

3.3. Drop-out analysis

To investigate possible reasons for drop-outs in this study, we compared baseline data of those who performed the six month testing ($N = 70$) with those who quit ($N = 27$) before the last sampling.

Interestingly, we observed significant differences between finishers and drop-outs (Fig. 2). Finishers showed significant lower MN frequencies (-20% , $p = 0.034$), a better physical performance in the 6 minute walking test ($+24\%$, $p = 0.002$) and also at the chair rise test ($+21\%$, $p = 0.023$) at baseline.

4. Discussion

4.1. Baseline characteristics

The aim of the present study was to investigate the effect of either a strength training, a strength training and nutritional, or a cognitive training intervention on chromosomal damage in PBMCs in a cohort of elderly institutionalized, women and men.

Although more women than men participated in this study (87.6% female), the gender distribution was representative for the group of people living in retirement homes in Vienna (KWP, Kuratorium-Wiener-Pensionisten-Wohnhäuser, 2014a).

The mean age of the study population (women 82.9 ± 6.0 years; men 84.9 ± 6.7 years) reflects the high age of subjects from institutionalized facilities and was almost seven years higher than the present life expectancy for men in Austria (StatisticsAustria, 2014; KWP, Kuratorium-Wiener-Pensionisten-Wohnhäuser, 2014b).

Although only very limited data are available for this age group (8th, 9th and 10th decade), our results seem to be within the given range for the CBMN-assay (Fenech, 2007). Notably, our data on MN formation in the elderly clearly demonstrate that there is a great heterogeneity in this age group, with the mean value (26.3 ± 12.0 MN/1000 BN cells) being at the upper limit of the expected range for the CBMN-assay

Table 3

The impact of 3 (T2) and 6 (T3) months of intervention [resistance training (RT), resistance training and supplementation (RTS) and cognitive training (CT)] on physical performance and vitamin status.

Intervention	RT				RTS				CT			
	T1	T2	T3	p	T1	T2	T3	p	T1	T2	T3	p
Subjects [number]	35	23	26		29	27	22		33	22	22	
Performance parameters												
Chair rise [repetitions]	11.9 ± 3.3	13.6 ± 3.7	14.6 ± 3.7	0.002	13.0 ± 4.4	14.3 ± 4.6	16.1 ± 5.4	0.001	11.9 ± 4.2	11.8 ± 3.5	11.3 ± 3.3	0.829
6 minute walking [m]	373 ± 87	394 ± 89	422 ± 91	0.021	357 ± 101	399 ± 158	396 ± 143	0.015	373 ± 102	374 ± 117	383 ± 111	0.650
Handgrip [kg]	19.8 ± 6.6	20.4 ± 6.2	20.2 ± 6.1	0.610	19.1 ± 6.4	19.4 ± 6.2	19.4 ± 5.9	0.629	16.7 ± 6.0	17.2 ± 7.0	16.3 ± 6.8	0.316
Vitamin status												
B12 [pmol/l]	346 ± 268	388 ± 349	384 ± 352	0.738	452 ± 432	799 ± 802	1047 ± 934	0.006	388 ± 433	607 ± 925	439 ± 629	0.956
Folic acid [nmol/l]	204 ± 116	186 ± 111	228 ± 123	0.678	154 ± 33	231 ± 150	216 ± 83	0.018	192 ± 142	207 ± 129	206 ± 70	0.204

Data are means ± standard deviation; p-values are calculated using Friedman test for differences between time points; significant differences are highlighted using bold formatting.

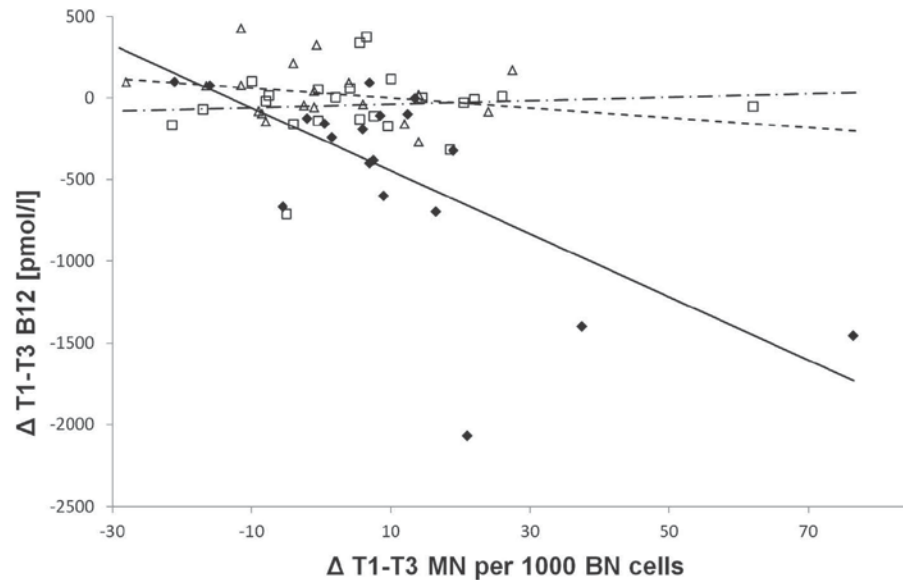


Fig. 1. Correlation between six month change (T1–T3) of plasma B12 status and MN frequency. Filled square – RTS group (full line), empty square – RT group (dotted line), triangle – CT group (broken line); RTS: $r = -0.584$, $p = 0.009$; RT: $r = 0.081$, $p = 0.715$; CT: $r = -0.259$, $p = 0.284$.

(0–30 MN/1000 BN cells) (Fenech, 2007). Considering the individual range, we observed MN frequencies from 5.5 MN/1000 BN cells up to 98 MN/1000 BN cells. Deviating from what has been reported on younger age groups, no significant difference between men and women was observed. However, as described in the literature (Fenech et al., 1994; Norppa and Falck, 2003), the total number of MN and cells with MN was about 10% higher in women than in men (data not shown).

Besides gender, it is well known that aging highly influences the formation of MN. Most studies focusing on the incidence of MN over the human lifespan reported a linear relationship until the age of 60–70 years (Fenech and Bonassi, 2011; Wallner et al., 2012; Bonassi et al., 2001; Peace and Succop, 1999). So far, only very little data are available on study cohorts in their 80s or 90s, demonstrating hints for opposite observations (Bolognesi et al., 1997; Bonassi et al., 1995; Wojda et al., 2007; Fenech, 1998). In our subjects of very old institutionalized elderly, at or above life expectancy, we did not observe any age related effects. Interestingly, even a tendency for lower MN frequency with higher age occurred. Investigating the same subjects, we recently showed that there is a leveling-off of the MN frequency at about 60–70 years of age (Franzke et al., 2014). These findings indicate that there might be a threshold of genome instability that limits the upper rate of MN formation. If the “survivors” are able to reach that high age of 85 years or above, they seem to be either more resistant to MN formation or cells with MN are more likely to undergo apoptosis (Wojda et al., 2006, 2007).

