The effect of aerobic exercise and vitamin D supplementation on inflammatory factors, anti-inflammatory proteins, and lung function in male smokers: A randomized controlled trial

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Research article

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Abstract

Background

This study aimed to investigate the effect of aerobic exercise and vitamin D supplementation on inflammation (TNF- α , IL-6, CC16, SP-D, and CC16/SP-D ratio) and lung function (FEV₁, FVC, and FEV₁/FVC ratio) in male smokers.

Methods

After applying inclusion criteria, a total of 40 healthy male smokers were recruited in this study. The subjects were randomly divided into four groups as follows: Aerobic Exercise + vitamin D Supplementation (AE + VitD, N = 10), Aerobic Exercise (AE, N = 10), vitamin D Supplementation (VitD, N = 10), and Control (C, N = 10). The subjects in the AE + VitD and AE groups performed aerobic exercise training (running) up to 50% of heart rate (HR) max, three times a week for four weeks. Subjects in AE + VitD and VitD groups received 6000 IU/w vitamin D3 for four weeks. The subjects in control group did not receive any intervention. Serum tumor necrosis factor (TNF)- α , interleukin (IL)-6, Clara cell protein (CC16), surfactant protein (SP)-D, CC16/SP-D ratio, and lung function (FEV₁, FVC, and FEV₁/FVC ratio) were measured before and after four weeks of intervention.

Results

Serum levels of TNF- α , IL-6, CC16, and SP-D decreased significantly in AE + VitD and AE groups after four weeks ($P < 0.05$), but serum SP-D level in the AE group did not decrease significantly. In addition, FEV₁ and FVC increased significantly ($P < 0.05$) in AE + VitD and AE groups after four weeks of intervention. However, AE + VitD and AE did not have a significant effect on CC16/SP-D ratio and FEV₁/FVC ratio. Furthermore, serum levels of TNF- α , IL-6, and CC16 decreased significantly after vitamin D supplementation ($P < 0.05$).

Conclusions

The results of present study demonstrates that aerobic exercise combined with vitamin D supplementation can reduce inflammatory factors and anti-inflammatory proteins in the blood and improve lung function after four weeks. Further larger trials with higher sample size and longer duration are suggested to confirm these results.

Trial registration

Retrospectively registered. IRCT20180513039637N4. Registration date: 2020/10/20. URL: <https://www.irct.ir/search/result?query=IRCT20180513039637N4>

Background

Evidence suggests that tobacco-related diseases and deaths are growing in the world [1]. The most common type of tobacco use is cigarette smoking (CS) [2]. Long term cigarette smoking (LTCS) causes various diseases by affecting the organs of the body [3]. But the most well-known effect of toxic substances of cigarettes is on the respiratory system, which results in the pulmonary epithelial and cell membrane damages, and alter mucosal permeability [4, 5]. These alterations cause such diseases as lung cancer and chronic obstructive pulmonary disease (COPD) [3, 6, 7]. Also, smoking reduces pulmonary function by decreasing FEV₁ and FVC [8].

LTCS causes the release of inflammatory cells (e.g., macrophages, neutrophils, and lymphocytes) and inflammatory mediators (e.g., TNF- α and IL-6) from epithelial and smooth muscle cells and initiates the inflammatory process [5, 9, 10]. On the other hand, toxic substances pass through the alveolar and capillaries and enter the bloodstream. These toxins are identified by immune system receptors and trigger the inflammatory process via NF- κ B in the bloodstream [5]. NF- κ B has an important role in triggering inflammation by activating the expression of genes for inflammatory factors such as IL-6 and TNF- α [11]. Also, lung epithelial cells release anti-inflammatory proteins such as Clara cell protein (CC16) and surfactant protein D (SP-D) during inflammation and disease [12, 13]. Studies have also shown that CS increases serum levels of CC16 and SP-D [13, 14]. Increasing CC16 and SP-D in serum and decreasing them in epithelial cells make lungs more vulnerable to damage [12]. In addition, the serum CC16/SP-D ratio is a valid and sensitive marker for the diagnosis of lung epithelial cell injury [12].

