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Experimental Investigations

EVALUATION OF ACUTE EXOGENOUS HYPOXIA IMPACT ON THE FRACTION OF EXHALED NITRIC OXIDE IN HEALTHY MALES

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ОЦЕНКА ЭФФЕКТА ОСТРОЙ ЭКЗОГЕННОЙ ГИПОКСИИ В ОТНОШЕНИИ ФРАКЦИИ ОКСИДА АЗОТА В ВЫДЫХАЕМОМ ВОЗДУХЕ

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ABSTRACT

INTRODUCTION: Exogenous hypoxia increases ventilation and contracts the pulmonary vessels. Whether those factors change the values of nitric oxide in exhaled air has not yet been evaluated. **OBJECTIVE:** To examine the effect of exogenous normobaric hypoxia on the values of the fraction of nitric oxide in exhaled breath (FeNO). **SUBJECTS AND METHODS:** Twenty healthy non-smoker males at mean age of 25.4 (SD = 3.7) were tested. The basal FeNO values were compared with those at 7 min. and 15 min. after introducing into the hypoxic environment (hypoxic tent), imitating atmospheric air with oxygen concentration corresponding to 3200 m above sea level. Exhaled breath temperature was measured at baseline and at 10-12 min. of the hypoxic exposition. Heart rate and oxygen saturation were registered by pulse-oximetry. **RESULTS:** All the subjects had FeNO values in the reference range. The mean baseline value was 14.0 ± 3.2 ppb, and in hypoxic conditions - 15.5 ± 3.8 ppb (7 min.) and 15.3 ± 3.6 ppb (15 min.), respectively, as the elevation is statistically significant ($p = 0.011$ and $p = 0.008$). The values of exhaled breath temperature were $33.79 \pm 1.55^\circ\text{C}$ and $33.87 \pm 1.83^\circ\text{C}$ ($p = 0.70$) at baseline and in hypoxic conditions, respectively. Baseline oxygen saturation in all subjects was higher than that, measured in hypoxia ($96.93 \pm 1.29\%$ vs. $94.27 \pm 2.53\%$; $p < 0.001$). **CONCLUSIONS:** Exogenous hypoxia leads to an increase of FeNO values, but does not affect the exhaled breath temperature.

Key words: hypoxia, nitric oxide, breath temperature, saturation

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РЕЗЮМЕ

ВВЕДЕНИЕ: Экзогенная гипоксия увеличивает объём вентиляции и вызывает контракции пульмональных сосудов. До настоящего времени не исследовано влияние данных факторов на изменение оксида азота в выдыхаемом воздухе. **ЦЕЛЬ:** Исследовать эффект экзогенной нормобарической гипоксии в отношении показателей фракции оксида азота в выдыхаемом воздухе (FeNO). **МАТЕРИАЛ И МЕТОДЫ:** Обследованию были подвергнуты двадцать здоровых некурящих мужчин, средний возраст которых составлял 25.4 г. (SD = 3.7). Исходные показатели FeNO были сопоставлены с показателями, полученными через 7 и 15 мин. после помещения в гипоксическую среду (гипоксическую палатку), которая имитировала атмосферный воздух с концентрацией кислорода, соответствующей концентрации на высоте 3200 м над уровнем моря. Температура выдыхаемого воздуха была измерена в начале и по истечении 10-12 мин. гипоксической экспозиции. Частота сердечных сокращений и сатурация кислорода были зарегистрированы при помощи записывающего пульсоксиметра. **РЕЗУЛЬТАТЫ:** Все лица имели показатели FeNO в рамках референтного диапазона. Средний исходный показатель составлял 14.0 ± 3.2 ppb, а в гипоксических условиях - 15.5 ± 3.8 ppb (7 мин.) и 15.3 ± 3.6 ppb (15 мин.), соответственно, при этом повышение было статистически значимым ($p = 0.011$ и $p = 0.008$). Показатели температуры выдыхаемого воздуха составляли $33.79 \pm 1.55^\circ\text{C}$ и $33.87 \pm 1.83^\circ\text{C}$ ($p = 0.70$), соответственно в начале и в условиях гипоксии. Исходная сатурация кислорода у всех лиц была выше измеренной в гипоксической палатке ($96.93 \pm 1.29\%$ vs. $94.27 \pm 2.53\%$);

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$p < 0.001$). **ЗАКЛЮЧЕНИЕ:** Экзогенная гипоксия приводит к увеличению показателей FeNO, но не изменяет температуру выдыхаемого воздуха.