At baseline, the subjects of the present study demonstrated a low physical performance compared to reference values for maintaining physical independence at this age (Werle et al., 2009; Rikli and Jones, 2013). They relatively performed best at the chair rise test where they reached $104 \pm 37\%$ of the reference for their age. Nonetheless, our results demonstrated the relatively weak physical condition of our study participants that performed $90 \pm 30\%$ in the handgrip strength test and only $68 \pm 18\%$ in the 6 minute walking test, compared to normative values. In the elderly, aerobic fitness seems to be of great importance to prevent genomic instability. Mota et al. (Mota et al., 2010) clearly demonstrated that elderly subjects with higher $\text{VO}_{2\text{max}}$ showed less DNA damage and higher mitochondrial activity. As we observed a negative correlation between MN frequency and the 6 minute walking test, our results confirm this aspect, demonstrating a link between chromosomal damage and aerobic fitness (Franzke et al., 2014).

According to the definitions of the World Health Organization (WHO), our subjects showed a lower red blood cell folate status but an appropriate plasma vitamin B12 level (de Benoist, 2008).

4.2. Intervention effects

After six months of strength training alone, combined with dietary supplementation, or cognitive training, our subjects showed a tendency for decreased chromosomal damage, independently of the intervention. We observed reductions in MN frequency between 10–15% (Table 2), but due to the high standard deviation the results were not significant. Only very scattered observations about the effect of exercise on MN formation are available and their conclusions are inconsistent, whereas in institutionalized elderly they are missing completely. Goon et al. (Goon et al., 2008) compared people performing Tai Chi ($N = 35$; age = 58.5 years) for at least one year with sedentary people ($N = 35$; age = 57.0 years) and observed significantly lower chromosomal damage in the first cohort. Similarly, Huang et al. (Huang et al., 2009) observed lower MN frequencies in Japanese metal workers with higher physical activity. Studies of very intense or very long exercise regimes showed contrary results. Endurance trained athletes did not show higher MN frequency after an ironman triathlon (Reichhold et al., 2008) but acute exhaustive sprint exercise increased the MN frequency significantly (Schiffel et al., 1997). However, there seems to be a dose-dependent effect of exercise on DNA damage since very prolonged exhausting and/or unaccustomed exercise could impair the balance between ROS production and the antioxidant defense system, therefore possibly leading to chronic oxidative stress. As Radak et al. (Radak et al., 2013) pointed out, a moderate level of oxidative stress is essential for the adaptive response after exercise. To our knowledge, we were the first to perform an intervention study to investigate the effect of six month resistance exercise on chromosomal damage in the very oldest of our society.

At the six-month time point, only the RT and the RTS group improved significantly at the chair rise and the 6 minute walking test. There was no change at the handgrip strength test for any group (Table 3).

Although the CT group had neither a physical exercise intervention nor a nutritional supplementation, they surprisingly showed similar tendencies for decreased chromosomal damage compared to the RT

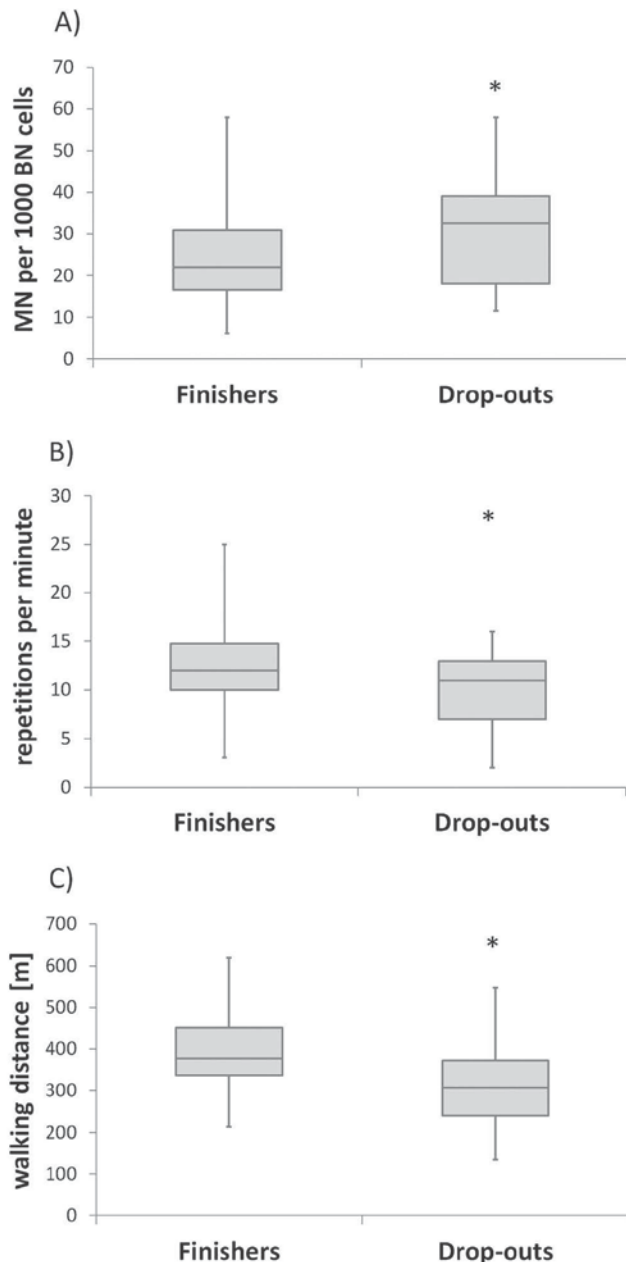


Fig. 2. Comparison of baseline data: study finishers vs. drop-outs. Boxplot A) MN frequency ($p = 0.034$), boxplot B) chair rise test ($p = 0.023$), boxplot C) 6 minute walking test ($p = 0.002$).

and the RTS groups. Similar to physical activity, socialization and psychological stress seem to have an impact on DNA damage (Gidron et al., 2006; Møller et al., 1996; Bouayed et al., 2009). Notably, in elderly institutionalized people, oxidative stress is higher when compared to free-living subjects (Maugeri et al., 2004). Social isolation and the psychosocial environment have severe impacts on the subjects' wellbeing, influencing stress parameters and the endocrine system and therefore affecting DNA stability (Arnetz et al., 1983; Epel, 2009). As the participants of the current study met twice a week for 60 min and performed either strength training or cognitive exercises, these social events might have improved resistance against chromosomal and DNA damage in the CT group to a similar extent as in the RT and RTS groups. We hypothesize therefore that being a part in this study motivated the subjects of the CT group, which seems to induce similar biochemical changes for their body as in the physical training groups.

