The State of the Nitric Oxide Cycle in Respiratory Tract Diseases

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This review describes the unique links of the functioning of the nitric oxide cycle in the respiratory tract in normal and pathological conditions. The concept of a nitric oxide cycle has been expanded to include the NO-synthase and NO-synthase-independent component of its synthesis and the accompanying redox cascades in varying degrees of reversible reactions. The role of non-NO-synthase cycle components has been shown. Detailed characteristics of substrates for the synthesis of nitric oxide (NO) in the human body, which can be nitrogen oxides, nitrite and nitrate anions, and organic nitrates, as well as nitrates and nitrites of food products, are given. The importance of the human microbiota in the nitric oxide cycle has been shown. The role of significant components of nitrite and nitrate reductase systems in the nitric oxide cycle and the mechanisms of their activation and deactivation (participation of enzymes, cofactors, homeostatic indicators, etc.) under various conditions have been determined. Consideration of these factors allows for a detailed understanding of the mechanisms underlying pathological conditions of the respiratory system and the targeting of therapeutic agents. The complexity of the NO cycle with multidirectional cascades could be best understood using dynamic modeling.

1. Introduction

Nitric oxide (NO), until recently, was known mainly as a toxic gas in the atmosphere of large cities and was considered primarily, even almost totally, from the point of view of environmental pollution [1].

However, a large number of studies in the field of vascular physiology, pathophysiology, neurology, biochemistry, pharmacology, and immunology have convincingly shown that this molecule is synthesized in living organisms and has a broad spectrum of bioregulatory action [2–4].

In 1991, Gustafsson et al. found nitric oxide in exhaled air in animals and healthy people [5], and further changes in NO level in exhaled air were detected in a number of respiratory system diseases (bronchial asthma, bronchiectasis, systemic connective tissue diseases, sleep apnea syndrome, pulmonary tuberculosis, complications after lung transplantation, and cystic fibrosis) [2, 3, 6]. The evidence based on the contribution of NO to the pathogenesis of many respiratory tract diseases has been accumulated [7].

Currently, it is believed that NO is formed from arginine by NO synthase and enters into many competing reactions that are presented in Scheme 1.

1.1. NO in Respiratory Tract Pathologies. Exhaled nitric oxide (FeNO) is a sensitive, reproducible, and noninvasive marker of eosinophilic airway inflammation. Accordingly, FeNO is widely used to diagnose and monitor the effectiveness of therapy in the treatment of asthma. In patients with asthma, the high content of FeNO which decreased in response to treatment by corticosteroids was revealed. An increase in the FeNO content in mild and moderate bronchial asthma (BA) during exacerbation in comparison with the control and a group of patients with severe BA has been shown. In
In patients in the latter group, the concentration of FeNO was at the level of control values. Currently, it is associated with a direct inhibitory effect of oral and inhaled glucocorticosteroids on NOS, as well as changes in the level of cytokines and a modification of the inflammatory response [10–12].

FeNO measurement has been shown to be an alternative diagnostic tool compared to conventional lung function tests for the diagnosis of bronchial asthma [10, 11]. Analysis of NO metabolites revealed that the concentration of nitrotyrosine in exhaled breath condensate (EBC) increases with mild asthma. In the same study, its level was reduced in moderate and severe BA compared to the control group. The nitrotyrosine content correlated with the level of FeNO only in mild BA. At the same time, the study of another nitric oxide metabolite, nitrosothiol (RS-NO), showed an increase in its concentration with moderate asthma compared to the control group. The authors suggest that a decrease in the concentration of NO in exhaled air in patients with CF may be due to the peculiarities of NO metabolism in airway secretions [23].

Interesting data were obtained by studying the possibility of using indicators of FeNO and inflammatory markers (IM) in EBC (pH, nitrites, nitrates, hydrogen peroxide (H₂O₂), 8-isoprostanate, and Th1/Th2 cytokines) to detect (exacerbate) CF and by studying the ability of these noninvasive IMs to indicate the CF severity [24]. It was found that in CF, the concentration of interferon (IFN-c) and nitrite in EBC was significantly higher, while the levels of FeNO were lower compared to the control. When using multivariate logistic regression models, the presence of CF was best indicated by 8-isoprostanate, nitrite, and IFN-c. Exacerbation of CF was best indicated by 8-isoprostanate and nitrite. The most significant biomarkers of CF severity were FeNO and pH of the condensate. Thus, the authors believe that this combination of different exhaled IMs can indicate the presence (exacerbation) of CF and the severity of the disease in children [24].

