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The effects of pulsed magnetic field on the key elements responsible for synthesis and destruction of elastin-collagen in diabetic lung tissue

Cagil Coskun[1], Onur Tokgun[2], Murat Tokus[1], Figen Cicek[1], Isil Ocal[1], Ismail Gunay[1]

[1]Cukurova University, Faculty of Medicine, Department of Biophysics, Adana, Turkey
[2]Pamukkale University, Faculty of Medicine, Department of Medical Genetic, Denizli, Turkey

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Abstract

Changes in the expression levels of genes responsible for synthesis-destruction of elastin-collagen and the occurrence of lung diseases are in correlation. Although, diabetes-related complications are important health problems, the mechanism by which diabetes exerts this effect is unclear. On the other hand, although the effect of pulsed magnetic field (PMF) in lung diseases has been shown, its mechanism of action is unknown. The study aimed to determine the effects of PMF on the key regulator elements of the synthesis-destruction of elastin-collagen at the transcriptional level. Rats were divided into groups as control, control + PMF 10Hz, diabetes, diabetes + PMF 10Hz. PMF groups were exposed to 10 Hz PMF for four weeks. In diabetic conditions, ELN, ELANE, and COL1A1 genes were dysregulated at the transcriptional level as their levels were 14.23±2.56; 7.62±1.37 and 0.24±0.04, respectively. Dysregulated ELN gene expressions were decreased to 6.17±1.97 by PMF application. There were no meaningful changes in gene expressions in control + PMF 10 Hz groups. The present study shows that ELN, ELANE, and COL1A1 may play a key role at the transcriptional level in the mechanism of diabetic-lung diseases. In addition, it may be said that "PMF shows its effect by re-regulating the expression level of ELN gene in diabetic lung tissues". In future studies, ELN gene-targeted PMF application methods may be developed. Moreover, PMF application might not affect the genes that are responsible for elastin-collagen synthesis-destruction in healthy lungs when the PMF is applied on different tissues for the treatment of various diseases.

Keywords: Elastin, collagen, diabetes, gene expression, pulsed magnetic field

Introduction

Extracellular matrix (ECM) of lung tissue has a pivotal role in the maintaining of a healthy and functionally lung by its major components, elastin, collagen, and proteoglycans. They provide biomechanical properties of the lungs such as tensile strength, elasticity, and compliance [1-3]. Given the importance of critical roles, it is not surprising to say that their destructions, abnormal accumulations, or changes in their proportional compositions cause some lung diseases such as pulmonary fibrosis, emphysema, and chronic obstructive pulmonary disease (COPD) [4-6]. Therefore, the balance between the synthesis and destruction of elastin and collagen tissue should be in a constant manner for maintaining a proper healthy lung. On the other hand, dysregulations at the transcriptional levels of the coding genes of ECM synthesis and destruction proteins such as ELN (elastin), COL1A1 (collagen), ELANE (elastase), and MMP-1 (Matrix metalloproteinase 1), MMP-13 (Matrix metalloproteinase 13) are highly correlated with these diseases [7-10]. Considering the new treatment methods based on targeting RNA regulation [11], it is thought the modulation of these dysregulated genes to the normal level could be important for treatment of lung disease or preventing from them.

Diabetes mellitus, a complex and global medical problem worldwide, is one of the leading causes of death [12]. In addition, approximately %25 of the total health expenditures are spent on diabetes in the USA [13]. Therefore, preventing or treating the effects of diabetes and its related complications is highly crucial.

***Corresponding Author:** Cagil Coskun, Cukurova University, Faculty of Medicine, Department of Biophysics, Adana, Turkey
E-mail: ccoskun@cu.edu.tr

Diabetes causes serious complications by affecting many different systems and their subsystems in the body [14]. Diabetes can cause and increase the risk for lung diseases such as COPD pulmonary fibrosis, emphysema, or decreased lung function, though the exact molecular mechanism by which diabetes leads to these diseases is unknown, yet [15-18].

Pulsed magnetic field (PMF) application, a non-invasive and relatively cheap method, is widely used in the treatment of many diseases including neuropathic pain, bone healing, and nerve regeneration [19,20]. Its efficiency has also been demonstrated for lung diseases that PMF increased the functional capacity of lung tissue in COPD patients and also showed its therapeutic effect on patients with bronchial asthma child [21,22]. Although the PMF has been put forward as an alternative complementary method for the treatment of lung diseases, the exact mechanism by which PMF affects the lung tissue is still unknown. The present study aimed to primarily reveal the mechanism of action of PMF in the diabetic lungs by focusing on the transcriptional levels of the key elements of ECM in the lung tissue. Because PMF application modulates the gene expression levels and shows its therapeutic effect by regulating certain genes at the transcriptional level [23], it was hypothesized that PMF regulates the genes involving the synthesis and destruction of elastin and collagen in diabetic conditions.

