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Vitamin D and its receptor in skeletal muscle are associated with muscle disease manifestation, lipid metabolism and physical fitness of patients with myositis

Lucia Vernerová[1], Martina Vokurková[1], Nikoleta Alchus Laiferová[2], Michal Nemec[2], Maja Špiritović[1,3], Oksana Mytiai[2,4], Sabína Oreská[1,5], Martin Klein[1,5], Kateřina Kubínová[1,5], Veronika Horváthová[1,6], Tereza Kropáčková[1,5], László Wenchich[1], Michal Tomčík[1,5], Jozef Ukropec[2], Barbara Ukropcová[2,4] and Jiří Vencovský[1,5*]

Abstract

Background Low levels of vitamin D have been associated with several autoimmune diseases. A growing body of evidence supports the association of vitamin D with skeletal muscle damage, regeneration, and energy and lipid metabolism. The aim was to analyse vitamin D and its receptor (VDR) in the muscle tissue of patients with idiopathic inflammatory myopathies (IIM) and to relate them to clinical parameters and muscle lipid and energy metabolism.

Methods Forty-six patients with IIM and 67 healthy controls (HC) were included in the study. 27 IIM patients participated in a 24-week exercise intervention. Muscle biopsies were obtained from 7 IIM patients before/after training, 13 non-exercising IIM controls, and 21 HC. Circulating concentrations of 25(OH)D and 1,25(OH)D were measured. Gene expression of VDR and CYP27B1, the enzyme converting 25(OH)D to hormonally active 1,25(OH)D, was determined by qPCR in muscle tissue and primary muscle cells. Lipid oxidative metabolism was assessed in muscle tissue (mRNA, qPCR) and primary muscle cells (radioactive assays).

Results Lower levels of active 1,25(OH)D were observed in IIM patients compared with HC (mean ± SD: 125.0 ± 45.4 vs. 164.7 ± 49.2 pmol/L; $p < 0.0001$). 25(OH)D was associated with CRP ($r = -0.316$, $p = 0.037$), MITAX ($r = -0.311$, $p = 0.040$) and HAQ ($r = -0.390$, $p = 0.009$) in IIM. After 24 weeks of training, active 1,25(OH)D was associated with MMT8 ($r = 0.866$, $p < 0.0001$), FI-2 ($r = 0.608$, $p = 0.013$) and HAQ ($r = -0.537$, $p = 0.032$). Gene expression of both VDR and CYP27B1 in primary muscle cells decreased after training ($p = 0.031$ and $p = 0.078$, respectively). Associations of VDR mRNA in muscle tissue with MMT-8 (IIM: $r = -0.559$, $p = 0.013$), serum CK (HC: $r = 0.484$, $p = 0.031$), myoglobin (IIM: $r = 0.510$, $p = 0.026$) and myostatin (IIM: $r = -0.519$, $p = 0.023$) were observed. The expression of VDR in differentiated muscle cells correlated negatively with the complete oxidation of palmitic acid ($r = -0.532$, $p = 0.028$). Muscle mRNA of carnitine palmitoyl transferase 1 (CPT1) (downregulated in IIM, $p = 0.001$) correlated positively with serum 1,25(OH) vitamin D ($r = 0.410$, $p = 0.042$).

*Correspondence:
Jiří Vencovský
vencovsky@revma.cz
Full list of author information is available at the end of the article

Conclusion Reduced biologically active vitamin D in circulation suggests its impaired metabolism in IIM. Serum vitamin D levels and gene expression of its receptor and activating enzyme in muscle tissue were modified by regular exercise and associated with disease manifestations, physical fitness, and muscle lipid metabolism of IIM patients.

Keywords Myositis, Vitamin D, Muscle, Physical activity, Lipid metabolism, Mitochondria

Introduction

Muscle weakness and fatigue, elevated serum muscle enzymes, mononuclear cell infiltration, and myofiber degeneration/fibrosis represent clinical and histological features common to all subtypes of idiopathic inflammatory myopathies (IIM) [1]. Based on the particular combination of key features, the IIMs can be divided into dermatomyositis (DM), polymyositis (PM), and inclusion body myositis (IBM). Recently, other subsets of IIM have been defined such as clinically amyopathic dermatomyositis (CADM), antisynthetase syndrome (ASyS), and immune-mediated necrotizing myopathy (IMNM) [2]. After initial immunosuppressive treatment, inflammatory symptoms are suppressed in most patients, but only a few recover previous muscle function and their physical limitations persist [3]. Therefore, in addition to inflammation, an important role of non-immune mechanisms, such as endoplasmic reticulum stress [4], hypoxia [5, 6], autophagy [7], and metabolic dysfunction [8] has been suggested.

Recent studies have associated vitamin D deficiency with several autoimmune diseases such as rheumatoid arthritis [9], multiple sclerosis [10], systemic lupus erythematosus [11], and IIM [12, 13]. In IIM, vitamin D levels were correlated with circulating muscle enzymes, the presence of anti-Jo-1 and anti-Mi-2 antibodies, and the absolute number of total T and Treg cells [13]. Patients with IIM with extremely low vitamin D levels at the time of diagnosis also frequently had a heliotrope rash, and gastrointestinal and hepatic involvement [13]. Interestingly, VDR agonists with their emerging anti-inflammatory properties have been suggested as candidates for the future treatment of IIM [14].

Vitamin D is partially produced in the skin after UVB-mediated conversion of 7-dehydrocholesterol, and obtained from food (cholecalciferol). In the liver, cholecalciferol is hydroxylated into prohormone 25-hydroxyvitamin D (25(OH)D; calcidiol). Since serum 25(OH)D best reflects vitamin D production and absorption and has a relatively long half-life, its concentrations are most commonly used as an indicator of vitamin D status [15]. In the kidneys and several other tissues [16], 25(OH)D is converted by CYP27B1 (25-hydroxyvitamin D 1-alpha-hydroxylase) into biologically active metabolite 1,25-dihydroxy vitamin D (1,25(OH)D; calcitriol) [17]. Calcitriol activates the nuclear vitamin D receptor (VDR), a member of the ligand-induced transcription factor family. After binding of 1,25(OH)D to VDR, a complex with the retinoid X receptor (RXR) is formed and binds to specific DNA sequences for transcriptional modulation of target genes involved in calcium/phosphate homeostasis, cellular proliferation/differentiation, immune and mitochondrial function [18]. The 1,25(OH)D-VDR complex also shows rapid, non-genomic effects on intracellular signalling and calcium flux [19].

It has long been accepted that vitamin D plays a critical role in the regulation of Ca^{2+} and phosphate homeostasis and is therefore critical for bone function. However, the discovery of a VDR in skeletal muscle cells provided evidence of the important role of this hormone also in skeletal muscle function and metabolism [20]. Serum concentrations of 25(OH)D are associated with muscle strength and muscle mass, and the incidence of injury across a broad range of age groups [21–23]. Many studies show that muscle weakness and low physical performance associated with vitamin D deficiency can be prevented or restored by adequate vitamin D supplementation [24, 25]. In vitro experiments in human skeletal muscle myoblasts showed a positive effect of vitamin D treatment on myogenesis and mitochondrial oxidative capacity [26]. However, the effect of additional factors such as age, medical condition, physical exercise, or protein consumption needs to be considered in the interpretation of the results [27–29].

Limited contractile activity, muscle disuse, and glucocorticoid therapy promote lipid accumulation, lipotoxicity, inflammation, and metabolic dysfunction that contribute to muscle atrophy and functional deterioration in IIM [30–32]. In our previous work, we showed the reduced capacity of skeletal muscle cells derived from patients with IIM to adapt to increased lipid load, which was linked to reduced mitochondrial function and fat oxidative metabolism [33]. Others showed that muscle cells exposed to saturated fatty acid in vitro displayed excessive fat accumulation and cellular damage. This effect was reversed by 1,25(OH)D treatment, in parallel with increased ATP production and expression of genes related to mitochondrial biogenesis and lipid oxidative metabolism, such as PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1-α), CPT1 (carnitine palmitoyl transferase 1) and PPARα (peroxisome proliferator-activated receptor-α) [34]. VDR silencing reduced

mitochondrial oxidative phosphorylation in C2C12 muscle cells [35]. These results suggest that reduced vitamin D levels could contribute to reduced oxidative capacity and deteriorated lipid metabolism in the skeletal muscle of patients with IIM.

In the present study, the potential role of vitamin D in disease manifestations related to skeletal muscle function and metabolism was investigated in patients with idiopathic inflammatory myopathies. Apart from 25(OH)D, serum levels of biologically active 1,25(OH)D, a vitamin D metabolite that skeletal muscle cells are exposed to *in vivo*, were analysed and compared to those of healthy individuals. The association of vitamin D status with clinical and physical activity parameters was assessed. Gene expression of vitamin D receptor and 25(OH)D activating enzyme CYP27B1 in muscle tissue and primary muscle cells was evaluated, together with their relation to muscle function parameters and lipid metabolism.

Methods

Study design and population

A total of 46 IIM patients with established disease and 67 healthy controls (HC) matched by age, gender, and body mass index were recruited. Of these, 27 patients with IIM attended 24-week physiotherapy program (personal training, PT) focused on activities of daily living and resistance and stability training for muscle strength and endurance. 19 IIM patients (mostly living outside of Prague) represented a non-exercising control patients group (CG). The intervention included physical exercise for 1 hour per session twice a week performed under the supervision of a physiotherapist and for the remaining five days a week an individual home exercise for 30 min per session according to an instruction manual (a detailed description of the physical therapy program and all outcome measures is available in [36], intervention trial registration number ISRCTN35925199). Peripheral blood collection and clinical examination were performed before and after the training intervention. All IIM patients were recruited from the inpatient and outpatient wards of the Institute of Rheumatology in Prague. Patients with IIM were diagnosed according to EULAR/ACR criteria [37] and ENMC criteria for IMNM [38] and were divided into 3 groups – PM, which also contained patients with antisynthetase syndrome, DM, and IMNM. Patients with inclusion body myositis were excluded. All study participants were ≥18 years of age, and each of them signed an informed consent form. The study design and informed consent form were approved by the local ethics committee at the Institute of Rheumatology in Prague.