Previous studies have demonstrated the non-pharmacological effect of exercise training on reducing lung diseases [5, 15]. Regular exercise can reduce inflammatory factors by activating the anti-inflammatory signaling pathway. This anti-inflammatory property of exercise training improves pulmonary rehabilitation, reduces lung diseases (e.g., dyspnea, airway hyperresponsiveness, exercise-induced bronchospasm, and asthma), and increases strength, endurance capacity, and quality of life [5, 15, 16]. Also, the results of Krüger *et al.* and Toledo-Arruda *et al.* showed that aerobic exercise can reduce CS-induced inflammation [17, 18]. Furthermore, recent scientific evidence suggests that consumption of certain foods and nutrients improves lung function and reduces risks of COPD [11]. Interestingly, recent evidence demonstrates that vitamin D [defined as 1,25-dihydroxyvitamin D (1,25(OH)₂D) $\geq$ 20 ng/ml] deficiency causes lung disease and decrease lung function (FEV₁ and FVC) [19]. Vitamin D is reported to have anti-inflammatory properties, and it can reduce CS-induced inflammation [19] by regulating the proliferation and function of immune cells through the vitamin D receptor (VDR), which is a member of the steroid hormone receptor family [20]. Agrawal *et al.* demonstrated that vitamin D supplementation can reduce the inflammatory effects [21].

Although some studies have confirmed the positive effects of aerobic exercise and vitamin D on reducing inflammation, few research has been conducted in this issue and the results are controversial. As far the researchers investigated, no study has examined the effect of aerobic exercise and vitamin D supplementation on inflammation and lung function in tobacco smokers so far. Therefore, this study aimed at evaluating the effect of aerobic exercise and vitamin D supplementation on inflammatory factors, anti-inflammatory proteins, and lung function in male smokers.

Methods

In this experimental study, 40 healthy male smokers were recruited from Tabriz city, Iran. Inclusion criteria were as follows: at least one-year experience of smoking cigarettes, no prior history of specific diseases such as diabetes and cardiovascular and lung disease, nutritional allergies, and medication use, no prior history of using anti-inflammatory agents, β_2 -agonists, any supplements (i.e. vitamins, proteins drinks, amino acids, etc.), or medication before or during the study, and no history of doing any regular exercise or physical activity in the past six months. Information on personal and family history of atopic diseases, diet, sociodemographic data, and frequency and duration of cigarette smoking were collected by a general questionnaire. After reviewing the personal and family questionnaire information and medical examinations, 40 healthy male smokers were selected. All subjects were informed about the objectives of the study and a written consent was obtained.

The study was approved by the Research Ethics Committee of Tabriz University of Medical Sciences, Iran which are in accordance with the Helsinki Declaration (Ethical code: IR.TBZMED.REC.1399.727). Also, the protocol of the study was registered in the Iranian registry of clinical trials (IRCT code: IRCT20180513039637N4).

Subjects were randomly divided into four groups: Aerobic Exercise + vitamin D supplementation (AE + VitD, N = 10), Aerobic Exercise (AE, N = 10), vitamin D supplementation (VitD, N = 10), and Control (C, N = 10). Randomization was carried out on an individual basis using Randomizer software. Opaque and sealed envelopes were used for allocation concealment. The groups were encoded, and the codes were sealed by an independent monitor.

All subjects were requested to follow their daily CS behavior and regular diet throughout the period of the study. Also, they were not allowed to use supplements such as omega-3 s that have anti-inflammatory properties [22]. Body weight (with 10 g measurement accuracy) was measured using a digital scale (OMRON, BF: 508, Finland). Body fat was measured using an Inbody 720 body Composition Analyzer with an accuracy of 0.5% (Inbody Co. Ltd. Seoul Korea). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, and Cooper test was used to estimate the maximum oxygen consumption (VO_2 max) of the subjects [23].

