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ORIGINAL ARTICLE

Vitamin D supplementation does not enhance resistance training-induced gains in muscle strength and lean body mass in vitamin D deficient young men

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Abstract

Purpose Vitamin D (Vit-D) supplementation has been shown to increased muscle strength in young adults. It remains unclear if Vit-D supplementation enhances the efficacy of resistance training (RT). This study tested the hypothesis that Vit-D supplementation would enhance the RT-induced increases in muscle strength and lean body mass (LBM) in Vit-D deficient young men.

Methods Thirty-nine men (baseline serum 25(OH)D < 50 nmol L^{-1}) were quasi-randomly assigned to one of the two groups that performed a 12-week supervised RT program concomitant with either Vit-D (8000 IU daily; VD) or placebo (PLC) supplementation.

Results During 12-week RT, energy and nutrient (except Vit-D) intake and training loads did not differ in the two groups. Serum 25(OH)D levels increased from 36.3 ± 9.2 to 142.4 ± 21.9 nmol L^{-1} ($P < 0.05$) in VD group and remained unchanged between 36.3 ± 8.9 and 29.4 ± 6.6 nmol L^{-1} ($P > 0.05$) in PLC group. Muscle strength (1-repetition maximum) increased ($P < 0.05$) to an equal extent in the two groups in 5 exercises performed on RT equipment, whereas strength gains in chest press and seated row were greater ($P < 0.05$) in PLC compared to VD group. Total and regional LBM (measured by DXA scan) increased ($P < 0.05$) equally in the two groups. Android fat mass decreased ($P < 0.05$) in VD group only.

Conclusion Vit-D supplementation does not enhance the efficacy of RT in terms of muscle strength and LBM gains in Vit-D deficient young healthy men.

Keywords Serum 25-hydroxyvitamin D · High daily vitamin D dose · Muscle strength · Body composition · Quasi-randomized controlled trial

Abbreviations

ANOVA	Analysis of variance
BM	Body mass
BMI	Body mass index
CK	Creatine kinase
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
LBM	Lean body mass
PLC	Placebo group
PTH	Parathormone
1RM	1-Repetition maximum
5RM	5-Repetition maximum
RT	Resistance training
VD	Vitamin D group
VDR	Vitamin D receptor
Vit-D	Vitamin D

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Introduction

Vitamin D (Vit-D) receptors (VDR), that are considered the absolute determinants of Vit-D physiological functions (Boland 2011), have been detected in almost every human tissue, including skeletal muscle (Zittermann 2003), where Vit-D acts via both genomic and non-genomic pathways (Girgis 2020; Pojednic and Ceglia 2014). Evidence suggests that Vit-D directly stimulates muscle protein synthesis in Vit-D deficient rats (Birge and Haddad 1975; Wassner et al. 1983) and increases intramyonuclear VDR concentration and muscle fiber size in Vit-D-deficient humans (Ceglia et al. 2013). In murine myotubes (Salles et al. 2013) and human myoblasts (Romeu Montenegro et al. 2019), the stimulating effect of Vit-D on protein synthesis is associated with activation of Akt/mTOR-dependent pathway.

Increases in muscle mass and strength that occur as a result of resistance training (RT) are largely based on changes in muscle protein synthesis (Gonzalez 2016; Phillips 2015). Besides the direct impact through muscular VDR, Vit-D may stimulate muscle protein synthesis indirectly through modulating the levels of anabolic hormones in blood. In men, muscle mass correlates with testosterone levels (Lafranco and Minetto 2013) and suppression of serum testosterone concentration attenuate RT-induced muscle hypertrophy (Kvorning et al. 2006). On the other hand, increased levels of serum testosterone and growth hormone improve muscle adaptations to RT (Gharahdaghi et al. 2019; Rønnestad et al. 2011). VDR expression occurs in human testis (Habib et al. 1990) and positive association have been observed between serum 25(OH)D (marker of Vit-D status) and testosterone levels in men (Pilz et al. 2011; Wehr et al. 2010). Insulin-like growth factor 1 (IGF-1) may stimulate muscle hypertrophy (Haddad and Adams 2004; Yin et al. 2020), and both RT (Shelmadine et al. 2009; Tsai et al. 2015) and Vit-D supplementation (Ameri et al. 2013; Soliman et al. 2008) may increase serum IGF-1 levels.

Recent meta-analysis concluded that Vit-D supplementation increases upper and lower body muscle strength in young healthy women and men (Tomlinson et al. 2015). Considering that the most effective strategy to enhance muscle strength and mass is RT (Phillips 2015; Westcott 2012), the combined impact of Vit-D supplementation and RT on muscle strength and body composition, especially in Vit-D deficient subjects, may be greater than the impact of Vit-D or RT alone. The only two randomized double-blind placebo-controlled studies, that have investigated the potential additive effect of Vit-D supplementation during RT on muscle function in young adults (Agergaard et al. 2015; Carrillo et al. 2013), concluded that Vit-D compared to placebo did not induce greater increases in muscle strength. However, in both studies, participants' serum 25(OH)D concentrations were higher than the level of 50 nmol L^{-1}, which is defined as cut-off for Vit-D sufficiency (Ross et al. 2011; Trummer et al. 2016). Moreover, in one study (Carrillo et al. 2013), serum 25(OH)D levels in Vit-D and placebo supplemented groups did not differ.

Therefore, the purpose of this study was to test the hypothesis that in Vit-D deficient young healthy men Vit-D supplementation compared to placebo ingestion would enhance the increases in muscle strength and lean body mass (LBM) during 12-week supervised RT program.

Materials and methods

Participants

Young males were invited to participate in the study through the University of Tartu mail lists. The basic criteria of inclusion were the absence of chronic illnesses, non-usage of supplements containing Vit-D, absence of experience in participation in competitive sport, and non-participation in any systematic recreational RT program within the previous 12 months.