Moreover, the effect of PMF on the gene expression levels of elastin-collagen synthesis and destruction elements which should be strongly balanced in healthy patients is also unknown. Because PMF is getting more used for the treatment of diseases apart from lung diseases, it's highly important to determine its effect on gene expression levels of ECM structures in healthy individuals. Therefore, the present study focused to determine the effects of PMF on the ECM balance of lung tissue at the transcriptional level in both diabetic and control tissues.

Materials and Methods

All animal procedures and animal care of the study were approved by the local ethic committee of Çukurova University the Faculty of Medicine Experimental Medicine Research and Application Center, Turkey (Approval No:24.08.2017-7).

Experimental groups and diabetes mellitus model

In the present study, 32 Wistar Albino rats were divided into 4 groups (n=8) as; Control, Control + PMF 10Hz, Diabetes, and Diabetes + PMF 10Hz and used as experimental animals. To create an experimental diabetes model, a single dose of (45 mg/kg) streptozotocin (STZ) (Sigma S0130) was injected intravenously through the tails of rats in diabetes groups, as described before [24]. Rats belonging to control groups were injected with equivalent physiological saline. Diabetes mellitus is highly correlated with hyperglycemia, and rats with high blood glucose concentration (>250 mg/dl) are described as diabetic [25]. Therefore, the blood glucose levels of each rat were measured regularly, starting 3 days after injections, every week using a glucometer until the end of the PMF applications.

Pulsed magnetic field application

In the present study, magnetic field applications were carried out throughout a similar system that was used before [26]. Rats in magnetic fields groups (Control + PMF 10Hz and Diabetes + PMF 10Hz) were exposed to 1.5 mT, 10Hz PMF for an hour a day for 4 weeks. 1.5mT PMF was used because of its previously demonstrated therapeutic efficiency [26]. The applied magnetic field program consisted of four consecutive trains, each with have 4 min duration with 1 min interval, that was repeated 3 times for an hour. During the magnetic field applications, 4 rats were kept in containers.

Tissue preparation

Animals were euthanized with high-dose isoflurane inhalation after 4 weeks of magnetic field applications. With the help of sterile surgical instruments, the lung tissue of each rat was dissected, removed, and cleaned in PBS medium. After the shock freezing processes performed in liquid nitrogen, the tissues were labeled and stored at 80°C until the further subsequent analysis. Lung tissues stored under suitable conditions were homogenized using Nucleosol (Macherey-Nagel, Duren, Germany) solution in a Dounce homogenizer (Sigma, D8938), and RNA isolation was carried out by considering the instructions of the kit manufacturer. The concentration of the isolated RNA samples measured by using a Nanodrop (Nanodrop 2000c, Thermo Scientific, USA), was in a range between 1.5–7 µg/µl. Therefore, each RNA sample was diluted to 1 µg/µl with RNase free-water to use at cDNA synthesis. Because it's highly vulnerable to degradation, isolated RNA samples were immediately converted to cDNA. For this purpose, High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) was used by taking into account the instructions of the kit manufacturer. The cDNA samples were stored at -20°C to be used in further gene expression analysis.

Real-time PCR experiments

In the present study, targeted genes (ELN, ELANE, COL1A1, MMP-1, and MMP-13) which play a crucial role in balancing between synthesis and destruction of lung extracellular matrix, expression levels were determined by analyzing the real-time PCR experiments. The primer pairs that amplify targeted gene regions were designed by considering certain criteria [27]. Moreover, a GAPDH gene was also used to normalize the gene expression data. Table 1 shows the primers used in the present study.

Ct (Cycle threshold) values belonging to target genes in each group were obtained by real-time PCR experiments using synthesized cDNA, designed primers, and GoTaq qPCR master mix (Promega, WI, USA). After the preparation of the reaction mix considering the kit's manual, the mix was exposed to the following thermal conditions; 95 °C for 2 min for a cycle, 95 °C for 15s, and 60 °C for the 60s for 40 cycles in Biorad CFX96 thermal cycler. To get melt curves, the temperature was gradiently increased from 60 °C to 95 °C at the end of the RT-PCR reaction. In addition, gene expression levels of each group were determined by the comparative delta-delta CT method (2-ΔΔCt) [28].

The experimental procedure of the study is briefly demonstrated in Figure 1.

