Effect of Vitamin D Supplementation and/or Physical Training on Cigarette Smoke Induced COPD in Rats

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Abstract

Background: Chronic obstructive pulmonary disease (COPD) is a major health problem with increasing morbidity and mortality. Vitamin D deficiency has been established as exceedingly prevalent in many of chronic lung disease populations and exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance.

Aim of Work: The purpose of the present study was to investigate the effect of vitamin D supplementation and/or physical training on pulmonary functions, lung inflammation, antimicrobial production and matrix degradation in a rat model of COPD.

Methodology: Forty male Albino rats were used in this study and divided into 5 groups, 8 rats each: Group 1: Control group, Group 2: (COPD group): COPD rats maintained untreated for the experimental period, Group 3: (Vit. D+COPD): COPD rats were treated with vitamin D injection 1, 25 (OH) D3 was administered intraperitoneally (i.p.) at dose 0.5 µg/kg of body weight (BW), 3 times a week for 8 weeks, Group 4: (COPD+ Exercise): COPD rats performed daily exercise program and group 5: (COPD+Vit D+exercise) COPD rats treated with vitamin D injection (i.p.) at a dose of 0.5 µg/kg, 3 times a week for 8 weeks and performed daily exercise program. After 8 weeks of treatment, pulmonary functions were tested and blood samples were withdrawn for measuring vitamin D and Ca2+ levels and the lung tissues were excised to measure interleukin 12 (IL12), tumor necrosis factor alpha (TNF alpha), metalloproteinase-9 (MMP-9) and cathelicidin.

Results: Peak expiratory flow (PEF), forced vital capacity (FVC), vitamin D and Ca2+ levels were significantly reduced in COPD rats after 12 weeks of exposure to cigarette smoke. Vitamin D supplementation and swimming training for 8 weeks improved PEF, FVC, vitamin D and Ca2+ significantly as compared to untreated COPD. Combined vitamin D treatment and physical training significantly improved FVC level as compared with each treatment separately. The improvement was associated with significant reduction in inflammatory markers and MMP-9 as compared to COPD untreated rats. The antimicrobial cathelicidin was significantly increased in COPD rats and was further increased on vitamin D treatment but not with exercise training.

Conclusion: Our results showed that COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplement or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuate lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a synergistic effect and produced a significant improvement as compared to each therapy separately. We can conclude that vitamin D supplement has a beneficial effects as a therapy in cases of COPD and it is better added to rehabilitation training programs for better results.

Key Words: COPD – Vitamin D – Physical training – Cathelicidin.

Introduction

CHRONIC obstructive pulmonary disease (COPD) is a major health problem with increasing morbidity and mortality; in 2020, COPD will be the 3rd leading cause of mortality worldwide and the 5th leading source in terms of burden of disease [1]. COPD is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [2-4].

Exposure to cigarette smoke is known to significantly increase the risk for the development of COPD [5,6]. The exact pathogenesis has yet to be discovered; however, numerous cellular elements have demonstrated involvement in the pathophysiology of COPD. These include macrophages, neutrophils and cytokines such as interleukin (IL)-4, IL-5 and IL-13, along with interferon-gamma [6-8].

The alveolar wall destruction and loss of elastic recoil that occur in COPD are believed to be the
Vitamin D is a steroid hormone that is synthesized in the epidermal keratinocytes under influence of UV-B light (290-315nm) or acquired in the diet. Dietary sources include supplemented dairy products, fish oil, fish liver and eggs. It is estimated that approximately 3 percent of the human genome is regulated directly or indirectly by the vitamin D endocrine system [10,11].

Recent research has revealed new sites of action that may force the re-examination of vitamin D and its role in human physiology. Vitamin D receptors (VDRs) have been found in organs not typically believed to be involved with bone metabolism, including the pancreas, gonads, liver, heart, brain and breast, as well as the hematopoietic and immune systems [10].

