

The effects of vitamin D combined with thermotherapy and resistance exercise on inflammatory factors, thyroid hormones and MSTN in elderly patients with sarcopenia

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Abstract: This study investigated the impact of vitamin D (VD) combined with thermotherapy and resistance exercise (RE) on elderly patients with sarcopenia, thereby offering novel insights into evidence-based management strategies for sarcopenia. 140 sarcopenia patients were admitted between May 2023 and August 2024. Participants were randomized to receive either VD combined with thermotherapy and resistance exercise (treatment group) or thermotherapy and resistance exercise alone (control group). Skeletal muscle mass and handgrip strength were evaluated before and after the intervention using a body composition analyzer and a hand dynamometer. In addition, the levels of blood glucose, blood lipids, inflammatory factors and thyroid hormones were measured. Both groups exhibited significant improvements in skeletal muscle mass, handgrip strength and glucose/lipid metabolism post-treatment, with the treatment group demonstrating superior enhancements ($P < 0.05$). After treatment, the level of inflammatory factors in the treatment group was lower, while the level of thyroid hormone was higher ($P < 0.05$). There was a significant negative correlation between 25-hydroxyvitamin D3 [25(OH)D3] and myostatin (MSTN) in both groups ($P < 0.05$). The combination of VD with thermotherapy and RE effectively enhances skeletal muscle function, suppresses inflammatory responses and regulates endocrine function in elderly patients with sarcopenia.

Keywords: Inflammatory factors; Resistance exercise; Sarcopenia; Thyroid hormones; Vitamin D

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INTRODUCTION

Sarcopenia, a progressive skeletal muscle disorder, is closely associated with aging and frequently coexists with a range of chronic diseases (Sayer & Cruz-Jentoft, 2022). By 2022, the prevalence of sarcopenia among the elderly had surged to 29%, highlighting its growing significance (Cho *et al.*, 2022). As awareness of this condition increases, sarcopenia has been recognized not only as a major clinical issue but also as a pivotal challenge to achieving healthy aging (Xie *et al.*, 2021). Despite its parallels with osteoporosis—both involving the progressive decline of skeletal muscle mass and function—sarcopenia has historically been overlooked in clinical practice. It was not until 2016 that it was formally classified as a distinct disease on an international scale (Gielen *et al.*, 2023). This delayed recognition has left research on sarcopenia in its infancy, with few substantial advancements to date.

Age is a well-established risk factor for sarcopenia. As individuals grow older, a decline in exercise capacity precipitates a reduction in both muscle mass and strength (Lisco *et al.*, 2023). This decrease in exercise ability further contributes to a weakening of muscle strength, creating a self-perpetuating cycle: weakened muscle strength further limits physical activity, which in turn accelerates muscle deterioration, ultimately culminating in sarcopenia (Papadopoulou, 2020). Consequently, exercise-based

therapies, particularly resistance exercise (RE), have become a cornerstone in the clinical management of sarcopenia. Beyond physical activity, vitamin D (VD) deficiency has also been identified as a significant contributor to the development of sarcopenia (Bollen *et al.*, 2022). While both RE and VD supplementation for sarcopenia treatment have been extensively studied individually (Chang & Choo, 2023; Shen *et al.*, 2023), the synergistic effects of combining these therapies remain largely uncharted.

Advances in research have gradually shed light on the multifaceted physiological changes driving sarcopenia. For instance, the activation of chronic inflammatory pathways, mitochondrial dysfunction and abnormal muscle cell death and repair processes are now understood to disrupt skeletal muscle structure and function. Research has demonstrated marked upregulation of interleukin (IL)-1 β and tumor necrosis factor-alpha (TNF- α) in patients with sarcopenia (Chang *et al.*, 2023). Additionally, endocrine dysregulation, which influences metabolism and protein synthesis, has been implicated in the progression of this condition (Gupta & Kumar, 2022). Among endocrine factors, thyroid hormones, which play a central role in regulating endocrine metabolism, have shown a preliminary association with sarcopenia (Chen *et al.*, 2023).