Based on our experience in studying NO metabolism in various diseases of the respiratory tract, it seems promising to search and study the most significant metabolites of nitric oxide and their relationships with each other and with other disease markers. These studies may turn out to be the most diagnostically valuable approach in understanding the pathogenetic mechanisms of the development of respiratory pathologies, including CF.

Despite the fact that to date, there is a huge amount of evidence on the generation of NO and its metabolites in various respiratory pathologies, the exact contribution of NO and/or its metabolites to the inflammatory diseases of the
The Pathways of Nonenzymatic Synthesis of NO from NO-Synthase-Independent Component of the Nitric Oxide Cycle. Despite the fact that chemical reactions accompanied by the release of nitrogen oxides were studied in inorganic chemistry for a long time, their place in physiological processes, before the discovery of endogenous NO synthesis, seemed impossible.

The following nitrogen oxides are known: nitrogen oxide (I) $N_2O$, nitrogen oxide (II) NO, nitrogen oxide (III) $N_2O_3$, nitrogen oxide (IV) NO$_2$, and nitrogen oxide (V) $N_2O_5$, in which oxidation states from +1 to +5 are exhibited. Oxides NO, NO$_2$, $N_2O_3$, and $N_2O_5$ quite easily turn into each other, and these transformations are considered as nonenzymatic reactions of nitric oxide. Almost all of these reactions are reversible reactions [8]. The forward and reverse reactions have different rate constants ($k$), and, depending on the microenvironment conditions, concentrations of substrates and products differ [8].

In this regard, it is possible to regulate the concentration of nitric oxide in specific local conditions in vivo. The dissolution coefficients of NO, in particular, are different for the hydrophobic and hydrophilic phases. Therefore, the phase...
component of the regulation of these reactions is of particular importance (Figure 2).

The following reactions are known for producing NO. For example, nitric oxide is formed in the reaction of HNO3 interaction with some metals [29], in particular, with copper, which is included in the active centers of a number of enzyme systems:

\[ 3\text{Cu} + 8\text{HNO}_3 \rightarrow 3\text{Cu(NO}_3)_2 + 2\text{NO} + 4\text{H}_2\text{O} \quad (1) \]

The source of nitric oxide are other nonenzymatic reactions:

\[ \text{FeCl}_2 + \text{NaNO}_2 + 2\text{HCl} \rightarrow \text{FeCl}_3 + \text{NaCl} + \text{NO} + \text{H}_2\text{O} \]
\[ 2\text{HNO}_2 + 2\text{HI} \rightarrow 2\text{NO} + \text{I}_2 + 2\text{H}_2\text{O} \quad (2) \]

These reactions occurring in vivo are usually not considered. However, a number of authors registered the release of NO in the presence of hydrochloric acid, in particular, when the nitrite of saliva hits the stomach [30, 31].

There is a small amount of information collected from the study of the role and changes in the content of other nitrogen oxides in patients with lung diseases.

In particular, the effect on NO2 of the lungs as an air pollutant, which causes the formation of free radicals, lipid peroxidation, oxidative damage to proteins, increased proliferation, production of proteases by macrophages, etc., has been explored [32]. It is also shown that the short-term effect of NO2 has a negative impact on the parameters of lung function, and it increases the concentration of isoprostane-8 in EBC among students without respiratory pathologies. A direct correlation was found between the concentration of NO2 and the content of isoprostane-8 and the inverse association between FEV1 (forced expiratory volume in one second) and NO2 [33]. The elevated NO2 concentrations in the air have been shown to increase the frequency of asthma exacerbations and rhinoconjunctivitis in the urban population [34, 35].

1.3. Organic Nitrates as a Substrate for the Synthesis of NO in the NOS-Independent Component of the Nitric Oxide Cycle. A number of substances which increase NO availability have been applied successfully as medicine. Nitroglycerine (NG) (1,2,3-trinitroksipropan), being an ester of glycerin and nitric acid and a pharmacological medicine from the group of organic nitrates, has been used for over 100 years. The main property of these drugs is the ability to cause relaxation of vascular smooth muscle. Organic nitrates, as well as sydnonimines (molsidomine), are nitrovasodilators. Organic nitrates (nitroglycerin, isosorbide dinitrate, and isosorbide mononitrate) are the most common in clinical practice [36]. The effects of these compounds, mediated by the functioning of nitrate/nitrite reductase components, correspond to the proposed concept of the nitric oxide cycle. It is believed that the main active compound in the application of organic nitrates is NO or S-nitrosothiols, followed by activation of guanylate cyclase (GC). It is through the reaction with GC that the role of NO as a signaling molecule is realized. The activation of GC leads to the transformation of the Mg+2-2-GMP complex into cGMP and subsequent activation of cGMP-dependent protein kinases, which ends by the regulation of phosphodiesterase and ion channel activity [37].