Vitamin D deficiency has been established as exceedingly prevalent in many of chronic lung disease populations [12]. COPD patients without any glucocorticoid use had significantly decreased 25-hydroxyvitamin D levels when compared with age-matched controls [13]. Children with a diagnosis of wheezy bronchitis had more than two-and-a-half times the incidence of rickets than the age-matched controls. Also it was noted a 10 times higher incidence of wheezy bronchitis when severe rickets was present [14]. Limited studies [15,16] in patients with chronic lung disease suggest that bone mineral density is correlated with lung function, whereas another study [17] was unable to confirm this.

Recent studies have shown that vitamin D has pleiotropic protective effects [18,19]. 1,25 (OH)2D3 (1,25-dihydroxyvitamin D3), an active metabolite of vitamin D is also a potent regulator of the immune response in Th1 cell-directed diseases [20,21]. Sunder and colleagues [22] have recently shown that VDR deficiency invokes lung inflammation and alterations in lung function. Hence, understanding the molecular mechanisms of dietary vitamin D for the treatment of lung disease and their exacerbations are an emerging area of research [23].

Skeletal muscle dysfunction is common in patients with advanced COPD and it contributes importantly to limiting their functional capacity and quality of life [24]. The role of exercise training in pulmonary rehabilitation of patients with severe COPD has been studied [25]. Exercise training in COPD patients results in positive effects in dyspnea and exercise tolerance [25,26], however, the mechanisms by which exercise affect the pulmonary functions in COPD have not been determined.

**Aim of work:** Accordingly, the purpose of the present study was to investigate the effect of vitamin D supplementation and/or physical training on pulmonary functions (peak expiratory flow rates (PEF) and forced vital capacity (FVC) in a rat model of COPD induced by 3 months exposure to cigarette smoke. The effect of vitamin D supplementation and physical training on vitamin D level and calcaemic state, lung inflammation as determined by lung expression of TNF-α and IL-12 was measured. The effect of vitamin D and physical training on the antimicrobial protein cathelicidin and tissue breakdown as indicated by measuring lung metalloproteinase 9 (MMP-9) was also investigated.

**Material and Methods**

**Experimental animals and groups:**

Forty male Albino rats, weighing 100-120g, were used in this study. This work was conducted in the Physiology department, Cairo University from May 2013 – October 2013. The rats were kept under standard conditions. Placed in cages, at 20±5°C, average humidity, and normal light/dark cycles. Standard chow and water were available ad libitum.

**Rats were divided into 5 groups:**

Group 1: Control group (8 rats): These are normal rats serving as control rats for the different values measured in the other groups.

Remaining rats were exposed to passive cigarette smoke for 3 months in order to develop COPD and then tested by pulmonary function tests to examine the development of COPD.

32 male rats were daily exposed to the smoke resulted from burning of 12-15 cigarettes. COPD was induced by cigarette smoking exposure technique: Cigarette smoke (CS) exposure was achieved by same procedure which had been used in a previous study [27] with some modification in periods of exposure and number of cigarettes. Popular Egyptian filter-tipped cigarette were used containing (25mg) tar and (1.8mg) nicotine.

The exposure to the CS was initial progressive concerning the burned material mass and the exposure time in order to permit the biological adaptation of the animals and to avoid accidents such as smoke intoxication that could determine death of rats.
The cigarette smoke exposure lasts for 3 months. The rats were randomly divided into 4 groups after developing COPD:

Group 2: (COPD group): Eight rats were included in this group and maintained untreated for the experiment period.

Group 3: (Vit.D+COPD): Eight COPD rats were treated with vitamin D injection. 25(OH) D₃ was administered intraperitoneally (i.p.) at dose 0.5 µg/kg BW, 3 times a week for 8 weeks [28].

Group 4: (COPD+Exercise): Eight COPD rats performed daily exercise program.

The exercise protocol consisted of swimming exercise (1hr/day, 5 days/week) [29] for 8 weeks in a swimming tank filled with water at a temperature of 36ºC. Daily swimming period was divided into 2 sessions each formed of 30 minutes separated by 1 hour rest. At the completion of each period of swimming exercise, the rats were removed from the water, carefully dried and returned to their cages. The exercised rats underwent a swimming programme consisting of gradually increasing periods of swimming in the first 4 days the duration of exercise was gradually increased from an initial period of 15 min to the maximum permissible period of 30 min.