Clinical disease activity was evaluated by the disease activity core set measures proposed by International Myositis Assessment & Clinical Studies Group (IMACS): myositis disease activity assessment (MYOACT), patient and physician global activity using visual analogue scales (VAS), myositis intention-to-treat activity index (MITAX), and the myositis damage index (MDI) [39]. Physical functioning was evaluated by MMT-8 (manual muscle test scores of individual eight muscle groups), Functional index-2 (FI-2), Health assessment questionnaire (HAQ), and Medical outcomes study 36-item short form health survey (SF-36: the physical component score (PCS) and the mental component score (MCS)) [40]. Anthropometric parameters and body composition were assessed by Dual energy X-ray absorptiometry (DEXA) (iDXA Lunar). Laboratory analyses of biochemical parameters for clinical practice (serum C-reactive protein (CRP) and markers of muscle impairment creatine kinase (CK), lactate dehydrogenase (LD), and myoglobin, insulin, lipid profile), and both vitamin D isoforms were performed in an accredited laboratory of Clinical Biochemistry and Hematology of the Institute of Rheumatology using Beckman Coulter AU 680 (Beckman Coulter, USA), Roche cobas e601 (Roche, Switzerland) and Liaison XL (Diasorin, Italy) analytical systems.

Muscle biopsy and skeletal muscle cell cultures

Muscle biopsies were obtained from 20 chronic IIM patients (7 patients before and after 24-week training, 13 control/nonexercising IIM patients), and 21 healthy controls. Samples of *musculus vastus lateralis* (100 - 150 mg) were taken by Bergström needle under local anaesthesia, cleaned from blood, snap frozen in liquid nitrogen, and stored at −80°C. Approximately 80 mg of tissue was used to establish a primary muscle cell culture, as previously described [41]. Briefly, satellite cells were isolated and propagated to myoblasts in collagen-coated flasks (culture medium: Dulbecco's Minimum Essential Medium supplemented with 20% foetal bovine serum and antibiotics), harvested at ~80% confluence and stored in liquid nitrogen. In a subpopulation of patients (before/after 24-week training/healthy controls n=7/7/7), cells were exposed to palmitic fatty acid (100 μM), coupled to a fatty acid-free BSA (in 5:1 molar ratio), or to BSA alone (control cells) for the 3 last days of differentiation. Palmitate treatment represents a chronic metabolic challenge and tests the capacity of muscle cells to handle saturated fatty acid overload [33].

Radiometric fatty acid oxidation (Fox) assay

The assay was described in detail previously and characterizes the metabolic adaptability by measuring a capacity to increase fat oxidation with increasing substrate load [33]. Briefly, differentiated muscle cells (myotubes) were pre-incubated in glucose- and serum-free media,

followed by a 3-hour incubation with [1- 14 C]-labeled palmitate (0.5 mCi/ml; ARC, USA), with/without a fatty acid challenge provided by 100μM „cold"/non-labeled palmitate (Sigma, USA), coupled to a fatty acid–free BSA. Following incubation, the medium was transferred into the 48-well custom-made CO_2-trapping plate. CO_2 released from media by perchloric acid was collected in 1M NaOH. Acidified media were spun twice/4°C, and radioactivity of supernatant containing intermediate Fox metabolites (ASP/Acid-Soluble-Products) was measured (Ecolite/Germany; TriCarb2910TR/Perkin-Elmer/USA). Cells were washed twice (ice-cold-PBS), harvested (0.25ml 0.05%SDS), and used to measure protein (BCA, Thermo-Fisher-Scientific, USA) and to detect neutral lipids content by thin layer chromatography (TLC), as described in [33]. Data were normalized to the protein content of the corresponding well. The impact of IIM and the 24-week training on fatty acid oxidation, lipid accumulation, and mitochondrial OxPHOS proteins was reported elsewhere [33], and in this work, these results were used solely for the correlation analyses.

Enzyme-linked immunosorbent assay (ELISA) and multiplex immunoassay

Myostatin and clusterin concentrations in serum were measured using commercially available ELISA kits (R&D Systems, Minneapolis, USA, and BioVendor, Brno, Czech Republic, respectively) according to the manufacturer's protocol. The absorbance was detected at 450 nm on a microplate reader (Tecan Sunrise, Salzburg, Austria). Bio-Plex Human Cytokine 27-Plex Panel (Bio-Rad, Hercules, USA) was used to determine cytokine concentrations in serum and analyzed by Bio-Rad Bio-Plex 200 Systems reader (Hercules, USA) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qPCR) analysis

The muscle samples were processed by homogenization of the tissue using a bead homogenizer (Retsch, Germany). Total RNA was isolated from 30 – 50 mg of frozen muscle using TRIzol Reagent (Thermo Fisher Scientific, Waltham, USA) or Qiazol (Qiagen, USA). Samples were treated with DNase I (New England BioLabs, France) and quantified with NanoDrop 2000c (Thermo Fisher Scientific, Waltham, USA). cDNA was obtained by reverse transcription with a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, USA) in a thermal cycler (Bio-Rad MyCycler, Hercules, USA). qPCR was carried out by TaqMan Gene Expression Assays (Thermo Fisher Scientific, Waltham, USA) in the QuantStudio 7 Flex qPCR system (Thermo Fisher Scientific, Waltham, USA). qPCR data are expressed as $2^{(-\Delta Ct)*}1000$. After the evaluation of four housekeeping genes using NormFinder software (MOMA, Aarhus, Denmark), the average of ACTB and RPL13 genes was used as an endogenous control in muscle tissue samples. For VDR expression in muscle cell cultures, RPL13 and β−2-microglobulin were used as an internal control.

Immunoblotting

Muscle cell protein lysates (40 μg) containing Complete protease inhibitor cocktail (Roche, Germany) were mixed with a loading dye (240mM Tris-HCl pH 6.8, 40% glycerol, 8% SDS, 5% β-mercaptoethanol, and 0.4% bromophenol blue), incubated (96°C for 5 min), separated on the 10%-SDS-PAGE and transferred to PVDF membrane (Millipore, USA). After blocking (Odyssey blocking buffer, LI-COR, USA), membranes were incubated overnight with an OxPHOS human antibody cocktail (Abcam/UK,1:250). Appropriate IRDye 680RD or 800CW antibodies (LI-COR, USA, 1:10000) were used to visualize protein content (LI-COR, USA). Protein ladder 10–180 kDa (Thermo-Fisher-Scientific, USA) was used. The relative content of individual OxPHOS protein complexes was calculated as % of the sum of protein signals for all five complexes (%OxPHOS).

Statistical analysis

Student's t-test or Mann-Whitney U test was used for the comparison of two groups of subjects. One-way ANOVA or the Kruskal-Wallis test and corresponding posthoc analyses were conducted for comparison of data obtained from three or more groups of subjects. The associations of serum and gene expression levels with clinical and laboratory markers were assessed by Pearson's product-moment correlation coefficient or Spearman's rank correlation coefficient, as applicable. Correlations were adjusted for body mass index, glucocorticoid dose equivalent to prednisone, and vitamin D supplementation using the partial correlation technique [42]. The analyses and the graphs were performed using GraphPad Prism version 9 (GraphPad Software , Boston, USA) and IBM SPSS version 22 (IBM SPSS, NY, USA).

Results

Characteristics of the study population

The characteristics of all IIM patients and HC enrolled in the study are summarized in Table 1. From the total of 46 IIM patients recruited in the study, 27 participated in a 24-week personal training (PT) and 19 patients represented a control patients group (CG). 7 patients from the PT group underwent a skeletal muscle biopsy before and after the training period along with an additional 13 non-training patients (CG) and 21 healthy controls who had a single muscle biopsy. Full clinical data for each patient

Table 1 Characteristics of the study population

	All	
	Healthy Controls	**IIM**
Number	67	46
PM/ASyS/DM/IMNM	NA	10/7/20/9
Gender, female/male (% female)	56/11 (84)	39/7 (85)
Age [years]	50.9 ± 14.7	56.7 ± 12.4 *
BMI [kg/m^2]	27.3 ± 4.5	27.6 ± 6.1
Biochemical markers		
CRP [mg/L]	3.2 ± 3.2	6.1 ± 13.1
CK [µkat/L]	1.7 ± 0.9	7.7 ± 13.2 **
LD [µkat/L]	2.9 ± 0.6	4.5 ± 2.1 ****
Myoglobin [µg/L]	NA	258 ± 421.8
Autoantibodies, N (%)		
ANA/Jo-1/HMGCR/TIF1/Mi2/ MDA5/NXP2/SAE/SRP/Ro52/Ro60/ SSA/La/PMScl/Ku/U1RNP	NA	29 (63)/10 (22)/2 (4)/3 (7)/3 (7)/ 1 (2)/3 (7)/1 (2)/2 (4)/20 (44)/8 (17)/ 3 (7)/2 (4)/5 (11)/3 (7)/1 (2)
Clinical features (at the time of examination)		
Disease duration, years	NA	6.5 ± 6.0
Muscle weakness, N (%)	NA	38 (83)
Rash, N (%)	NA	6 (13)
Arthritis, N (%)	NA	2 (4)
Raynaud´s phenomenon, N (%)	NA	10 (22)
Mechanic's hands, N (%)	NA	4 (9)
Interstitial lung disease, N (%)	NA	16 (35)
Cardiac involvement, N (%)	NA	9 (20)
Dysphagia, N (%)	NA	11 (24)
Chronic kidney disease, N (%)	NA	0
Malignancy, N (%) [a]	NA	3 (7)
Disease activity (range)		
HAQ (0–3)	NA	1.1 ± 0.8
MMT-8 (0–80)	NA	57.9 ± 12.4
MYOACT (0–1)	NA	0.059 ± 0.057
MITAX (0–1)	NA	0.212 ± 0.166
MDI extent (0–1)	NA	0.071 ± 0.055
SF-36 PCS (16.6–57.9)	NA	29.5 ± 12.3
SF-36 MCS (5.5–63.6)	NA	42.9 ± 12.1
Human Activity Profile (adjusted score) (0–94)	NA	50.8 ± 19.7
Treatment		
Daily GC dose (mg)	NA	12.7 ± 13.2
DMARDs, N (%)	NA	33 (72)
Vitamin D supplementation dose, IU/day	NA	1700 (500) [b]
Body Composition		
Lean Body Mass [kg]	NA	40.3 ± 6.9
Body Fat [%]	NA	42.8 ± 7.3

