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„Effect of Vitamin D supplementation and resistance training on aerobic performance, gait speed and timed up and go test of older adults: A randomized controlled intervention study.“

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Abstract

Background: Many of today's societies are facing new challenges because the proportion and number of older people is growing. In fact, ageing is associated to health deterioration, partly linked to lifestyle habits, inappropriate diet, physiological strength loss, etc. which leads to functional limitations, lower quality of life and morbidity ("World Health Organization", 2004). Strength training (ST) is proven to positively influence physical performance of older adults. However, it is still unclear whether vitamin D (VD) supplementation would exert additional effects. Therefore, this thesis aims to investigate the effects of combining VD supplementation and ST on gait speed (GS), timed up and go (TUG) and 6 minutes walking distance (6MWD) of older adults.

Methods: The study involved 100 healthy community-dwelling participants (70.6 ± 4.6 yrs, 33.3% females). They were randomly assigned to one of the three intervention groups. The Vitamin D monthly (VDM) group received a high dose of vitamin D (VD) once per month (50,000 IU VD + 400 mg calcium/day), the VD daily (VDD) group received a daily, but lower dose (800 IU VD + 400 mg calcium/day) and the Control group (CON) received placebo (calcium 400 mg/day) for the whole length of the study. After four weeks of supplementation only, all three groups performed progressive ST for two sessions per week over a period of further 10 weeks. A two-way mixed ANOVA was used to explore time, group and time*group interaction effects on TUG, GS and 6MWD.

Results: Time effects were found in primary outcomes showing better general performances in GS ($p=0.016$) and 6MWD ($p=0.011$) irrespectively from group allocution. No interaction effects were observed. Actual VD serum concentration did not play any observable role in neither the performance nor the changes in the anthropometric values (deltas) that were examined in this thesis.

Conclusion: The current study supports previous findings that ST improves physical performance of older adults. However, according to our results, VD does not further enhance the effectiveness of a ST program on functional and endurance performances for older people.

Zusammenfassung

Hintergrund: Viele der heutigen Gesellschaften stehen vor neuen Herausforderungen, weil der Anteil älterer Menschen an der Bevölkerung zunimmt. Tatsächlich ist das Altern mit einer Verschlechterung der Gesundheit verbunden, die teilweise mit dem Lebensstil wie schlechten Ernährungsgewohnheiten sowie mit einer physiologischen Abnahme der Muskelkraft zusammenhängt, was zu funktionellen Einschränkungen, geringerer Lebensqualität und Morbidität führt (World Health Organization, 2004). Es ist erwiesen, dass Krafttraining (ST) die körperliche Leistungsfähigkeit älterer Erwachsener positiv beeinflusst. Es ist jedoch noch unklar, ob eine Vitamin D (VD)-Supplementierung zusätzliche Effekte bewirken kann. Daher zielt diese Arbeit darauf ab, die Effekte einer Kombination von VD-Supplementierung und ST auf die Gehgeschwindigkeit (GS), den Timed Up and Go (TUG) Test und die 6-Minuten-Gehdistanz (6MWD) von älteren Erwachsenen zu untersuchen.

Methodik: Die Studie umfasste 100 gesunde, selbstständig lebende Teilnehmer*innen (70,6 ± 4,6 Jahre, 33,3 % Frauen), die randomisiert einer der drei Interventionsgruppen zugewiesen wurden. Die Vitamin-D-Monatsgruppe (VDM) erhielt einmal pro Monat eine hohe Dosis Vitamin D (VD) (50.000 IE VD + 400 mg Kalzium/Tag), die VD-Tagesgruppe (VDD) erhielt eine tägliche, aber niedrigere Dosis (800 IE VD + 400 mg Kalzium/Tag) und die Kontrollgruppe (CON) erhielt über die gesamte Studiendauer ein Placebo (Kalzium 400 mg/Tag). Nach vier Wochen der reinen Supplementierung führten alle drei Gruppen über einen Zeitraum von weiteren 10 Wochen zwei Mal pro Woche ein progressives ST durch. Eine two-way mixed ANOVA erlaubte die Untersuchung von Zeit-, Gruppen- und Zeit*Gruppen-Interaktionseffekte auf die Variablen TUG, GS und 6MWD.

Ergebnisse: Es wurden Zeiteffekte in den primären Outcomes gefunden, die unabhängig von der Gruppenzuordnung bessere allgemeine Leistungen in GS (p=0,016) und 6MWD (p=0,011) zeigten. Es wurden keine Interaktionseffekte beobachtet. Die tatsächliche VD-Serumkonzentration spielte weder für die Leistung noch für die Veränderungen der in dieser Arbeit untersuchten anthropometrischen Werte (Deltas) eine beobachtbare Rolle.

Schlussfolgerung: Die aktuelle Studie unterstützt frühere Befunde, dass ST die körperliche Leistungsfähigkeit von älteren Erwachsenen verbessert. Nach unseren Ergebnissen verbessert VD jedoch nicht die Effektivität eines ST-Programms auf die funktionelle Leistung und die Ausdauerleistung bei älteren Menschen

Table of Contents

List of figures

List of tables

Abbreviations

1RM: 1 repetition maximum.

5RM: 5 repetition maximum.

4mGS: 4 meters gait speed.

6mGS: 6 meters gait speed.

6MWD: 6 minutes walking distance.

6MWD: 6 minutes walking distance.

ANOVA: Analysis of Variance.

BIA: Bioelectrical impedance analysis.

BMI: Body mass index.

CON: Control group.

MM: Muscle mass.

RE: Resistance training

ST: Strength training

Sup: Supplementation

TUG: Timed up and go.

VDD: Vitamin D daily.

VDM: Vitamin D monthly.

1. Introduction

1.1 Ageing and physical activity

Many of today's societies are facing new challenges because the proportion and number of older people is growing. In fact, ageing is associated to health deterioration, partly linked to lifestyle habits, inappropriate diet, physiological strength loss, etc. which leads to functional limitations, lower quality of life and morbidity (World Health Organization, 2004). According to this latter organization, being physically active generally prevents older adults from various early mortality causes, such as coronary heart disease, high blood pressure, stroke, cancers, etc. Active persons have a higher functional health, a lower risk of falling (which is a major public health concern in that age group (Kannus, Parkkari, Niemi & Palvanen, 2005), and a lower risk of functional limitations. As the World Health Organization explained in 2004, the ability of older adults to maintain their functional capacities as long as possible is an important factor not only in containing the needs and cost of health services, but also to assure them a certain quality of life. Functional performance and quality of life are indeed linked to each other (World Health Organisation, 2004).

According to Bonder and Dal Bello-Haas (2018) functional performance is a wide ranging term, which takes account of the mobility, the lower and upper extremities functioning and generally to the capacity to sustain activity of daily living. In this thesis this term is associated with the physical ability, capacity or performance of an individual to carry out specific tasks that are supposed to be correlated to daily living activities. It has been established in 1995 that a bad functional performance can predict consequent disabilities on the midterm (Goldspink, 2012). Therefore, it is interesting to focus on how to maintain proper functional performance as long as possible. The possible direct reasons how ageing negatively impacts physical activity is shown in figure 1, inspired by World Health Organization (2004), Goldspink (2012), Young and Skelton (1994), Aagaard, Suetta, Caserotti, Magnusson and Kjær (2010), and Rantanen et al. (1999).

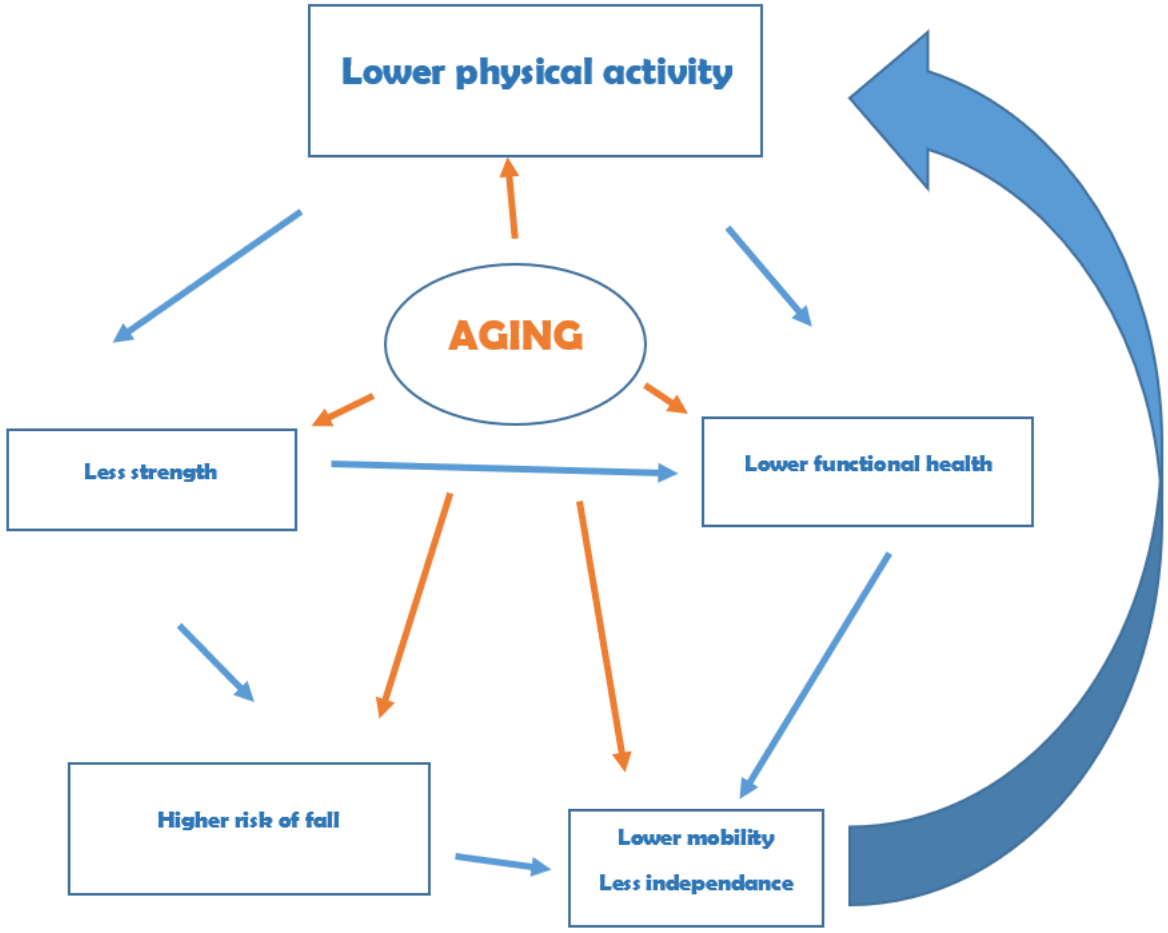

Figure 1: The possible negative impact of ageing on physical activity levels.

1.2 Muscle strength in the elderly

This previous statement is important to note, as one of the characteristics of ageing is the constant reduction of muscle strength, muscle power (Young & Skelton, 1994; Aagaard, Suetta, Caserotti, Magnusson & Kjær, 2010) muscle mass (MM) and muscle quality (Keller & Engelhardt, 2013), which often leads to a (pre-)sarcopenic state. Conforming to a consensus of the European Working Group on Sarcopenia (2010), sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal MM and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death (Cruz-Jentoft et al., 2010). In 2017, a meta-analysis highlighted the fact that non-sarcopenic patients are supposed to have a four times lower risk of mortality than the ones suffering from sarcopenia (Beaudart, Zaaria, Pasleau, Reginster & Bruyère, 2017). Thus, higher muscle strength should be able to preserve older people from disabilities for a longer period of time (Rantanen et al., 1999). In order to maintain muscle strength, strength training (ST) seems to be the matter of choice as it can fight against weakness, MM, frailty (Seguin & Nelson, 2003; Vikberg et al., 2019) and

avoid falls that often leads to important injuries or death in older population. (Uusi-Rasi, 2012). This reduced capacity of the muscle to generate strength can be in particular linked to the decrease of the type 2 muscle fibres size in the older muscle (Lexell & Taylor, 1998; Nilwik et al., 2013). Justly, according to that same paper, ST increases specifically those type of fibres, triggering therefore the muscle size and strength. It has been suggested that two or three training days per week are enough to gain MM and muscle strength, and indeed maintain independence and vitality in the elderly. Therefore, some studies involving older adults showed that a well-planned resistance training can prevent a global muscular frailty, improve strength and physical functioning (Peterson, Rhea, Sen & Gordon, 2010; Beaudart et al., 2017). The American College of Sports Medicine adds more specific guidelines, such as the number of sets per exercise (1 to 3) and the necessity to train all major muscle groups. Importantly, the progressive aspect of training needs to be considered, by allowing elderlies to start with lower intensities and lighter weights and carefully evolve towards heavier weights or/and faster concentric movements. Working with older adults always makes the safety concern a priority: according to Reid and Fielding (2013), adapting higher intensity to older and frail populations have been shown to be secure, adequate and well-endured. As muscle weakness is associated to poorer functional performance (Papa, Dong & Hassan, 2017) and as ST is suited to improve muscle strength, resistance training could thus be considered as a trigger to enhance functional performance of older adults.

As stated by Ades et al. (1995), functional performance and endurance capacity are closely linked to one another. As the following explanations and example suppose, muscle strengthening could possibly improve both. The next chapter will therefore focus on the influence of ST on endurance and its possible functional consequences.

1.3 Strength training's influence on endurance and functional performance

1.3.1 Endurance and aerobic capacity.

Endurance training has been proven to increase the level of functional capacity (Green & Crouse, 1995). Endurance is by definition "the ability to sustain an activity over a period of time" (Miller-Keane Encyclopaedia, 2003). Numerous factors such as physiological and anthropometric factors influence the endurance performance (Figure 2).

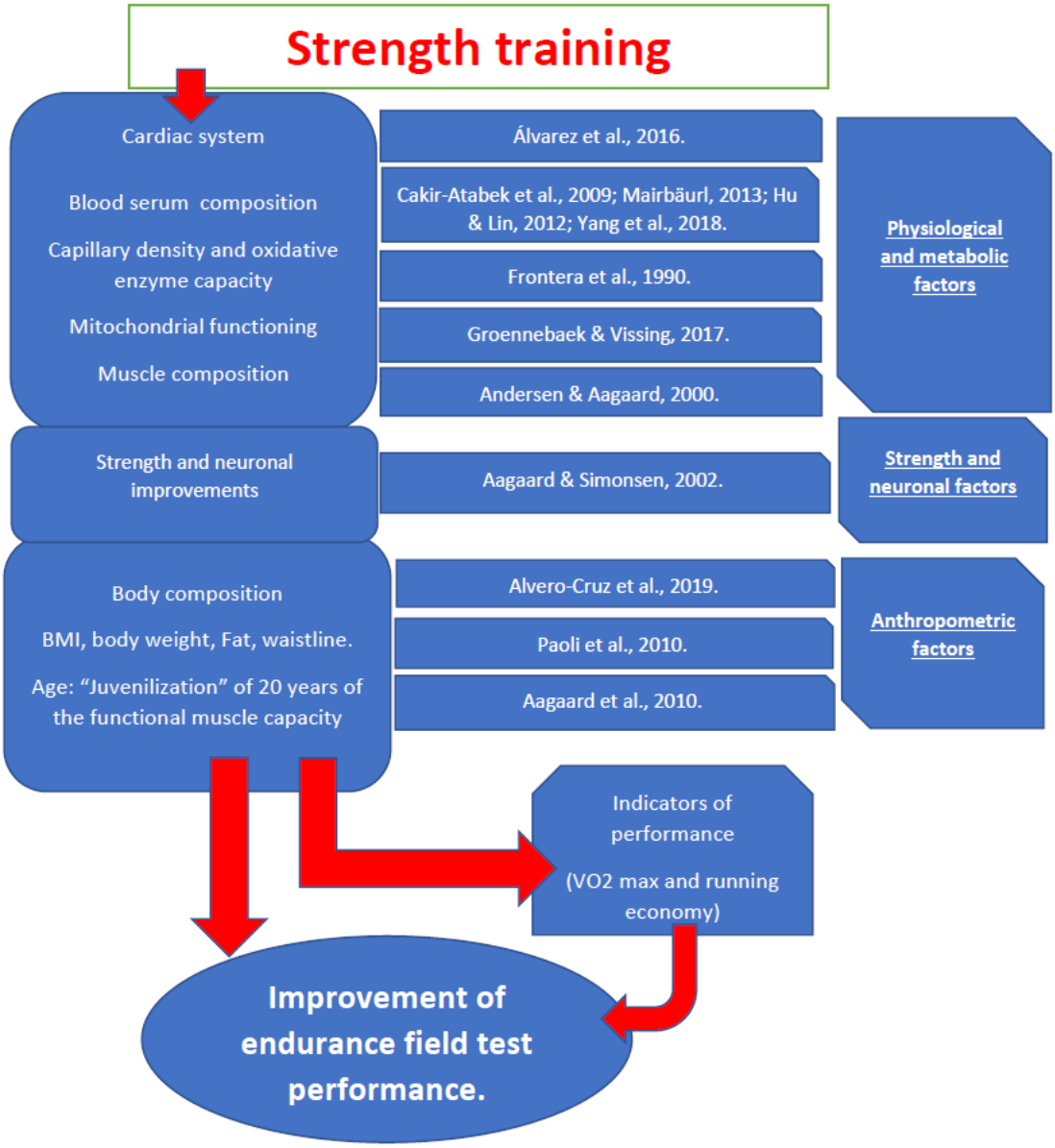

Figure 2: Possible influences of ST on endurance field test performance.

As aerobic capacity involves -by definition- the oxidative functioning of the organism, the capacity of the cardiovascular system to bring oxygen to the muscle is highly relevant among other physiological aspects influencing the endurance performance. It is tied to lung function, cardiac system efficiency, and red blood cells concentration and functioning (Buick, Gledhill, Froese, Spriet & Meyers, 1980; Mairbäurl, 2013; Yang et al., 2018). Furthermore, the blood microcirculation network inside the muscle cell (or capillary density), the oxidative enzyme work load, the type of cell and its oxygenation capacity (which is linked to mitochondrial activity and to oxidative enzyme activities) play important roles in endurance capacity (Adai & Montani 2010; Gollnick & Saltin, 1982; Klitgaard et al., 1990; Nystoriak & Bhatnagar, 2018).

As the longevity of an effort requires a constant supply of oxygen to the muscle, endurance is closely linked to the general aerobic capacity. Aerobic capacity is defined as the capacity of the cardiorespiratory system to supply oxygen to the muscle, and the capacity of the muscles to utilize oxygen (ACSM, 2013). Aerobic capacity is in fact considered not only to be the most essential part of physical activities (Rankovic et al., 2010), but also an important determinant of functional capacity in the elderly (Ades et al., 1995). Maintaining a certain level of aerobic capacity is indeed in line to a person old age's independent lifestyle (Fleg, 2012). As functional capacity is linked to general health in keeping with Callander, Schofield, Shrestha and Kelly (2011), we can therefore conclude that aerobic capacity has an important role in general health prevention. To estimate the aerobic capacity or general endurance capacity, some physiological measurable performance indicators exist. Aerobic capacity is generally estimated by measurement of the VO2max (or VO2peak), which is thus a central parameter for endurance performance (Jones, 1998). However, VO2max is not the only measurable indicator capable of estimating endurance performance. Some other parameters have proven their importance, especially in high endurance performance, such as the running economy (the VO2 required to run at a certain speed), the lactate threshold or others (Jones, 1998).