Ключевые слова: гипоксия, оксид азота, температура выдыхаемого воздуха, сатурация

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INTRODUCTION

The fraction of exhaled nitric oxide (FeNO) has been in the scope of scientific attention for the last two decades. Nitric oxide is being synthesized from L-arginine by the NO synthase (NOS) enzyme family having 3 isoforms – neuronal, inducible and endothelial (nNOS, iNOS, eNOS). Most studies focus on the elevated FeNO found in bronchial asthma where there is prevalent eosinophilic inflammation. This test is non-invasive, easy to obtain and useful not only in diagnosis but even more in monitoring treatment and predicting the therapeutic response to inhaled corticosteroids. In bronchial asthma the increased FeNO is believed to be due to activation of the iNOS in the airway epithelium.

AIM

In the present study we aimed to investigate the changes in FeNO applying exogenous hypoxia in healthy male subjects. As a factor that is known to alter ventilation and pulmonary vascular tone, we tested how those changes would affect NO production in the airways.

SUBJECTS AND METHODS

Twenty healthy males between the age of 21 and 34 (mean $\pm$ SD 25.4 ± 3.7) were tested. The main spirometry parameters were in the normal range: FVC – $97.94 \pm 7.50\%$ and FEV_1 – $99.63 \pm 11.62\%$ of predicted values. Mean BMI of all the subjects was 24.1 ± 3.5 , two of them were overweight (25.6 and 27.8) and other two – slightly obese (30.5 and 33.1). According to our inclusion criteria the subjects had to be healthy adult males who do not smoke and do not have any cardiovascular, respiratory or hematologic disturbance or any other pathologic condition that could cause a change in their oxygen regime, affect the results or compromise the safety of the enrolled volunteers. All subjects received thorough information about the nature of the study. A signed informed consent was obtained from all the subjects prior to any other actions related to the study protocol which included a single visit. A physical examination was carried out including an ECG reviewed by a cardiologist.

FeNO measurements were performed with a NIOX MINO device (Aerocrine, Solna, Sweden) at baseline – two maneuvers, before and after spirometry and at 7th and 15th min. during hypoxic exposure. Spirometry was done with Ultima PFX (Medical Graphics, St Paul, USA). Exogenous hypoxia was created by a hypoxicator (AltiPro 8850 Summit+, Altitude Tech, Canada) in a special tent. The hypoxic environment consisted of air, corresponding to atmospheric air at 3200 m above sea level (approx. $FiO_2 = 14\%$). Exhaled breath temperature (EBT) was measured by X-halo breath thermometer (DELMEDICA, Singapore) at baseline and at 10-12 min. after entering the hypoxic tent. Heart rate and hemoglobin oxygen saturation were registered by a pulse oximeter (CMS50F, Contec Medical Systems, Qinhuangdao, China) during the whole visit and average values for both from 10 minutes before entering the tent and the entire time during hypoxia (15 minutes) were later evaluated. For the statistical processing of data we used the program SPSS. The normal distribution was checked with Kolmogorov-Smirnov test and the differences between the sets of values were compared using paired samples T-test.

The study was approved by the Institutional Ethics Committee with all related documents – study protocol, inclusion and exclusion criteria, informed consent.

RESULTS

All the differences of the measured parameters between the two moments in the time scale (hemoglobin oxygen saturation, exhaled breath temperature and fraction of exhaled nitric oxide) had normal data distribution (checked with Kolmogorov-Smirnov non-parametric test). Baseline oxygen saturation in all subjects was higher than that, measured in hypoxia ($96.93 \pm 1.29\%$ vs. $94.27 \pm 2.53\%$; $p < 0.001$) – Fig. 1. The slightest drop in SpO_2 was 0.2%, but there were two subjects with a drop over 4% and one with 8.4%. As compared to baseline (14.0 ± 3.2 ppb), the FeNO in hypoxic conditions was significantly higher – 15.5 ± 3.8 ppb at 7 min and 15.3 ± 3.6 ppb at 15 min, respectively ($p = 0.011$ and $p = 0.008$) – Fig. 2. At 7th min thirteen

subjects had an elevation, two a decrease and five – no change in the FeNO. The results of exhaled breath temperature were 33.79 ± 1.55 °C and 33.87 ± 1.83 °C ($p = 0.70$) at baseline and in hypoxic conditions, so no statistically significant difference was observed - Fig. 3.

DISCUSSION

In this study we found a slight elevation in FeNO in hypoxic conditions, corresponding inversely to saturation level and not accompanied by a change in EBT.

The time point of the 7th minute was chosen because we wanted to evaluate the rapid changes in FeNO. The first 7 participants in the study had

Figure 1. Average baseline oxygen saturation compared to average saturation during hypoxia.

Figure 2. Changes in FeNO in response to the hypoxic stimulus.