However, the molecular process of the biotransformation of NG to NO has not yet been studied. Many works consider only the effects associated with an already formed NO. It has been shown that the biotransformation products in NG tissues are 1,2-glycerol dinitrate, 1,3-glycerol dinitrate, nitrite anion, and NO or S-nitrosothiols. A number of intracellular enzymes are considered as the alleged participants in the NG reduction reaction, namely, glutathione S-transferase, cytochrome P450 reductase, cytochrome P450, and xanthine oxidase [38]. The role of mitochondrial aldehyde dehydrogenase, as a participant in NG biotransformation, was also noted [38]. In addition, it was shown that under conditions...
of acute hypoxia, the release of NO from organic nitrates increases, clarifying their selective effect in ischemic foci [39].

The influence of the use of organic nitrates on the NO cycle in inflammatory lung diseases has not been studied. The effect of NO donors on pulmonary arterial hypertension has been shown [40, 41]. Intravenous use of 1,2-propanediol on a model of pulmonary embolism in rabbits has been found to increase the concentration of FeNO, unlike inorganic nitrite, and prevent lung hypertension [42]. A dose-dependent increase in FeNO in healthy lambs after intravenous administration of a trinitroglycerin or a nitroprusside has been noted [43].

1.4. Microbiota as a Participant in the Nitric Oxide Cycle. Natural microbiota can have a significant effect on the metabolism of many substances and processes in the host organism [44]. The transformation, in particular, of the respiratory microbiota with the consolidation of representatives of conditionally pathogenic and pathogenic flora is associated with exacerbations and severity of many chronic diseases of the respiratory tract [45, 46].

It is known that a number of bacteria are able to release gaseous nitrogen oxides. Electron acceptors with high redox potential, such as nitrite and nitrate anions, are often used as reaction substrates. The reduction of nitrate to gaseous nitrogen oxides, the so-called nitrate respiration or denitrification, is a process of anaerobic respiration of bacteria. A series of enzymes is involved in the chain of reactions shown in Scheme 2: nitrate reductase, NO-forming nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, respectively [45–47].

Denitrifying bacteria are widespread. The ability to denitrify was found in representatives of more than 40 genera of eubacteria and only in one group of archaeabacteria. Among eubacteria, gram-negative proteobacteria of the genera *Pseudomonas*, *Alcaligenes*, *Paracoccus*, *Hyphomicrobium*, and *Thauera*, as well as some representatives of gram-positive bacteria of the genus *Bacillus*, have the ability to denitrify [47].

Theoretically, these pathways for the formation of nitrite and NO are also relevant in the human body. However, this issue remains virtually unexplored. Before the discovery of the endogenous synthesis of NO from arginine, the presence of nitrite in the human body was associated exclusively with the activity of the microflora of the gastrointestinal tract, although the presence of nitroso compounds in human tissues and fluids was known for a very long time [8]. A number of studies have been shown that oral microflora can reduce nitrates to nitrites and, lastly, to NO with subsequent regulation of blood pressure [48, 49].

Dietary and metabolic nitrates enter saliva from the blood through their active accumulation in the salivary glands and are reduced to nitrite and NO in the oral cavity by the action of local microbiota. It has also been shown that when consuming foods rich in nitrates, the formation of NO in the oral cavity leads to an increase in FeNO. The authors suggest that such data may be misinterpreted as an enhancement in inflammatory activity in the respiratory tract [50].

Also, the avalanche-like growth of NO metabolites in the blood during septic shock may be related to the degree of bacteriuria and/or the type of microflora [51]. Accordingly, the microbiota of the skin, gastrointestinal and respiratory tracts, etc., as well as their quantitative and qualitative changes in various physiological/pathophysiological processes, affect the nitric oxide cycle. More and more studies are devoted to studying the composition of the microbiota of the respiratory tract in various lung diseases [52–55].