1.3.2 Possible influence of ST on aerobic performance

First of all, ST is able to influence relevant *endurance physiological factors***.** Thereby, ST is not generally associated to a gain in endurance capacity as it doesn't directly improve parameters such as the number of type 1 muscle fibres or mitochondrial density by increasing the general muscle volume (Chilibeck, Syrotuik & Bell, 1999). Gettman and Pollock (1981) stated that contrary to aerobic exercise programs, circuit weight trainings (which is an "aerobic-orientated" type of ST) could only help maintaining initial levels without improving the aerobic fitness. However, specifically for elderly people, counterarguments can be considered. Traditional high-load resistance exercise has been associated with an improved mitochondrial oxidative function and a muscle mitochondrial biogenesis (Groennebaek & Vissing, 2017). This could be explained by the stress generation resulting from an intense ST. In fact, the muscle energetic stress is according to Piantadosi and Suliman (2012), one of the physiological stimuli influencing mitochondrial biogenesis. This is in line with a study by Granata, Jamnick and Bishop (2018), seeing exercise intensity and volume as determining factors playing a role in the mitochondrial adaptations. Metabolically, ST influences *the muscle composition* by replacing the level of very fatigable Type IIX fibres with type IIA fibres (Andersen & Aagaard, 2000), which may thus reduce the general muscle fatigability and therefore improves general muscle oxidative capacity.

It is proven that ST affects the *blood cell composition* by enhancing the number of red blood cells, thereby improving oxygen supply to muscles during exercise (Cakir-Atabek et al., 2011; Mairbäurl, 2013). The changes seem to positively affect blood cell composition towards a better aerobic capacity in inactive men by increasing the haematocrit (Hu, Finni, Xu, Zou & Cheng, 2011). In a study by Yang et al. (2018), ST was shown to acutely influence haemoglobin and haematocrit levels. However, the long terms effects and health implication of ST on red blood cells status still needs to be better understood (Hu & Lin, 2012).

Moreover, the ST's influence on endurance performance can be linked to the *increased oxidative citrate synthase enzyme activity* (which plays a role in the Krebs circle) and *increased fibre capillarization* noted after a 12 weeks ST (Frontera et al. 1990). This is in line with a study from Andersson et al. (2017), which showed an increase in muscle oxidative capacity and in glucose tolerance with ST. Furthermore, *general heart function* can be influenced by ST. As reported by Álvarez et al. (2016), high intensity training produces heart rhythm adaptations in insulin-resistant patients by lowering beats per min during exercise, after exercise and at rest. In addition, ST *influences muscle factors and neuronal activation capacities.* This is essential as a certain amount of strength is required for any type of physical activity. As shown in figure 2, various factors influence an endurance performance. Among others, neuronal activations and muscle functioning are relevant. In fact, strength parameters must be taken into account as every type of performance – aerobic and anaerobic – requires a certain amount of strength to be conducted. Evidently, muscle strengthening exercises improve the level of resistance against which a performance can even start. This is highly relevant for older people, for which endurance performances can in fact be impaired just because of a lack of strength to even start it. Vincent (2002) claims that with a greater lower limb strength aerobic capacity can be increased, as the gain in muscle strength allows activities to be performed longer at a higher intensity. However, this seems to be true only in the older and weaker population, where a possible relationship between lower muscular strength and VO2peak is shown, supposing that the influence of leg strength on those aerobic improvements is linked to the initial level of strength. The lack of muscle strength could therefore possibly explain a plateau above which no other performance is possible. This is in line with older findings suggesting that the muscle strength deterioration can eventually explain the decrease in aerobic capacity in elderly (Braith & Vincent, 1999; Noakes, 1988; Flegg & Lakatta, 1998).

A decrease in muscle function is linked to neuronal modification in the muscle functioning (Macaluso & De Vito, 2004). However, with resistance training, an increase in muscle strength is generally linked firstly to neuronal improvements, especially in the elderly (Aagaard & Simonsen, 2002). This specific improvement in muscle functioning is one of the most important influence of ST as the intensity of training demands a higher degree of muscle activation as

other activities. According to Beattie (2017), the most important physiological parameters in cycling that indicates a level of performance (Cycling economy, power output at VO2max and the anaerobic function) are to some degree influenced by neuromuscular factor, and that ST is thus a way to improve it.

Those specific strength and neuronal factors mentioned above are highly relevant for the current thesis as they can be positively influenced by ST. Likewise, endurance-relevant anthropometric factors have been shown to be influenced positively by ST.

Indeed, ST has proven to influence positively *the body composition,* which influences the endurance performance (Alvero-Cruz et al., 2019). In fact, ST showed to influence the body composition: fat percentage, fat mass and body mass index (BMI) showed a significant correlation with race time ($P < 0.05$). After 3 different training programs, Sillanpää et al. (2008) demonstrated that the percentage of fat mass was reduced from 5 to 8%, as much in a ST program, as in the endurance program and the ST combined with endurance program. A high intensity training seems to be relevant as well to induce efficient changes in body composition: in a study by Paoli et al. (2010), the high intensity circuit training group showed the most notable anthropometrical changes throughout time: waistline, fat mass percentage and the body weight were reduced.

Age: correspondingly, in a paper relating the ultra-endurance performance (Lepers, Rüst, Stapley & Knechtle, 2013), the age after 40 years old is shown to influence highly the racing times, especially after 70. This is in consonance with studies by Fleg et al. in 2005, and Hollenberg, Yang, Haight and Tager in 2006 and by Jackson, Sui, Hébert, Church and Blair in 2009 in which the peak vo2max capacity has been reduced by over 20% every 10 years for healthy over 70 years old people. Even though age is not modifiable, the effect that time has on physical performance can be in some measure reverse in the older population: an explosive ST intervention study lasting 12 weeks by Caserotti et al. (2008) demonstrated how this type of training could improve women's capacity to limit age's influence on muscle function. The researchers even talk about a "juvenilization" of 20 years of the functional muscle capacity (Aagaard, Suetta, Caserotti, Magnusson & Kjær, 2010).

This influence ST possibly has on physiological, neuronal, strength and anthropometric factors could explain why ST interventions were associated to enhancement in endurance performance indicators. In fact, studies have shown improvement in those specific measurable performance indicators of endurance mentioned previously: in a study by Vincent et al. in 2002 for example, elderly persons aged from 60 to 83 years old have been assigned either to a control group (n=16) or to a specific type of resistance training (low intensity (n=24) or high intensity (n=22)). In addition to a muscle strength enhancement for both training groups, their

aerobic capacity (VO2maxpeak) and treadmill time increased considerably, reaching an over 20% improvement for every trained participant regardless of their workout intensity.

The running economy and the cost of running (concerns both the aerobic and anaerobic metabolism) of all groups going through a flywheel ST and a strength explosive program improved (respectively: Giovanelli et al, 2017; and Festa et al, 2019). The flywheel intervention even showed an improvement in 2 and 10km running times for the recreational runners assigned in the lower intensity group.

The theoretical transfer from ST towards endurance capacities is shown in figure 2.

1.3.3 Consequences for functional capacity

Endurance capacity is important to consider mostly because of the link it has with physical activities and functional capacity. This is well known for many years now (Ades et al. 1995): however, applying high performances testing to assess the endurance capacity linked to daily living activities in the elderly doesn't seems as relevant as the field tests (Marcinik et al., 1991).

Some studies have in fact shown the effect of ST on field tests related to endurance: among others, walking endurance post training performance is influenced by improvements in back strength after a resistance training intervention, in older obese adults suffering from chronic low back pain (Vincent, 2014). Just as Borges and Carvalho (2014) who found an improvement in a 6 minutes walking distance (6MWD) assessment for patient going under a ST during hospitalization.

When specifically focusing on functional field tests, the influence ST has is shown in other papers: the study from Beyer et al (2007) has proven strong improvement in functional capacities (performance in chair rise test) for older men after a 36-week training in which various exercises were associated with ST. An above 10% improvement in maximal walking speed, sit - to - stand test, and in stair climbing were shown in frail elderly women. This is in line with studies showing that strength related trainings in the elderly has improved improve 10m and 30m walking speed (Caserotti et al., 2008) and 12% gain in maximal horizontal walking speed (Fiatarone et al., 1994; Suetta et al., 2004).

In conclusion, ST has shown to improve endurance capacities through physiological, molecular and anthropometric factors, influencing the aerobic and metabolic capacities. Those improvements seem to be mostly relevant for older and frail people and have therefore shown functional improvement in specific field tests due probably at first, to the neuronal and muscular enhancement that avoided the low initial muscular strength to be a limiting factor.

However, strength and aerobic decline is not the only supposed characteristic of ageing explaining the negative consequence on functional capacities and on the quality of life. As specific body function and metabolic systems get older, the elderly person face various micronutrients deficiencies. VD deficiency is one of them. Yet, a sufficient VD level is considered important for general health and for a good muscle functioning. Lacking VD may induce various concerns.

1.4 Vitamin D

1.4.1 Main sources of vitamin D

VD deficiency is not only considered to be the most spread nutritional deficiency in the world but also to be positively correlated with morbidity and mortality (Khayyatzadeh, Bagherniya, Abdollahi, Ferns & Ghayour-Mobarhan, 2019). Sun exposure is by far the main source of VD (Calvo et al. 2005). Yet from October to March, at a 48° latitude (Vienna, Austria), there is not enough UVB radiation from the sun to endogenously build VD (Spiro & Buttriss, 2014). VD is synthesized from 7-dehydrocholesterol in the epidermis layer of the skin. For the process to be started, the skin needs to be exposed to a specific wavelength of ultraviolet B radiation. 7-dehydrocholesterol is then converted into Vitamin D3 through a cascade process (Ceglia, 2009).

The synthesis level is dependent on many various factors such as the sun exposure, the season of the year, the latitude, the degree of skin pigmentation, the age and the use of sun scream or specific clothes. According to Ross and colleagues (2011), for the same time and intensity of UVB exposure, persons with a *darker skin* have a significantly lower VD concentration in blood. *Sunscreen* is theoretically used to protect from sun UVB radiation. However, due to the way it is used (not on the total skin surface exposed to sun for example), sunscreen is not always considered as a direct influence on VD levels (Springbett et al., 2010). *Latitude* alone does not consistently predict the average serum 25OHD level of a population, as many other factors have to be taken into account: the height of the atmosphere (50 percent less at the poles), cloud coverage (more intense at the equator than at the poles), ozone coverage and the duration of sunlight in summer versus winter. Those are factors contributing to the complexity of the relationship. In fact, *the season* has shown to influence the cutaneous synthesis and the level of VD in blood as well (Lucas et al., 2005). By favouring greater UVB radiation on the skin, higher altitude is linked to a higher VD synthesis (Ross, Taylor, Yaktine & Del Valle, 2011). However, according to the same authors, many other factors such as genetic factors, the local air pollution, the cultural differences in clothing practices, the urban settings and the cloudiness of the weather play a role.

In this respect, *diet* is considered to be an important influencing component VD levels. Especially specific food such as fatty fish, eggs, bakery products, some types of mushrooms and dairy, are rich exogenous sources of VD (Rui, 2016). Facing the lack of VD in parts of the population, some national health authorities decided to supplement daily comestible products with VD: this was the case in Canada for example, where milk and margarine is fortified with VD (Ross, Taylor, Yaktine & Del Valle, 2011). The International Unit (IU) is used to measure the VD quantity in the diets or in supplementation. To quantify the level of circulating active 25OHD, nanomoles per litre (nmol/L) or nanograms per millilitre (ng/mL) are used, with 1 nmol/L equalling to 0.4 ng/mL.

1.4.2 Vitamin D supplementation and blood concentration

The level of response to a given supplementation seems to depend on various factors and is subject of continuous research: the route of administration (Zheng, Cui, Hong & Yao, 2015), origins of the person, the biological scope (Morley, 2019), the initial VD status (the higher, the lower is the response (Aloia et al., 2008)), the duration of the supplementation, the age of the subject and many more. The dosage timing is under investigation as well, as daily, weekly, monthly and annual dosage have been investigated to verify which is the most relevant strategy to increase the VD blood levels. The studies differ in their results, however Ish-Shalom et al. (2008) did not find differences linked to the dosage timing: they hypothesised that a higher dosage reduces a possible influence of timing.

Another affecting factor is the type and amount of food and of fat consumed aside VD sources. A bond has been established between those components and the absorption of VD even though no optimal dosage of fat intake has been detected (Weber, 1981; Johnson et al., 2005; Mulligan and Licata, 2010).

The specific VD form that is measured in blood influences the results as well. Calcitriol (1.25-dihydroxycholecalciferol) for example, when used for such, has limitations: blood level concentration is influenced by many other influencing factors besides supplementation. This specific substance is only detectable a few hours in the blood, which makes it difficult to assess the effect of the nutritive intervention properly. Therefore, the fifteen days lasting 25OHD substance seems more relevant: it is said to be the most used reference and it is considered to be the most effective biomarker of VD status (Uusi-Rasi et al., 2012). Further unclarified factors affect the relationship between the supplementation and the 25OHD blood levels (Ross, Taylor, Yaktine & Del Valle 2011). In fact, the molecular type of VD administrated has been under investigation as well. Between the two specific primary form of VD that exist (VD2

and VD3) the state of the research supposes that at higher doses, the VD3 supplementation is more effective to elevate serum levels than its counterpart.

1.4.3 Recommendations

As discussed above, VD deficiency is supposed to be the most spread vitamin deficiency in the world (Khayyatzadeh, Bagherniya, Abdollahi, Ferns & Ghayour-Mobarhan, 2019). In fact, 1 milliard people should have less than 20 ng/ml (50 nmol/l), and 88% of the world population is considered to be under 30ng/ml (75 nmol/l) (Holick, 2007). The same trends have been noted in Austria with more than 60% of the older population not reaching the recommended values of 25(OH)D above 20 ng/ml (50 nmol/l) (Österreichischer Ernährungsbericht, 2012; German Nutrition Society, 2012). Yet, Dawson‐Hughes et al. 2010 claims among other that this threshold should be raised to 30 ng/ml (75nmol/l). For this thesis, deficient, insufficient and sufficient VD levels will be defined as shown in table 1.

Table 1: VD levels categorisation defined as such in this manuscript

	ng/mL	nmol/L
Deficient	<20	<50
Insufficient	20-29.9	50-74.9
Sufficient	≥30	≥75
Based on the opinion of Österreichischer Ernährungsbericht (2012), Holick (2007), Ross, Taylor, Yaktine & Del Valle, (2011), Kotlarczyk et al., (2017).		

The recommendations to compensate the population's deficiencies are various. Therefore, the recommended intake of VD varies according to the country, to the initial serum levels and to the population concerned. In Australia for example, 200 IU/day is recommended for the youngest populations, and up to 400 IU/day for the elderly. (National Health and Medical Research Council). In the USA adults above the age of 70 years are advised to reach an intake of 800 IU/day (IOM (USA), 2011). If sun exposure is lacking, the European Food Safety Authority (2002) as well as the Nutrition Societies for Germany, Switzerland and Austria (2012) also recommended an intake of 800 IU/day to rise and maintain 25OHD levels above 20 ng/ml (50 nmol/l) and to counteract the lack of endogenous synthesis in the skin.

However, Aloia et al. (2008) insist with the fact that the VD supplementation must be thought according to the complexity of the relationship between intake and actual blood levels variations. In addition, it needs to be adapted to the individual initial VD status to reach sufficient blood concentrations of VD. For those under 22 ng/ml (55 nmol/L), a dosage of 5000 IU/day would be necessary to reach a level above 30 ng/ml (75 nmol/L), as daily dose of 3800

IU should be enough for those above 22 ng/ml (55nmol/L). According to the European Food Safety Authority (2002), supplementations have still to be understood as a secondary source of VD because the most effective process to rise the blood VD level remains a frequent sun exposure. Yet, as the skin morphology deteriorates with age and the 7- dehydrocholesterol levels are reduced with time, the VD synthesis is limited in older populations (MacLaughlin & Holick, 1985). In addition, *elderly people* may not have the same ability to assimilate VD when supplemented (Ross, Taylor, Yaktine & Del Valle 2011). This may explain - along with the fact that they are more likely to spend less time outdoor (Perry et al., 1999; Morley, 2019) - that older adults in modern societies are at higher risk of VD deficiency and therefore face greater consequences of low VD status, such as an increased risk to face fractures due to poorer bone metabolism. Those parameters need to be considered when supplementation strategies are investigated. Recommendations of 800 IU/day should therefore be re-evaluated according to Carlberg (2014) to 1000 IU/day if not more for people at risk. This is also supported by Holick et al. (2011) and Ross et al. (2011) claiming that at least 1500 IU/day would be necessary to increase and maintain 25(OH)D blood levels above the 30 ng/ml (75 nmol/L) threshold. Yet, in a paper from the Journal of Clinical Endocrinology and Metabolism, Holick et al., (2011) claims that if older persons are deficient, an initial 6000 IU/day during the first eight weeks is to be added to the previous recommendations.

Upper limits have been under examination as well and various opinions are discussed: even though a study by Jetty et al. (2016) found no negative impact of administering up to 100,000 IU/week for statin intolerant VD-deficient people, there may be danger of high doses. The Institute of Medicine (2011) states 4000 IU/day as the maximal intake recommended. If higher dosages are administered for a longer period of time, the high VD plasma concentration may cause VD toxicity which can result in hypercalcemia, vomiting, abdominal pain, dehydration amongst others (IOM, 2011). However, for that to happen, concentration levels would have to reach levels above 50 ng/ml (125 nmol/l) (Marcinowska-Suchowierska et al., 2018). Serum 25(OH)D concentrations considered to be toxic have to reach 150 ng/ml (375 nmol/l). This would be possible with an approximately 10000 IU intake per day. Negative effects of higher doses (60000 IU/month) when compared to lower doses (24000 IU/month) on functional mobility have been found by Bischoff-Ferrari et al. (2016). Concerning other specific upper limits, the European Food Safety Authority (2002) fixed thresholds for younger children of 2000 IU/day and for teenagers and adults of 4000 IU/day. The National implementation was examined in 14 different European countries in which even high consumers have been noted to be under the recommended threshold (Agostoni et al., 2012).