Table 1. Primers used in the real-time PCR experiments

Primer name	Primer sequence (5'→3')	Amplification Length	NCBI accession code
R-ELNF	GTAGGCCTTGGAGGTGTGTC	199 bp	NM_012722.1
R-ELNR	CATAGGGCTTCGGGGGTTTT		
R-ELANEF	CTTTGAGAACGGCTTTGACC	211 bp	NM_001106767.1
R-ELANER	CACATTGAGCTCTTGGAGCA		
R-COL1A1F	GAGAGAGCATGACCGATGG	284 bp	NM_053304.1
R-COL1A1R	AGTTCCGGTGTGACTCGTG		
R-MMP1F	ACAGTTTCCCCGTGTTTCAG	224 bp	NM_001134530.1
R-MMP1R	CCCACACCTAGGTTTCCTCA		
R-MMP13F	ACCCAGCCCTATCCCTTGAT	215 bp	M_133530.1
R-MMP13R	GGCCCAGAATTTTCTCCCTCT		
R-GAPDHF	AAGATGGTGAAGGTCGGTGT	178 bp	NM_017008.4
R-GAPDHR	TGACTGTGCCGTTGAACTTG		

Statistical Analyses

One-way ANOVA analyses with the posthoc Tukey's test were performed using GraphPad 7.0 (GraphPad Software, San Diego, CA) to determine the difference between groups. Based on the Brown-Forsythe test the homogeneity of variances assumption has been met ($p<0.05$), and the Shapiro-Wilk test showed that there is no deviation from the normality assumption ($p<0.05$). Moreover, $p<0.05$ was set as the significance level.

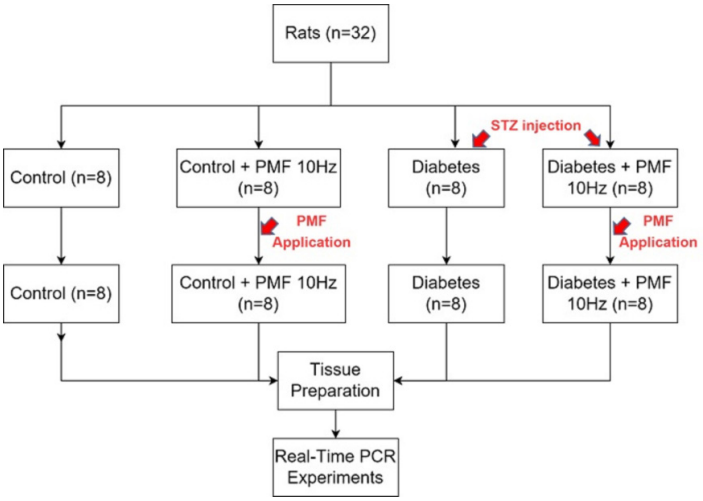

Figure 1. Diagram demonstration of the method section in the study

Results

In the present study, hyperglycemia (blood glucose level ≥250 mg/dl) was developed in each rat belonging to diabetes groups, from starting 3rd days of STZ injection. This diabetic condition continued until the end of the PMF applications. Blood glucose levels of control groups were in a normal physiological range of 80-100 mg/dl during the whole experimental procedure.

ELN, ELANE, COL1A1, MMP-1, and MMP-13 relative gene expression levels belonging to each group were obtained by comparing them with that of control's (Figure 2-6). Table 2 is also demonstrated the gene expression data values.

As a result of the analyses of the real-time PCR experiments, it was determined that the ELN gene expression level in the diabetes group was highly and significantly increased when compared with that in controls ($p<0.05$) (Figure 2). On the other hand, 10 Hz PMF application (Diabetes + PMF 10 Hz group) reversed this effect and the ELN gene expression level significantly decreased compared with the untreated diabetes group ($p<0.05$). In addition, PMF application to the healthy group (Control + PMF 10 Hz group) did not make any significant difference when compared to the control group.

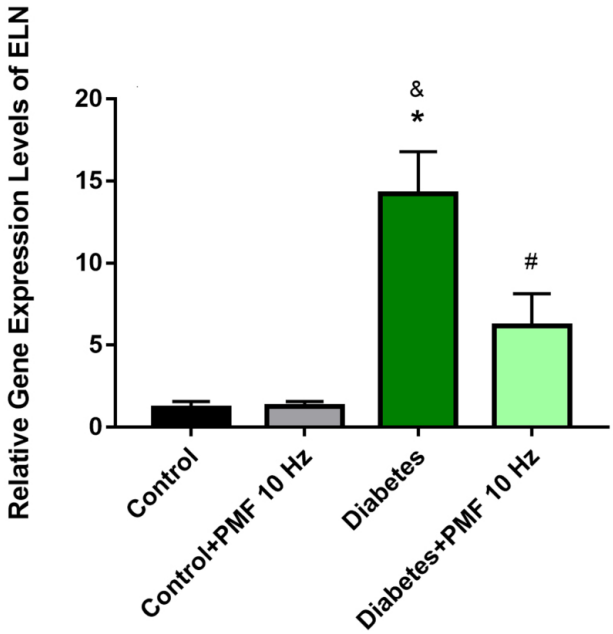

Figure 2. ELN gene expression levels of each group."*"p<0.05 vs. control group,"&"p<0.05 vs. control + PMF 10 Hz group,"#"p<0.05 vs. diabetes group. Data are presented as mean ± SEM (n=8)