Group 5: (COPD+Vit.D+Exercise) eight COPD rats treated with vitamin D injection (i.p.) at dose 0.5 µg/kg, 3 times a week for 8 weeks and performed daily exercise program similar to the protocol tried by group 4.

After 8 weeks, the animals were transferred to National Research Centre, Cairo, Egypt where pulmonary functions were assessed and blood samples were withdrawn by capillary tubes and left to clot to get the serum for measurement of vitamin D and Ca²⁺. The animals were then sacrificed and their chests were opened and the lungs were excised for measurement of IL12, TNF alpha, cathelicidin and MMP-9.

**Measurement of pulmonary function:**

Rats were placed in a specific body plethysmograph made of plexi glass. Rats head protruded through a neck collar made of a dental latex dam into a head exposure chamber that ends with a flow head connected to spirometer (AD Instruments spirometer, ML 140) which is a precision differential pressure transducer for measuring respiratory variables. It measures differential pressure across fine gauze mounted in a flow head [30].

**Estimation of vitamin D and calcium:**

Blood samples were withdrawn and left to clot for 20 min then centrifuged at 12,000 rpm for 10 min then the separated serum was kept frozen at -80 ºC till analysis. Serum samples were examined for 25(OH) D levels by Enzyme-linked immunosorbent assay (ELISA) by kit supplied by (Immundiagnostic USA) briefly, monoclonal antibody identify 25, OH vitamin D was used in the assay. The samples were incubated with the detection antibody after the extraction step. Then Peroxidase-conjugated antibody was then added into microplate well, forming a complex of 25-hydroxy vitamin D-detection antibody-peroxidase conjugate. Tetramethylbenzidine (TMB) was used as a substrate, the colour density developed is proportional to vitamin D concentration. Finally, to terminate the reaction stop solution was added and the microplate were read by elisa reader at 520 nm [31]. Serum calcium concentrations were measured by standard laboratory methods.

**Measurement of TNF-α and IL-12:**

TNF-α and IL-12 in lung tissues were measured by using ELISA (quantikine R&D system USA) according to the manufacturer’s instructions [32,33].

**Detection of MMP-9 & cathelicidin gene expression using real time:**

PCR (RT-PCR):

RNA extraction:

Total RNA was isolated from lung tissue homogenates using RNasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer’s instruction. The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (GeneQuant 1300, Uppsala, Sweden). RNA quality was confirmed by gel electrophoresis.

cDNA synthesis:

First-strand cDNA was synthesized from 4 µg of total RNA using an Oligo (dT) 12-18 primer and SuperscriptTM II RNase Reverse Transcriptase; This mixture was incubated at 42 ºC for 1h, the kit was supplied by Super Script Choice System (Life Technologies, Breda, the Netherlands).

Real-time quantitative polymerase chain reaction (PCR):

Real-time PCR (RT-PCR) amplification was carried out using 10 µL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equiv-
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Conclusion: Our results showed that COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplement or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuate lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a synergistic effect and produced a significant improvement as compared to each therapy separately.

Table (1): Changes in pulmonary functions (PEF and FVC) in untreated COPD rats and in COPD rats after 8 weeks of either vitamin D supplementation, exercise training or both.

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| Control | COPD | COPD+ Vit D | COPD+ exercise | COPD+ vit D+exercise |
| PEF (mL/min) | | | | |
| Mean±S.D. | 13.65±1.04² | 6.78±1.15³ | 10.36±0.87³ | 8.80±0.89⁴ |
| FVC (mL) | | | | |
| Mean±S.D. | 9.24±0.78⁴ | 4.81±0.85⁴ | 7.90±0.54⁵ | 6.21±0.56⁵ |

- (n=8).
- Values are Mean±SD.
- Values with different letters in the same row are significantly different from each other (p<0.05).
- Values with the same letters in the same row are insignificantly different from each other (p>0.05).
Fig. (1): Effects of vitamin D supplement and physical training for 8 weeks on pulmonary function (PEF) in COPD rats (Mean±SD).