1.4.4 Vitamin D metabolism

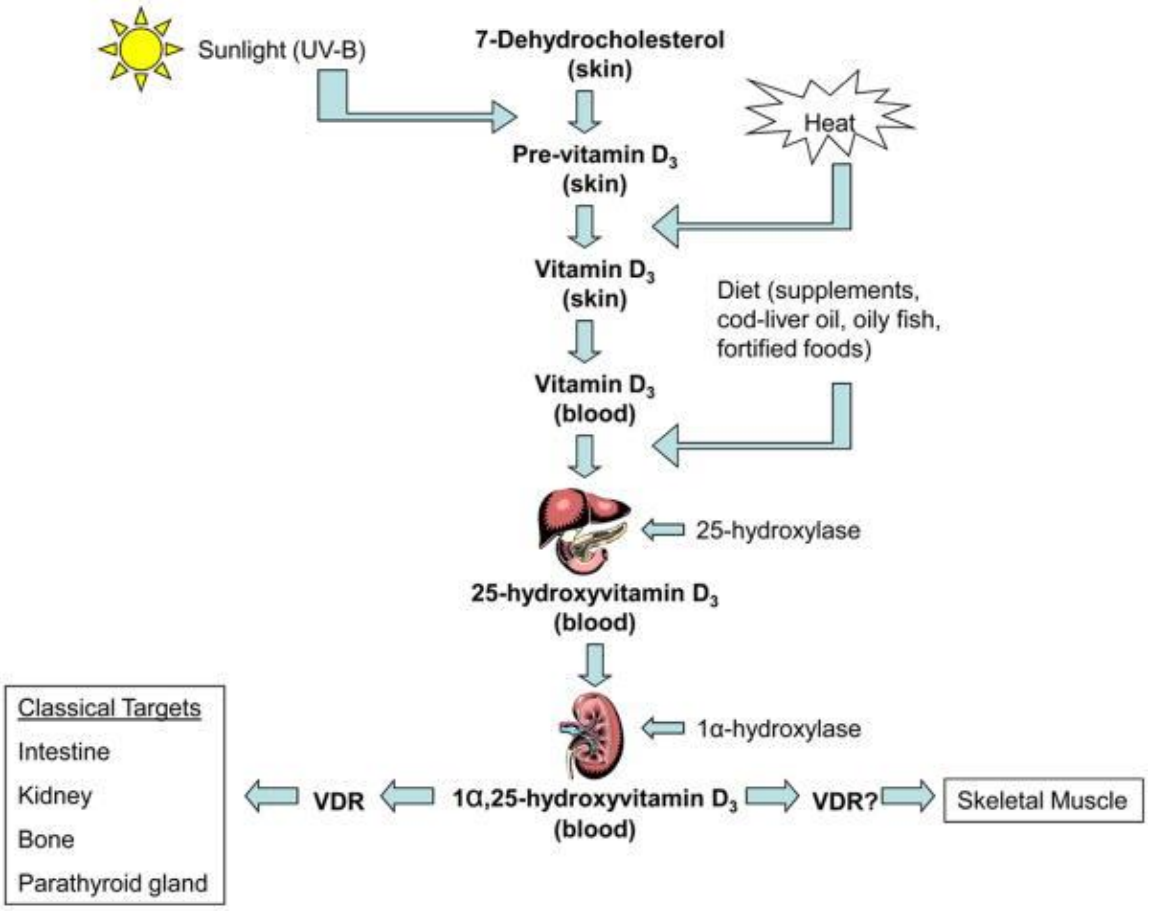

Figure 3: Vitamin D and Its Role in Skeletal Muscle from Ceglia, 2009.

The metabolic pathway of VD is described above in figure 3 from its pre-hormonal form to its most functional form (Calcitriol or $1,25(OH)_2$ VD). It involves the liver and the kidney, to then target tissues such as bones, intestine, kidney, skin and muscle cells (Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013). The VD can also be stored in the organism either into adipose tissues, or in skeletal muscle (Ross et al., 2011). As explained above, the capacity of the body to produce VD_3 in the epidermis layer of the skin is primarily subject to the UVB radiation on the skin surface and the availability of 7-dehydrocholesterol (Ross, Taylor, Yaktine & Del Valle, 2011). VD has no biological effect before is goes through the liver and the kidney hydroxylation's process. Thereby it is transported in the blood by a VD binding protein. Both liver and kidney degradation stages are facilitated by specific Cytochrome enzymes (CYP) that firstly convert VD into 25-hydroxyvitamin D and then into an active hormone, $1,25(OH)_2$ VD.

1.4.5 Roles of vitamin D in the body

The major role of calcitriol is to elevate the calcium and the phosphate levels in the plasma. As they both are vital for a good bone health, VD is therefore considered traditionally primarily for its influence on osseous metabolism (DeLuca, 1979). In addition, the neuromuscular junction, the nerve transmission, the hormonal secretion and the vasodilatation benefit from a higher plasma calcium too, making VD an indirect influencer of various physiological processes. VD is seen as influencing the immune system (Ross, Taylor, Yaktine & Del Valle, 2011), playing a role in inflammatory processes (Barbalho, de Alvares Goulart & Gallhardi Gasparini, 2019) and for cancer cell inhibition (Deeb, Trump & Johnson, 2007). The presence of the VD receptor (VDR) in cells, makes researchers claim that there is a targeted function of VD on those specific cells, even though the precise action is not always elucidated. In fact, VDR is present in calcium and phosphate regulating cells, in T cells involved in the immune system (Ross, Taylor, Yaktine & Del Valle, 2011) and in muscle cells (Bischoff et al., 2001).

1.5 Vitamin D and muscle function

In fact, VD is physiologically influencing the muscle cells and therefore muscle functioning via two mechanisms:

Firstly, through a molecular mechanism, in which VD's active form 1,25 $(OH)_2$ can bind to specific muscle cells nuclear receptors and thus influence expression of various genes. There is growing evidence that VD is able to up-regulate actin and myosin protein synthesis. Furthermore, 1,25 $(OH)_2$ has an activating role in the mitogen-activated protein kinase (MAPK) inside myoblasts, which shows an influence on gene expression linked to proliferation and differentiation of muscle cells (Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013).

The second mechanism is characterized by the binding of 1,25 $(OH)_2$ to non-nuclear VDR, leading to complex intracellular signal transduction pathways influencing various parameters inside the muscle cell such as the phosphate and calcium uptake, thereby playing an important indirect role in muscle contraction (Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013; Rui, 2016). As a matter of fact, the indirect role of the VD on muscle function seems very important, as calcium plays (as said above) a major role in muscle contraction through three main mechanisms: firstly, calcium plays a role in the nerve transmission is relevant as the muscle function is dependent on the nervous system. Secondly, through its activity inside the muscular junction allows the contraction signal to reach the muscle contractile units (Van der Kloot, 1978). Finally, by binding inside the muscle cell to troponin C, calcium plays an essential role in the contraction-relaxation circle. It allows the myosin head to be detached from the actin

complex which permits a new binding, resulting in a possible shorter and more contracted muscle (Szent-Györgyi, 1975).

1.5.1 Vitamin D, physical performance and supplementation

In recent cross-sectional studies, VD baseline status has been shown to be associated positively to physical performance and negatively to frailty status and strength decline in the very old (Beaudart et al., 2017, Vaes et al., 2019; Granic et al., 2017). In addition, in a study by Bischoff-Ferrari et al. (2004) an association between VD status and lower extremity function regardless of the participants' activity level was found. A recent meta-analysis described a correlation between 25OHD (pre-active form of VD in the blood) and walking speed among adults (Annweiler et al., 2017). This is in line with other studies in which frailty status, timed up and go (TUG) and gait speed (GS) were correlated to a VD blood levels (Vaes et al., 2019), GS as well as handgrip strength was particularly linked to lower VD statues for older males (Granic et al., 2017).

There seems to be a consensus concerning the fact that the initial VD levels correlate with functional performance: however, as correlation does not imply causation, it doesn't mean that the VD supplementation would help deficient people to perform better. In a recent provocative paper, Morley (2019) states that VD potential beneficial effects are over-rated, as there might be a link that healthier people are more outside, therefore more exposed to the sun light and as a consequence probably more active too. Therefore, cross sectional studies tried to verify possible effects of VD supplementation on muscle function and tried to find out who would benefit from a supplementation. The results are much more contrasted:

In randomized control trials (RCT) after a one year follow up study no difference in muscle strength between a VD-supplemented group and a control group were found, even though the participant were given 20000 IU twice a week (Grimnes, Emaus, Cashman, Jorde, 2017). In another study, VD supplementation that was able to enhance VD blood concentration of sedentary men aged between 65 and 90 years did not lead to any identifiable improvements in functional tests. The authors concluded that the results did not support VD supplementation as a viable solution to prevent physical function decline (Levis & Gómez-Marín, 2017). Ranathunga et al. (2019) ended up with the same conclusion, as even though the high monthly doses administered (12000 IU, 24000 IU or 48,000 IU) during one year helped to increase the fasting plasma concentration of 25OHD of the three groups, no effects neither on TUG times nor on handgrip strength were found in an over 70 years old population. A meta-analysis from Tabrizi et al. (2019) investigating twelve RCTs found similar findings about TUG times, handgrip strength or back muscle strength, concluding that VD supplementation does not influence

muscle functionality. Hansen et al. (2015) found that-compared with placebo a high-dose cholecalciferol did not translate to better physical functioning or fewer falls.

Those results are somewhat surprising, considering the numerous roles VD is supposed to have in the musculoskeletal system according to the general literature (Moon et al., 2019) and according to a specific recent article from Dzik and Kaczor (2019) concluding that VD deficiency is possibly influencing the mechanism that lead to muscle atrophy. Sato, Iwamoto, Kanoko and Satoh (2005) found likewise an increased relative number and dimension of type II fibres following a VD treatment in older females, which is supposed to be caused by the three to six month 1-hydroxyvitamin D supplementation.

Some other findings are more shaded: when compared to a control group, a small effect of VD supplementation did improve muscle strength of elderly women, but it did not lead to better TUG times or mobility parameters (Abshirini, Mozaffari, Kord ‐ Varkaneh, Omidian & Kruger, 2019). Yet, some outcomes testify a possible influence of VD supplementation on functional parameters as well: a meta-analysis (Dewansingh et al., 2018) noticed a general small but significant improvement in functional tests after giving participants VD supplementation for several months with higher doses leading to better results.

In two other studies (Bray et al., 2008; Levis & Gómez-Marín in 2017), the same trend appeared: improvement in the Short Physical Performance Battery tests (SPPB), fast GS and VD levels were shown for the insufficient VD baseline participants (< 30ng/ml (75 nmol/L)). This inclines to think VD supplementation to be at higher relevancy to improve physical function when addressed to initially physically weaker people or VD deficient populations.

We can therefore admit that investigations will tend to show that the poorer VD initial levels are correlated to poor level of functional fitness. However, cross sectional supplementation studies show a large variety of results: the level of supplementation required to reach a satisfactory VD blood concentration and its influence on functional performance remains unclear. Yet, if VD supplementation does have an influence on functional performance, it seems to be true only for deficient or weaker population (Morley, 2019).

1.5.2 Vitamin D and ST.

To maximize the possible effects VD has on muscle functioning, it would be interesting to consider the combination between ST and VD supplementation. One of the physiological reasons why this combination could have additive effects is that VD is involved in the recovery of muscle (Moon et al., 2019) and promotes myogenesis inside the muscle cell after muscle

wasting conditions (Garcia, King, Ferrini, Norris & Artaza, 2011; Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013). As ST is leading to a muscle wasting/recovery process, it is plausible that the effects would be shown more clearly if VD would be given as a supplementation during a ST program, to optimize its positive effect on muscle tissues and therefore on muscle performance.

1.5.3 Literature overview – Effects of Vitamin D supplementation and resistance training

To check already published studies on ST and VD supplementation a short literature review about studies performed during the last 15 years was done. Studies were identified on PubMed, using the advanced researched parameters in the title and abstract sections: “exercise” and “vitamin D” were used as search terms and “Clinical Trial” and “Aged: 65+ years” were used as filters. The titles from the ones written after 2005 were read to determine whether or not the articles discussed studies with ST intervention included next to VD supplementations and if they were focusing on the outcome measures used in the thesis (TUG, GG, 6MWD, muscle mass, body fat, physical function). Information about the remaining nine studies, their VD supplementation and resistance training protocols and their outcome results, can be found in table 2.

- Enhancement of VD levels and anthropometric factors

This systematic review shows a general VD enhancement due to the interventions: Fielding et al. (2017) with a daily consumption of 800 IU VD managed to enhance plasma levels from VD insufficient elderlies (<25 ng/ml) by 7.4 ng/ml for the supplemented group. Similar results were observed by Seino et al. (2018) as supplementation significantly increased the mean serum level of 25(OH)D by 4.7 ng/mL, 95% CI: 1.6-7.9) even though the daily consumption was only 200 IU.

In the study from Bell et al. (2017) plasma levels of 22.4 ng/mL (57.1 nmol/L) were achieved for the supplemented group (1000 IU after 19 weeks ($P< 0.001$) whereas no changes were observed for the control group ($P< 0.05$). The same trend was observed in a study in which participants received 800 IU/day (Uusi-Rasi et al, 2015). The average serum 25(OH)D plasma level increased in the supplemented groups from 25.1 ng/mL at the start, to 37.0 ng/mL after two years while no significant changes were found in the control group. Concerning the effects on anthropometric factors, VD combined with resistance training seems to influence positively the fat free mass (FFM). This is verified in various studies: as compared with resistance

exercise (RE) only, RE and supplementation significantly increased FFM (gain of 1.7 kg, $p < 0.001$), relative skeletal MM (Rondanelli M et al, $p = 0.009$), appendicular MM ($p < 0, 05$) of elderly sarcopenic adults (Yamada M et al., 2019), and whole-body, appendicular and leg lean soft tissue mass of community-dwelling adults (Seino et al.,2018).

Table 2: Overview of RCTs that incorporated vitamin D supplementation and resistance

Author, year (ref)	Age (Ø)	Groups (n)	Sex (F/M), Fitness, Health	Vitamin D						Resistance training			Outcome results
				VitD & Ca	Protein intake	Extra amount	Timing	Sup day	Placebo	Frequncy and study length	Intensity	Training	
Yamada M et al., 2019	84	-Ex+Nu (28) Ex (28) Nu (28) Co (28)	Community dwelling	800 IU (20 µg)	10.0g WP	Ex+Nu Nu groups for 12 weeks	NA	daily	YES	12 wks.	8 exercises. 3 sets. 20 reps for each exercise.	RE Slow mvt BW or an EB. +Home exercises daily	Ex+Nu inc. appendicular MM (P < 0.05), (sarcopenic older). No change from pre for any grp p=0.09 for the 5-m maximum walking time
Seino et al., 2018	73	RE+VD+DP (n = 41). RE only (n = 41).	NA	200 IU VD	Dairy protein (10.5 g/day)	NA	NA	daily	Yes, RE only	2x/wk. for 12 wks.	NA	RE	RE+DP+VD and RE: -Maximum GS and TUG imp Prot+VD inc. MM. No inc imp in functional outcomes.
Englund et al., 2019	79	Ex+VD Ex (149 in 2g)	M-I+VD ins F/M (46.3%/53.7)	800 IU VD	20g	No	NA	daily	Yes, No VD	3x/wk. for 6 months.	NA 15 to 17 (BS)	L RE	At the end: 2 int grp had better body composition, and thigh composition. VD supp: - lower intermuscular fat (p = 0.049) -higher muscle density (p = 0.018).
Fielding et al., 2017	79	Ex+VD Ex (149 in 2g)	F/M (46.3%/53.7) Unt,. M-I+VD ins	800 IU VD	20g	NA	NA	daily	Yes, No VD	3x/wk for 6 months.	NA 15 to 17 (BS)	L RE	Both gps imp in GS time. No difference between EX+VD and Ex only. (p=0.06)
Bell et al., 2017	73	Ex+ Nu (25) CT (24)	Men (49) Unt., Healthy	1000 IU 400 mg calcium (6+12 weeks).	30 g twice/ day	NA	500 IU 1h after bf + 500 IU 1h prior to bed.	daily	Yes, No VD	2/wk. for 12 wks.	65% 1RM 4 ex, 3 sets, 10–12 reps To 80% 1RM 4 ex, 3 sets 6–8 reps	First 3 wks: familiarization. To the end: intensification.	Time-dependent increases in TUG, 6 min walk test P < 0.001; no changes after P1; Imp after P2. The TUG time decreased by ~0.3 s (SUPP: -7% and CON: -3%; P = 0.004), + 6 min walk test inc by ~25 m (P < 0.001). 2%; CON = 92 ± 2%.
Kirsti Uusi-Rasi, 2015.	74	CT (102) D+(102) Ex+(103) D+Ex+	Home-dwelling women 409	800 IU (20 µg)	NA	NA	NA	daily	2 Placebo grp = maintain	P1: 2/wk. (12 first months) P2: 1/month	30% to 60% (1RM) to 60% to	Pin-loaded Weight machines,	-VD without **ex associated with no diff physical functioning.**

		(102)						their pre level of pa	(Remaining 12 months).	75% (1RM) (METs).	Pulleys, and FW.	-Exercise imp. physical functioning. TUG time deteriorated significantly more in the VD alone compared with the placebo alone (P = .01)
Bunout et al., 2006	76	RE+ VD+(24) VD+Ca+ (24) Re+Ca+ (24) Ca+ (24)	-(F/M) 10/86 -Healthy, VD deficient	VD 400 IU + Ca: 800 mg Or Ca: 800mg	NA	NA	NA	dail y	Yes	2/wk/ 9months	Intensity individually set by coach (according to BS and progression)	Chair stands, modified squats, step-ups in a stair arm pull-ups (3 progression steps) using EB	-Trained subjects had significant imp TUG (more in trained subjects supp with vitamin D) After follow up: GS higher for supplemented groups than non-sup. (838 ± 147 and 768 ± 127 m/12 min, p = 0.02).
Rondanelli M et al., 2016	80	Dietary supplement group (n = 69) Placebo group (n = 61)	F/M (77/53) Sarcopenic	100 IU	whey protein (22 g)	NA	NA	dail y	Yes	5 times/wk. for 12 wk.	~12–14 on BS	≤8 rep /mvt/ex (~5min)	Ffm inc in int group: p<0.001. No change in the placebo: p= 0.316 Compared with pa and placebo, sup + pa inc Ffm (1.7kg gain, P < 0.001) % skeletal Mm (P = 0.009).
Oesen et al., 2015.	83	RT (41) RTS, (36) CT (40)	F/M (133/14) Healthy	800 IU	20.7 g protein	After each training session, same supp amount	Morning	dail y	Yes	2/wks (on non-consecutive days) for 6 month.	Adapted EB	P1: 4 wks low external RE P2: 1-2 ex /6 muscle grp 1 set 15 reps. (60min)	Time effect on: 6 MWD and GS time (p<0.05) No supplementation effect on performance.

Ref (reference), n (group sample size), F/M (females/males), Sup day (supplementation day), VD (vitamin d supplementation), PS(2), CT control, Supp (supplemented group), Dairy protein = DP (HRmax),HR ; M-l (Mobility-limited), VD ins (insufficient), EB (Elastic Band), Ffm (Fat free mass), MM (Muscle mass) BW (Bodyweight), P1 (Phase 1), P2 (Phase 2), NA (not available), bf (breakfast), pre (pre-intervention), post (post-intervention), g (grams), M+E (morning and evening), TD (training days), w (weeks), d/w (days per week), U (upper body), L (lower body), LBM (lean body mass), Wks (weeks), RT (Resistance training), CT (Cognitive training), FW (Free weight), BS (Borg Scale), pre (pre training), pa (physical activity), Unt (untrained), inc (increase), imp (improve), grp (group), fqcy (frequency), supp (supplementation).

- Training and functional outcomes

In most of the included studies, training has been the main parameter explaining an increase of functional outcomes. Interestingly, time effects (independent of group allocation) have been observed for GS (Seino et al., 2018; Oesen et al., 2015; Fielding et al.,2017; Bell et al.,2017), 400-meter walk (Fielding et al., 2017), 6MWD (Oesen et al., 2015; Bell et al., 2017) and TUG (Bunout et al., 2006). Nevertheless, no change from baseline for any of the intervention groups (exercise and supplementation, exercise alone, supplementation alone and control) were observed for the 5‑meters maximum walking time (Yamada et al., 2019).

- Interaction effects

When the focus is set on the influence VD supplementation on outcomes after a RE, only Bunout et al. (2006) observed a time*group effect concerning a change in TUG time. It was concluded that training is associated with a better TUG performance after VD supplementation compared to training performed alone. In contrast, Uusi-Rasi et al. (2015) found that in their experimental design, TUG time was increased for the group having VD supplementation only compared to the placebo group that had neither supplement nor training. This latter study shows that the VD supplementation only influences the 12 minutes walking test. Bunout et al. (2005) identified a VD supplementation-dependent influence on 12 minutes walking test: independently from the participation in training, the subjects being supplemented had better 12 minutes walking distances at the end of the follow up. However, most of the other studies including the paper from Kirsti Uusi-Rasi et al. (2015) revealed no association between “VD supplementation without exercise” and changes in physical functioning.