One week after the briefing session, the subjects began consumption of vitamin D supplement and aerobic exercise. AE + VitD and AE groups performed aerobic running for six weeks (30 minutes for three sessions a week) on the standard track racetrack. In the first three weeks, AE + VitD and AE groups ran at 50–60% of their individually determined maximum heart rate (HR). In the second three weeks of the study, the intensity and duration of the exercise increased to 60–70% HR max. POLAR (T31C, Finland) HR monitors were used to estimate subjects' HR and maintaining determined intensity during exercise. All exercise sessions were held between 8–10 am. Subjects in VitD and C groups were requested to perform their usual daily activities. Simultaneously with aerobic exercise, vitamin D3 supplementation started in VitD and AE + VitD groups. Subjects in AE + VitD and VitD groups received 6000 UI/week (1000 IU/day =

25 microgram, except Fridays) oral vitamin D3 in tablets form for six weeks. Tablets were produced and supplied by Health Aid Company (England).

To determine the baseline and final levels (after six weeks) of IL-6, TNF- α , CC16, SP-D, and 1,25-dihydroxyvitamin D (1,25(OH)₂D), blood samples were collected after 12-hour overnight fasting from the subjects' antecubital vein 24 hours before the start of the study (10 ml) and 24 hours after the last aerobic exercise session (10 ml). Samples were centrifuged at 3,000 rpm for 15 minutes, and serum was subsequently distributed in 1.8-mL aliquots and stored at - 80 °C. IL-6, TNF- α , CC16, and SP-D were analyzed by ELISA using commercial kits (East Biopharm, USA).

FEV1 and FVC were measured before and after the study with an Easy One portable spirometer (nidd Medical Technologies, Zürich, Switzerland) following standard recommendations [24–26]. FEV1 and FVC were expressed as the percentage.

Statistical analysis

Statistical analysis was performed using SPSS (version 22). Descriptive data were reported as means and standard deviations. Normal distribution of data was verified with the Kolmogorov-Smirnov test. Paired sample t-test was used to compare within-group differences. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to analyze between-group differences, respectively. If the value of F-ratio was significant, Bonferroni post hoc test was used to identify the differences between groups. P-value less than 0.05 was considered to be statistically significant.

Results

A total of 40 participants were recruited, with 91.6% of participants completing the study (Fig. 1; Consolidated Standards of Reporting Trials (CONSORT) diagram). The anthropometric characteristics, VO₂ max, and duration of cigarette consumption are demonstrated in Table 1. Based on the results of one-way ANOVA, there was no significant difference in anthropometric characteristics, VO₂ max, and cigarette consumption between the four groups ($P \geq 0.05$).

Table 1
Anthropometric characteristics, VO₂ max, and smoking duration in the studied groups

Variables	AE + VitD (n = 10)	AE (n = 10)	VitD (n = 10)	C (n = 10)	P ^a
Age, year	30.4 ± 4.08	31.3 ± 4.00	30.1 ± 3.72	31.7 ± 3.83	0.77
Height, m	1.74 ± 0.06	1.75 ± 0.07	1.74 ± 0.09	1.76 ± 0.07	0.96
Weight, kg	76.00 ± 9.00	74.00 ± 7.34	75.10 ± 9.1	76.20 ± 6.44	0.92
BMI, kg.m ²	24.90 ± 1.57	24.29 ± 0.75	25.19 ± 3.47	24.98 ± 1.70	0.80
Fat, %	14.50 ± 2.54	13.90 ± 1.85	14.90 ± 2.51	15.20 ± 1.93	0.59
VO ₂ max, %	41.22 ± 2.52	40.28 ± 3.25	39.30 ± 3.43	40.40 ± 2.41	0.54
History of smoking, (year)	6.90 ± 2.37	7.87 ± 2.62	7.65 ± 3.10	8.40 ± 5.31	0.85
Data are presented as Mean ± SD					
^a One-way ANOVA					

