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Effect of vitamin D and physical activity on DNA damage in Elderly People

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Contents

List of abbreviations

$25(OH)D_3$	25 hydroxyvitamin D
$1,25(OH)_2D$	1,25- dihydroxy vitamin D
PTH	parathyroid hormone
FGF23	fibroblast growth factor 23
DBP	vitamin D binding protein
VDR	vitamin D receptor
CYP	Cytochrome P-450 enzymes
ER	Endoplasmic reticulum
UV	Ultraviolet
25OHD	25 hydroxyvitamin D
IU	International units
MMSE	Mini-Mental State Examination
ROS	Reactive oxygen species
FPG	Formamidopyrimidine-DNA Glycosylase
DNA	Deoxyribonucleic acid
RS	Reactive species
NF-kB	Nuclear factor kappa B
PPAR-	Peroxisome proliferator-activated receptor
MDA	Malondialdehyde
GSH	Glutathione
PBL	Peripheral blood lymphocytes
DSB	Double-strand breaks
SSB	Single-strand breaks
H_2O_2	Hydrogen peroxide
NaCl	Natrium Chloride
PBS	Phosphate-buffered saline
LMP	Low melting point
EDTA	Ethylenediaminetetraacetic acid
NaOH	Sodium hydroxide
KCl	Potassium chloride
KOH	Potassium hydroxide
CON	Control group
VDD	Vitamin D_3 daily group
VDM	Vitamin D_3 monthly group
T1	Before the main intervention phase
T2	Post loading phase
T3	Post-supplementation and exercise phase
SD	Standard deviation

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1. Introduction

Due to the association of numerous diseases, the interest in vitamin D, particularly its deficit, has expanded greatly over the past few decades [1], [2], [3], [4] Globally, it appears that the big prevalence of vitamin D deficiency and low vitamin D intake is the main issue [5]. Given the fact that vitamin D has a huge impact on bone health, muscle strength, immune and cognitive function, it is essential for individuals to maintain adequate levels. Furthermore, the hormonal form and role of vitamin D has been proven in various physiological processes [6]. The extensive amount of research that has been conducted recently on vitamin D, its functions and importance towards overall health, has indicated that people who have vitamin D deficiency should receive therapy to maintain bone health and overall good health. Therapy can be crucial, especially for patients with a high risk of deficiency such as: older adults, people living in nursing homes or patients with diseases of the nervous or digestive system, which can potentially negatively impact the absorption and metabolization of vitamin D [7], [8].

Nevertheless, aging and the associated loss of muscle mass are considered to negatively impact the human body, particularly the DNA in the older population [9]. Vitamin D has been studied in relation to its potential effect on DNA damage, thus studies concluded that lower vitamin D status is associated with greater-induced DNA damage [10]. Even though the exact mechanism and implications are still explored, it is widely known that vitamin D has many antioxidant properties, it can affect gene expression, bone health, muscle function as well as modulate the immune system [10], [11]. Nevertheless, the relationship between aging and vitamin D is only beginning to be elucidated.

This master's thesis was created within the "NutriAging" study, which was conducted by the Department of Nutritional Sciences at the University of Vienna. The trial examined the effect of vitamin D supplementation and resistance training on DNA damage in older populations. For this study 101 subjects were recruited, who were divided into three groups (two interventional groups and one control group). The study included healthy participants between the ages of 65 and 85, who have not had any regular strength training or vitamin D supplementation at the time of recruitment. During the study participants underwent a supplementation phase for four weeks, after which a resistance training for 10 weeks was added to their still ongoing supplementation intervention.

The control group included participants, who did not supplement vitamin D, rather only with 200 mg calcium daily. The second group received a high dose of vitamin D supplementation of 50 000 IU once a month and a daily dose of 200 mg of calcium. Participants in the third group received a daily supplementation of 800 IU and 200 mg of calcium. Whole blood samples were collected and processed using the comet assay method to analyze the extent of DNA damage.

This master's thesis aims to explore how vitamin D and calcium supplementation (a daily intake of 800 IU vs a monthly intake of 50.000 IU vs only calcium Supplement), alongside resistance training, impacts DNA damage- and by extension the aging process in elderly populations.

1.1 Vitamin D

Vitamin D_3, which is the natural form of vitamin D, is a fat- soluble Vitamin that shows characteristics of a hormone. There are two ways of getting vitamin D in our bodies: through endogenous synthesis or through a dietary intake. The endogenous vitamin D_3 synthesis is the main source of vitamin D and occurs by ultraviolet light exposure of 7-Dehydrocholesterol within the skin. This results in its conversion into 25 hydroxyvitamin D ($25(OH)D_3$) by several enzymes which are located either in the endoplasmic reticulum (ER) (e.g., CYP2R1) or in the mitochondria (e.g., CYP27A1, CYP27B1 and CYP24A1).

Vitamin D is transported in the bloodstream bound to vitamin D binding protein first to the liver, where the first step of the vitamin D Metabolism occurs.

Here vitamin D is hydroxylated at the 25- position resulting in the production of $25(OH)D_3$ [12].

Many cytochrome P-450 enzymes (CYPs), such as: CYP2R1, CYP27A1 and CYP2D25 are believed to be responsible for the conversion of vitamin D into $25(OH)D_3$ [13]. It has been discussed that CYP2R1 is the most essential vitamin D 25-hydroxylase due to the large amount of evidence that patients with a mutation of the CYP2R1 also have a $25(OH)D_3$ deficiency [14], [15] . Nevertheless, the measurement of $25(OH)D_3$ is globally established as one of the greatest biomarkers to assess the vitamin D status of a population [10].

Furthermore, $25(OH)D_3$ is further transported by DBP to the kidneys where $25(OH)D_3$ is being converted either into $24,25(OH)_2D_3$ by CYP24A1 or into the active metabolite 1,25-dihydroxy vitamin D ($1,25(OH)_2D$) by CYP27B1. Both enzymes are tightly controlled [12].

One of the root causes for the stimulation of the $1,25(OH)_2D_3$ synthesis in the kidney is the elevated levels of parathyroid hormone (PTH), which is caused by hypocalcemia, thus the expression of CYP27B1 is stimulated by PTH and low levels of calcium in serum. On the other hand, $1,25(OH)_2D_3$ is inhibited by the fibroblast growth factor 23 (FGF23) as well as by calcium, phosphate and $1,25\ (OH)_2D$ itself. The regulation of CYP24A1 occurs in the exact opposite way: it is being stimulated by $1,25\ (OH)_2D$, FGF23, calcium and phosphate and inhibited by PTH [16].

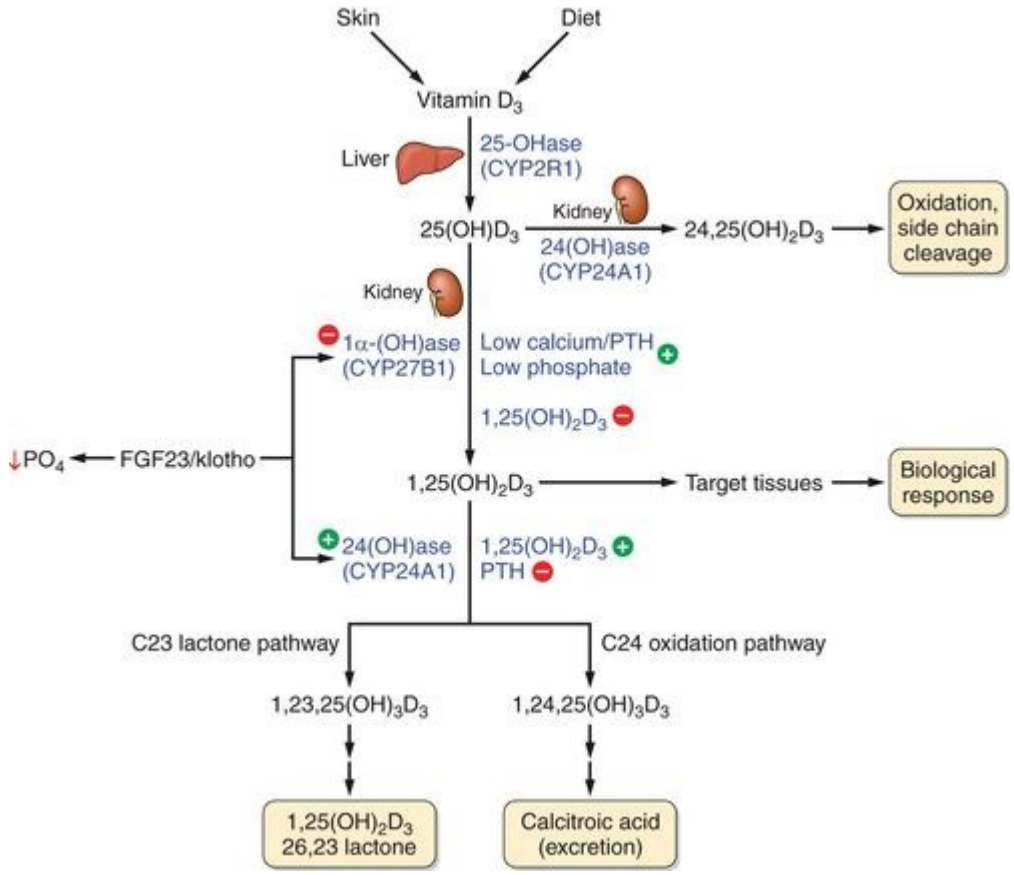


Figure 1:Metabolic pathway of vitamin D, [12]

Figure 1 Metabolic pathway of vitamin D displays the whole vitamin D metabolism step by step, starting with the vitamin D_3 production in the skin or intake throughout the diet, then converting into its first metabolite $25(OH)D_3$ in the liver. Further, $25(OH)D_3$ is transported by DBP to the kidneys and there converted either into $24,25(OH)_2D_3$ or into 1,25- dihydroxy vitamin D ($1,25(OH)_2D$) [12].

1.2 Sources of vitamin D

Vitamin D can be obtained mainly through exposure to sunlight. In this case type B UV (UVB) radiation with a wavelength of 290-320 nanometers plays the key role. UVB converts the 7-dehydrocholesterol to previtamin D_3, which afterwards becomes vitamin D_3.

The synthesis of vitamin D depends on season, time of the day, cloud cover, melanin content in the skin, clothing, and sun- screen. Melanin in the skin blocks UVB from reaching 7- Dehydrocholesterol which leads to restricted production of vitamin D3 [17], [18]. Older people, as well as people with dark skin, produce less vitamin D from sunlight [19].

Vitamin D can also be delivered by the diet. However, only a few foods are rich in vitamin D but are not widely consumed. Table 1 displays levels of vitamin D found in several food sources.

Food	Vitamin D content µg /100 g
Herring	7.80-25.00
Salmon	16.00
Egg yolk	5.60
Mackerel	4.00
Margarine	2.50-7.50
Champignons	1.90

Table 1: Vitamin D content of selected foods (according to Souci/Fachmann/Kraut, 2008),[20]

There are plenty of foods on the market that are fortified with vitamin D. However, a diet primarily consisting of food fortified with vitamin D is not recommended. The focus is on the body's own capability of forming vitamin D [20]. Vitamin D used for fortification is often D2 (ergocalciferol). D2 is produced by UVB light in plants and fungi (e.g., mushrooms).
The difference between D2 and D3 is in the double bond between C22 and C23 and the methyl group at C24 in the side chain as figure 2 shows below [16].

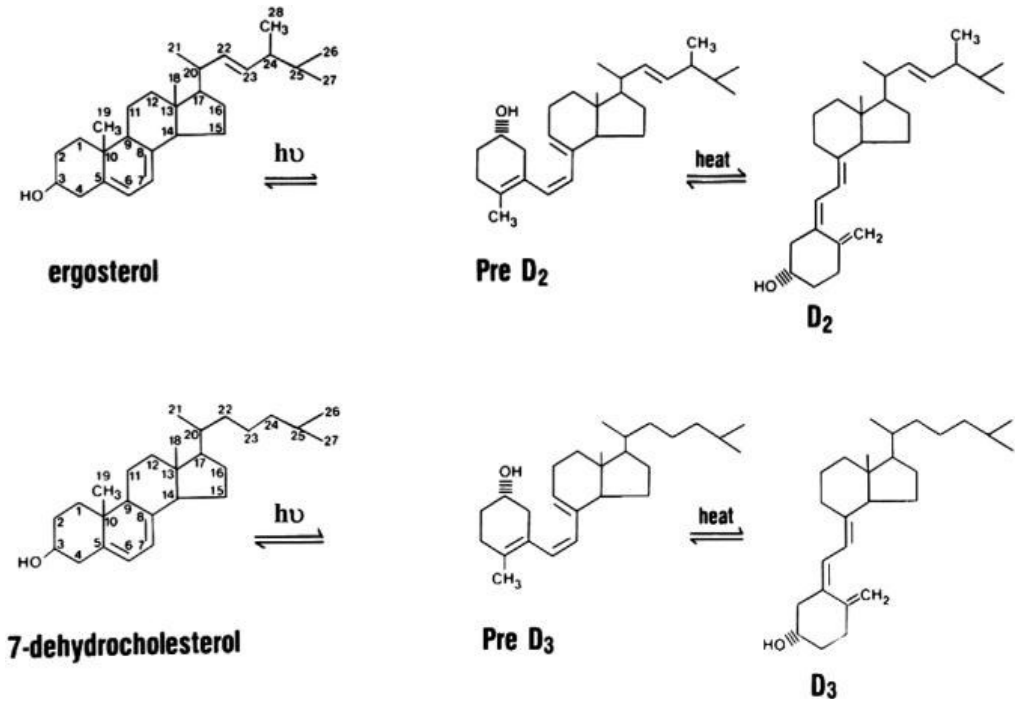


Figure 2: Production and Metabolism of Vitamin D2 and D3 [16]

Previously, it was considered that the 2 forms (vitamin D2 and D3) were equivalent and interchangeable, however recent studies have proven that D3 is the more powerful form of vitamin D [21]. The presence of the double bond of C22 and C23 and the methyl group in the side chain of vitamin D_2 is said to lower its affinity for the vitamin D binding protein (DBP) in plasma. This leads to a more rapid clearance from the blood circulation resulting in limited conversion into 25 hydroxyvitamin D (25OHD). On the other hand, vitamin D3 is shown to more efficiently raise the serum 25- hydroxyvitamin D [16].

The actions of the hormonally- active form of vitamin D $1,25(OH)_2D_3$ are mediated by the vitamin D receptor VDR, a nuclear receptor, to which vitamin D binds and thus controls gene expression [22]. VDR is part of the steroid receptor family which includes receptors for thyroid hormone, retinoic acid, adrenal steroids, and others [23].

Vitamin D is mainly stored in the fat and muscle tissue of the human body, but smaller amounts are also found in the liver. The overall storage capacity is relatively large and contributes to the vitamin D supply during the winter [20].

1.3 Function of vitamin D

The primary functions of $1,25(OH)_2D_3$ are the absorption of intestinal calcium and phosphorus and homeostasis. The hormonally active metabolite of vitamin D3 plays a crucial role in the stimulation of transcellular intestinal calcium transport by activating bone calcium mobilization and enhancing the renal reabsorption of calcium. Calcium and bone homeostasis are strongly linked, as bones are the major storage component of calcium in the body [11].

Vitamin D elevates the plasma calcium and phosphorus concentrations which leads to mineralization of the skeleton but also prevents hypocalcemic tetany [10].