Data are expressed as mean ± SD

IIM idiopathic inflammatory myopathies, *PM* polymyositis, *ASyS* anti-synthetase syndrome, *DM* dermatomyositis, *IMNM* immune-mediated necrotizing myopathy, *BMI* body mass index, *NA* not applicable, *CRP* C-reactive protein, *CK* creatinine phosphokinase, *LD* lactate dehydrogenase, *HAQ* health assessment questionnaire, *MMT-8* manual muscle test 8, *MYOACT* myositis disease activity assessment visual analogue scales, *MITAX* myositis intention to treat activity index, *MDI* muscle damage index, *SF-36* Medical Outcomes Study 36-Item Short Form Health Survey, *PCS* physical component summary, *MCS* mental component summary, *GC* glucocorticoids, *Daily GC dose* mg of prednisone equivalent, *DMARDs* disease-modifying antirheumatic drugs

Table 1 (continued)

* $p < 0.05$

** $p < 0.01$

**** $p < 0.0001$

[a] diagnosed and treated 9, 8 and 1 year before sample acquisition

[b] data represent median (IQR). For a detailed breakdown of the study subgroups characteristics, see Supplementary File 1

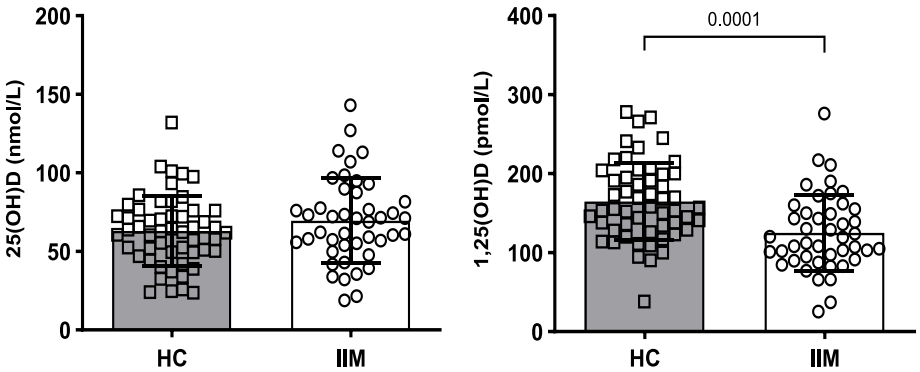

Fig. 1 Vitamin D levels in myositis patients (IIM) and healthy controls (HC). An unpaired t-test was used to compare the means between groups. Columns represent mean, whiskers represent SD

subgroup according to the biological material available for subsequent analyses are shown in Supplementary Table S1.

The levels of biologically active 1,25(OH)D are significantly decreased in myositis patients

Serum levels of 25(OH)D and 1,25(OH)D were analyzed in 46 IIM patients and 67 age-/sex-matched healthy controls. Decreased levels of active 1,25(OH)D were observed in IIM patients compared to HC (125.0±45.4 vs. 164.7±49.2 pmol/l; $p<0.0001$). No difference was found for 25(OH)D (Fig. 1). There was no significant difference in either 25(OH)D or 1,25(OH)D serum levels between myositis subgroups PM, DM, and IMNM (Suppl. Fig. 1). The 24-week training program did not alter serum 25(OH)D or 1,25(OH)D levels (Suppl. Fig. 2). No season-related difference in 25(OH)D or 1,25(OH)D serum levels in either IIM patients or healthy controls was observed (Suppl. Fig. 3). Also, no difference in 25(OH)D or 1,25(OH)D serum levels was observed between patients positive and negative for the 3 most abundant autoantibodies – antinuclear (ANA), anti-Jo-1 and anti-Ro-52 (data not shown).

Serum 25(OH)D and 1,25(OH)D levels are associated with clinical parameters and physical activity

The correlation analyses of circulating 25(OH)D and 1,25(OH)D were performed in a cohort of IIM patients before ($N=46$) and after ($N=27$) the training intervention. Since a significant association of 25(OH)D serum levels with BMI ($r=-0.424$, $p=0.004$), borderline association with daily GC dose ($r=-0.273$, $p=0.074$) and an association of 1,25(OH)D with a vitamin D supplementation daily dose ($r=0.342$, $p=0.025$) were observed, another set of correlation analyses adjusted for these three parameters was performed. At the baseline, significant associations of 25(OH)D with inflammatory markers (CRP, TNF, IL-8), disease activity (MITAX), overall function (HAQ), and physical activity (HAP, SF-36 PSC) were observed (Table 2). The associations remained significant even after correction for BMI, GC, and vitamin D supplementation daily dose. In the intervention group after 24 weeks of training, a positive association of active 1,25(OH)D with muscle strength (MMT-8) and endurance (FI-2) and overall function (HAQ), independent of BMI, GC, and vitamin D supplementation was observed. In the group of non-exercising patients, only borderline association of 1,25(OH)D with FI-2 ($r=0.435$, $p=0.062$) and MMT-8 ($r=0.435$, $p=0.062$) was observed, and the significance was lost after correction for BMI + GC dose + vitamin D supplementation dose. The correlation analysis of training-induced changes (Δ) in studied parameters did not show statistically significant associations (Suppl. Table 2).

Vitamin D receptor and vitamin D activating and deactivating enzymes (CYP27B1 and CYP24A1) gene expression in muscle decreases after training and is associated with muscle function parameters

We did not observe a statistically significant difference in gene expression of VDR and CYP27B1 in muscle tissue and primary muscle cells in IIM compared to

Table 2 The heat map of 25(OH)D and 1,25(OH)D vitamin D associations with clinical parameters in myositis patients before and after the exercise intervention

	Pre-PT				Post-PT			
	Vitamin D		1,25-OH vitamin D		Vitamin D		1,25-OH vitamin D	
Correlation coefficient	Spearman	Partial correlation	Spearman	Partial correlation	Spearman	Partial correlation	Spearman	Partial correlation
BMI (kg/m^2)	-0.424 #	-	0.065	-	-0.424	-	0.090	-
GC dose (mg/day)	-0.273	-	-0.208	-	-0.163	-	-0.555 #	-
vitamin D supplementation dose (IU/day)	0.005	-	0.342 #	-	-0.165	-	-0.042	-
CRP (mg/L)	-0.316 #	-0.283	-0.066	-0.249	-0.083	0.038	-0.084	-0.100
MITAX	-0.311 #	-0.359 #	-0.161	-0.231	NA	NA	NA	NA
HAQ	-0.390 #	-0.312 #	-0.213	-0.288	-0.265	-0.213	-0.394	-0.537 #
HAP AAS	0.385 #	0.315 #	0.107	0.305	0.115	-0.078	0.306	0.332
SF-36 PCS	0.492 #	0.414 #	0.175	0.312 #	0.249	0.307	0.359	0.176
FI-2	0.075	0.008	0.065	0.180	0.230	0.083	0.487 #	0.608 #
MMT-8	0.183	0.186	0.150	0.257	0.207	0.083	0.667 #	0.866 #
Myoglobin (µg/L)	-0.020	0.060	-0.107	0.073	-0.087	0.050	-0.398	-0.402
TNF (pg/mL)	-0.402 #	-0.391 #	0.051	0.029	0.233	0.098	-0.091	-0.192
IL-8 (pg/mL)	-0.372 #	-0.329 #	-0.055	-0.046	0.247	0.362	0.194	0.255
Insulin (mIU/L)	-0.310 #	0.022	-0.028	0.108	-0.167	0.145	0.017	-0.248
TG (mmol/L)	-0.286	-0.074	0.041	0.017	NA	NA	NA	NA

PT physiotherapy program, *BMI* body mass index, *GC dose* mg of prednisone equivalent, *CRP* C-reactive protein, *MITAX* myositis intention to treat activity index, *DXA FM* total fat mass by densitometry, *HAQ* health assessment questionnaire, *HAP AAS* human activity profile adjusted activity score, *SF-36 PCS* The 36-Item Short Form Health Survey physical component score, *FI-2* functional index 2, *MMT-8* manual muscle test of 8 muscles, *TG* triglycerides, *NA* not available

# $p < 0.05$

HC. However, after the 24-week training, gene expression of both VDR and CYP27B1 in primary muscle cells decreased significantly (Fig. 2). Associations of VDR gene expression with myoglobin (Fig. 3A IIM, Fig. 3E HC), MMT-8 (Fig. 3D IIM), myostatin (Fig. 3B IIM, Fig. 3F HC), CK (HC: r=0.484, p=0.031), and lipid profile (Fig. 3G, H HC) were observed in muscle tissue. Lower CYP27B1 gene expression in the muscle was also associated with higher MMT-8 (Fig. 4B IIM), clusterin (Fig. 4C IIM), TGFβ mRNA (Fig. 4D IIM), lower myoglobin (Fig. 4F HC), and lower VDR (Fig. 4A IIM, Fig. 4E HC). Moreover, lower CYP27B1 correlated with better metabolic parameters in IIM patients, such as higher lean body mass (r=−0.651, p=0.0026), basal metabolic rate (r=−0.630, p=0.0038), and lower 120-min blood glucose levels in oral glucose tolerance test (oGTT) (r=0.532, p=0.023).

