Biogerontology
https://doi.org/10.1007/s10522-019-09821-6

RESEARCH ARTICLE

# Hypercapnic hypoxia as a potential means to extend life expectancy and improve physiological activity in mice

**Vladimir P. Kulikov · Pavel P. Tregub · Ilya S. Osipov · Arseniy I. Trukhanov**

Received: 15 April 2019 / Accepted: 10 July 2019


**Abstract** The application of combined hypoxia and hypercapnia (hypercapnic hypoxia) during respiratory exercises results in a maximum increase in resistance to acute hypoxia and ischemic tolerance of the brain. The results of those researches allow the assumption that hypercapnic hypoxia is a promising method for prophylaxis, treatment, and rehabilitation, as well as a means to increase life expectancy. The study was conducted to verify the hypothesis that it is possible to extend the life span through regular courses of respiratory exercises with hypercapnic hypoxia. In the present experimental research carried out on mice, the geroprotective effect of regular hypercapnic-hypoxic exercises ($PO_2$—90 mm Hg and $PCO_2$—50 mm Hg) was assessed in the context of the average life expectancy and the main criteria of its quality (reproductive function, muscle strength, and behavior). Results suggest that with regular training, life span is extended significantly by 16%. This result was accompanied by improved reproductive and cognitive functions, increased motor and search activities, and physical stamina in old age mices. This important phenomenon is accompanied by improved reproductive and cognitive functions, high motor function and search activity, as well as better physical stamina in old-aged mices. Recurring respiratory training under combined hypoxia and hypercapnia (hypercapnic hypoxia) during the lifetime significantly extended the life span of mice in the experiments.

**Keywords** Hypoxia · Hypercapnia · Hypercapnic hypoxia · Rejuvenation · Lifespan · Healthy longevity

V. P. Kulikov · P. P. Tregub (✉) · I. S. Osipov
LLC "Altai Medical Institute of Postgraduate Education", Polzunova st., 34a, Altai Region, Barnaul, Russia 656043
e-mail: pfiza_asmu@mail.ru

P. P. Tregub
"Professor VF Voyno-Yasenetsky Krasnoyarsk State Medical University" of the Ministry of Health of Russia, Partizan Zheleznyak st., 1, Krasnoyarsk, Russia 660022

A. I. Trukhanov
Federal Scientific Clinical Center for Reanimatology and Rehabilitation, Lytkino, 777, Moscow, Russia 141534

## Introduction

Highland hypoxia is well known for contributing to human life extension as well as increasing reproductive activity (Agadzhanian et al. 1995; Matsubayashi and Okumiya 2012; Boretto et al. 2018). Hypoxia has been proven to be effective in increasing the general resistance (Lukyanova et al. 2009) of individual organs and tissues (Chen et al. 2005; Yang et al. 2009). There are numerous data on the preventive and therapeutic efficacy of hypoxia against cardiovascular pathology (Shatilo et al. 2008; Neckár et al. 2002), trophic disorders (Obrenovitch 2008), nervous system conditions (Sharp et al. 2004), and respiratory diseases

Published online: 15 July 2019

(Lin et al. 2011). It is logical to assume that the use of hypoxic exposure clearly has high geroprotective potential for healthy longevity. However, the practical application of hypoxic therapy is limited due to the necessity of long-term exposure (1–6 h) and several sessions (at least 7) (Yang et al. 2009; Neckár et al. 2002).

The simplest and most physiological means of inducing hypoxia in a person during breathing exercises is by rebreathing the additional dead space volume (ADSV), during which part of the exhaled air with low oxygen content is inhaled again (Fierstra et al. 2013). The excess of carbon dioxide, which occurs during rebreathing, is usually corrected by using a chemical $CO_2$ absorbent (Serebrovskaya and Xi 2016). However, it is the therapeutic use of carbon dioxide that is gaining increasing interest. The pronounced neuroprotective efficiency of permissive hypercapnia has previously been established (Zhou et al. 2010; Tao et al. 2013). In the scientific literature, there has even emerged a special term, "therapeutic hypercapnia" (Tao et al. 2013; Laffey et al. 2000). The main neuroprotective mechanisms of hypercapnia are probably the carbon dioxide-induced inhibition of the apoptosis of nerve cells (Zhou et al. 2010; Tao et al. 2013), its antioxidant effect (Zakynthinos et al. 2007), and the stimulation of angiogenesis (Siafakas et al. 2001).