Figure 3. EBT in hypoxia compared to baseline.

an additional FeNO measurement at the 1st minute in the hypoxic tent, but the results did not differ from baseline. Therefore we presumed this is too early to observe any changes and skipped this measurement for the other subjects. If we compare the FeNO results at 7th and 15th min of hypoxia, we see that they do not differ significantly and there is a slight tendency to diminishing. This led us to the conclusion that at 15 min. some kind of a “steady state” has been reached already, in terms of NO synthesis triggered by exogenous hypoxia, so we picked 15 minutes as an end point.

We presume that the observed increase in FeNO following hypoxic exposure is caused by mechanisms, different than those, responsible for elevated FeNO in bronchial asthma. It can be speculated that instead of activating the iNOS, the rise of NO in exhaled breath over minutes of hypoxic exposure is more likely to be due to stimulation of the eNOS. In a paper by Persson¹ it is stated that endogenous NO acts as a modulator of the hypoxic vascular pressor response. Gustafsson² suggests that NO plays regulatory role regarding vascular tone and at the same time takes part in pulmonary defence mechanisms. The above mentioned studies included rabbits and guinea pigs. It seems plausible that the generalized pulmonary vasoconstriction provoked by inhaling air with decreased pO_2 is being counteracted by an increase of eNOS activity and NO production, since the general vasoconstriction does not possess adaptive properties in this case. It can be assumed that NO synthesized in the airways takes part in the adaptation to hypoxia from an acute perspective. Several epidemiological studies examined the differences in FeNO in high altitude residents and lowlanders and the results are

controversial – Ayamara children are found to be protected from high altitude pulmonary hypertension, but it seems NO is not related to this fact³, while Tibetan adults had actually higher values of FeNO⁴ than lowlanders. Similar to the conditions of our protocol are described in a paper by Seys⁵ where asthmatic subjects underwent a one hour normobaric hypoxia exposure which resulted in slightly lower levels of FeNO ($P < 0.04$) as compared to baseline. This result that is opposite to our findings shows that there is a substantial difference in the mechanisms of NO synthesis in health and disease and the respiratory reaction to acute hypoxia as long as NO is concerned requires further investigations.

As shown in the results tendencies in decreasing saturation and elevating FeNO are present as triggered by hypoxia, however the responses in the tested group are heterogenous. It is interesting to point out that the top three results in oxygen desaturation (8.4%; 4.5% and 4.3% while mean desaturation was 2.7% for the whole group) belong to subjects with no elevation of FeNO. These findings, though scarce in number of tested persons, support the thesis that NO plays a role in hypoxic adaptations. Another interesting finding is that in the subgroup of thirteen subjects who react with elevating FeNO in the first 7 minutes there is a marked tendency of no further increase with mean difference between FeNO at 7th and 15th minute of -0.77 ppb and only one person having a higher value in the end of the test. In the other subgroup of 7 subjects, however, changes between 7th and 15th min are in the opposite direction with a mean increase of 1.29 ppb and only one person having a decrease. In other words, almost all the tested people had an elevation of FeNO triggered by hypoxia, just in some this response is delayed in time. The reason why we do not have a significant desaturation in all the subjects who have a prolonged FeNO increase is probably because SpO₂ depends on many other factors. We could not establish a significant inverse correlation between changes in FeNO and SpO₂ due to the small group size and the heterogenous responses but the tendency is clear.

Within the protocol FeNO testing was performed only once at a time, because it requires 2 minutes for a complete test and thus we cannot measure it twice in a particular minute. Moreover there is enough evidence for the very good repeatability of the NIOX MINO device results.^{7,8}

The exhaled breath temperature is expected to decrease in the hypoxic environment which was

not observed in our study. It can be explained with one of the technical characteristics of the X-halo breath thermometer which requires certain time to reach a thermal equilibrium i.e. to perform a measurement. The measurement of EBT in the hypoxic tent started after the FeNO testing at 7 min so the result we receive is obtained after 10-12 minutes of exposure. That reflects a relatively late moment regarding the physiological changes we are discussing when directly entering a hypoxic environment, prepared in advance. We hypothesize that in those first 10 minutes the curve of EBT changing is biphasic and there is actually a primary pulmonary vasoconstriction that is transitory, but powerful enough to provoke an increase in NO synthesis. In its turn this brings the vascular tone to normal, hence the EBT similar to baseline levels. Such an idea worths testing EBT with a more sensitive and fast responsive method to better understand its dynamics after applying exogenous hypoxia. The lack of such a method is one of the limitations of our study. We also do not have a direct measurement of the pulmonary vascular tone, e.g. by ultrasound, so the speculations we are making about the changes in the EBT reflecting changes in the pulmonary vascular tone are just deductive.

CONCLUSIONS

Exogenous hypoxia causes a mild increase of the FeNO in healthy males. Assessing the cause-effect relationship between the latter and the pulmonary vascular tone demands further investigations. After 10 min of breathing air with FiO₂ $\approx$ 14% the EBT is similar to the baseline value.

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