Some pathogens, including *P. aeruginosa*, have a genetically determined ability to denitrify. The ability of microorganisms to use a wide range of electron acceptors to generate ATP provides them with metabolic flexibility in transitional environments, as these organisms live in different habitats. Kolpen et al. demonstrated the relationship between nitric oxide metabolites and the respiratory tract microbiota [56, 57]. In particular, it was demonstrated that in cystic fibrosis in the anaerobic zones of endobronchial mucus, denitrification processes occur. The level of N₂O was used as a marker of denitrification. The significant generation of N₂O was found in the sputum of patients with CF with chronic *P. aeruginosa* infection. In this process, there was a decrease in initially high levels of NO₃⁻ and NO₂⁻ in sputum. According to the authors, these data indicate that denitrification can serve as an alternative metabolic pathway that allows *P. aeruginosa* to successfully develop in airway microniches with a lack of oxygen in CF patients [56].

It has been shown that the ability of microorganisms to perform denitrification correlates with their pathogenicity in CF. In the study of infectious isolates from 32 patients with CF, it was found that all the studied pathogens (*P. aeruginosa*, *Achromobacter xylosoxidans*, *Burkholderia multivorans*, and *Stenotrophomonas maltophilia*) grow under anaerobic conditions with the consumption of NO₃⁻. However, denitrification recorded for N₂O production was detected for *P. aeruginosa*, *Achromobacter xylosoxidans*, and *Burkholderia multivorans*, but was not found in *S. maltophilia* isolates. The ability to conduct denitrification may contribute to the pathogenicity of infectious isolates since complete denitrification promotes the most rapid anaerobic growth. The inability of *S. maltophilia* to multiply during denitrification and, therefore, to grow in an anaerobic environment with CF may explain its low pathogenicity in these patients [57].

Further study of the possible relationships between the composition of the microbiota and the components of the nitric oxide cycle in lung diseases seems promising for understanding the mechanisms of pathogenesis, as well as targeted treatment and prevention of respiratory diseases.

1.5. Inorganic Nitrates and Nitrites as a Substrate for the Synthesis of NO in the NOS-Independent Component of the Nitric Oxide Cycle. Nitrate and nitrite anions have been considered by a number of researchers as stable metabolites of nitric oxide and are present in living organisms in micromolar
amounts [58]. In addition, a different, but a significant amount of these ions comes from food and water. Poisoning with an excessive content of nitrates and nitrites proves their active participation in metabolic processes in a living organism, by embedding in the links of the nitric oxide cycle [59].

It is known that eukaryotes, in particular, fungi and liver cells of animals are capable of releasing N₂O, especially in an environment with nitrites. Unlike bacteria, as described above, this process, however, is not associated with obtaining energy and is carried out to detoxify the body from nitrites [46]. In plants, the assimilative reduction of nitrate to nitrite is used in biosynthesis reactions [60]. In humans, these processes are poorly understood. Some studies have shown that nitrates and nitrites can be substrates for the synthesis of NO. The generation of nitric oxide by the skin when applying the nitrite solution to it was noted [61]. Matsunaga and Furchgott demonstrated the myorelaxation of an isolated rabbit aorta in a medium with sodium nitrite [62].

The main applicants for nitrite and nitrate reductase activity are the same groups of enzymes that are considered as in NG biotransformation. It has been shown that hem-containing proteins are capable of converting nitrates to NO with various rate constants (Figure 3). Among the currently studied hemoproteins, neuroglobin has the highest rate of conversion of nitrite to nitric oxide (Figure 3) [63, 64].

Neuroglobin is one of the vertebrate globin proteins involved in maintaining the gas homeostasis of the cell. It is an intracellular hemoprotein expressed in the central and peripheral nervous system, cerebrospinal fluid, retina, and endocrine tissues [64].

It is shown that hemoproteins are involved in NO₂ reduction under hypoxic conditions at the site of protein expression similar to bacterial nitrite reductases. Poisoning by nitrates and nitrites is manifested primarily in methemoglobinemia with associated clinical symptoms, which is associated with the oxidation of heme iron under the action of excess nitrates/nitrites [64].

In our work studying the content of metabolites of the nitric oxide cycle in various diseases of the respiratory organs and comorbid conditions, it has been shown that among NO metabolites, nitrate anions have the highest concentration [65, 66]. The levels of nitrite anion vary from undetectable values (especially in EBC) to 20% of the total concentration of nitrate and nitrite anions. The ratio of nitrate to nitrite anions varies depending on the pathophysiological conditions [65, 66]. The concentrations also vary significantly depending on the biological environment under study, from units of μM in EBC to hundreds of mM in urine [67].