The initial number of young men who responded and who met the basic criteria of inclusion was 60. They received detailed information regarding the aims, duration, and procedures of the study the protocol of which was approved by the Research Ethics Committee of the University of Tartu. Potential participants were informed that subjects with a baseline serum 25(OH)D level exceeding 60 nmol L^{-1} and those with a serum level above 50 nmol L^{-1} 1 month after baseline measurement will be excluded from the remainder of the study. After giving written informed consent, subjects donated baseline venous blood sample. According to the 25(OH)D level criteria, 8 men were excluded from the study after their baseline blood samples were analysed, and another 3 men were excluded 1 month later, i.e., at the end of the preparatory phase (Fig. 1). During the preparatory phase, 8 men withdrew from the study on their own initiative. Finally, 2 more men were excluded from the study during the main phase due to illness and loss of motivation to continue training. Thus, the number of men who completed the study, i.e., participated in at least 80% of the training sessions, was 39. Their age, height, body mass (BM), and body mass index (BMI) (mean ± SD) at the beginning of the preparatory phase were 23.7 ± 2.5 years, 1.83 ± 0.06 m, 79.7 ± 9.7 kg, and 23.7 ± 2.5 kg m^{-2}, respectively.

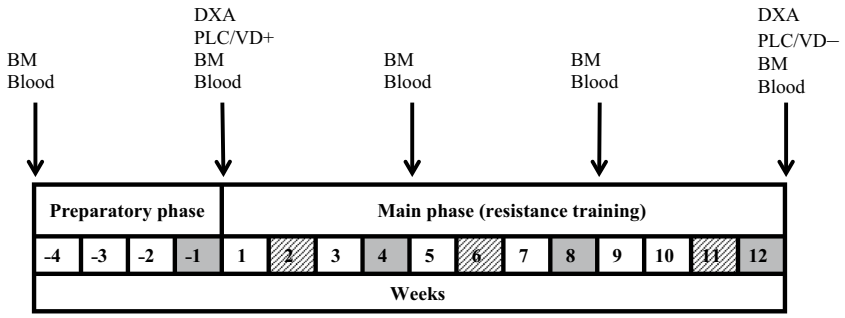

Fig. 1 Study protocol. Arrows indicate time points for measuring body mass (BM) and body composition (DXA), venous blood sampling (Blood), and beginning (PLC/VD+) and end (PLC/VD−) of placebo or vitamin D supplementation. Grey and striped cells indicate the weeks during which muscle strength was assessed or 4-day food diaries were filled, respectively

Table 1 Anthropometric characteristics and serum vitamin D concentrations across groups at the beginning of the main phase of the study

Parameter	PLC ($n=18$)	VD ($n=21$)
Age (years)	23.3 ± 2.3	24.0 ± 2.6
Height (m)	1.84 ± 0.05	1.83 ± 0.06
Body mass (kg)	78.7 ± 9.6	80.1 ± 10.6
BMI (kg m^{-2})	23.2 ± 2.5	23.9 ± 2.7
25(OH)D (nmol L^{-1})	36.32 ± 8.90	36.33 ± 9.22

Data are presented as mean ± SD. *PLC* placebo group, *VD* vitamin D group, *BMI* body mass index

Experimental design

This double-blind placebo controlled quasi-randomized study was carried out in two (preparatory and main) phases (Fig. 1). The total duration of the study was 16 weeks and it was scheduled for the winter-spring period of a year, i.e., from December to March.

The purpose of the 4-week preparatory phase was to teach the participants to perform strength exercises included in the training program technically correctly, and to accustom them to train in a regular basis. This phase also aimed in standardizing the training status of the participants and to overcome neural and learning adaptations usually occurring within the first few weeks of RT (Hulmi et al. 2015). During the preparatory phase, the participants trained 2−3 times a week, did not consume any supplements, but learned to record their habitual diet and enter the data into the web-based Nutridata platform (National Institute for Health Development, Estonia).

The 12-week main phase began with dividing the participants into placebo (PLC) and vitamin D supplemented (VD) groups by listing their body mass from the lowest to the highest and then assigning them to alternate groups. There were no significant differences between the groups for age, height, body mass, or body mass index (Table 1). During the main phase, the participants trained regularly 3 times a week. Muscle strength was tested, body mass was measured, and venous blood samples were collected at weeks −1, 4, 8, and 12 (Fig. 1). At the beginning and end of the main phase body composition of the participants was assessed. Three times during the main phase (Fig. 1), the participants filled 4-day food diaries, including one weekend day, in the web-based Nutridata platform.

Administration of dietary supplements

Both Vit-D and placebo supplements were gelatine capsules containing vitamin D_3 solution in edible oil and pure edible oil, respectively. The two supplements (product codes ST45851 and ST47202 for Vit-D and placebo, respectively; Diafarm A/S, Vejle, Denmark) were indistinguishable from each other. In a double-blind manner, participants in the VD group administered 8000 IU of vitamin D_3 daily during the 12-week main phase of the study, whereas participants in the PLC group ingested placebo during the same period. A calculation based on data from Owens et al. (2014) predicted that a daily dose of 8000 IU would raise mean serum 25(OH)D level to approximately 120 nmol L^{-1} in 4 weeks and maintain this or slightly higher level for the next 8 weeks. According to Pludowski et al. (2018), minimal 25(OH)D concentrations required for triggering extra-skeletal effects range between 75 and 125 nmol L^{-1}, and athletes are recommended to maintain serum 25(OH)D level above 100 nmol L^{-1} (Larson-Meyer and Willis 2010; Ogan and Pritchett 2013). Via e-mail and in person during training sessions, all our participants were frequently reminded to administer the supplement capsules regularly.

To standardise the potential impact of nutritional factors on early recovery processes, participants in both groups ingested 25 g of whey-based supplement (Whey 80, Elite Fitness OY, Helsinki, Finland) after each training session during the main phase of the study. A 25 g portion of the supplement contained 20 g of pure whey protein, 1.25 g

of carbohydrates, 1.75 g of fats and provided 100 kcal of energy. At the end of a workout, the supervisor of training gave each portion of the supplement personally to each participant with instruction to dissolve the powder in approximately 0.5 L of water and to drink the solution within the following 30 min. We considered this standardisation measure important, because post-workout protein ingestion influences muscle protein synthesis (Moore et al. 2009) and post-workout changes in muscle protein synthesis correlate with the level of muscle hypertrophy achieved as a result of RT program of several weeks (Damas et al. 2016).

Resistance training program

All training sessions in the preparatory and main phases of the study were conducted under the direct supervision of a research team member at the University of Tartu Sports Club. Depending on each individual's daily schedule, the participants came to training sessions either in the mornings or in the evenings. Each training session started with a 10−15-min light general warm-up. The warm-up exercises were performed on rowing ergometer, cycle ergometer, and 200 m indoor running track.