Alongside calcium, prominent functions of vitamin D relate to the possible prevention of osteoporosis, high bone turnover, bone loss, and fracture incidence, especially among older individuals [24], [25]. Older subjects often tend to indicate lower levels of vitamin D levels due to lack of exposure to sunlight, as well as reduced mobility and physical activity. Another important factor is that the capacity of endogenous vitamin D synthesis tends to decrease with age because the kidneys are less able to synthesize $1,25(OH)_2 D_3$. This leads to a decline in the absorption of calcium and could potentially be the underlying cause of age-

related bone loss [12]. Consequently, this indicates that the supplementation of vitamin D may reduce the higher risk of bone fractures in older subjects [24].

The main causes of age-related increases in bone turnover are decreasing estrogen levels, declining physical activity and changes in calcium and vitamin D metabolism [3]. When there is an inadequate amount of vitamin D in the body, calcium absorption is also lower which leads to a compensatory increase in parathyroid hormone (PTH) levels. This stimulates a constant bone resorption which results in accelerated bone loss [2]. Vitamin D has been shown to play an important role in maintaining muscular strength. In older individuals, it has been proven that muscular weakness and decreased physical performance are related to lower levels of vitamin D [25].

Additionally, vitamin D is pivotal in influencing the growth and differentiation of a variety of tissues, monitors gene transcription, regulates the parathyroid gland and supports the immune system [4], [10].

1.4 Requirements and deficiency of vitamin D

1.4.1 Prevalence of vitamin D deficiency

Vitamin D deficiency is a very common issue worldwide that is primarily caused by inadequate exposure to sunlight as well as adequate absorption of vitamin D from the digestive tract. People who have a milk allergy or are lactose intolerant, as well as ovo-vegetarians and vegans, often have lower levels of vitamin D [18], [26].

It is considered that around one billion people globally are vitamin D deficient or insufficient, regardless of their origin and age [8]. Prevalence is particularly high among elderly people [27]. Vitamin D deficiency has been established as a 25(OH)D concentration of less than 20ng/ml., while vitamin D insufficiency has been established as a 25(OH)D of 21-29ng/ml [28]. Relative to these established findings, it is considered that 20-60% of the population in the U.S., Canada and Europe is vitamin D deficient [28], [29], [30] .

Furthermore, research has indicated that there is a strong correlation between vitamin D deficiency and several acute and chronic illnesses including preeclampsia, autoimmune disorders, cardiovascular disease, and type 2 diabetes [5]. The concentration of 25(OH)D in the blood serum is currently the most-established marker for the assessment of the vitamin

D supply [10], which could be measured either in ng/ml or nmol/l. One nmol/L = 0.4 ng/mL and 1 ng/mL = 2.5 nmol/L [27].

Stage	Serum 25(OH)D (ng/ml)	Serum 25(OH)D (nmol/l)
Vitamin D sufficiency	30-100	75-250
Vitamin D insufficiency	20.1-29.9	50-74.9
Vitamin D deficiency	<20	<50

Table 2: Vitamin D deficiency, insufficiency, sufficiency [31]

Table 2 provides definitions of vitamin D deficiency, insufficiency, and sufficiency according to the Endocrine Society's Clinical Practice Guidelines.

1.4.2 Recommendations and guidelines for vitamin D

Since food sources (Tab. 1) with vitamin D are typically consumed less often, the prominent source of vitamin D intake is via cutaneous synthesis. Consequently, the potential risk factors for long-term vitamin D deficiency rise with the decrease in opportunities for cutaneous synthesis [6].

The endogenous synthesis of vitamin D in the skin is very easily affected by multiple factors, including individual responsiveness, skin tone, exposure to sunlight, age, latitude, and several other determinants. This makes it difficult for the majority of organizations and specialists to provide specific guidelines on exactly how much sun exposure is needed for sufficient vitamin D synthesis [18]. However, it is recommended to spend approximately 5 to 25 minutes a day in the sun with major parts of the body uncovered and without sunscreen [7], [29], [32]. Supplementing vitamin D is only recommended if the daily vitamin D intake cannot be achieved either through the body's own vitamin D synthesis through exposure to sunlight or obtained from the diet [20]. However, with frequent exposure to the sun, the daily recommended vitamin D intake can be achieved without taking a supplement. For optimal bone health, most specialists agree that the ideal level of 25(OH)D is 75nmol/L (30 ng/mL) or higher [7]. The German, Austrian and Swiss (D-A-CH) reference values for nutrient intake recommend a daily vitamin D intake of 20 µg (800 IU) for adults in the absence of endogenous synthesis [33].

Meanwhile, the National Institute of Health (NIH) recommends adolescents and adults a daily vitamin D intake of 600 IU. Seniors over 70 are advised to take a higher dose of vitamin D starting from 800 IU [18]. The International Osteoporosis Foundation also suggests a vitamin D intake of 800-1000 IU for adults aged 50 and older every day [7].

Generally speaking, many countries around the globe and other health societies often have different guidelines for vitamin D intakes [32]. The Endocrine Society supports the thesis that adults might need around 1500 – 2000 IU vitamin D per day to maintain serum 25(OH)D levels above 75 nmol/L (30ng/mL)[28]. On the other hand, the United Kingdom government states that citizens aged 4 years and older should take 400 IU vitamin D per day [34].

Vitamin D could also be potentially toxic when given in large amounts. Its toxicity results in hypercalcemia since vitamin D is responsible for the calcium absorption in the gastrointestinal tract, as well as in hypercalciuria and high serum 25(OH)D levels [26].

However, vitamin D overdoses and potential undesirable effects are only possible through excessive oral intake, mainly from supplements, and not through excessive sun exposure of the skin [20], [26]. As reported by the National Institute of Health, the upper safe limit of vitamin D is 4,000 IU per day [18].

1.4.3 Vitamin D and DNA damage

It is widely known that vitamin D and its role acting as a steroid hormone helps maintaining bone and muscle health. However, more recent research suggests that vitamin D is not only important for the musculoskeletal system, but is also involved in immune response regulation, and the prevention of inflammation and autoimmunity, which can be factors for increased oxidative stress and DNA damage. Consequently, the maintenance of DNA integrity is a potential outcome of optimal vitamin D levels. This particular function of vitamin D fosters the prevention of DNA damage and regulation of cell growth rate [10].

Furthermore, vitamin D has been shown to reduce oxidative damage to DNA. There are several animal studies that reported a decrease in different markers for oxidative stress. A regiment with vitamin D is shown to prevent telomere shortening and inhibit telomerase activity. In general, vitamin D is also able to regulate the cell cycle to prevent the formation of damaged DNA and promote apoptosis [35]. Vitamin D in its active hormonal form

$1,25(OH)_2D_3$ is believed to be one of the main controllers of systemic inflammation, oxidative stress and subsequently the ageing process in humans. Data indicates that low levels of vitamin D damage mitochondrial functions which results in increased oxidative stress and systemic inflammation. Additionally, it is well proven that hypovitaminosis D could also accelerate a few age-related diseases, such as type 2 diabetes, hypertension, memory disorders, osteoporosis, colorectal cancer, and others [36].

However, there is no significant evidence to support the hypothesis that vitamin D can be directly linked to preventing DNA damage in humans. Nevertheless, there is some indication that vitamin D sufficiency leads to less oxidative stress and improves mitochondrial and endocrine functions and thus it reduces the risk for metabolic and endocrine problems, infections, and autoimmune disorders [36]. Vitamin D also seems to affect the mortality rate in healthy adults and adults in a stable phase of disease [37]. This, on the other hand, directly impacts the quality of life and overall health.

1.5 Calcium Metabolism and Function

Calcium is a nutrient closely related to bone metabolism and health. The majority of total body calcium is to be found in bones and teeth in the form of calcium hydroxyapatite [38]. However, calcium is also present as an intracellular messenger in cells and tissues throughout the body. Ionized calcium is in extracellular fluid, muscle, and other tissues, where it oversees vascular contraction and vasodilatation, muscle function, intracellular signaling, hormonal homeostasis and nerve transmission [26]. The approximate calcium content in the adult human body is 1 kg. Almost all the calcium is stored in the skeleton, which also serves as a reservoir of calcium for all the other vital calcium-functions throughout the body [39]. Calcium is primarily responsible for tissue rigidity, elasticity, and strength [26].

Calcium metabolism is closely connected to the parathyroid hormone (PTH)- vitamin D endocrine system. Calcium absorption can occur both transcellularly, through active transport, or paracellularly, through a passive diffusion. The active transport is tightly regulated by vitamin D and the intestinal vitamin D receptor (VDR). The transcellular transport takes place mainly in the duodenum where VDR is most concentrated.

The passive diffusion transports calcium between mucosal cells and takes place mainly in the presence of a higher calcium intake [26].

In order to maintain adequate levels of calcium in serum, calcium is released from bones. Vitamin D is important for calcium absorption in the body, therefore bone metabolism is highly affected by vitamin D deficiencies. This process increases the PTH secretion which releases the enzyme 1α-hydroxylase in the kidney. 1α-hydroxylase is responsible for the conversion of vitamin D to its active hormonal form $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ and induces the calcium absorption from the gut [26].

However, calcium absorption depends on multiple factors, such as bioavailability of the calcium source, digestibility and solubilization, as well as vitamin D intake [39]. Recent studies have suggested that the mean calcium absorption in men and non-pregnant women is approximately 25% of the total calcium intake [40]. It is believed that calcium absorption differs inversely with calcium intake when the intake is very low. Aging is another factor that slows down calcium absorption. There are plenty of reports of an inverse correlation between age and calcium absorption, especially in older women after menopause [41], [42].

Metabolic problems and conditions also exert a significant influence on calcium absorption. For instance, severe obesity is associated with higher calcium absorption [43] and energy restriction is related to decreased calcium absorption as weight loss is linked to bone loss [44].

1.5.1 Guidelines and Recommendations for Calcium

Calcium requirements and recommendations are different for each age group. However, the remainder of this work will be centered on the requirements and recommendations for adults aged 65 and older.

Adults over the age of 65 are most likely to experience risk fractures due to the loss of bone mass and density, as well as muscle loss associated with aging [45].

The meta-analysis by Tang et al. [46] indicated a lower bone density loss and a decreased risk of fracture when supplementing with 1,200 mg calcium per day, in combination with vitamin D. Another study demonstrated a similar outcome with a supplement of 1,200 mg calcium daily and an additional calcium intake of 900mg/day from the diet [47].

However, the mentioned studies above did not observe any dose-response correlation between the calcium intake and risk of fracture. Additionally, there is no clear proof that a

calcium intake of more than 1,000 mg provides any further benefit regarding overall bone health and homeostasis in the elderly [48]. Consequently, the reference value for calcium intake of German, Austrian and Swiss societies for adults aged 65 and older is 1.000 mg calcium per day [49]. Additionally, it has been indicated that the reference values of calcium intake require good vitamin D serum concentration. Particularly for the age group of 65 and up, it is recommended to supplement calcium together with vitamin D for obtaining optimal bone health [48].

1.6 The importance of weight training in the Elderly

Strength training has been shown to improve strength, range of motion and mobility of the skeletomuscular system. Additionally, weight training prevents the loss of muscle mass which may preserve bone density and reduce the risk of fractures and osteoporosis [50]. Weight training regimen for elderly individuals (>60 years) can increase muscle strength which leads to the reduction in the prevalence of sarcopenia (the loss of skeletal muscle mass and strength due to ageing) as well as bone fractures in older populations [51]. It is widely known that by having a more active lifestyle the number of fall episodes and their consequences are reduced [52]. After the age of 50 the prevalence of falls and injuries rises. Particularly endangered are seniors, aged 65 or more [53]. The latest research has shown that physical exercise has a positive impact on falls and fall-related effects, especially weight training seems to be verry efficient for maintaining adequate muscle mass in the elderly. Training programs differ regarding their intensity, number of sets and repetition as well as duration and weight. Therefore, exercise programs should be individually customized for each person, having in mind age, sex, and other health aspects [52].

Bone density and muscle strength decrease gradually from the 30th year. From the 60th year of life there is a rapid decrease of 15% observed and by the 8th decade of life this may be up to 30%. This reduction of muscle mass and strength leads to a loss in functionality and balance capacity. Meanwhile the risk of injuries and degenerative illnesses increases [54]. Clinical studies suggest that 20 to 30 minutes of weight training, 2 to 3 times per week, has a positive impact on risk factors for cardiovascular disorders, diabetes, and osteoporosis [55], [56]. Furthermore, resistance training shows a positive effect in maintaining functional strength and increasing muscle mass in the older population with pre-sarcopenia, by

increasing hypertrophy (muscle mass) on the one hand and promoting neuronal adaptation (inter- and intramuscular coordination) on the other hand [50], [57]

1.7 Weight training and its effect on DNA-Damage

Physical activity has shown to positive influence the quality of life by improving functional performance and meanwhile decreasing morbidity. Those changes vary with the intensity, time and type of training provided [58].

However, it is widely known that exercise may also increase oxidative stress and oxidative damage to DNA by enhancing the production of reactive oxygen species. ROS on the other hand can possibly connect and react with organic structures, resulting in changes in the cell's structure and functions [59]. The main damage ROS can do to DNA is predominantly single-strand breaks and modified bases [60]. Despite that, trials have reported an overall increase in the antioxidant activity of cells and a significant decrease in DNA strand breaks after completing physical exercise training. Lipid peroxidation levels were also lower after physical exercise training. In addition to that cell adaptation and general improved resistance to stress were reported. Taking those aspects into consideration it could be stated that exercise may decrease DNA damage and thus lower the risk of developing cell mutations [61], [62].

The study of Soares et al. [62] also illustrates a similar finding that DNA damage decreases after 16 weeks of combined physical exercise training (aerobic and strength training). The results indicated a significant decrease in single breaks and FPG-sensitive sites. This might be because of a decrease in ROS production and/or an increase in antioxidant protection.

This hypothesis was also tested in trials in different animal tissues and cells [9], [63].

The research indicates that regular physical activity causes low-to-moderate ROS production, which could be beneficial since it enhances the overall antioxidant capacity of the cell [62]. Another phenomenon, connected to DNA damage and limiting the quality of life during aging is telomere dysfunction. Telomere uncapping, a structural change at the end of the telomere that leads to its progressive shortening, occurs in all the tissues during aging and could enhance the rate of cell turnover[64]. These findings raise the importance of regular physical activity in the prevention of DNA damage which is linked to aging and some diseases that occur with aging such as: diabetes mellitus [65], cardiovascular diseases [66], and cataracts [67].

1.8 Cellular oxidative stress and free radicals

Oxidative stress is one of the most researched topics lately. It is believed that oxidative stress is closely connected with various human diseases. Because our body produces and releases reactive oxygen species all the time, our organism has developed an antioxidant system and defense to prevent the free radicals from damaging biomolecules such as lipids, proteins, and nucleic acids. Normally those two processes (production of reactive oxygen species: antioxidant defense) balance each other. However, one speaks of "oxidative stress" when the balance is upset, either by an overproduction of free radicals or by a deficiency in the antioxidant system [68].