As Table 2 suggests, AE + VitD ($P=0.05$), AE ($P=0.05$), and VitD ($P=0.05$) resulted in a significant decrease in serum levels of TNF- α and IL-6 compared to the baseline, but the serum levels of TNF- α and IL-6 did not reduce in the control group ($P=0.05$). In addition, comparison of the mean group differences of TNF- α and IL-6 showed no significant differences between the groups at the basic level and after the intervention (Table 2; $P=0.05$).

Table 2

Baseline and Week-4 values of inflammatory markers, anti-inflammatory proteins, lung function, and 1,25-dihydroxy vitamin D

Variables	AE + VitD (n = 10)	AE (n = 10)	VitD (n = 10)	C (n = 10)	P [#]
TNF- α , (ng/ml ⁻¹)					
Baseline	73.2 $\pm$ 12.4	75.4 $\pm$ 15.0	73.9 $\pm$ 13.5	74.1 $\pm$ 10.6	0.001
Week 4	55.0 $\pm$ 11.4	66.8 $\pm$ 16.6	67.7 $\pm$ 11.1	74.9 $\pm$ 9.4	0.001
P [#]	0.001	0.009	0.020	0.394	
IL-6, (pg/ml ⁻¹)					
Baseline	5.87 $\pm$ 1.61	6.30 $\pm$ 2.02	5.95 $\pm$ 2.11	6.16 $\pm$ 1.39	0.001
Week 4	4.98 $\pm$ 1.37	5.93 $\pm$ 1.67	5.70 $\pm$ 1.98	6.31 $\pm$ 1.37	0.001
P	0.004	0.039	0.046	0.097	
CC16, (ng/ml ⁻¹)					
Baseline	7.19 $\pm$ 1.28	7.34 $\pm$ 1.07	7.24 $\pm$ 1.81	7.25 $\pm$ 1.05	0.001
Week 4	6.42 $\pm$ 1.06	7.11 $\pm$ 1.02	6.87 $\pm$ 1.08	7.34 $\pm$ 0.97	0.001
P	0.001	0.005	0.025	0.688	
SP-D, (ng.ml ⁻¹)					
Baseline	69.80 $\pm$ 13.7	71.83 $\pm$ 12.9	70.75 $\pm$ 10.4	71.20 $\pm$ 11.5	0.001
Week 4	62.06 $\pm$ 9.4	69.50 $\pm$ 11.1	68.4 $\pm$ 8.3	72.3 $\pm$ 11.2	0.001
P	0.011	0.085	0.084	0.240	
CC16/SP-D					
Baseline	0.104 $\pm$ 0.014	0.102 $\pm$ 0.010	0.101 $\pm$ 0.005	0.102 $\pm$ 0.009	0.001
Week 4	0.104 $\pm$ 0.104	0.102 $\pm$ 0.008	0.099 $\pm$ 0.005	0.101 $\pm$ 0.009	
P	0.817	0.810	0.393	0.903	0.856
FEV ₁ , %					