Only the RTS group showed significant changes in vitamin B12 and folate status after six months of consuming the provided supplement. It is well known, that these two nutrients are strongly linked to cell differentiation and therefore correlate particularly with the MN frequency (Fenech and Bonassi, 2011; Ni et al., 2012). Although our subjects showed a sufficient B12 plasma level at baseline, we observed a significant negative correlation between the six month change of B12 and the six-month-change of the MN frequency in the RTS group ($r = -0.584$, $p = 0.009$) (Fig. 1). Consequently, an increase in vitamin B12, due to supplementation, is associated with a decrease in chromosomal damage in our study cohort of institutionalized elderly. This observation underlines the importance of a well-balanced diet and a sufficient supply of nutrients in this age-group. Therefore it could be discussed to recommend a supplementation of B12 and folic acid in this very high age group in order to prevent chromosomal damage.

4.3. Drop-out analysis

Compliance with a treatment, either medical, nutritional or physical, is of great importance for the success of a therapy and for human intervention studies (Rich et al., 1996; Hubbard et al., 2012). Therefore, we decided to compare the baseline data of the study finishers to the drop-outs.

Interestingly, the finishers differed significantly from the drop-outs in MN frequency, in the 6 minute walking test and the chair rise test (Fig. 2). These results indicate, that elderly institutionalized study participants with better physical performance and a lower cellular mutation rate are more likely to finish an intervention program than same-aged subjects with lower fitness and higher chromosomal damage from the same study cohort. These parameters can therefore be discussed as predictors for lifestyle interventions.

5. Conclusion

To our knowledge, we were the first to investigate the effect of a six-month lifestyle intervention of cognitive training, resistance training alone or combined with a nutritional supplement on resistance against chromosomal damage in highly aged institutionalized elderly, as measured by the CBMN-assay. In this very heterogenic age group, we observed a tendency for a reduction of the MN frequency and of cells with MN of up to -15% and -20% respectively. Unexpectedly, the participants of the CT group also demonstrated similar tendencies for chromosomal damage, whereas only the RT and RTS groups improved their physical performance. After six months of consuming the provided supplement, the RTS group showed significant higher vitamin B12 and folate levels and their B12 increase correlated significantly with a reduction of the MN frequency. Interestingly, those subjects with higher chromosomal damage and lower physical performance at baseline were more likely to quit the study before the last samplings.

Our results from studying a cohort of very old institutionalized elderly led us to several conclusions: 1) Higher B12 plasma levels, due to an oral supplementation containing vitamin B12, are negatively linked to chromosomal damage. 2) Compliance with a human intervention study seems to be influenced by the subjects' baseline physical condition. 3) In this age group either physical or cognitive training could achieve similar biochemical changes which tend to enhance resistance against genomic instability. 4) Social events and social well-being might be an important factor in institutionalized elderly's health.

With the increasing life expectancy and consequently more people moving to residential care homes, further studies of the elderly, especially of the very oldest of our society, are demanded to investigate the underlying mechanisms of exercise and nutrition on cellular mutation.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.exger.2015.03.001>.

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Review

Super DNaging—New insights into DNA integrity, genome stability and telomeres in the oldest old

Bernhard Franzke^a, Oliver Neubauer^{a,b}, Karl-Heinz Wagner^{a,c,*}

^a University of Vienna, Research Platform Active Ageing, Althanstraße 14, 1090 Vienna, Austria

^b Queensland University of Technology, Faculty of Health, School of Biomedical Sciences, Institute of Health and Biomedical Innovation (IHBI), Tissue Repair and Regeneration Group, 60 Musk Avenue, Kelvin Grove Campus, Brisbane, QLD 4059, Australia

^c University of Vienna, Faculty of Life Sciences, Department of Nutritional Sciences, Althanstraße 14, 1090 Vienna, Austria

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ABSTRACT

Reductions in DNA integrity, genome stability, and telomere length are strongly associated with the aging process, age-related diseases as well as the age-related loss of muscle mass. However, in people reaching an age far beyond their statistical life expectancy the prevalence of diseases, such as cancer, cardiovascular disease, diabetes or dementia, is much lower compared to “averagely” aged humans. These inverse observations in nonagenarians (90–99 years), centenarians (100–109 years) and supercentenarians (110 years and older) require a closer look into dynamics underlying DNA damage within the oldest old of our society. Available data indicate improved DNA repair and antioxidant defense mechanisms in “super old” humans, which are comparable with much younger cohorts. Partly as a result of these enhanced endogenous repair and protective mechanisms, the oldest old humans appear to cope better with risk factors for DNA damage over their lifetime compared to subjects whose lifespan coincides with the statistical life expectancy. This model is supported by study results demonstrating superior chromosomal stability, telomere dynamics and DNA integrity in “successful agers”. There is also compelling evidence suggesting that life-style related factors including regular physical activity, a well-balanced diet and minimized psycho-social stress can reduce DNA damage and improve chromosomal stability. The most conclusive picture that emerges from reviewing the literature is that reaching “super old” age appears to be primarily determined by hereditary/genetic factors, while a healthy lifestyle additionally contributes to achieving the individual maximum lifespan in humans. More research is required in this rapidly growing population of super old people. In particular, there is need for more comprehensive investigations including short- and long-term lifestyle interventions as well as investigations focusing on the mechanisms causing DNA damage, mutations, and telomere shortening.

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Contents

1. Introduction	00
2. DNA integrity	00
3. Genome stability	00
4. Telomeres	00
5. Role of mitochondrial DNA damage and replication errors in ageing skeletal muscle	00
6. Concluding remarks of the literature review	00
7. Behavioral strategies for successful “DNaging”	00
8. Conclusion	00
Conflict of interest	00
Acknowledgements	00
References	00

* Corresponding author at: Department of Nutritional Sciences University of

Vienna Althanstraße 14 A-1090 Vienna, Austria. Fax: +43 1 4277 9549.

E-mail address: karl-heinz.wagner@univie.ac.at (K.-H. Wagner).

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1. Introduction

Predictions from the World Health Organization [1] estimate that the global number of people aged 85 years and older is going to increase by 351% from 2010 to 2050. Particularly the sub-group of centenarians will increase 10-fold, which makes the oldest old group (aged 85 years or older) the fastest growing segment of the population in developed countries [1].

Especially the group of elderly aged around the statistical life-expectancy of humans (developed countries: men around 72 years, women around 80 years) is most susceptible to disease and disability [2]. With this bio-demographic development the burden of chronic, age-related diseases such as cardio-vascular diseases, type 2 diabetes, cancer, dementia and physical impairments associated with the age-related loss of skeletal muscle and function, is steadily increasing [3].

