International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Short Communication

Effect of Intermittent Normobaric Hypoxia Training on Oxygen Tension in the Subcutaneous Tissue

Levashov Mikhail, Yanko Roman, Chaka Elena, Plotnikova Lidiia

Department of Clinical Physiology of Connective Tissue, A. A. Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine

Corresponding Author: Levashov Mikhail

ABSTRACT

It was investigated the dynamics of oxygen tension (PO_2) in the subcutaneous tissue of male Wistar rats exposed to intermittent normobaric hypoxia (INH). Monitoring of the tissue PO_2 was performed in situ using the open platinum electrode. It was evaluated the rate of change and the time to establish a relative steady state of tissue PO_2 in the transitional stages of breathing: air – 10 % hypoxic gas mixture – air. Hypoxia was accompanied by cyclic changes in oxygen tension in the subcutaneous tissue of rats. When animals were transferring to breathe of hypoxic gas mixture, PO_2 began to decrease already in the first seconds of observation. It was found that the time to reach the new relative steady state of PO_2 in the rats' subcutaneous tissue after transition from air breathing to 10 % hypoxic gas mixture breathing ranged from 60 s to 360 s and after transition from hypoxic gas mixture breathing to air breathing – from 30 s to 240 s. We have noted an increase in tissue PO_2 duration from 60 s to 390 s after period of hypoxia. For determining the optimum parameters of INH training it is necessary to take into account the phase nature of PO_2 changing and the inter individual variations in setting time of PO_2 steady state in the tissue after transition from air breathing to hypoxic gas mixture breathing and vice versa.

Key words: intermittent normobaric hypoxia, tissue oxygen tension

INTRODUCTION

Currently, in experimental and clinical studies use different regimens of intermittent normobaric hypoxia training (INH). [1-3] The main parameters that determine the differences in the individual regimens of INH training are: the content of oxygen in the inspired hypoxic gas mixture (HGM), the duration of phases of air and hypoxic gas mixture breathing in one cycle of INH exposure, the number of breathing cycles "hypoxic gas mixture - air" in a single session, and the total duration of exposure to INH. It is considered that the lower limit for safe human-level reduction of oxygen in the hypoxic gas mixture during medical and wellness treatments of INH is 10±1%. [4] The gas mixtures are most often used with the same oxygen content in the experimental investigations. Although the number of researchers employed hypoxic gas mixtures with an oxygen content up to 7-8%. [5,6] The upper limit of the oxygen content in the normobaric hypoxic gas mixtures are less clearly defined. However, most researchers believe that to achieve a physiologically relevant effect of INH training the maximum oxygen content in hypoxic gas mixture should not exceed 14-15%, which corresponds to the middle level of mountain. [1,3,7] Another important parameter is the duration of HGM breathing. It varies within a wide range - from several minutes to 1-2 hours. It is mainly determined by the oxygen content in the inspired gas mixture. The minimum duration of the hypoxic gas mixture breathing in clinical setting is usually varied from 2-3 to 5-10 min. The duration of hypoxic gas mixture breathing in experimental studies reaches up to 10-30 min and in some studies - 60 min or more. The duration of atmospheric air breathing is generally the same as breathing of hypoxic gas mixture. [4] Number of hypoxic gas mixture breathing phases per one session of INH may be vary from 1 to 10 or more. The total duration of INH training in experimental conditions vary over a wide range (from several days to 1 month). The duration of the INH therapy course in clinical setting vary from 12-14 to 28-30 days.

Attempts have been made to develop a universal method for the quantitative assessment of the degree of hypoxia effect on the body. [3] However, they face a number of difficulties arising due to impossibility of taking into account all the possible factors associated with the impact of INH on the entire organism, and the individual characteristics of responses to hypoxia. However, the researchers are involved in the study of physiological mechanisms of the therapeutic and health-improving actions of INH, have always a problem with choosing the most suitable regimen of hypoxic exposure, provides the most pronounced positive physiological effect. The main requirement for optimal parameters of INH training is next. The time of hypoxic gas mixture breathing, on the one hand, must be so short that damaging effects of hypoxia can't be realized. But on the other hand, it must be prolonged as much as possible to provide the maximum activation of more compensatory and adaptive mechanisms which aim to reduce the damaging effect of low PO_2.