Similar results were obtained when analyzed ELANE gene expression levels between groups (Figure 3) as the gene expression level was significantly increased in diabetic condition when compared to the control group ($p<0.05$). PMF application reversed and decreased its expression in the diabetes + PMF 10 Hz group, however, the decrease was not significant. Although the PMF application increased the ELANE gene expression level in healthy

groups, the change was not statistically significant as compared to the control's level.

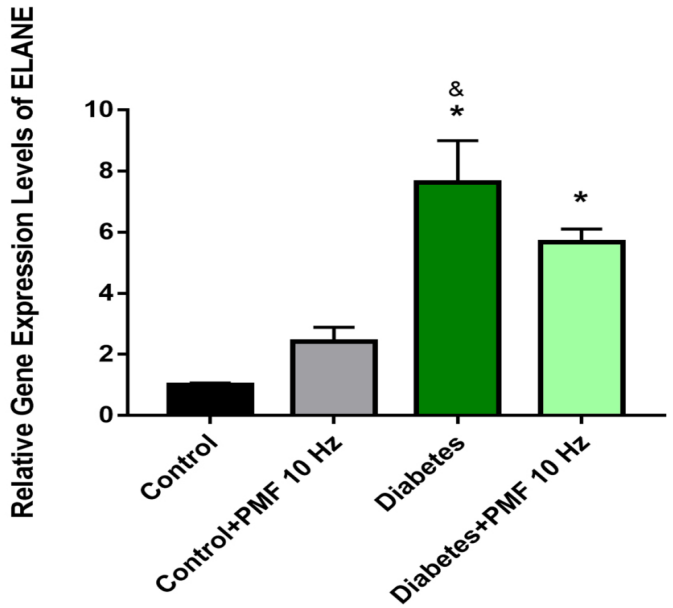

Figure 3. ELANE gene expression levels of each group."*"p<0.05 vs. control group,"&"p<0.05 vs. control + PMF 10 Hz group. Data are presented as mean±SEM (n=8)

The gene encoding collagen tip I protein, COL1A1 gene expression level significantly decreased in both diabetes and diabetes + PMF 10 Hz group when compared to the control group (p<0.05) (Figure 4). However, PMF exposure did not make any significant difference in diabetic conditions when compared with that in the diabetes group. Although COL1A1 gene expression level decreased in healthy individuals by PMF application, the difference was not significant as compared to the control group.

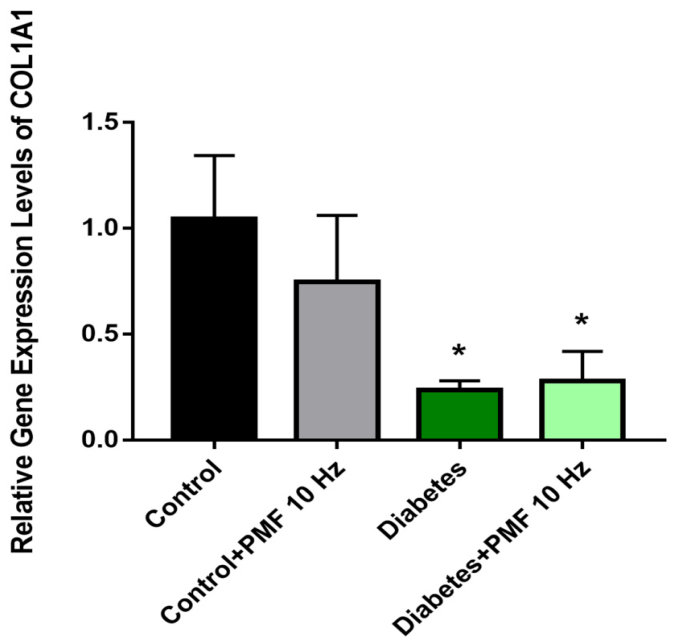

Figure 4. COL1A1 gene expression levels of each group." * " p<0.05 vs. control group. Data are presented as mean ± SEM (n=8)

Statistical analyses of the result of RT-PCR experiments revealed that MMP-1, which encodes collagenase, gene expression level increased in the diabetes group, but the change was not significant (Figure 5). In addition, magnetic field applications in both control and diabetes groups did not make any significant difference when compared to their control groups.