Our recent researches have shown that, compared with the individual use of hypoxia and hypercapnia, the application of combined hypoxia and hypercapnia (hypercapnic hypoxia) during respiratory exercises results in a maximum increase in resistance to acute hypoxia (Tregub et al. 2013) and ischemic tolerance of the brain (Tregub et al. 2015). The results of those two research results allow the assumption that hypercapnic hypoxia is a promising method for prophylaxis, treatment, and rehabilitation, as well as a means to increase life expectancy and to improve physiological activity in old age.

The new findings about the therapeutic potential of hypercapnia and hypercapnic hypoxia provide encouragement for those who support the idea that carbon dioxide plays a key role in life extension. This idea is based on data on the life expectancy of animals that are exposed to hypercapnia and hypoxia on a regular basis during their lifetime (e.g., whales, turtles, and mole rats) (Krivoruchko and Storey 2010; Muradian 2013). However, no direct studies have been carried out to assess the efficiency of hypercapnic hypoxia in increasing life span.

In the present research, experimental trials on laboratory mice were carried out to test the hypothesis that it is possible to extend the life span through regular courses of respiratory exercises with hypercapnic hypoxia. Considering that the modern concept of healthy longevity implies not only life extension but also reduced intensity of physiologic aging (Jafari 2015), we also studied important variables in the physical development, the reproductive function, and behavior of the animals during the aging process.

## Methods

### Animals and cage keeping conditions

Experiments were carried out on 36 BALB/c mice (19 males and 17 females) (Institute of Cytology and Genetics SD RAS, Novosibirsk, Russia). All of the mice were born within one week and the exact date of birth of each animal was reliably known.

The mice were housed in plastic cages at room temperature ($\sim$ 23 °C) and under natural light cycle during their lifetime. They were kept in four standard rodent cages, providing at least 40 $sm^2$ of space per animal. The mice had free access to drinking water and a full ration with a good balance of trace elements and vitamins (Van Zutphen et al. 2001). During the pregnancy and lactation period, dried milk was additionally fed in the diet of females. The mating opportunities of the animals were not limited (Harrison et al. 2009), and in 2 weeks infant mice were housed in separate cages.

### Animal groups and experimental scheme

At the age of 2 weeks, the animals were randomized into two experimental groups by using Statistica 6.0 (StatSoft, Inc., USA). The randomization followed the principle of equal gender composition. Each cage was given a sequence number. The test group contained animals from cages 1 and 3, and the control group included mice from cages 2 and 4. At the beginning of the experiment, there were 9 mice (4 females and 5 males) in cage 1; 10 mice (5 females and 5 males) in cage 2; 9 mice (4 females and 5 males) in cage 3; and 8 mice (4 females and 4 males) in cage 4. Thus, there

were 9 females and 9 males in the control group, and 8 females and 10 males in the test group.

The groups differed in the partial pressure of oxygen ($P_{CO2}$) and carbon dioxide ($P_{CO2}$) during the respiratory impacts. In the test group (HH), the composition of inhaled air was maintained at $PO_{2}$ = 90 mmHg and $PCO_2$ = 50 mm Hg throughout the respiratory impacts. In the control group, the mice underwent similar experimental procedures, except for alterations in the composition of the inhaled air during the respiratory impacts.

The respiratory training started after the mice had reached the age of 3 months. Before the exposure, the mice were visually evaluated, their body mass was measured, and their muscle strength, physical fatigability, and behavior were assessed. In the course of the experiment, the animals underwent daily visual examination, and cases of birth and death among the posterity were recorded.

During their lifetime, the mice in both groups underwent 21-day sessions of respiratory impacts, at 30 min daily and with a 2-month interval between sessions. The number of exposures and the duration of the interval between sessions were selected based on our previous researches (Tregub et al. 2013; Kulikov et al. 2009), with 30-min exposures determined as the most optimal (Kulikov et al. 2015). The day after each exposure session, all animals underwent a reassessment of their muscle strength, physical fatigability, and behavior.

### Application of respiratory impacts

For respiratory intervention, we used a flow-type chamber, as previously described (Tregub et al. 2015). The experimental groups of mice breathed a gas mixture with a composition that was dependent on the prescription for the group. The control group was placed in the chamber under similar conditions; however, instead of a gas mixture, atmospheric air was pumped in by the compressor. The gas composition in the chamber was controlled by using a Microlux O2 + CO2 gas analyzer (Microlux Ltd, Russia).