Considerable evidence implies that free radicals take part in numerous biological processes. Those are molecules that carry an unpaired electron in their outer orbit, which results in high reactivity. Because they are highly unstable, they interact very quickly with non-radical substances. Free radicals are often employed with the term "reactive species" (RS). Reactive oxygen species (ROS) are highly reactive molecules, formed from O_2. The most common ROS are superoxide radicals, hydrogen peroxide and the hydroxyl radical. Mitochondria are believed to be one of the main intracellular sources of ROS in our body because the oxygen metabolism occurs there [69]. The second major source of ROS is thought to be the ischaemia reperfusion phenomenon because of medical interventions, shocks or during excessive physical exercise [70]. When hemoglobin is oxidized, which occurs mainly when physical activity is happening, it produces free radicals (Methemoglobin and $O_2^{\cdot -}$). Other reasons of the production of ROS during exercise are the increased body temperature, catecholamine and lactic acid which could convert $O_2^{\cdot -}$ into hydroxyl radicals$^{\cdot}$ [71], [72]

The presence of ROS and especially their instability and high reactivity is thought to contribute to many different processes in our body such as lipid peroxidation and protein degradation. This interaction leads further to DNA-strand breaks which results in damaging the cell and its homeostatic environment [69], [73]. As a result ROS can stimulate the process of apoptosis into healthy cells and induce inflammation. All those factors can further influence the process of cell aging and promote the pathology of some diseases such as cataract, cancers, and others [74].

To limit those harmful consequences, the human body has developed a complex protection system with the help of numerous antioxidants. This includes, among others, antioxidant

enzymes (catalase, superoxide dismutase or glutathione peroxidase) and non-enzymatic antioxidants (vitamin E, A, C, glutathione etc.) [71] .

Oxidative stress is defined as a disruption in the balance between the presence of free radicals and antioxidants in the body. This imbalance is thought to contribute further to the production of tissue harm and thus DNA damage [75]. DNA strand breaks and base repair damage are the two most common phenomena that are negatively influenced by ROS.

Recent research has indicated a strong correlation between oxidative stress and inflammation which could potentially play an important role in the progression of some diseases. For instance, inflammatory macrophages produce glutathionylated peroxiredoxin-2, which can activate the production of tumor necrosis factor- alpha [76]. However oxidative stress could possibly activate other transcription factors as well, such as NF-kB or PPAR-y. Those factors may express more than 500 different genes, including those for growth factors, inflammatory cytokines, or cell cycle regulatory molecules [77].

1.8.1 Oxidative stress and training

Oxidative stress is strongly related to physical activity since it is well known that physical exercise induces oxidative stress in the body by enhancing mitochondrial superoxide production and the production of reactive oxygen species (ROS) in general [78]. Free radicals, on the other hand, react with different cellular components such as proteins, polyunsaturated fatty acids, and cell membranes and thus it might lead to damage the cell [79]. Enhanced oxidative stress during or after physical activity is usually assessed by measuring lipid - and DNA damage as well as assessing the antioxidant redox status by evaluating glutathione levels [78]. However, whether physical activity affects oxidative stress positively or negatively, depends on the training load, intensity, and quantity of training. Studies have indicated that exhaustive and very intense physical activity enhances the toxicity of free radicals in the body, which may result in the promotion and development of injuries, chronic fatigue, and diseases [71]. The argument that physical activity increases the production of ROS is provided mainly by studies with very intensive training periods. It has been proven that overtraining could lead to enhancing the ROS and thus oxidative stress which could possibly result in training fatigue and injuries [80].

Even though ROS, produced during physical exercise, might play a crucial role in muscular fatigue, aging process, and some diseases, it has been proven that free radicals have some positive benefits on our organism, especially on the immune system and metabolic functions. Atabek et al. [79] has also shown in his study that Malondialdehyde (MDA), a product of lipid peroxidation, used as a quantitative marker for oxidative stress in this study, has significantly decreased after strength training. Another study from Peters et al. [81] showed that 6 weeks of exercise training led to a significant decrease in oxidative stress markers and meanwhile the ratio of blood GSH/oxidized GSH has improved. Furthermore, it should be stated that free radicals are constantly produced and play an important role in our body and, as such, they are a key role in the organism's natural immune system [72].

Considerable evidence implies that physical activity leads not only to an improved lipoprotein profile, but also to enhanced antioxidant enzyme activity [82]. Furthermore, it could be stated that moderate physical activity has mainly positive effects on oxidative stress by increasing the capability and efficacy of the antioxidant system [71], while the negative impact of training on oxidative stress is seen mainly within very intense exercise [83].

2. Materials and Methods

2.1 Experimental Design

In the present thesis, the effect of vitamin D and calcium supplementation in combination with strength training was examined on DNA damage in the elderly population. The samples and results were obtained as part of the "Nutri Aging" Study.

The "Nutri Aging" study was a randomized placebo-controlled double blinded trial that was conducted between January and July of 2019. The study started with a screening period in January 2019, assessing the inclusion and exclusion criteria (t0). The subjects were randomly assigned to the following groups: control group (CON), the vitamin D_3 daily group (VDD) and the vitamin D_3 monthly group (VDM).

The study period consisted of two phases: a four- week vitamin D_3 supplementing phase followed by a ten-week combined strength training and supplementation phase.

	4 weeks	10 weeks
Group 1 (CON)	• Calcium daily 400mg	• Calcium daily 400mg • Strength training 2x per week
Group 2 (VDD)	• Vit.D 800 I.E. daily • Calcium daily 400 mg	• Vit. D daily 800 I.E • Calcium daily 400 mg • Strength training 2x per week
Group 3 (VDM)	• Vit D. (50.000 I.E.) 1x monthly • Calcium daily	• Vit. D (50.000 I.E.) 1x monthly • Calcium daily • Strength training 2x per week

Table 3: Interventional groups

Blood samples were collected prior to the main intervention (T1), after the loading phase (T2) and after the supplementation + exercise phase (T3) [84].

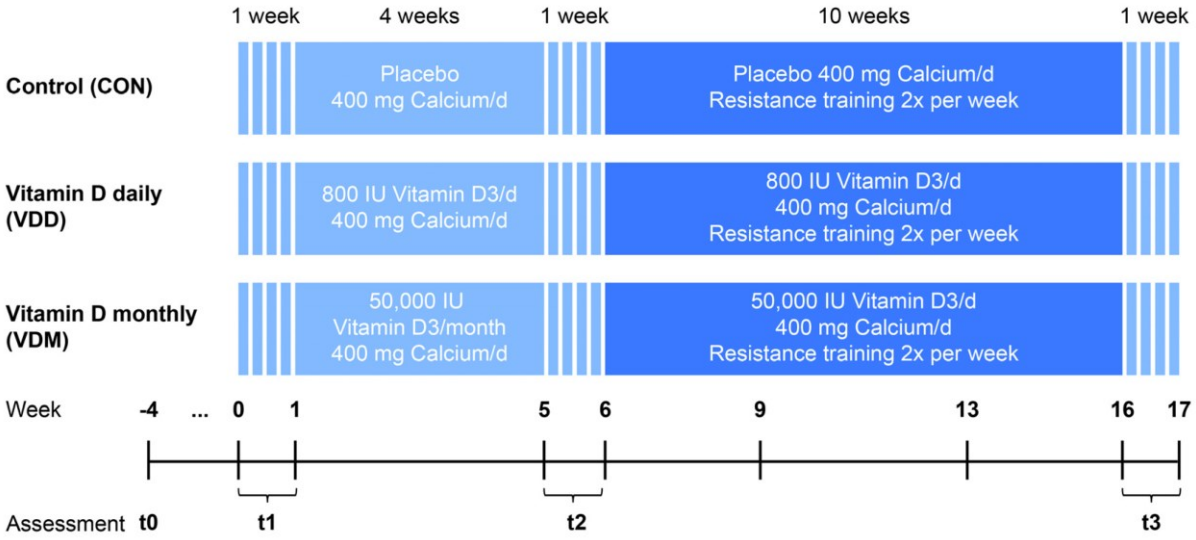


Figure 3: Study timeline [94]

2.2 Recruitment of participants

Participants were recruited via newspaper, radio, and online advertisement. Each potential participant must have met all the following criteria to be enrolled in the study:

1. Age between 65 and 85 years.
2. Having an independent lifestyle (not living in a retirement home and not receiving aid by a home carer).
3. MMSE (Mini Mental State Examination) Score > 23.
4. Signing an informed consent form.

Any potential participant who had met any of the following criteria would have been excluded from participation in the study:

1. 25(OH)D level > 30 ng/mL at screening (t0).
2. The need for walking aid.
3. Chronic conditions that would oppose sports training therapy.
4. Medical conditions such as:
    - severe cardiovascular disease (decompensated chronic cardiac insufficiency, extreme or symptomatic aortic stenosis, unstable angina pectoris, untreated arterial hypertension, cardiac arrhythmia); diabetic retinopathy; osteoporosis or osteopenia with Vitamin D and/or calcium substitution; kidney disease; kidney stones; disorder of the parathormone level; intake of cardiac glycosides; intake of diuretics; disturbance of the calcium levels; regular intake of cortisone or antibiotics (last six months)
5. Regular resistance exercise (> 1x/week) in the last six months before study inclusion

2.3 Interventions

2.3.1 Nutritional Interventions

Participants were assigned to either the control group (CON), vitamin D daily group (VDD) or vitamin D monthly group (VDM).

The control group received a supplementation with calcium only (400mg/daily). The VDD group is supplemented with 400 mg of calcium and 800 IU of vitamin D3 daily. The VDM

group took 400 mg calcium daily plus once every four weeks a dose of 50, 000 IU vitamin D3. The monthly dose of vitamin D3 was dispensed in the laboratory during the assessments every four weeks (at T1, T2, weeks 9 and 13). The rest of the supplements were dispensed to the participants in boxes. Participants were instructed to take the supplements always at the same time of the day, with a meal [84].

2.3.2 Weight training intervention

The training program was conducted as per guidelines of the American College of Sports Medicine and the American Heart Association [85]. The strength training program was completed over a period of 10 weeks in a total of 20 training sessions of 60 to 90 minutes.
The training took place twice a week on two non-consecutive days (at least 48 hours apart) under the guidance of qualified fitness trainers. The training sessions were conducted in selected fitness centers in Vienna. During the first two weeks the training was conducted at a lower intensity in order for the participants to get familiar with the exercises. The intensity increased gradually in the following 8 weeks. Participants trained in small groups (up to five) and were guided carefully by a qualified trainer. Each training session started with a five-minute warm-up on a cross trainer or a bicycle and then followed by mobility exercises, focusing on joint movement.
For the purpose of this Master thesis the following physical functional parameters were included in the thesis and later on in the statistics: 6-minute walking test and 30-second Arm curl dominate hand.

2.3.2.1 6-Minute Walking Test

The 6-Minute Walking Test was performed to measure the aerobic endurance of the study participants. The examination site was a 30m marked route on which the test subjects had to walk back and forth as quickly as possible in six minutes to be able to cover as long a distance as possible. The number of meters they managed during this period was recorded [84], [86].

2.3.2.2 30-second Arm curl dominate hand

For the 30-seconds Arm Curl test men used a 3.6 kg dumbbell, while women used a 2.3 kg dumbbell. Participants were instructed to practice the test without any additional weight to get comfortable. During the test, they were encouraged to perform as many proper arm curls as possible in 30 seconds while seated. The total number of correctly completed repetitions was recorded for further analysis [84], [86] .

2.4 Definition and Background of Comet Assay

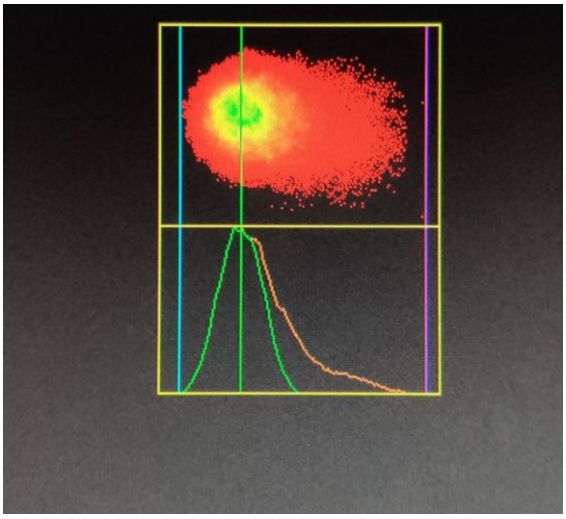
Comet assay is also known as a single gel electrophoresis and is believed to be one of the most common methods to detect DNA damage in eukaryotic cells and thus to measure DNA repair activity. DNA repair activity is a very important factor and marker for the vulnerability of the cells towards mutation, cancer, and many diseases.

Furthermore, comet assay counts as one of the most frequently used in vitro methods in genotoxicology and DNA damage studies. The method may be applied in many different areas, including environmental monitoring, human biomonitoring, early drug-research selection etc. [87], [88].

Even though several cell types have been used over the years, for example nasal and lens epithelial cells, whole blood leukocytes and peripheral blood lymphocytes (PBL) are the most common types of cells used for the performance of comet assay. In the past years the use of whole blood has been implemented more frequently because the procedure with whole blood requires a smaller sample volume (20 µl) and takes less time, thus it appears to reduce some cell damage that occurs during the isolation process of the lymphocytes [89]. Nevertheless, PBLs are still the preferable type of cells because of their longer lifespan and due to the fact that they circulate throughout the whole body [90].

The Comet Assay is a very simple and sensitive method, in which agarose-based cell suspensions are embedded and then lysed and run through electrophoresis under neutral or alkaline conditions. The concept of the assay is that damaged DNA migrates from the nucleoid body (“the comet head”) under the influence of an electric field and forms a DNA tail (the “comet tail”). After the damage migrates, the intact DNA remains in the comet’s head, while the intensity and length of the comet’s tail reflects the extent of the DNA damage. The resulting image is reminiscent of a “comet” with a head and tail, as shown in

Figure 3 below. Calculating tail lengths and intensity could be useful for comparing DNA damage between different experimental groups.



The comet metaphor has its origin in astronomy where the “comet” represents the head, which is composed of intact DNA, while the tail is made of damaged (single-strand or double-strand breaks) DNA [88].

Figure 4: Comet head and comet tail [Varcheva,2021, unpublished]

The first attempt for comet assay was made in 1976 by Cook et al. [91] where they lysed the cells with nonionic detergent and sodium chloride. As a result of this treatment all the membranes, cytoplasm, and other cell components including some of the histones were solubilized by the salt and only the nucleotide was left intact, composed of RNA, proteins, and DNA. With this assay, Cook et al. concluded that structures that look like nuclei but are also low in protein may be released by lysing cells in non-ionic detergents and high salt-solutions. However, one of the most important conclusions from this experiment was that the salt concentration highly affects the protein content of the nucleoids. High-molarity sodium chloride removes almost all the histones.

Later, in 1984 two Swedish researchers Östling and Johanson developed the comet assay based on the steps and methods described above. In their attempt, they placed gel cells already embedded in agarose on a microscope slide, which then underwent a high salt treatment with lysis. An ionized radiation in an electric field with a pH of 9.5-10 was used to detect DNA breaks. The staining of the cells was performed with acridine orange. Because the image, obtained under the microscope, looked like a “comet”, the name comet assay was given. Nevertheless, with their experiment, only double-strand breaks were formed and detected due to using a neutral pH solution [92].

A few years later, the method was modified by Singh et al. [93] by increasing the pH of the electrophoresis to above 13 and that is the comet assay which is widely used today. Performing the Comet Assay in alkaline conditions has been shown to significantly increase the specificity and sensitivity of the assay. The alkaline comet assay detects not only double-strand breaks but also single-strand breaks and alkali-labile sites.

Double-strand breaks (DSBs) seem to be easier to detect as they result in DNA fragments, which can be formed at neutral pH. Single-strand breaks (SSBs), however, appear only after the two strands of the DNA are denatured or the DNA helix is unwound. For this purpose, a pH of at least 12 is needed. Single-strand breaks are most often induced either by treatment with H_2O_2 or by exposure to X, γ- rays and oxidized purines [93], [94].