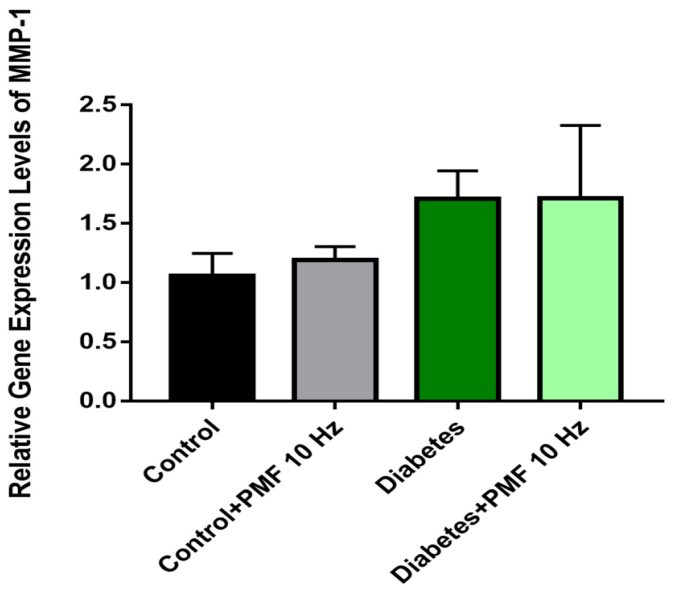

Figure 5. MMP-1 gene expression levels of each group. Data are presented as mean ± SEM (n=8)

MMP-13, another collagenase encoding gene, expression levels increased in diabetic conditions but the change was not statistically significant (Figure 6). PMF application decreased this expression level under the control's level but the change was not also statistically significant. As similar, the PMF application did not change the gene expression level in the control group.

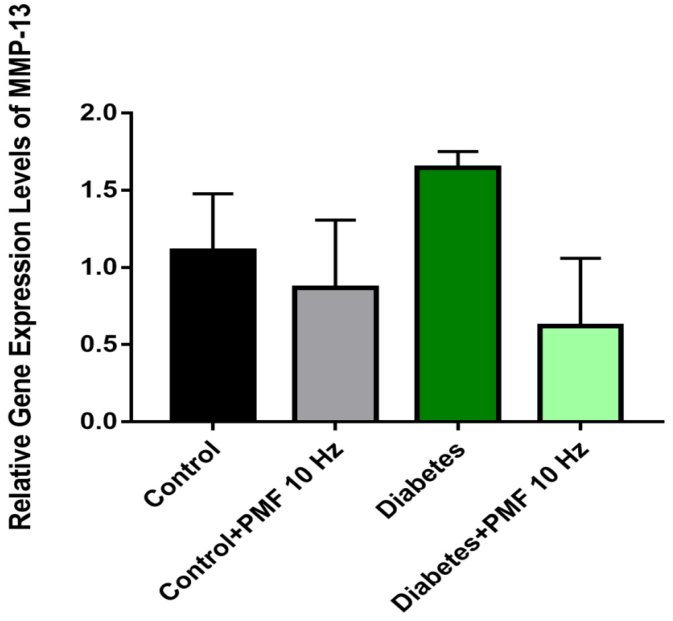

Figure 6. MMP-13 gene expression levels of each group. Data are presented as mean ± SEM (n=8)

Melting curve graphics obtained at the end of the real-time PCR experiments for each primer pair are also demonstrated in Figure 7. The melt curve analyses clearly show that primers used in all targeted gene expression, are specific and give specific amplification without the formation of primer-dimer.

Table 2. Relative gene expression data values of each gene. Data are mean ± SEM, n=8.

Gene	Control	Control+PMF 10 Hz	Diabetes	Diabetes + PMF 10 Hz
ELN	1.17±0.4	1.27±0.31	14.23±2.56(*.&)	6.17±1.97(#)
ELANE	1.002±0.06	2.41±0.48	7.62±1.37(*.&)	5.67±0.42(*)
COL1A1	1.044±0.29	0.74±0.31	0.24±0.04(*)	0.28±0.14(*)
MMP-1	1.056±0.19	1.189±0.11	1.705±0.24	1.712±0.61
MMP-13	1.107±0.37	0.87±0.43	1.645±0.10	0.62±0.44