The aim of this study was to investigate the dynamics of oxygen tension in the subcutaneous tissue for determine the optimal parameters of intermittent normobaric hypoxia training.

METHODS

It was studied the dynamics of oxygen tension (PO_2) in the subcutaneous tissue of 23 male Wistar rats aged 3 months during breathing atmospheric air and 10% hypoxic gas mixture. Continuous registration of hronoamperogram, reflecting the dynamics of oxygen tension in the subcutaneous tissue was performed in situ by an open platinum electrode. [1] Electrode was placed in paravertebral region of the lower third of the rats back. Initially it was recorded the baseline oxygen tension in subcutaneous tissue while rat breath of atmospheric air. After that the rat was connected with the source of 10.0% hypoxic gas mixture. For this purpose the rats' head was placed in a sealed breathing mask. Rat breath of HGM until PO_2 in the tissue reached a new steady state and hronoamperogram curve achieve on a "plateau". Then the animal was connected to breath of atmospheric air, which lasted until the establishment of the initial of steady state of PO_2 in the tissue.

The following indicators were determined: the oxygen tension in the rats' subcutaneous tissue in relative steady state when breathing atmospheric air and hypoxic gas mixture, the difference in steady state PO_2 values when rat breath air and hypoxic gas mixture (ΔPO_2), the time to reach the relative steady state PO_2 after transition from air breathing to hypoxic gas mixture breathing and vice versa (t, s), the rate of change in PO_2 while transient phases of breathing air - hypoxic mixture - air (ΔPO_2 / t). The studies were performed with an interval of 15 min for determine the dynamics of the individual variations in oxygen tension in the rats' subcutaneous tissue while repeated cycles of hypoxic gas mixture breathing.

All procedures with animals were carried out in accordance with the principles of bioethics and European Convention for the Protection of Vertebrate Animals used for
Experimental and Other Scientific Purposes (Strasbourg, 1986).

Data analysis

The obtained data were processed by methods of variation statistics using the software Statistica 6.0 for Windows and Microsoft Exel program 2010 to check the normality of the distribution of digital arrays used Pearson criterion. Statistical significance was declared when $p \leq 0.05$.

RESULTS

When we studied the dynamics of oxygen tension in the rats' subcutaneous tissue, we proceeded from the fact that the impact of INH on the body is cyclical in nature. At the same time in each cycle of INH we can distinguish 4 consistently developing phases: "the phase of normoxia" (the source a relatively steady state of PO_2 when breathing of atmospheric air), "the phase of hypoxygenation" (the process of PO_2 decreasing after transition from air breathing to hypoxic gas mixture breathing), "the phase of hypoxia" (the relatively steady state of PO_2 when breathing of HGM), "the phase reoxygenation" (the process of PO_2 increasing after transition from hypoxic gas mixture breathing to air breathing), which completes the restoration of a relatively steady state of PO_2 in tissue.

The oxygen tension in the subcutaneous tissue was 27,6 ± 2,9 mm Hg in condition of a relatively steady state when the rats breathe with atmospheric air. When animals were transferring to breathe of 10.0 % hypoxic gas mixture, PO_2 began to decrease already in the first seconds of observation. It is reflected in the nature of the dynamics of the registered hronoamperogram (Figure 1).

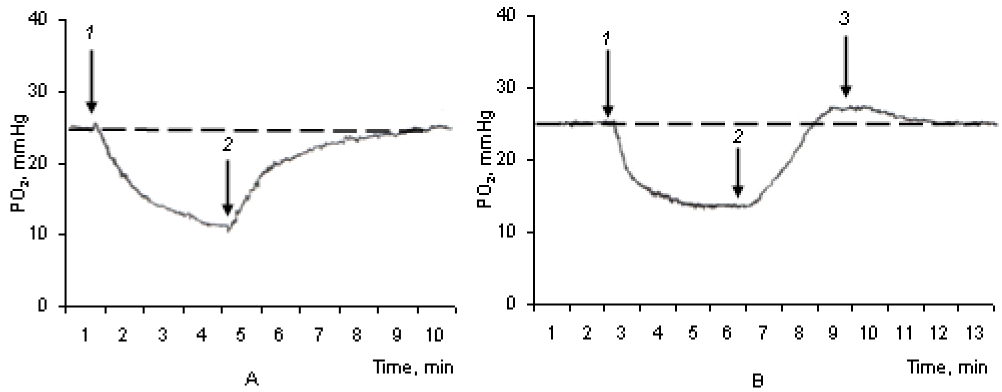

Figure 1: Two types (A, B) of oxygen tension changes in the rats' subcutaneous tissue during the breathing cycle "air – 10% of the hypoxic gas mixture - air". The arrows indicate: 1 - the beginning of hypoxic gas mixture breathing, 2 - the beginning of air breathing, 3 - "overshunt".