Comet assay is not only a standard method for assessing DNA damage and for evaluating the DNA repair ability of cells but also for testing novel chemicals for genotoxicity, human biomonitoring, and molecular epidemiology [88]. This method is widely used and enjoys great popularity among researchers because it offers many advantages, for instance- it can be applied in eukaryotic and prokaryotic cells, it is highly sensitive (detects between 50 and 15 000 breaks per cell on average, results are available on the same day, fresh or frozen samples can be used, and it is a non-invasive technique) [94].

The comet assay is often considered as the “gold standard” for assessing and measuring single and double-strand DNA breaks at the cellular level. Even though comet analysis is a very simple method, there are many different steps in the protocol and some critical points where one should be particularly careful. The preparation of the reagents is the basis for the quality of the method. Therefore, each solution should be freshly prepared and kept at the appropriate temperature in advance.

2.5 Methodology of the Comet Assay

Figure 4 represents the steps and the methodology of the method Comet Assay.

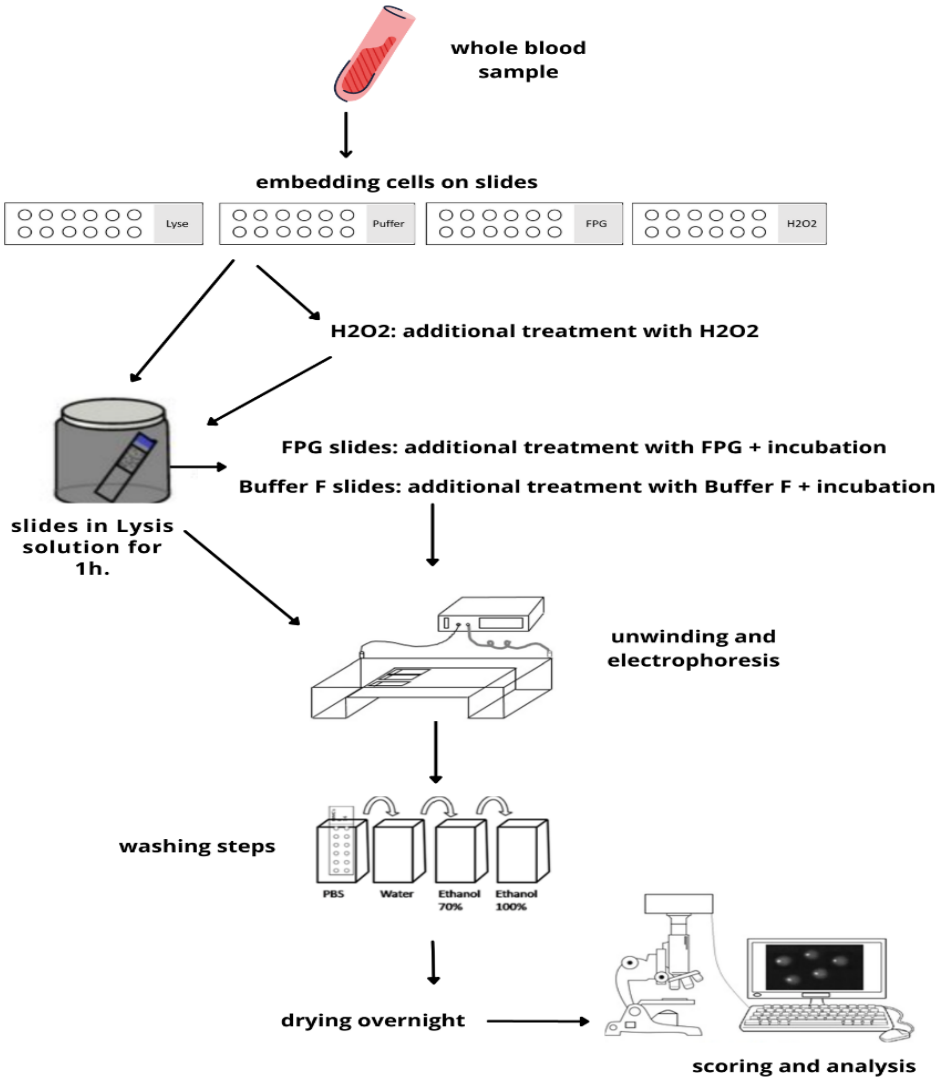


Figure 5: How to perform Comet Assay [Varcheva,2021, unpublished], illustrated with Canva

Initially, the suspension of cells is mixed with agarose to form a gel on a microscope slide. All slides undergo a one-hour treatment with lysis, which is mixed with a detergent (Triton X-100) and NaCl >2M to remove cell and nuclear membranes as well as histones from the DNA. Some of the slides undergo an additional treatment with H_2O_2, FPG or Buffer F. Electrophoresis is needed for the DNA to wander towards the anode. Different protocols have different requirements but most often an electrophoresis is used at voltage of 25V and

most commonly 300 mA, which correlates directly to the volume of the electrophoresis buffer.

2.6 Reagents for the performance of comet assay

2.6.1 Solution for coating the slides

1g ultra-pure agarose is dissolved in 100 ml distilled water in the microwave until the solution becomes clear. The solution is then transferred to two small 50 ml beakers and placed in a water bath at 60 °C. Slides are slowly immersed into the fluid; the bottom of the slides is wiped, and then they are left drying horizontally overnight. The slides are then stored in the fridge until use.

2.6.2 Agarose solution for gel spots

160 mg of low melting point agarose was dissolved in 20 ml PBS in 50 ml volumetric flasks in the microwave. The solution must be clear, and the agarose must be completely dissolved before transferring it and aliquoting it into small bottles. The aliquots were stored in the refrigerator until use. Before using them, the aliquots were heated in the microwave until they melted and then held into the water bath at 37 °C. 5 µl whole blood was mixed thoroughly with 120 µl of LMP agarose and applied to a slide. The slides were then dried on the surface until the gel was stable and then incubated in the lysis at 4 °C for one hour.

2.6.3 Lysis

The lysis is used to show the simple strand breaks or also the basis damage of the DNA. The lysis treatment impairs the DNA the least with an average tail damage of 3%. The lysis solution in this protocol is prepared weekly with a pH of 10 with the following reagents and is held in the fridge until use:

	1L
2.5NaCl	146,1 g
0.1M EDTA	29,2 g
10mM Tris	1,211g
NaOH	8g

Table 4: Lysis reagents

NaCl, EDTA, Tris and NaOH are mixed with distilled water with a magnetic stirrer in a 1000ml beaker until a clear solution is obtained. Then the pH is assessed by adding 10M NaOH dropwise until the pH is adjusted at 10 (+/- 0.01). The fluid was then transferred to a 1000 ml volumetric flask, and the rest was filled with distilled water up to the mark. The lysis is stored in the refrigerator until use. Shortly before using the lysis solution, 200ml lysis is mixed with 2ml Triton X 100 in a 400 ml beaker, using a magnetic stirrer.
The lysis incubation completely removes the cytoplasmic and nuclear membrane so that the nuclear DNA is free in the agarose and not attached by any cellular components.

2.6.4 H_2O_2 – stock solution (0.1M)

10 ml of distilled water was measured with a 10 ml- pipette in a tube. 103 µl from the water were withdrawn with a pipette and discarded. Then 103 µl H_2O_2 (stored in the refrigerator in the dark) were added and mixed well. This solution could be prepared once per week and held in the fridge until use. However, to ensure good quality of the method, the stock solution was prepared daily.

2.6.5 H_2O_2 – solution (100 µM)

200 ml distilled water was filled into a wide necklace with a lid. 100 µl H_2O were withdrawn and discarded. 100 µl H_2O_2 stock solution were then added with a pipette and mixed well. This solution was freshly prepared daily before each test.
The H_2O_2 slides underwent an additional treatment with 100 µM H_2O_2, incubated for 15 minutes, before they were incubated for an hour in the lysis solution.
H_2O_2 represents the strongest treatment and leads to the most powerful damage of DNA. H_2O_2 is commonly applied in comet assay as this allows for assessing the antioxidant status of the cells by testing their resistance towards H_2O_2. When cells undergo treatment with H_2O_2 the main outcome is the strand breaks, although some bases are also being oxidized. Challenged cells with H_2O_2 show strand breaks which depend on the individual level of antioxidant defenses in the cells [68].

2.6.6 FPG and Buffer F

After an incubation period of 1 hour in the lysis at 4 °C, some of the slides underwent an additional treatment with Buffer F and FPG.

FPG, short for Formamidopyrimidine-DNA-Glycosylase, is a bacterial repair enzyme, used for the detection of oxidized bases from damaged DNA. Certain repair enzymes uncover base modifications that do not lead to the increase of DNA migration in the standard comet assay. FPG removes oxidized and alkylated purine bases. This leads to an additional denaturation of the DNA. The FPG-treated slides were examined in comparison with only enzyme buffer F-treated slides [95]. Throughout this comparison, a statement about the amount of existing base modifications can be formed.

2.6.7 Aliquoting FPG

40 µl FPG (FPG - stock for preparing the aliquots) and 360 µl buffer F were mixed.

Aliquots of 5 µl each were added into 1.5 ml tubes and frozen at -20 °C.

2.6.8 Buffer F- stock Solution

The Buffer F stock solution, pH 8, was prepared at the beginning of the study with the following detergents:

	2L
40 mM HEPES	190,6 g
0,1 M KCL	149,12 g
0,5 mM EDTA	2,92 g
0,2 mg/ml BSA	4g

Table 5: Buffer F stock solution reagents

HEPES, KCL and EDTA were mixed with distilled water with a magnetic stirrer in a 2000ml beaker until a clear solution is obtained. BSA was added and the pH value was adjusted to exactly 8 (+/- 0,01) with 4M KOH. The fluid was transferred into a 2000 ml volumetric mark

and filed up to the mark with distilled water. At the end the solution was aliquoted in tubes, each 45ml and frozen and held at - 20 °C until use.

2.6.9 Buffer F- working solution

The stock solution (45ml), pH 8, was being thawed in a water bath at 37 °C. Then it was transferred to a 500 ml beaker, filled with more distilled water and the pH was adjusted to exactly 8 (+/-0.03) with 1M KOH. Afterwards it was transferred into a 500 ml volumetric flask and filled with distilled water to the 500 ml mark. It was stored in the refrigerator until use.

2.6.10 Electrophoresis solution

The electrophoresis solution, with a pH 13, was prepared daily at the beginning of the trial with the following detergents:

0.5L	2L
6g NaOH (0.3M)	24g NaOH (0.3M)
0.145g EDTA (0.001M)	0.58g EDTA (0.001M)

Tab 6: Electrophoresis solution reagents

NaOH was dissolved with the corresponding amount of EDTA in a matching beaker with distilled water and a magnetic stirrer. Then transferred to either a 500 ml or 2L measuring cylinder (depending on the amount) and filled up to the mark with distilled water. Both solutions were stored in the refrigerator.
The electrophoresis was performed for 30 minutes with a 25V and a power between 300-350mA.

2.6.11 Neutralization

Following the electrophoresis, the slides underwent three neutralization steps, each lasting for 5 minutes, starting with distilled H_20, following with 70% Ethanol and then 100%

ethanol. The slides were then kept in special containers in the fridge, ready for evaluation and counting.

2.6.12 Gel Red

For the preparation of Gel Red for counting 3 µl of Gel Red were mixed in 10 ml of distilled water in a tube and refrigerated until use. Gel Red was prepared on a weekly basis.

2.6.13 Counting of cells

The counting process was performed in a dark room with only artificial light sources permitted.
The fluorescent dye Gel Red was pipetted onto the gel spots, and these were covered with a glass and examined under the fluorescence microscope.
The DNA fragments are shown under the fluorescence microscope as a diffuse tail behind the nucleus also known as “comet”, where the name of the method comes from. The undamaged DNA stays in the Comet’s head and the impaired DNA fragments move into the Comet’s tail.

Per subject 2 gel spots (50 cells per gel point) were counted because it is established that an overall count of 100 cells is necessary to obtain the overall damage of a cell.
The mean value of the damage from the two gels is calculated and exported by the COMET IV program to an Excel sheet for further evaluation and comparison of the values.

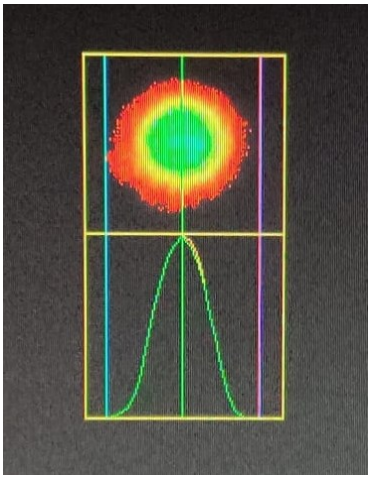


Figure 6: Lysis treatment [Varcheva,2021, unpublished]

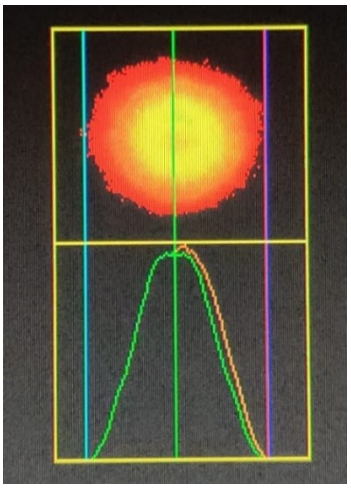


Figure 7:Buffer F treatment [Varcheva,2021, unpublished]

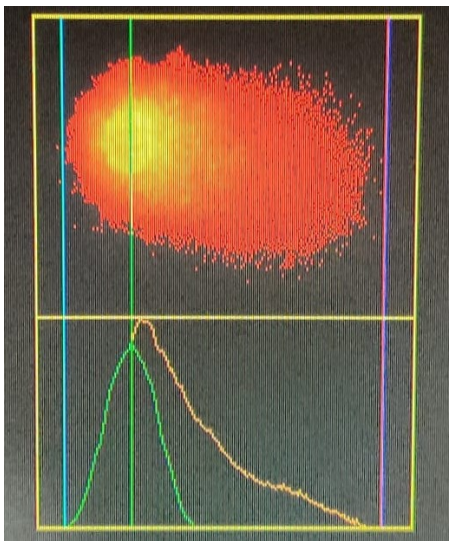


Figure 9: H_2O_2 Treatment [Varcheva 2021, unpublished]

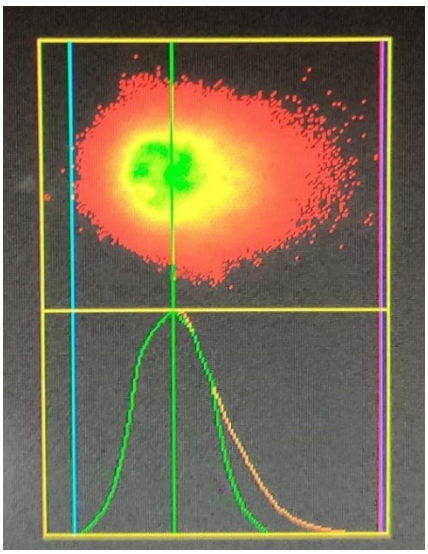


Figure 8: FPG treatment [Varcheva, 2021, unpublished]

2.7 Statistical analysis

SPSS for Windows was used for the statistical evaluation of the data. The
statistical analysis started with a test for normality. The Kolmogorov-Smirnov-Test was performed for checking the normal distribution of the most important variables. Since the data turned out to not be normally distributed, non-parametric tests were used for further evaluation.