Against the backdrop of a rapidly aging global population, the incidence of sarcopenia continues to rise, posing a

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significant public health challenge (Chen & Wu, 2024). Modern clinical research emphasizes not only symptom management but also a deeper understanding of the underlying physiological mechanisms. Therefore, the present study seeks to evaluate the effects of combining VD supplementation with thermotherapy and RE in elderly patients with sarcopenia, with special attention paid to changes in thyroid hormones, inflammatory markers and myostatin (MSTN) levels. By exploring these mechanisms, this research aims to provide novel insights and evidence-based recommendations for the treatment of sarcopenia.

MATERIALS AND METHODS

Study design and principles

G-Power 3.1.9.2 software was used to calculate the sample size required for the sample. A two-tailed test was set with $\alpha=0.5$, effect size=0.4, $\beta=0.1$ and the calculation results showed that the minimum sample size was 58. A minimum of 64 patients per group were required to account for a 10% dropout risk. Randomization was performed using the PEMS3.1 software's "Completely Randomized Design (for two or more groups)" module, generating random numbers for 140 participants divided equally into two groups. Eligible patients were sequentially enrolled and randomly assigned to either the treatment or control group. The study design and workflow are depicted in fig. 1.

Study participants

A total of 140 sarcopenia patients diagnosed and treated in our hospital between May 2023 and August 2024 were recruited, with 70 participants assigned to the treatment group and 70 to the control group. The baseline information of the study population is shown in table 1. Inclusion criteria included: age ≥ 65 years; diagnosis of sarcopenia according to established criteria (Sayer *et al.*, 2024); 25-hydroxyvitamin D3 [25(OH)D3] levels ≤ 30 ng/L; and provision of written informed consent. Exclusion criteria comprised: neurological or skeletal disorders hindering study participation; chronic cardiac or pulmonary insufficiency (e.g., acute exacerbations of chronic obstructive pulmonary disease, New York Heart Association (NYHA) classification III or IV); malignancy; peripheral arterial ischemia; acute or chronic infectious diseases; and use of VD supplements within the preceding three months.

Methods

Control group: Participants in the control group underwent a combination of thermotherapy and RE. Thermotherapy involved the application of warm paraffin wax to the limbs 30 minutes prior to RE sessions, administered every other day for 30 minutes per session over six months. The RE regimen consisted of seated knee extension, seated knee flexion, chest press, shoulder press and lower back muscle training, performed every other day for 30 minutes per session. Exercise intensity was maintained at 40-50% of one-repetition maximum (1RM) for a duration of 24 consecutive weeks.

Treatment group: In addition to the interventions provided to the control group, participants in the treatment group received daily oral supplementation of vitamin D3 tablets (Nycomed Pharma AS, X19990196) at a dosage of 600 IU for six months.

Assessment of skeletal muscle improvement

To evaluate changes in skeletal muscle mass, a body composition analyzer was employed to measure appendicular skeletal muscle (ASM) before and after the intervention. The appendicular skeletal muscle index (ASMI) was derived by dividing ASM by the square of the patient's height ($ASM/height^2$). Additionally, handgrip strength (HGS) was measured using a hand dynamometer, with the dominant hand used for assessment.

Laboratory tests

Before and after treatment, fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) levels were determined using a glucose meter, while total cholesterol (TC) and triglycerides (TG) were analyzed with a biochemical analyzer (BS-2000, Mindray). Additionally, inflammatory markers, including IL-1 β (CSB-E04620h), IL-6 (CSB-E04638h), IL-8 (CSB-E04641h), TNF- α (CSB-E09315h) and interferon-gamma (IFN- γ) (CSB-E08636h), were quantified using ELISA at baseline (T_0), 3 months (T_1) and 6 months (T_2) of treatment. All kits were purchased from Wuhan Huamei Biological Engineering Co., LTD. Thyroid function was assessed by measuring free triiodothyronine (FT₃), total triiodothyronine (TT₃), free thyroxine (FT₄), total thyroxine (TT₄) and thyroid-stimulating hormone (TSH) using an immunoluminescence analyzer (e 411, Cobas).

Statistical analysis

Statistical analyses were performed with SPSS 23.0. Normally distributed continuous data are expressed as mean $\pm$ standard deviation, analyzed by independent/paired t-tests for group comparisons. Longitudinal measurements were evaluated through repeated-measures Analysis of Variance (ANOVA) with Bonferroni post-hoc adjustment. Categorical variables appear as frequency counts (percentages), with χ^2 tests assessing distribution differences. Repeated measures analysis of variance (RM-ANOVA) was used for longitudinal data and Bonferroni correction was used for post-hoc pairwise comparison. A mixed-effects model was used for comparison between groups.