Aging is considered as a degenerative and multi-factorial process caused by accumulating molecular and cellular damage that leads to cell and tissue dysfunction [4–6]. Proposed mechanisms that contribute to the aging process and the development of chronic, age-associated diseases include increased levels of DNA damage, genotoxicity, oxidative stress, and incidence of shorter telomeres [7–11]. Many theories have been proposed to explain the phenomenon of aging, yet none has been able to fully explain the mechanisms that drive the process of aging [12]. The number of aging theories, focusing on particular mechanisms, increased within the last decades (e.g., the somatic mutation theory, the wear-and-tear theory, the free radical/oxidative stress/mitochondrial theory and the rate-of-living theory) [13]. In parallel to the different aging hypotheses, integrative approaches and “network” theories of aging were developed and gained more and more importance. Based on these integrative theories, aging is a multi-factorial process involving complex interactions between biological and molecular mechanisms, which may be at least as important as single actions [12,14–17]. All theories in common is the idea of with age linearly accumulating processes leading to

cellular damage, tissue dysfunction and finally to death of the organism. Importantly, however, people exceeding the statistical life-expectancy, and especially the very oldest age-groups – nonagenarians (90–99 years), centenarians (100–109 years) and super-centenarians (110 years and older) – demonstrate a different picture of age-related diseases compared to study cohorts at or below life-expectancy [18]. This phenomenon of “successful aging” contradicts the theories of aging where age linearly correlates with the accumulation of reactive oxygen species (ROS), DNA damage, mitochondrial dysfunction, and shortening of the telomeres [19].

In this review we therefore investigate the impact of DNA damage for successful and healthy aging in the oldest old of our society and try to explore the crucial difference between elderly individuals at or below life-expectancy and the “super old” – nonagenarians, centenarians and super-centenarians. We focus on reports about DNA damage (double/single strand breaks, DNA repair, antioxidant defense, mitochondrial DNA), mutagenicity (sister chromatid exchange, acentric fragments, nondisjunction, aneuploidy, chromosomal loss, formation of micronuclei) and telomeres (telomere length, telomerase activity) within the oldest age-groups, summarized in Tables 1–3. Furthermore, recognizing the importance of mitochondria and, especially, mitochondrial (mt)DNA stability and integrity for aging [4] as well as the functionality of skeletal muscle in elderly individuals [20] we discuss findings on mtDNA damage in skeletal muscle of aging humans. The primary focus was on data of human studies; however, animal models were additionally included for discussing underlying mechanisms.

Finally we examined potential life-style based strategies for successful “DNAging” and reaching the individually predetermined lifespan.

2. DNA integrity

The process of aging has shown to be linked to increased DNA damage [21]. These findings are supported by the free radical

Table 1
Summary of available studies concerning DNA integrity in elderly at or beyond life-expectancy compared to younger age-groups.

DNA integrity				
Reference	Subjects	Age	Markers/methods	Main results
Hyland et al. [28]	Young controls: n = 18 Nonagenarians: n = 138	Young controls = 47.4 years Nonagenarians = 90.4 years	Comet assay in PBMCs; FRAP	Higher plasma antioxidant capacity in study group; similar level of DNA damage in PBMCs
Chevanne et al. [29]	Fibroblasts from young (n = 4), old (n = 4), and centenarian (n = 3) donors were cultured	Young = 18–22 years Old = 68–76 years Centenarians = 99–102 years	DNA strand breaks; DNA repair; Glutathione peroxidase activity; Glyceraldehyde-3-phosphate dehydrogenase activity	Less sensitivity of cells from centenarians to H ₂ O ₂ induced DNA damage; comparable levels of DNA strand breaks in all age-groups
Chevanne et al. [30]	PBMCs from young (n = 5), old (n = 3), and centenarian (n = 4) donors were cultured	Young = 19–26 years Old = 69–75 years Centenarians = 100–107 years	Comet assay and DNA repair	Cells from centenarians show similar DNA repair capacity as cells from young donors and improved compared to cells from old subjects
King et al. [31]	PBMCs from young (previous study), mid-aged (previous study), old (previous study), and very old (n = 31) subjects	Young = 35–39 years Mid-aged = 50–54 years Old = 65–69 years Very old = 75–80 years	DNA strand breaks and repair (ELISA); antioxidant enzyme activity	Basal DNA damage and DNA repair of the very old were comparable to young cells; increased level of GPx and CAT in the very old
Humphreys et al. [32]	PBMCs from young (n = 40), old (n = 35), and very old (n = 22) subjects	Young = 20–35 years Old = 63–70 years Very old = 75–82 years	Comet assay; DNA repair (OGG1)	Increased oxidative base damage in old age; improved DNA repair in the oldest group
Franzke et al. [84]	6 months lifestyle intervention in institutionalized elderly; resistance training (RT) (n = 34), RT & supplement (RTS) (n = 30), cognitive training (CT) (n = 32)	Age = 65–98 years Mean age = 83.1 years	Comet assay; antioxidant enzyme activity; functional parameters	Significantly increased basal DNA damage in RT and RTS groups; improved resistance against H ₂ O ₂ induced DNA damage in all groups; increased CAT and SOD activity in RT and RTS groups

Five cross sectional studies comparing young vs. old subjects and one lifestyle intervention study investigated “super old” humans.

Main conclusions from comparing very old with old and young humans:

- Increased antioxidant defense capacity in the very old compared to “normal” old
- Subjects above life-expectancy demonstrated improved DNA repair capacity compared to elderly below life-expectancy
- DNA damage and repair of the oldest groups was similar to youngest groups

Table 2

Summary of available studies concerning genome stability in elderly at or beyond life-expectancy compared to younger age-groups.

Genome stability				
Reference	Subjects	Age	Markers/methods	Main results
Franceschi et al. [40]	Control group: <i>n</i> = 25 Centenarians: <i>n</i> = 9	Control group = 20–79 years Centenarians—age not defined	Spontaneous chromatid breaks; Spontaneous sister chromatid exchange (SCE); Micronuclei (MN) frequency (CBMN-assay)	Centenarians show consistent genomic stability; significantly lower SCE-frequency in centenarians; MN frequency consistently increased with age
Erceg et al. [41]	Elderly group: <i>n</i> = 106 Control group: <i>n</i> = 42	Elderly group = 65–95 years Control group = 5–50 years	Samples were scored for all chromosomes, ring chromosomes, acentric fragments and chromatid or chromosome breakages	Comparable low percentages of chromosomal aberration were observed in the oldest subjects of 80 years or above and younger controls
Bonassi et al. [42]	Data were gathered from the Molecular Epidemiology Data Base (MEDB) project Females: <i>n</i> = 947 Males: <i>n</i> = 1184	Total sample: <25 years until >64 years	SCE; Chromosomal aberrations (CA), MN frequency	Gender independent leveling off of MN frequency occurred after 45–54 years; in females a decrease after 55 years of age was observed
Franzke et al. [43]	Elderly, institutionalized group: <i>n</i> = 105 Younger adults: <i>n</i> = 76	Elderly group = 83 ± 6 years; Younger adults = 32 ± 11 Years	MN frequency (CBMN-assay)	A leveling off of the MN frequency after the age of 60 years was observed
Wojda et al. [44]	Centenarians: <i>n</i> = 53 Younger adults: <i>n</i> = 74	Centenarians = 100–108 years; 69–78 years: <i>n</i> = 20; 60–68 years: <i>n</i> = 14; 40–50 years: <i>n</i> = 20; 21–30 years: <i>n</i> = 20	MN frequency (CBMN-assay)	A significant decrease in the MN frequency after the age of 70 years in women; a leveling-off was observed in men
Wojda et al. [78]	Centenarians: <i>n</i> = 52 Younger adults: <i>n</i> = 71	Centenarians = 100–108 years; Younger adults = 21–78 years	G-band karyotype data sheets were reviewed to identify hypoploid, hyperploid, and polyploid cells, and structural aberrations of chromosomes.	Chromosomal aberrations in women were higher than in men until the age-group of 60–70 years; centenarian males demonstrated a leveling-off; in centenarian females a reduction of genetic damage after 60–70 years
Franzke et al. [83]	6 months lifestyle intervention in institutionalized elderly; resistance training (RT) (<i>n</i> = 35), RT & supplement (RTS) (<i>n</i> = 29), cognitive training (CT) (<i>n</i> = 33)	Age = 65–98 years Mean age = 83.1 years	MN frequency (CBMN-assay); functional parameters; vitamin B12; folate	Significantly reduced frequency of nucleoplasmic bridges (CT group) and apoptotic cells (RTS and CT group); tendency for reduced MN frequency in all groups; increased plasma B12 status due to supplementation was linked to decreased chromosomal damage