The new relatively steady state of PO_2 in tissues was achieved when PO_2 decreased (ΔPO_2) to 12.62 ± 0.32 mm Hg (45.6 ± 2.6% from baseline). The PO_2 reduced most significantly during the first 45-60 s of breathing with hypoxic gas mixture (up to 61.5 ± 4.4% from the maximum decreasing of PO_2). The tempo of PO_2 changes have been slowed subsequently. Individual variations of ΔPO_2 ranged from 9.33 to 15.8 mm Hg (33.8 - 57.1% of the initial level). The transitional period of the establishment of a new steady state of PO_2 in the subcutaneous tissue was completed already during the first 180 s of hypoxic gas mixture breathing in 69.8% of cases. Its completion was recorded in 91.3% of cases after 300 s (Figure 2). The minimum time to reach a steady state of PO_2 was 65 s, after rats transition from air breathing to 10.0% of HGM breathing. The maximum duration of the transitional period from the beginning of hypoxic gas mixture breathing to the establishment of a new steady state of PO_2 in rats' subcutaneous tissue reached up to 360 s.

The dynamics of regenerative processes in the transition from 10.0% of HGM breathing to air also had a number of features. The duration of the recovery period of initial PO_2 level was less than in transition from air to hypoxic gas mixture breathing and recovery rate was higher. The minimum time to reach the initial level of PO_2 was 30 s, and the maximum – 240 s. That was, respectively, less for 50% and 33% than after transition from air breathing to 10.0% of HGM breathing. In 66.2% of the cases within the first 120 s of air breathing PO_2 reached baseline. This effect was observed in 90.4% of cases after 180 s. The highest rates of PO_2 increasing were within 30-90 s after transition from breathing of hypoxic gas mixture to air breathing. The maximum rates of PO_2 increasing were observed in the first 30-90 s after transition from hypoxic gas mixture breathing to air breathing.

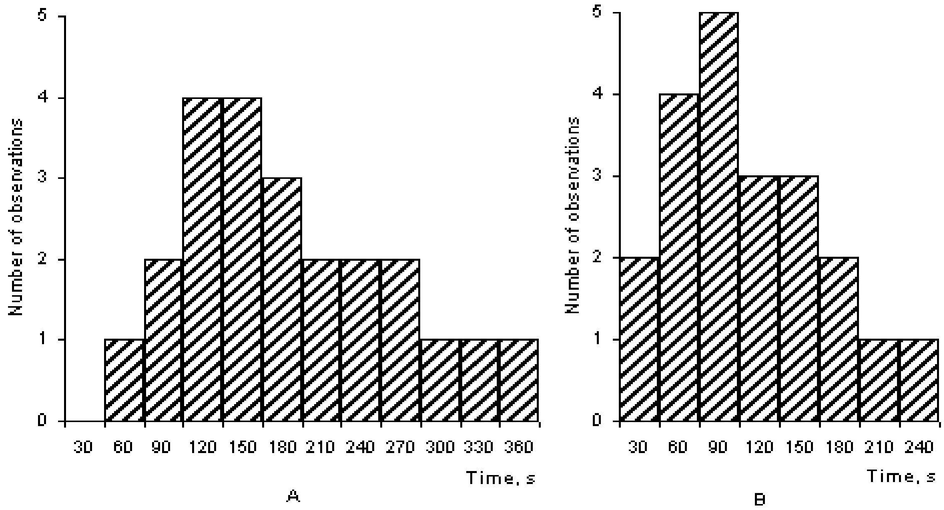

Figure 2: Histogram of the time to reach a new of PO_2 steady state equilibrium in the rats' subcutaneous tissue in the transition from air breathing to 10.0% hypoxic gas mixture breathing (A) and vice versa (B).