### Assessment of muscular strength and physical fatigability

A string was fixed horizontaly at a height of 75–80 cm was used to determine the muscle strength and physical fatigability of the mice. The animals were enabled to grip the string with their front paws and they hung in this position until they were exhausted or fell down; thus, the "holding time" was recorded (Jackson and Broadhurst 1982). Twenty minutes later, the activity was repeated and the mean value was calculated based on the two indices, as well as the difference between the recorded holding time in the first and second attempts.

### Open field and dark/light box test

The procedure of behavioral testing in an open field entailed preparation to minimize the animal stress and for the test itself (Hall 1936). During the test, the following data were recorded: horizontal motor activity, based on the number of sections crossed; vertical motor activity, based on the number of "risings" onto the hind legs; and time of exploration of "burrows" (holes in the floor) per second throughout the whole test period. The test allowed measuring the emotional reactivity and behavior of the animal, as well as its strategy of exploratory/defensive behavior (Hall 1936; Gould et al. 2010).

The dark/light box test was used to evaluate the conditioned passive avoidance reflex (CPAR) in mice, which is based on their inborn tendency toward confined and enclosed spaces (Bourin and Hascoët 2003). The experimental chamber consisted of two compartments: a bigger illuminated section and a smaller dark section with an electroconductive floor. The time of the first entry of the animal into the smaller chamber and the total time of its stay there were recorded over a period of 3 min. After 3 min, the mouse, while still in the dark compartment, received an electrical pain stimulus of 0.45 MA and 30 V until it ran out into the illuminated section. Within 24 h after the CPAR was acuired, it was tested by placing the mouse into the lighted chamber. In the evaluation of CPAR maintenance, the total time of stay in the dark section was considered, as was the difference in the time of stay in the dark compartment until the formation of CPAR and during its testing ($\Delta t$).

The open field and dark/light box behavioral tests were carried out once after four training cycles.

### Statistical analysis

Statistical analysis was done by using the Statistica 6.0 software (StatSoft, Inc., USA). The hypothesis of normalcy of distribution was confirmed with the use of the Shapiro–Wilk test. Some data did not follow the normal distribution law; thus, independent groups were compared by applying the nonparametric Mann–Whitney U test, and related groups by using the Wilcoxon signed-rank test. The statistical power was 80% ($\beta \leq 0.2$). Values of $p < 0.05$ were considered statistically significant. The data are presented as medians ± 25/75 percentiles. The survival rate of mice was analyzed by the Kaplan–Meier method with the help of the log-rank test by using Cox's F test to calculate the p-level (Harrison et al. 2009).

## Results

### Life span

During the experiments the animals died mainly from natural causes, having reached chronological and physiological old age. Some of the animals died in puberty fights for dominance. As a result, the number of mice that were included in the different training cycles varied throughout the experiment period (Table 1). The body mass indices of the mice did not differ in the animals of experimental and control groups over the course of study (data are not reported).

**Table 1** Number of mice subjected to different training cycles

| No. of training cycles | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| HH[a] | | | | | | | |
| Males | 10 | 9 | 9 | 8 | 7 | 5 | 4 |
| Females | 8 | 7 | 3 | 3 | 2 | 1 | 0 |
| Control[b] | | | | | | | |
| Males | 9 | 9 | 8 | 7 | 3 | 0 | 0 |
| Females | 9 | 7 | 4 | 4 | 1 | 0 | 0 |

[a]Hypercapnic hypoxia group

[b]Control group

The maximum number of full sessions of exposure to hypercapnic hypoxia carried out during the research was seven training cycles. The statistical analysis of the survival rate of mice (Fig. 1) showed that the respiratory exercises under hypercapnic hypoxia extended the mean life span of mice by 16% ($p = 0.04866$). The results indicated that the average life expectancy was 457 days in the test group and 384 days in the control group.

### Causes of death and reproductive activity in mice

During the experiments, two cases of acute cerebral blood flow disturbance in female mice at age 6 months were recorded in the control group, compared with one case in the test group (a female at age 12 months). Stroke was diagnosed based on typical neurologic symptoms: unidirectional rotation along the longitudinal or lateral body axis, unilateral muscle spasticity, and blepharoptosis. Autopsy proved the cause of death.