We have studied the relationship of the components of the nitric oxide cycle in Chernobyl clean-up workers with COPD during antioxidant therapy with N-acetylcysteine (NAC) [68]. In all patients, NO₃⁻ and NO₂⁻ were measured in EBC before and after antioxidant therapy with NAC at a dose of 600 mg per day for 3 months, taken in addition to standard therapy. A change in the nature of the relationship between the content of nitrate and nitrite anions during treatment was revealed. Prior to the course of antioxidant therapy, a nonlinear relationship was observed, and at the end of therapy, a linear relationship was registered between the levels of metabolites. Thus, the findings indicate a different role of nitrite and nitrate anions in the nitric oxide cycle [68].

Due to the role of low oxygen concentration in the activation of nitrite reductase components of the NO cycle, we studied the effect of hypoxic conditions on the cycle parameters. Interval hypoxic training (IHT) was used as a model of hypoxia. Hypoxic training is used to improve physical

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**Figure 3**: The action of globin proteins in the human nitrite reductase system. The figure shows the formation rate constants ($k$) of nitric oxide for various hemoproteins (Hb, Mb, and Ngb) of their nitrite anion. Neuroglobin has the highest rate of conversion of nitrite to nitric oxide (marked in red).
performance and improve the function of vital systems in extreme conditions and is also used in the treatment of various diseases. In children with mild bronchial asthma, during a three-week IHT, an increase in the total concentration of nitrates and nitrites in EBC was found compared to the initial level. At the same time, they showed an improvement in the clinical state assessed by standardized assessment scales of the therapy [69].

1.6. Dynamic Modeling of NO Cycle. Taking into account the huge range of physiological effects of NO, there is obviously a need for the regulation of its cycle. For this purpose, a detailed understanding of the relationships of its significant components, the consistent patterns of their change, and the possibility of the numerical prediction of their concentrations are required. In this regard, there is a need for simulation modeling of NO cycling. It will give the chance to develop much more effective algorithms for the targeted effects of therapeutic agents and predict response changes in physiological parameters. Therefore, one of the main goals of modeling is the resolution of a huge number of apparent contradictions in the accumulated experimental data on NO metabolites [70–72].

The NO cycle is a complexly regulated system in which most reactions are reversible, depending on a large number of conditions. Also, numerous loops of negative and positive feedback are present in the NO cycle. Therefore, nonlinear modeling is required for an adequate approximation of such a system [70–72]. For dynamic modeling, an accurate numerical estimate of the activity of NO-synthase and nitrite reductase systems is required, depending on the presence/level of hypoxia, the intracellular concentration of ions, the ratio of hydrophobic and hydrophilic phases, the presence/absence of inflammation, etc. Creating a dynamic model of the NO cycle will allow the evaluation of possible modulating effects on the system in order to maintain or enhance the protective and physiological effects of the components of the cycle and/or limit their damaging effects.

2. Conclusion

The nitric oxide cycle is a highly regulated system of key importance in the functioning of the body. The formation of nitric oxide is possible without the participation of NO-synthases. Nitrate and nitrite anions can be considered as substrates in the nitrite and nitrate reductase units of the nitric oxide cycle.

Clarifying the role of the significant components of nitrite and nitrate reductase systems in the nitric oxide cycle, the mechanism of their activation and deactivation (participation of enzymes, cofactors, homeostatic indicators, etc.) under various conditions allows detailing the principles of NO cycle control. This specification will serve as the basis for the targeted effects of therapeutic agents in a variety of pathological processes of the respiratory system associated with an imbalance of multidirectional production cascades and NO modification. In the future, to understand the interrelationships of significant components of the NO cycle, the patterns of their change, and the possibility of the numerical prediction of their concentrations, there arises a need for dynamic modeling. Describing the NO cycle and its relationship to oxidative stress will enable the development of algorithms for effective diagnosis and treatment of respiratory tract diseases.

Conflicts of Interest

The authors declare that there is no potential conflict of interest associated with this manuscript.

Authors’ Contributions

Each of the authors contributed equally to the writing of the manuscript.