Nonparametric tests for related samples were applied to evaluate changes between different interventional groups. A Mauchly-Test was used for testing sphericity of samples, thus either greenhouse-Geisser or Huyn-Feldt-corrected values were further used. The Levene test was applied to confirm whether variances are homogeneous and equal.

Furthermore, the test of within subject effects between different groups and variables was utilized to examine interaction effects. Post-hoc analysis was carried out for the purpose of determining which groups exactly differ from each other.

Last but not least a Spearman correlation was used for variables, which are not normally distributed, and Pearson correlations were applied for all normally distributed variables.

$P < 0.05$ was considered a statistically significant.

2.8 Results

2.8.1 Gender Distribution

In this interventional study 231 participants had interest at the baseline, but data was obtained only from 101 subjects. 33 women and 68 men were selected to start the trial.

2.8.2 Distribution of the groups

Overall, 378 people were interested in the trial, however, 231 fulfilled the eligibility criteria and only 101 proceeded further with screening and 100 were included.

Age groups	**Gender**	**Interventional groups**			
65-69.9 years		Control	VDD	VDM	In total
	Women	6	5	6	17
	men	14	12	13	39
	In total	20	17	19	**56**
70-74.9 years	Women	2	3	3	8
	men	5	4	7	16
	In total	7	7	10	**24**
75-79.9 years	Women	2	2	2	6
	men	3	4	3	10
	In total	5	6	5	**16**
80-85 years	Women	0	0	2	2
	Men	1	0	1	2
	In total	1	0	3	**4**
In total	Women	10	10	13	33
	Men	23	20	24	67
	In total	33	30	37	100

Table 7: Distribution of subjects according to age, gender, and intervention

Table 7 shows the distribution of subjects according to their age, gender, and interventional group. Participants were between the ages of 65 and 85, with a mean age of 70.63 ± 4,53 years. Almost half of the participants, 55.4% or 56 participants in total, were between the age of 65 and 70 years. The second largest category was 70-75 years of age, of which were 24 (25%) of the subjects. 16 participants (15.8%) were in the age group between 75-79.9 and only 4 (3.96%) were between the age of 80 and 85 years.

Blood samples were taken at the beginning of the main intervention (T1), after the supplementation-only phase (T2) and after the exercise plus supplementation phase (T3).
The distribution of the groups was not exactly even with 37 participants (24 male and 13 female) (37%) allocated to the monthly group, 30 (20 male and 10 female) (30%) to the vitamin D daily group and 33 (23 male and 10 female) (33%) in the control group.

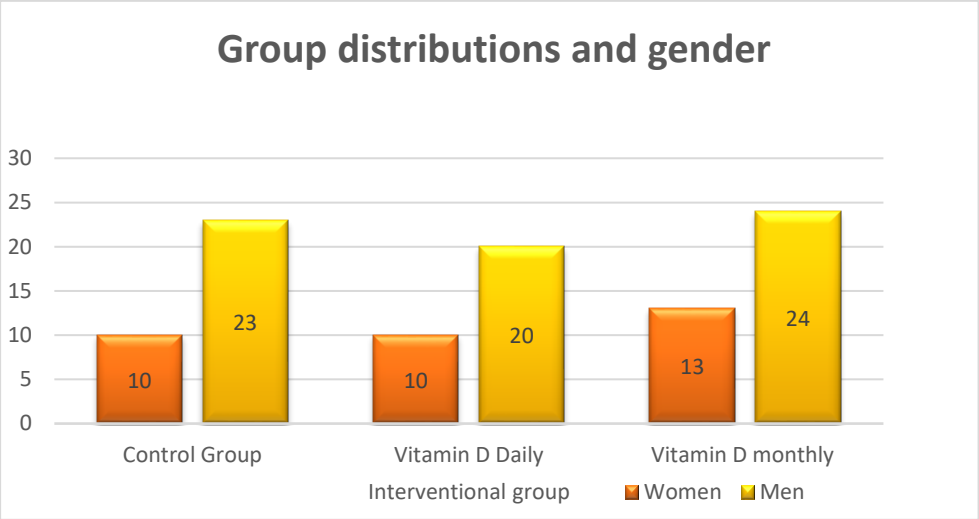


Figure 10: Clustered bar count of intervention groups divided in sex.

As shown in figure 10, interventional groups were not evenly distributed between both sexes. In all three interventional groups the majority of participants were male.
Overall, there were 16 participants who dropped out during the study.
One participant withdrew his consent prior to T1. Furthermore, three participants dropped out during the first phase of the study due to loss of interest in the study. During the second phase (supplementation and training phase) another 12 participants quit the study due to medical issues. Overall, 85 subjects successfully finished the trial.

2.8.3 Vitamin D plasma levels throughout the study (T1-T3)

Furthermore, it was tested whether the vitamin D plasma levels of the subjects in the different groups differs during the interventions and throughout the study. Supplementation of vitamin D, 50.000 IU monthly in the monthly group, and 800 IU daily in the daily group, started after T1 assessment. At the time of T1 12 of the subjects had a deficiency of vitamin D, 34 were insufficient and 39 subjects started the trial with vitamin D sufficiency.

There was a significant difference between vitamin D plasma concentrations during T1 between groups ($p<0.001$).

Table 8 illustrates the mean vitamin D concentration in ng/mL for each group at T1, T2 and T3.

	Intervention group	Mean	SD	N
Vitamin D plasma level T1	CON	21.49	5.77	27
	VDD	23.10	7.10	27
	VDM	23.34	8.20	31
	overall	22.66	7.11	85
Vitamin D plasma level T2	CON	22.13	5.16	27
	VDD	24.44	5.67	27
	VDM	24.68	5.36	31
	overall	23.76	5.46	85
Vitamin D plasma level T3	CON	24.89	9.64	27
	VDD	27.85	6.78	27
	VDM	31.91	7.30	31
	overall	28.40	8.40	85

Table 8: Vitamin D Plasma level in intervention groups throughout the study

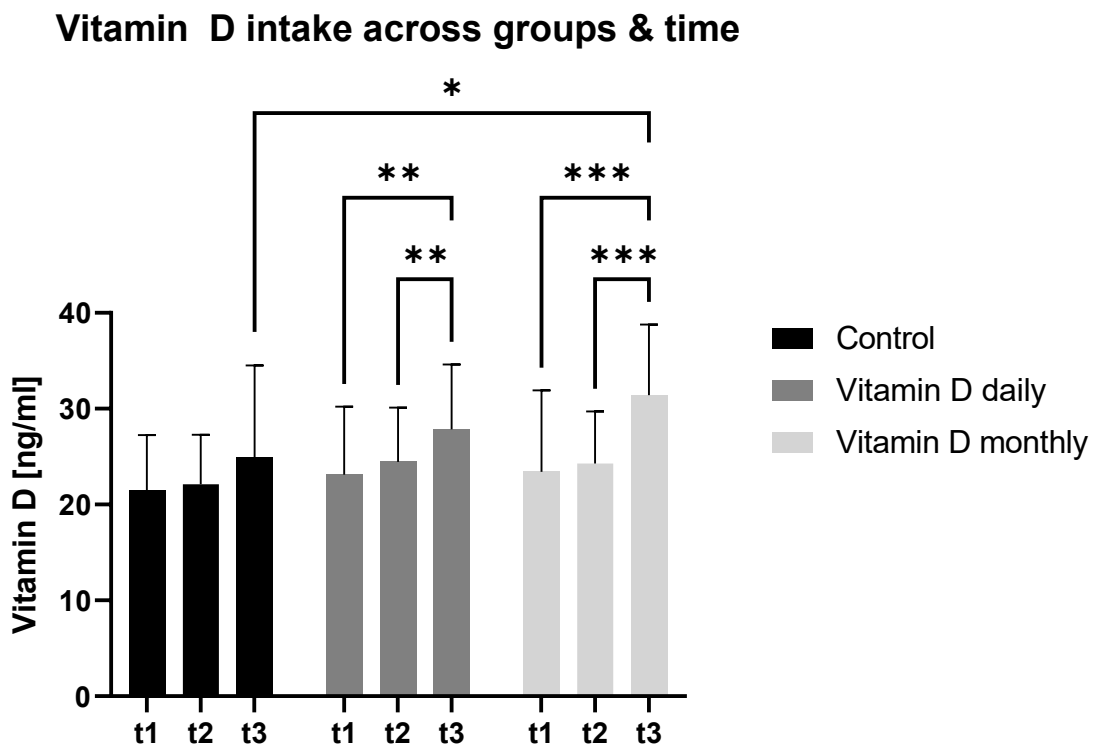


Figure 11: Plasma Vitamin D Plasma level throughout the study.

A mixed ANOVA with time and group effects showed that there was no significant change in the vitamin D plasma levels in the control group while the intervention but there was a significant change in both groups that supplemented vitamin D for the vitamin D daily group (p= 0.001) and for the vitamin D monthly group (p= 0.000).

Between baseline tests and T2 there were no significant changes in the vitamin D plasma levels over the first 4 weeks of Interventions.

From T2 to T3 there was a significant vitamin D plasma level increase both in the monthly (p = 0.000) and in the daily group (p = 0.006).

As expected, there was a significant change in the vitamin D plasma level between T1 and T3 in the monthly vitamin D group (p = 0.000) and the daily vitamin D group (p = 0.003), but not in the control group.

This indicates that the greatest increase in vitamin D concentration between T1 and T3 was in the monthly vitamin D group, where participants supplemented 50 000 IU vitamin D_3 per month, followed by the vitamin D daily group with a daily supplementation of 800 IU Vitamin D_3. The control group did not show any significant change in the vitamin D concentration over the course of the study.

Additionally, a Mauchly-test showed no sphericity (p = 0.001), thus Huynh-Feldt-corrected values were further used. A Levene Test proved that variances are homogeneous and equal.
A test for within-subjects effects indicates that there is no interaction effect between vitamin D plasma concentration at T1, T2, T3 and the intervention groups.
Moreover, a post-hoc analysis was conducted for the purpose of determining which groups exactly differ from each other, irrespective of any particular time frame.

Bonferroni, Multiple comparisons	Intervention group	Intervention group	Sig p-Value
	CON	VDD	0.347
		VDM	0.017
	VDD	CON	0.347
		VDM	0.703
	VDM	CON	0.017
		VDD	0.703

Table 9: Bonferroni Post Hoc Analysis Intervention groups

A significant difference was observed between the CON and the VDM group (p = 0.017), while no statistically significant differences were found among the remaining groups overall.

2.8.4 Vitamin D Level at T1, T2, T3 and Gender:

Moreover, it was tested whether the vitamin D plasma levels differs between men and women at different examinations. Vitamin D plasma levels at T1 in the female group were 22.56 (ng/ml) ± 5.58, 24.10 (ng/ml) ± 5.33 at T2 and 26.64 (ng/ml) ± 7.82 at T3. The male subjects showed common values with a concentration of 22.28 (ng/ml) ± 8.23 at T1, 23.81 (ng/ml) ± 5.56 at T2 and 29.25 (ng/ml) ± 8.62 at T3.

	Females (mean ± SD)	Males (mean ± SD)
Vitamin D plasma level T1 (ng/ml)	22.56 ± 5.58	22.28 ± 8.23
Vitamin D plasma level T2 (ng/ml)	24.10 ± 5.33	23.81 ± 5.56
Vitamin D plasma level T3 (ng/ml)	26.64 ± 7.82	29.25 ± 8.62

Table 10: Mean Values of Vitamin D supply at T1, T2, T3 according to gender

First, a Mauchly-test for sphericity was performed, which showed that there is no sphericity between those variables, thus Huynh-Feldt-corrected values were further used.
A Levene Test was used to prove that variances for all three groups (vitamin D concentration at T1, T2, T3) are equal thus there is a homogeneity between groups.
T-Test for independent samples showed that there is no significant difference in the vitamin D concentration between males and females at T1 ($p = 0.288$), T2 ($p = 0.571$) and T3 ($p = 0.181$).

2.8.5 DNA damage between sex at baseline

Furthermore, it was examined whether the DNA damage between men and women differ from one another at the time of the baseline.
Both Table 11 and the figures below show the DNA damage parameter comparison between both sexes at T1.

	Females Mean ± SD	Males Mean ±SD	P-Value
Tail Intensity Lysis_T1 %	3.77 ± 0.76	3.55 ±0.89	0.331
Tail Intensity H_2O_2_T1 %	12.18 ±2.50	11.33 ± 2.04	0.075
Tail Intensity Buffer_T1 %	4.62 ± 0.93	4.37 ±0.91	0.213
Tail Intensity FPG_T1 %	9.41 ± 2.05	8.66 ±1.48	0.066

Table 11: DNA damage of males and females at T1.

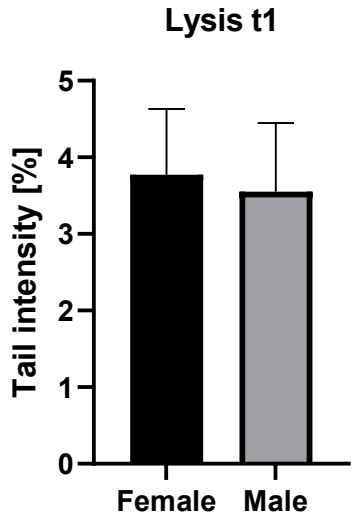


Figure 12: Lysis for females and males at baseline.

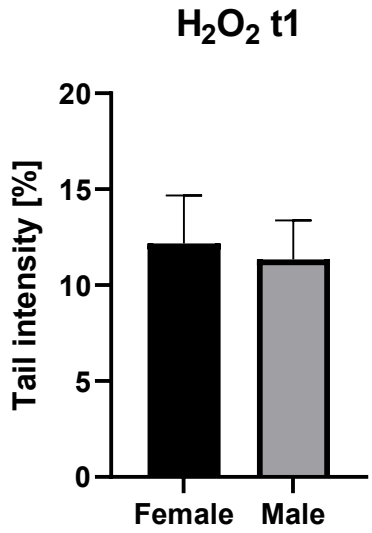


Figure 13: H202 for females and males at baseline.

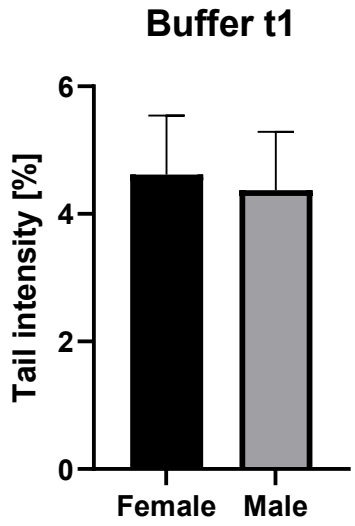


Figure 14: Buffer for females and males at baseline.

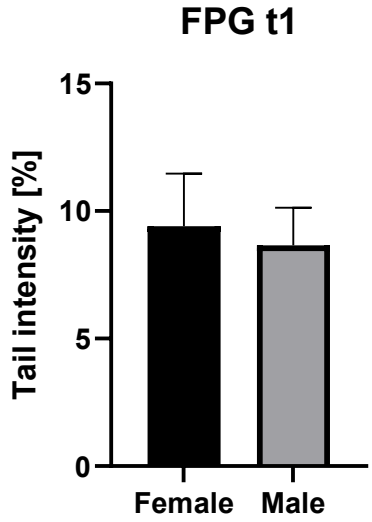


Figure 15: FPG for females and males at baseline.