Bivariate correlations were determined via Pearson's coefficient. Statistical significance threshold was set at $\alpha=0.05$.

RESULTS

Improvement in skeletal muscle parameters

The two groups showed no significant differences in pre-treatment ASM, ASMI, or HGS ($P>0.05$). Post-treatment,

both groups demonstrated significant improvements in ASM, ASMI and HGS ($P < 0.05$). While ASM and ASMI did not differ between the groups ($P > 0.05$), the treatment group achieved a notably higher increase in HGS compared to the control group ($P < 0.05$, Table 2).

Changes in glucose and lipid metabolism

After the intervention, both groups exhibited reductions in FBG, HbA1c, TC and TG levels compared to baseline. Notably, the treatment group showed significantly greater reductions in these parameters than the control group ($P < 0.05$, Table 3).

Changes in inflammatory factors

At T_0 , the detection outcomes of inflammatory factors in the two groups of patients did not show any significant differences ($P > 0.05$). During T_1 and T_2 , the inflammatory factors in both groups exhibited a continuous downward tendency. However, the treatment group exhibited significantly lower levels of IL-1 β , IL-6, IL-8, TNF- α and IFN- γ at both T_1 and T_2 compared to the control group ($P < 0.05$, Table 4).

Changes in thyroid hormones

At T_1 , levels of FT $_4$, TT $_4$ and TSH remained unchanged in both groups ($P > 0.05$), whereas FT $_3$ and TT $_3$ levels increased significantly compared to baseline (T_0) ($P < 0.05$). By T_2 , both groups showed significant elevations in FT $_3$, TT $_3$, FT $_4$, TT $_4$ and TSH levels compared to T_0 ($P < 0.05$). Intergroup comparisons revealed no differences in thyroid hormone levels at T_0 ($P > 0.05$). However, the treatment group demonstrated higher FT $_3$ levels at both T_1 and T_2 , as well as higher TT $_3$ levels at T_2 , compared to the control group ($P < 0.05$, Table 5).

Relationship between 25(OH) D3 and MSTN

In the treatment group, 25(OH) D3 levels progressively increased with treatment, while MSTN levels declined ($P < 0.05$). In the control group, MSTN levels decreased over time ($P < 0.05$), although no significant change in 25(OH)D3 was observed at T_1 compared to T_0 ($P > 0.05$). By T_2 , 25(OH)D3 levels in the control group rose significantly ($P < 0.05$, table 6). Correlation analysis indicated a significant negative association between 25(OH)D3 and MSTN in both the treatment and control groups ($P < 0.05$, Table 6, Fig. 2).

DISCUSSION

The pathogenesis of sarcopenia involves a complex interplay of chronic inflammation, hormonal dysregulation and impaired muscle protein synthesis (Montero-Erasquin & Cruz-Jentoft, 2023). This study investigated the therapeutic potential of combining VD with thermotherapy and RE, focusing on their effects on inflammatory responses, thyroid hormones and MSTN, as shown in this study.

Regarding the enhancement of skeletal muscle, both groups exhibited significant increases in ASM, appendicular ASMI and HGS following the intervention, underscoring the effectiveness of thermotherapy combined with RE in enhancing skeletal muscle health. The benefits of RE as a cornerstone treatment for sarcopenia have been extensively validated in previous studies (Smith *et al.*, 2022; Papadopoulou *et al.*, 2021). RE exerts its effects by applying mechanical load to muscles, which enhances muscle mass and volume. Key mechanisms include improved recruitment efficiency and discharge frequency of motor units, leading to better neural control over muscle activity (Cuyul-Vasquez *et al.*, 2023), as well as the promotion of the hypertrophy and proliferation of type II muscle fibers (fast-twitch fibers), which optimize muscle synergy, exercise efficiency and power output (Tezze *et al.*, 2023).

When paired with thermotherapy, this approach further elevates muscle temperature and flexibility, reducing the risk of exercise-related injuries and enabling elderly patients to better withstand high-intensity RE. Supporting this, Liu *et al.* demonstrated that thermotherapy enhances blood circulation and oxygen delivery, facilitates mitochondrial function recovery and suppresses the activity of the ubiquitin-proteasome system, thereby reducing muscle protein degradation (Liu *et al.*, 2023).