It is important to note that even though for those studies VD was given every day to the participants, they used various type of VD supplementation quantity, from 100 IU/day to 1000 IU/day. The length of supplementation varies as well from 12 weeks to 2 years, and nutrients accompanying the VD supplementation were heterogenic. Nevertheless, the available information about the effects of VD shows that the supplementation strategies and dosages used in various studies quoted above were effective to impact the 25(OH)D level. However, most studies - with the exception of Bunout et al. (2006) – did not show an effect of supplementation on physical functioning, irrespective of VD was added to a RE program or not (no additive effect detected). Yet, VD is positively associated with significant improvements in body composition parameters and seems to enhance the positive effect of training on FFM. The quality and the intensity of the resistance training is difficult to assess as well, as some studies have been combining resistance exercises with other type of exercises (balance for example), and they used free weights, bodyweight or elastic bands as tools. The progression

and intensities were assessed by either subjective (Borg scale) or objective parameters (number of repetitions). Nevertheless, the positive functional outcomes of training have been proven in many of those studies. For a combined effect of VD and ST, the same ambiguous conclusion was noted by a meta-analysis conducted by Antoniak and Greig (2017). They found that taking VD supplementation in addition to a ST helps improving lower limb strength compared to control groups going only through a ST program. However, surprisingly, no effects on functional performances were shown. As suggested in the conclusion of this meta-analysis further evidence would be needed.

1.6 Aims

The aims of this study are therefore to investigate the link between VD supplementation, VD serum levels, functional performance and anthropometric outcomes after a ST program. The first step has been to determine whether one of the two VD supplementation strategies would influence the VD plasma levels and whether these supplementation strategies have any impact on TUG, 6MWD and GS test results after a 10 weeks ST program. In addition, the influence of the intervention on secondary outcomes (BMI, weight, relative and absolute body fat, and MM) is investigated.

2. Methods

2.1 NutriAging project

With the aim to establish clearer nutritional and behavioral guidelines for the elderly population in Europe, the EU-funded, bi-national project investigating the influence of nutrition and physical activity on ageing is to be conducted. The NutriAging Project makes the Comenius university of Bratislava (project cross-border leader) and the University of Vienna (project leader) cooperate on studies focusing on micro- and macronutrients such as protein, calcium, omega 3 fatty acids and VD intake (Wagner & Muchová, 2019). This paper is based on the study performed in 2019 investigating how VD status and intake combined with ST affect health status and physical performance of an older Viennese population.

2.2 Study design

The study was designed as a randomized, placebo-controlled, double-blind intervention trial. All the participants were randomly assigned into 3 different groups: the first one received

placebo preparation consisting of a calcium supplement (CON), the second one received calcium and VD daily (VDD) and the third one received in addition to calcium a higher monthly dose of VD (VDM).

As the aim was to determine the effects of VD supplementation alone on physical performance, all the groups went through 4 weeks of only VD supplementation (with pre and post investigation: T1 and T2) followed by a 10 weeks progressive resistance training program (with pre and post intervention investigation: T2 and T3).

2.3 Study procedures/timing

The first examination took place before start of the study (T0) with all the interested elderlies to be examined for eligibility considering the inclusion and exclusion criteria. At the beginning of the study (T1) the participants went through a general assessment that provided baseline data before any intervention. Between the first (T1) and the second (T2) investigation, the participant went through the nutritional intervention according to the group they were assigned to. Therefore, T2 provides only the data about the effect of the placebo's intake, VDD or VDM. The data that examines the combined effect of VD and ST is gathered at T3, after a 10 weeks ST program.

2.3.1 Assessment schedule and organization

Every participant had to go through various examinations at the Centre of Sport Science and University Sports (Auf der Schmelz 6, 1150 Vienna, Austria) before (T1), and after both the nutritional only intervention (T2) and the ST intervention (T3). Blood sampling, anthropometric values and physical performances from each individual were assessed within one week at the indicated time points.

This large number of examinations and those 3 different testing phases required a precise organization: the participants were therefore divided in 7 subgroups named from "A" to "G". The "A" group went through all their examinations the first week of each phase, the group "B" the second week, and so on until group "G" went through all their assessments as well. The beginning of each week was generally used for blood and anthropometric assessments and to familiarize the participants with most of the physical tests. The end of the weeks was left to assess the various tests. Many assessments and tests have been performed, this paper however focuses only on three of the physical performance tests which are analyzed and described precisely in this paper. The TUG, GS (4 and 6 meters) and 6MWD.

2.3.2 Study sampling

At first, the Institute of Sport Science of the University of Vienna made their study known by online, newspaper and radio announcements targeting the elderly population in Vienna and surroundings. The interested persons came to at least one information meeting in January or early February 2019 at the Centre of Sport Science and University Sports. After a short presentation of the study, the potential participants had one week to decide whether they were willing to be involved in the study. To widen the number of potential participants, information sheets were given to the ones attending the meetings to be shared with relatives that could be interested in taking part of the project. To discuss possible specific questions and to control for eligibility, the motivated subjects were invited to plan for their first individual appointment with the study physician.

2.4 Participants

To be part of this study, every motivated candidate had to give a written consent after having received clear information about risks, benefits, purposes and general procedures of the study. They had on the one hand to fulfill all the inclusion criteria: they were accepted if they were between 65 and 85 years old, living in or close to Vienna, in good mental health (Mini-Mental-State above 23) and physically independent (not being dependent of any walking assistance nor receiving home care or living in a retirement home).

On the other hand, meeting any of the following exclusion criteria would not allow them to participate:

- Initial VD levels above 30ng/ml (75 nmol/l).
- Specific diseases or illness contraindicating intensive ST such as diabetic retinopathy, osteoporosis or osteopenia with VD and/or calcium substitution, disturbance of the calcium levels, disorder of the parathormone level (calcium regulation), kidney disease, kidney stones, intake of diuretics (thiazides), regular intake of cortisone or antibiotics (last six months), severe cardiovascular disease (decompensated chronic cardiac insufficiency, extreme or symptomatic aortic stenosis, instable angina pectoris, untreated arterial hypertension, cardiac arrhythmia, intake of cardiac glycosides).
- Frailty index above 2.
- Regular resistance exercise training for more than once a week in the last 6 months.

2.5 Randomization and blinding

Once every criterion has been validated by the study physician, every participant has been randomly assigned to one of the three specific intervention groups. Concerning the group allocation, participants and conductors of the analysis were blinded to the assessments and intervention. This diminish the likelihood of change in behaviors that would result from the awareness of the group allocation.

Influential variables such as age, the VD status and gender have been considered by the randomization program from the Medical University of Graz for stratification to ensure a proper arbitrarily group allocation according to the group age, VD status and gender.

2.6 Ethic and legislative objections

The Vienna University Ethics Commission did approve the present study protocol (Reference number: 00405). The project and the specific study are following the Austrian laws (Among which the Data Protection Act, the Doctors Act and the CISA), the declaration of Helsinki (World Medical association) and the ICH-GPC guidelines.

2.7 Interventions

2.7.1 Nutritional Intervention

The study included two different interventions: a nutritional intervention and an exercise intervention. The different tests were conducted before (T1) and after (T2) the nutritional intervention only and separately after (T3) the combination of both interventions.

The nutritional intervention consisted of 3 different intake dosages of VD (VDD, VDM and CON). The subjects had dosage containers for 28 days, which was renewed when needed. It started at T1 and every participant had to take their dosage according to the allocation group until the end of the study (T3):

-The VDD group received 800 IU of Cholecalciferol (which are commonly known as Vitamin D3 tablets) and a 200 mg calcium tablets to be taken every day.

-The VDM group had two 20000 IU and two 5000 IU capsules of Cholecalciferol once per month at the laboratory and a 200 mg calcium administrated every day.

-The CON group took two pills every day containing 200 mg of calcium each day.

The Calcium tablets were purchased from Mamisch GmbH Prorenal (Ludwigshafen, Germany), the cholecalciferol was provided by Vitactiv Natural Nutrition, FeelGood Shop BV, (Venlo, Netherlands), and the VD capsules by Gall-Pharma GmbH (Judenburg, Austria). Every group had different intake or dosage; however, they all went through the same exercise intervention.

2.7.2 Resistance training and exercise intervention

The training took place in three different fitness gyms in Vienna. They all were accessible by public transport and located in different areas of Vienna (22th, 20th, 14th (Holmes Place) and 3rd (Star Fitness) district). The ST was divided into 2 main parts: the familiarization and the progressive resistance training (or intensification).

Familiarization training

The familiarization consisted of four training sessions during the first two weeks, which allowed the participant to familiarize with the specific exercises, to learn a correct execution technique and to estimate the initial training weight for each exercise. For this first phase, weights for each exercise were selected according to either a certain percentage of bodyweight or an estimated low intensity weight (Table 3).

Table 3: Familiarization phase

Exercise	**Box Squat**	**Leg Curl**	**Lat Pulldown**	**Dumbbell shoulder**	**Leg Press**	**Front Plank**	**Rowing**	**Chest press**
Women	3 to 5 kg	15% of BW	25% of BW	2 to 5 kg	30% of BW	5 reps/leg with hands on a box	25% of BW	5 to 10 kg
Men	5 to 10 kg			3 to 5 kg				10 to 20 kg
BW=bodyweight, reps= repetitions								

Lower resistance and higher repetitions allowed better learning of a correct and safer technique. Every subject was introduced to four out of the eight exercises the first day, introduced to the other four the next training day. The training routine on the third training day included all exercises with 2 sets/exercise.

5RM

To estimate the 1 repetition maximum (1RM) before the intensification program, an adapted 5RM evaluation was conducted for the leg curl, lateral pulldown, leg press and chest press: they are frequently used to assess maximal strength and rely to the specific muscle strength (Peterson et al., 2010). To keep the session short enough and as the muscle involved are similar, the lateral pulldown was considered to estimate rowing intensity as well.

The participants were mostly unexperienced in ST, thus the 5RM was not conducted for exercises that depend upon a specific multi joint technique such as the goblet squat (Flanagan, Salem, Wang, Sanker, & Greendale 2003) or movements that may lead to injury if done with heavier weights (dumbbell shoulder press). Therefore, the 4 exercises assessed were A2 (leg curl), B1 (lateral pull down), C1 (leg press) and D1 (chest press).

This evaluation happened during the third training day. A proper specific warm up was necessary for each exercise as 5RM is a very intense effort. In fact, 5RM should represent approximately 85% of the 1RM. The way the 5RM has been assessed for each exercise during this particular session is described as follows in table 4:

Table 4: Testing the 5 Repetition maximum procedure

	Set	Repetitions	Rest	Weights
Warm up	1	10 to 15	1 minute	Estimated weights for 20RM
	2	5 to 7	2 minutes	Estimated weights for 10RM
To 5RM	3 (+n if necessary)	5*	At least 3 minutes between each set	Estimated weights for 5RM
*If the 6th repetition is achieved, weight is increased for the next set, until the last repetition possible is the 5th one. RM = repetition maximum.				

Intensification training

The intensification training consisted of twenty training sessions, spread in two nonconsecutive training days a week. The workout took place in small groups per trainers (n<5), for ten weeks in a row. Three sets per exercise were performed. The starting weight was approximately 75% of the 1RM. As soon as 12 repetitions could be performed in a given exercise, the weight was increased to achieve progression. The general procedure can be found in table 5.

Table 5: General procedure of the training program

	Familiarization				Intensification
Timing	1st Week		2nd Week		3rd week to 10th week
	1st session	2nd session	1st session	2nd Session	
Days	Mo, Tue or Wed	Wed, Thu or Fri	Mo, Tue or Wed	Wed, Thu or Friday	2 days per week
Repetitions	10 to 20		8 to 12	5 to 6	8 to 12
Intensity (%1RM)	40 to 50		50 to 70	60 to 80 max	50 to 70
Sets/exercise	2		2	-	3
Exercises	As and Bs	Cs and Ds	All exercises (As,Bs,Cs and Ds)	5RM (A2, B1,C1, D2)	All exercises (As, Bs,Cs and Ds.)
Mo=Monday; Tue=Tuesday; Wed=Wednesday; Thu=Thursday; Fri=Friday. 1RM= 1 repetition maximum; Max= maximum; A1= Leg Curl; A2= Box Squat. B1= Dumbbell Shoulder Press; B2= Lateral Pull down. C1= Leg Press; C2= Front plank. D1= Lower back rowing; D2= Chest.					

The warm-up consisted of a 5 minutes lasting individual cross-trainer or bicycle routine, followed by mobilization and balance exercises led by the trainers. Every training group could then start the program. Subjects were encouraged to train in pair, as the exercises consisted in an alternation of agonist/antagonist supersets, which allowed the participant to rest during the exercise rotation (1 to 2 minutes) and to change from one exercise to the other alternating with the paired subject. Eight exercises were chosen in order to involve all major muscle group by using guided machines, free weight as well as body weights:

- Superset A: Quadriceps (Box squat) and hamstring/gastrocnemius (leg curl)
- Superset B: Shoulder (Dumbbell Shoulder press) and higher back muscles (Lateral pull down)
- Superset C: Quadriceps (Leg press) and posterior muscle chain and chore muscles (front plank with alternating single leg hip extension)
- Superset D: Chest (Chest press) and lower back muscles (Rowing)

In order to ensure a sufficient specific muscle rest between exercises, supersets involving lower limb muscles (A and C) were performed alternating with supersets involving upper limb musculature (B and D). To ensure a dynamic concentric phase, the participants were inquired to reach the full range of motion in about one second. To have a controlled eccentric movement, this phase was prolonged to about 3 seconds. The total sessions lasted generally between 50 and 90 minutes, depending on the machine availability and the individual motivation. Training diaries allowed a general individual follow-up: questions were asked at the beginning to evaluate the current state of the person (mood, motivation, sleep length and quality) and potential pain, unusual comfort or illness. This same diary permitted weight and repetitions to be annotated for each exercise after each set, to keep record of every session. After a short cool down routine at the end of every session, participants were asked to scale the training session’s intensity from 1 to 10. All the registered information was recorded also online by the respective trainers.

The training exercises are in line with the current literature: similar type of intense training have been tested and were noted as being suitable for older people (Bell et al., 2017). In fact, according to the American College of Sports Medicine Guidelines (ACSM, 2018), resistance training bouts should involve 8 to 10 exercises with 10 to 15 repetitions each. Those sessions should find place at least two nonconsecutive days per week. All-important muscle groups should be targeted. This should have beneficial general health and mobility consequences in the elderly. Warming up and cooling down appears relevant as well. The session should target a moderate intensity. However, high intensity training can be performed if the settings are adapted with competent supervisions and experienced subjects (Nelson et al., 2019).

To ensure a safe practice, the trainers need to be aware of various risks when leading the sessions (Colado & Garcia-Masso, 2009). The progressive aspect is essential as well to achieve specific strength improvements while considering the injury risks (Maren et al., 2019). Falling may represent a high risk during the sessions as well, therefore trainers were instructed to take care that nothing inappropriate was laying on the ground. Adapted clothes and especially suited shoes were advised, no jewelry or unsuitable watches should be worn during the sessions. In addition, any machine had to be checked before use. Including balance training and taking account of advices makes ST considered as a low risk activity for elderly, which has more positive consequences than risks. However, high-intensity training (higher speeds or heavier weights) may increase the injury hazard (Colado & Garcia-Masso, 2009; Liu & Latham, 2010). Yet, injury rates are not greater for the elderly men nor for post menopause women compared with younger subjects (Coleman et al., 1996; Warren & Schmitz, 2009, Liu & Latham, 2010).

2.8 Outcomes

All fitness test stations were set up at the Centre of Sport Science and University Sports (Auf der Schmelz 6, Vienna). The same routine was respected at every assessment: balance, followed by dynamic strength and walking speed tests. The last station assessed the aerobic capacity with the 6MWD. To avoid intraday variability and weather's influence on the performance, the tests took always place in the same indoor hall between 8 and 11 o'clock in the morning. As the tests of interest in this thesis are walking tests, the tester had to insist on the fact that it would be not allowed to run, and that by definition at least 1 foot had to be on the ground at each time of the test. Two to three days before the testing, every participant had familiarization trials: the TUG and GS tests were already explained, shown if necessary, and driven out in real conditions. An explanation sheet was given to the participant for the 6MWD.

2.8.1 Timed up and go test

The TUG assesses of the dynamic strength the under extremity, the dynamic balance and general coordination. As described by figure 4 and 5, the TUG test consists in rising (STEP 1) from a standard chair (43 cm high), walking to a cone (STEP 2)on the floor 3 meters away, turning around (STEP 3) it and return walking (STEP 4) to sit down (STEP 5) as fast as possible as shown in figure bellow (4). The start signal was given by a tester, which started the stopwatch when the participant lost contact to the chair and stopped it when he/she was back on it. The first trial was a familiarization attempt; the recorded time was the best of the two following evaluations. A special attention was given to the starting position: a line was drawn

between the two front feet of the chair to indicate where the first foot's heel and the back-foot's front had to be placed, respectively in front and behind that line. The subject had to avoid any contact with the back of the chair. No help was allowed for the walk (Podsiadlov & Richardson, 1991).

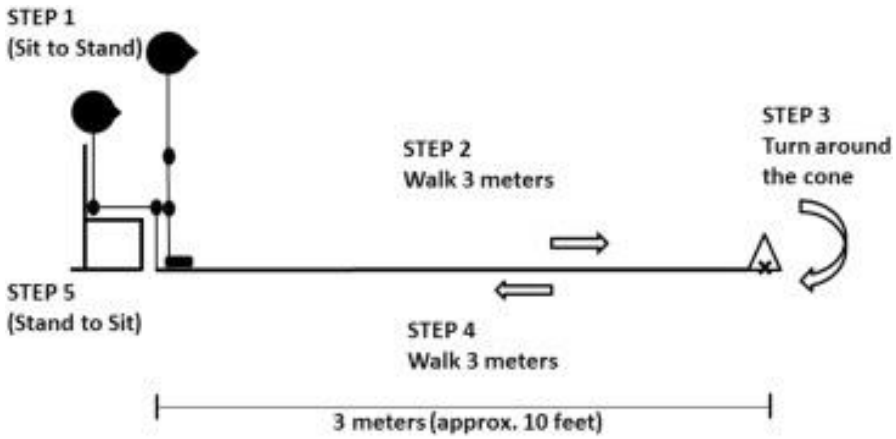

Figure 4: Timed Up and Go (TUG) test, descriptive image (Sebastiao, Sandroff, Learmonth & Motl, 2016). Used with permission.

2.8.2 4 and 6m-GS test

The participants were told to walk as fast as possible from the starting line to the final point. A walking distance of 4 and 6 meters was timed by using electronic timer (Brower Timing System USA) which assessed the time accurately to 1/100 and was transferred to meters per second.

As shown by the figure 6 and 7, a 2-meter acceleration zone and 2-meter slow down zone were established. The participant started therefore 2 meters before the first accession point and was asked to slow down only once he/she crossed the last timer, 6 meters further away. Two GS values were recorded per person for each one of the two trials: one for the first 4 meters only, one for the 6 meters. A short break was given to the participant between the two conducted trials (Mijnarends et al., 2013).

