ORIGINAL ARTICLE

Vitamin D supplementation attenuates oxidative stress in paraspinal skeletal muscles in patients with low back pain

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Abstract

Purpose The aim of the study was to evaluate markers of oxidative stress and vitamin D receptor in paraspinal muscles in low back pain patients with vitamin D deficiency, with normal level of vitamin D, and after 5 weeks of vitamin D supplementation.

Methods Patients were divided into three groups: supplemented (SUP) with vitamin D, placebo with normal concentration of vitamin D (SUF), and the placebo group with vitamin D deficiency (DEF). The concentration of serum vitamin D was measured before and after the supplementation with vitamin D (3200 IU/ day for 5 weeks). Markers of lipid and protein peroxidation, the activity of antioxidant enzymes, and protein content of vitamin D receptor was determined in multifidus muscle of patients.

Results Vitamin D supplementation increased serum level of $25(OH)D_3$ ($p < 0.001$). In paraspinal muscle level of 8-isoprostanes and protein carbonyls was higher in DEF group as compared to the SUP group ($p < 0.05$). Antioxidant enzyme activity and vitamin D receptor in paraspinal muscle altered between the groups with different serum vitamin D concentration. The cytosolic superoxide dismutase and glutathione peroxidase activities were significantly higher in DEF group as compared to the SUP group ($p < 0.05$).

Conclusions An attenuation of markers of free radical damage of lipids and proteins was observed in participants supplemented with Vitamin D. Antioxidant enzyme activities in skeletal muscle differ among patients with different serum vitamin D concentration. Monitoring oxidative stress and VDR protein content might be useful for future studies on the mechanism(s) of vitamin D action in muscle.

Keywords Vitamin D · VDR · Skeletal muscle · Oxidative stress · Low back pain

Abbreviations

ANOVA	Analysis of variance
CAT	Catalase
Cu/ZnSOD	Copper/zinc-dependent dismutase
GPx	Glutathione peroxidase
IU	International unit
H_2O_2	Hydrogen peroxide
LBP	Low back pain
LSD	Least significant difference
MnSOD	Manganese-dependent superoxide dismutase
PLIF	Posterior lumbar interbody fusion

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ROS	Reactive oxygen species
SEM	Standard error
SOD	Superoxide dismutase
VAS	Visual Analog Scale
VDR	Vitamin D receptor

Introduction

Chronic low back pain (LBP) is becoming one of the most common musculoskeletal disorders in modern society and reduces spontaneous activity of patients. LBP reduces the spontaneous activity of patients, also requires expensive treatment, which causes a major economic burden for individuals, families, communities and the national economy (Murray et al. 2013). LBP worsens with age and can become an chronic illness. The greatest likelihood of LBP occurs in the 5th–6th decade of life (Shmagel et al. 2016). Pain sensation may affect muscle structure by compromising muscle function (Falla and Farina 2008). LBP affects paraspinal muscles, coordination of which is required to ensure optimum control of the dynamics of the intervertebral and postural stability during voluntary movements of the torso undertaken at spontaneous physical activity (Reeves et al. 2007). Comprised function of paraspinal muscles in LBP leads to macroscopic muscle degeneration of the multifidus muscle (Freeman et al. 2010). Several studies have shown lowered cross-sectional area in this muscle in patients with chronic LBP when compared to healthy controls, indicating the multifidus atrophy (Danneels et al. 2000).

The mechanisms underlying muscle atrophy are largely unknown. Age-dependent atrophy is associated with increases in oxidative damage to DNA, lipids, and protein in human skeletal muscle (Mecocci et al. 1999). In addition, disuse atrophy has been shown to promote an increase in reactive oxygen species (ROS) generation in skeletal muscle (Muller et al. 2006). The key difference between oxidative stress associated with disuse atrophy and for example exercise-induced oxidative stress is the duration of the exposure. Oxidative stress following exercise is transient in nature, while disuse atrophy results in chronic exposure to elevated ROS (Wiggs 2015). Therefore, it is very likely that atrophy of multifidus muscle in LBP patients may be associated with prolonged oxidative stress due to immobilization of these muscles.