Data are presented as Mean $\pm$ SD,

□ Inter-group comparisons with ANCOVA

paired sample T-test

Variables	AE + VitD (n = 10)	AE (n = 10)	VitD (n = 10)	C (n = 10)	P [#]
Baseline	87.29 ± 7.51	88.30 ± 10.54	87.55 ± 8.48	86.70 ± 8.48	0.001
Week 4	91.70 ± 5.86	91.80 ± 8.23	89.21 ± 7.85	86.44 ± 11.30	0.001
P	0.001	0.021	0.121	0.534	
FVC, %					
Baseline	94.39 ± 7.41	94.81 ± 12.69	92.85 ± 6.93	93.15 ± 10.10	0.001
Week 4	97.97 ± 6.94	97.65 ± 9.51	93.95 ± 7.99	92.55 ± 10.73	0.004
P	0.002	0.035	0.313	0.133	
FEV ₁ /FVC, %					
Baseline	92.45 ± 2.59	93.42 ± 3.97	94.20 ± 4.08	92.89 ± 3.59	0.001
Week 4	93.78 ± 3.71	94.17 ± 3.99	95.06 ± 4.11	93.31 ± 4.22	0.934
P	0.153	0.397	0.643	0.478	
1,25(OH) ₂ D, (ng/ml ⁻¹)					
Baseline	23.90 ± 5.72	23.38 ± 6.65	24.51 ± 7.82	24.73 ± 7.60	0.001
Week 4	27.48 ± 6.85	24.15 ± 5.51	27.55 ± 8.05	24.64 ± 7.18	0.004
P	0.002	0.237	0.001	0.924	
Data are presented as Mean ± SD,					
□ Inter-group comparisons with ANCOVA					
# paired sample T-test					

As shown in Table 2, there was a significant difference between groups in CC16 and SP-D values before (baseline) and after (four weeks) the intervention ($P \leq 0.05$). In addition, inter-group difference of CC16/SP-D ratio at the baseline was significant ($P \leq 0.05$); however, no significant difference was observed between groups after four weeks ($P \geq 0.05$). AE + VitD ($P = 0.001$), AE ($P = 0.005$), and VitD ($P = 0.025$) reduced the levels of CC16 compared to baseline values; but no significant intra-group difference in the CC16/SP-D ratio was observed between the studied groups. Furthermore, serum SP-D level decreased significantly only in the AE + VitD ($P = 0.011$) group and no significant difference was observed in any of the groups (Table 2; $P \geq 0.05$).

According to the results, after four weeks of intervention, FEV₁ ($P = 0.001$) and FVC ($P = 0.001$) significantly increased in the AE + VitD and AE groups, but none of the interventions had a significant effect on the FEV₁/FVC ratio ($P \geq 0.05$). In addition, a significant difference was observed between the groups in the amount of FEV₁ and FVC before and after the intervention ($P \geq 0.05$). Also, inter-group difference of FEV₁/FVC ratio at the baseline was significant ($P \geq 0.05$); but no significant difference was observed between groups after four weeks ($P \geq 0.05$).

Furthermore, changes of serum 1,25-dihydroxyvitamin D (1,25(OH)₂D) were significant between the groups ($P \geq 0.05$). Also, the serum levels of 1,25-dihydroxyvitamin D ($P = 0.001$) increased only in the AE + VitD and VitD groups; but AE ($P = 0.41$) and AE + VitD ($P = 0.86$) groups did not have an effect on serum levels of 1,25-dihydroxyvitamin D.

Discussion

In the present study, AE + VitD, AE, and VitD reduced inflammatory factors)TNF- α and IL-6 (in the bloodstream, but no significant difference was observed in the control group after four weeks. Previous studies have demonstrated that physical activity [7, 18] and vitamin D supplementation [19, 20] can reduce inflammatory cytokines. Exercise training moderates inflammatory process by activating anti-inflammatory signaling pathways [5]. Exercise training inserts its anti-inflammatory properties by increasing systemic levels of anti-inflammatory cytokines such as IL-1RA, and prevents the secretion of pro-inflammatory factors [5]. On the other hand, prolonged exercise plays an important role in reducing the production of the pro-inflammatory factors by decreasing expression of Toll-like receptors (TLRs) on monocytes [5]. In addition, vitamin D supplementation reduces inflammation by inhibiting the proliferation of T-cells and the production of cytokines [20].