**Effect of COPD, vitamin D supplementation, and exercise training on vitamin D levels and serum Ca2+ level in rats:**

As expected from previous researches the present results showed deficiency of vitamin D and a hypocalcaemic state in rats with COPD and the levels of vitamin D and Ca2+ were significantly reduced in COPD rats as compared to control rats. It can be seen from Table (2) and Figs. (3,4) that vitamin D supplementation and/or exercise corrected the deficiency and the values of these two parameters were back to normal control values.

Table (2): Effects of vitamin D supplement and physical training for 8 weeks on serum levels of vitamin D and Ca2+ in COPD rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COPD</th>
<th>COPD+ Vit D</th>
<th>COPD+ exercise</th>
<th>COPD+ Vit D+exercise</th>
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<tbody>
<tr>
<td>Vit D (ng/ml)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean±S.D.</td>
<td>52.25±8.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.00±5.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.41±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.95±5.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.46±9.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean±S.D.</td>
<td>10.18±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.40±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.25±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.48±0.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.75±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- *(n=8).*
- Values are mean±SD.
- Values with different letters in the same row are significantly different from each other (*p*<0.05).
- Values with the same letters in the same row are insignificantly different from each other (*p*>0.05).

Changes in inflammatory markers TNF alpha and IL12 in lung tissues in untreated COPD rats and in COPD rats after 8 weeks of either vitamin D supplementation, exercise training or both:

The results of the present work confirmed that COPD is an inflammatory disease. As shown in Table (3) and Figs. (5,6), lung TNF alpha and IL12 were significantly increased in COPD rats as compared to control rats. Treatment with i.p. injection of vitamin D, for 8 weeks reduced the inflammation and the levels of the inflammatory markers were significantly decreased as compared to the untreated COPD rats. Physical training induced also an anti-
Effect of Vitamin D Supplementation and/or Physical Training

inflammatory effect and significantly reduced the level of TNF alpha and IL-12 as compared to the untreated COPD rats but the reduction was significantly less than that produced by vitamin D treatment. Combined treatment with vitamin D and physical training induced a further significant decline in the levels of inflammatory markers as compared to each treatment alone.

Table (3): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory markers (TNF alpha and IL-10), matrix degradation (MMP-9) and antimicrobial (cathelicidin) in COPD rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COPD</th>
<th>COPD+ Vit D</th>
<th>COPD+ exercise</th>
<th>COPD+ vit D+exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12 (pg/ml) Mean±S.D.</td>
<td>30.56±4.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.66±19.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.93±7.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.86±5.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>44.99±4.60&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml) Mean±S.D.</td>
<td>46.60±9.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266.56±59.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>133.24±8.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.63±17.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>102.46±9.18&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMP-9 Mean±S.D.</td>
<td>1.35±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.51±1.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.42±0.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.62±0.35&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.68±0.56&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cathelicidin Mean±S.D.</td>
<td>0.14±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.39±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84±0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- (n=8).
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- Values with different letters in the same row are significantly different from each other (p<0.05).
- Values with the same letters in the same row are insignificantly different from each other (p>0.05).

![Fig. (5): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory marker (IL12) in COPD rats (Mean±SD).](image)

**Effect of COPD, vitamin D supplementation, and exercise training on mRNA of lung matrix (MMP-9):**

Table (3) and Fig. (7) show that in rats developed COPD there is enhanced degradation of lung parenchyma as indicated by elevated level of MMP-9 in lung tissues. Treatment of COPD rats with vitamin D for 8 weeks or exercise significantly reduced destruction of lung tissue by attenuation of MMP-9 production as compared to the untreated rats. It can be observed that vitamin D supplementation caused a more significant reduction in MMP-9 production than did exercise training. Combining both therapy together induced a better improvement and a significant reduction in MMP-9 in lung tissues as compared to each therapy alone.