Six cross sectional studies comparing young vs. old subjects and one lifestyle intervention study investigated “super old” humans.

Main conclusions from comparing very old with old and young humans:

- Genomic instability increased until the age of 60–70 years
- After the age of 60–70 years chromosomal aberrations stabilized or reduced
- In subjects above life-expectancy chromosomal damage similar to younger cohorts was observed

theory of aging, suggesting that the production of ROS by mitochondria accumulates over the lifespan and, therefore, lead to a state of chronic oxidative stress at old age [22]. As antioxidant defense mechanisms and DNA repair capacity seem to be impaired in the elderly, DNA damage has been proposed to be a consequence of aging [23]. However, the free radical theory of aging has not conclusively been proven; and whether ROS are the primary cause of aging or a consequence remains a matter of debate [24]. Furthermore, evidence has been increasing in the recent years that ROS are not only damaging agents, but also act as signaling molecules for initiating adaptive responses to physiological stimuli such as associated with exercise [25–27].

Studies in nonagenarians and centenarians concerning DNA damage are still rare, but revealed interesting results as human individuals in this age group represent an excellent model for healthy aging [18]. Importantly, DNA damage has been shown to be lower in “successful agers” (reaching an age of 85+ years), compared to people below their statistical lifespan [28–30], indicating that for these individuals the free radical theory of aging is not applicable. Previous studies suggested that age-related increased DNA damage is accompanied by enhanced DNA repair mechanisms, which might, at least to a certain extent, explain that there is no further increase of DNA damage after the age of 70–80 years [31,32]. Notably, cells of the oldest humans (centenarians) showed improved antioxidant defense and DNA repair activity

compared to younger elderly and were even similar in their responses as cells of young adults (19–26 years) [29,30].

Therefore, the maintenance of antioxidant defense and repair mechanisms with aging appears to play a critical role in preventing ROS-mediated DNA damage in immune-competent cells such as PBMCs, which are routinely exposed to ROS during immune responses and at sites of inflammation. The preservation of DNA integrity with age especially in immune cells may, in turn, contribute to prevent or attenuate the age-associated decrease in immune competence, also referred to as immune-senescence [33]. Considering that persistent DNA damage has been shown to induce the secretion of pro-inflammatory cytokines [34], enhanced DNA-protecting mechanisms might also play an important role in counteracting the development of chronic low-grade inflammation with aging, termed “inflamm-aging” [35].

Conclusively, successful agers seem to have better DNA repair capacity and superior antioxidant defense mechanisms compared to elderly below the statistical life-expectancy. Furthermore, “super old” individuals often show cell characteristics similar to much younger cohorts.

3. Genome stability

Cytogenetic aberrations, such as acentric fragments, nondisjunction, aneuploidy, chromosomal loss, and the formation of

Table 3
Summary of available studies concerning telomere length in elderly at or beyond life-expectancy compared to younger age-groups.

Telomeres				
Reference	Subjects	Age	Markers/methods	Main results
Halaschek-Wiener et al. [54]	Healthy old subjects: $n = 134$ Control group: $n = 47$	Healthy old = 85 years and older; Control group = 40–50 years	Telomere length (flow FISH)	Healthy aged individuals showed significantly more telomeres with “normal” length, indicating an age-related selection towards an optimal length
Terry et al. [55]	Centenarians: $n = 38$	Healthy ($n = 19$) and physically limited ($n = 19$) centenarians = 97–108 years	Quantitative polymerase chain reaction (QPCR)	Healthy individuals demonstrated significantly longer telomeres than physically limited subjects
Fitzpatrick et al. [56]	1136 subjects were observed between 1992 and 1998	Mean age = 73.9 years	Southern blot	Subjects within the shortest quartile of telomere length were 60% more likely to die than those within the longest quartile
Ponsot et al. [57]	Physically active young ($n = 16$) and old ($n = 26$) women and men	Young = 25 ± 4 years; Old = 75 ± 4 years	Southern blot adapted for muscle tissue	No difference in telomere length in muscle tissue of physically active young and old adults
Bendix et al. [58]	548 same-sex twins were observed to examine the link between telomere length, physical ability and cognitive function.	Women and men aged 73–94 years	Southern blot	Those twins who reported better physical ability also demonstrated significant longer telomeres
Tedone et al. [59]	Semisuper-centenarians: $n = 29$; centenarians: $n = 59$; centenarians’ offspring: $n = 70$; matched offspring of parents who both died at an age in line with life expectancy: $n = 28$; matched offspring of both non-long-lived parents: $n = 35$	Semisuper-centenarians = 105–109 years; Centenarians = 100–104 years; centenarians’ Offspring = 70.6 ± 6.9 ; matched offspring of parents who both died at an age in line with life expectancy = 73.1 ± 7.0 ; matched offspring of both non-long-lived parents = 73.1 ± 3.5	Quantitative polymerase chain reaction (QPCR)	Centenarians showed no difference in telomere length compared to the younger groups. The transition from centenarians to semisuper-centenarians was accompanied by a considerable loss of telomere length.
Ishikawa et al. [60]	Pituitary tissue samples: $n = 65$	Subjects were aged between 0 and 100 years	Southern blot	Telomere length showed a significant decrease until the 61–75 years group, leveled off or increased slightly in advanced age groups.
Nakamura et al. [61]	White and gray brain matter samples: $n = 72$	Subjects were aged between 0 and 100 years	Southern blot	Telomere length decreased significantly until the 70–79 years group, leveled off or increased in advanced age-groups.
Kimura et al. [62]	Leukocyte telomere length was analyzed in exceptionally old ($n = 82$) and younger individuals ($n = 99$)	Old = 99–104 years; Young = 23–74 years	Genome-wide telomere length analyses by the terminal restriction fragment length (TRFL) and single molecule telomere length analysis (STELA) of the X and Y chromosomes in leukocytes	Old subjects demonstrated shorter telomeres with a remarkable over-representation of ultra short telomeres