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References

[1] L. B. Borisova and A. I. Burkhanov, “Experimental study of the toxicity of nitric oxide,” Gigiena i sanitariya, no. 4, pp. 89–90, 1985.
[2] K. Ashutosh, “Nitric oxide and asthma: a review,” Current Opinion in Pulmonary Medicine, vol. 6, no. 1, pp. 21–25, 2000.
[3] A. Dhir and S. K. Kulkarni, “Nitric oxide and major depression,” Nitric Oxide, vol. 24, no. 3, pp. 125–131, 2011.
[4] X. Han, M. P. Fink, T. Uchiyama, R. Yang, and R. L. Delude, “Increased iNOS activity is essential for pulmonary epithelial tight junction dysfunction in endotoxemic mice,” American Journal of Physiology Lung Cellular and Molecular Physiology, vol. 286, no. 2, pp. L259–L267, 2004.
[5] L. E. Gustafsson, A. M. Leone, M. G. Persson, N. P. Wiklund, and S. Moncada, “Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans,” Biochemical and Biophysical Research Communications, vol. 181, no. 2, pp. 852–857, 1991.
[6] P. J. Barnes and M. G. Belvisi, “Nitric oxide and lung disease,” Thorax, vol. 48, no. 10, pp. 1034–1043, 1993.
[7] H. Sugiuira and M. Ichinose, “Nitrate stress in inflammatory lung diseases,” Nitric Oxide, vol. 25, no. 2, pp. 138–144, 2011.
[8] A. A. Nedospasov, “Is N2O3 the main nitrosating intermediate in aerated nitric oxide (NO) solutions in vivo? If so, where, when, and which one?,” Journal of Biochemical and Molecular Toxicology, vol. 16, no. 3, pp. 109–120, 2002.
[9] A. F. Vanin, R. R. Borodulin, and V. D. Mikoyan, “Dinitrosyl iron complexes with natural thiol-containing ligands in aqueous solutions: synthesis and some physico-chemical characteristics (a methodological review),” Nitric Oxide, vol. 66, no. 66, pp. 1–9, 2017.
[10] K. Alving, E. Weitzberg, and J. M. Lundberg, “Increased amount of nitric oxide in exhaled air of asthmatics,” The European Respiratory Journal, vol. 6, no. 9, pp. 1368–1370, 1993.
[11] S. A. Kharitonov, D. Yates, R. A. Robbins, R. Logan-Sinclair, E. A. Shinebourne, and P. J. Barnes, “Increased nitric oxide in exhaled air of asthmatic patients,” The Lancet, vol. 343, no. 8890, pp. 133–135, 1994.
[12] B. Gaston, J. Drazen, C. B. E. Chee, M. E. B. Wohl, and J. S. Stamler, "Expired nitric oxide concentrations are elevated in patients with reactive Airways disease," *Endothelium*, vol. 1, pp. 87–92, 1993.

[13] S. A. Khartitonov and P. J. Barnes, "Biomarkers of some pulmonary diseases in exhaled breath," *Biomarkers*, vol. 7, no. 1, pp. 1–32, 2002.

[14] C.-m. Xie, F.-j. Chen, X.-y. Huang, Y.-l. Liu, and G.-p. Lin, "Importance of fractional exhaled nitric oxide in the differentiation of asthma—COPD overlap syndrome, asthma, and COPD," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 11, no. 11, pp. 2385–2390, 2016.

[15] N. Kubysheva, S. Soodaeva, L. Postnikova, V. Novikov, A. Maksimova, and A. Chuchalin, "Associations between indicators of nitrosative stress and levels of soluble HLA-I, CD95 molecules in patients with COPD," *COPD*, vol. 11, no. 6, pp. 639–644, 2014.

[16] N. I. Kubysheva, L. B. Postnikova, S. K. Soodaeva et al., "The significance of soluble molecules of cellular adhesion, nitric oxide metabolites, and Endothelin-1 and their associations as markers of progression of inflammation in COPD," *Sovremennye tehnologii v medicine*, vol. 9, no. 2, pp. 105–117, 2017.

[17] M. F. Beg, M. A. Alzoghbi, A. A. Abba, and S. S. Habib, "Exhaled nitric oxide in stable chronic obstructive pulmonary disease," *Annals of Thoracic Medicine*, vol. 4, no. 2, pp. 65–70, 2009.

[18] N. Bazezgi, T. A. Gerds, E. Budtz-Jørgensen, J. Hove, and J. Vestbo, "Exhaled nitric oxide measure using multiple flows in clinically relevant subgroups of COPD," *Respiratory Medicine*, vol. 105, no. 9, pp. 1338–1344, 2011.