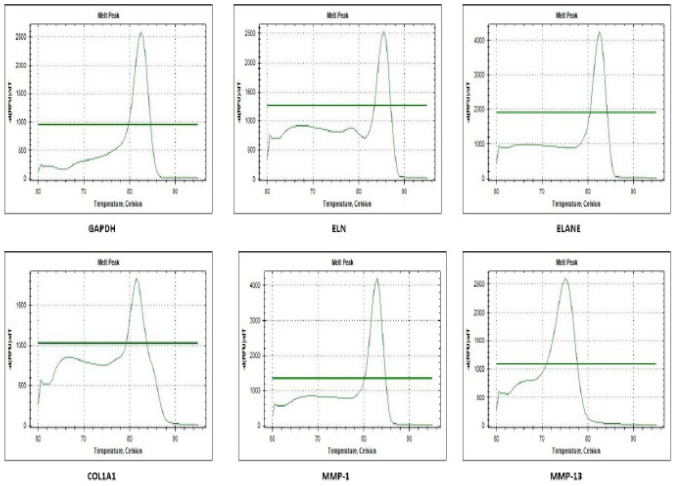

Figure 7. Melting curve plots were obtained after amplification of each gene (given as change in relative fluorescence unit versus temperature)

Discussion

The present study was conducted to investigate (1) to determine the expression levels of the certain genes that are responsible for synthesis or destruction of elastin and collagen in lung tissues at diabetic conditions, (2) to test the hypothesis that "pulsed magnetic field application shows its effect by re-regulating the genes that could be dysregulated in diabetes, (3) to evaluate whether PMF has any possible effect over the balanced level of elastin and collagen at the transcriptional level in controlled conditions. For these purposes, the expression level of targeted genes (ELN, ELANE, COL1A1, MMP-1, and MMP-13) for each group (Control, Control + PMF 10Hz, Diabetes, Diabetes + PMF 10Hz) were determined by the real-time PCR experiments.

As a result, it was determined that ELN gene expression increased significantly in the diabetes group. Previous studies showed that the gene expression increased in some lung diseases such as fibrosis and chronic obstructive lung disease [10,29,30]. Therefore, it may be asserted that the increase of ELN in the lung tissue at the transcriptional level, may be one of the mechanisms that cause lung diseases in diabetic conditions. Previous studies conducted on STZ-induced diabetic rat models have controversial results that ELN gene expression decreased in skeletal muscle [31]. However, it is well known that a specific gene can have a tissue-specific gene expression pattern [32]. In the present study, it was determined that the application of PMF to the diabetic rats (diabetes + PMF 10Hz group) significantly decreased the ELN gene expression as compared to the diabetes group. The decrease of the gene indicated that 10 Hz PMF application re-regulated the ELN gene which was dysregulated in diabetic conditions in lung tissue. This result may claim the acceptance of the hypothesis of the present study that PMF shows its effect by re-regulating certain genes at the transcriptional levels in diabetic conditions. In addition, the result of the unchanged gene expression level the in control + PMF 10 Hz group demonstrated that the magnetic field application has no noteworthy change on the ELN gene expression in the healthy lung tissue. Besides all these, it has been previously stated that the increase or decrease in ELN gene expression may not always correlate with the elastin fiber density in the tissue [29]. Therefore, further molecular genetic studies are necessary to better understand the correlation between the ELN gene expression and elastin fiber density after PMF application in diabetic lung tissue.

ELANE gene expression is highly increased in the diabetes group when compared to the level of control in the present study. Although there is no known previous study showing the ELANE gene expression in diabetic lung tissue, Xu et al. demonstrated the increase of the gene expression in neutrophils in diabetic obese patients [33]. Therefore, it can be claimed that the increase in ELANE in the present study may reveal the mechanism of occurrence for diabetic lung diseases. Previous studies showed that ELANE increased at the transcriptional level in emphysema which is caused by the destruction of elastin fibers [34]. Therefore, in the present study, the increase in the elastase coding gene ELANE, which is an enzyme that plays a crucial role in the degradation of elastin, might cause lung diseases by the destruction of elastin fibers. The dysregulation or increase of ELANE in the diabetes group was re-regulated and decreased by magnetic field application though the change was not statistically significant as compared to the diabetes group. Therefore, the hypothesis of the study was not statistically accepted for the ELANE gene even the PMF slightly regulated the ELANE at the transcriptional level. The fact that ELANE gene expression did not change statistically in the control + PMF 10 Hz group, magnetic field application did not affect this gene in healthy individuals.

In the present study, COL1A1 which is the gene that encodes collagen type-1 protein decreased at the transcriptional level in diabetic lung tissues. The result is compatible with the previous studies that Van Lunteren et. al. demonstrate the decreasing of the COL1A1 expression in diaphragm and lung tissues in STZ-induced animal models [35, 36]. Lehti et. al. also demonstrate the same changes in muscle tissues in a similar animal model [31]. Nevertheless, it is necessary to determine the relationship between the decrease in COL1A1 gene expression and its protein level in further studies. Pulsed magnetic field application did not change the COL1A1 gene

expression in diabetes groups. Therefore, it can be claimed that PMF had no regulatory effect over COL1A1 in diabetic conditions.