All studies ($n = 9$) are cross sectional analyses comparing young vs. old subjects. No data regarding lifestyle interventions in “super old” humans are available. Main conclusions from comparing very old with old and young humans:

- Telomere length decreased until about 60–80 years of age.
- After the age of 60–80 years telomere length stabilized or increased.
- Healthy and physically active individuals demonstrated telomeres comparable to much younger cohorts.
- Even in “super-agers”, the end of the human lifespan is accompanied by a significant reduction of telomere length.

micronuclei are elevated in the elderly and in subjects with age-related diseases such as cancer, cardio-vascular diseases and diabetes [36,37]. It is well reported that chromosomal damage increases over the human lifespan and shows a linear relationship until the age of 60–70 years [38,39]. Only a few investigations on subjects within the oldest age-groups of nonagenarians or centenarians have been performed so far. Available data indicate that this particular group of the oldest old might be more resistant to mutagenicity than elderly below or at their statistical life-expectancy. Franceschi et al. [40] reported a significant lower frequency of sister chromatid exchange in cells from centenarians compared to younger age-groups (23–79 years) which suggests a high genomic stability in the oldest old. In support of these data, Erceg et al. [41] observed similar chromosomal aberrations in subjects between 80 and 100 years compared to the younger middle-aged control group. In subjects between 65 and 80 years of age, frequencies of cytogenetic aberrations about twice as high were observed compared with younger control groups [41].

Bonassi et al. [42] were the first to report rather a reduction of the micronucleus frequency in the latest age classes. By analyzing data of 181 subjects (20–98 years), we recently demonstrated a leveling-off of the micronucleus frequency beyond the age of 60–70 years [43]. Our findings are supported by the results of Wojda et al. [44], which also indicate no further increase in the micronucleus frequency of centenarians and a plateau-like effect of chromosomal aberrations after the age of 60 years.

Genome stability of human cells decreases with age. However, comparable to the data on DNA damage (as discussed in the previous chapter), no further deterioration of genome stability has been observed in subjects over their statistical life-expectancy. Available data even suggest a trend for improved genome stability in this age-group.

4. Telomeres

Telomeres have been proposed as protective ends of the chromosomes, constituting of repeated DNA sequences [45]. Their

length, as well as their replicative capacity contribute to the stability of the genome and to DNA damage [46]. Telomere length maintenance and preservation of telomerase activity are inversely correlated with the aging process [47,48] and age related chronic diseases are characterized by shorter telomeres [11,49,50]. Moreover, a decline in telomere length is associated with morbidity and mortality in the elderly; however, in the oldest old this correlation cannot be observed [51–53]. In people exceeding life expectancy, a lower variance in telomere length was observed compared to younger elderly [54]. Halaschek-Wiener et al. [54] concluded that there might be a selection towards an individual, optimal telomere length in the healthy oldest old (85–97 years). Especially in people over their statistical life-expectancy a significant relationship between health status and telomere length occurs. This is supported by healthy centenarians demonstrating significantly longer telomeres than individuals, with two or more clinical symptoms including hypertension, congestive heart failure, myocardial infarction, peripheral vascular disease, dementia, cancer, stroke, chronic obstructive pulmonary disease, or diabetes type 2 [55].

In a six-year follow-up study [56] samples of 1136 subjects were analyzed for telomere length, health related parameters, and mortality. The participants within the shortest quartile of telomere length demonstrated a 60% higher mortality risk than those within the longest quartile [56]. This supports the concept that health status has a remarkable influence on telomere dynamics in the elderly [56], particularly in elderly individuals beyond life-expectancy [54] including centenarians [55].

Furthermore, telomere maintenance until old age seems to be influenced by physical activity. Ponsot et al. [57] observed no difference in telomere length in skeletal muscle of old (70–83 years) compared with young (20–31 years) subjects with comparable physical activity level. In a twin-study, Bendix et al. [58] described, that siblings (73–94 years) with better physical condition (measured using a physical ability score questionnaire) also displayed longer telomeres. Notably, they observed that with a one-unit-increase of physical ability, leucocyte telomere length increased by 0.066 kb, which equals approximately three years of telomere shortening [58].

Telomere maintenance seems to be an important determinant for healthy aging and longevity. This concept is supported by the fact that centenarians show no further shortening of the telomere length compared to younger controls [59], independently of the investigated tissue sample [60,61]. However, the transition from centenarians to super-centenarians is accompanied by a significant decrease (–30% over 5 years) in telomere length, indicating that at this super old age the replicative mechanisms for telomere maintenance might have reached their functional limit [59,62].

Taken together, significantly delayed characteristics of “DNAging” and/or improved replicative, antioxidant and anti-mutagenic defense mechanisms have been observed in successfully aged individuals (nonagenarians, centenarians, super-centenarians) compared to younger elderly at or beyond life-expectancy. These superior attributes of genetic stability in the oldest old are reflected by longer and/or more stable telomeres, which are even improved by a healthy lifestyle and also in absence of diseases.

5. Role of mitochondrial DNA damage and replication errors in ageing skeletal muscle

In the previous sections of this review, we described data on the stability of the nuclear genome in association with ageing and longevity. In the following, we discuss the evidence suggesting the involvement of mtDNA damage and replication errors in aging. Considering that mtDNA damage and mutations have been proposed as a critical cellular mechanism underlying the age-

associated loss of skeletal muscle mass, strength and functionality [20,63], a particular focus is on damage to and/or mutations of mtDNA in skeletal muscle of elderly humans.

Mitochondria contain their own genetic material, mtDNA, which encodes 13 proteins that are components of the oxidative phosphorylation system [4,5,64]. Due to the central functions of mitochondria in cellular energetics including ATP production and the regulation of metabolic signaling pathways and apoptosis, mtDNA damage and mutations are associated with several human mitochondrial diseases, age-related diseases and phenotypes associated aging [4,5,64]. Over the past years, there has been mounting data showing that somatic mtDNA mutations increase with age in various tissues in mammals [65], including humans [20,65] and, in particular, in human skeletal muscle [66]. Furthermore, it has been shown that mtDNA mutates at higher rates than nuclear DNA [66]. The higher susceptibility of mtDNA to mutations is thought to be a consequence of several factors such as its close proximity to the inner mitochondrial membrane as a main site of ROS generation, its lack of protective histones and introns, and its limited DNA repair systems compared to nuclear DNA [4,5,67]. According to the mitochondrial free radical theory of aging [21,68] (proposed in extension upon the free radical theory of aging [22,24]), the primary molecular mechanism underlying aging is an increased mitochondrial ROS production causing somatic mtDNA mutations [69]. This theory is based on observations and correlative data from cell culture, invertebrate, and mammalian models suggesting that oxidative damage to mtDNA accumulates with age [21,24], which, in turn, has been assumed to contribute to mitochondrial dysfunctions and a further increase in ROS generated from the defective mitochondrial respiratory chain [24]. However, neither the mitochondrial free radical theory of aging nor the hypothesis that somatic mtDNA mutations are predominantly induced by ROS, have been conclusively proven [4,5,20,64].