The active form of vitamin D, 1,25-dihydroxy vitamin D, is a major calcium-regulating hormone that is essential for maintenance of calcium and bone homeostasis. Vitamin D acts through binding to the vitamin D receptor (VDR) that belongs to the nuclear receptor superfamily (Dusso et al. 2005). In situ studies on human skeletal muscles confirm the presence of VDR in this tissue (Bischoff et al. 2001) and confirm its action in vitamin D uptake in muscle

(Girgis et al. 2014). Moreover, it has been presented that VDR$^{-/-}$ mice exhibit abnormal skeletal muscle development (Endo et al. 2003).

Vitamin D insufficiency has been shown to be associated with increased falling tendency in adults, lower muscle strength, abnormal muscle physiology, and muscle mitochondrial defects in humans and animals (Bouillon and Verstuyf 2013), and evolving type II muscle fiber atrophy (Girgis et al. 2013). The latest study shows that vitamin-D supplementation reverses those changes (Dawson-Hughes et al. 1997), and can improve the pain and disability in patients with chronic LBP (Ghai et al. 2017). Sorensen and coworkers (Sorensen et al. 1979) have observed an increase in the relative number and cross-sectional area of fast-twitch muscle fibers in elderly osteoporotic women treated with vitamin D for 3 months. The mechanism(s) of vitamin D influence on atrophy is not fully understood. Studies show that to achieve an effect on the skeletal muscle, vitamin D needs to be bound to the VDR receptor (Ceglia 2009). Some studies imply, that vitamin D has an antioxidant potential (Bhat and Ismail 2015). Another study shows that hypovitaminosis of vitamin D may cause the diffuse pain in bones and muscles, weakness, and paresthesia (Johansen et al. 2013), or play a role in the development of modic changes via the increased susceptibility to inflammation in the vertebral end plates (Albert et al. 2008). Although, it has been reported that rats treated with vitamin D show reduced tissue damage and attenuated oxidative stress after exhaustive exercise (Ke et al. 2016), there is still lack of direct results showing possible connection between vitamin D, disuse and oxidative stress in human skeletal muscle.

The purpose of this study was to investigate oxidative stress and VDR protein content in paraspinal muscles in LBP patients with vitamin D deficiency, normal level of vitamin D, and after 5 weeks of vitamin D supplementation. We examined the effect of vitamin D supplementation on lipid and protein peroxidation, VDR protein content and antioxidant enzyme activity in paraspinal muscle.

Materials and methods

LBP patients

Subjects

The study population was selected from among consecutive patients qualified for lumbar spine surgery utilizing static or dynamic implants (posterior lumbar interbody fusion—PLIF). All patients were classified by medical and MRI examination. Inclusion criteria for PLIF surgery were: discopathy, segmental spinal pathology, segmental instability, etc. All patients had experienced LBP for at least 2 years.

All patients were Caucasian. Pregnant or lactating women were not included. All patients were informed of the surgical techniques, and informed consent was obtained. The study was approved by the local institutional Bioethical Committee in Gdansk (No. NKBBN/120/2012) and conformed to the Declaration of Helsinki guidelines. Nineteen women and nineteen men participated in the study (48.2 ± 9.9 years of age, BMI 29.9 ± 3.7).

Study design

First, patients were randomly assigned into two groups: supplemented with 3 200 IU (SUP) of $25(OH)D_3$/day for 5 weeks (Vigantol, Merck), and placebo group supplemented with vegetable oil. Patients stayed blinded to the content of the vial which they were given. In addition, patients were instructed not to change their regular diet. Blood samples were taken at the baseline and after 5 weeks of supplementation for the determination of serum vitamin D concentration. Then, based on the baseline serum vitamin D level, placebo group was divided into placebo group with normal concentration of vitamin D (SUF) with $25(OH)D_3$ level above 21 ng/mL (above 50 nmol/L), and the placebo group with vitamin D deficiency (DEF) with $25(OH)D_3$ serum level between 10 and 20 ng/mL (30–49 nmol/L). The choice of 50 nmol/L (20 ng/mL) as the border line was made according to the vitamin D deficiency level defined by Endocrine Society (Holick et al. 2011). There were no significant differences in age and BMI between the groups (Table 1). LBP intensity was measured before and after the supplementation by Visual Analog Scale (VAS, 100 mm horizontal scale, 0 = no pain; 100 = worst imaginable pain) (Revill et al. 1976).