In the control group, six cases of spontaneous mammary cancer in females were recorded (Fig. 2); this is typical in adult mice and, as a rule, appears as B cell lymphoma (Anisimov et al. 2007). Such tumor was first observed in a 10-month-old female in the control group. There was only one case of tumor formation in the test group, in a female at age 13 months. The histopathological analysis of tumors

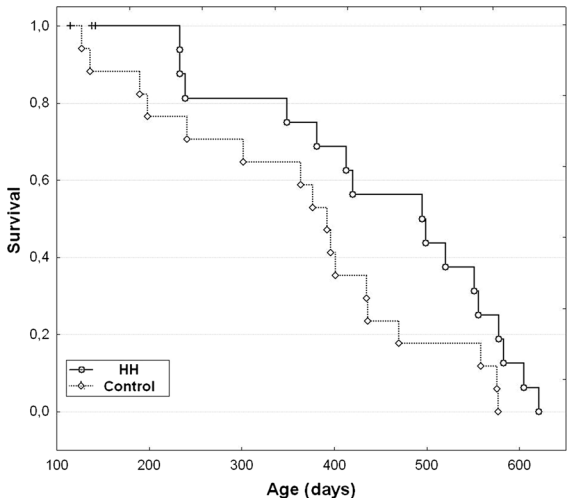


**Fig. 1** Survival rate of mice during the experiments (according to the Kaplan–Meier method). The data are expressed as life span in days. *Control* control group, *HH* hypercapnic hypoxia

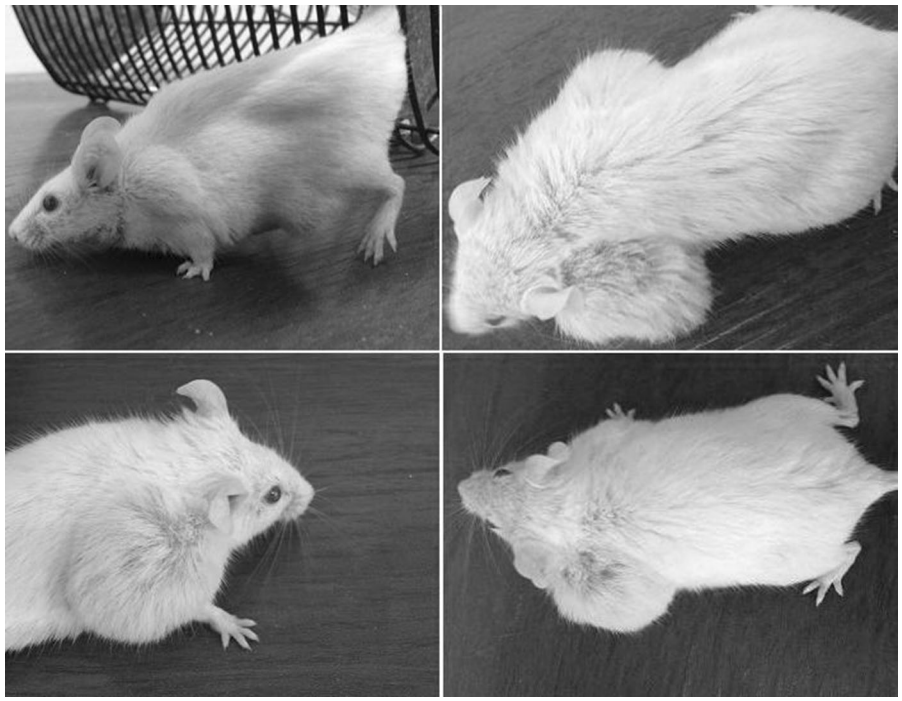

**Fig. 2** Female mice in the control group with spontaneous tumors

in mice was not carried out as these data were not of interest or required within the given experiment. However, the significant difference in the number of tumors in animals of different groups could not be ignored.

The important integrative indices of reproductive function in mice are the average number of pups born per female and the number of young mice that live to 4 weeks (Gustin et al. 2008). After the first training cycle, the mean birth rate was higher in the group consisting of mice that underwent respiratory training under hypercapnic hypoxia (Fig. 3a). The birth rate in the HH group after the second and third exposure sessions did not differ from the indices in the control group; however, the birth rate was significantly higher after the fourth and fifth cycles.

The survival indices of pups that lived to 28 days showed a similar trend to the birth rate (Fig. 3b). The number of mouse pups that survived in the group exposed to hypercapnic hypoxia was also higher compared with the control group after the first, fourth, and fifth training cycles. Besides, after the third exposure session, more mouse pups survived in the HH group than in the control group, despite the fact that the birth rate in the HH group in this cycle did not differ from that in the control group.

### Muscle strength and physical fatigability

The initial indices of muscle strength and physical fatigability did not differ between the groups (Figs. 4, 5). After the second cycle of respiratory training, the physical fatigability in the HH group was lower than that in the control group and remained at a low level until the fifth training cycle. After the third and fourth training cycles, the holding time on the string increased in the HH group compared with the control group.