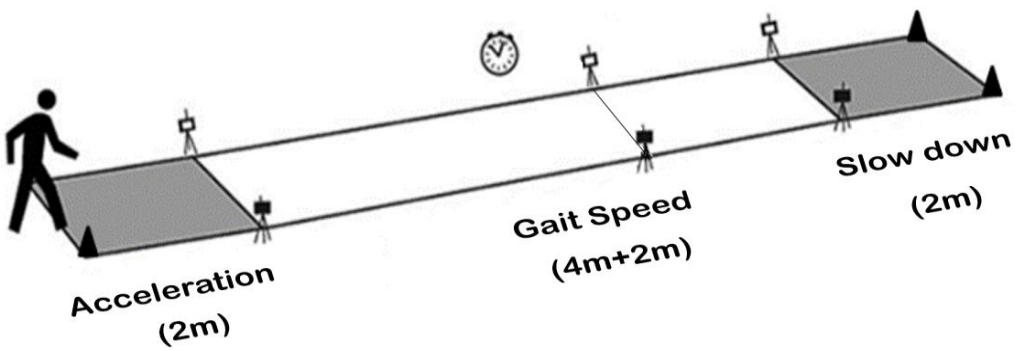

Figure 5: Gait speed (4 and 6 meters) descriptive image (adjusted from: Karpman & Benzo, 2013).

2.8.3 6 Minutes Walking Distance

The 6MWD assesses the functional aerobic endurance capacity. It was always performed after the TUG test and the 4 and 6m GS test. An explanation sheet was given the days before to make the participant know how the test will be conducted. It is explained to the participant that the aim of the test is to walk as far as possible in 6 minutes. The participants must walk back and forth around 2 cones positioned at 30 meters from each other as shown in figure 8 and 9. It took place in a « hallway » on a hard surface and they could adapt their speed as they wish. If needed, the participant could have a break. Only one subject performed the test at the time, to avoid any concurrent influence on the performance (Grindrod et al., 2006). A starting point was defined at one cone and a starting signal was given when the test started. The distance was marked every five meters. Two face to face 30m long measuring tape were used on the track between the 2 cones to precisely assess where the performer stopped after 6 minutes walking. As soon as the 6 minutes were finished, the participant had to stop. The distance covered was reported. Next to which the pre- and post-heartbeat frequency and post blood oxygen saturation (SpO2) were annotated. Every participant was wearing a heartbeat belt (Suunto T6d) right underneath the chest and a pulse oximetry was used to measure the blood oxygen saturation before and after the walk. At last, the participants were asked to rate the effort by using a Borg-Scale (Borg, 1982) between 6 (no effort) and 20 (maximal effort imaginable). No warm-up has been performed. (Steffen et al., 2002; American Thoracic Society, 2002).

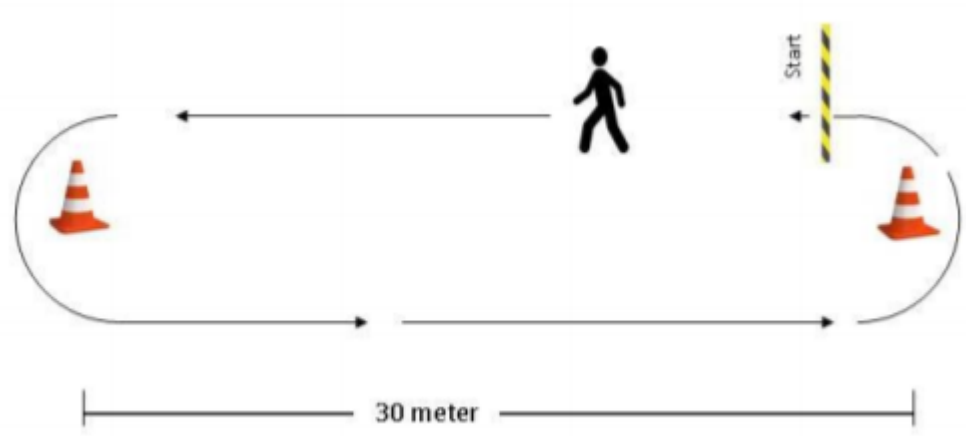

Figure 6: 6 minutes walking distance (6MWD) descriptive image (EMPCHILD).

2.8.4 Vitamin D status

VD status was analyzed six times per participant during the whole length of the study. At T0, at T1, four weeks after T1, at T2, four weeks after T2, six weeks after T2 and at T3. 25(OH) was the specific VD form targeted by the analyses as it comprises the most used reference and is considered to be the most effective biomarker of VD status (Uusi-Rasi et al., 2012). The assay “Access 25(OH) VD” (Immunoassay Systems, Beckman Coulter Life Sciences, Brea, California, USA) was used. Beckman immunoassay is proven reliable (limit of blank, limit of detection, limit of quantitation) by Madenci et al. (2017). To come out with a clear amount of 25(0H) in the sample, the automated procedures undergoes two incubation steps and one final chemiluminescent measurement: The first incubation consists of allowing a binding between the immobilized monoclonal anti 25(OH)D and the 25(OH)D analogue-alkaline phosphatase. The second incubation step will wash away unwanted unbound materials while holding the materials bound to the solid phase to allow the last analyze. The chemiluminescent substrate Lumi-Phos*530 can then be added into the vessel. A luminometer records the light produced by the reaction. The concentration of 25(OH)D in the sample equals to the inverse proportion of the light production.

2.9 Anthropometric measurements: Height, weight and BIA

For height measurements, the assessed subject was asked to keep an upright standing position, while having the heels, glutes, upper back and the back of the shoulder head on the height scale (model 437, seca GmbH & Co. KG, Hamburg, Germany).

The weight was measured using a model 877 scale (seca GmbH & Co. KG, Hamburg, German). Jackets, shoes and heavy trousers were asked to be taken off. Both the height and weight assessments were conducted in the mornings while being in a state of fasting. Body mass index (BMI) could then be calculated with those values as followed: BMI = (bodyweight (kg)) / (height $(m)^2$). To assess the absolute (in kg) and relative (% of body mass) fat mass and MM, a bioelectrical impedance analysis (BIA) was conducted at the three different time points (T1, T2, T3).

The bio impedance analysis allows a constant electrical current, which evaluates the total resistance/impedance (Z) from which the resistance (R) and the reactance (Xc) can be determined. The specific use of the bio impedance analyzer (Nutriguard-MS Leupamed, Germany) is described previously (Walter-Kroker et al., 2011). Three other body composition elements can be estimated. The Total body water (TBW) using high frequencies 100 signaling

(100kHz), the body cell mass (BCM) using a medium frequency signaling (50kHz) and the extra-cellular water with a low frequency signaling (5kHz).

For means of standardization, the tester had to place the electrodes at the same place every time: the first electrode lying right above the fissure of the wrist was attached at the ulna's head level. On the hand, the second electrode lies between the second and the third metacarpal bone and is attached slightly distal from the base joint of the middle finger. On the toe, the electrode lies on the soft tissue between the second and third metatarsal bones and was attached distal from that line. The last electrode was fixed at the inner's ankle's level and lying on the back of the foot distal from the attachment.

Other considerations: The skin was cleaned before the application of the electrode with an alcoholic solution (Cutasept). The distance between the electrodes was about 4 cm. Each double cable had a marked clip: the one for the foot had a red sleeve, for the hand a yellow sleeve. Moreover, red clips were applied onto distal electrodes, and black ones onto proximal. Bones were drawn on the electrodes to furtherly facilitate the correct positioning. The cable lied hanged during the measurements to avoid knots. Once the tester made sure the cable wasn't touching any metal pieces and registered the height of the participant on the software, the body analysis could start. The participants had to be in a fasting state, having avoided alcohol consumption for the last 24 hours and sporting activities for the last 12 hours.

The subjects had to lie down on the back in a supine position, with bare feet and hands. To avoid the thighs from touching each other, the legs had to be set apart. The arms were at 30° distant from the body, and no contact to metal (Jewelry, watches, bed frames) was allowed, to avoid bias in the results.

2.10 Statistical analysis

All the following statistical information were analysed using the 25th version of IBM SPSS Statistics (IBM Corporation, New York, USA).

To start the investigations, a one-way analysis of variance (ANOVA) was used to verify potential differences between the groups at baseline. Changes over time, differences between intervention groups, and time*group interactions were assessed by a two-way mixed ANOVA. The homogeneity of covariances ($p > 0.001$) was assessed by Box's M test and the homogeneity of variance was admitted with the Levene Test ($p<0.05$). The Greenhouse-Geisser estimate of sphericity was used as significant reference when a Maulchy-Test showed a sphericity violation ($p<0.05$). Even when assumptions such as the normal distribution are violated ANOVA is proven to be robust against type 1 and type 2 errors: therefore, when an explorative data analysis showed potential non-normally distributed data (Kolmogorov-Smirnova ($n >30$) or Shapiro-Wilk ($n < 30$) with p values under 0.05, the QQ diagram and the

Histogram were visually analysed. If the visual distributions were satisfactory or tented towards a kurtosis, the whole data set was admitted as normally distributed. In other cases, the data needed to be transformed, either through the “sqrt”, “log10” or “inverse” transformation. When no transformation gave any satisfactory normally distribution, the analysis was driven by a Kruskal-Wallis non-parametrical test. Outliers have been verified. Bivariate correlation tests using Pearson correlation verified potential interaction between primary and secondary factor and time changes depending on group allocution, gender, VD status etc.

To image the different changes that happened through time, graphics have been drawn using SPSS IBM. As the table (8) includes non-parametric values (Spo2 pre and Spo2 post), median and interquartile range were calculated for all different values to have a coherent and comparable overview.

3. Results

3.1 Participant flow

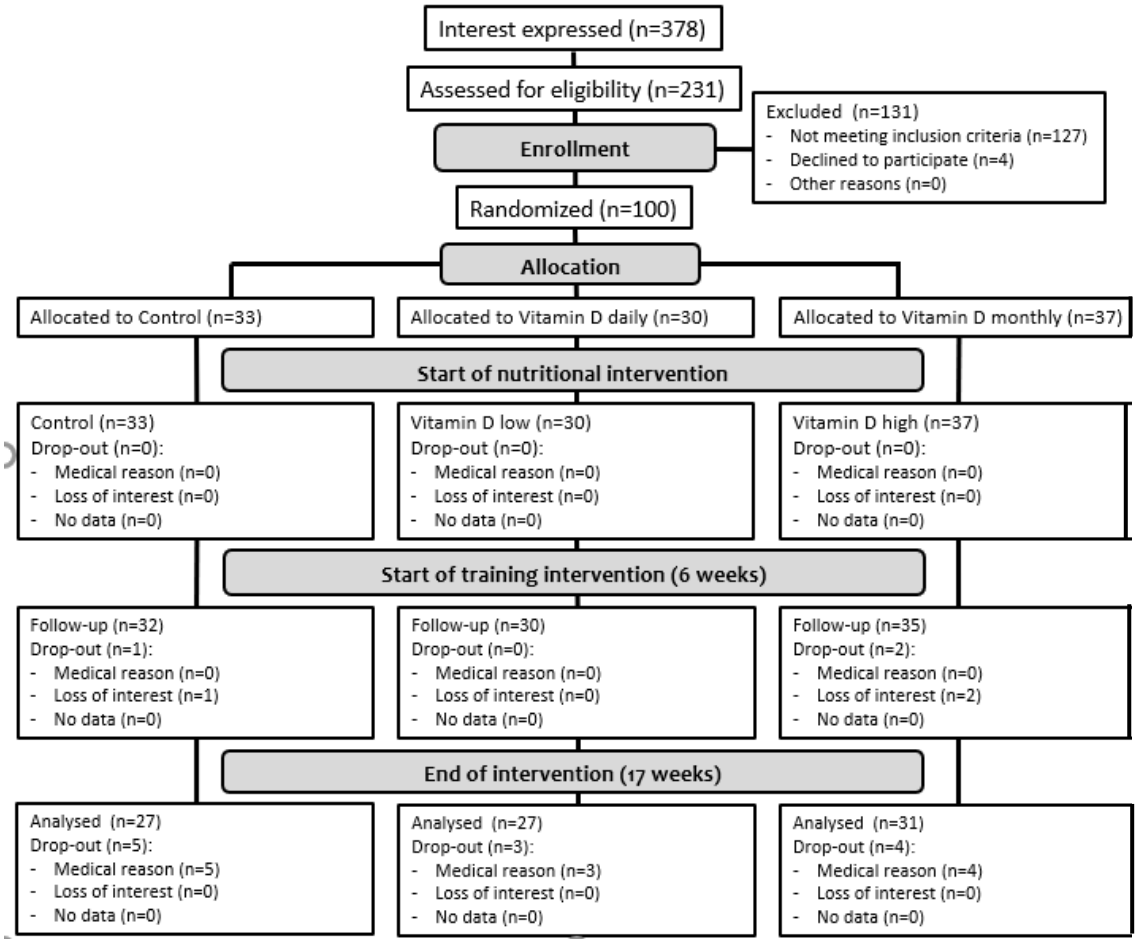

Figure 10: Participant flow

Recruitment for the study was made in the end of 2018 and early 2019 through radio, newspaper and online announcements: 378 people responded with interest concerning the participation in the study. 231 were then assessed for eligibility. Four of them declined to participate, and 127 did not meet the inclusion criteria previously mentioned.

Among the hundred participants left which started the experimental part of the study and were allocated to the intervention groups (control group, n=33; VDD, n=30; VDM, n=37), 15% stopped before the end of the 17th weeks. During the nutritional intervention, 3 involved subjects stopped as they lost interest in participating in the study. All of the dropouts due to medical reasons happened during the ST period between the 6 and the 17th week. 27 people finished the study in both control and VDD groups, and 31 in the VDM group, resulting in 85 complete data at the end of the intervention and examinations.

3.2 Baseline Characteristics

3.2.1 Baseline anthropometric and vitamin D results

The study population included 100 participants, among which 33 were women and 67 were men. The group was in average 70.6 years old (± 4.6), weighed 81.2 kg (± 16.0) and 1.72 cm (± 0.10) tall. The mean BMI in kg/m² was 27.3 with a standard deviation of 4.7. In kilogram per person, the mean MM was 54.9 ± 10.6 and 23.1 ± 9.7 for the body fat, which represents in general 27.8 (± 8.1) % of the total body weight (the values were available only for 98 participants). The mean VD blood concentration of the study population was 22.66 ng/mL (± 7.11). All the three intervention groups are described as Control (CT), VD Daily (VDD) and VD Monthly (VDM) and their baseline anthropometric and VD values are indicated in table 6, with the correspondent mean and standard deviation values. Furthermore, a one-way multivariate analysis of variance was run to verify if there was any difference between the intervention groups with respect to the baseline anthropometric results.

Table 6: Baseline anthropometric characteristics and 25(OH)D serum levels

	Total	**CT**	**VDD**	**VDM**	***p-value***
Gender [female/male, (% female)], n=100	**33/67 (33%)**	10/23 (30.3%)	10/20 (33.3%)	13/24 (35.1%)	*0.911*
Age [years], n=100	**70.6 ± 4.6**	70.3 ± 4.8	70.5 ± 4.5	71.0 ± 4.5	*0.781*
Body weight [kg], n=100	**81.2 ± 16.0**	77.0 ± 16.2	84.0 ± 14.6	82.7 ± 16.4	*0.174*
Height [m], n=100	**1.72 ± 0.10**	1.72 ± 0.10	1.72 ± 0.09	1.72 ± 0.11	*0.932*
BMI [kg/m²], n=100	**27.3 ± 4.7**	25.9 ± 4.5	28.3 ± 4.4	27.7 ± 4.9	*0.114*
Muscle mass [kg], n=98	**54.94 ± 10.62**	52.91 ± 10.36	56.62 ± 10.16	55.35 ± 11.21	*0.376*
Bodyfat [kg], n=98	**23.13 ± 9.67**	21.05 ± 9.53	23.97 ± 9.21	24.28 ± 10.18	*0.335*
Bodyfat [%], n=98	**27.78 ± 8.13**	26.57 ± 7.87	28.01 ± 8.06	28.66 ± 8.49	*0.564*
Vitamin D [ng/mL], n=100	**22.66 ± 7.11**	21.49 ± 5.77	23.10 ± 7.09	23.34 ± 8.19	*0.514*

Shown values: n = sample size, mean ± standard deviation, *p*-value: significance between groups (Chi² Test, one factor ANOVA). CT (control group), VDD (vitamin D daily), VDM (vitamin D monthly), BMI (body mass index).

3.2.2 Baseline results of the functional tests

A one-way multivariate analysis of variance was run to verify if there was any difference between groups in baseline functional performance. The three tests were assessed: TUG, 4mGS and 6mGS and the 6MWD (with associated pre and post effort heart rate, pre and post percentage of oxygen saturation and BORG results). The mean TUG performance for all the group was at baseline 4.80 ± 0.87 m, the GS for 4 meters and GS for 6 meters were respectively 2.42 ± 0.40 and 1.61 ± 0.28 m/s. The average 6MWD distance was 628.7 ± 91.9 m. There is in fact no statistically significant difference neither in the tests themselves nor in the associated results between the tasted groups at baseline. The different mean and standard deviation for those functional performance assessments and their associated results are readable in table 7.

Table 7: Baseline performance in the sport motoric tests.

	Total	**CT**	**VDD**	**VDM**	***p-value***
Timed up and go [s], n=100	**4.80 ± 0.87**	4.75 ± 0.67	4.76 ± 0.86	4.86 ± 1.03	*0.839*
Walking speed for 4 m [m/s], n=100	**2.42 ± 0.40**	2.40 ± 0.33	2.45 ± 0.41	2.41 ± 0.44	*0.893*
Walking speed for 6 m [m/s], n=100	**1.61 ± 0.28**	1.60 ± 0.25	1.64 ± 0.29	1.59 ± 0.31	*0.775*
6MWD [m], n=100	**628.7 ± 91.9**	621.2 ± 83.1	634.5 ± 93.1	630.6 ± 100.0	*0.841*
Heart rate before 6MWD [bpm], n=99	**84 ± 13**	82 ± 11	86 ± 15	85 ± 13	*0.420*
$Sp0_2$ before 6MWD [%], n=98	**87 ± 3**	97 ± 4	97 ± 1	97 ± 2	*0.736*
Heart rate post 6MWD [bpm], n=100	**136 ± 21**	132 ± 19	139 ± 20	138 ± 22	*0.345*
$Sp0_2$ post 6MWD [%], n=98	**97 ± 1**	98 ± 1	97 ± 2	98 ± 2	*0.479*
BORG post 6MWD [-], n=99	**14 ± 2**	14 ± 2	14 ± 2	14 ± 2	*0.911*

Shown values: n = sample size, mean ± standard deviation, *p*-value: significance between groups (one factor ANOVA). CT (control group), VDD (vitamin D daily), VDM (Vitamin D monthly), 6MWD (Six minutes walking distance). $Sp0_2$ (peripheral capillary oxygen saturation)

3.3 Differences between intervention groups

3.3.1 TUG

There was no statistically significant interaction between the intervention and time on TUG times, F(3.514, 144.068) = 0.822 partial η2 = 0.020, p = 0.500. The main effect of time did not show a statistically significant difference in mean TUG times, F(1.757,144.068) = 3.084, partial η2 = 0.036, p = 0.055. The main effect of group did not show any statistically significant difference in TUG times between intervention groups $F(2, 82) = 0.031$, $p = 0.970$, partial $\eta^2 = 0.001$.