More recent data indicated that ROS and cumulative oxidative damage might be secondary to other mechanisms causing mtDNA mutations [4,5]. An alternative hypothesis is that the majority of age-associated increases in mtDNA mutations are due to a clonal expansion of mtDNA replication errors [4,5,64,70]. Since the replication of mtDNA occurs independently of the cell cycle, a particular mtDNA molecule (either normal or mutated) will be replicated several times or not at all during a single cell cycle, resulting in the loss of somatic mtDNA mutations in some cells and a clonal expansion in others [4,5,64]. This mitotic segregation of mutated mtDNA is suggested to cause a mosaic occurrence of mitochondrial respiratory chain deficiency and dysfunction within different cells of the same tissue [4,5], as observed in aged skeletal muscle [66]. First experimental evidence for a causal role of mtDNA mutations in aging were provided by studies in mtDNA mutator mice with defects in the proofreading activity of mitochondrial DNA polymerase γ (Pol γ) that caused a premature aging syndrome [71].

Data on mtDNA integrity and mutations in skeletal muscle tissue of elderly humans are limited. However, a few human studies have been performed in this context, which provide important observational evidence on the characteristics and the potential functional consequences of mtDNA mutations in ageing human skeletal muscle. Analyses of skeletal muscle biopsy samples from humans have shown that there is an increase in mtDNA mutations with aging [72–74], and that mtDNA mutations are correlated with mitochondrial respiratory chain deficiencies [66,72,74] and abnormalities in skeletal muscle [72–74]. The observed age-related mtDNA alterations in human skeletal muscle included point mutations [66,73] and large-scale deletions [66,74]. In a study involving 10 healthy, elderly individuals ranging in age from 69 to 87 years (in addition to 10 younger individuals included

as control group [75], randomly deleted mtDNA was predominantly detected in the oldest individuals (i.e., beyond 80 years of age). Data supporting the concept that mtDNA mutations expand clonally to cause mosaic respiratory chain deficiency [5], especially in aged human skeletal muscle, include the findings of Fayet et al. [66]. In their study, that involved 14 individuals without muscle disease aged 69–82 years, muscle fiber segments with a deficiency in the (partially mitochondrial encoded) respiratory chain enzyme cytochrome c oxidase (COX) were associated with mtDNA mutations whereas no mtDNA mutations were detected in normal fibers. These data [66] suggested that the focal accumulation of mtDNA mutations caused impaired mitochondrial function in individual muscle cells; despite the overall level of mtDNA mutations in muscle tissue was low. In agreement with the concept that mtDNA mutations must be present above a certain threshold level to cause respiratory chain dysfunction in individual cells [5], the findings of Bua et al. [74] indicated a manifestation in electron transport system abnormalities in single muscle fibers in which more than 80% of the total mtDNA contained mtDNA-deletion mutations. The number of vastus lateralis muscle fibers, obtained from 12 individuals with no known mitochondrial myopathies, exhibiting respiratory chain deficiencies increased from an estimated 6% at age 49 to 31% at age 92 years [74]. Importantly, the latter study also demonstrated that age-associated accumulations of mtDNA mutations were not only co-localized with mitochondrial electron transport chain abnormalities, but also with areas of fibers displaying abnormal morphology, indicative of structural atrophy. These observations [74] suggest that mtDNA mutations may play a causative role in the age-associated loss of muscle fibers, referred to as sarcopenia [63].

Studies using mouse models provide further support that mtDNA mutations in skeletal muscle may contribute to sarcopenia by inducing mitochondrial dysfunction, activation of apoptosis and a decline in the satellite cell pool [76]. However, the underlying mechanisms and the role of mtDNA mutations in human ageing in general, and in sarcopenia in particular, are still incompletely understood and will, therefore, remain an active area of research.

Collectively, increasing evidence suggests that mtDNA mutations increase with aging in human skeletal muscle. Mitochondrial DNA damage and mutations have repeatedly been shown to coincide with mitochondrial aberrations and dysfunction in aged muscle tissue.

6. Concluding remarks of the literature review

Achieving an age beyond the statistical life-expectancy, to a certain extent, appears to be affected by a genetic component [6,18]. In successful agers as nonagenarians, centenarians or super-centenarians, the incidence of age-related diseases is remarkably low [77]. Both, the “regular” aging process and the chronic disease development are accompanied by increased DNA damage, chromosomal damage, and telomere loss [23,36,39,47,49]. In previous studies, no further increase in DNA damage has been observed in healthy individuals above life-expectancy. Furthermore, an increasing amount of data suggests that chromosomal stability, DNA repair activity, and antioxidant defense capacity in successfully aged subjects is comparable to much younger cohorts [28,30,41,54,59,78,79]. These observations indicate that the oldest old humans cope better with risk factors for DNA damage over their lifetime than subjects whose lifespan coincides with the statistical life-expectancy. Their superior resilience until old age might originate from a naturally improved antioxidant defense, combined with better DNA repair capacity and higher telomerase activity compared to “normal” agers. These genetically controlled mechanisms appear to prevent or, at least, minimize telomere shortening, DNA strand breaks and chromosomal aberrations, all of

which consequently protects from cellular damage, cell malfunction and senescence.

In accordance with the different network theories of aging [12,14–17], our review of the literature lead us to the conclusion that successful “DNAging” is a multifactorial process. It appears that a combination of molecular and cellular mechanisms, many of which are genetically controlled, preserves DNA integrity and genome stability in very old individuals [6,18]. However, there is also compelling evidence that a healthy lifestyle can significantly contribute to achieving the maximum lifespan and remaining independently into old age [28,30,41,54,59,78–80].

7. Behavioral strategies for successful “DNAging”

Along with a genetic predisposition to reach super old age [59,81], protective mechanisms against oxidative stress and DNA damage, can be enhanced by individual lifestyle factors including nutritional behavior, physical activity and the psycho-social environment [82–84]. The existing data about the effects of physical activity on DNA damage are inconsistent, which, to a certain extent, is due to differences in the acute exercise interventions, exercise training programs or the training status of the individuals [85,86]. The most conclusive picture that emerges from data about exercise on DNA damage is that strenuous and – in relation to the training status – 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 [86–88]. Especially in the elderly, where DNA repair capacity might have already been reduced [85,89], very intensive and unaccustomed exercise might lead to increased oxidative DNA damage [26]. 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, particularly to adaptations in endogenous antioxidant defense and other cell-protective mechanisms [26,27].