These effects synergize with the anabolic actions of RE, thus optimizing the overall effectiveness of RE. The treatment group, which received VD supplementation in addition to thermotherapy and RE, achieved significantly greater improvements in HGS compared to the control group. Furthermore, this group demonstrated superior outcomes in glucose and lipid metabolism and inflammatory markers, highlighting the enhanced efficacy of the combined therapy in the treatment of sarcopenia. Research has confirmed that the inflammatory response is one of the keys to activating sarcopenia (Lenska-Micieik *et al.*, 2023). Munem *et al.* demonstrated that VD significantly decreases serum levels of pro-inflammatory cytokines (Munem *et al.*, 2023). We propose that the anti-inflammatory properties of VD may disrupt this pathological cascade, fostering a conducive microenvironment for muscle repair. These findings are consistent with research by Ilchovska *et al.* (Ilchovska & Barrow, 2021), highlighting the critical role of VD's immunomodulatory capabilities in the management of sarcopenia. Nevertheless, the potential influence of VD on thyroid function remains a subject of debate. This study revealed that VD supplementation increased FT $_3$ levels, potentially through VDR-mediated protection of thyroid cells or by enhancing peripheral tissue sensitivity to thyroid hormones (Hantusch *et al.*, 2024). While the exact mechanisms remain to be fully elucidated, the normalization of thyroid function may indirectly support muscle metabolism, necessitating further investigation.