The "over shunt" phenomenon - exceeding the initial level of the hronoamperogram, was observed in 38% of cases after transition from hypoxic gas mixture breathing to atmospheric air breathing. This fact testified that when animals are transitioned from breathing of hypoxic gas mixture to air breathing the oxygen tension in the rats' subcutaneous tissue exceeded the values that were recorded in the initial state. Duration of "over shunt" ranged from 60 s to 390 s, and amplitude from 10% to 62% of the PO_2 reduction while rats breath of HGM. Analysis of the PO_2 dynamics in repeated cycles of exposure to INH identified a number of specific laws (Figure 3).

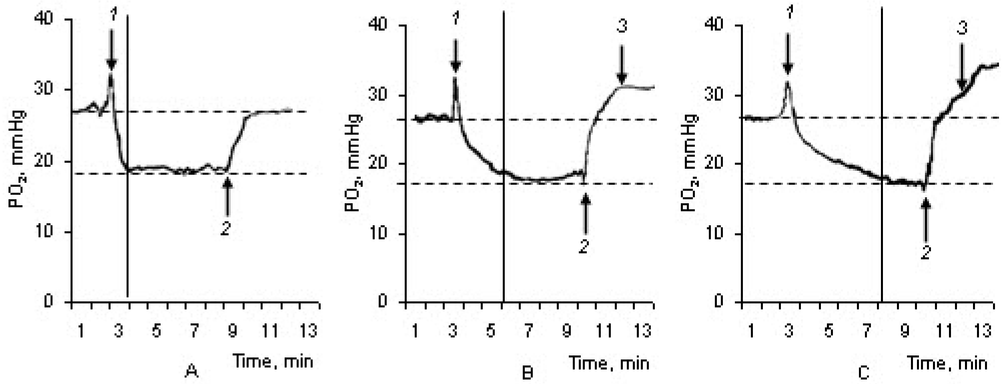

Figure 3: Dynamics of oxygen tension in the rats' subcutaneous tissue over 3 cycles (A, B, C) of breathing "air - hypoxic gas mixture - air". The arrows indicate: 1 - the beginning of breathing hypoxic mixture, 2 - the beginning of air breathing, 3 - "overshunt".

The decline of PO_2 in tissue was most rapidly when the rats breath of 10.0% HGM in the first cycle of INH exposure. Hronoamperogram reached a plateau within 60-90 s. The rate of PO_2 decline have been gradually slowed in subsequent cycles of hypoxic gas mixture breathing. The steady state of PO_2 during breathing HGM was achieved within 210 s in the 2nd cycle, and within 360 s - in the 3rd cycle of INH exposure. This pattern of PO_2 dynamics are reflected in corresponding changes in the time and speed parameters of hronoamperogram. Time to reach the initial level of PO_2 after transition from 10.0% HGM breathing to air breathing was reduced to 45-30 s in 2-d and 3-rd cycles of INH exposure. However, the state of relatively steady state of PO_2 have not been achieved, and there was a further increase in tissue PO_2 ("over shunt" phenomenon). In this regard, the recovery of baseline PO_2 occurred with a delay reaches 240 – 390 s. The frequency, duration and severity of this effect increased in repeated cycles of INH exposure.

Based on the results of our studies, we used the same time parameters for rats INH training. Animals breathed alternately at least 10 minutes of 10% hypoxic gas mixture and atmospheric air for 2 hours daily. The total duration of the training was 28 days. Oxygen tension in the subcutaneous tissue was measured in situ before and after 14 and 28 days of INH training. After 14 days of INH training oxygen tension in the subcutaneous tissue was 20.0% higher than before the start of training (Fig. 4)

The rate of increase in PO_2 was significantly slowed down if the training lasted up to 28 days. On average, PO_2 increased by only 5.4% over the next 14 days of training. The total increase of tissue oxygen tension was 25.4% after 28 days of INH training. Thus, the use of such time parameters of the INH training allowed to achieve the maximum values of tissue PO_2 increase in minimal time.