Vitamin D receptor and circulating vitamin D levels are related to muscle oxidative capacity and lipid metabolism in cultured muscle cells

There was a decline of VDR mRNA expression related to 3-day palmitate challenge in primary myotubes, irrespective of the disease or training state of the IIM patients (Fig. 5A, B).

The expression of VDR in differentiated muscle cells correlated negatively with the complete oxidation of saturated palmitic fatty acid (CO_2 production) (Fig. 6A), total palmitate disposal (oxidative + non-oxidative palmitate disposal) (Fig. 6B), non-oxidative palmitate disposal (Fig. 6C), and relative content of complex V (% of the sum of all five OxPHOS complexes) (Fig. 6F). Moreover, the expression of VDR correlated positively with the relative content of complex I and complex IV (Fig. 6D, E).

The expression of genes important for the mitochondrial oxidative capacity, carnitine palmitoyl transferase (CPT1, the rate-limiting enzyme of mitochondrial fatty acid oxidation) (Fig. 7A) and frataxin (the nuclear-encoded mitochondrial protein), was downregulated in the skeletal muscle of IIM patients (n=12, p=0.035). Muscle mRNA levels of CPT1 correlated positively with serum 1,25(OH) vitamin D levels (Fig. 7B) and negatively with levels of 25(OH) vitamin D (Fig. 7C). mRNA expression of MYH7, the marker of oxidative muscle fibers, did not differ between IIM patients and controls (p>0.1, data not shown). Due to the limited amount of

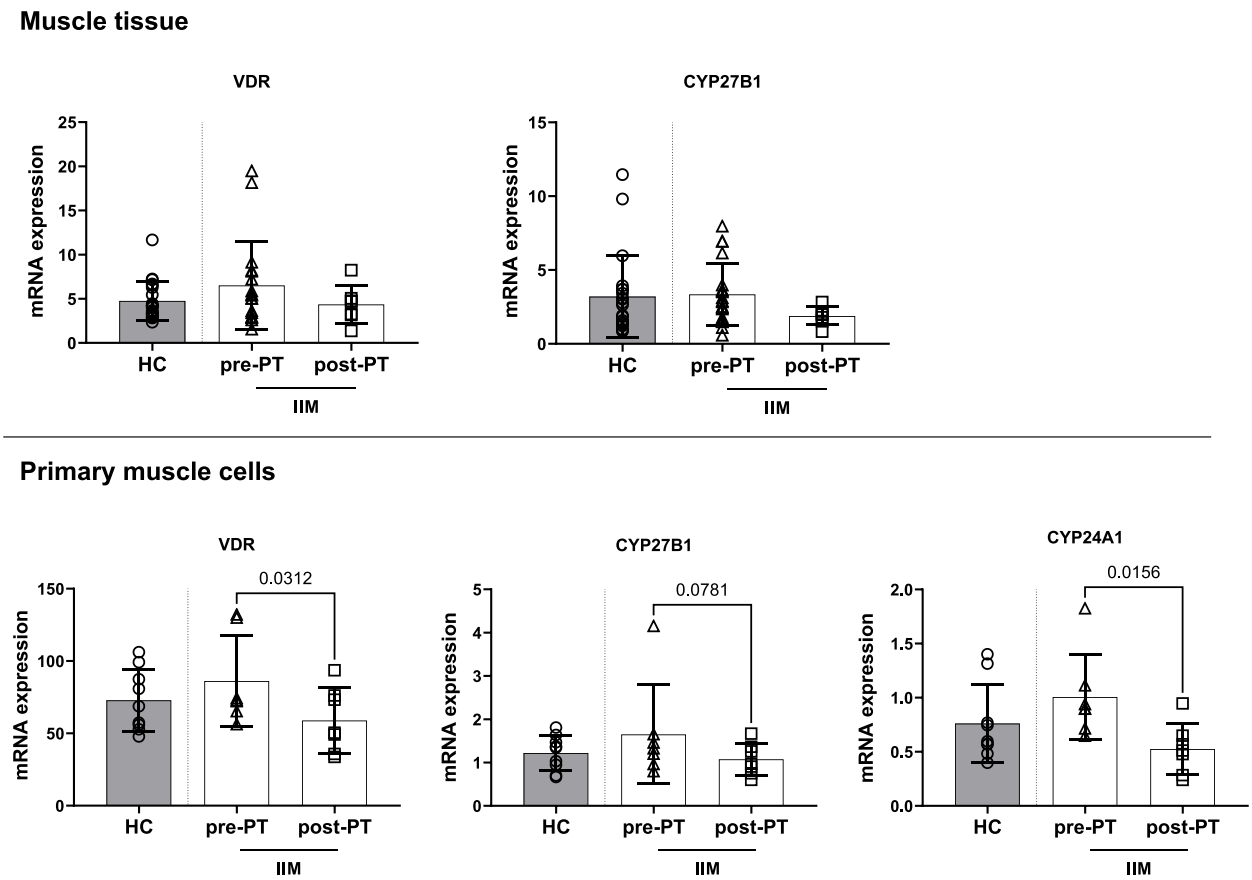

Fig. 2 Vitamin D receptor (VDR), CYP27B1, and CYP24A1 gene expression in muscle tissue and in primary myotubes derived from myositis patients before and after training intervention (pre- and post-PT) and healthy controls (HC). Mann–Whitney (HC vs pre-PT IIM) and Wilcoxon (pre- vs post-PT IIM) tests were used to compare the means between groups. Columns represent mean, whiskers represent SD

biological material obtained, the impact of training intervention on the expression of these genes could not be determined.

Circulating 1,25(OH) vitamin D levels correlated positively with in vitro parameters of lipid metabolism in human primary myotubes, including total fatty acid disposal (r=0.656, p=0.0012), the relative proportion of lipid oxidative metabolism (expressed as a percentage of total fatty acid disposal, r=0.573, p=0.0067), and incomplete fatty acid oxidation (the production of intermediate metabolites of Fox; r=0.587, p=0.0051).

Discussion

Active vitamin D is decreased in IIM and correlates with clinical parameters and physical activity

Our study on vitamin D in idiopathic inflammatory myopathies shows decreased levels of biologically active 1,25(OH)D in IIM patients compared to healthy individuals, even though the levels of commonly measured 25(OH)D were comparable with controls. Vitamin D deficiency in IIM has been reported by two studies so far [12, 13], however, only 25(OH)D levels were assessed. We have not found a significant difference in vitamin D levels between the myositis subgroups similar to Azali et al. [12]. Yu et al. [13] showed the correlation of vitamin D levels with muscle enzymes, the presence of anti-Jo-1 and anti-Mi-2 antibodies, and the absolute numbers of total T and Treg cells suggesting a role of vitamin D in the immunological pathogenesis of IIM. In the presented study, higher 25(OH)D was significantly associated with lower inflammation (CRP, TNF, IL-8), lower disease activity (MITAX), better overall function (HAQ), and physical activity parameters (HAP, SF-36 PCS) in IIM patients despite the 25(OH)D levels comparable to healthy controls. These associations highlight the importance of vitamin D from the clinical perspective. Moreover, the correlations were independent of BMI, glucocorticoid treatment, and vitamin D supplementation dose. The correlation analyses were adjusted for these parameters, as increased BMI and steroid use have been associated with 25(OH)D deficiency [43–45]. An inverse correlation between 25(OH) vitamin D and CRP