![Fig. (6): Effects of vitamin D supplement and physical training for 8 weeks on lung inflammatory marker (TNFalpha) in COPD rats (Mean±SD).](image)

**Effect of COPD, vitamin D supplementation, and exercise training on mRNA of antimicrobial cathelicidin in lung tissue:**

Table (2) and Fig. (8) showed that COPD was associated with a significant increase in the level of antimicrobial cathelicidin. Vitamin D supplementation for eight weeks alone or in combination with exercise training induced a further significant increase in its value. Exercise alone had no effect on the lung level of cathelicidin as compared to COPD untreated group.
Discussion

Chronic obstructive pulmonary disease (COPD) has been defined as a preventable and treatable pathologic condition characterized by partially reversible airflow limitation [35]. It is well known that cigarette smoke is one of the most important risk factors for COPD, and it can accelerate the development of COPD in humans [36]. In this study, we found that exposure to cigarette smoke caused COPD after 12 weeks in male albino rats; it produced a significant reduction in pulmonary function as detected by a significant decrease in mean values of PEF and FVC as compared to control group.

Short term exposure to noxious gases as cigarette smoke (days) [37] resulted in a pulmonary inflammatory infiltrate, increased mucus production, and pulmonary edema. Long term induction protocols (weeks or months) [38] produced, in addition to the inflammatory infiltrate, emphysema and pulmonary remodeling characterized by fibrosis, and thickened bronchiole and arterial walls.

In the present study, we found that vitamin D was deficient in rats with COPD and that the dose of vitamin D supplemented in our study was enough to raise the blood vitamin D level to be comparable with the control value. Exercise protocol performed in COPD rats also was able to significantly increase vitamin D level as compared to COPD untreated rats. In rats with COPD treated with both vitamin D and exercise, there was a significant increase in vitamin D levels as compared with the COPD untreated rats but the increase was insignificant when compared with vitamin D or exercise treated rats.

There are several factors that could account for vitamin D deficiency in COPD patients: Poor diet, a reduced capacity of aging skin for vitamin D synthesis, reduced outdoor activity and therefore sun exposure, an increased catabolism by glucocorticoids, impaired activation because of renal dysfunction, and a lower storage capacity in muscles or fat due to wasting [38]. Many steps of the vitamin D pathway (intake, synthesis, storage, metabolism) can potentially be disturbed in COPD patients.

In agreement with our results, Forli et al., [12] found vitamin D deficiency (in their study defined as below 20ng/ml) in more than 50% of a cohort waiting for lung transplantation. In an outpatient study on patients with COPD in Denmark, 68% of the participants had osteoporosis or osteopenia [40]. A recent study showed that vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D binding gene [41].

Moderate endurance exercise increases serum 1,25(OH)2D3 level [42,43]. Also, Sato et al., [44], reported that immobilization, in contrast to endurance exercise, 1,25-(OH)2D3 are suppressed. However, Maimoun et al., [45], reported that in exercise trained rats, serum 1,25-(OH)2D3 concentration was not affected.

In this study vitamin D supplementation, exercise training or both significantly improved pulmonary functions compared with COPD untreated group.

In agreement with our results, a strong relationship between serum levels of vitamin D and lung function (FEV1 and FVC) was found [46,47]. In another study, 25(OH)D was correlated with FEV1 [48]. Ferrari et al., [49] also demonstrated that the maximal exercise capacity and carbon monoxide transfer in the single breath method were both positively correlated with serum 25(OH)D concentrations.
A cross-sectional study found that higher plasma levels of vitamin D are associated with increased bone mineral density and exercise capacity in people with COPD [50]. Evidence also showed that high dose vitamin D supplementation improved respiratory muscle strength and exercise capacity in people with COPD [51]. Epidemiological studies revealed a dose-dependent association between serum 25(OH)D levels and pulmonary function so that adequate vitamin D supplementation may extend beyond its protection against osteoporotic fractures [52].