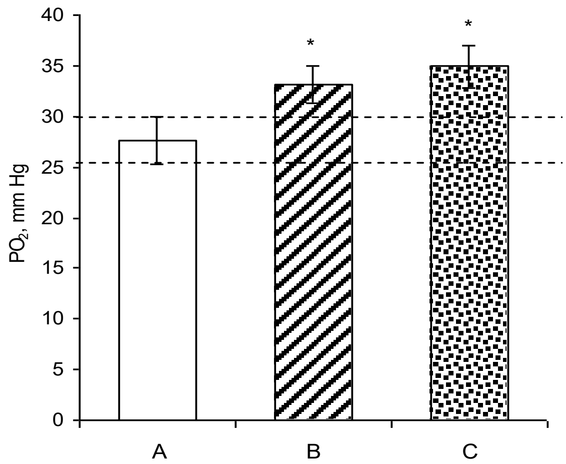

Figure 4: Oxygen tension in the rats' subcutaneous tissue before (A), after 14 days (B) and after 28 days of INH training (C).
*Indicates significant difference from the baseline value ($p<0.05$)

DISCUSSION

The degree of PO_2 decrease in the inspired gas mixture, the duration and the frequency of hypoxic gas mixtures and air breathing to a large extent determine the physiological effects of INH exposure to body. [8-11] The results of histomorphological studies of heart and lung tissues, the state and nature of changes of the pro - and antioxidant systems of the body in different modes of hypoxygenation and reoxygenation are confirming this fact. [9,12-15] However, the issues relating to the choice of optimal parameters of INH exposure regimen, as well as information content of individual indicators, which are used for their assessment, required further study and clarification. The results of our researches showed that the settling time of the PO_2 relative steady state in the rats' subcutaneous tissue after transition from air breathing to breathing 10.0% hypoxic gas mixture varies over a wide range (from 1 to 6 min). This fact may be due to inter-individual differences in the degree of activation of adaptive mechanisms, which are included under the influence of short-term hypoxia. Among these mechanisms can be noted genetically determined differences in the resistance of animals to hypoxia, degree of pulmonary non-uniformity ventilation and blood circulation, the severity of hypoxic vasoconstriction and intensity of local microcirculation.

It was found that the breathing of hypoxic gas mixtures in most cases (> 90%) allow to achieve a new steady state of PO_2 in the subcutaneous tissue within for 4-6 min of the first INH cycle. However, the rate of decrease in oxygen tension in the tissues slowed down in phase of hypoxic gas mixture breathing in repeated cycles of INH. This fact can be attributed to inclusion of adaptation mechanisms to hypoxia. In this regard, it should be recognized expedient to increase the duration of hypoxication phase in repeated cycles of INH an additional 1-2 min. Thus, the maximum duration of the hypoxic gas mixture breathing may be reach to 8 min. Extending the hypoxic gas mixture breathing enhances the completion of the transition process – hypoxication phase. This allow to increase the probability of reaching the relatively steady state at the lowest possible values of PO_2 in the tissue, which provides a more clear effect of hypoxia on the body.

Analysis of the oxygen tension dynamics in the phase of reoxygenation showed that the recovery of the initial level of PO_2 in the rats' subcutaneous tissue has been more rapid pace (for 3-4 min). However, further increase of PO_2 in the tissue (the phenomenon of "over shunt") led to an increase in the time to achieve of the steady state. It is obvious that in this period to start the next cycle of INH training is not suitable, because it does not provide the maximum possible of PO_2 reduction. In this regard, there is need to increase the duration of reoxygenation phase until it reaches the initial steady state of PO_2. Given the fact that duration of "over shunt" can reach 4-6 min, it is advisable to continua the phase of atmospheric air breathing during each cycle of INH training to 10 min.

Considering the physiological nature of "over shunt" phenomenon it should be noted that it reflects the severity of the effect of "posthypoxic hyperoxia" - an important component of the sanogenic mechanism of INH training. Increasing the PO_2 in the tissue in the aftereffects period of hypoxia is a consequence of the inclusion of a set of functional and structural adaptation mechanisms aimed at maintaining oxygenation and ensuring the necessary level of metabolism in hypoxic conditions. This effect, to some extent, explains the mechanism of positive action of INH training. It confirms the validity of the use of intermittent normobaric hypoxia for therapeutic and prophylactic purposes. Securing this effect by repeated cycles of breathing hypoxic gas mixtures is one of INH training purposes.

CONCLUSION

Our results indicate that in determining the optimum parameters of INH training must take into account the impact of the phase character and different duration of oxygen tension changes in the tissues during the transition from air breathing to hypoxic gas mixture and vice versa.