[19] C. Brindicci, K. Ito, O. Resta, N. B. Pride, P. J. Barnes, and S. A. Kharitonov, "Exhaled nitric oxide from lung periphery is increased in COPD," *The European Respiratory Journal*, vol. 26, no. 1, pp. 52–59, 2005.

[20] B. Balint, S. A. Kharitonov, T. Hanazawa et al., "Increased nitrotyrosine in exhaled breath condensate in cystic fibrosis," *The European Respiratory Journal*, vol. 17, no. 6, pp. 1201–1207, 2001.

[21] K. L. Jones, A. H. Hegab, B. C. Hillman et al., "Elevation of nitrotyrosine and nitrate concentrations in cystic fibrosis sputum," *Pediatric Pulmonology*, vol. 30, no. 2, pp. 79–85, 2000.

[22] N. Anil, M. Singh, A. Rajwanshi, and H. Vohra, "Induced sputum nitrites correlate with FEV1 in children with cystic fibrosis," *Acta Paediatrica*, vol. 99, no. 5, pp. 711–714, 2010.

[23] H. Grasemann, I. Ioannidis, R. P. Tomkiewicz, H. de Groot, B. K. Rubin, and F. Ratjens, "Nitric oxide metabolites in cystic fibrosis lung disease," *Archives of Disease in Childhood*, vol. 78, no. 1, pp. 49–53, 1998.

[24] C. M. H. T. Robroeks, P. P. R. Rosia, D. van Vliet et al., "Biomarkers in exhaled breath condensate indicate presence and severity of cystic fibrosis in children," *Pediatric Allergy and Immunology*, vol. 19, no. 7, pp. 652–659, 2008.

[25] A. Van der Vliet, J. P. Eiserich, and C. E. Cross, "Nitric oxide: a pro-inflammatory mediator in lung disease?*, *Respiratory Research*, vol. 1, no. 2, pp. 67–72, 2000.

[26] S. Soodaeva, T. Li, I. Klimanov et al., "The cycle of the nitric oxide metabolism in patients with COPD and chronic cerebrovascular diseases (CCVD)," *European Respiratory Journal*, vol. 42, article P646, Supplement 57, 2013.

[27] S. Soodaeva, T. Li, I. Klimanov et al., "Nitric oxide metabolism in COPD comorbidities," *European Respiratory Journal*, vol. 44, article P3822, Supplement 58, 2014.

[28] S. K. Soodaeva, I. A. Klimanov, T. V. Li et al., "Changes in nitric oxide metabolism in co-morbidity of chronic obstructive pulmonary disease and chronic cerebral ischemia," *Russian Pulmonology*, no. 1, pp. 31–34, 2012.

[29] J. E. House, *Inorganic Chemistry*, Elsevier, 2nd edition, 2013.

[30] H. H. Björne, J. Petersson, M. Phillipson, E. Weitzberg, L. Holm, and J. O. Lundberg, "Nitrite in saliva increases gastric mucosal blood flow and mucus thickness," *The Journal of Clinical Investigation*, vol. 113, no. 1, pp. 106–114, 2004.

[31] C. Duncan, H. Dougall, P. Johnston et al., "Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate," *Nature Medicine*, vol. 1, no. 6, pp. 546–551, 1995.

[32] J. Kleinerman, "Some effects of nitrogen dioxide on the lung," *Federation Proceedings*, vol. 36, no. 5, pp. 1714–1718, 1977.

[33] B. Hashemzadeh, E. Idani, G. Goudarzi et al., "Effects of PM1.5 and NO2 on the 8-isoprostane and lung function indices of FVC and FEV1 in students of Ahvaz City, Iran," *Saudi Journal of Biological Sciences*, vol. 26, no. 3, pp. 473–480, 2019.

[34] F. Cibella, G. Cuttitta, R. Della Maggiore et al., "Effect of indoor nitrogen dioxide on lung function in urban environment," *Environmental Research*, vol. 138, pp. 8–16, 2015.

[35] A. Möller, R. M. Agius, F. de Vocht et al., "Long-term exposure to PM2.5 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort," *Environmental Health Perspectives*, vol. 121, no. 10, pp. 1232–1238, 2013.

[36] T. Yamamoto and R. J. Bing, "Nitric oxide donors," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 225, no. 3, pp. 200–206, 2000.