After chronic exercise, enzyme activity and DNA repair mechanisms have been shown to be up-regulated [90]. In a study cohort of institutionalized elderly (65–98 years) basal DNA damage was increased after six months of regular exercise, while antioxidant defenses against H_2O_2 induced oxidative stress were improved [84] and chromosomal damage was reduced [83]. Investigating groups of young sedentary, young physically active, old sedentary, and old physically active men, Radak et al. [91] observed improved DNA repair mechanisms in skeletal muscle after an acute exercise bout in young and old physically active subjects only, but not in the sedentary. Parise et al. [92] also reported that resistance exercise training decreased oxidative damage to DNA in older adults. These findings support data from animal studies, suggesting that exercise training appears to reduce oxidative damage to nuclear and mtDNA [93] and to increase the activity of the DNA repair enzyme uracil DNA glycosylase (UDG) in the nuclei and in the mitochondria in old rats [94]. Furthermore, exercise training has also been shown to enhance mitochondrial biogenesis and up-regulate the expression of genes and proteins contributing to the function and integrity of mtDNA [95–97]. These adaptive mechanisms may prevent or delay mitochondrial defects in skeletal muscle with age. Importantly, muscle mitochondrial function is closely related to aerobic capacity, which in turn is inversely related to premature mortality [96,98,99]. A recent study involving octogenarian athletes, who have trained and remained physically active their entire lives, showed that endurance training had remarkably positive effects on mitochondrial function, skeletal muscle oxidative capacity and cardiovascular health [100]. This supports the idea that regular and lifelong exercise training represents an effective lifestyle intervention for maintaining a

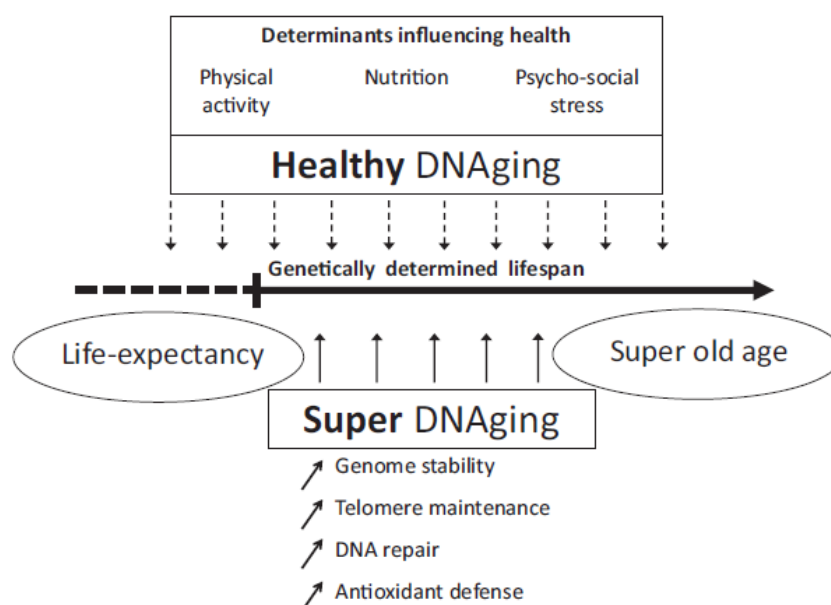


Fig. 1. Hypothesized model of factors that may explain why super old agers surpass life-expectancy from a DNA point of view. Dashed line arrows indicate influence of health behavior to possibly reach one's "genetically determined" age. Full line arrows indicate potential factors for reaching super old age.

functional capacity and reducing the risk of disability and mortality even in individuals beyond 80 years of age [100]. Accordingly, elderly subjects with higher fitness levels demonstrated no difference in telomere length compared to a younger cohort [57], as well as shorter telomere length [58] and less chromosomal damage [43] compared to untrained controls of the same age.

Similar to physical activity, socialization and psychological stress appear to have strong impacts on DNA damage [101–104]. In institutionalized elderly oxidative stress was higher when compared to free-living subjects [105]. Social isolation and the psychosocial environment can have significant effects on oxidative stress markers and the endocrinal system, which in turn might affect DNA stability [106,107]. Recently, a social intervention program in institutionalized elderly (without additional physical activity) has been shown to result in similar protective effects by reducing DNA and chromosomal damage to levels as observed after the exercise intervention [83,84].

Furthermore, in the elderly, especially under institutionalized conditions, nutrient density is often low due to malnutrition and loss of appetite [108,109]. There is sufficient evidence that deficiencies in vitamins and minerals correlate with DNA damage, DNA repair mechanisms, cellular mutation, and telomere shortening [110–112]. Therefore, diet quality also plays a key role in preventing age-related diseases by minimizing DNA damage and enhancing repair capacity [113]. Vitamins folate and B12, in particular, are discussed as factors affecting genome stability and DNA integrity and for maintaining telomere length at a physiological level [38,112,114]. Folate and vitamin B12 are important for converting uracil (dUMP) to thymidine (dTTP) in DNA methylation. Deficiency of vitamin B12 decreases folate availability and increases the uracil incorporation into DNA. The latter results in an increased susceptibility of DNA for strand breaks, increases in genome instability and an impaired telomerase activity [112,115,116]. Although the intake of physiological amounts of antioxidant micronutrients and phytochemicals is important for the functionality of antioxidant defenses, micronutrient supplementation does not prolong the maximum lifespan [117].

Caloric restriction is another diet-related strategy that potentially increases lifespan, as shown by studies in mammals. The

beneficial mechanisms underlying dietary restriction appear to be, in part, due to reduced DNA damage, improved DNA repair and reduced mitochondrial ROS production [118]. Animal studies suggested that caloric restriction has the potential to lower the mitochondrial ROS production by slowing down metabolism and consequently possibly increasing the lifespan [118]. However, further research is warranted to verify the beneficial effects of caloric restriction on DNA and longevity in humans.

Taken together, numerous studies involving different age-groups have been demonstrating that lifestyle-related factors including physical activity, diet and reduced psycho-social stress, essentially contribute to reductions in DNA damage. Based on these data, it is feasible to suggest that a healthy lifestyle is crucial for longevity and healthy "DNAging", as summarized in Fig. 1.

8. Conclusion

In this review we investigated the relationship between DNA damage and longevity by focusing on data on DNA strand breaks, DNA repair, oxidative stress, mutagenicity, chromosomal damage, telomere length and muscular mtDNA damage in individuals at or beyond statistical life-expectancy. These data suggest that super old subjects beyond the statistical life-expectancy appear to be "genetically/naturally predisposed". Available data on DNA damage in the oldest humans indicate the major involvement of hereditary factors. However, independently of the individual aging-potential, living a healthy lifestyle has been shown to importantly contribute to healthy "DNAging" by improving genome stability, antioxidant defense mechanisms, DNA repair and telomere maintenance. Therefore, a healthy lifestyle and behavior over the whole life time is recognized as a strong component for maintaining physiological functionality into old age and remaining independent and healthy over the maximum lifespan (Fig. 1).

Considering the relatively small number of studies on this topic that have been performed up to now, more research on the super-aging-cohort is required. In particular, there is need for more retrospective data about their early-life lifestyle for better understanding the complex mechanisms to achieve an age beyond life-expectancy.

Conflict of interest

The authors declare that there are no conflicts of interest.

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