The RT program consisted of 7 exercises (Levinger et al. 2009) which were carried out on RT equipment: chest press (Technogym, Cesena, Italy), leg press (David, Helsinki, Finland), lateral pull-down, triceps push-down, seated row, knee extension, and biceps curl (Precor, Woodinville, USA). The order of the training exercises during a training session was not fixed, except for knee extension and leg press which had to be performed with an upper body exercise(s) in between them. The concentric and eccentric phases of each exercise lasted approximately 1−2 s. The rest interval between repetition sets of the same exercise was 1−2 min and between different exercises 3 min (Agergaard et al. 2015). Participants had to perform all sets of a particular exercise sequentially and thereafter move to another exercise.

During the first 2 weeks of the preparatory phase, there were 2 training sessions a week and from the third week of preparatory phase until the end of the study 3 sessions a week. The number of sets performed during a training session and the number of repetitions per set varied throughout the study (Table 2). During the preparatory phase, the training weights to be used by each participant were determined by the supervisor. The training weights used in the main phase were determined as a percentage of an individual 1-repetition maximum (1RM) performance (Table 2).

Assessment of muscle strength

1RM-s were calculated from 5-repetition maximum (5RM) values as described by Baechle and Earle (2008). 5RM was defined as the heaviest weight a participant was able to lift five times with a proper technique and without compensatory movements. 5RM tests (Baechle and Earle 2008) for each exercise were performed at weeks −1, 4, 8, and 12 (Fig. 1). On the basis of the estimated 1RM-s, the individual training weights were determined (Table 2).

5RM testing protocol begun with 1 set of 10 repetitions followed by 1-min rest period. The initial load was

Table 2 Distribution of training loads during the preparatory and main phases of the study

Phase	Week	SPW	Sets	Reps	Training load (weight used)
Preparatory	− 4	2	2	15−20	Chosen by supervisor
	− 3				
	− 2	3	3	12−15	
	− 1	*3*	*1*	*12–15*	
Main	1	3	3	12−15	60%−75% of the 1RM measured at week -1
	2				
	3				
	4	*3*	*1*	*12–15*	
	5	3	3	8−12	60%−75% of the 1RM measured at week 4
	6				
	7				75%−85% of the 1RM measured at week 4
	8	*3*	*1*	*8–12*	
	9	3	3	8−12	75%−85% of the 1RM measured at week 8
	10				
	11				
	12	*3*	*1*	*8–12*	

1RM test weeks are in italics; *1RM* 1-repetition maximum; *SPW* number of training sessions per week; *Sets* number of sets of each exercise performed during each training session; *Reps* number of repetitions per one set of each exercise

determined individually for each participant by the supervisor based on his expert opinion and participant's previous progress. Then load was increased, assuming that participant could be able to perform 8–10 repetitions with these weights. After 2-min rest period, near 5RM load was achieved in the same manner, with the participant performing 6–8 repetitions followed by 2–4-min rest period. Then, the load was increased once more, and the 5RM test was carried out. If the participant failed to achieve 5 repetitions, a 2–4-min rest was provided and the load was decreased by 2.5–4.5 kg for upper body exercises and 5–10 kg for lower body exercises (Baechle and Earle 2008). The 5RM-s were determined in the following order: lateral pull-down, triceps push-down, leg press and chest press in the first training session of RM testing weeks, and seated row, knee extension, and biceps curl in the second training session. In the first and second training sessions of RM testing week, in addition to the 5RM tests, the participants performed 1 set of all the remaining training exercises (Table 2). In the third training session of RM testing week, 1 set of each training exercise was performed (Table 2).

Blood sampling and biochemical analyses

Five blood samples were taken from antecubital vein of the participants: at baseline (i.e., prior to the preparatory phase), at the end of the preparatory phase (i.e., immediately prior to the main phase), and after weeks 4, 8 and 12 during the main phase of the study (Fig. 1). The blood samples were taken following two resting days, on Monday or Tuesday morning, after approximately 12-h overnight fast. Blood was collected into 5-mL Vacutainer serum tubes and left to clot for 10 min at room temperature after which the tubes were centrifuged (Eppendorf 5804R, Eppendorf AG, Hamburg, Germany) for 10 min at 3000 rpm (2000*g*) at 4 °C. After centrifugation, the tubes were still maintained at 4 °C and transported to the United Laboratories of the Tartu University Hospital for analysis.

Serum 25(OH)D concentration was measured in all the five blood samples, and the concentrations of testosterone, growth hormone (GH), insulin-like growth factor-1 (IGF-1), cortisol, parathormone (PTH), calcium (Ca), ionized Ca, urea, and creatine kinase (CK) were measured in samples obtained immediately prior to the main phase, and after weeks 8 and 12 (Fig. 1).

Serum concentrations of 25(OH)D, IGF-1 and GH were measured by chemiluminescence immunoassay method using IDS-iSYS Multi-Discipline Automated Analyzer (Immunodiagnostic Systems Limited, Copenhagen, Denmark). Concentrations of testosterone, cortisol, PTH, and CK were measured by electrochemiluminescence immunoassay "ECLIA" on Cobas 6000 analyzer (Roche Diagnostics GmbH, Tokyo, Japan). The Cobas 6000 analyzer was also used for measuring serum Ca (photometrical NM-BAPTA method) and urea (kinetic test with urease and glutamate dehydrogenase) concentrations. Ionized Ca levels were measured by ion-selective electrode method using analyzer Prime ES (Roche, Mannheim, Germany).

Anthropometric measurements and assessment of body composition

Body height of the participants was measured at the beginning of the main phase to the nearest 0.005 m using a stadiometer (Seca bodymeter 206; Seca GmbH, Hamburg, Germany). Nude BM was measured four times during the study (Fig. 1), always in the morning after overnight fast and to the nearest 0.001 kg using an electronic scale (CH3G-150I Combics; Sartorius AG, Goettingen, Germany).

Body composition of the participants was analysed using dual energy X-ray (DXA) scanner Hologic Discovery W (Hologic, Inc., Marlborough, USA) before and after the main phase of the study. Lean mass of trunk, arms and legs as well as android fat mass and percentage and total fat mass and percentage were measured.

Dietary intake monitoring

During the whole study period, the participants were told not to change their eating habits or use any dietary supplement other than those that were given them by the research team member who supervised their training sessions. During the 2nd, 6th, and 11th weeks of the main phase of the study (Fig. 1), the participants filled 4-day food diaries in the Nutridata platform. All the 4-day periods included 1 resting (weekend) day, i.e., a day off from training and work or studies. The participants provided access to their data loaded into the Nutridata platform to one research team member who performed the analysis of these data at the individual as well as at the group level.