Blood analysis and collection

Blood samples were taken from the antecubital vein into the vacutainer tubes with silica clot activator, at the baseline and after 5 weeks of supplementation. The samples were centrifuged at 2000 rpm for 10 min at 4 °C. The separated serum samples were frozen and kept at − 80 °C until later analysis. The tubes containing the serum samples were number-coded

to blind the laboratory personnel regarding treatment group and the sequence of sample collection.

Serum $25(OH)D_3$ level was determined before and after supplementation by enzyme immunoassay method using 25-OH Vitamin D total ELISA kit (DE1971, Demeditec Diagnostics, Germany), according to the manufacturer's instructions. The intra-assay coefficients of variability (CVs) and inter-assay CVs reported by the manufacturer were 2–8 and 4–9%, respectively.

Human muscle sample

After 5 weeks of supplementation, multifidus muscle samples were obtained from all the patients during PLIF surgery. All muscle samples were taken between the tenth thoracic and fifth lumbar vertebrae. Multifidus muscle specimens of 40–150 mg were collected and immediately frozen at − 80 °C.

Muscle homogenization

The tissue samples were reconstituted in ice-cold lysis buffer containing 50 mM of TRIS HCl, 1 mM of EDTA, 1.15% of KCl, 0.5 mM of DTT, 0.005% BHT, 0.2% protease inhibitor cocktail (Sigma P834) and phosphatase inhibitor tablets PhosSTOP (Roche, Italy). Final homogenate concentration was 4%. Samples were centrifuged at 750g for 10 min at 4 °C and the supernatant was divided into serial aliquots for enzyme activity, western blot and markers of oxidative stress. The tubes containing homogenates were number-coded to blind the laboratory personnel regarding treatment group and the sequence of sample collection. Protein concentration was determined using the Bradford protein assay (Sigma B6916) according to the manufacturer's instructions.

Assays

Vitamin D receptor in muscle homogenates was determined using immunoassay kits ELISA Kit for Vitamin D Receptor (VDR) (SEA475Hu, Cloud-Clone Corporation, USA). A biomarker of lipid peroxidation, skeletal muscle 8-isoprostanes content was determined with 8-isoprostane ELISA

Table 1 The characteristics of non- and supplemented LBP patients

Variable	All subjects ($n = 38$)	DEF group ($n = 14$)	SUF group ($n = 10$)	SUP group ($n = 14$)
Age (years)	48.2 (± 9.9)	49.8 (± 9.6)	45.8 (± 9.8)	48.2 (± 10.6)
BMI (kg/m^2)	29.9 (± 3.7)	30.3 (± 3.3)	27.9 (± 2.8)	28.5 (± 4.6)
Sex				
Male	19	8	5	6
Female	19	6	5	8

Values are means ($\pm$ SD)

BMI body mass index

Kit (516351, Cayman Chemicals, USA). The protein oxidation in skeletal muscle, expressed as carbonyl groups, was measured by an enzyme-like immunosorbent assay BioCell Protein Carbonyl Assay Kit (Biocell Corporation LTD, New Zealand). All of the ELISA were performed according to the manufacturer's instructions.

Antioxidant enzymes activities

Antioxidant enzymes activities were measured in muscle homogenates. Glutathione peroxidase (GPx) activity was determined with Cayman GPx Assay Kit (703102, Cayman Chemicals, USA) according to the manufacturer's instructions.

Catalase (CAT)

Muscle catalase activity was determined in multifidus muscles by measuring the kinetic decomposition of H_2O_2, according to Aebi (Aebi 1984). Briefly, 20 µL of supernatant from the 750 g of spin was added to a cuvette containing 970 µl of phosphate buffer (50 mM with 5 mM of EDTA, and 0.05% Triton X-100 at pH 7.4). 10 µL of 1M H_2O_2 was added to the cuvette and mixed to initiate the reaction. Absorbance was measured at 240 nm for 1 min at 25 °C. All of the samples were analyzed in duplicate. Catalase activity was expressed as micromoles per minute per milligram of protein.