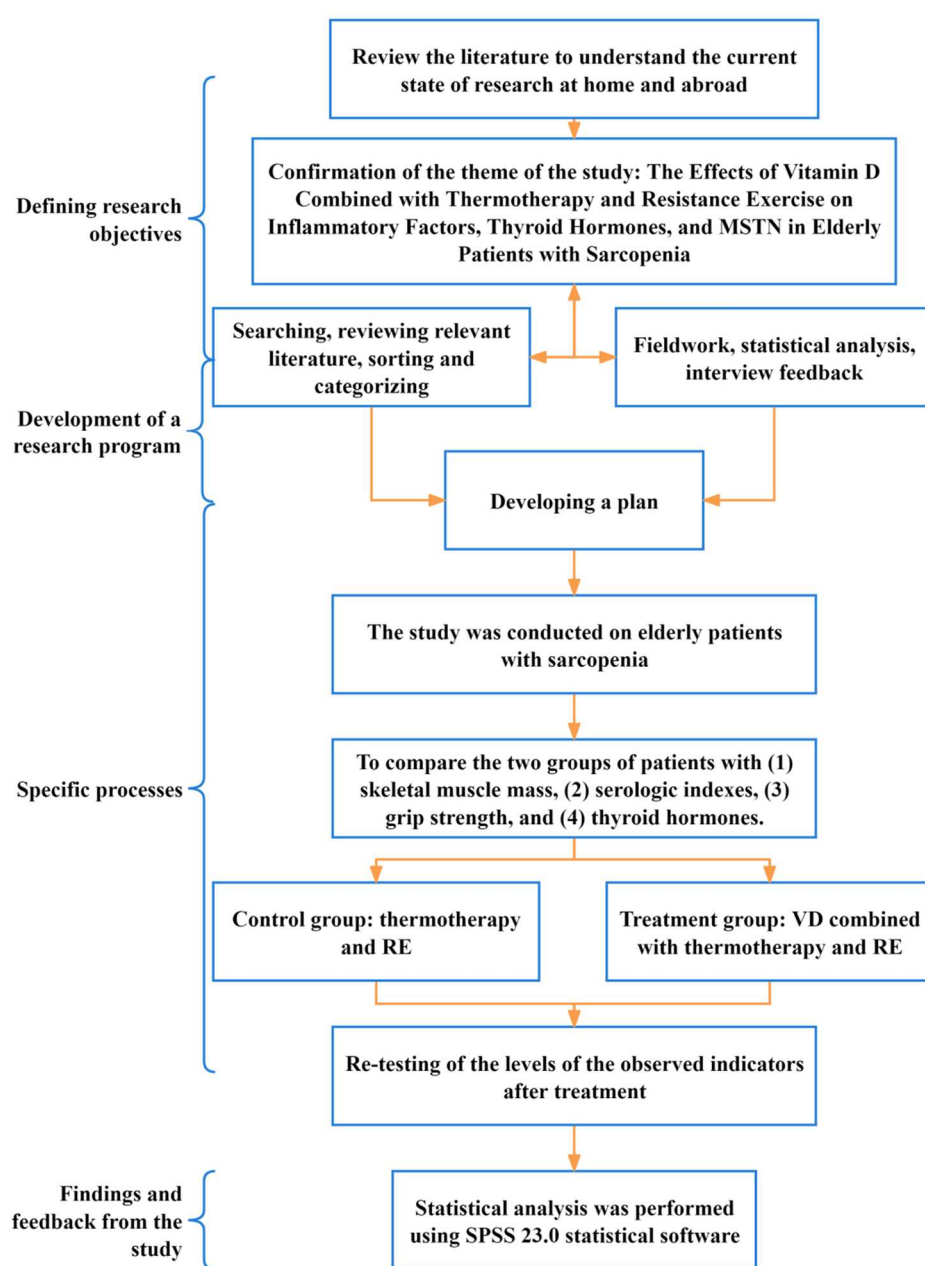


Fig. 1: Flow of this study.

Table 1: Baseline information of the study population

Groups (n=70)		Control	Treatment	Statistical value (t or χ^2)	P
Sex	Age	69.91±2.83	69.34±2.93	1.175	0.242
	Male	42 (60.00)	37 (52.86)	0.726	0.394
	Female	28 (40.0)	33 (47.14)	1.393	0.166
Body mass index (kg/m ²)	Body mass index (kg/m ²)	23.53±1.51	23.91±1.71	0.530	0.467
	Smoking	46 (65.71)	50 (71.43)	1.036	0.309
	Drinking	41 (58.57)	35 (50.00)	0.722	0.396
Combined disease	Diabetes	29 (41.43)	34 (48.57)	0.136	0.712
	Hypertension	22 (31.43)	20 (28.57)	0.357	0.550
	Hyperlipidemia	18 (25.71)	15 (21.43)	0.548	0.459
	Coronary heart disease	11 (15.71)	8 (11.43)		

Table 2: Skeletal muscles

Groups (n=70)	Control	Treatment	t	P
ASM (kg)	Before	15.53±1.26	15.87±1.49	1.422
	After	16.93±1.31*	17.25±1.41*	1.358
ASMI (kg/m ²)	Before	6.32±1.01	6.24±1.01	0.470
	After	7.09±1.17*	7.32±1.12*	1.147
HGS (kg)	Before	28.27±3.15	27.61±3.87	1.097
	After	33.87±3.10*	35.41±2.54*	3.197

Note: vs. before treatment *P<0.05.

Table 3: Glucose/lipid metabolism

Groups (n=70)	Control	Treatment	t	P
FBG (mmol/L)	Before	6.91±1.72	6.58±1.15	1.332
	After	6.24±1.47*	5.63±1.69*	2.280
HbA1c (%)	Before	8.41±1.94	8.85±1.47	1.219
	After	7.25±1.68*	6.65±1.20*	2.451
TG (mmol/L)	Before	1.72±0.30	1.73±0.22	0.064
	After	1.52±0.26*	1.40±0.20*	3.003
TC (mmol/L)	Before	5.50±0.70	5.61±0.79	0.892
	After	4.80±0.79*	4.44±0.79*	2.687

Note: vs. before treatment *P<0.05.