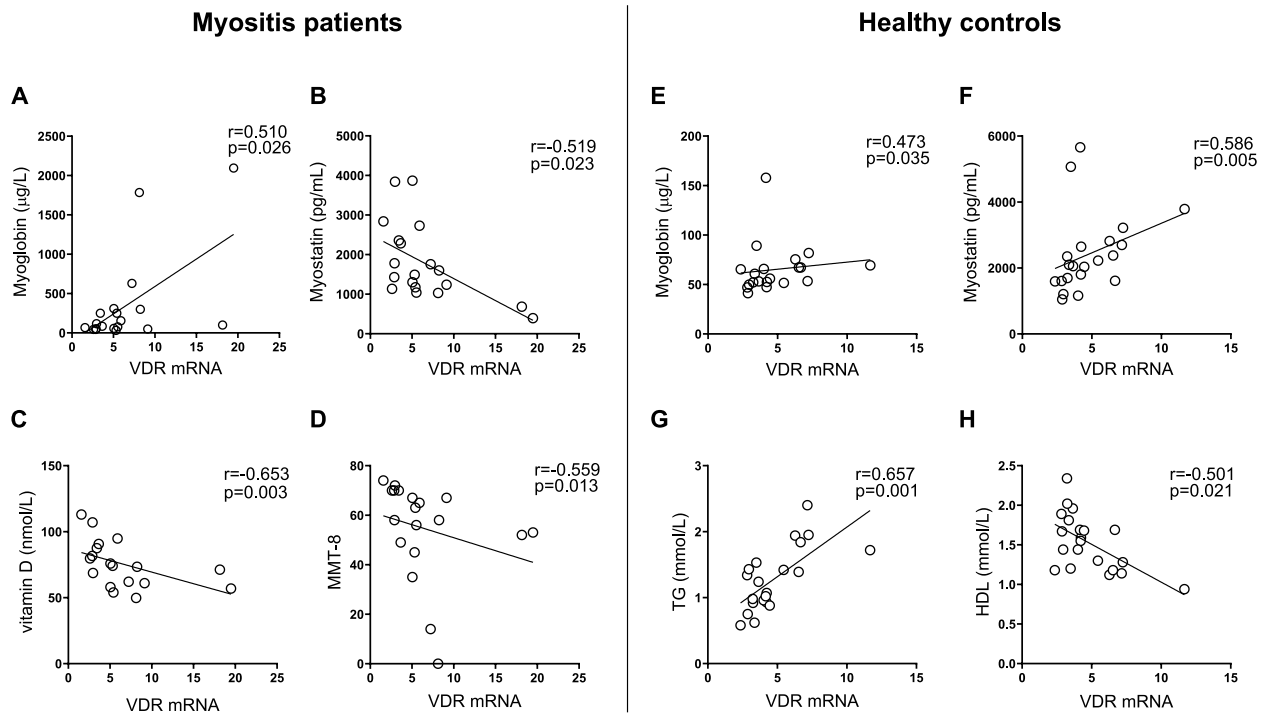

Fig. 3 Vitamin D receptor (VDR) gene expression in muscle tissue correlates with serum myoglobin (**A**, **E**), serum myostatin (**B**, **F**), vitamin D levels (**C**), muscle strength (**D**), and serum lipids (**G**, **H**) in myositis patients and healthy controls (baseline levels). The bivariate relationships between variables were assessed using the Spearman correlation coefficient. MMT-8, manual muscle testing of 8 muscles, TG—triglycerides, HDL, high-density lipoprotein

in a large patient cohort has been reported and this effect was more pronounced in patients with inflammatory diseases compared to patients with non-inflammatory diseases [46]. The protolerogenic activity of vitamin D relies on its capability to control the maturation, differentiation, and activation of immune cells through VDR that is either constitutively present or inducible in the majority of the immune cells [47–49].

Implementing physical activity and exercise in the treatment of IIM has been shown to reduce impairment and improve patients' quality of life [50]. Specifically, the improvement of muscle strength and endurance [36], aerobic and mitochondrial capacity [31], and reversed metabolic dysfunction [33, 51] represent the contribution of exercise to both systemic and intrinsic muscle adaptations in IIM patients. Even though vitamin D status depends largely on sunlight exposure and dietary or supplementary intake, recent studies have found that endurance and resistance exercise can also influence circulating levels of 25(OH)D in various study populations [52]. However, the intensity of exercise, the initial vitamin D deficiency, BMI, and seasonal factors may play a role in the observed inconsistent effect of exercise on 25(OH)D levels [52]. In our study, a 24-week training intervention (a tailored program focused on activities of daily living, resistance, and stability training provided indoors) did not affect either 25(OH)D or 1,25(OH)D serum levels in IIM patients. However, higher levels of 1,25(OH)D correlated with higher muscle strength and endurance parameters (MMT-8 and FI-2, respectively), and the overall function evaluated by HAQ. These findings suggest a possible connection of 1,25(OH)D with physical fitness improvement. For instance, in a study by Marantes et al. [53] no consistent association between 25(OH)D and measurements of muscle mass or strength was found. However, low 1,25(OH) (2) D was associated with low skeletal mass and low knee extension moment.

VDR and CYP gene expression in skeletal muscle is associated with muscle fitness indicators

The gene expression of VDR and 25(OH)D metabolizing enzymes (CYP27B1 and CYP24A1) in primary muscle cells derived from IIM patients' muscles significantly decreased after 24-week training to levels comparable with healthy controls. As the muscle strength and endurance of patients participating in the training intervention increased [36] without changes in vitamin D levels, we may suggest that the local metabolism of vitamin D in muscle tissue of IIM patients reflects the changes in overall physical fitness and a lower

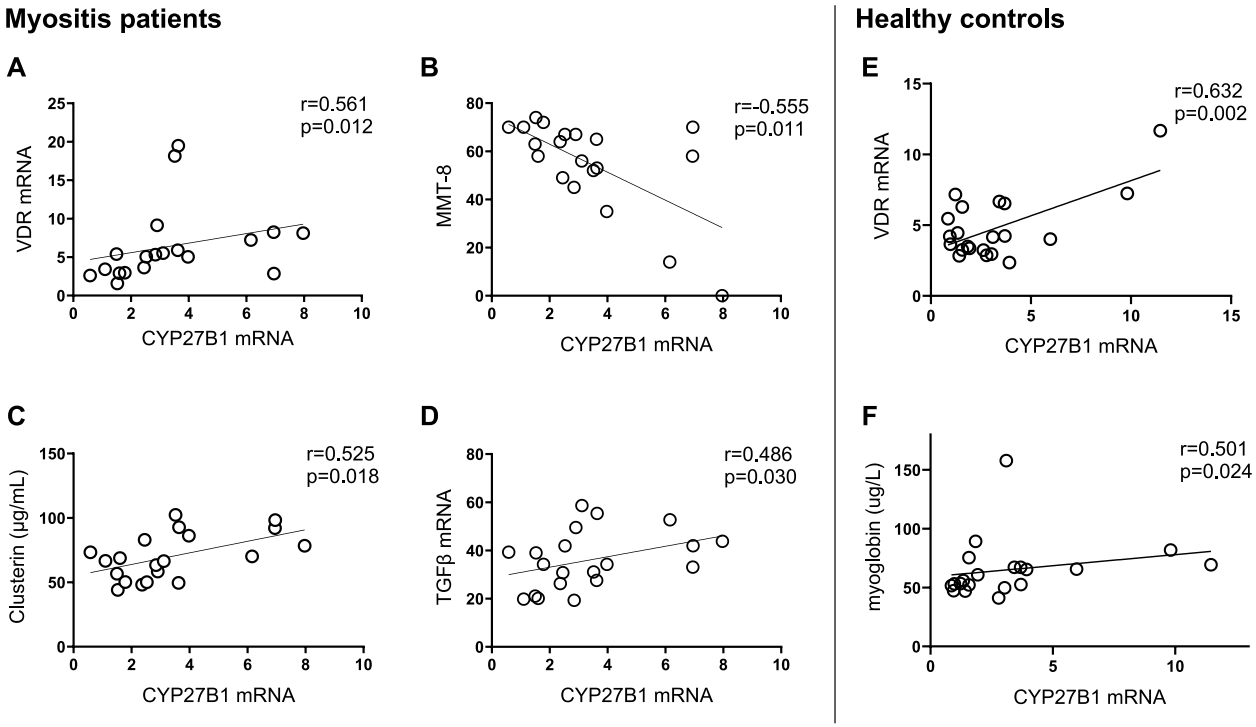

Fig. 4 Vitamin D 1A hydroxylase (CYP27B1) gene expression in muscle tissue correlates with VDR (**A**, **E**), muscle strength assessed by MMT-8 (**B**), and indicators of muscle damage/dysfunction (**C**, **D**, **F**) in myositis patients and healthy controls (baseline levels). The bivariate relationships between variables were assessed using the Spearman correlation coefficient. MMT-8, manual muscle testing of 8 muscles

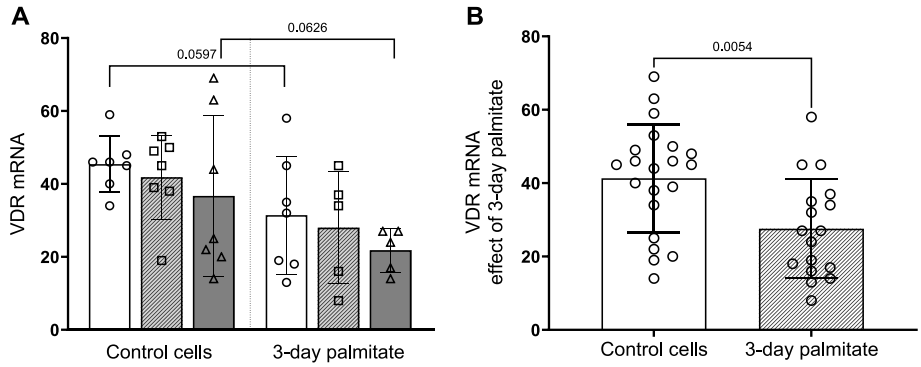

Fig. 5 Vitamin D receptor expression in primary myotubes exposed to a 3-day treatment with saturated fatty acid. Myotubes were treated with 100 µM palmitic fatty acid coupled to BSA (3-day palmitate) or to BSA alone (control cells). Data are presented as means ± SEM, $n = 21$ per group. **A** Comparison between IIM before PT- white; IIM after PT- light grey; healthy controls—dark gray, $n = 7$ per group. Paired t-test was used to asses the effect of stimulation in each group of subjects. **B** global effect of palmitate independent from IIM or training effect, $n = 21$. Unpaired t-test was used to compare the means between groups. PT – personal training

requirement for vitamin D. This may be supported by the correlation of lower VDR mRNA with higher 25(OH)D levels, higher muscle strength measured by MMT-8, lower release of myoglobin into circulation, and higher serum myostatin. The latter has been shown to correlate with the improvement in muscle inflammation in IIM [54, 55]. Lower expression of vitamin D activating enzyme 25-hydroxyvitamin D 1-alpha-hydroxylase encoded by CYP27B1 gene in muscle tissue correlated with lower expression of VDR, suggesting a mutual regulation that could be expected based on their function. Similarly to VDR, lower expression of CYP27B1 correlated with higher muscle strength. Moreover, lower CYP27B1 mRNA was associated with