Statistical analysis

Statistica 13.3 software was used for performing statistical analysis. Data are presented as means ± SD. All data were checked for normal distribution using Kolmogorov–Smirnov test. A two-way mixed-model analysis of variance ANOVA with a between factor of group (VD vs PLC) and within factor being time was used to evaluate the differences within and between the groups for BM, body composition, blood parameters, nutrition data, and 1RM-s. In case a significant main effect was observed, Tukey's honestly significant difference post hoc analysis was used to locate differences between the means. The mean values of different parameters registered at a single time point were compared using Student's *t* test for independent variables. A Pearson product

moment coefficient of correlation (r) was applied to determine the relationships between variables. Significance was set at $P < 0.05$ level.

Results

Daily energy and nutrient intake

Energy and nutrient intake of the participants is shown in Table 3. No main effect of group or time or group by time interaction occurred for any of the dietary parameters measured ($P > 0.05$).

Serum 25(OH)D

There were significant main effects of group ($F = 365.79$) and time ($F = 151.24$) and a significant group by time interaction ($F = 232.32$) for serum 25(OH)D levels (in all cases $P < 0.0001$). At weeks −4 and 0, serum 25(OH)D concentrations did not differ between the two groups (Fig. 2). However, from week 0 to week 4 a significant increase in serum 25(OH)D level (from 36.3 ± 9.2 to 101.3 ± 17.7 nmol L^{-1}; $P < 0.0001$) occurred in VD group, whereas no change was evident in PLC group (36.3 ± 8.9 and 31.8 ± 8.7 nmol L^{-1}, respectively; $P > 0.05$). In VD group, from week 4 to week 8, serum 25(OH)D concentration further increased significantly ($P < 0.0001$) and reached the level of 134.6 ± 19.5 nmol L^{-1}. Peak serum 25(OH)D concentration in VD group (142.4 ± 21.9 nmol L^{-1}) occurred at week 12, but this value did not differ significantly ($P > 0.05$) from that registered at week 8. Compared to PLC group, average serum 25(OH)D concentrations were 3.2, 4.4, and 4.8 times higher at weeks 4, 8, and 12, respectively (in all cases $P < 0.0001$). In PLC group, a continuous gradual decrease in 25(OH)D level occurred throughout the whole study period from 45.0 ± 10.4 nmol L^{-1} at week −4 to 29.4 ± 6.6 nmol L^{-1} at week 12 ($P = 0.001$).

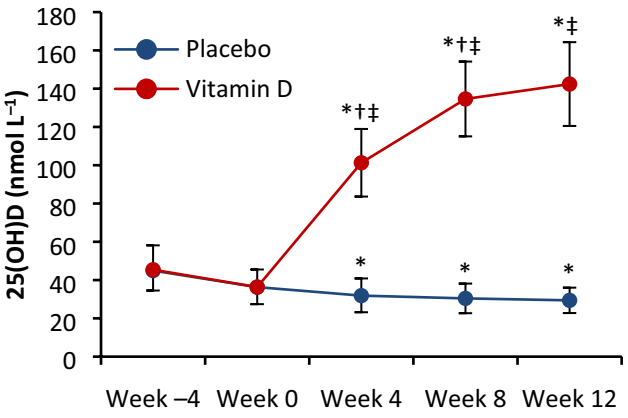

Fig. 2 Serum 25(OH)D concentrations. Data are presented as means ± SD, $n = 18$ in placebo and $n = 21$ in vitamin D group. Significantly different ($P < 0.05$): *from Week −4; †from previous week; ‡from placebo group

Table 3 Energy and nutrient intake

Variable	Group	Week 2	Week 6	Week 11
Energy intake (kcal)	PLC	2314 ± 525	2476 ± 619	2317 ± 414
	VD	2404 ± 597	2193 ± 519	2323 ± 627
Protein (%)	PLC	16.6 ± 3.2	16.2 ± 3.6	16.5 ± 3.5
	VD	17.4 ± 3.6	18.1 ± 3.3	18.7 ± 4.8
Protein (g kg^{-1})*	PLC	1.24 ± 0.33	1.23 ± 0.36	1.17 ± 0.26
	VD	1.30 ± 0.34	1.23 ± 0.31	1.32 ± 0.34
Fat (%)	PLC	35.0 ± 7.4	36.2 ± 6.8	36.4 ± 5.5
	VD	38.3 ± 3.3	35.8 ± 5.4	34.1 ± 2.6
Fat (g kg^{-1})	PLC	1.22 ± 0.42	1.28 ± 0.51	1.17 ± 0.30
	VD	1.30 ± 0.37	1.07 ± 0.23	1.11 ± 0.32
Carbohydrates (%)	PLC	45.5 ± 7.1	47.0 ± 7.3	44.9 ± 5.5
	VD	42.9 ± 4.6	45.1 ± 7.1	44.3 ± 5.8
Carbohydrates (g kg^{-1})	PLC	3.47 ± 1.03	3.61 ± 1.05	3.31 ± 1.09
	VD	3.28 ± 1.05	3.19 ± 1.20	3.27 ± 1.13
Vitamin D (μg)	PLC	4.89 ± 3.98	3.21 ± 1.23	4.03 ± 3.61
	VD	3.29 ± 1.66	3.36 ± 1.22	2.97 ± 1.43
Calcium (mg)	PLC	862 ± 428	823 ± 294	792 ± 208
	VD	896 ± 478	817 ± 350	929 ± 378

Data are presented as means ± SD, $n = 14$ in PLC (placebo) and $n = 16$ in VD (vitamin D) group

*The amounts of protein in the table do not include 20 g of whey which was consumed only on the training days after each workout

Muscle strength

A significant main effect of time for 1RM occurred in all seven exercises: leg press ($F = 360.94$), knee extension ($F = 300.65$), chest press ($F = 391.50$), triceps push-down ($F = 225.06$), biceps curl ($F = 169.81$), lateral pull-down ($F = 250.02$), and seated row ($F = 290.31$) (in all cases $P < 0.0001$). However, in none of the exercises, main effect of group for 1RM was observed (in all cases $P > 0.05$), and a significant group by time interaction for 1RM occurred only in chest press ($F = 5.78$; $P = 0.001$) and seated row ($F = 5.55$; $P = 0.001$). In all the seven exercises in both groups, consistent gradual increases in 1RM occurred throughout the 12-week RT program, i.e., from week 0 to week 12 (Table 4). In chest press and seated row, significantly greater increases in 1RM were observed in PLC group compared to VD (32.6 ± 6.6 kg vs 26.1 ± 8.5 kg; $P = 0.014$ and 22.4 ± 5.8 kg vs 16.7 ± 4.5 kg; $P = 0.001$, respectively), whereas in the remaining five exercises, no significant between group differences in strength gains occurred (Fig. 3).