As lysis was not normally distributed (p = 0.013) a Mann-Whitney test was used. With a p= 0.331 for the treatment with lysis, no significant difference between males and females for lysis treatment was shown at T1.

T-Test for independent samples showed a p= 0.075 for the treatment with H_2O_2, p= 0.213 for Buffer and p= 0.066 for the treatment with FPG. Those p-values indicate that there is no significant difference in DNA damage between both genders at baseline.

2.8.6 Comet Assay parameters

Table 12 illustrates the comet assay parameters (Tail Intensity lysis, H_2O_2, buffer and FPG) for T1, T2 and T3. During the comet assay implementation no additional samples were compromised, thus the only missing samples were the dropouts, as described earlier under chapter 7.5.2.

	N	Mean	SD
VB_Tail Intensity Lysis_1 [%]	100	3.63	.88
VB_Tail Intensity Lysis_2 [%]	97	3.45	1.01
VB_Tail Intensity Lysis_3 [%]	89	3.32	1.54
VB_Tail Intensity H2O2_1 [%]	100	11.61	2.23
VB_Tail Intensity H2O2_2 [%]	97	10.40	2.01
VB_Tail Intensity H2O2_3 [%]	85	9.80	2.06
VB_Tail Intensity buffer_1 [%]	100	4.45	.92
VB_Tail Intensity buffer_2 [%]	97	4.01	.99
VB_Tail Intensity buffer_3 [%]	85	3.70	.98
VB_Tail Intensity Fpg_1 [%]	100	8.91	1.72
VB_Tail Intensity Fpg_2 [%]	97	8.30	1.45
VB_Tail Intensity Fpg_3 [%]	85	7.84	1.50

Table 12: Comet Assay Parameters throughout the study

2.8.7 DNA damage throughout the course of Intervention

Furthermore, it was analyzed whether there is a significant change between DNA damage throughout the course of the study. For this purpose, one-way Anova Test with repeated measurements for T1, T2, T3 between all DNA damage parameters was performed.

The sphericity was tested with a Mauchly-Test which showed that there is no sphericity between the DNA damage parameters. However, analysis showed that there are highly significant results between the time points (T1, T2, T3) and the interaction between them (p- value of 0.000).

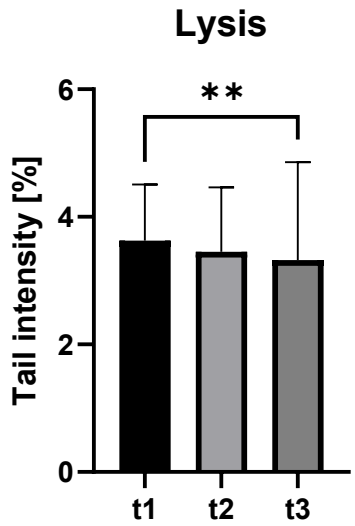


Figure 16: Lysis tail intensity throughout the study.

With a p= 0.001 for the treatment with lysis, a significant decrease in the tail intensity was observed between T1 and T3.

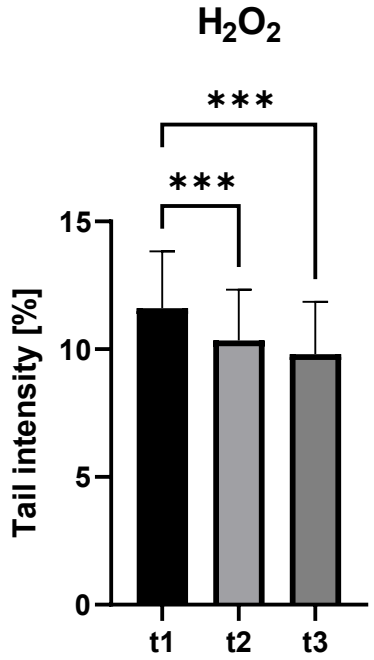


Figure 17: H202 tail intensity throughout the study.

For H_2O_2 a highly significant difference between T1 and T2 was observed, as well as between T1 and T3 (p= 0.000).

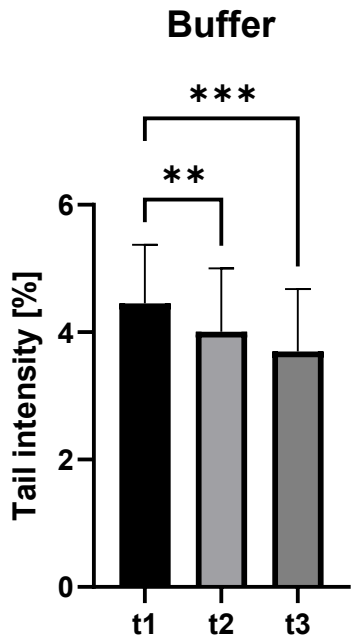


Figure 18: Buffer tail intensity throughout the study.

Tail Intensity Buffer and FPG were also significantly reduced from T1 to T3 (p= 0.000).

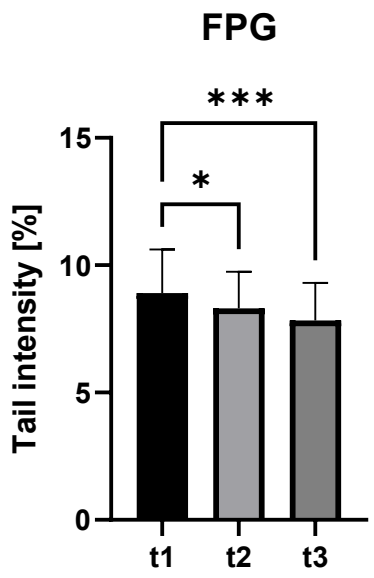


Figure 19: FPG tail intensity throughout the study.

Thus, it can be stated that DNA damage decreased significantly over the course of the treatment. All four DNA damage parameters decreased following both the 4-week and 10-week intervention periods.

2.8.8 DNA damage and Vitamin D intervention according to interventional group

Furthermore, it was analyzed whether there is a significant reduction in DNA damage between T1, T2 and T3 according to the interventional groups.

	Intervention group	Mean	SD	N
VB_Tail Intensity Lyse_1 [%]	Control group	3.57	.93	27
	Vitamin D daily	3.65	.88	27
	Vitamin D monthy	3.70	.98	31
	Gesamt	3.64	.92	85
VB_Tail Intensity Lyse_2 [%]	Control group	3.40	.99	27
	Vitamin D daily	3.43	.89	27
	Vitamin D monthy	3.63	1.24	31
	Gesamt	3.49	1.05	85
VB_Tail Intensity Lyse_3 [%]	Control group	2.95	.68	27
	Vitamin D daily	2.97	.87	27
	Vitamin D monthy	3.22	1.15	31
	Gesamt	3.05	.93	85

VB_Tail Intensity H2O2_1 [%]	Control group	11.60	2.60	27
	Vitamin D daily	11.96	2.59	27
	Vitamin D monthy	11.45	1.73	31
	Gesamt	11.66	2.30	85
VB_Tail Intensity H2O2_2 [%]	Control group	9.92	1.30	27
	Vitamin D daily	10.89	2.35	27
	Vitamin D monthy	10.42	2.30	31
	Gesamt	10.41	2.07	85
VB_Tail Intensity H2O2_3 [%]	Control group	9.19	1.48	27
	Vitamin D daily	10.18	1.86	27
	Vitamin D monthy	10.01	2.55	31
	Gesamt	9.80	2.06	85
VB_Tail Intensity Puffer_1 [%]	Control group	4.47	.81	27
	Vitamin D daily	4.45	.87	27
	Vitamin D monthy	4.47	.95	31
	Gesamt	4.46	.87	85
VB_Tail Intensity Puffer_2 [%]	Control group	3.88	.87	27
	Vitamin D daily	4.08	.89	27
	Vitamin D monthy	4.07	1.23	31
	Gesamt	4.01	1.02	85
VB_Tail Intensity Puffer_3 [%]	Control group	3.54	.87	27
	Vitamin D daily	3.61	1.02	27
	Vitamin D monthy	3.91	1.02	31
	Gesamt	3.70	.98	85
VB_Tail Intensity Fpg_1 [%]	Control group	8.53	1.70	27
	Vitamin D daily	9.14	2.08	27
	Vitamin D monthy	9.02	1.42	31
	Gesamt	8.90	1.74	85
VB_Tail Intensity Fpg_2 [%]	Control group	7.91	1.10	27
	Vitamin D daily	8.75	1.54	27
	Vitamin D monthy	8.41	1.57	31
	Gesamt	8.36	1.45	85
VB_Tail Intensity Fpg_3 [%]	Control group	7.38	1.02	27
	Vitamin D daily	7.83	1.67	27
	Vitamin D monthy	8.24	1.53	31
	Gesamt	7.84	1.47	85

Table 13: DNA-Damage parameters according to interventional groups

Table 13 displays descriptive statistics of all four DNA damage parameters according to the three interventional groups: control group, vitamin D daily group and vitamin D monthly group.

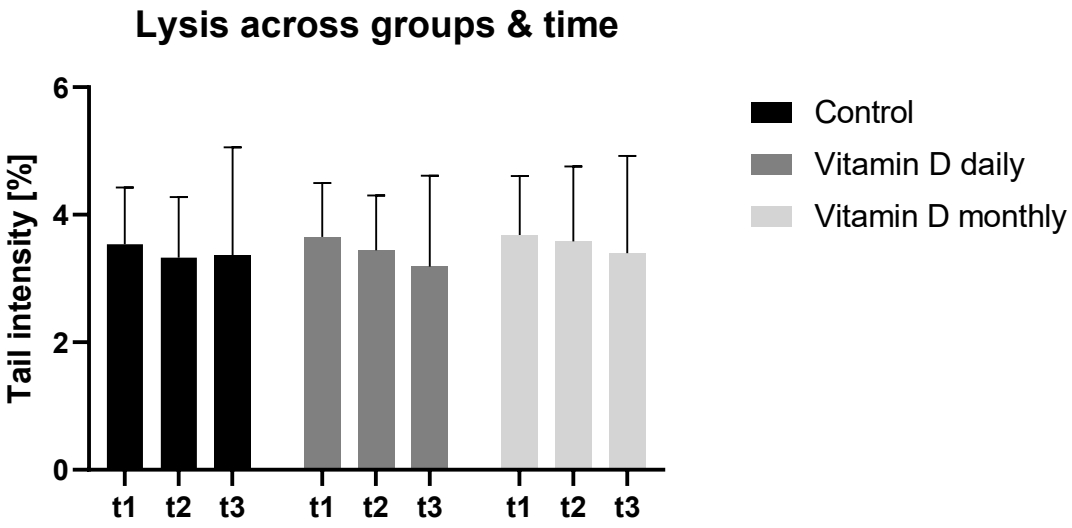


Figure 20: Tail Intensity Lysis throughout the study.

With a mixed anova with time and group effects no significant change in the tail intensity of lysis between groups was observed.

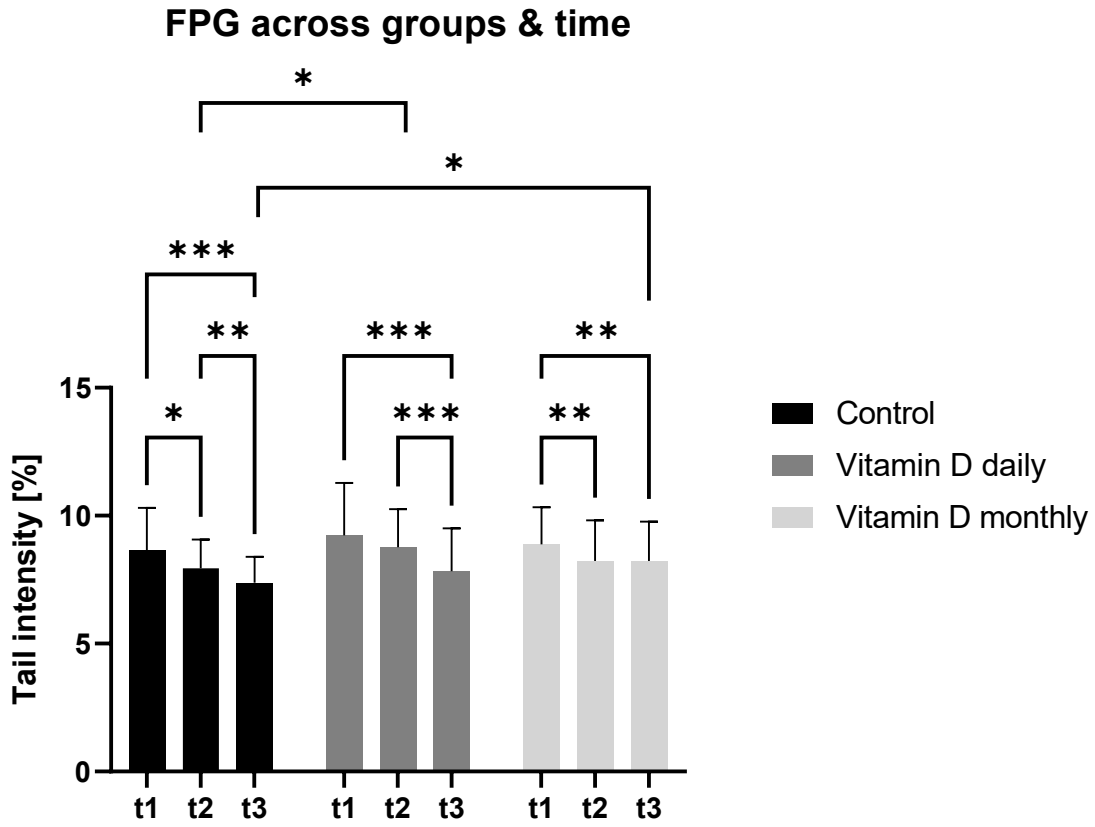


Figure 21: Tail Intensity FPG throughout the study.

When comparing FPG parameters among the groups a different trend was observed: between T1 and T3 there was a significant change in all three interventional groups: CON (p-value= 0.001), VDD (p-value= 0.000), VDM (p-value= 0.002), however, the FPG intensity tail was most significantly reduced after a daily supplementation of vitamin D.

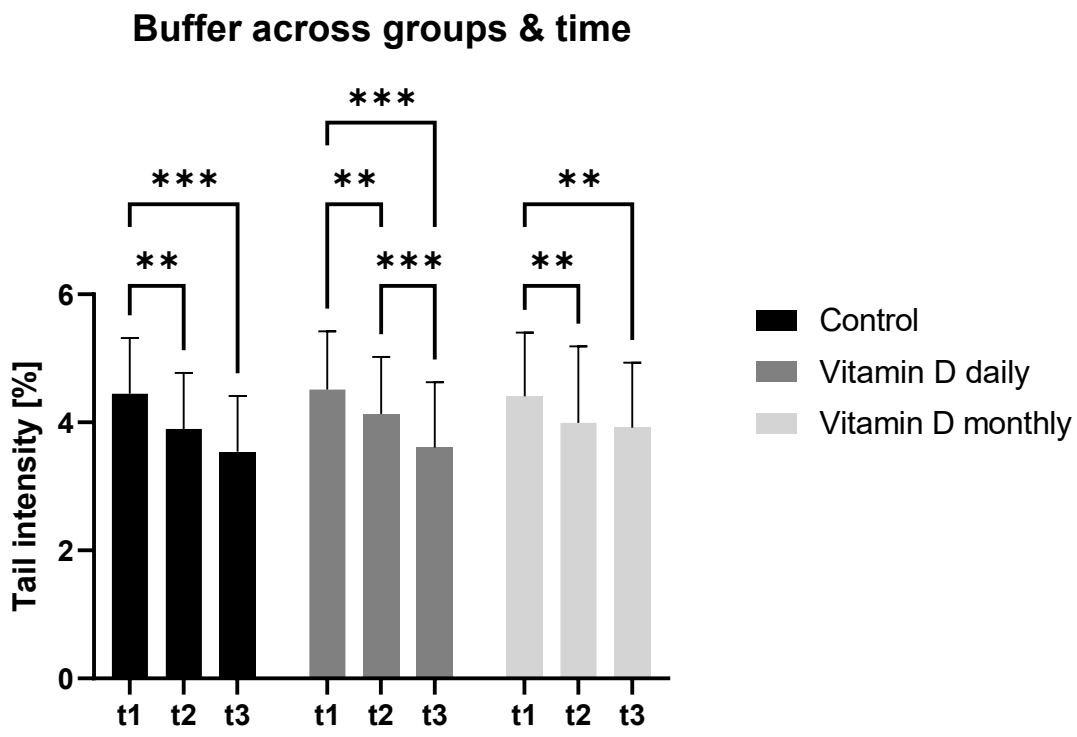


Figure 22: Tail Intensity buffer throughout the study.