However Shaheen et al., [53] reported that total vitamin D intake was positively associated with forced expiratory volume in 1 s (FEV1); they did not confirm a positive association between blood 25(OH)D concentrations and adult lung function. They suggested that the apparent relationships with dietary vitamin D are likely to be explained by other highly correlated nutrients in the diet.

Also, Bjerk et al., [54], reported that short-term vitamin D supplementation in patients with COPD had no discernible effect on a simple measure of physical performance [55].

As regard the effects of physical exercise on pulmonary functions, there was a controversy. Maltais et al., [56] reported improvement of pulmonary functions FEV1 and demonstrated physiologic gain following 12 weeks of exercise in persons with severe COPD. Similar improvement in physiologic parameters have been confirmed by several additional studies [57,58].

On the other hand, Flo et al., [59], reported worsening of pulmonary emphysema induced by exercise training. Their hypothesis is that the increase in mechanical forces on the connective tissue may contribute to the worsening of pulmonary function. One alternative explanation for the worsening of emphysema induced by exercise was the presence of exercise-induced oxidative stress. It has been demonstrated that strenuous aerobic exercise is associated with oxidative stress and tissue damage [60,61]. However, moderate exercise training is associated with adaptive responses in at least some antioxidant capacities [62].

The beneficial effect of vitamin D supplementation and or exercise on pulmonary functions could depend on the calcemic effects of vitamin D.

In the present study it was observed that the calcium levels in the blood of COPD rats were significantly lower than normal control. Treatment of the COPD rats for 8 weeks with vitamin D improved the calcemic state of the rats to the normal control values. Also in the exercise trained group or combined vitamin D and exercise group calcium levels were increased to be insignificantly changed as compared to the control rats.

The vital capacity and total lung capacity were found to decline with an increasing number of thoracic vertebral fractures as a direct consequence of vitamin D deficiency and hypocalcemia [63]. Nuti et al., observed 3030 ambulatory COPD patients and found a strong association between COPD severity and fractures [64]. Kyphosis related to osteoporosis caused limitation in rib mobility and inspiratory muscle function and correlated with a reduction in FEV1 and FVC [65]. The altered properties of the thoracic skeleton could result in failure of the respiratory muscles contributing to the pathophysiology of COPD.

It was seen from the results of this study that exercise also improved pulmonary functions in COPD rats and we can find that also exercise improved the calcemic state in COPD rats. Blood Ca2+ levels were increased but not significantly in exercise trained groups as compared with untreated COPD rats.

In agreement with our results, Yeh et al., reported that in exercise trained rats plasma ionized calcium slightly increased [42]. Previous studies suggest that moderate endurance exercise increases serum 1,25(OH)2D3 level, decreases urinary calcium excretion [43]. By using a flat-bed treadmill exercise, Yeh and co-workers found that the endurance exercise trained female Sprague-Dawley rats had higher duodenal active, but not passive, calcium absorption than the control [42]. Although exercise-enhanced intestinal calcium absorption is likely mediated by an increase in serum 1,25-(OH)2D3 level, exercise may also stimulate calcium absorption by changing intestinal motility and epithelial permeability [66,67].

Acute exacerbations of COPD are an important cause of hospitalization and lead to a faster decline in FEV1 [68]. Exacerbations are triggered by viruses, bacteria, atypical strains, or a combination of these [69,70].

In the present study it was found that vitamin D treatment enhances the innate immune system by inducing the production of antimicrobial cathelicidin and was associated with a significant increase in its lung production.

The occurrence of exacerbations, which are very often caused by bacterial or viral infections, increases the severity of COPD and causes a higher
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dehth rate in humans [71]. 1,25(OH)2D3 is a direct regulator of antimicrobial peptides, such as cathelicidin (camp) and defensin β2 (def/β2) genes that are driven by vitamin D response elements (VDRE)-containing promoters, revealing the potential therapeutic role of vitamin D3 analogs against opportunistic infections, including the infections in the respiratory tracts which occurs in patients with COPD susceptible to exacerbations [72].