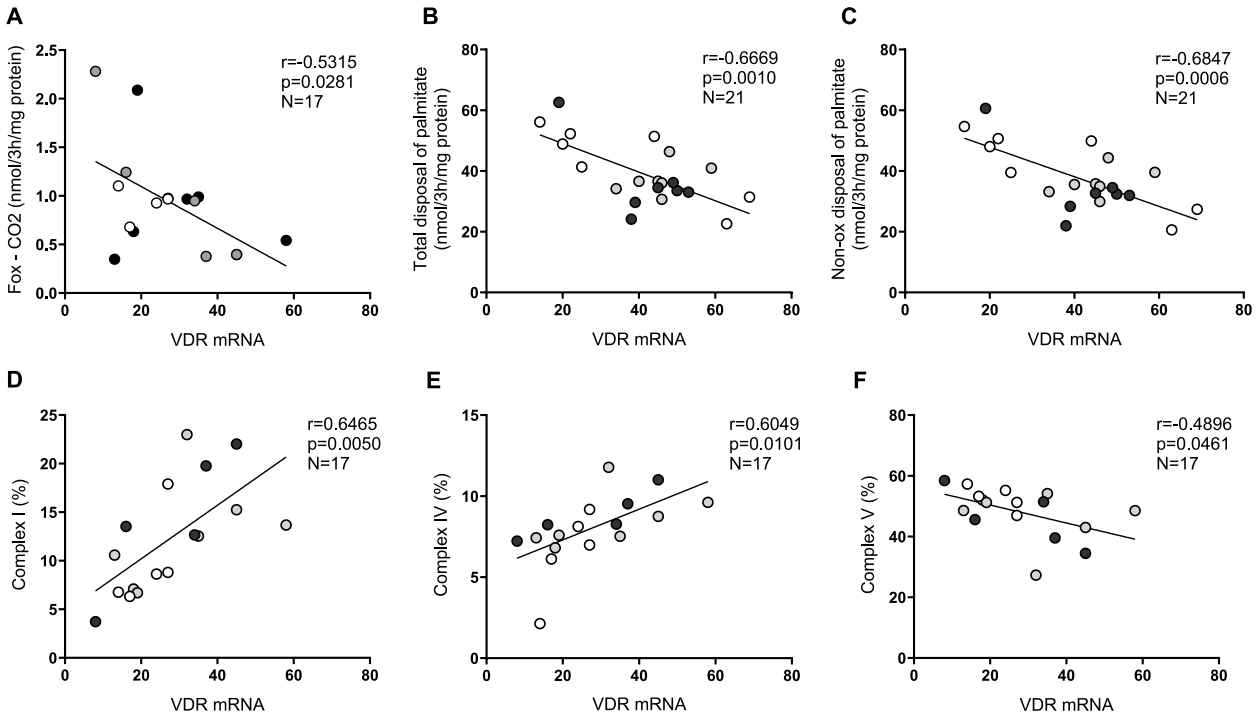

Fig. 6 Vitamin D receptor expression in primary myotubes correlates with characteristics of lipid metabolism in vitro. Lipid metabolism was assessed by radioactive assays, using saturated14C-labeled palmitic fatty acid, and normalized to protein content. VDR, Vitamin D receptor; Fox, fatty acid oxidation. Individual complexes of mitochondrial oxidative phosphorylation (OxPHOS) are presented as % from the total signal, obtained as a sum of signals of all 5 protein complexes (immunoblotting). The bivariate relationships between variables were assessed using the Pearson correlation coefficient. Points colour scheme: white – HC, light grey—IIM before PT, dark grey – IIM after PT PT – personal training

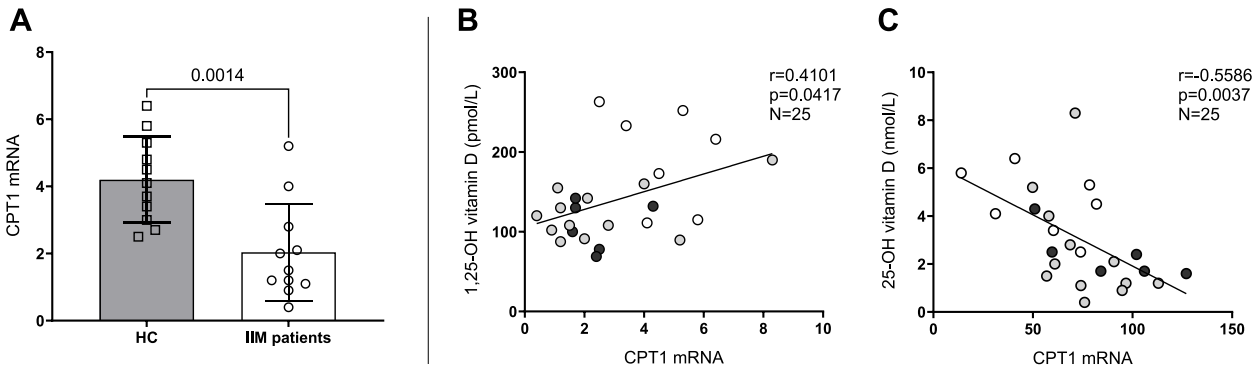

Fig. 7 Carnitin palmitoyl transferase 1, CPT1, the rate-limiting enzyme in mitochondrial fatty acid oxidation is decreased in IIM skeletal muscle (**A**), and correlates with vitamin D levels (**B**, **C**). An unpaired t-test (**A**) and Pearson correlation coefficient (**B**, **C**) were used to assess the variables relationships. Points colour scheme: white – HC, light grey—IIM before PT, dark grey – IIM after PT. PT – personal training

lower TGFβ mRNA in muscle tissue and lower clusterin levels in serum. We have previously shown upregulated clusterin in the serum and muscle of IIM patients with higher disease activity [56]. TGFβ is considered a negative regulator of adult skeletal muscle [57] through reduction of myoblast fusion [58] and contribution to muscle fibrosis [59].

Vitamin D and VDR correlate with parameters of muscle oxidative capacity and lipid metabolism in vitro

There is some evidence linking vitamin D, its regulation, and signalling to the functioning of the lipid system in muscle cells. VDR knockdown models have demonstrated the crucial role of vitamin D for mitochondrial oxidative phosphorylation capacity, supporting also

its role in muscle regeneration [60]. VDR silencing in murine C2C12 muscle cells reduced ATP production and mitochondrial respiration, without the impact on mitochondrial protein content and markers of mitochondrial fission [35]. Differentiated C2C12 muscle cells exposed to palmitic fatty acid displayed excessive fat accumulation and cellular damage, which was reversed by 1,25(OH) vitamin D treatment [34]. Vitamin D has increased ATP levels and activation of AMPK and SIRT1 as well as gene expression related to mitochondrial biogenesis and lipid oxidative metabolism such as NRF1, PGC-1α or CPT1, PPARα, and others [34]. Sinha et al. [61] demonstrated an increase in the maximal mitochondrial oxidative phosphorylation rate measured by 31P-NMR spectroscopy in vitamin D deficient humans after vitamin D treatment. Similarly, the mitochondrial oxygen consumption rate coupled to the generation of ATP was increased after 1,25(OH) vitamin D treatment in muscle cells in vitro [62]. These observations support the role of vitamin D in modulating / stimulating mitochondrial oxidative phosphorylation.

In this work, VDR mRNA expression was reduced in muscle cells exposed to saturated palmitic fatty acid and correlated negatively with in vitro parameters of lipid metabolism and relative content of complex V, which plays a decisive role in the ATP-producing capacity of mitochondria. The gene expression of the rate-limiting enzyme of mitochondrial fatty acid oxidation CPT1 was downregulated in the skeletal muscle of IIM patients; however, it correlated positively with serum 1,25(OH) vitamin D levels. Moreover, circulating 1,25(OH) vitamin D levels correlated positively with in vitro parameters of lipid metabolism in human primary myotubes in this study.

We also observed higher VDR gene expression in muscle from healthy controls (suggesting a compensatory mechanism for insufficient vitamin D signalling) associated with higher serum TG and lower HDL levels. Low vitamin D levels could result in an increase in triglycerides, total cholesterol, and low-density lipoprotein and a decrease in high-density lipoprotein cholesterol levels [63–65]. Interestingly, mice fed with a high-fat diet displayed downregulated vitamin D-related metabolic genes and changes in gene-specific methylation in the liver [66]. In our previous work, we showed that muscle cells from patients with IIM display reduced capacity to adapt to the saturated fatty acid exposure, compared to healthy controls [33]. It is plausible to speculate that a diet higher in saturated fat can deteriorate lipid metabolism also via the negative impact on vitamin D metabolism/signaling in the skeletal muscle of IIM patients. Overall, our data support the existence of a feedback loop linking vitamin D deficiency in patients with IIM to impaired lipid metabolism.

Study limitations

A relative heterogeneity of the study population and a limited availability of biological material resulted in a small sample size in some analyses. Low numbers of individuals per group precluded the realization of the correlation analysis in the individual subgroups. We made an effort to compensate for this limitation by carefully matching the cohort of healthy controls. All patients involved in the study had an established disease and were receiving treatment at the time of sample collection. Even though the analyses were adjusted for therapeutics with the presumed highest impact where possible, the effect of treatment on the presented results cannot be excluded. The adaptive response to training in health and disease could be better discerned if exercise interventions were also performed in healthy control subjects and repeated muscle biopsies obtained from non-exercising IIM patients. Protein levels of VDR and 25(OH)D metabolizing enzymes (CYP27B1 and CYP24A1) would help to specify the suggested response of muscle tissue and primary muscle cells to vitamin D deficiency. We plan to address at least some of these limitations in our future studies.