The time of achievement the relatively steady state PO_2 in the tissue can be used as a criterion for selecting the optimal duration the air and hypoxic gas mixtures breathings during INH training in sports and rehabilitation medicine. It allows to take into account the inter-individual variations in the dynamics of PO_2 in impact cycles of INH that are caused by different rates of activation of adaptive mechanisms and degree of the posthypoxic increase oxygen tension effect.

The PO_2 in tissue can not reach the relative steady state in phases of air breathing and hypoxic gas mixture when time parameters of INH have been selected incorrect. In this case, the effect of INH in subsequent cycles will be carried out in the transient gas-dynamic, i.e. with less severe degrees of hypoxia. Increasing the duration of breathing 10.0% HGM and atmospheric air up to 8 to 10 min in the cycle of INH training allows to achieve a relative steady state PO_2 in subcutaneous tissue in more than 90% of cases. This time parameter can be used as a base figure in determining the exposure regimen of INH training.

ACKNOWLEDGEMENTS

The authors would like to extend particular gratitude to prof. Vadim Berezovskii from AA Bogomoletz Institute of Physiology for his help with methods and interpretation.

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Berezovskii VA, Levashov MI. Introduction to orotherapy. Kiev, 2000. [In Russian].
2. Berezovskii VY, Gorban EM, Levashov MI, et al. Technology of increase of body resistance by means of hypooxygenation. Method. guid. of MHS of Ukraine. Kiev, 2000. [In Ukrainian].
3. Berezovskii VA. Natural and experimental orotherapi. Donetsk. Kiev, 2012. [In Ukrainian].
4. Strelkov RB, Chizhov AYa. Intermittent Normobarical hypoxia in prophylaxis, treatment and rechabilitation. Ekaterinburg. 2001. [In Russian].
5. Bolgova TV, Dubova MG, Rozova EV, et al. Morphometric analysis of mitochondrial apparatus lungs and the myocardium at different modes of intermittent hypoxia. News morphology. 2014; 20:352-357. [In Ukrainian].
6. Saharchuk II, Slipchenko VG, Denisenko GT, et al. The effectiveness of periodic individual dosed hypoxic training in the clinic of internal deseases. in: Kolchinskaya AZ, ed. Interval hypoxic training (efficacy, mechanism of action). Kiev.1992. [In Russian].
7. Levashov MI. Benefical effects of Intermittent normobaric hypoxic training on respiratory function in patients with chronic pulmonary diseases. In: Xi L, Serebrovskaya TV, eds. Intermittent Hypoxia and Human Diseases. Springer-Verlag, London; 2012:115-126.
8. Levashov MI, Berezovskii VY, Chaka EG, et al. Effect of Intermittent Normobaric Hypoxia on Total Oxygen Consumption and efficiency of cardio-respiratory mechanisms of oxygen supply in patients with a high risk of chronic obstructive pulmonary diseases. Fiziol Zh. 2013; 59:57-64. [In Ukrainian].
9. Gonchar O, Mankovska I. Antioxidant system in adaptation to Intermittent Hypoxia. J. Biol. Sci. 2010; 10:545-554.
10. Suzuki YJ, Jain V, Park AM, et al. Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. Free Radic. Biol. Med. 2006; 40:1683-1692.
11. Zong P, Setty S, Sun W, et al. Intermittent hypoxic training protects canine myocardium from infarction. Exp. Biol. Med. 2004; 229:806-812.
12. Chen L, Einbinder E, Zhang Q, Hasday J, et al. Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats. Am. J. Respir. Crit. Care Med. 2005; 172:915-920.
13. Joyeux-Faure M, Stanke-Labesque F, Lefebvre B, et al. Chronic intermittent hypoxia increases infarction in the isolated rat heart. J. Appl. Physiol. 2005; 98:1691-1696.
14. Ramond A, Godin-Ribuot D, Ribuot C, et al. Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia. Fundam. Clin. Pharmacol. 2013; 27:252-261.
15. Rozova K, Gonchar O, Mankovska I. Benefits and Risks of Different Regimen of Intermittent Hypoxic Training. In: Xi L, Serebrovskaya TV, eds. Intermittent Hypoxia and Human Diseases. Springer-Verlag, London; 2012:273-279.

How to cite this article: Mikhail L, Roman Y, Elena C et al. Effect of intermittent normobaric hypoxia training on oxygen tension in the subcutaneous tissue. Int J Health Sci Res. 2017; 7(7):299-305.

****