Table 4: Inflammatory factors

Groups (n=70)	Control	Treatment	t	P	Groups (n=70)
IL-1 β (pg/mL)	T ₀	9.83±1.56	9.78±1.98	0.187	0.852
	T ₁	7.91±1.39*	7.37±1.61*	2.092	0.038
	T ₂	6.43±1.28* [#]	5.38±1.21* [#]	5.032	<0.001
IL-6 (pg/mL)	T ₀	7.86±0.97	7.95±1.03	0.520	0.604
	T ₁	6.74±1.04*	6.12±1.20*	3.276	0.001
	T ₂	5.53±1.04* [#]	4.21±1.20* [#]	6.938	<0.001
IL-8 (pg/mL)	T ₀	45.23±6.79	46.45±8.21	0.956	0.341
	T ₁	36.33±6.22*	33.17±5.76*	3.119	0.002
	T ₂	27.74±5.56* [#]	23.77±5.00* [#]	4.450	<0.001
TNF- α (pg/mL)	T ₀	16.26±3.22	16.70±3.46	0.776	0.439
	T ₁	14.06±2.08*	13.10±3.10*	2.153	0.033
	T ₂	11.67±2.26* [#]	10.08±1.56* [#]	4.839	<0.001
IFN- γ (pg/mL)	T ₀	7.57±1.83	7.46±1.88	0.362	0.718
	T ₁	6.75±1.49*	6.18±1.32*	2.395	0.018
	T ₂	5.71±1.16* [#]	5.02±0.97* [#]	3.841	<0.001

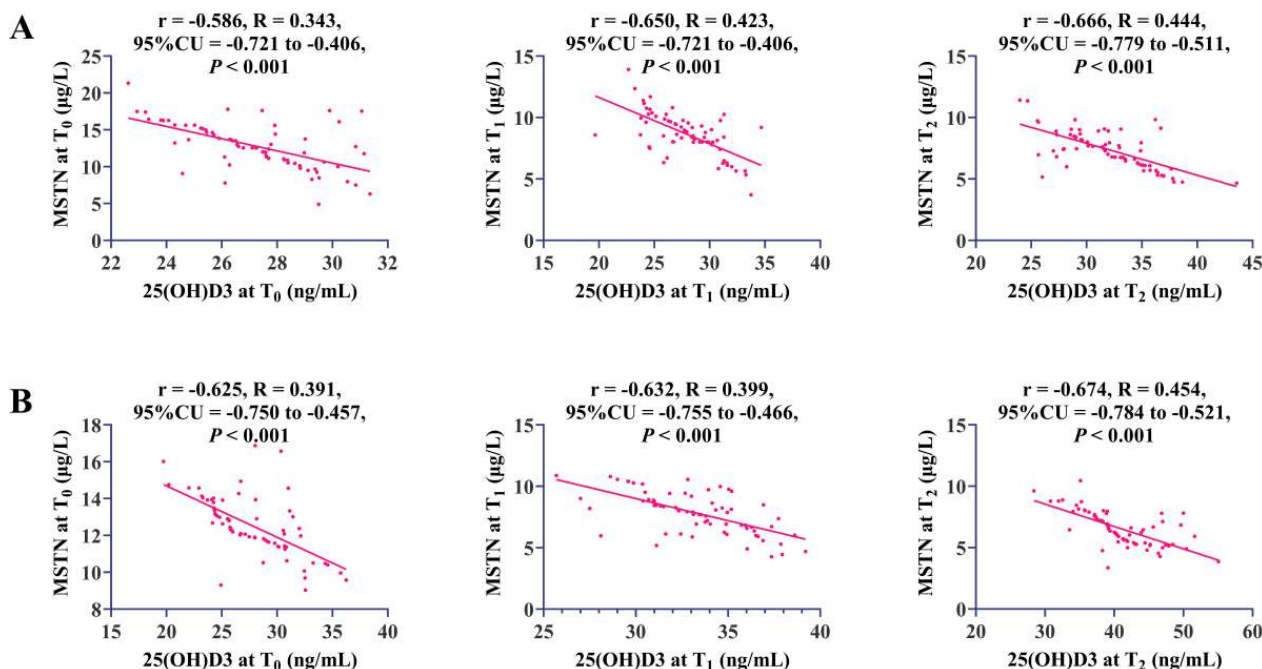
Note: vs. T₀ *P<0.05, vs. T₁ [#]P<0.05.**Table 5:** Thyroid hormones