Conclusions

Lower levels of the biologically active form of vitamin D in circulation suggest an impairment of its metabolism in IIM. Vitamin D serum levels are associated with inflammatory markers, disease activity, muscle function, and metabolic parameters, indicating an important role of vitamin D in disease manifestations and physical fitness of IIM patients. Moreover, we suggest that the positive association of VDR and CYP27B genes in muscle tissue of IIM patients with increased muscle functional decline indicators may represent i) a response of the organism to decreased availability of active 1,25(OH)D, and ii) a mechanism to limit further inflammation- and atrophy-induced muscle damage characteristic for IIM. Lower levels of circulating vitamin D in IIM patients and their associations with impaired lipid metabolism in muscle cells in vitro indirectly indicate that reduced vitamin D may contribute to altered fatty acid metabolism in skeletal muscle, mitochondrial dysfunction, oxidative stress, muscle weakness, and atrophy, which are typical features for patients with IIM.

Abbreviations

IIM	Idiopathic inflammatory myopathies
HC	Healthy controls
DM	Dermatomyositis

PM	Polymyositis
ASyS	Antisynthetase syndrome
IMNM	Immune-mediated necrotizing myopathy
VDR	Vitamin D receptor
25(OH)D	25-hydroxy vitamin D
1,25(OH)D	1,25-dihydroxy vitamin D
CPT1	Carnitine palmitoyl transferase 1
MYOACT	Myositis disease activity assessment
MITAX	Myositis intention-to-treat activity index
MDI	Myositis damage index
MMT-8	Manual muscle test scores of individual eight muscle groups
FI-2	Functional index-2
HAQ	Health assessment questionnaire
HAP AAS	Human activity profile adjusted activity score
SF-36	Medical outcomes study 36-item short form health survey
PCS	Physical component summary
MCS	Mental component summary
DEXA	Dual energy X-ray absorptiometry
CRP	C-reactive protein
CK	Creatine kinase
LD	Lactate dehydrogenase
ELISA	Enzyme-linked immunosorbent assay
PT	Personal training
CG	Control patients group
GC	Glucocorticoids
DMARDs	Disease-modifying antirheumatic drugs
SD	Standard deviation
BMI	Body mass index
CYP27B1	25-hydroxyvitamin D 1-alpha-hydroxylase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03516-9.

Supplementary Material 1.

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Authors' contributions
LV and BU were responsible for the study concept and design and drafted the manuscript. JV supervised the study implementation and manuscript preparation. JV, MK, SO, and MT were responsible for patient recruitment and clinical assessment. MS, SO, and MT organized and performed the training intervention. MK and KK performed the muscle biopsies. LV, NAL, MV, OM, MN, VH, and TK conducted the in vitro experiments and laboratory analyses. LW was responsible for analyses of biochemical parameters for clinical practice including both vitamin D isoforms. LV carried out the statistical analysis. LV, JU, BU, and JV are responsible for data interpretation. All authors reviewed the manuscript and gave their final approval of the version to be published.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethics approval and consent to participate: all relevant study documentation and amendments were approved by the independent Ethics Committee of the Institute of Rheumatology Prague with reference numbers 5675/2015 and 5688/2015. The study was conducted following the principles outlined in the Declaration of Helsinki, the Guidelines of the International Council for Harmonisation (ICH) on Good Clinical Practise (GCP) Guideline E6 (R2) (EMA/CPMP/ICH/ 135/95) European Union (EU) Directive 95/46/EC, and other applicable regulatory requirements. Patients provided informed written consent before enrolment in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Institute of Rheumatology, Na Slupi 4, Prague 12850, Czech Republic. [2]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia. [3]Department of Health Care Disciplines and Population, Faculty of Biomedical Engineering, Czech Technical University, Prague, Czech Republic. [4]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia. [5]Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic. [6]Faculty of Science, Charles University, Prague, Czech Republic.

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References

1. Lundberg IE, Fujimoto M, Vencovsky J, Aggarwal R, Holmqvist M, Christopher-Stine L, et al. Idiopathic inflammatory myopathies. Nat Rev Dis Primers. 2021;7(1):86.
2. Vencovsky J, Alexanderson H, Lundberg IE. Idiopathic inflammatory myopathies. Rheum Dis Clin North Am. 2019;45(4):569–81.
3. Loell I, Lundberg IE. Can muscle regeneration fail in chronic inflammation: a weakness in inflammatory myopathies? J Intern Med. 2011;269(3):243–57.
4. Nagaraju K, Casciola-Rosen L, Lundberg I, Rawat R, Cutting S, Thapliyal R, et al. Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and dysfunction. Arthritis Rheum. 2005;52(6):1824–35.
5. Grundtman C, Tham E, Ulfgren AK, Lundberg IE. Vascular endothelial growth factor is highly expressed in muscle tissue of patients with polymyositis and patients with dermatomyositis. Arthritis Rheum. 2008;58(10):3224–38.
6. Niinikoski J, Paljarvi L, Laato M, Lang H, Panelius M. Muscle hypoxia in myositis. J Neurol Neurosurg Psychiatry. 1986;49(12):1455.
7. Girolamo F, Lia A, Amati A, Strippoli M, Coppola C, Virgintino D, et al. Overexpression of autophagic proteins in the skeletal muscle of sporadic inclusion body myositis. Neuropathol Appl Neurobiol. 2013;39(7):736–49.
8. Loell I, Raouf J, Chen YW, Shi R, Nennesmo I, Alexanderson H, et al. Effects on muscle tissue remodeling and lipid metabolism in muscle tissue from adult patients with polymyositis or dermatomyositis treated with immunosuppressive agents. Arthritis Res Ther. 2016;18(1):136.
9. Liu Y, Wen H. Impact of vitamin D deficiency on clinical parameters in treatment-naive rheumatoid arthritis patients. Zeitschrift fur Rheumatologie. 2018;77(9):833–40 Einfluss eines Vitamin-D-Mangels auf klinische Parameter bei therapienaiven Patienten mit rheumatoider Arthritis.
10. Oliveira SR, Simao ANC, Alfieri DF, Flauzino T, Kallaur AP, Mezzaroba L, et al. Vitamin D deficiency is associated with disability and disease progression in multiple sclerosis patients independently of oxidative and nitrosative stress. J Neurol Sci. 2017;381:213–9.
11. Ospina-Caicedo AI, Cardona-Rincon AD, Bello-Gualtero JM, Valle-Onate R, Romero-Sanchez C, Chalem-Choueka P, et al. Lower levels of vitamin D associated with disease activity in Colombian patients with systemic lupus erythematosus. Curr Rheumatol Rev. 2019;15(2):146–53.
12. Azali P, Barbasso Helmers S, Kockum I, Olsson T, Alfredsson L, Charles PJ, et al. Low serum levels of vitamin D in idiopathic inflammatory myopathies. Ann Rheum Dis. 2013;72(4):512–6.
13. Yu Z, Cheng H, Liang Y, Ding T, Yan C, Gao C, et al. Decreased serum 25-(OH)-D level associated with muscle enzyme and myositis specific