MMP-1 and MMP-13 are important genes in the degradation of collagen fibril structures[37]. Although their expression levels are slightly increased in diabetes, the differences were not statistically significant in this study. Previous studies conducted on STZ-induced models demonstrated that expression of the genes increased in glomerulus and aorta tissues [32,38], but decreased in ventricles [32]. Therefore, it can be claimed that tissue-specific expression patterns of these genes are also different. On the other hand, previous studies demonstrated that MMP-1 and MMP-13 gene levels increased in lung diseases such as emphysema and fibrosis [9, 39-41]. In this study, PMF did not significantly affect MMP-1 and MMP-13 gene expression levels. Therefore, the hypothesis of the present study is not accepted for these genes that PMF did not re-regulate the genes in diabetic conditions. On the other hand, PMF application did not significantly change these genes at the transcriptional level in control groups. It can therefore be claimed that pulsed magnetic field applications may not cause any changes in the expression levels of the genes in healthy lung tissue when the PMF is applied for the parts of the body except the lungs.

Conclusion

In conclusion, the present study showed the first time changes on transcriptional levels of the regulatory elements of the lung connective tissues in diabetic and healthy conditions with the presence or absence of PMF application. ELN, ELANE, and COL1A1 gene expression levels were dysregulated in diabetic conditions. Taking into account the changes of the genes that are responsible for the synthesis and construction of connective tissues, the result may have clarified the underlying mechanism of diabetic lung diseases. In future studies, new treatment methods targeting these genes can be developed for such diseases. After PMF application, the dysregulated ELN gene in diabetes was re-regulated back. Therefore, the hypothesis that "pulsed magnetic field application may show its therapeutic effect by re-regulating the genes involved in the synthesis and destruction of elastin-collagen in lung tissues in diseases" is highly acceptable for the ELN gene. The result may promote focusing on the new treatment methods that collocation of PMF application and targeted ELN gene in future studies. On the other hand, the present study also demonstrated the PMF application had no meaningful changes in all targeted genes in healthy individuals. Therefore, it may be claimed that even in cases where PMF application is used for different treatment purposes on different tissues, this application might not affect the genes responsible for elastin-collagen synthesis-destruction on healthy lung tissue.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
This study was supported by Cukurova University Scientific Research Council (TSA-2019-12160).

Ethical approval
All animal procedures and animal care of the study were approved by the local ethic committee of Çukurova University the Faculty of Medicine Experimental Medicine Research and Application Center, Turkey (24.08.2017; No:7).

References

1. Suki B, Stamenovic D, Hubmayr R. Lung parenchymal mechanics. Compr Physiol. 2011;1:1317-51.
2. Dunphy SE, Bratt JA, Akram KM, et al. Hydrogels for lung tissue engineering: Biomechanical properties of thin collagen-elastin constructs. J Mech Behav Biomed Mater. 2014;38:251-9.
3. Pelosi P, Rocco PR, Negrini D, et al. The extracellular matrix of the lung and its role in edema formation. An Acad Bras Cienc. 2007;79:285-97.
4. Laurent GJ. Lung collagen: more than scaffolding. Thorax. 1986; 41:418-28.
5. Maclay JD, McAllister DA, Rabinovich R, et al. Systemic elastin degradation in chronic obstructive pulmonary disease. Thorax. 2012;67:606-12.
6. Zhou Y, Horowitz JC, Naba A, et al. Extracellular matrix in lung development, homeostasis and disease. Matrix Biol. 2018;73:77-104.
7. Chen Y, Jiang P. Identification of key genes associated with idiopathic pulmonary fibrosis using bioinformatics analysis. 2016.
8. Imai K, Dalal SS, Chen ES, et al. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. Am J Respir Crit Care Med. 2001;163:786-91.
9. Nkyimbeng T, Ruppert C, Shiomi T, et al. Pivotal role of matrix metalloproteinase 13 in extracellular matrix turnover in idiopathic pulmonary fibrosis. PLoS One. 2013;8:73279.
10. Blaauboer ME, Boeijen FR, Emson CL, et al. Extracellular matrix proteins: a positive feedback loop in lung fibrosis? Matrix Biol. 2014;34:17-8.
11. Sahu I, Haque A, Weidensee B, et al. Recent Developments in mRNA-Based Protein Supplementation Therapy to Target Lung Diseases. Mol Ther. 2019; 27:803-23.
12. Tao Z, Shi A, Zhao J. Epidemiological Perspectives of Diabetes. Cell Biochem Biophys. 2015;73:181-5.
13. American Diabetes A. Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care. 2018;41:917-28.
14. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther. 2008; 88:1254-64.
15. Ehrlich SF, Quesenberry CP, Jr., Van Den Eeden SK, et al. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. Diabetes Care. 2010;33:55-60.
16. Hsia CC, Raskin P. Lung function changes related to diabetes mellitus. Diabetes Technol Ther. 2007; 9 Suppl 1:73-82.
17. Litonjua AA, Lazarus R, Sparrow D, et al. Lung function in type 2 diabetes: the Normative Aging Study. Respir Med. 2005;99:1583-90.
18. Khateeb J, Fuchs E, Khamaisi M. Diabetes and Lung Disease: A Neglected Relationship. Rev Diabet Stud. 2019;15:1-15.
19. Shupak NM, Prato FS, Thomas AW. Therapeutic uses of pulsed magnetic-field exposure: A review. URSI Radio Science Bulletin. 2003:9-32.
20. Mert T. Pulsed magnetic field treatment as antineuropathic pain therapy. Rev Neurosci. 2017;28:751-8.
21. Sadlonova J, Korpas J, Vrabec M, et al. The effect of the pulsatile electromagnetic field in patients suffering from chronic obstructive pulmonary disease and bronchial asthma. Bratisl Lek Listy. 2002;103:260-5.
22. Sadlonova J, Korpas J, Salat D, et al. The effect of the pulsatile electromagnetic field in children suffering from bronchial asthma. Acta Physiol Hung. 2003; 90:327-34.
23. Coskun C, Ocal I, Gunay I. A Low-Frequency Pulsed Magnetic Field Reduces Neuropathic Pain by Regulating NaV1.8 and NaV1.9 Sodium Channels at the Transcriptional Level in Diabetic Rats. Bioelectromagnetics. 2021;42:357-70.
24. Mert T, Ocal I, Guzel AI, et al. Clodronate changes neurobiological effects of pulsed magnetic field on diabetic rats with peripheral neuropathy. Electromagn Biol Med. 2013;32:342-54.
25. Craner MJ, Klein JP, Renganathan M, et al. Changes of sodium channel expression in experimental painful diabetic neuropathy. Ann Neurol. 2002;52:786-92.