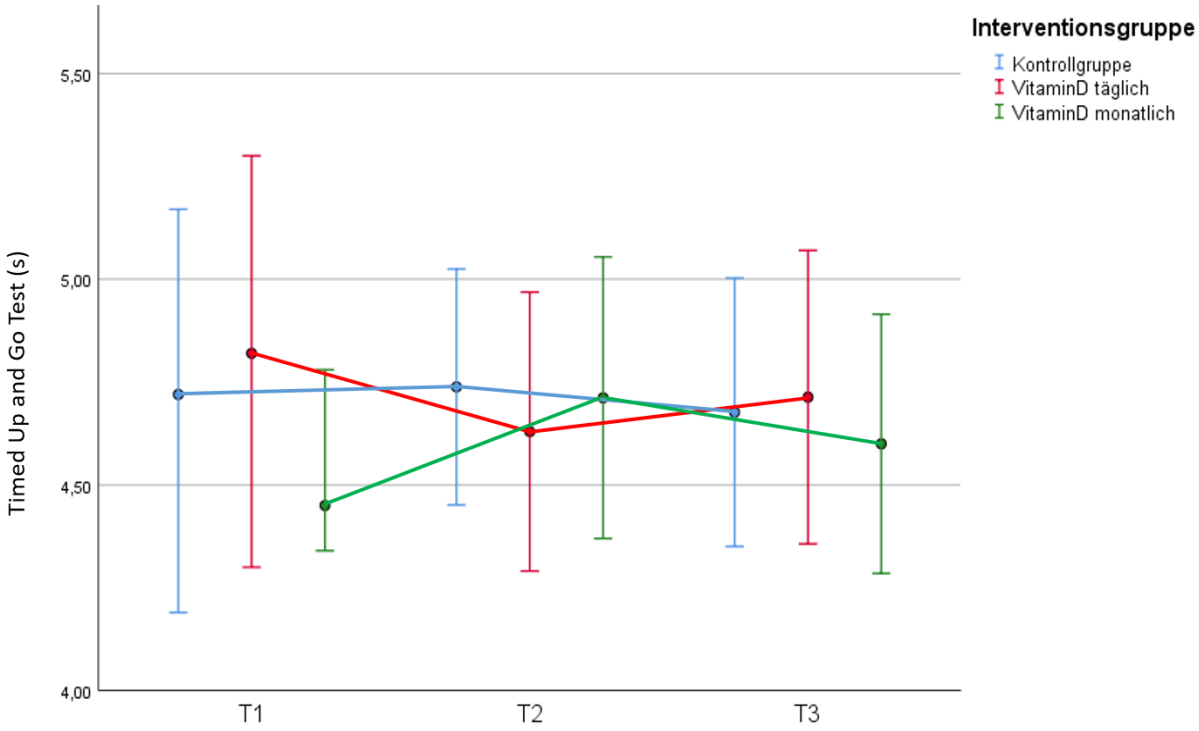

Figure 11: Mean TUG time [s] and 95% confidence interval for all interventions group (Kontrollgruppe: CON; Vitamin D täglich: VDD; Vitamin D monatlich; VDM).

3.3.2 Gait speed

4m gait speed

There was no statistically significant interaction between the intervention and time on GS measurement on the 4 meters distance times, F(4,164) = 0.741: partial η2 = 0.018. p= 0.565. The main effect of time did not show a statistically significant difference in mean GS times F(2,164) = 2.584: partial η2 = 0.031. p= 0.08. The main effect of group did not show any statistically significant difference neither between intervention groups F(2, 82) = 0.318, *p* = 0.728, partial η^2 = 0.008.

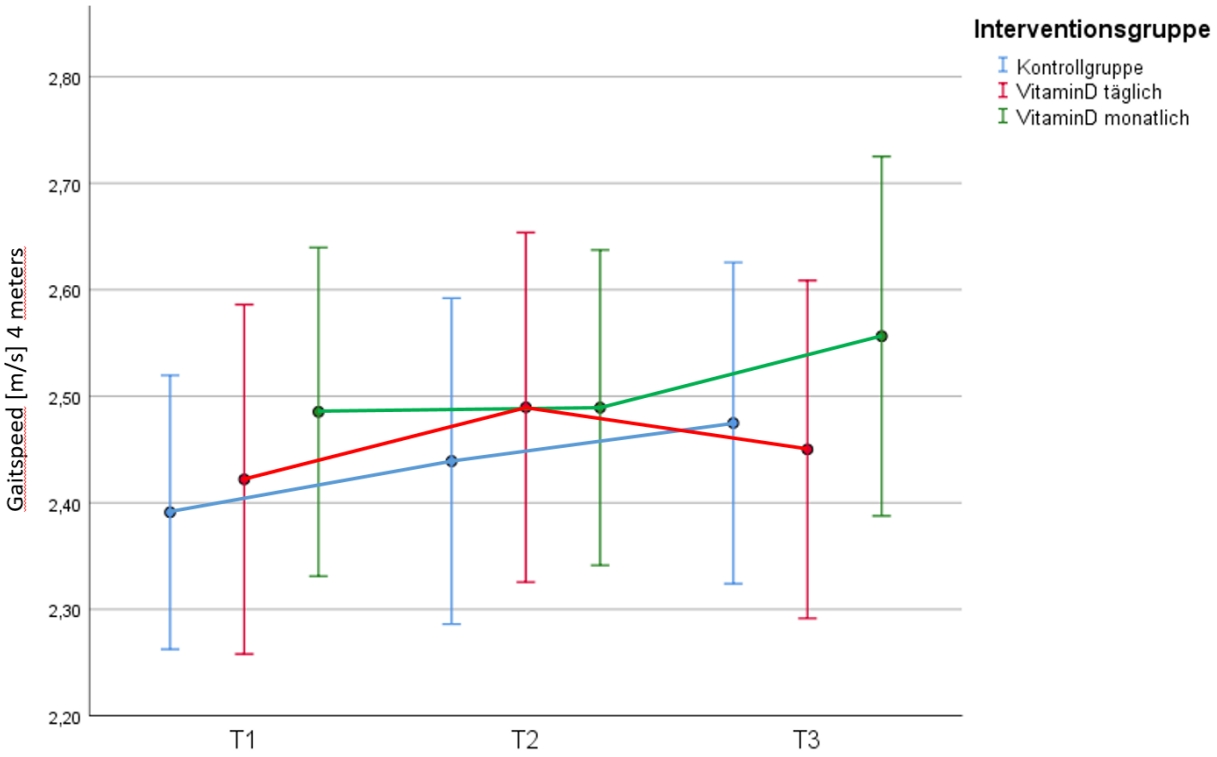

Figure 12: Mean 4 meters gait speed [m/s] and 95% confidence interval for all interventions group (Kontrollgruppe: CON; Vitamin D täglich; VDD, Vitamin D monatlich; VDM).

6m gait speed

A significant change in time was noted irrespectively from group allocation: F(2,164) = 4,220, partial η2 = 0.049, p = 0.016. A pairwise comparison followed by Bonferroni post hoc test was needed. The time difference is detected between T1 and T3 and comprised 2.98% (p = 0.02). Yet no interactions were seen between time and intervention group F(4,164) = 1.183. partial η2 = 0.028. p= 0.320, and no differences were detected between the groups F(2,82) = 0.196, partial η2 = 0.005; p= 0.823.

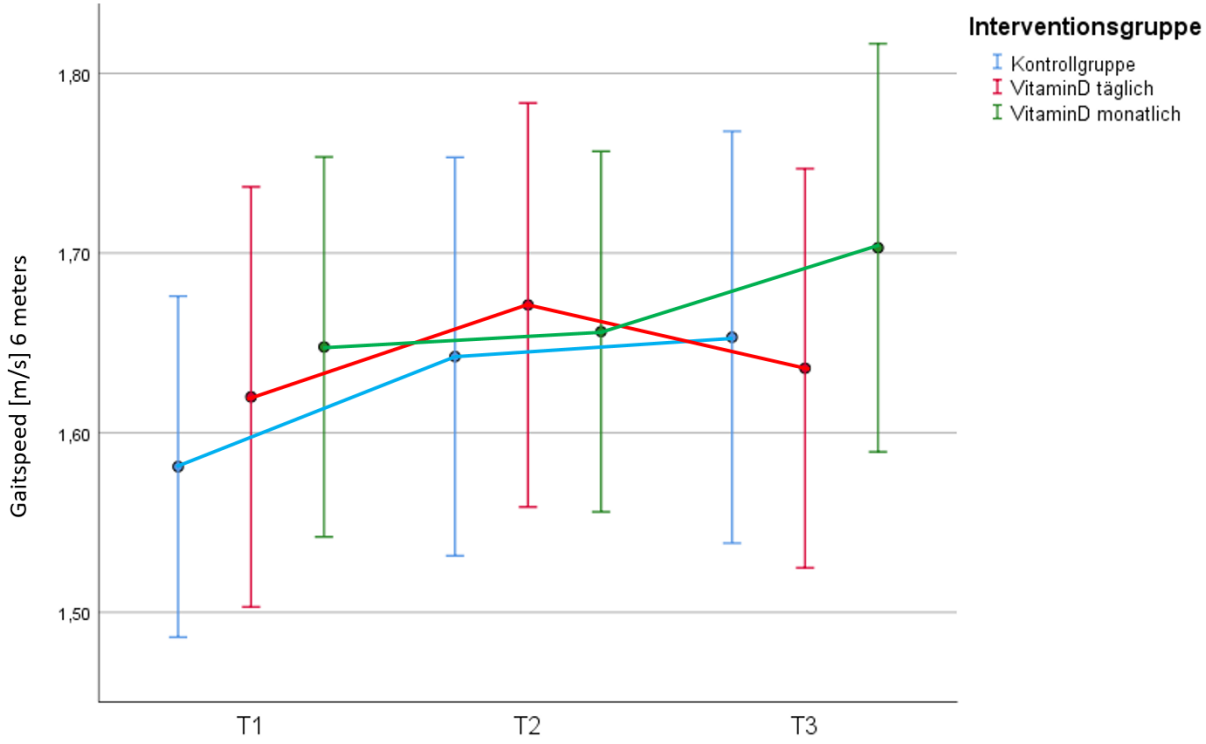

Figure 13: Mean 6 meters gait speed [m/s] and 95% confidence interval for all interventions group (Kontrollgruppe: CON; Vitamin D täglich: VDD; Vitamin D monatlich; VDM).

3.3.3 6MWD

A significant time effect was shown F(2,162) = 4.649, partial η2 = 0.054, p = 0.011. Bonferroni post hoc analysis proved a significant change between T1 and T3 p < 0.05 (1.80%) and between T1 and T2 p = 0.031 (1.58%). No significant interaction effect was shown F(4,162) = 0.370, partial η2 = 0.009, p= 0.830. Between the groups, there is no significant difference F(2,81) = 0.530, partial η2 = 0.013, p= 0.591.

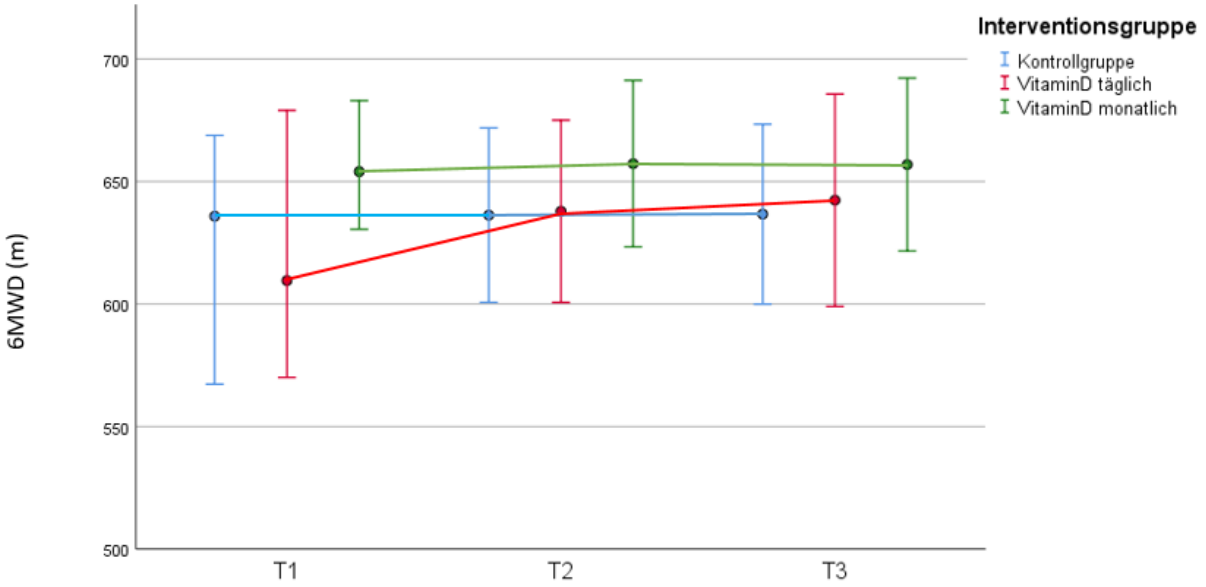

Figure 14: Mean 6MWD distance [m] and 95% confidence interval for all interventions group (Kontrollgruppe: CON; Vitamin D täglich: VDD; Vitamin D monatlich: VDM)

6MWD parameters (Hf pre and post, Spo2 pre and post)

For the pre 6MWD heart frequency change, a multi variance analysis showed no significant changes over time regardless of the group allocution, after a Greenhouse-Geisser correction: $F(1.640,131.196) = 1.099$, partial $\eta 2 = 0.014$, $p = 0.326$. No significant interaction effect was shown $F(3.280, 131.196) = 0.455$: partial $\eta 2 = 0.011$, $p= 0.732$, and no significant difference between the groups was established $F=(2,80)=2.584$, partial $\eta 2 = 0.061$.

To measure the possible differences over time, group or time*group in the post 6MWD heart frequency, an ANOVA for repeated measures was used. No significant time effect $F(2,160) = 0.503$, partial $\eta 2 = 0.006$, $p = 0.606$, no different time changes between groups $F(2,80) = 0.318$, partial $\eta 2 = 0.008$, $p = 0.215$, and no group effect $F(4,160) = 1.467$, partial $\eta 2 = 0.728$.

A Kruskal-Wallis H test was conducted to determine if there were differences in Spo2 measurements before the 6MWD between intervention groups. Distribution of Spo2 results were similar for all groups, as assessed by visual inspection of a boxplot. No statistically significant differences were admitted: $\chi 2(2) = 0.194$, $p = 0.908$.

A Kruskal-Wallis H test was used to determine if there were differences in Spo2 Post 6MWD scores between intervention groups. Distribution of Spo2 results were similar for all groups, as assessed by visual inspection of a boxplot. No statistically significant differences were admitted: $\chi 2(2) = 2.404$, $p = 0.301$.

The summary of the median and interquartile range, mean differences between deltas from different time points and p-values from TUG, 4-6mGS, 6MWD and latter associated values (HF pre and post, Spo2 pre and post) can be found in table 8 on the next page.

Table 8: Results of performance tests. (Median and interquartile range).

Parameter	**Group**	**Median (interquartile range)**					**P-value**		
		T1	T2	T3	**Delta (T2-T1)**	**Delta (T3-T2)**	Time	group	Time x group
TUG [sec]	CON VDD VDM	4.72 (1.04) 4.82 (1.10) 4.45 (0.62)	4.71 (1.00) 4.44 (1.31) 4.57 (1.03)	4.45 (1.42) 4.86 (1.15) 4.41 (0.92)	-0.15 (0.57) 0.12 (0.66) -0.11 (0.52)	-0.07 (0.32) -0.25 (0.53) -0.09 (0.67)	0.008	0.921	0.451
GS 4m [m/s]	CON VDD VDM	2.40 (0.37) 2.45 (0.64) 2.52 (0.69)	2.56 (0.74) 2.63 (0.67) 2.40 (0.51)	2.52 (0.55) 2.52 (0.71) 2.44 (0.52)	0.02 (0.34) 0.08 (0.32) 0.03 (0.41)	0.02 (0.41) -0.02 (0.29) 0.10 (0.26)	0.079	0.728	0.565
GS 6m [m/s]	CON VDD VDM	1.54 (0.29) 1.59 (0.46) 1.66 (0.43)	1.69 (0.53) 1.75 (0.42) 1.63 (0.36)	1.67 (0.39) 1.61 (0.52) 1.65 (0.39)	0.04 (.018) 0.04 (0.19) 0.02 (0.20)	0.06 (0.25) -0.04 (0.23) -0.05 (0.18)	0.016	0.823	0.320
6MWD	CON VDD VDM	635.80 (125.2) 609.55 (121.7) 654.00 (92.0)	642.70 (12.38) 618.40 (152.88) 660.00 (89.18)	627.60 (105.11) 623.28 (169.27) 638.00 (109.07)	6.72 (35.13) -1.89 (48.38) -0.90 (50.26)	18.80 (39.60) 1.30 (48.20) 10.00 (42.80)	0.011	0.591	0.830
HF pre	CON VDD VDM	79.00 (15) 81.50 (21) 85.50 (16)	80.00 (20.00) 85.50 (16.25) 86.50 (17.25)	79.00 (16.00) 82.50 (13.50) 84.50 (11.25)	-2.00 (19.00) 2.00 (21.00) 0.05 (13.00)	0.00 (17.00) -2.00 (11.00) -1.5 (9.75)	0.326	0.082	0.732
Spo2 pre	CON VDD VDM	98 (2) 97.00 (2) 98 (2)	97.00 (2.00) 97.00 (2.00) 97.00 (1.25)	97-00 (2.00) 97.00 (1.50) 97.00 (2.25)	0.00 (3.00) 0.00 (2.00) -1.00 (2.00)	0.00 (2.00) 0.00 (2.00) 0.00 (2.75)	.908.	(KWTEST)	(KWTEST)
HF post.	CON VDD VDM	124.00 (36) 139.50 (25) 142.00 (38)	133.00 (42.00) 136.00 (21.75) 140.50 (32.50)	138.00 (30.00) 136.50 (30.25) 132.50 (31.25)	-1.00 (21.00) -2.00 (16.50) -3.5 (11.25)	3.00 (17.00) 2.00 (12.00) 1.00 (10.75)	0.606	0.728	0.215
Spo2 post.	CON VDD VDM	98.00 (1) 97.00 (2) 98.00(2)	98.00 (3.00) 97.00 (2.50) 97.00 (3.00)	97.00 (1.00) 97.00 (1.50) 97.00 (2.00)	0.00 (2.00) 0.00 (1.50) 0.00 (2.50)	0.00 (2.00) 0.00 (2.50) 0.00 (2.75)	0.301	(KWTEST)	(KWTEST)

Shown values: n= sample size; median(interquartile range) for each group for each time points; p-value: time = differences between time points regardless of groups. group = differences in groups regardless of time. Time x group = interaction between time and group. P values are significant when marked as p<…, p=…. p≤0.05 or 0.01 or 0.001.

3.4 Differences in changes of the parameters between the study phases

To verify is there was any statistical difference between the various changes in the tests results when the supplementation period and the ST period are juxtaposed, deltas from TUG, 6MWD and GS were compared. A T-test for dependant variables analysis assessed that there is no significant difference in the test's changes between the supplementation phase (T2-T1) and ST intervention phase (T3-T2) in the TUG ($p=0.426$), the 6MWD ($p=0.194$), 4GS and 6mGS ($p=0.760$; $p=0.347$ respectively).

Intervention group's influence

To verify if the results differ according to the intervention group allocation, a T-test for dependent variable was driven out. It manifests no statistically significant difference from any of the intervention groups between the changes in the tests results during the supplementation period and the ST period ($p>0.05$) for all tests regardless of the group allocation.

Changes according to Vitamin D levels at T2.

A T-test for dependent variable showed no statistically significant difference in the performances variations for neither the group that reached 30ng/ml or the one underneath, between the changes in the tests results during the supplementation period and the ST period ($p>0.05$).

No significant difference was shown between the changes in the performance during the supplementation phase and during the supplementation plus training phase, and this regardless of whether the group allocations according to the intervention or the actual VD levels at T2.

3.5 Test improvement linked to actual VD serum level

No influence of intervention group allocation has been found of any of the tests. Tests results will therefore be analyzed under the scope of the actual VD serum levels to verify possible links between VD blood concentration at T1 and T2 and test improvement.