Superoxide dismutase (SOD)

SOD activity was determined in multifidus muscle by measuring the kinetic consumption of O_2^{-} by superoxide dismutase in a competitive reaction with cytochrome c, as described by Flohe and Otting (1984). Briefly, 10 µL of supernatant after 750 g centrifugation was added to a cuvette containing 980 µl of medium (50 mM of phosphate buffer, 0.1 mM of EDTA, pH 7.8, with partially acetylated cytochrome c (25 mg/100 mL) and xanthine 0.5 µM). Ten microliters of xanthine oxidase (0.2 U/mL) was added to initiate the reaction, and absorption at 550 nm was measured for 3 min at 30 °C. One unit of SOD activity was defined as the amount of enzyme which caused a 50% inhibition in cytochrome c reduction. In a separate cuvette, manganese-dependent dismutase (MnSOD) activity was measured on the same sample analyzed under identical conditions with the addition of 10 µL of 0.2 M KCN (prepared fresh at pH 8.5–9.5). Copper/Zinc-dependent dismutase (Cu/ZnSOD) activity was calculated by subtracting MnSOD activity from total SOD activity. All of the samples were analyzed in duplicate. The SOD activities were expressed as units per milligram protein.

All kinetics were measured in a temperature-controlled Cecil Super Aquarius CE 9200 spectrophotometer.

Statistical calculations

Statistical analyses were performed using a software package (Statistica v. 12.0, StatSoft Inc., Tulsa, OK, USA). The results are expressed as mean ± SEM. The differences between the means before and after time points, inside groups, were tested using the paired Student's t test. The differences between groups at the same time point were tested using one-way ANOVA, if a difference was detected in the ANOVA model, the significant differences were determined using the least significant difference (LSD) post hoc test. Associations among measured parameters were analyzed using Pearson's linear regression model. The results were statistically significant when $p \leq 0.05$.

Results

Serum 25(OH)D_3 concentration was significantly different between DEF and other groups before and after the supplementation. In DEF group, it was 38.4 ± 2.8 and 38.2 ± 2.1 nmol/L before and after supplementation with placebo, respectively. In SUF group, it was 74.1 ± 3.0 and 67.7 ± 4.2 nmol/L before and after supplementation with placebo, respectively. In SUP group, serum 25(OH)D_3 concentration significantly increased from 52.8 ± 3.0 to 86.6 ± 3.2 nmol/L after supplementation with vitamin D ($p < 0.001$; Fig. 1).

Mean VAS before and after the supplementation did not differ significantly between the groups (Table 2). However, in the SUP group we observed substantial reduction in pain intensity, which was 15.5 mm in VAS. In SUF and DEF
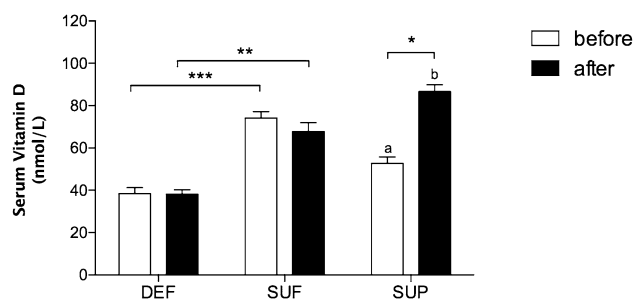

Fig. 1 The concentration of vitamin D in serum of LBP patients. Results were expressed as mean ± SEM. DEF ($n = 14$), SUF ($n = 10$), SUP ($n = 14$). *$p < 0.001$—difference between the means before and after time points, inside groups. **$p < 0.001$—difference between the indicated results/mean. ***$p < 0.001$—difference between the indicated results/mean. [a]$p < 0.001$—difference between the indicated results/mean and SUF before. [b]$p < 0.001$—difference between the indicated results/mean and DEF after

Table 2 VAS score before and after the supplementation in LBP patients

|	Before	After	p
DEF	47.5 (± 5.9)	47.2 (± 7.0)	N.S
SUF	56.2 (± 5.5)	48.5 (± 9.1)	N.S
SUP	53.1 (± 6.3)	37.4 (± 6.5)	N.S

Values are means (± SEM)

groups, pain intensity was reduced by 7.7 mm and 0.2 mm in VAS, respectively.

The level of 8-isoprostanes in the multifidus muscle was significantly higher in DEF group as compared to the SUP group ($p < 0.05$) and it was 39.9 ± 7.5 and 20.8 ± 2.4 ng/mg. In SUF group, the concentration of 8-isoprostanes was 26.1 ± 4.0 ng/mg (Fig. 2a). Protein carbonyl groups concentration in muscle was the highest in DEF group and it measured 0.83 ± 0.04 nmol/mg. It was lower in SUF and SUP groups (0.77 ± 0.04 and 0.72 ± 0.04, respectively) and the significant difference between DEF and SUP groups was observed ($p < 0.05$) (Fig. 2b).