In agreement with our results, Vazirmia et al., [73] reported a beneficial effect of 1,25(OH)2D on the innate immunity and they also found an elevation of the antimicrobial cathelicidin in cases treated with vitamin D.

Several interventional studies examining the effect of vitamin D supplementation on the risk of influenza [74,75] and in patients with active tuberculosis showed a significantly reduced risk for influenza an improved immunity against mycobacteria [76,77]. There was also a higher rate of tuberculosis symptom improvement in children [78].

Several mechanisms could explain vitamin D’s potentially beneficial effects on infectious diseases. In addition to its antimicrobial effect, vitamin D can affect the inflammatory response. In the present study, the increase of lung production of inflammatory mediators in untreated COPD rats relative to healthy controls are in agreement with those previously reported in patients with stable COPD of similar severity and body mass index [79]. Studies have also reported increased levels of circulating cytokines (interleukin (IL)-6 and -8 and tumour necrosis factor-α (TNF-α) and acute phase reactant protein (C-reactive protein (CRP), both of which reflect systemic inflammation, in the peripheral circulation of stable COPD patients [80,81].

In agreement with our results, it was reported that 1,25D (1,25-dihydroxyvitamin D) decreases TNF-alpha [82]. Other studies showed that vitamin D can modulate the activity of various immune cells and inhibit inflammatory responses [83,84]. It was reported that vitamin D-induced inhibition of IL-12 release by dendritic cells has a profound effect on T lymphocyte differentiation [85]. Vitamin D-binding protein has immunomodulatory functions pertinent to the lung, and is associated with activation of macrophages and neutrophil chemotaxis [19].

In agreement with our results, regular exercise was noted to protect against diseases associated with chronic inflammation [86]. On the other hand, American Thoracic Society reported that exercise induced oxidative stress and could, inversely, induce abnormal exercise-induced inflammation in COPD. Plasma inflammatory mediators TNF-alpha and IL-6 were not significantly modified by training. Pulmonary rehabilitation can induce peripheral muscle adaptations without decreasing the levels of systemic or local muscle inflammation [87].

In our study it can be observed that matrix metalloproteinase-9 (MMP-9) is significantly elevated in lung tissues of COPD rats and a causative role has been suggested in the development of COPD [88]. The effect of vitamin D on extracellular matrix homeostasis not only in bone tissue, but also within the lung may have a role in COPD development. In the present study, vitamin D supplementation significantly reduced MMP-9 production in lung tissues of treated COPD rats as compared with untreated controls.

In agreement with our results, Boyan and Schwartz [89] found vitamin D to be an autocrine regulator of extracellular matrix turnover and growth factor release via matrix metalloproteinases. Also Bahar-Shany et al., [90] reported that vitamin D attenuates MMP-9 production in keratinocytes and they suggested that vitamin D deficiency may lead to a reduced attenuation of MMP-9 activity resulting in enhanced degradation of lung parenchyma.

Our results showed that exercise training reduced lung production of MMP-9, suggesting that exercise training may be important not only in pulmonary rehabilitation in COPD but also as an adjuvant in the prevention and progression of lung destruction due to cigarette smoking.

Few studies investigated the effect of physical training on lung production of MMP-9 in COPD. Toledo et al., [91] found a decrease in TIMP 1 in mice exposed to cigarette smoke that was reversed by aerobic exercise.

Conclusion:

We can see from the results of this work that, COPD is an inflammatory disease and it is associated with vitamin D deficiency. Vitamin D supplementation or rehabilitation by physical training each separately improved the pulmonary functions, reduced inflammation, and attenuate lung parenchymal degradation. Vitamin D in addition induced an antimicrobial protection, however vitamin D supplement had a slightly better effects as compared with exercise training. Combination of both vitamin D supplementation and exercise training had a
synergistic effect and produced a significant improvement as compared to each therapy separately. We can conclude that vitamin D supplement has a beneficial effect as a therapy in cases of COPD and it is better added to rehabilitation training programs for better results.

References


Effect of Vitamin D Supplementation and/or Physical Training


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