3.5.1 Influence of baseline VD levels on performance adaptation

The next calculations were performed to test the hypothesis that the initial VD level influences the outcomes of ST on functional outcomes. Therefore, groups were made according to their

levels at baseline (in 3 equally distributed groups and in interquartile groups, and at T2 (above 30ng/ml).).

Interactions effect between time and equally distributed VD groups for functional tests.

The baseline VD level allowed to divide the participants into 3 equally distributed groups according to their relative VD status: lower initial VD status, moderate initial VD status, and higher initial VD status. No interaction between group and time were detected neither in the 6mGS (p=0.446), 4mGS (p=0.990), the 6MWD (p=0.873) nor in the TUG test (p=0.345).

Interactions effect between time and interquartile groups for functional tests.

Similar results appeared in all of those tests when the groups were divided into quartiles (first 25%, 50%, and last 25% of the distribution) considering the *VD initial* level of participants. There are no significant differences between the changes in performance tests of 6MWD (p=186), the 6mGS (p=0.919), the 4mGS (p= (0.762) and the TUG (p=0.274) between the groups.

3.5.2 Influence of VD level at the start of the training period

The T2 VD levels allowed to make two distinctive groups: one group with the participants that achieved sufficient 25(OH)D levels after the initial supplementation period (>30ng/ml, n=11) and another one which did not (<30ng/ml, n=74). An ANOVA for repeated measure was carried out to verify if there was a different adaptation effect between these two groups.

TUG:

No significant change in time was noted: $F(1.86,154.35) = 0.494$, partial $\eta2 = 0.006$, $p = 0.598$. No differences were detected neither between time or intervention group $F(1.86,154.35) = 1.226$. partial $\eta2 = 0.015$. $p= 0.294$, nor between the groups $F(1,83) = 1.863$: partial $\eta2 = 0.022$; $p= 0.176$.

6mGS:

No significant change in time was shown: $F(2,166) = 1.04$: partial $\eta2 = 0.012$, $p = 0.355$. No differences were seen neither between time or intervention group $F(2,166) = 0.215$. partial $\eta2 = 0.003$. $p= 0.807$, nor between the groups $F(1,83) = 0.685$: partial $\eta2 = 0.008$; $p= 0.410$.

4mGS:

No significant change in time was shown: $F(2,166) = 0.92$: partial $\eta2 = 0.011$, $p = 0.402$. No differences were seen neither between time or intervention group $F(2,166) = 0.971$. partial $\eta2 < 0.001$. $p= 0.971$, nor between the groups $F(1,83) = 0.339$: partial $\eta2 = 0.004$; $p= 0.562$.

6MWD:

No significant change in time was shown: $F(2,164) = 1.599$: partial $\eta 2 = 0.019$, $p = 0.205$. No differences were seen neither between time or intervention group $F(2,164) = 0.083$. partial $\eta 2 = 0.001$. $p = 0.920$, nor between the groups $F(1,82) = 0.024$: partial $\eta 2 < 0.001$; $p = 0.878$.

3.6. VD increase and training adaptation

As neither the baseline VD levels nor the fact that participant reached 30ng/ml of VD blood concentration at T2 showed a difference in time*group or between the groups, the hypothesis remains that the individual mean VD increase is correlated to the test improvements.

During the supplementation period

A correlation test was carried out to verify if VD delta between T2 and T1 (ΔVD T2-T1) were correlated to the TUG, 6MWD, 4mGS and 6mGS changes in performance. Table 9 lists the significant levels of correlations (p values).

Table 9: Level of correlation between VD changes and changes in performance (supplementation period)

Variables	ΔVD T2-T1
ΔTUG T2-T1	p=0.908
Δ6MWD T2-T1	p=0.076
Δ6mGS T2-T1	p=0.490
Δ4mGS T2-T1	p=0.338

There is no correlation between any of the 25(OH)D serum variables (between T1 and T2) and any of the functional performance tests. The change between T1 and T2 in VD serum levels is not linked to the change in the performance tests.

During ST period

A correlation test verified if VD changes between T3 and T2 (deltas) were correlated to the TUG, 6MWD, 4 meters and 6 meters GS changes in performance (delta). The table 10 lists the significant levels of the correlation (p values).

Table 10: Level of correlation between VD changes and changes in performance (ST period)

Variables	ΔVD T3-T2
ΔTUG T3-T2	p=0.218
Δ6MWD T3-T2	*p=0.029** *(r= -0.238)*
Δ6mGS T3-T2	p=0.446
Δ4mGS T3-T2	p=0.477
* Significant level at p<0.05.	

The fact that the participants had different changes in VD serum levels between T2 and T3 is not linked with changes in performance. However, a greater increase in VD serum level during the training phase was weakly and negatively associated with a change in 6MWD performance (r=-0.24, p=0.029).

3.7 Anthropometrics results, intervention groups and vitamin D

All mean values and standard deviation, the mean differences between deltas between different time points and p-values from VD, bodyweight, BMI, MM, relative and absolute fat mass can be found in table 11.

VD:

A significant change over time was found after Greenhouse-Geisser correction F(1.73, 141.98) =30.35, $p < 0.001$, partial $\eta 2 = 0.270$) showing general increase in VD levels regardless of the group allocation. A post-hoc test using Bonferroni correction showed a 18.79% increase in the supplementation only period (between T1 and T2) and a 26.92% ($p < 0.001$) increase over the total study period from T1 to T3 ($p < 0.001$).

A significant difference between the intervention groups was found: F(2, 82) = 4.05, p = 0.021, partial $\eta 2 = 0.090$. A Bonferroni post-hoc correction revealed a difference between the CON and the VDM group (p=0.017).

No statistically significant interaction was found concerning the time*group interaction; Greenhouse-Geisser F(3.46,141.98)=1.95, p= 0.115, partial $\eta 2=0.045$.

Bodyweight:

A statistically significant time effect was found: F(22.15,293.13)=6.196, p=0.004, partial $\eta 2=0.070$ (Greenhouse-Geisser correction). After a Bonferroni post-hoc analysis, a significant time change between T1 and T3 was visible (p=0.011): 0.9% body weight was lost over the whole study period. Neither a significant group effect F(2:82)=0.98, p=0.379, partial $\eta 2 = 0.023$ nor time*group interaction F(3.49,142.99)=0.56, p=0.669, partial $\eta 2 = 0.013$ (Greenhouse-Geisser correction) was found.

BMI

A statistically significant time interaction was found, F(1.74,142.79)=5.08, p=0.010, partial $\eta 2=0.058$ (Greenhouse-Geisser correction). After a post-hoc analysis corrected by Bonferroni, a significant time change between T1 and T3 (p=0.024) was shown: mean BMI regardless of group allocation was 0.65% lower at the end of the study compared to before. No group effect

was detected, F(2,82)=1.79, p= 0.173, partial η2 = 0.042. No statistically significant interaction between group and time was found after Greenhouse-Geisser correction, F(3.48,142.79)=0.19, p=0.924, partial η2 = 0.005.

Muscle mass

Concerning a change in MM, a significant time effect is manifest without regard to the group distribution F(2,160)=6.54, p=0.002, partial η2=0.076. A Bonferroni-corrected post-hoc analysis revealed a significant difference between T2 and T3 (p=0.001), with increases of 1.01% MM. No group effect was found F(2,80)=0.73, p=0.484, partial η2=0.018, and no interaction between group and time was detected, F(4,160)=1.18, p=0.350, partial η2=0.027.

Absolute fat mass

For the absolute change in fat mass, a significant time effect was found irrespective of group distribution following a Greenhouse-Geisser correction, F(1.69,135.18)=21.01, p<0.001, partial η2=0.208. A Bonferroni-corrected post-hoc analysis revealed two significant time differences: between T1 and T3 (p<0.001) and T2 and T3 (p<0.001), with 4.83% and 3.53% loss of body fat, respectively. No group effect was found F(2,80)=0.550, p=0.579, partial η2=0.014, and no interaction between group and time was detected, Greenhouse-Geisser correction F(3.38,165.18)=0.33, p=0.833, partial η2=0.008.

Relative fat mass

A mean change of relative fat mass throughout the study has been assessed by a statistically significant time effect (p<0.001). Post-hoc analysis by Bonferroni shows significant time difference between T1 and T3, and between T2 and T3. Regardless of group allocation, a 4.64% and a 3.67% loss of relative fat mass is observed, respectively.

No significant group effect was found, F(2,80)=0.366, p=0.695, partial η2=0.009. No statistically significant interaction between group and time effect was manifest, Greenhouse-Geisser correction F(3.37,134.96)=0.377, p=0.793, partial η2=0.009.

Table 11: Results Vitamin D and anthropometric parameters.

Parameter	Group	Mean ± Standard deviation.					P-value		
		T1	**T2**	**T3**	**Delta (T2-T1)**	**Delta (T3-T2)**	Time	group	Time x group
Vitamin D [ng/mL], N=85	CON VDD VDM	20.9±5.9 22.8±7.3 23.3±8.7	21.9±5.4 24.4±6.9 25.2±4.8	24.9±9.6 28.9±6.8 31.9±7.3	1.0±4.5 1.6±6.4 1.9±8.0	3.0±7.2 3.4±5.7 6.7±6.3	<0.001	0.021	0.115
Bodyweight [kg], n=85	CON VDD VDM	77.1±17.6 83.7±14.9 81.6±16.5	76.9±17.4 83.0±14.6 81.2±16.4	76.8±17.9 82.6±15.1 80.7±17.0	-0.2±1.4 -0.6±1.7 -0.4±1.8	-0.0±1.6 -0.4±1.6 -1.3±1.6	0.004	0.379	0.669
BMI [kg/m²], n=85	CON VDD VDM	26.2±5.0 28.4±4.8 26.8±4.5	26.1±4.8 28.1±4.7 26.7±4.4	26.1±5.1 26.1±4.9 26.6±4.6	-0.1±0.5 -0.2±0.6 -0.1±0.6	-0.0±0.6 -0.1±0.6 -0.1±0.6	0.010	0.173	0.924
Muscle mass [kg] n=83	CON VDD VDM	52.8±11.1 56.2±11.2 56.1±10.3	53.0±11.2 55.8±10.0 55.8±10.5	53.5±11.1 56.3±10.0 56.4±10.9	0.3±1.3 -0.3±1.7 -0.4±1.2	0.5±1.5 0.5±1.1 0.7±1.3	0.002	0.484	0.350
Absolute Fat mass [kg] n=83	CON VDD VDM	52.7±11.1 56.1±10.4 56.2±11.2	53.0±11.2 55.8±10.0 55.8±10.5	53.5±11.1 56.3±9.9 56.4±10.9	-0.4±1.3 -0.3±1.6 -0.2±1.3	-0.6±1.3 -0.7±1.2 -1.0±1.6	<0.001	0.579	0.833
Relative Fat mass [%], n=83	CON VDD VDM	27.0±8.1 28.3±8.4 26.8±6.8	26.6±7.9 28.0±8.5 24.1±6.6	25.7±8.6 27.2±8.2 25.4±7.3	-0.4±1.3 -0-2±1-7 -0-2±1-2	-0.9±1.7 -0.8±1.1 -1.2±1.1	<0.001	0.695	0.793

Shown values: n= sample size; mean ± standard deviation for each group for each time points; p-value: time = differences between time points regardless of groups. group = differences in groups regardless of time. Time x group = interaction between time and group. P values are marked in bold when significant.

Besides the significant group difference between VDD and VDM in VD serum levels, no other group effects on any of the anthropometric parameters mentioned above have been found. Anthropometric results will therefore be analyzed under the scope of the actual VD serum levels to determine whether a link between VD blood concentration and changes in anthropometric variables is existing.

3.8 Anthropometric results linked to actual 25(OH)D serum levels

3.8.1 Influence of VD level at the start of the training period

An ANOVA for repeated measures was carried out to find eventual differences between the group of subjects that had reached a 30 ng/ml concentration at T2 (n=11), and the one which did not (n=74).

Weight:
No significant change over time was detected: F(1.74,144.05) = 2.613, partial η2 = 0.031, p = 0.084. No time*group interactions F(1.74,144.05) = 0.010. partial η2 < 0.001, p= 0.983, and no differences between the groups F(1,83) = 0.282, partial η2 < 0.015; p= 0.261 were found.

BMI:
No significant change in time was shown: F(1.76,145.89) = 1.328: partial η2 = 0.016, p = 0.267. No differences were seen neither between time nor intervention group F(1.76,145.89) = 0.003. partial η2 < 0.001. p= 0.994, nor between the groups F(1,83) = 0.867: partial η2 = 0.010; p= 0.354.

Muscle mass:
No significant change in time was shown: F(2,162) = 1.77: partial η2 = 0.021, p = 0.174. No differences were seen neither between time nor intervention group F(2.162) = 0.948. partial η2 < 0.012. p= 0.390, nor between the groups F(1,81) = 1.064: partial η2 = 0.013; p= 0.305.

Relative body fat:
No significant change in time was shown: F(1.70,137.65) = 8.01: partial η2 = 0.090, p = 0.001. No differences were seen neither between time nor intervention group F(1.70.137.65) = 1.01. partial η2 = 0.012. p= 0.356, nor between the groups F(1,81) = 0.124: partial η2 = 0.002; p= 0.726.

Absolute body fat:
No significant change in time was shown: F(1.70,138.25) = 5.88: partial η2 = 0.068, p = 0.006. No differences were seen neither between time nor intervention group F(1.70,138.25) = 1.05: partial η2 = 0.013, p = 0.345, nor between the groups F(1,83) = 0.113: partial η2 = 0.001; p= 0.738.

3.9 Correlations

Correlations between anthropometric measurements and test performance have been calculated. The detailed p values and correlation coefficients can be found in the annex.

3.9.1 Anthropometric data and performance tests

BMI measurements at T1, T2 and T3 are weakly and negatively correlated to the 4mGS and 6mGS at T1, T2 and T3 ($p=<0.05$; $-0.49 \leq r \leq -0.20$). Negative and low correlation with BMI

measurements at T1 at T2 were identified for the T1 to T2 delta TUG score, the Spo2 post at T2, and T3 and the HF post at T1 ($p<0.05$; $r \leq +0.29$).

Similar levels and directions of correlations were found for the BMI measurements and the Spo2 post at T3, and with the delta score of the TUG from both the supplementation period and the total study length.

Delta TUG from T3-T1 showed a negative and small correlation as well with the BMI at T1. 6MWD at all three measurements is moderately and negatively associated to BMI (T1, T2 and T3, $p \leqslant 0.001$; $r > -0.5$), and the correlation levels between TUG times at T1 and BMI are moderately and positively correlated with the BMI (T1, T2 and T3, $p \leqslant 0.05$; $r \geqslant +0.5$). TUG times at T2 and T3 are weakly correlated to BMI at all three measurements periods, ($p \leqslant 0.001$; $+0.30 \leqslant r \leqslant +0.49$). All the exact significant coefficient and correlation levels can be found in the annex.

Weight: low and negative correlations were observed between *the weight* of the participants at T3 and 6MWD at T1, T2, T3 ($p<0.05$; $-0.30 \leq r \leq -0.49$). The same strength of correlation was shown between weight at T2 and the 6MWD at T2. Small and negative correlation happens between weight at T1 and T2 and 6MWD at T1, T3, delta TUG scores from T1 to T3 and Delta TUG scores from T1 to T2. A small ($r \leq 2.9$) but significant ($p<0.05$) positive correlation is found between TUG times at T1 and weights of the participants at T1 and T2. A weak level of correlation ($r= +0.37$) is shown for the same variable when the weight at T3 is considered. A higher weight was weakly and negatively correlated to the delta times between T1 and T3 for the TUG test ($p=0.003$; $r= -0.32$).

Muscle mass: a small positively correlation is shown between *muscle mass* of the subjects at T1, T2, T3 and the GS times at T1, T2, T3, the 6mGS times at T1, T2, T3, and the post 6MWD Borg scale subjective measurement at T2 ($p <0.05$; $r \leqslant 0.29$). 6MWD at T3 were negatively correlated ($p<0.05$) to MM at T1 and T2 (small correlation, $r<0.29$), and at T3 (weak correlation ($r=-0.32$). MM at T1 is positively weakly correlated to 6MWD subjective Borg Scale ($r=+0.32$) and the correlation is considered small when that same variable is compared to the MM at T2 and T3. Finally, MM at T2 is positively correlated to the Spo2 levels at the last examination.

The absolute body fat mass: It is strongly negatively correlated to the 6MWD ($p<0.001$; $r \geq 0.6$). The absolute fat mass is weakly and negatively correlated at T1, T2 and T3 with the 4mGS and 6mGS at all 3 tests periods ($p<0.001$; $+0.30 \leq r \leq +0.49$). The body fat at T1 and T2 shows a low and negative correlation with SPo2 post at the second examination ($p<0.001$; $+0.30 \leq r \leq +0.49$). A positive and weak correlation is to be noted between the absolute body fat mass measured at T1, T2 and T3 and the TUG times at T2 and T3 ($p<0.001$; $r = \pm 0.30$ and ± 0.49).

The delta of the 6MWD between T2 and T3, the Spo2 test post T3 and the VD levels at T3 are all negatively correlated with all 3 body fat time measurements ($p<0.05$; $r\leq0.29$). Body fat at T3 and Spo2post T2 are negatively correlated with one another ($p<0.05$). The strength of correlation is small ($r=-0.23$). Low and negative correlation are identified between the absolute body fat at T1 and T2 with the HF pre at T1 and the Spo2 pre at T2. Delta TUG times from T1 to T2 correlate with absolute fat mass at T1 ($p=0.049$, $r=-0.20$).

The relative fat mass: a negative strong correlation between the *relative fat mass* measurements at T1 T2 and T3 are shown with the 4mGS and 6mGS, and with the 6MWD ($p<0.05$; $r\geq0.5$). The fat percentage measurements at T1, T2 and T3 are strongly positively correlated with the 3 different TUG times measurements. A negative and small correlation exist between the first two body fat measurements and the 6MWD delta from T1 to T3 and the HF post at T2 ($p<0.05$; $r\leq0.29$). A similar small and negative correlation exists between body fat measurements at T2 and T3 and HF post at t3. Spo2 post at T2 is moderately associated to body fat percentages values at T1 and T2, and a small correlation exists as well when the T3 body fat percentage values are looked at.

The summary of the correlation results between anthropometric measurements and various tests parameters is to be found in the tables in the annex.

3.9.2 Correlations analysed according to gender and age.

Age: it is positively and moderately correlated ($p<0.05$) to TUG times at T1 ($r= 0.33$), at T2 ($r=0.41$), at T3 ($r=0.44$). A small moderate negative correlation exists between age and 4mGS at T1 ($r=-0.32$) and T3 ($r=-0.35$), between 6mGS at T1 ($r=-0.32$) and T3 ($r=-0.36$) and a small negative correlation between 4mGS at T2 ($r=-0.28$) and 6m GS at T2 ($r=-0.29$). Negatively small and moderate correlation ($p<0.05$; $r=-0.24$) are shown between age and muscle mass at T1 and T2 and between age and the 6MWD. A positive and small correlation happened between the delta TUG times from T1 to T3 ($p=0.046$; $r= +0.22$). A higher BMI at T1, T2 and T3 are negatively and weakly correlated to a better 6MWD for the younger age group ($p<0.05$, $r=-0.4$).