In all three interventional groups a significant reduction in the damage caused by buffer was also detected. DNA damage decreased significantly from baseline T1 to T3 for all three groups (p= 0.000 for CON and VDD; p= 0.001 for VDM)

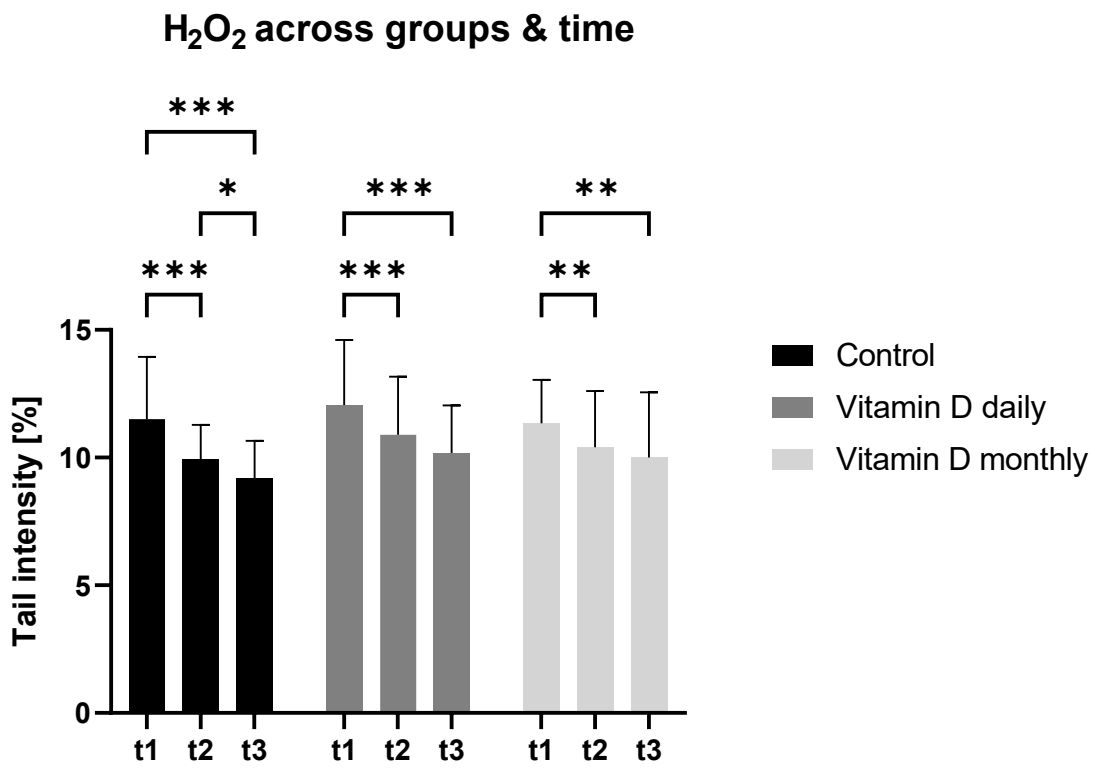


Figure 23: Tail Intensity H202 throughout study.

Also, for H_2O_2 a significant reduction in the damage in all three groups between T1 and T3 was observed with a p= 0.000 for CON and VDD and p= 0.001 for VDM.

Further an Anova Test was performed, and Sphericity was tested.

A Levene Test was performed for the analysis of the homogeneity of variance. It has been shown that all DNA damage parameter variances are approximately equal and homogeneous ($p > 0.05$).

A Within-Subject effects test between DNA damage parameters x timepoints x interventional groups indicated no significant interaction effect between DNA damage parameters and time points according to the intervention groups ($p = 0.628$). However, there was a statistically significant interaction (p- Value = 0.000) between DNA damage and time points in each group.

2.8.9 Correlation between DNA damage, Vitamin D Concentration, and functional parameters throughout the study

With a correlation analysis it should be evaluated whether there is any significant correlation between vitamin D plasma concentration, functional parameters (30-second arm curl dominate hand test; 6-minutes walking test) and DNA damage throughout the study for each interventional group at T1 and T3.

First, it was evaluated whether applicable DNA damage parameters are normally distributed. Spearman correlation was used for variables, which are not normally distributed, and Pearson correlation was used for all other variables which are normally distributed.

Bivariate, significant strong correlations were found between vitamin D plasma concentrations and FPG tail intensity at T1 ($p = 0.024$, $r = 0.226$) and between vitamin D concentration and H_20_2 at T3 ($p= 0.005$, $r= 304$).

Between vitamin D plasma concentration and functional parameters there were no significant correlations found neither during baseline nor at T3.

2.8.10 Correlation between DNA damage and Vitamin D Concentration according to Intervention groups

Furthermore, the correlation between DNA damage parameters and vitamin D plasma concentration was analyzed according to the intervention groups. For this purpose, delta values (T3-T1) for each parameter were calculated.

For the control group (Tab. 14) there were no significant correlations between vitamin D Delta values and any of the DNA damage and functional parameters.

CON Correlations								
		VitDdelta	six6minDelta	Deltalysis	DeltaH2O2	DeltaBuffer	DeltaFPG	DeltaArmCurl
VitD delta	Pearson Correlation	1	-.346	-.155	-.208	-.318	.020	-.236
	P Value		.077	.439	.297	.106	.922	.246
	N	27	27	27	27	27	27	26

Table 14: Pearson correlation between vitamin D delta, DNA damage parameters and functional parameters in the control group.

For the vitamin D daily group (Tab. 15) there were also no significant correlations between vitamin D delta values and any of the DNA damage and functional parameters.

VDD Correlations								
		VitDdelta	six6minDelta	Deltalysis	DeltaH2O2	DeltaBuffer	DeltaFPG	DeltaArmCurl
VitD delta	Pearson Correlation	1	-.105	.160	-.005	-.087	.098	.213
	P Value		.609	.426	.981	.667	.627	.296
	N	27	26	27	27	27	27	26

Table 15: Pearson correlation between vitamin D delta, DNA damage parameters and functional parameters in the vitamin D daily group.

For the monthly vitamin D group there was a bivariate significant correlation between vitamin D delta and the six-minute walking test (p=0.014 r=-0.437) and H_2O_2 (p=0.050, r= -0.356).

VDM Correlations								
		VitDdelta	**six6minDelta**	Delta lysis	**DeltaH2O2**	DeltaBuffer	DeltaFPG	DeltaArmCurl
VitDdelta	Pearson correlation	1	-.437*	-.009	-.356*	.205	.035	-.134
	P Value		.014	.963	.050	.268	.854	.473
	N	31	31	31	31	31	31	31

Table 16: Pearson correlation between vitamin D delta, DNA damage parameters and functional parameters in the vitamin D monthly group.

Also, by dividing the groups according to gender, no significant correlation was found between any of the DNA damage parameters and the vitamin D concentration in each intervention group.

2.8.11 DNA damage and Functional Parameters

First, it was tested whether all variables are normally distributed. Spearmen correlation was applied for all not normally distributed parameters and Pearson correlation was ran for all normally distributed variables.

6-Minute Walking Test

There was a significant difference (p = 0.000) between both sexes in the walking test results at T1. Women covered an average of 584.5 ± 73.8 m and the men a distance of 650.4 ± 92.5 m.

The result in meters was then checked for correlation with DNA damage.

Overall, there was no significant correlation between the 6-Minute Walking Test and any of the DNA damage parameters at T1, T2 or T3.

However, when dividing the data into gender, significant correlations were observed between some of the Comet Assay parameters and the 6-minute walking test.

For the women there was a significant positive correlation between the six-minute walking test and buffer F (r=0.426, p =0.014) as well as H_20_2 (r = 0.455, p = 0.008) at T1.

A significant positive correlation was recorded for females between the six-minute walking test and lysis (r= 0.413, p = 0.019) and buffer F (r = 0.438, p = 0.012) at T2 and buffer (r =0.550, p = 0.002) for T3.

For males there were no significant correlations between six-minute walking test and any of the DNA damage parameters during T1, T2 or T3

30-seconds Arm curl dominate hand

There was a significant difference (p = 0.001) between both genders in the 30-second Arm curl dominate hand test results at T1. Women covered an average of 17.55 ± 3.36 repetitions and men 18.96 ± 3.98 repetitions.

Spearman correlation was used as 30-seconds Arm curl dominate hand T1, T2, T3 were not normally distributed.

Overall, there was no significant correlation between the 30-second arm curl dominate hand Test and any of the DNA damage parameters at T1, T2 or T3.

For females a significant positive correlation between Arm curl of the dominate hand and buffer F (r = 0.376, p = 0.037) was detected at T2.

For the male group there were no significant correlations found.

3. Discussion

This master's thesis, which was completed as part of the Nutri Aging Study, addressed the question whether vitamin D3 supplementation and resistance training have an influence on DNA damage in older populations.

Overall, 101 participants fulfilled all necessary criteria. 33 women and 68 men were randomized in the study. Participants were between 65 and 85 years of age, however the mean age was 70.63 ± 4,53.

Over the course of the study, it has been shown that the vitamin D plasma levels do not differ between gender with a $p = 0.288$ for vitamin D levels at T1, $p = 0.571$ at T2 and $p = 0.181$ for T3.

No significant differences at the time points between both genders were observed and not as an interaction effect (gender x time). Thus, it can be stated that vitamin D plasma levels were not gender specific.

Results from other studies, that evaluated whether vitamin D measured in plasma differs between men and women are inconsistent. Different trials, conducted in different countries and regions concluded that women are more prone to a vitamin D deficiency. Age is considered an important factor for lower serum levels of vitamin D, which was established in studies, conducted in USA with a target group of people over 65 years of age [96], [97].

However, findings from a study, conducted in the United Arab Emirates concluded that vitamin D deficits are prevailing in younger girls, aged 15-18 [98]. Similar discoveries were obtained by a study with Brazilian young girls, aged 12-17 [99].

Furthermore, the Nutri Aging study demonstrates clear evidence that vitamin D plasma concentration has gradually and constantly improved over the course of the study in both intervention groups with vitamin D supplementation. The control group, which supplemented calcium only, did not show any significant change ($p = 0.097$) in the serum 25(OH)D status over the course of the study. Although the study started in winter time and finished in summer, there was a strong significant change in the vitamin D levels in both groups that supplemented vitamin D (VDD, $p = 0.001$ and VDM, $p = 0.000$).

Between baseline and T2 only a monthly supplementation of 50.000 IU showed a significant change ($p = 0.012$) in the serum 25(OH)D status. Between T2 and T3, which lasted for 10

weeks, both groups that supplemented with vitamin D led to a significant increase in the 25(OH)D serum status (VDD p= 0.006; VDM p = 0.000).

As expected, there was a significant change in vitamin D plasma level between T1 and T3 and T2 to T3 in both vitamin D groups (VDM p =0.000 and VDD p = 0.003)

Nevertheless, speaking of short supplementation of 4 weeks from T1 to T2 only the vitamin D monthly group showed a significant increase in the serum status of 25(OH)D (VDM p= 0.012; VDD p = 0.144; CON p =0.926).

Other trials assessing the vitamin D supply also concluded that concentrations of 25(OH)D increased significantly after a supplementation of 50.000 IU/weekly compared to a daily supplementation of 4.000, 2.000 or 800 IU[100]. A monthly supplementation dose of 50.000 IU of vitamin D shows a rapid and safe increase in the serum 25(OH)D status [101], which was demonstrated as a result also in this thesis.

In the present Nutri Aging study, DNA damage was measured and evaluated by the following Comet Assay parameters: Lysis, Buffer F, H_2O_2 and FPG.

Results showed no significant difference between DNA damage-related parameters and gender at T1 (Lysis p = 0.331; H_2O_2 p = 0.075; Buffer p =0.213; FPG p = 0.066).

The results of the analysis demonstrated highly significant findings (p = 0.000) between the DNA damage- related parameters, the time points (T1, T2, T3) and the interaction between them. Therefore, it can be stated that DNA damage decreased significantly over the course of the treatment.

The current results also suggest some DNA damage reduction according to the different intervention groups. Lysis (VDD p = 0.000; CON p = 0.009; VDM p = 0.002)- and FPG (CON p = 0.001; VDD p= 0.000; VDM p = 0.002)-related DNA damage decreased significantly within all three groups; however, the highest reduction was observed within the daily vitamin D group for both Comet Assay treatments (FPG and H_2O_2).

In all three interventional groups a significant reduction in DNA damage caused by H_2O_2 and buffer was observed. When treated with H_2O_2 there was a significant decrease (p= 0.000) in DNA damage from T1 to T3. The damage caused by Buffer also decreased significantly from T1 to T3 (p= 0.000).

Therefore, it can be concluded that vitamin D supplementation in general led to a decrease in DNA damage and daily supplementation in particular with 800 IU vitamin D achieves

greatest reduction in DNA damage, caused by FPG and Lysis, in comparison to a monthly supplementation with 50.000 IU vitamin D or no supplementation at all.

Those findings align with other trials, that suggest that vitamin D may reduce oxidative DNA damage, as supplementation was shown to decrease different markers of oxidative damage [35], [102].

However, due to the scarcity and the limitations of human-based data, the optimal vitamin D intake needed to minimize DNA damage remains uncertain and must be further investigated.

The study also explored a potential link between the participants' physical activity and DNA damage. To assess this connection, two functional tests were implemented: 30-second Arm curl dominate hand test and 6-minute walking test, which were analyzed for their correlation with DNA damage and vitamin D plasma levels.

Data results suggest a bivariate, significant positive correlation between vitamin D plasma concentrations and FPG tail intensity at T1 ($p = 0.024$, $r = 0.226$) and H_20_2 at T3 ($p= 0.005$, $r= 304$).

Between vitamin D plasma concentration and functional parameters there were no significant correlations found neither during baseline nor at T3.

For further correlations analysis between DNA damage and vitamin plasma levels according to all three intervention groups delta values for each parameter were calculated.

For the monthly vitamin D group a negative bivariate significant correlation between vitamin D delta and the six-minute walking test ($p=0.014$ $r=-0.437$) and H_2O_2 ($p=0.050$, $r= -0.356$) was detected. These findings suggest that a monthly intake of vitamin D (50.000 IU) leads to a decrease in DNA damage caused by H_2O_2.

Overall, analysis revealed that there was no significant interaction between increased 25(OH)D levels and changes in physical performance. This aligns with intervention studies that found no additional benefit of vitamin D3 intake during various types of exercise on physical functioning, muscle strength or aerobic capacity [103], [104].