The protein content of VDR in muscle was 65.6 ± 7.9 ng/mg in SUF group. In SUP group VDR was 63.1 ± 5.7 ng/mg. The lowest VDR protein level was observed in DEF group and it amounted 46.9 ± 5.9 ng/mg (Fig. 3). The difference in protein content of VDR between groups did not reach the significance, however, positive correlation between increased VDR level and increased concentrations of vitamin D in serum of supplemented patients was observed ($r = 0.36$, $p < 0.05$).

Total SOD activity also did not differ between the groups. SOD activity in DEF group was 31.32 ± 3.383 U/mg, in SUF group 28.25 ± 1.66 U/mg, and in SUP group 26.60 ± 1.99 U/mg (Fig. 4a). Mitochondrial superoxide
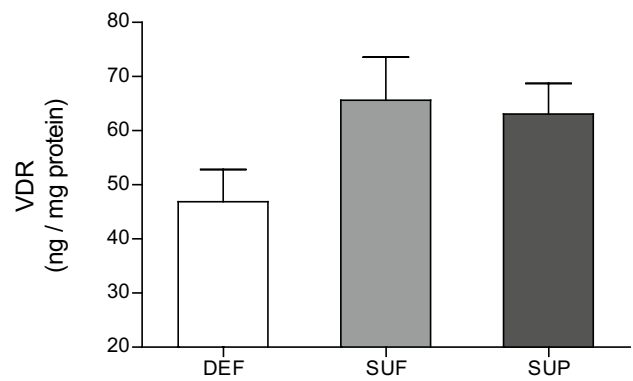
Fig. 3 The level of VDR in skeletal muscle of LBP patents. Results were expressed as mean ± SEM. DEF ($n = 14$), SUF ($n = 10$), SUP ($n = 14$)

dismutase MnSOD also did not show significant difference between the groups. The activity of MnSOD was 11.21 ± 0.91 U/mg in DEF group, 10.93 ± 1.63 U/mg in SUF group and 13.02 ± 1.09 U/mg in SUP group. Cu/ZnSOD activity in muscle was 20.12 ± 2.81 U/mg, 17.31 ± 1.53 U/mg and 12.41 ± 1.63 U/mg in muscle of DEF, SUF and SUP groups, respectively. The activity of Cu/ZnSOD was significantly lower in SUP group as compared to DEF group ($p < 0.05$; Fig. 4b).

The activity of GPx in muscle was significantly lower in SUP group when compared with SUF and DEF groups ($p < 0.005$). GPx activity was 659.7 ± 45.5 nmol/min/mg in SUP group, 1114.0 ± 108.8 nmol/min/mg in SUF group and 1092.0 ± 107.9 nmol/min/mg in DEF group (Fig. 4c).