Table 4 One-repetition maximum (1RM) strength (kg) during 12-week resistance training

Variable	Group	Week 0	Week 4	Week 8	Week 12
Leg press	PLC	219.1 ± 52.9	262.2 ± 44.6*	297.0 ± 47.6*†	336.0 ± 52.7*†
	VD	212.5 ± 39.0	256.6 ± 39.3*	289.2 ± 44.4*†	326.6 ± 0.1*†
Knee extension	PLC	84.4 ± 23.5	106.8 ± 27.7*	134.7 ± 31.8*†	154.9 ± 33.2*†
	VD	92.4 ± 19.3	120.6 ± 21.5*	145.2 ± 26.6*†	162.9 ± 5.0*†
Chest press	PLC	65.8 ± 11.4	77.3 ± 12.9*	88.4 ± 12.6*†	98.4 ± 13.2*†
	VD	69.8 ± 11.9	80.9 ± 12.4*	88.3 ± 11.1*†	95.9 ± 12.1*†
Triceps push-down	PLC	62.9 ± 9.7	70.9 ± 9.2*	77.8 ± 11.1*†	83.6 ± 10.1*†
	VD	62.6 ± 10.6	71.1 ± 10.3*	76.1 ± 8.6*†	82.7 ± 10.5*†
Biceps curl	PLC	62.7 ± 9.8	69.9 ± 9.3*	75.2 ± 9.6*†	81.4 ± 9.2*†
	VD	63.3 ± 9.4	70.4 ± 8.0*	75.6 ± 6.5*†	81.2 ± 7.3*†
Lateral pull-down	PLC	65.3 ± 13.6	71.4 ± 12.5*	78.2 ± 13.3*†	83.6 ± 13.8*†
	VD	65.1 ± 9.2	73.0 ± 8.4*	77.7 ± 9.1*†	82.8 ± 10.1*†
Seated row	PLC	66.1 ± 10.6	75.4 ± 11.4*	81.5 ± 13.0*†	88.5 ± 11.8*†
	VD	69.5 ± 7.5	75.8 ± 7.4*	82.3 ± 8.9*†	86.2 ± 8.6*†

Data are presented as means ± SD, $n = 18$ in PLC (placebo) and $n = 21$ in VD (vitamin D) group

*Significantly different from Week 0 ($P < 0.05$)

†Significantly different from previous week ($P < 0.05$)

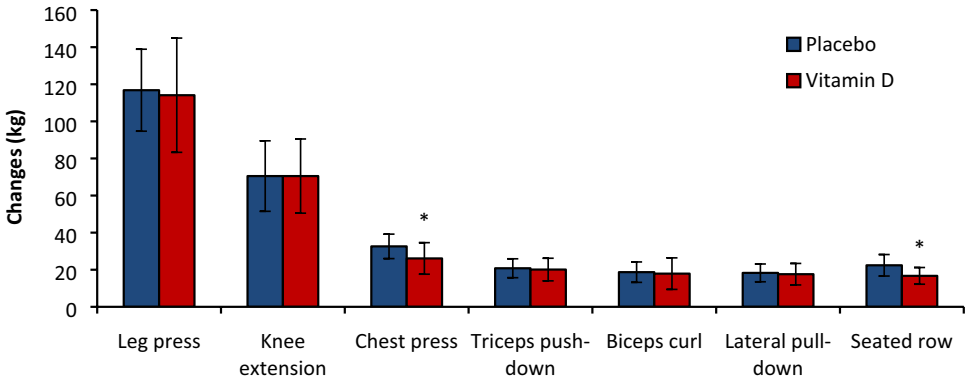

Fig. 3 Changes in 1-repetition maximum during 12-week resistance training program. Data are presented as means ± SD, $n = 18$ in placebo and $n = 21$ in vitamin D group. *Significantly different ($P < 0.05$) from placebo group

Body mass and composition

Body mass ($F = 6.77$), total body fat mass ($F = 30.04$), total body fat % ($F = 70.06$), android fat mass ($F = 12.62$), android fat % ($F = 28.02$), total lean mass ($F = 125.1$), trunk lean mass ($F = 63.42$), lean mass of arms ($F = 66.4$), and lean mass of legs ($F = 45.81$) all showed a significant main effect of time (in all cases $P < 0.001$). No main effect of group or group by time interaction for body mass or for any body composition parameter occurred ($P > 0.05$). During the 12-week RT program, i.e., from week 0 to week 12, a small increase ($P = 0.006$) in body mass and a decrease in android fat mass ($P = 0.013$) occurred in PLC and VD groups, respectively (Table 5). However, there were no significant between-group differences in the magnitude of changes of these two parameters ($P > 0.05$). Total fat mass and android fat % decreased, whereas total lean mass, trunk lean mass, lean mass of arms and also that of legs all increased in both groups without between-group differences ($P > 0.05$) in the magnitude of change in any of these parameters (Table 5).

At week 0, correlation analysis of pooled data of PLC and VD groups revealed significant relationships between serum 25(OH)D level and total lean mass ($r = 0.432$, $P = 0.006$), trunk lean mass ($r = 0.427$, $P = 0.007$), lean mass of arms ($r = 0.378$, $P = 0.018$), and lean mass of legs ($r = 0.390$, $P = 0.014$). On the other hand, serum 25(OH)D level did not correlate with BMI, total fat mass or android fat mass (in all cases $P > 0.05$).