autoantibodies in patients with idiopathic inflammatory myopathy. Front Immunol. 2021;12:642070.
14. Crescioli C. Vitamin D receptor agonists: suitable candidates as novel therapeutic options in autoimmune inflammatory myopathy. Biomed Res Int. 2014;2014:949730.
15. Hansen KE, Johnson MG. An update on vitamin D for clinicians. Curr Opin Endocrinol Diabetes Obes. 2016;23(6):440–4.
16. Bikle DD, Patzek S, Wang Y. Physiologic and pathophysiologic roles of extra renal CYP27b1: case report and review. Bone reports. 2018;8:255–67.
17. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004;80(6 Suppl):1678S-1688S.
18. Carlberg C. Vitamin D and its target genes. Nutrients. 2022;14(7):1354.
19. Carlberg C, Campbell MJ. Vitamin D receptor signaling mechanisms: integrated actions of a well-defined transcription factor. Steroids. 2013;78(2):127–36.
20. Montenegro KR, Cruzat V, Carlessi R, Newsholme P. Mechanisms of vitamin D action in skeletal muscle. Nutr Res Rev. 2019;32(2):192–204.
21. Grimaldi AS, Parker BA, Capizzi JA, Clarkson PM, Pescatello LS, White MC, et al. 25(OH) vitamin D is associated with greater muscle strength in healthy men and women. Med Sci Sports Exerc. 2013;45(1):157–62.
22. Redzic M, Lewis RM, Thomas DT. Relationship between 25-hydoxyvitamin D, muscle strength, and incidence of injury in healthy adults: a systematic review. Nutr Res. 2013;33(4):251–8.
23. Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K. Calcium and vitamin d supplementation decreases incidence of stress fractures in female navy recruits. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2008;23(5):741–9.
24. Chiang CM, Ismaeel A, Griffis RB, Weems S. Effects of vitamin D supplementation on muscle strength in athletes: a systematic review. J Strength Cond Res. 2017;31(2):566–74.
25. Barbagallo M, Veronese N, Di Prazza A, Pollicino F, Carruba L, La Carrubba A, et al. Effect of calcifediol on physical performance and muscle strength parameters: a systematic review and meta-analysis. Nutrients. 2022;14(9):1860.
26. Romeu Montenegro K, Carlessi R, Cruzat V, Newsholme P. Effects of vitamin D on primary human skeletal muscle cell proliferation, differentiation, protein synthesis and bioenergetics. J Steroid Biochem Mol Biol. 2019;193:105423.
27. Chang MC, Choo YJ. Effects of whey protein, leucine, and vitamin D supplementation in patients with sarcopenia: a systematic review and meta-analysis. Nutrients. 2023;15(3):521.
28. Zhang JL, Poon CC, Wong MS, Li WX, Guo YX, Zhang Y. Vitamin D supplementation improves handgrip strength in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. Front Endocrinol. 2022;13:863448.
29. Prokopidis K, Giannos P, Katsikas Triantafyllidis K, Kechagias KS, Mesinovic J, Witard OC, et al. Effect of vitamin D monotherapy on indices of sarcopenia in community-dwelling older adults: a systematic review and meta-analysis. J Cachexia Sarcopenia Muscle. 2022;13(3):1642–52.
30. Schakman O, Kalista S, Barbe C, Loumaye A, Thissen JP. Glucocorticoid-induced skeletal muscle atrophy. Int J Biochem Cell Biol. 2013;45(10):2163–72.
31. Alemo Munters L, Dastmalchi M, Katz A, Esbjornsson M, Loell I, Hanna B, et al. Improved exercise performance and increased aerobic capacity after endurance training of patients with stable polymyositis and dermatomyositis. Arthritis Res Ther. 2013;15(4):R83.
32. Meex RCR, Blaak EE, van Loon LJC. Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2019;20(9):1205–17.
33. Nemec M, Vernerova L, Laiferova N, Balazova M, Vokurkova M, Kurdiova T, et al. Altered dynamics of lipid metabolism in muscle cells from patients with idiopathic inflammatory myopathy is ameliorated by 6 months of training. J Physiol. 2021;599(1):207–29.
34. Chang E, Kim Y. Vitamin D ameliorates fat accumulation with AMPK/SIRT1 Activity in C2C12 skeletal muscle cells. Nutrients. 2019;11(11):2806.
35. Ashcroft SP, Bass JJ, Kazi AA, Atherton PJ, Philp A. The vitamin D receptor regulates mitochondrial function in C2C12 myoblasts. Am J Physiol Cell Physiol. 2020;318(3):C536–41.
36. Spiritovic M, Hermankova B, Oreska S, Storkanova H, Ruzickova O, Vernerova L, et al. The effect of a 24-week training focused on activities of daily living, muscle strengthening, and stability in idiopathic inflammatory myopathies: a monocentric controlled study with follow-up. Arthritis Res Ther. 2021;23(1):173.
37. Lundberg IE, Tjarnlund A, Bottai M, Werth VP, Pilkington C, Visser M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Ann Rheum Dis. 2017;76(12):1955–64.
38. Allenbach Y, Mammen AL, Benveniste O, Stenzel W, Immune-Mediated Necrotizing Myopathies Working G. 224th ENMC International Workshop: clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14–16 October 2016. Neuromuscular disorders : NMD. 2018;28(1):87–99.
39. Isenberg DA, Allen E, Farewell V, Ehrenstein MR, Hanna MG, Lundberg IE, et al. International consensus outcome measures for patients with idiopathic inflammatory myopathies. Development and initial validation of myositis activity and damage indices in patients with adult onset disease. Rheumatology. 2004;43(1):49–54.
40. Rider LG, Werth VP, Huber AM, Alexanderson H, Rao AP, Ruperto N, et al. Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: Physician and Patient/Parent Global Activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire (HAQ)/Childhood Health Assessment Questionnaire (C-HAQ), Childhood Myositis Assessment Scale (CMAS), Myositis Disease Activity Assessment Tool (MDAAT), Disease Activity Score (DAS), Short Form 36 (SF-36), Child Health Questionnaire (CHQ), physician global damage, Myositis Damage Index (MDI), Quantitative Muscle Testing (QMT), Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis care, research. 2011;63 Suppl 11(0 11):S118-57.
41. Ukropcova B, McNeil M, Sereda O, de Jonge L, Xie H, Bray GA, et al. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. J Clin Investig. 2005;115(7):1934–41.
42. Brown BL, Hendrix SB. Partial Correlation Coefficients†. In Wiley StatsRef: Statistics Reference Online (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggeri and J.L. Teugels). 2014. https://doi.org/10.1002/9781118445112.stat06488.
43. Earthman CP, Beckman LM, Masodkar K, Sibley SD. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. Int J Obes. 2012;36(3):387–96.
44. Lagunova Z, Porojnicu AC, Vieth R, Lindberg FA, Hexeberg S, Moan J. Serum 25-hydroxyvitamin D is a predictor of serum 1,25-dihydroxyvitamin D in overweight and obese patients. J Nutr. 2011;141(1):112–7.
45. Skversky AL, Kumar J, Abramowitz MK, Kaskel FJ, Melamed ML. Association of glucocorticoid use and low 25-hydroxyvitamin D levels: results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. J Clin Endocrinol Metab. 2011;96(12):3838–45.
46. Kruit A, Zanen P. The association between vitamin D and C-reactive protein levels in patients with inflammatory and non-inflammatory diseases. Clin Biochem. 2016;49(7–8):534–7.
47. Baeke F, Korf H, Overbergh L, van Etten E, Verstuyf A, Gysemans C, et al. Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. J Steroid Biochem Mol Biol. 2010;121(1–2):221–7.
48. Calton EK, Keane KN, Newsholme P, Soares MJ. The impact of vitamin D levels on inflammatory status: a systematic review of immune cell studies. PLoS ONE. 2015;10(11):e0141770.
49. Holick MF, Mazzei L, Garcia Menendez S, Martin Gimenez VM, Al Anouti F, Manucha W. Genomic or non-genomic? A question about the pleiotropic roles of vitamin D in inflammatory-based diseases. Nutrients. 2023;15(3):767.
50. Alexanderson H. Exercise in myositis. Current treatment options in rheumatology. 2018;4(4):289–98.
51. Bertolucci F, Neri R, Dalise S, Venturi M, Rossi B, Chisari C. Abnormal lactate levels in patients with polymyositis and dermatomyositis: the

benefits of a specific rehabilitative program. Eur J Phys Rehabil Med. 2014;50(2):161–9.
52. Zhang J, Cao ZB. Exercise: a possibly effective way to improve vitamin D nutritional status. Nutrients. 2022;14(13):2652.
53. Marantes I, Achenbach SJ, Atkinson EJ, Khosla S, Melton LJ 3rd, Amin S. Is vitamin D a determinant of muscle mass and strength? Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2011;26(12):2860–71.
54. Vernerova L, Horvathova V, Kropackova T, Vokurkova M, Klein M, Tomcik M, et al. Alterations in activin A-myostatin-follistatin system associate with disease activity in inflammatory myopathies. Rheumatology. 2020;59(9):2491–501.
55. Mahoudeau A, Anquetil C, Tawara N, Khademian H, Amelin D, Bolko L, et al. Myostatin in idiopathic inflammatory myopathies: Serum assessment and disease activity. Neuropathol Appl Neurobiol. 2023;49(1):e12849.
56. Kropackova T, Vernerova L, Storkanova H, Horvathova V, Vokurkova M, Klein M, et al. Clusterin is upregulated in serum and muscle tissue in idiopathic inflammatory myopathies and associates with clinical disease activity and cytokine profile. Clin Exp Rheumatol. 2021;39(5):1021–32.
57. Burks TN, Cohn RD. Role of TGF-beta signaling in inherited and acquired myopathies. Skeletal muscle. 2011;1(1):19.
58. Girardi F, Taleb A, Ebrahimi M, Datye A, Gamage DG, Peccate C, et al. TGF-beta signaling curbs cell fusion and muscle regeneration. Nat Commun. 2021;12(1):750.
59. Ismaeel A, Kim JS, Kirk JS, Smith RS, Bohannon WT, Koutakis P. Role of Transforming Growth Factor-β in Skeletal Muscle Fibrosis: A Review. Int J Mol Sci. 2019;20(10):2446. https://doi.org/10.3390/ijms20102446.
60. Latham CM, Brightwell CR, Keeble AR, Munson BD, Thomas NT, Zagzoog AM, et al. Vitamin D promotes skeletal muscle regeneration and mitochondrial health. Front Physiol. 2021;12:660498.
61. Sinha A, Hollingsworth KG, Ball S, Cheetham T. Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. J Clin Endocrinol Metab. 2013;98(3):E509–13.
62. Ryan ZC, Craig TA, Folmes CD, Wang X, Lanza IR, Schaible NS, et al. 1alpha,25-Dihydroxyvitamin D3 regulates mitochondrial oxygen consumption and dynamics in human skeletal muscle cells. J Biol Chem. 2016;291(3):1514–28.
63. Dibaba DT. Effect of vitamin D supplementation on serum lipid profiles: a systematic review and meta-analysis. Nutr Rev. 2019;77(12):890–902.
64. Wang Y, Si S, Liu J, Wang Z, Jia H, Feng K, et al. The associations of serum lipids with vitamin D status. PLoS ONE. 2016;11(10):e0165157.
65. Surdu AM, Pînzariu O, Ciobanu DM, Negru AG, Căinap SS, Lazea C, Iacob D, Săraci G, Tirinescu D, Borda IM, Cismaru G. Vitamin D and Its Role in the Lipid Metabolism and the Development of Atherosclerosis. Biomedicines. 2021;9(2):172. https://doi.org/10.3390/biomedicines9020172.
66. Parsanathan R, Jain SK. Glutathione deficiency induces epigenetic alterations of vitamin D metabolism genes in the livers of high-fat diet-fed obese mice. Sci Rep. 2019;9(1):14784.

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