The activity of CAT in muscle did not differ significantly between the groups and was 55.39 ± 4.09, 53.21 ± 7.51 and 51.94 ± 3.72 µmol/min/mg in DEF, SUF and SUP groups, respectively (Fig. 4d).
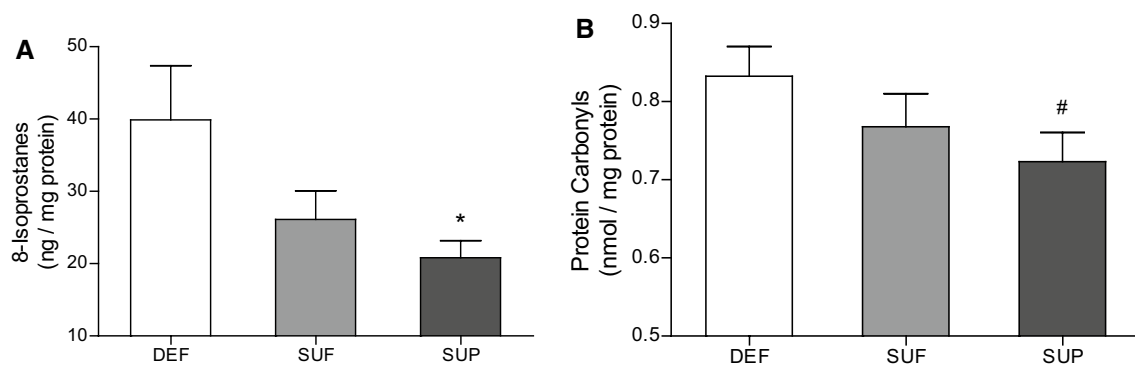
Fig. 2 The level of free radical damage in muscles of LBP patients. **a** Marker of lipid peroxidation- 8-izoprostanes. **b** Marker of protein peroxidation- carbonyl's group. Results were expressed as mean ± SEM. DEF ($n = 14$), SUF ($n = 10$), SUP ($n = 14$). *$p < 0.05$—difference between DEF and SUP groups, #$p < 0.05$—difference between DEF and SUP groups
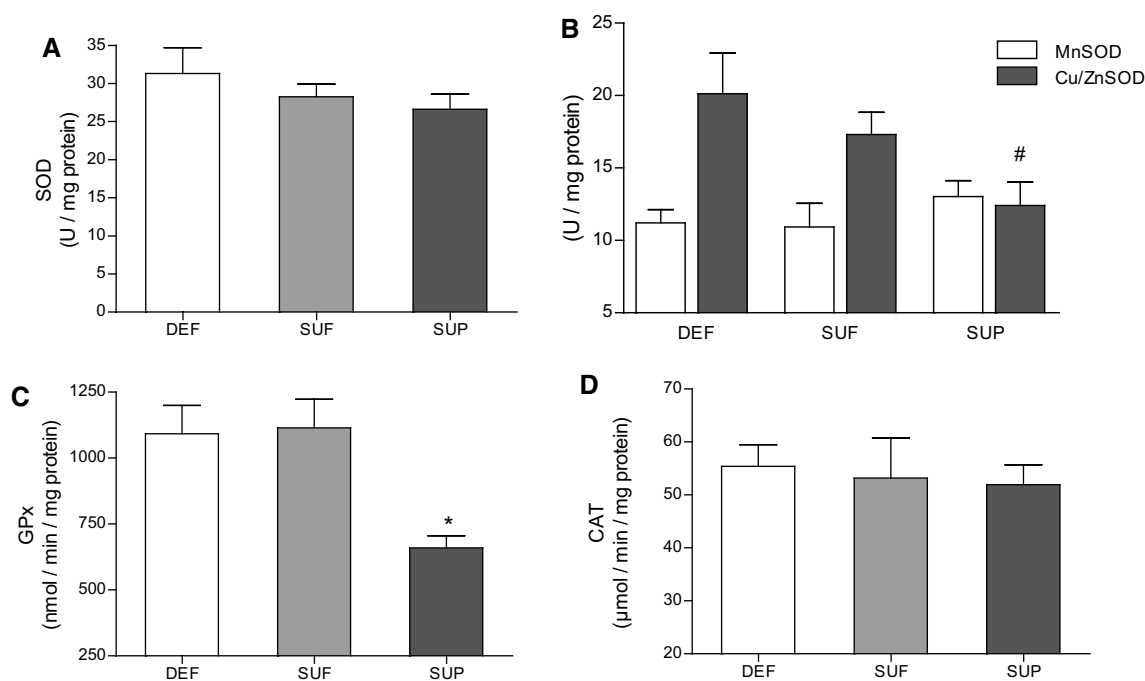
Fig. 4 The enzyme activities in skeletal muscle of LBP patients. **a** Total SOD activity, **b** Cu/ZnSOD and MnSOD activities, **c** GPx activity and (**d**) CAT activity. Results were expressed as mean ± SEM. For **a, b** plot: DEF ($n=12$), SUF ($n=9$), SUP ($n=12$) and for **c, d** plot: DEF ($n=14$), SUF ($n=10$), SUP ($n=14$). *$p < 0.005$—difference between the indicated result/mean and SUP. #$p < 0.05$—difference between the indicated result/mean and corresponding SUP

Discussion

The main finding of the present study was that LBP patients deficient in serum $25(OH)D_3$ showed higher oxidative stress and increased antioxidant enzyme activity (GPx and Cu/ZnSOD) in skeletal muscle. What is more, all patients with LBP had elevated level of markers of lipid and protein peroxidation, 8-isoprostanes and carbonyl groups, respectively, in muscle. In addition, the study showed that 5 weeks of vitamin D_3 supplementation with 3200 IU raised serum vitamin D by an average of 53 nmol/L in SUP group and placed the level of serum $25(OH)D_3$ above 85 nmol/L—the concentration indicated as optimal for adults by Endocrine Society (Holick et al. 2011) .