Blood hormones

There was a significant main effect of group for cortisol levels ($F = 4.43$; $P = 0.042$). Overall cortisol levels in PLC group (460.4 ± 94.0 nmol L^{-1}) were higher than in VD group (414.1 ± 82.4 nmol L^{-1}), but no main effect of time or group by time interaction was observed ($P > 0.05$) (Table 6). Main effect of time was evident for IGF-1 levels ($F = 3.42$;

Table 5 Body mass and body composition during 12-week resistance training

Variable	Group	Week 0	Week 12	Change
Body mass (kg)	PLC	78.74 ± 9.57	80.05 ± 8.61*	1.31 ± 1.85
	VD	80.13 ± 10.60	80.52 ± 10.15	0.39 ± 2.02
Total fat mass (kg)	PLC	16.74 ± 4.93	15.68 ± 4.95*	− 1.07 ± 0.86
	VD	17.33 ± 5.94	16.06 ± 5.40*	− 1.27 ± 1.62
Total fat (%)	PLC	21.3 ± 4.0	19.7 ± 4.3*	− 1.7 ± 0.8
	VD	21.6 ± 4.8	19.9 ± 4.4*	− 1.7 ± 1.5
Android fat mass (kg)	PLC	1.19 ± 0.48	1.12 ± 0.48	− 0.07 ± 0.10
	VD	1.24 ± 0.64	1.13 ± 0.58*	− 0.11 ± 0.19
Android fat (%)	PLC	22.9 ± 6.2	21.2 ± 6.3*	− 1.7 ± 2.1
	VD	23.0 ± 6.8	20.8 ± 6.2*	− 2.2 ± 2.5
Total lean mass (kg)	PLC	57.57 ± 5.34	59.79 ± 4.63*	2.23 ± 1.28
	VD	58.28 ± 5.48	60.12 ± 5.84*	1.84 ± 0.99
Trunk lean mass (kg)	PLC	28.07 ± 2.34	29.15 ± 1.96*	1.09 ± 0.83
	VD	28.45 ± 2.96	29.44 ± 3.11*	0.99 ± 0.80
Arms lean mass (kg)	PLC	6.73 ± 0.89	7.17 ± 0.79*	0.44 ± 0.31
	VD	6.72 ± 0.70	7.08 ± 0.83*	0.36 ± 0.30
Legs lean mass (kg)	PLC	19.20 ± 2.34	19.92 ± 2.13*	0.72 ± 0.59
	VD	19.72 ± 2.05	20.24 ± 2.09*	0.52 ± 0.56

Data are presented as means ± SD, $n = 18$ in PLC (placebo) and $n = 21$ in VD (vitamin D) group

*Significantly different from Week 0 ($P < 0.05$)

Table 6 Serum hormone concentrations during 12-week resistance training

Variable	Group	Week 0	Week 8	Week 12
Parathormone (pmol L^{-1})	PLC	4.78 ± 1.58	5.03 ± 1.78	4.80 ± 1.41
	VD	4.59 ± 1.98	3.78 ± 1.44	3.82 ± 1.87
Testosterone (nmol L^{-1})	PLC	23.8 ± 7.9	23.0 ± 6.1	22.3 ± 5.5
	VD	22.6 ± 6.2	22.3 ± 5.9	21.7 ± 7.5
Cortisol (nmol L^{-1})	PLC	465.7 ± 105.5	460.5 ± 81.5	455.1 ± 98.6
	VD	418.2 ± 83.4	400.2 ± 75.7	424.0 ± 89.6
Growth hormone (mU L^{-1})	PLC	0.943 ± 2.062	0.729 ± 1.029	0.770 ± 0.905
	VD	0.879 ± 1.658	0.457 ± 0.616	0.622 ± 1.256
IGF-1 (μg L^{-1})	PLC	218.6 ± 33.2	225.4 ± 42.2	225.3 ± 38.7
	VD	195.9 ± 39.8	205.1 ± 39.6	211.0 ± 48.0

Data are presented as means ± SD, $n = 18$ in PLC (placebo) and $n = 21$ in VD (vitamin D) group. *IGF-1* insulin-like growth factor-1

$P = 0.038$). Overall IGF-1 levels at week 12 (217.6 ± 44.0 μg L^{-1}) were higher than at week 0 (206.4 ± 38.2 μg L^{-1}). No main effect of group or group by time interaction occurred for IGF-1 levels ($P > 0.05$). No main effects of group or time or group by time interactions were observed for the levels of parathormone, testosterone and growth hormone (in all cases $P > 0.05$).

Blood metabolites and creatine kinase

Main effect of time occurred for ionized calcium ($F = 20.83$; $P < 0.0001$) and creatine kinase ($F = 11.62$; $P < 0.0001$) levels. In VD group only, a small but significant ($P < 0.001$) decrease in ionized calcium level was observed between weeks 0 and 8, and then, from week 8 to week 12, a small increase occurred in both groups (Table 7). Creatine kinase levels decreased ($P < 0.05$) between weeks 0 and 8 in both groups. No main effect of group or group by time interaction occurred for ionized calcium or creatine kinase levels ($P > 0.05$). No main effects of group or time or group by time interactions were observed for the levels of Ca and urea (in all cases $P > 0.05$).

Table 7 Metabolite concentrations and activity of creatine kinase in serum during 12-week resistance training

Variable	Group	Week 0	Week 8	Week 12
Ionized calcium (mmol L^{-1})	PLC	1.26 ± 0.07	1.19 ± 0.12	1.29 ± 0.04†
	VD	1.29 ± 0.06	1.18 ± 0.15*	1.30 ± 0.06†
Calcium (mmol L^{-1})	PLC	2.43 ± 0.06	2.41 ± 0.07	2.44 ± 0.09
	VD	2.48 ± 0.08	2.45 ± 0.10	2.47 ± 0.11
Urea (mmol L^{-1})	PLC	4.46 ± 1.55	4.55 ± 1.13	4.72 ± 1.48
	VD	4.82 ± 1.01	5.02 ± 1.50	5.14 ± 1.13
Creatine kinase (U L^{-1})	PLC	225.4 ± 127.0	127.2 ± 45.1*	174.8 ± 76.7
	VD	207.0 ± 116.1	133.4 ± 45.3*	148.8 ± 64.8

Data are presented as means ± SD, $n = 18$ in PLC (placebo) and $n = 21$ in VD (vitamin D) group

*Significantly different from Week 0 ($P < 0.05$)

†Significantly different from Week 8 ($P < 0.05$)

Discussion

In addition to purely scientific interest, the high prevalence of vitamin D deficiency previously observed among young Estonian men (Ööpik et al. 2017) prompted us to undertake this study. We hypothesized that Vit-D supplementation compared to placebo ingestion would enhance the increases in muscle strength and LBM during 12-week RT in Vit-D deficient young healthy men. The results show that Vit-D supplementation increased serum 25(OH)D levels in VD group, and RT increased 1RM in both groups in all seven exercises. However, contrary to our hypothesis, the magnitude of the increases in 1RM in most exercises was similar in the two groups. Moreover, in chest press and seated row, greater 1RM changes occurred in PLC group. RT, independently of group, increased total lean mass, trunk lean mass, lean mass of arms and legs, and decreased total fat mass, total fat percentage and android fat percentage. Android fat mass decreased only in VD group. Again, contrary to our hypothesis, the magnitude of the changes in the lean mass indices (and in the other body composition parameters listed above) was similar to the two groups.