The results of this study showed that AE + VitD, AE, and VitD can reduce CC16 after four weeks, but only AE + VitD could reduce serum SP-D levels after four weeks. Furthermore, interventions did not have a significant effect on CC16/SP-D ratio. CC16 is used as a marker of distal lung epithelial damage [27]. Although biological function of CC16 has not been completely discovered, it seems that it can also have direct anti-inflammatory properties through inhibiting the enzyme phospholipase A₂ and the protein prostaglandin D₂ release, which both are involved in the arachidonic acid inflammatory cascade [28]. Also, SP-D is a protein in pulmonary host defense that is released from the epithelial cells of the lungs to interact with alveolar macrophages and a variety of microorganisms [13, 29, 30]. We did not find any research on the effect of aerobic exercise and vitamin D supplementation on serum CC16 and SP-D levels. Only in one research, Moazami *et al.* reported a significant reduction in serum levels of CC16 after four weeks of aerobic exercise with methadone [31].

Based on the results of this study, AE + VitD and AE significantly increased FEV₁ and FVC after four weeks in male smokers. According to the results, physical activity can improve lung function [32–34]. Garcia *et al.* reported that moderate to high levels of regular exercise can increase lung function in smokers [35]. It seems that regular exercise prevents lung function decline by reducing inflammatory markers and

oxidative, and it can have a positive effect on respiratory muscle strength [35]. On the other hand, prior studies have shown an association between vitamin D and lung function. Vitamin D sufficiency can protect lung function against the inflammatory and oxidative effects of smoking [36]. However, the results of the present study showed that vitamin D supplementation had no effect on improving lung function. We speculate that four weeks of supplementation is not enough to see the positive effects on lung function. So, studies with longer durations are needed.

This is the first study that examined the effect of aerobic exercise and vitamin D supplementation on inflammatory and anti-inflammatory agents and pulmonary function. The results of this study should be interpreted considering the limitations. We did not study vitamin D supplementation and aerobic activity over longer periods of time (e.g., in 8–12 weeks).

In conclusion, the results of present study demonstrated that the interaction of aerobic exercise (running with intensity of more than 50% HR max for four weeks) and vitamin D supplementation (6,000 UI/week) could reduce inflammatory factors, increase anti-inflammatory proteins, and improve lung function in male smokers. Further larger trials with higher sample size and longer duration are suggested to confirm these results.

Abbreviations

Aerobic Exercise + vitamin D Supplementation (AE + VitD)

Aerobic Exercise (AE)

Vitamin D Supplementation (VitD)

Control (C)

Heart Rate (HR)

Tumor Necrosis Factor- α (TNF- α)

Interleukin-6 (IL-6)

Clara Cell Protein (CC16)

Surfactant Protein-D (SP-D)

Forced Expiratory Volume in one second (FEV₁)

Forced Vital Capacity (FVC)

Cigarette Smoking (CS)

Long Term Cigarette Smoking (LTCS)

Chronic Obstructive Pulmonary Disease (COPD)

1,25-dihydroxyvitamin D (1,25(OH)₂D)

Vitamin D Receptor (VDR)

Body Mass Index (BMI)

Maximum oxygen consumption (VO₂ max)

Analysis of Variance (ANOVA)

Analysis of Covariance (ANCOVA)

Toll-Like Receptors (TLRs).

Declarations

Ethics approval and consent to participate:

The protocol of the study was approved by the ethics committee of Tabriz university of medical sciences in accordance with the Helsinki Declaration (Ethical code: IR.TBZMED.REC.1399.727). A written consent was obtained from participants.

Consent to publish

Not applicable

Availability of data and materials

If requested, it will be available

Competing interests

No competing interests

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Authors' contributions

H.P and M.A designed the project and aided in interpreting the results; L.N directed the project, developed the theoretical framework, and drafted the manuscript; M.G performed the analysis, designed the figures and interpreted the results; H.P, H.N and L.N wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Figures

Figure 1

CONSORT Flow Diagram

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CONSORTstatement.doc](#)
- [CONSORTstatement.doc](#)