Previous studies showed that vitamin D supplementation with 20 000 IU alternating days for 12 weeks in vitamin D deficient individuals resulted in serum vitamin D average rise from 8.8 to 113.8 nmol/L and it induced the improved mitochondrial oxidative function (Sinha et al. 2013). In addition, the supplementation with 6000 IU for 12 weeks was sufficient for indoor athletes with vitamin D deficiency at baseline to reach an optimal vitamin D concentration (Flueck et al. 2016). On the other hand, there were reports of inefficient supplementation wherein over 80% of the participants' vitamin D level remained insufficient after supplementation with 2000 IU of the vitamin daily over a two-week period.

In addition, a supplementation of 800 IU daily over a duration of 1 year where was no sufficient in over 75% of the patients, who remained deficient in vitamin D (Bauman et al. 2005). A study on quantitative relation between steady-state cholecalciferol input and the resulting serum vitamin D concentration showed that a dose of 10 000 IU daily over five months was a safe intervention (Heaney et al. 2003). Therefore, the Endocrine Society set their tolerable upper limit of 10 000 IU daily (Holick et al. 2011). In the present study, we showed that 3200 IU of cholecalciferol over 5 weeks was enough to reach an optimal serum vitamin D level.

The latest study showed that vitamin-D supplementation in deficient LBP patients may lead to improvement in pain intensity and functional ability (Ghai et al. 2017). In our study patients supplemented with vitamin D experienced reduction in pain intensity by 15.5 mm in VAS, however, changes were not significant. LBP etiology is known to be multifactorial, including the loss of lumbar spinal stability through not sufficient activation of the deep lumbar stabilizing muscles such as multifidus (Danneels et al. 2002). Pain experience in LBP might reduce lumbar muscle activity due to pain-related nerve inhibition to prevent further tissue damage (Rkain et al. 2013). Decreased activation of the multifidus muscle in chronic LBP is a major cause of progressive atrophy of this muscle. This might be the reason for increased lipid and protein peroxidation in paraspinal

muscles in LBP patients as compared to healthy human muscle. Vitamin D deficiency seems to escalate these changes since we have observed the highest level of both 8-isoprostanes and protein carbonyls in the DEF group. Moreover, the group after supplementation presented the lowest free radical muscle damage. Our observations are consistent with several studies showing that vitamin D inhibits oxidative stress in various tissues (Nakai et al. 2014). Interestingly, the SUF group with the highest vitamin D level at baseline and almost as high as SUP group at the end of the intervention, had slightly higher protein carbonyls and 8-isoprostanes level than SUP group. This might suggest that the first period of vitamin D intervention has the most protective impact on muscle tissue and that vitamin D acts the most effective in pathological conditions. This observation might explain previous findings where in healthy elderly people, muscle strength declined with age, which was not prevented by vitamin D supplementation (Sato et al. 2005). In addition, another studies showed that the presence of vitamin D in optimal concentration might have positive preventive effects against abnormal muscle injury and performance (Foo et al. 2009), whereas induction of the skeletal muscle physiological response required higher amounts of vitamin D levels to achieve significant muscle performance (Stockton et al. 2011).

To our knowledge this is the first study showing the influence of vitamin D shifts on ROS generations in human skeletal muscles in pathological condition. We observed lower level of markers of lipid and protein peroxidation in the SUP group as compared to the DEF group, indicating lower free radical damage in the group after supplementation. In addition, there was a negative correlation between serum vitamin D concentration and muscular 8-isoprostane levels in all patients, which may indicate a preservative effect of vitamin D on skeletal muscle. This observation is consistent with results from a study using a diet-induced vitamin D-deficient rat model, which demonstrated mild oxidative stress in the vitamin D deficient rat muscle and an increase in protein oxidation as reflected by increased protein carbonyls in skeletal muscle. Moreover, the oxidative stress induced increase in lipid and protein peroxidation was altered upon treatment with $1,25(OH)_2D_3$ in the C2C12 murine muscle cell line (Bhat and Ismail 2015). Overall, our results support that there is an association of low levels of vitamin D and increased oxidative damage in human skeletal muscle.