All the blood samples from our participants were taken in the morning following two resting days. Fasting serum urea levels which steadily remained below 7.5 mmol L^{-1} and absence of elevations in creatine kinase activity suggest that the participants of both groups well tolerated training loads and that they started each consecutive week of the 12-week RT in a well recovered state (Viru and Viru 2001).

The magnitude of increases in 1RMs in upper and lower body exercises as well as the increases in lean mass and decreases in total fat mass and fat percentage in our participants were similar to the changes reported by others (Campos et al. 2002; Kraemer et al. 2009; Moore et al. 2007; Rønnestad et al. 2007; Snijders et al. 2015) who have studied young men employing similar RT concept and using DXA method for body composition analysis. Our observation that Vit-D supplementation did not augment the impact of RT on muscle strength in young men is in line with the previously published data (Agergaard et al. 2015; Carrillo et al. 2013). However, the novel aspect of the present study, which makes our findings unique, is the fact that our participants were Vit-D deficient [serum 25(OH)D concentration < 50 nmol L^{-1}]. Importantly, our PLC group remained Vit-D deficient throughout the whole 12-week supplementation and RT period, while the VD group revealed sufficient or even optimal Vit-D status at weeks 4–12. For comparison, the subjects studied by Carrillo et al. (2013) and Agergaard et al. (2015) were Vit-D sufficient during involvement in the supplementation and RT program. Furthermore, the Vit-D and placebo supplemented groups in the Carrillo et al. (2013) study actually did not differ in respect of serum 25(OH)D levels. Thus, we are the first to demonstrate that Vit-D supplementation in Vit-D deficient young healthy men does not improve the efficacy of RT in terms of muscle strength gain.

We are aware that no consensus regarding the cut-off for sufficient serum 25(OH)D concentration exists (Holick et al. 2011; Ross et al. 2011). However, there is broad agreement that 25(OH)D levels below 50 nmol L^{-1} should be prevented or treated (Trummer et al. 2016), and, according to Heaney (2011), distinction between Vit-D insufficiency and deficiency is not useful or necessary. Thus, we classify our PLC group as Vit-D deficient on the basis of serum 25(OH)D levels, that remained steadily low between 36.3 ± 8.9 (week 0) and 29.4 ± 6.6 nmol L^{-1} (Week 12) during RT. Considering bone health, serum 25(OH)D concentration should be at least 50 nmol L^{-1} (Ross et al. 2011), whereas the minimal serum 25(OH)D level required to trigger extra-skeletal effects is estimated to be in the range of 75–125 nmol L^{-1} (Pludowski et al. 2018). Optimal serum 25(OH)D levels have been defined as 100–200 nmol L^{-1} (Zittermann 2003) or 100–175 nmol L^{-1} (Cannell and Hollis 2008). Given these views (Cannell and Hollis 2008; Pludowski et al. 2018; Zittermann 2003), our VD group was in sufficient or even in optimal Vit-D status since week 4 of RT.

Our hypothesis for muscle strength and LBM was based in part on data showing that Vit-D supplementation may increase serum testosterone (Canguven et al. 2017; Pilz et al. 2011) and IGF-1 (Ameri et al. 2013; Marwaha et al. 2018; Soliman et al. 2008) levels. Testosterone levels in the physiological range correlate with RT-induced gains in LBM and muscle strength in elderly men (Gharahdaghi et al. 2019; Häkkinen and Pakarinen 1994). Manipulations of serum testosterone levels in young eugonadal men revealed that testosterone has positive impact on myonuclear number per fiber, cross-sectional area of muscle fibers and muscle volume (Sinha-Hakim et al. 2002), and that testosterone influences the RT-induced gains in muscle strength and lean mass of legs and decreases in body fat mass (Kvorning et al. 2006). Serum IGF-1 may exert hypertrophic effect on skeletal muscle (Hayakawa et al. 2015; Rubin et al. 2005; Yin et al. 2020). Recognizing that systemic hormones may not be the ultimate factor determining the impact of RT on muscle strength and body composition in young men (Morton et al. 2018), the absence of the influence of Vit-D supplementation on the outcomes of the 12-week RT in our participants may still partly be explained by similar hormonal milieu in VD and PLC groups.

We also assumed that Vit-D supplementation may augment gains in muscle strength and LBM due to direct, i.e., non-hormone-mediated impact of Vit-D on muscle protein synthesis. Translation initiation, a crucial step in muscle protein synthesis, is controlled by the Akt/mTOR pathway (Bodine et al. 2001) which is stimulated by insulin (Biolo et al. 1995) and leucine (Haegens et al. 2012). In vitro, Vit-D potentiates the effect of leucine and insulin on the Akt/mTOR pathway, leading to a 14–35% greater increase in the rate of protein synthesis (Salles et al. 2013; Romeu Montenegro et al. 2019). After each RT session, all our participants ingested 20 g of whey protein. Due to high leucine content, this amount stimulates muscle protein synthesis for the initial 4 h of recovery in young men with unknown vitamin D status (Witard et al. 2014), and acute increases in muscle protein synthesis correlate with the extent of muscle hypertrophy occurring as a result of prolonged RT program (Damas et al. 2016). Considering the findings of Salles et al. (2013), Romeu Montenegro et al. (2019) and Damas et al. (2016), we expected to see greater increases in LBM and muscle strength in our VD group compared to PLC group, but this was not the case. The effect of Vit-D supplementation on Akt/mTOR pathway activation in Vit-D deficient men involved in RT remains to be measured in further studies.