Groups (n=70)	Control	Treatment	t	P
FT ₃ (pmol/L)	T ₀	3.64±0.70	3.54±0.57	0.904
	T ₁	4.20±0.67*	4.46±0.51*	2.532
	T ₂	4.81±0.64* [#]	5.15±0.48* [#]	3.596
TT ₃ (nmol/L)	T ₀	1.38±0.13	1.40±0.17	0.666
	T ₁	1.61±0.17*	1.75±0.19*	4.879
	T ₂	1.76±0.13* [#]	1.86±0.19* [#]	3.610
FT ₄ (pmol/L)	T ₀	12.70±1.89	12.56±2.17	0.390
	T ₁	12.84±2.43	12.69±1.83	0.410
	T ₂	13.98±2.09* [#]	14.16±2.25* [#]	0.485
TT ₄ (nmol/L)	T ₀	93.02±15.14	94.65±17.92	0.583
	T ₁	93.44±17.00	94.19±14.39	0.282
	T ₂	113.74±17.41* [#]	116.65±14.57* [#]	1.074
TSH (mIU/L)	T ₀	2.18±0.89	2.12±0.82	0.439
	T ₁	2.13±0.78	2.08±0.81	0.310
	T ₂	2.61±0.83* [#]	2.72±0.74* [#]	0.876

Note: vs. T₀ *P<0.05, vs. T₁ [#]P<0.05.

Table 6: 25(OH) D3 and MSTN

Groups (n=70)		Control	Treatment	t	P
25(OH)D3 (ng/mL)	T ₀	27.24±2.21	27.84±3.63	1.176	0.242
	T ₁	27.96±3.02	33.38±2.95*	10.723	<0.001
	T ₂	31.90±3.94*#	40.86±5.22*#	11.472	<0.001
MSTN (μg/L)	T ₀	13.98±1.51	12.50±1.62	0.640	0.523
	T ₁	12.77±3.12*	7.79±1.68*	2.924	0.004
	T ₂	7.41±1.54*#	6.56±1.41*#	3.550	<0.001

Note: vs. T₀ *P<0.05, vs. T₁ #P<0.05.**Fig. 2:** Relationship between 25(OH) D3 and MSTN.

(A) Correlation analysis of 25(OH) D3 and MSTN during treatment in the control group. (B) Correlation analysis of 25(OH) D3 and MSTN during treatment in the treatment group.

MSTN, a negative regulator of muscle growth, inhibits the activation of muscle satellite cells when overexpressed (Godala *et al.*, 2024). This study is the first to report a significant reduction in serum MSTN levels following VD intervention, potentially mediated by VD's inhibition of the TGF- β /Smad pathway or its indirect modulation of MSTN production through calcium signaling (Li *et al.*, 2024). This effect complements the MSTN-suppressing action of RE, suggesting a synergistic mechanism underlying the combined therapy. However, future experiments are needed to test this hypothesis. We hypothesize that the therapeutic benefits of VD combined with thermotherapy and RE in sarcopenia may involve the following mechanisms: (1) Dual modulation of inflammation and metabolism: VD mitigates systemic inflammation, while RE enhances local IGF-1 secretion, collectively optimizing the muscle anabolic environment. (2) Dual suppression of MSTN: VD and RE downregulate MSTN through distinct pathways-molecular signaling and mechanical loading, respectively-thereby synergistically promoting muscle

fiber hyperplasia. (3) Enhanced exercise adaptability by VD: VD may improve mitochondrial function and calcium homeostasis, thereby increasing elderly patients' tolerance and responsiveness to RE. The observed significant negative correlation between 25(OH)D3 and MSTN further corroborates this hypothesis.

This study is the first to explore the effect of VD combined therapy on MSTN in patients with sarcopenia and to provide reliable reference and guidance for clinical practice through dynamic monitoring of inflammatory and endocrine indicators at multiple time points. However, key questions remain regarding the optimal dosage and duration of VD supplementation, as well as its precise regulatory mechanisms on thyroid hormones, which require validation through large-scale clinical trials. Future research should leverage advanced omics technologies to unravel the complex interactions between VD and exercise-related signaling pathways, laying the foundation for more personalized and effective treatment strategies.

CONCLUSION

The combination of VD with thermotherapy and RE, through its multi-target synergistic effects, represents a promising and efficient intervention strategy for sarcopenia in the elderly. Its clinical application merits further exploration and validation.

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Not applicable.

Authors' contributions

RS.W. conceived and designed the study, CM.Z. wrote and revised the manuscript, WX.L. collected the data, TT.K. analyzed the data, XX.Z. visualisation the data and supervised the study. All authors read and approved the final submitted manuscript.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

Approval for the study was obtained from the Shandong Provincial Taishan Hospital's ethics committee (No. KI20230212).

Conflict of interest

The authors declare that they have no competing interest.

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