26. Mert T, Altun I, Celik A, et al. Modulation of cytokine levels in ameliorative effects of pulsed magnetic field on an experimental model of Chronic Constriction Injury. Int J Radiat Biol. 2015;91:596-602.

27. Raymaekers M, Smets R, Maes B, et al. Checklist for optimization and validation of real-time PCR assays. J Clin Lab Anal. 2009;23:145-51.

28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402-8.

29. Deslee G, Woods JC, Moore CM, et al. Elastin expression in very severe human COPD. Eur Respiratory J. 2009;34:324-31.

30. Hoff CR, Perkins DR, Davidson JM. Elastin gene expression is upregulated during pulmonary fibrosis. Connect Tissue Res. 1999;40:145-53.

31. Lehti TM, Silvennoinen M, Kivelä R, et al. Effects of streptozotocin-induced diabetes and physical training on gene expression of extracellular matrix proteins in mouse skeletal muscle. Am J Physiol Endocrinol Metab. 2006;290:900-7.

32. Lu L, Zhang Q, Pu LJ, et al. Dysregulation of matrix metalloproteinases and their tissue inhibitors is related to abnormality of left ventricular geometry and function in streptozotocin-induced diabetic minipigs. Int J Exp Pathol. 2008;89:125-37.

33. Xu X, Su S, Wang X, et al. Obesity is associated with more activated neutrophils in African American male youth. Int J Obes (Lond). 2015;39:26-32.

34. Aggarwal N, Koepke J, Matamala N, et al. Alpha-1 Antitrypsin Regulates Transcriptional Levels of Serine Proteases in Blood Mononuclear Cells. Am J Respiratory Critical Care Med. 2016;193:1065-7.

35. Van Lunteren E, Moyer M. Gene expression profiling in the type 1 diabetes rat diaphragm. PLoS One. 2009;4:7832.

36. Van Lunteren E, Moyer M, Spiegler S. Alterations in lung gene expression in streptozotocin-induced diabetic rats. BMC Endocrine Disorders. 2014;14:5.

37. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog Mol Biol Transl Sci. 2017;147:1-73.

38. Surse VM, Gupta J, Tikoo K. Esculetin induced changes in Mmp13 and Bmp6 gene expression and histone H3 modifications attenuate development of glomerulosclerosis in diabetic rats. J Mol Endocrinol. 2011;46:245-54.

39. Rosas IO, Richards TJ, Konishi K, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. PLoS Med. 2008;5:93.

40. Konishi K, Gibson KF, Lindell KO, et al. Gene expression profiles of acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;180:167-75.

41. Woode D, Shiomi T, D'Armiento J. Collagenolytic matrix metalloproteinases in chronic obstructive lung disease and cancer. Cancers (Basel). 2015;7:329-41.