In elite male athletes, weekly 70,000 IU Vit-D dose led to a significantly lower ratio of 1,25$[OH]_2$D to 24,25$[OH]_2$D at week 6 of supplementation (Owens et al. 2017). The authors suggested that mega dose Vit-D supplements may be detrimental to Vit-D target tissues by increasing the production of 24,25$[OH]_2$D, which may block the activity of the VDR. Participants in our VD group administered 8000 IU of Vit-D daily. However, the total weekly Vit-D dose was 56,000 IU, which could be big enough to increase the 24,25$(OH)_2$D level. If this mechanism worked in our subjects, it may partly explain the ineffectiveness of Vit-D supplementation in respect of changes in muscle strength and body composition during 12-week RT.

One meta-analysis concluded that Vit-D supplementation (without any training intervention) may be effective in improving muscle strength in adults with baseline serum 25(OH)D levels $\leq$ 25 nmol L^{-1}, but not in those exhibiting higher 25(OH)D concentrations (Stockton et al. 2011). As our participants' mean serum 25(OH)D level was 36.6 nmol L^{-1} at week 0, the possibility remains that cumulative effect of Vit-D and RT on muscle strength and body composition also occur only in case of more severe Vit-D deficiency.

In two (chest press and seated row) of the seven exercises, our VD group showed smaller increases in 1RM compared to the PLC group. We are aware of only two studies, both conducted in postmenopausal women, which have reported similar findings. In participants involved in lifestyle-based weight loss interventions, Vit-D supplementation reduced leg but not upper body muscle strength compared to placebo (Mason et al. 2016). In Vit-D insufficient women, 3-month Vit-D supplementation reduced handgrip and knee flexion strength but did not influence elbow flexion, elbow extension or knee extension strength compared to placebo (Bislev et al. 2018). Like others (Mason et al. 2016; Bislev et al. 2018), we consider the adverse effects of Vit-D on some muscle strength indices, but not others, to be by-chance findings.

Energy intake, which is an important factor influencing adaptations to RT (Jäger et al. 2017), appeared to be relatively low in our participants. However, the method we used for assessment of energy intake is influenced by underreporting bias and may underestimate actual energy consumption by 15–30% (Poslusna et al. 2009; Ravelli and Schoeller 2020). Therefore, it seems plausible that our participants' actual energy intake was approximately 20% higher than the results of the analysis of their food diaries show. The methodological issue related to assessment of energy intake should not question the main conclusion of our study, because there is no reason to suspect that the magnitude of underreporting differed between the VD and PLC groups.

An inverse association between serum 25(OH)D levels and BMI is well documented (Rejnmark et al. 2017; Saneei et al. 2013), whereas there seem to be no consistent association between 25(OH)D and muscle mass (Marantes et al. 2011). However, in our participants at week 0, serum 25(OH)D levels did not associate with BMI, but correlated positively with total LBM as well as with lean mass of trunk, arms and legs separately. In an ex vivo study Abboud et al. (2013) demonstrated that muscle may function as a storage

depot for Vit-D, in which 25(OH)D may be preserved bound to the Vit-D binding protein, and as required, released into the circulation. Mason et al. (2019) suggested that this muscle-related mechanism may help maintain a relatively better Vit-D status in months when Vit-D supply through endogenous synthesis in skin is low. As muscle mass is a major component of LBM, our observation of a positive correlation between serum 25(OH)D and LBM is well consistent with this suggestion.

The daily dose of Vit-D of 4000 IU is usually defined as the tolerable upper intake level for adults (EFSA Panel on Dietetic Products 2012; Ross et al. 2011) and according to US Institute of Medicine serum 25(OH)D levels above 125 nmol L^{-1} "should raise concerns … about potential adverse effects" (Ross et al. 2011). However, The Endocrine Society accepts that daily intake of 10,000 IU may be needed to correct Vit-D deficiency, and considers serum 25(OH)D levels up to 375 nmol L^{-1} to be safe (Holick et al. 2011). In our VD group, the mean serum 25(OH)D concentration stabilized at the level of 135–140 nmol L^{-1}, which was approximately 15% higher than we expected, but still well below 375 nmol L^{-1}. As hypercalcemia is a sensitive marker of the potential harmful effects of Vit-D (Heaney et al. 2003), physiologically normal calcium levels in all our participants suggest that VD group well tolerated the daily dose of 8000 IU of Vit-D. On the other hand, Vit-D deficiency may lead to secondary hyperparathyroidism which may cause detrimental health consequences (Lips et al. 2017). In all our participants, serum PTH levels remained in a normal physiological range, suggesting that Vit-D deficient status did not increase the level of hyperthyroidism-related health risks in the PLC group.

Our study has some limitations. First, we were not able to measure serum 24,25$[OH]_2$D levels, which may have been significantly elevated in the VD group from week 4 due to high daily Vit-D doses and which may block the activity of the VDR (Owens et al. 2017), thus causing inefficacy of Vit-D supplementation in our participants. Therefore, further studies comparing the effects of different lower daily doses of Vit-D on RT outcomes in Vit-D-deficient subjects with concomitant measurements of serum 24,25$[OH]_2$D levels are warranted. The idea that lower doses may be more beneficial is to some extent supported by recent findings of Burt et al. (2019) who studied the impact of long-term Vit-D supplementation on bone health in healthy adults. Second, to standardize post-workout protein intake, we administered whey protein to all participants in both groups after each RT session. Therefore, consideration should be given to the possibility that the anabolic response to RT was already maximized by ingestion of whey, limiting or completely blocking the potential additional effects of Vit-D supplementation. However, Macnaughton et al. (2016) have shown in young men that after a whole-body RT session, ingestion of 20 g of whey does not maximize the rate of muscle protein synthesis, and 40 g of whey has a significantly stronger anabolic effect. The amount of whey ingested by our participants was 20 g and they practiced whole-body RT.

Conclusion

In conclusion, the results of this study show that in young healthy Vit-D deficient men participating in 12-week RT, daily Vit-D supplementation in amount of 8000 IU rapidly (within 4 weeks) eliminates Vit-D deficiency but does not enhance RT-induced muscle strength and LBM gains or total and regional fat mass reductions.

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Author contributions LS, MM, EU, and VÖ conceived and designed research. LS, ST, MM, EM, LT, FR, and ML performed experiments. LS, ST, MM, and LM collected and analysed the data. LS and VÖ wrote the manuscript. ST, MM, and EU revised the manuscript. All authors read and approved the final version of the manuscript.

Declarations

Conflict of interest The authors have no conflicts of interest to report.

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