Furthermore, we determined the impact of vitamin D on antioxidant enzyme activity in human paraspinal muscle. Oxidative stress induces higher production of hydrogen peroxide (H_2O_2) in mitochondria and/or cytoplasm and activates antioxidant enzymes, particularly CAT and GPx. We observed a decrease of GPx activity in group after supplementation with vitamin D. These data correspond with previous results demonstrating increased GPx activity in

vitamin D-deficient muscle in rats (Bhat and Ismail 2015). However, we did not find difference in CAT activity between groups. The lack of consistence in activity of both enzymes might be a consequence of different Km values for H_2O_2. CAT has a much higher affinity with H_2O_2 at greater concentrations than GPx (Singh et al. 2008), which makes GPx more sensitive to low grade changes.

SOD catalyses the dismutation of superoxide into oxygen and hydrogen peroxide and is known to be the first line of antioxidant defense in living cells. In this study, we did not observe the changes in SOD total activity, but only in the activity of cytosolic isoform of this enzyme—Cu/ZnSOD which was significantly higher in the group with vitamin D deficiency. Higher activity of Cu/ZnSOD in LBP patients in the DEF group corresponded with the higher oxidative damage in this group, and might suggest a compensatory role for Cu/ZnSOD during chronic states of increased oxidative stress. An increase in the activity of antioxidant enzymes is widely recognized to occur in response to increased ROS generation in muscle tissue as the adaptation to increased ROS activity (McArdle et al. 2001). In addition, the lack of changes in MnSOD activity might suggest that oxidative stress is generated from cytosolic sources. However, the results of lowest Cu/ZnSOD activity in SUP group are not consistent with previous studies showing the rise in Cu/ZnSOD activity in $1,25(OH)_2D_3$-treated muscle cells as well as vitamin D supplemented rat muscle suggesting the role of SOD in anti-oxidant effect of vitamin D (Bhat and Ismail 2015).

The mechanism for vitamin D-mediated changes in skeletal muscle is not fully understood. Vitamin D acts via specific biding to an intracellular receptor VDR, interacting with specific nucleotide sequences of over 60 target genes (Zhang et al. 2017). Therefore, VDR has become a field of interest of many studies. Our question of interest was whether muscular VDR expression differed between the groups. The highest VDR content was observed in the SUF group and lowest in the DEF group, which may suggest that the expression of VDR in skeletal muscle is dependent on serum vitamin D level. There was a trend toward higher VDR level in skeletal muscle in supplemented group and there was a positive correlation between the serum vitamin D concentration and the content of VDR in skeletal muscle of SUP group patients. The tendency of higher VDR level in SUF when compared to SUP group may suggest that the VDR expression is dependent on time of the exposition of muscular tissue to proper serum vitamin D concentration. The lower content of VDR in patients with vitamin D deficiency goes along with higher lipid and protein peroxidation as well as increased activity of Cu/ZnSOD and GPx that allows us to suggest that lower level of VDR in skeletal muscle may enhance ROS generation by cytoplasm and/or mitochondria. However, further studies are needed to find

the required time of exposition to optimal serum vitamin D concentration to achieve the highest VDR expression and to observe changes in skeletal muscle oxidative metabolism, and to find the mechanism triggered by vitamin D in skeletal muscle. The study has limitation: we did not monitor diet itself and other components of the diet that are proven to affect antioxidant status.

Conclusion

In summary, supplementation with vitamin D significantly enhanced the level of vitamin D in serum of LBP patients. Vitamin D deficiency increases Cu/ZnSOD and GPx activities in paraspinal muscle and leads to greater lipid and protein peroxidation. Our data suggest that higher ROS generation in skeletal muscle is related to serum concentration of vitamin D. The exact source of ROS generation in skeletal muscle of LBP patients is not fully elucidated but likely involves both cytosol and mitochondria. Supplementation with vitamin D to the sufficient serum vitamin D concentration in LBP patients decreases oxidative stress in skeletal muscle, and might have a beneficial influence on LBP intensity. We assume, that reduction of oxidative stress in multifidus muscle might have an impact on effective rehabilitation in LBP patients after the surgery.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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