However, when dividing data into gender, several significant correlations were observed for both functional tests only for the female participants. Male subjects did not show any significant interaction between the chosen functional tests and Comet Assay parameters.

There was a significant difference (p = 0.000) between both genders in the six-minute walking test results at T1. Women covered an average of 584.5 ± 73.8 m and men a distance of 650.4 ± 92.5 m.

Within the female group a significant positive relation was observed between the six-minute walking test and buffer F- related DNA damage (r = 0.426, p = 0.014) and H_2O_2-related DNA damage (r =0.455, p =0.008) at T1, as well as a positive correlation was shown for lysis (r = 0.413, p = 0.019) and buffer (r =0.438, p = 0.012) -related DNA damage at T2. Additionally, a significant positive correlation between the six-minute walking test and buffer F (r = 0.550, p = 0.002) was observed.

The 30-second Arm curl dominate hand test is used to assess the test subjects' upper body strength.

There was a significant difference (p < 0.001) between sex in the 30-second Arm Curl dominate hand test results at T1. Women covered an average of 17.55 ± 3.36 and men 18.96 ± 3.98 repetitions. Furthermore, in the female group a significant positive interaction between 30-second Arm curl and buffer F-related DNA damage (r = 0.376, p = 0.037) at T2 was detected.

Furthermore, according to the paper of Aschauer et al. [84] there was a significant increase (p <0.001) in different physical function parameters following both resistance training and nutritional intervention. These parameters included the 30-s Chair Stand Test, arm curl test (on both dominant as well as non-dominant side), as well as in gait speed and 6-min walk test. The improvements were especially noticeable in the second phase of the study, where participants underwent both nutritional and training intervention.

Moreover, the paper of Draxler et al. [86] also concluded that a Vitamin D supplementation, particularly 800 IU daily may have a positive effect on DNA damage, specifically on chromosomal stability parameters in elderly. Having said that these effects and results may be more noticeable in the elderly with a lower baseline vitamin D level, a factor that should be considered in future studies and research.

Altogether, these findings imply that both vitamin D supplementation and resistance training may have beneficial advantages on different aspects of health and function in elderly populations. While the data of this thesis focused on DNA damage linked to vitamin D supplementation, the referenced study by Aschauer et al. [84] and Draxler et al. [86]

highlight the possibility for resistance training to improve physical performance, which could complement the benefits of vitamin D supplementation in promoting healthy aging. Nevertheless, further research is required to explore the role of vitamin D in improving strength, balance and mobility, as well as in reducing DNA damage.

4. Conclusion

This master thesis has the aim of examining the effects of vitamin D supplementation in combination with resistance training in elderly people on DNA damage.

The findings of this thesis indicate a significant impact of vitamin D supplementation on both serum levels of 25(OH)D and DNA damage in older populations.

It is suggested that both forms of vitamin D supplementation - 800 IU per day and 50.000 IU once a month- lead to a significant increase in the 25-hydroxyvitamin D3 status of elderly people with vitamin D insufficiency prior to the beginning of the intervention. However, when discussing short-term supplementation, a higher dosage of vitamin D (50.000 IU per month) has demonstrated a stronger response than a daily supplementation of vitamin D.

Furthermore, the results from the present study indicate that after 17 weeks of nutritional and training intervention, there was a significant decrease in DNA damage parameters, including lysis, buffer, FPG and H_2O_2, as measured by the Comet Assay. The reduction in DNA damage was observed across all parameters over the course of the study with a highly significant p-value of 0.000 for each of the Comet Assay parameters for each time point.

Current findings also suggest DNA damage declined according to the different groups and interventions. Lysis and FPG-related DNA damage decreased significantly within all three groups; however, the highest reduction was noticeable in the daily vitamin D group. This could suggest that a daily intake of vitamin D may have a greater impact on DNA damage compared to monthly supplementation.

Although DNA damage parameters also decreased in the control group, VDD and VDM showed significantly stronger results than the CON. These findings conclude that the

combination of vitamin D supplementation and resistance training together has a stronger effect on reducing DNA damage compared to resistance training alone.
Moreover, the correlation between vitamin D levels, DNA damage and participant ‘s physical activity was examined throughout the study.

The analysis revealed a positive correlation between vitamin D plasma concentrations and DNA damage, caused by FPG at T1 and H_2O_2 at T3.

Although all other correlations are not clinically significant, current results suggest an increase in the 25(OH)D serum leads to an increase in the DNA damage by FPG at baseline and H_2O_2 at T3. Furthermore, no significant correlation was found between vitamin D plasma concentration and functional parameters.

When separating the analysis according to intervention groups a negative significant correlation was shown between vitamin D plasma levels and H_2O_2 and the six minute walking test in the monthly vitamin D group.

Finally, the study examined a potential link between participant’s physical activity and DNA damage. Overall, no significant interactions between increases in 25(OH)D levels and changes in performance were shown. However, when dividing the analysis into sex, females achieved some significant positive correlations between the six-minute walking and 30-second Arm curl test.
Despite the limited significant correlations observed, this master’s thesis found that a high-dose vitamin D supplementation (50.000IU) was significantly associated with reduced DNA damage, caused by H_2O_2. While the results were somewhat restricted, the study demonstrated a notable decrease in DNA damage among elderly population after 17 weeks of vitamin D supplementation.
These findings highlight the potential benefit of maintaining adequate vitamin D levels. However, further research into the mechanisms underlying the relationship between vitamin D, DNA damage and resistance training is needed.
In conclusion, this thesis provides compelling evidence for the benefits of a higher serum levels of 25(OH)D on both vitamin D status and DNA damage in older individuals.

5. Summary

The interest in vitamin D, particularly its deficiency, has indeed increased over the past few decades due to its association with numerous diseases and health outcomes. This has led to various studies investigating the effects of vitamin D deficiency and supplementation on various aspects of health. It is well known that vitamin D plays a crucial role in maintaining bone health, muscle strength, immune and cognitive function. Thus, it is considered that adequate levels are essential for preventing conditions such as osteoporosis, muscle weakness, sarcopenia, and several diseases.

Particularly important is the maintenance of adequate vitamin D levels for older people, as aging is associated with loss of muscle mass and this is considered to negatively affect the human body, particularly the DNA in older populations.

This master thesis aimed to evaluate the impact of vitamin D supplementation and resistance training on DNA damage in community-dwelling older adults.

Study participants were randomized into three groups: control group (only calcium supplementation 400 mg daily), daily vitamin D group (supplementation of 800 IU vitamin D and 400 mg calcium daily) and the monthly vitamin D group (supplementation of 50. 000 IU vitamin D monthly and 400 mg daily calcium). All participants began with a four-week nutritional intervention only and then underwent a 10-week resistance training intervention with two training sessions per week.

Whole blood samples were obtained at three timepoints: T1= baseline, prior to beginning of the study, T2= after the 4-week nutritional intervention and T3= after the nutritional and training intervention.

There was no significant difference in DNA damage between all three interventional groups at any of the time points throughout the study (T1, T2, T3). The findings of this thesis provide compelling evidence regarding the impact of vitamin D supplementation on both serum levels of vitamin D and DNA damage in elderly individuals with vitamin D insufficiency.

The results of the present analysis demonstrated a significant decrease in each of the four DNA damage parameters over the course of the treatment for the Tail Intensity for each of the Comet Assay parameters (Lysis, buffer F, FPG, H_2O_2 ; p 0.000). Nevertheless, the daily

vitamin D supplementation has indicated the strongest reduction in DNA damage, compared to the control group and the monthly vitamin D intake.

Unfortunately, analysis revealed a positive correlation between vitamin D plasma concentrations and DNA damage, caused by FPG at T1 and DNA damage by H_2O_2 at T3 which might imply that an increase in the 25(OH)D serum leads to an increase in DNA damage, caused by FPG and H_2O_2.Furthermore, no significant correlation was found between vitamin D plasma concentration and functional parameters.

When separating the analysis according to intervention groups a negative significant correlation was shown between vitamin D plasma levels and H_2O_2 and the six minute walking test in the monthly vitamin D group.

Furthermore, a significant correlation between DNA damage and changes in physical performance was detected only within female participants for the following functional tests, which were included in the analysis: six-minute walking test and 30-second Arm curl dominate hand test.

A positive correlation between the six-minute walking test and buffer F ($r = 0.426$, $p = 0.014$) and H_2O_2 ($r = 0.455$, $p = 0.008$) related damage at T1 as well as a positive correlation for the Lysis, ($r = 0.413$, $p = 0.019$) - and buffer F ($r = 0.438$, $p = 0.012$) -related DNA damage at T2 was found. Additionally, at time point T3 there was a significant positive interaction between the six-minute walking test and buffer F ($r = 0.550$, $p = 0.002$). For the 30-second Arm curl dominate hand test a significant positive correlation with buffer F-related DNA damage ($r = 0.376$, $p = 0.037$) at T2 was observed.

In conclusion, current findings suggest that vitamin D supplementation combined with resistance training positively impacts overall health outcomes in elderly population (65-85 years old) by significantly increasing 25(OH)D serum status and improving various functional parameters.

Additionally, a notable decrease in DNA damage was observed. Data suggests that both vitamin D supplementation and resistance training contribute to reducing DNA damage, as measured by different Comet Assay parameters in elderly people. These findings also

support the hypothesis that improvement in physical performance might complement the benefits of vitamin D supplementation and thus promoting healthy aging.

6. Zusammenfassung

Das Interesse an Vitamin D, insbesondere an dessen Unterversorgung, hat in den letzten Jahren deutlich zugenommen, da es mit zahlreichen Krankheiten und gesundheitlichen Folgen in Zusammenhang steht. Dieses gestiegene Interesse hat dazu beigetragen, dass verschiedene Studien immer wieder die Auswirkungen von Vitamin D-Mangel und-Supplementierung auf verschiedene Aspekte zur Gesundheit untersuchen.

Es ist bekannt, dass Vitamin D eine entscheidende Rolle bei der Aufrechterhaltung der Knochengesundheit, der Muskelkraft sowie der Immun- und kognitiven Funktion spielt. Daher wird davon ausgegangen, dass ausreichende Mengen zur Vorbeugung von Erkrankungen wie Osteoporose, Sarkopenie, und mehreren chronischen Krankheiten unerlässlich sind. Besonders wichtig ist die Aufrechterhaltung eines ausreichenden Vitamin-D-Spiegels bei älteren Menschen, da Alterung mit einem Verlust an Muskelmasse einhergeht. Darüber hinaus wird davon ausgegangen, dass sich dies negativ auf den menschlichen Körper, insbesondere auf die DNA bei älteren Menschen, auswirkt.

Das Ziel dieser Masterarbeit war es, den Einfluss von Vitamin-D-Supplementierung und Krafttraining auf die DNA-Schäden bei älteren Erwachsenen zu bewerten.

Die Studienteilnehmer wurden in drei Gruppen randomisiert: Kontrollgruppe (nur Kalziumaufnahme 400mg/ Tag), tägliche Vitamin-D-Gruppe (tägliche Supplementierung von 800 IE Vitamin D + 400 mg Kalziumaufnahme) und die monatliche Vitamin-D-Gruppe (monatliche Vitamin-D-Supplementierung von 50.000 IE + 400 mg Kalziumaufnahme täglich).

Alle Teilnehmer begannen mit einer reinen vierwöchigen Ernährungsintervention und absolvierten danach ein zehnwöchiges Krafttraining mit zwei Trainingseinheiten pro Woche.

Vollblutproben wurden zu drei Zeitpunkten entnommen: T1 (Ausgangswert vor Beginn der Studie), T2 (nach der 4-wöchigen Ernährungsintervention) und T3 (nach der Ernährungs-und Trainingsinterventionsphasen). Zu keinem Zeitpunkt der Studie (T1, T2, T3) gab es einen signifikanten Unterschied in der DNA-Schädigungen zwischen alle drei Interventionsgruppen.

Die Ergebnisse dieser Arbeit liefern gute Belege für den Einfluss einer Vitamin-D-Supplementierung sowohl auf den Serumspiegel von Vitamin D als auch auf DNA-Schäden bei älteren Menschen mit Vitamin D-Mangel.

Weitere Ergebnisse der vorliegenden Analyse zeigten eine signifikante Abnahme jedes der vier DNA-Schadensparameter im Verlauf der Intervention mit einem p-Wert von 0.000 für jeden der Comet-Assay-Parameter. Dennoch zeigte die tägliche Vitamin-D-Supplementierung im Vergleich zur Kontrollgruppe und der monatlichen Vitamin-D-Supplementierung die stärkste Reduktion der DNA-Schäden.

Leider ergab die Analyse eine positive Korrelation zwischen Vitamin-D-Plasmakonzentrationen und DNA-Schäden, die durch FPG in T1 sowie durch H2O2 in T3 verursacht wurden. Dies könnte darauf hindeuten, dass ein Anstieg des 25(OH)D-Serums zu einer Zunahme, der durch FPG und H2O2 verursachten DNA-Schäden führt. Darüber hinaus wurde keine signifikante Korrelation zwischen der Vitamin-D-Plasmakonzentration und funktionellen Parametern festgestellt.

Bei der Trennung der Analyse nach Interventionsgruppen zeigte sich eine negative signifikante Korrelation zwischen Vitamin-D-Plasmaspiegeln und H_2O_2 sowie dem Sechs-Minuten-Gehtest in der monatlichen Vitamin-D-Gruppe.

Darüber hinaus wurde bei beiden in die Analyse einbezogenen körperlichen Tests nur bei weiblichen Teilnehmern ein signifikanter Zusammenhang zwischen DNA-Schäden und Veränderungen der körperlichen Leistungsfähigkeit festgestellt. Es zeigte sich eine positive Korrelation zwischen dem Sechs-Minuten-Gehtest und Puffer F, - (r = 0,426, p = 0,014) und H2O2 (r = 0,455, p = 0,008) bedingten Schäden zu T1 sowie eine starke positive Korrelation Lyse (r = 0,413, p = 0,019) – und Puffer F (r = 0,438, p = 0,012) – bedingten DNA-Schäden zu T2. Darüber hinaus besteht zum Zeitpunkt T3 eine signifikante positive Korrelation zwischen dem Sechs-Minuten-Gehtest und Puffer F (r = 0,550, p = 0,002). Für den 30-Sekunden-Arm-Curl-Dominant-Hand-Test wurde eine signifikante positive Korrelation mit Puffer-F-bedingtem DNA-Schaden (r = 0,376, p = 0,037) bei T2 festgestellt.

Zusammenfassend lassen die aktuellen Ergebnisse darauf schließen, dass eine Vitamin-D-Supplementierung in Kombination mit Krafttraining die allgemeinen Gesundheitsergebnisse bei älteren Menschen (65–85 Jahre) positiv beeinflusst, indem sie den 25(OH)D-Serumstatus deutlich erhöht und verschiedene Funktionsparameter verbessert.

Darüber hinaus wurde ein deutlicher Rückgang von DNA-Schäden beobachtet. Daten deuten darauf hin, dass sowohl eine Vitamin-D-Supplementierung als auch Krafttraining zur Verringerung von DNA-Schäden beitragen, gemessen anhand verschiedener Comet-Assay-Parameter bei älteren Menschen. Diese Ergebnisse stützen zudem die Hypothese, dass eine Verbesserung der körperlichen Leistungsfähigkeit die Vorteile einer Vitamin-D-Supplementierung ergänzen und so ein gesundes Altern fördern könnte.

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