4. Discussion

4.1 Vitamin D

The mean VD level increased significantly over time. Surprisingly, this increase is irrespective of group allocation. No significant differences were found between the supplemented groups and the control group that did not receive any VD. It contrasts with many other papers who

prove a clear difference between the influence of VD supplementation and the control group. In a study with much higher daily supplementation in which the intervention group had already a mean sufficient VD baseline level of 23 ng/ml (57.5 nmol/L), Levis and Gómez-Marín (2017) managed to increase the mean blood levels. Thanks to a daily 4000 IU dose, the serum levels rose to 45.88 ng/ml (114.7 nmol/L). In comparison, the control group did not significantly increase its mean 25(OH) level (from 22.72 ng/ml (56.81 nmol/L) at baseline to 23.92 ng/ml (59.80 nmol/L) after 9 months). As well, in the study from Bunout et al. (2006), with a smaller VD supplementation (400 IU) for 9 months, the mean serum 25 (OH)D level increased from 12.4 ± 2.2 to 25.8 ± 6.5 ng/ml ($p < 0.001$) in the supplemented groups. For the control group, no changes in the levels were observed (13.1 ± 2.7 and 14.5 4.6 ng/ml at baseline and 9 months, respectively). Similar effects with a higher supplementation were observed in a study by Agergaard et al. (2015). They supplemented 1960 IU of VD every day for 12 weeks. The VD groups had a significantly higher 25(OH)D concentration increase at the end compared to the placebo in the elderly population of this study (44.48 ng/ml vs 26.68 ng/ml, respectively). Finally, Uusi-Rasi et al. (2015) found a significantly different VD level between the VD and placebo groups. The mean (25(OH)D serum level in the control group stayed at 27.5 (7.4) ng/mL at baseline and 27.5 (6.9) ng/mL after 2 years. However, the VD groups with a daily intake of 800 IU increased after 2 years their level from 25.1 (6.9) ng/mL at baseline to 37.0 (7.4) ng/mL. There is clearly a difference between their results and the ones from our study.

Many reasons could come in mind to explain why there is such a gap between our findings. The level of supplementation could be one of them. However, it does not seem to be a relevant limiting factor, as both 800 IU/day (Uusi-Rasi et al., 2015; Kotlarczyk et al., 2017) and 50000 IU per month (Ranathunga et al., 2017) are proven to be successfully increasing the blood level considerably. Our study had a supplementation period of 15 weeks, which is much shorter than the previously quoted that lasted two years, and one year (respectively). In addition, the fact that the study took place from February to July, a period in 2019 during which weather was exceptionally sunny, with moderate to high levels of UV radiation between the 21st of March and the 13th of May (Innsbruck Medical University). This could explain why the CON had an increase in the VD serum level during the time of the study. It may be a bias in the estimation of the VD supplementation efficiency compared to placebo. In fact, it was not possible to assess the time that the various groups spent outdoors during those months.

Therefore, the results must be taken with caution. However, the VDM was the only group which managed to rise the main VD level above the «sufficient» threshold which is 30 ng/ml (75 nmol/l) after the 15th week of the intervention. As there is no significant difference with the VDD group, we cannot conclude that this monthly protocol is statistically relevant compared to the daily protocol. Nevertheless, the hypothesis remains that a high monthly VD intake is a viable procedure to rise consequently the serum levels for VD insufficient elderly population.

In upcoming VD supplementation studies, a few considerations could therefore be taken into account: the period of the year during which the study is done is central to assess the actual influence of the supplementation. Moreover, the length and the quantity of the supplementation seems to be consistent and important parameters influencing the transfer into actual blood serum levels. In addition, according to Biondi et al. (2017) for obese people, persons with liver disease, people with intestinal malabsorption or kidney imperfections, a supplementation with an oral calcidiol [25(OH)D] instead of Vitamin D3 could be more adequate.

4.2 Functional tests

The study was designed to identify possible effects of supplementation strategies on TUG, 6MWD and GS tests after a 10 weeks ST program. However, the analysis of variance for repeated measurement showed no significant difference between the intervention groups. This is in line with previous studies mentioned in the systematic research. In fact, apart from Bunout et al. (2006), which found that GS was faster in the supplemented group, the general trend showed no additional effect of supplementation on 6MWD (Oesen et al, 2015; Bell et al, 2017), on maximal GS (Seino et al., 2018; Oesen et al., 2015; Fielding et al., 2017) regardless of 25(OH)D serum increase. Nevertheless, the fact that the TUG did not significantly improve over time in our experimental design after the training is surprising, as it did so in 2 comparable studies (Seino et al., 2018; Bell et al, 2017). As mentioned above, the VD supplementation partly failed to increase as expected the 25(OH)D serum levels. Therefore, the possibility remains that the serum levels - independently of the intervention group allocation – would influence the outcomes. This would have been in line with many studies that found a correlation between baseline VD levels and physical performances (Beaudart et al., 2017, Vaes et al., 2019; Granic et al., 2017), and walking speeds (Annweiler et al., 2017; Granic et al., 2017).

Another ANOVA for repeated measures was driven out between groups and performance tests. Groups were generated on our data base to divide the subjects in categories according to the VD levels at baseline: in interquartile range and in equally distributed groups. However, no significant difference was found. None of the group allocation proved that the initial baseline level influences the baseline performance nor the changes in performances from the beginning to the end of the supplementation period and from the beginning to the end of the training period.

As VD could be involved in the restoration of muscle (Garcia, King, Ferrini, Norris & Artaza, 2011; Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013), it is legitimate to think that the higher the VD levels are before the ST, the better the response to ST would be as it may be facilitated by a better muscle recovery. Therefore, the VD levels before the ST were checked (at T2) to verify if there was a possible influence of achieving 30 ng/ml pre training. In fact, 30

ng/ml of 25(OH)D in the blood is considered to be optimal (Dawson-Hughes et al. 2010). However, no time*group interactions were found between the groups that achieved this threshold at T2 and the ones who did not. This is unexpected, as VD is proven to play an important indirect role and a supposed direct role in muscle contraction (Girgis, Clifton-Bligh, Hamrick, Holick & Gunton, 2013; Rui, 2016; Moon et al., 2019). Two studies (Bray, Doherty & Montero-Odasso, 2008; Levis & Gómez-Marín, 2017) showed improvements in SPPB and fast GS for the insufficient VD baseline participants. Even though comparable baseline levels were chosen in our study, the lack of confirmation can be explained by the fact that the influence of VD supplementation on functional performance seems to be true only for deficient or weaker population (Morley, 2019). The fact that our population was independent and community-dwelling with a mean VD blood concentration of 22.66 ng/mL (with is considered as insufficient, not deficient) could be a bias in this specific field of research as well.

As the study period was divided into two main phases with post and pre-measurements before and after the supplementation and training plus supplementation phase, we tried to identify possible differences in the changes in tests performances between those two phases. Deltas from both periods were therefore compared. However, no significant differences between the means were found, meaning that the training intervention was not statistically more efficient than the supplementation period only in raising the general performances of any of the tests. This confirms the results obtained previously, showing no specific time difference between T2 and T3 only in the 6MWD and 6mGS in the ANOVA for repeated measures. We cannot therefore conclude that the ST program in itself was effective to improve the functional outcomes as such. However, the total intervention (from T1 to T3) did prove a general significant performance increase in 6MWD and 6mGS. This is considerably different from a similarly designed study (Bell et al., 2017), where no change happened after the supplementation phase and a time-dependent increase in TUG and in the 6 min walk test ($P < 0.001$) did appear after the “training plus supplementation phase” which followed the “supplementation phase only” similar to the current study.

Finally, the correlation test between mean VD values and performance tests did not show any association between any performance increase and VD serum increase. Only a small negative correlation between 6MWD and the VD increase occurred: a greater increase in VD serum level during the training phase was weakly and negatively associated with a change in 6MWD performance ($r=-0.24$, $p=0.029$).

4.3 Anthropometric results

The study intended to identify possible effects of supplementation strategies and VD levels on anthropometrics measurements. Some anthropometric factors were indeed associated to performance: a lower BMI has been associated to better 6MWD performances and positive changes in the TUG score. The same trend is shown with a heavier muscle mass correlated to better 6mGS and 6MWD at T3. On the contrary, having a higher absolute and relative fat mass influenced negatively the 6MWD, 4mGS and 6mGS results. The higher the fat mass, the lower was the post exercise heart frequency and peripheral blood oxygen concentration hinting to a lower exhaustion. When differences between intervention groups and VD levels groups were searched for, our results found no time group interaction or between groups effects. The ANOVA was carried out with both interventions groups and with groups built according to actual VD levels. Time effects were observed during the whole length of the study for the body weight (-0.9%; $p=0.011$), the BMI (-0.65 kg/m²; $p=0.024$) and only between T2 and T3 for the muscle mass ($p=0.001$), with 1.01% increase. The absolute and relative fat mass was significantly decreased between T1 and T3 ($p<0.001$) and T2 and T3 ($p<0.001$), with 4.83% and 3.53% absolute body fat lost, respectively. However, even though those changes are statistically significant, they remain very small changes. The fact that this intervention positively influenced anthropometric results is in line with the general considerations concerning the influence of ST on body composition. Sillanpää et al. (2008) for example showed a fat mass reduction from 5 to 8% after a ST program. In a study by Paoli et al. (2010), the high intensity circuit training group showed important fat mass percentage and body weight diminution as well. However, many studies have proven that when added to ST programs, VD has an additive effect influencing positively the body composition. This is verified in various results sections: as compared with RE only, RE and supplementation significantly increased FFM (1.7-kg gain, $p < 0.001$), relative skeletal MM ($p = 0.009$: Rondanelli et al., 2016) appendicular MM ($p < 0.05$) in elderly sarcopenic adults (Yamada et al., 2019), and whole-body, appendicular and leg lean soft tissue mass for community dwelling adults (Seino et al.,2018). Nonetheless, this additive effect has not been detected in our study as no differences were shown neither between the intervention group nor between groups made according to VD levels at baseline or at T2.

4.4 Limitations

The goal of this project and of this specific intervention was to increase the evidence needed to produce guidelines or recommendations for the general elderly population concerning VD supplementation and ST. A great focus was put to administer the respective supplementation amount to ensure similar treatments of all participants. Efforts were made to use reproducible,

replicable and viable tests. However, even though this study was carefully prepared and supervised by experienced PhD students and professors to avoid bias and measurement mistakes, the results must be interpreted with caution. The study lacks statistical power as only 100 participants took place in the study and 85 completed the study. The probability that our study detected a significant difference is therefore reduced (Biau, Kernéis & Porcher, 2008). This low number of participants per group (CON: n= 27, VDD: n= 27, VDM: n= 31) may explain why no statistically significant VD difference was found for the intervention group over time compared to the control group even though a general but not significant trend was noted. This weak statistical power is especially true for the group allocation according to 25(OH)D serum levels at T2 (which was conducted in addition to the initial randomization), as the group that achieved 30 ng/ml had only 11 subjects. In addition, to be representative of the population and transfer practical implications, not only a higher number of participants per group would have been needed but also a higher percentage of females (only 33% in our case). Our results need therefore to be examined in regard to the latest research on the topic.

5. Conclusion

The intervention combining a four weeks VD or calcium supplementation and a 10 weeks' progressive ST intervention with VD or calcium for elderly has shown significant influence on 6mGS and on the 6MWD. However, according to our results, neither the chosen VD dosage and administration regime nor the actual 25(OH)D serum enhancement seem to positively influence the effectiveness of a ST program on functional and endurance performance of older people. Muscle mass, fat mass, body weight and BMI were correlated with various performance tests variables, confirming the link between those anthropometric characteristics and the TUG, GS and the 6MWD. However, no enhancement of anthropometric values has been shown to be due to intervention group allocation nor to the VD parameters. Considering the various limitations and the differences noted with the current literature flow, these results are to be taken with caution.

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8. Erklärung

Ich erkläre, dass ich (Michel Guiborat) die vorliegende Arbeit selbstständig verfasst habe und nur die ausgewiesenen Hilfsmittel verwendet habe. Diese Arbeit wurde ausschließlich als Abschlussarbeit in Bezug auf den European Master in Health and Physical Activity eingereicht und weder in anderen Lehrveranstaltungen noch von anderen Personen (z. B. Arbeiten von anderen Personen aus dem Internet) vorgelegt.

Annex

Tables of significant correlations between anthropometric measurements and tests parameters.

Table annex 1: Weight and correlations.

Variables			TUG at T1	6MWD at T1	6MWDT at T2	6MWD at T3	ΔTUG T3-T1	ΔTUG T2-T1
Weight (kg).	P values	T1	0,004	0,008	0,004	0,009	0,010	0,011
		T2	0,003	0,009	0,002	0,004	0,014	0,019
		T3	0,001	0,005	0,003	0,003	0,011	0,003
	Level of correlation (r)	T1	0,282**	-0,263**	-0,288**	-0,282**	-0,279**	-0,259*
		T2	0,297**	-0,262**	-0,306**	-0,309**	-0,266*	-0,239*
		T3	0,366**	-0,301**	-0,324**	-0,321**	-0,274*	-0,318**
*, The correlation is at level 0.05 significant. **, The correlation is at level 0.01 significant.								

Table annex 2: BMI and correlations.

Variables			6MWD at T1	6MWD at T2	6MWD at T3	ΔTUG T3-T1	ΔTUG T2-T1	Spo2 post T2	Spo2 post T3	HF post T1	TUG at T1	TUG at T2	TUG at T3	4mGST 1	4mGS T2	4GG T3	6GG at T1	6mGS at T2	6GG at T3
BMI [kg/m²]	P values	T1	0.001	0.000	0.000	0.044	0.010	0.011	0.048	0.023	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001
		T2	0.000	0.000	0.000		0.022		0.023	0.031	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000
		T3	0.001	0.000	0.000	0.049	0.043	0.005	0.025		0.000	0.000	0.001	0.002	0.001	0.001	0.001	0.001	0.001
	Correlation level (r)	T1	-0.561**	-0.581**	-0.568**	-0.219*	-0.262*	-0.258*	-0.216*	-0.227*	0.541**	0.422**	0.347**	-0.376**	-0.377**	-0.340**	-0.392**	-0.380**	-0.345**
		T2	-0.564**	-0.591**	-0.592**		-0.233*	-0.287**	-0.248*	-0.219*	0.538**	0.433**	0.366**	-0.366**	-0.390**	-0.364**	-0.405**	-0.393**	-0.372**
		T3	-0.559**	-0.563**	-0.590**	-0.214*	-0.220*		-0.245*		0.524**	0.397**	0.357**	-0.357**	-0.345**	-0.356**	-0.348**	-0.343**	-0.364**

*, The correlation is at level 0.05 significant.

**, The correlation is at level 0.01 significant.

Table annex 3: Relative Fat mass correlations.

Variables			BORG 1	BORG 2	BORG 3	ΔVD T2 T3	4m GS at T1	4m GS at T2	4mGS at T3	6mGS at T1	6mGS at T2	6msGS at T3	6MWT T1	6MWT T2	6MWT T3	ΔSixMWD T1 T3	ΔSixMWD T2 T3
Fat mass (%):	p vales	T1	0.024	0.022	0.028	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.009
		T2		0.019	0.020	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.010
		T3	0.048	0.015	0.032	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.008
	Coefficient (r)	T1		-0.237*	-0.245*	-0.247*	-0.587**	-0.593**	-0.575**	-0.592**	-0.588**	-0.602**	-0.702**	-0.708**	-0.739**		-0.287**
		T2	-0.229*	-0.241*	-0.256*	-0.248*	-0.613**	-0.613**	-0.618**	-0.619**	-0.614**	-0.646**	-0.718**	-0.735**	-0.773**	-0.226*	-0.281*
		T3	-0.215*	-0.266*	-0.237*	-0.240*	-0.546**	-0.581**	-0.602**	-0.555**	-0.577**	-0.626**	-0.709**	-0.705**	-0.760**	-0.218*	-0.288**
**. The Correlation is at the level 0.01 significant. *. The Correlation is at the level 0.05 significant.																	

Table annex 4: Relative fat mass correlations (2).

Variables			Spo2 post T1	Spo2 post T2	Spo2 post T3	VD at T3	TUG at T1	TUG 2	TUG 3	Δ TUG T1 to T2
Fat Mass (%)	P value	T1		0.003	0.030	0.023	0.000	0.000	0.000	0.024
		T2		0.002	0.030	0.018	0.000	0.000	0.000	
		T3	0.013	0.020	0.040	0.022	0.000	0.000	0.000	0.048
	Coefficient (r)	T1		-0.303**	-0.240*	-0.249*	0.562**	0.476**	0.452**	-0.229*
		T2		-0.319**	-0.237*	-0.256*	0.579**	0.498**	0.485**	
		T3	-0.270*	-0.251*	-0.225*	-0.249*	0.568**	0.485**	0.467**	-0.215*
**. The Correlation is at the level 0.01 significant. *. The Correlation is at the level 0.05 significant.										

Table annex 5: Absolute fat mass correlations.

Variables			4mGSat T1	4mGSat T2	4mGSat T3	6m GS at T1	6m GS at T2	6m GS at T3	6MWT at T1	6MWT at T2	6MWT at T3	∆6MWD T3-T2	Spo2 post T2
Fat mass [kg]	P values	T1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.002
		T2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.001
		T3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.039
	Correlation level (r)	T1	-0.458**	-0.476**	-0.429**	-0.478**	-0.482**	-0.448**	-0.625**	-0.646**	-0.663**	-0.217*	-0.309**
		T2	-0.472**	-0.485**	-0.461**	-0.490**	-0.494**	-0.481**	-0.630**	-0.664**	-0.688**	-0.217*	-0.328**
		T3	-0.423**	-0.463**	-0.453**	-0.440**	-0.464**	-0.471**	-0.640**	-0.648**	-0.681**	-0.216*	-0.225*
*, The correlation is at level 0.05 significant.													
**, The correlation is at level 0.01 significant.													

Table annex 6: Absolute fat mass correlations (2).

Variables			Spo2 post T3	HF pre T1	Spo2 pre 2	VD at T3	TUG 1	TUG 2	TUG 3	∆Tug T1 to T2
Fat mass (kg)	P values	T1	0.021	0.037	0.040	0.020	0.000	0.000	0.000	0.049
		T2	0.015	0.034	0.042	0.028	0.000	0.000	0.000	
		T3	0.021			0.025	0.000	0.000	0.000	
	Correlation level (r)	T1	-0.256*	-0.211*	-0.212*	-0.256*	562**	476**	452**	-0.203*
		T2	-0.264*	-0.215*	-0.209*	-0.238*	0.579**	498**	0.485*	
		T3	-0.251*			-0.243*	0.568**	0.485**	0.467**	
*, The correlation is at level 0.05 significant.										
**, The correlation is at level 0.01 significant.										

Table annex 7: Absolute muscle mass correlations.

Variables			4mGS at T1	4mGS at T2	4mGS at T3	6mGS at T1	6mGS at T1	6mGS at T3	6MWT at T3	ΔTUG T1 to T2	6MWD BORG at T1	6MWD BORG at T2	Spo2 pre0. at T3
MM (kg)	P values	T1	0.005	0.008	0.019	0.013	0.010	0.011	0.010		0.002	0.016	
		T2	0.002	0.005	0.013	0.005	0.006	0.008	0.010		0.006	0.017	0.047
		T3	0.026	0.026	0.017	0.044	0.029	0.011	0.006	0.001	0.008	0.035	
	Correlation level (r)	T1	0.279**	0.271**	0.258*	0.250*	0.263*	0.279*	-0.280**		0.316**	0.250*	
		T2	0.305**	0.282*	0.268*	0.281**	0.278**	0.287	-0.294**		0.279**	0.244*	0.217*
		T3	0.241*	0.241*	0.258*	0.219*	0.237**	0.276	-0.312**	-0.363**	0.288**	0.230*	

*, The correlation is at level 0.05 significant.

**, The correlation is at level 0.01 significant.