[37] A. Tomasi, T. Ozden, and V. Skulachev, "Free radicals, nitric oxide, and inflammation: molecular, biochemical, and clinical aspects," in NATO: *Life and Behavioural Sciences*, vol. 344, pp. 71–88, IOS Press, Amsterdam, 2003.

[38] Z. Chen, J. Zhang, and J. S. Stamler, "Identification of the enzymatic mechanism of nitroglycerin bioactivation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 12, pp. 8306–8311, 2002.

[39] P. Agvald, L. C. Adding, A. Artlich, M. G. Persson, and L. E. Gustafsson, "Mechanisms of nitric oxide generation from nitroglycerin and endogenous sources during hypoxia in vivo," *British Journal of Pharmacology*, vol. 135, no. 2, pp. 373–382, 2002.

[40] B. R. Buca, L. Mittel-Tartaj, R. V. Lupușor, G. E. Popa, C. Rezuș, and C. E. Lupușor, "New nitric oxide donors with therapeutic potential," *Revista Medico-Chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi*, vol. 120, no. 4, pp. 942–946, 2016.

[41] J. Neidecker, "Pulmonary hypertension: the role of nitric oxide in adults," *Revista Española de Anestesiología y Reanimación*, vol. 48, no. 10, pp. 457–459, 2001.

[42] K. F. Nilsson and L. E. Gustafsson, "Treatment with new organic nitrates in pulmonary hypertension of acute experimental pulmonary embolism," *Pharmacology Research & Perspectives*, vol. 7, no. 1, 2019.

[43] M. Husain, C. Adrie, F. Ichinohe, M. Kavosi, and W. M. Zapol, "Exhaled nitric oxide as a marker for organic nitrate tolerance," *Circulation*, vol. 89, no. 6, pp. 2498–2502, 1994.
by reduction of sweet nitrate,” *The Journal of Investigative Dermatology*, vol. 107, no. 3, pp. 327–331, 1996.

[62] K. Matsunaga and R. F. Furchgott, “Interactions of light and sodium nitrite in producing relaxation of rabbit aorta,” *The Journal of Pharmacology and Experimental Therapeutics*, vol. 248, no. 2, pp. 687–695, 1989.

[63] M. Tiso, J. Tejero, S. Basu et al., “Human neuroglial functions as a redox-regulated nitrite reductase,” *Journal of Biological Chemistry*, vol. 286, no. 20, pp. 18277–18289, 2011.

[64] M. Brunori and B. Vallone, “A globin for the brain,” *The FASEB Journal*, vol. 20, no. 13, pp. 2192–2197, 2006.

[65] S. K. Soodaeva, N. Kubysheva, I. Klimanov et al., “Oxidative stress indicators in patients with an exacerbation of COPD,” *European Respiratory Journal*, vol. 52, article PA933, Supplement 62, 2018.

[66] S. Soodaeva, N. Kubysheva, L. Postnikova et al., “Cytokine status and nitrosative stress indicators in patients with an exacerbation of COPD,” *European Respiratory Journal*, vol. 46, article PA855, Supplement 59, 2015.

[67] J. Zhao, J. Wang, Y. Yang, and Y. Lu, “The Determination of nitrate and nitrite in human urine and blood by high-performance liquid chromatography and cloud-point extraction,” *Journal of Chromatographic Science*, vol. 53, no. 7, pp. 1169–1177, 2015.

[68] I. Klimanov, S. Soodaeva, A. Lisitsa, and T. Marchenko, “The investigation of stable nitric oxide (NO) metabolites correlation in exhaled breath condensate (EBC) in Chernobyl clean-up workers (CCUW) with COPD,” *European Respiratory Journal*, vol. 38, article p4186, Supplement 55, 2011.

[69] I. Klimanov, S. Soodaeva, A. Chuchalin, N. Geppe, and T. Bogdanova, “Changes of total nitrite/nitrate levels in breath condensate in children with bronchial asthma during the adaptation of hypoxia,” *European Respiratory Journal*, vol. 24, 2004.

[70] B. E. Keller, “Mathematical modeling,” *International Journal of Radiation Oncology • Biology • Physics*, vol. 2, no. 7-8, p. 823, 1977.

[71] G. Ciobanu and G. Rozenberg, *Modelling in Molecular Biology*, Springer Science & Business Media, 2012.

[72] A. Stéphanou and V. Volpert, “Hybrid modelling in biology: a classification review,” *Mathematical Modelling of Natural Phenomena*, vol. 11, no. 1, pp. 37–48, 2016.