### Open field and dark/light box test

The open field test showed that the horizontal motor activity of the mice in the HH group was lower, whereas the vertical motor activity and the time of exploration of burrows were higher compared with the control group (Fig. 6).

The dark/light box testing of the mice after the fourth training cycle showed that in the HH group, the time of stay in the dark compartment after the

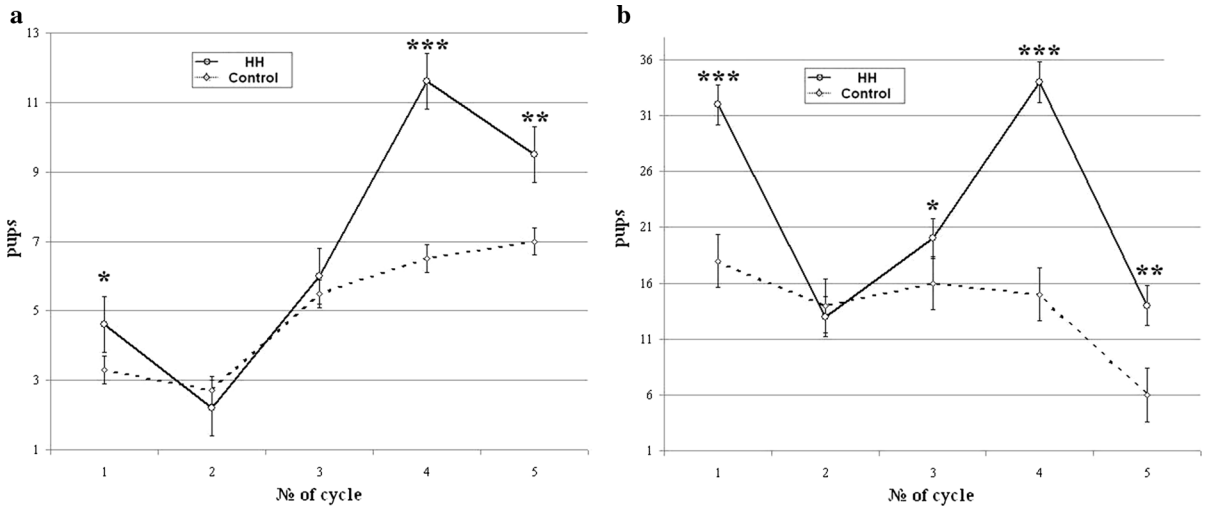


**Fig. 3** Birth rate during the experiment, expressed as the average number of pups born per female (**a**). Survival curves for posterity born during the experiment (**b**). The data are presented as medians (25/75 percentile). *p < 0.05, differences from control group; **p < 0.01, differences from control group; ***p < 0.001, differences from control group. *Control* control group, *HH* hypercapnic hypoxia

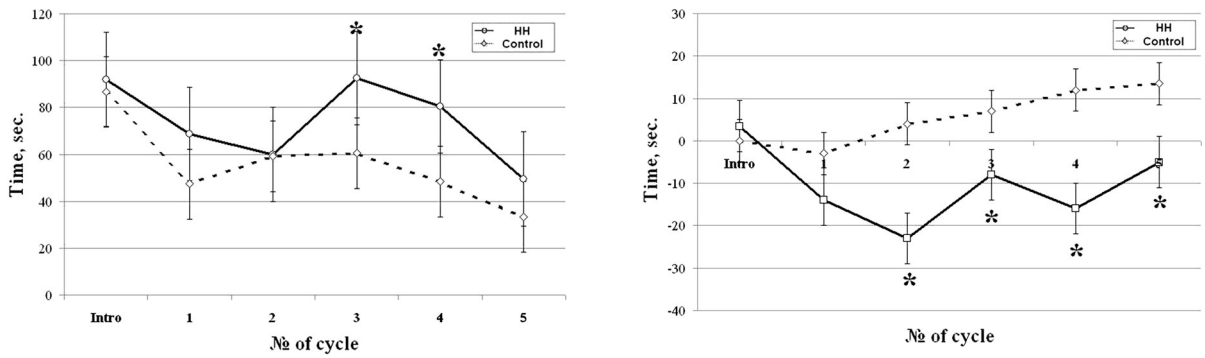


**Fig. 4** Dynamics of the average holding time on the string. *p < 0.01, differences from control group. *Control* control group, *HH* hypercapnic hypoxia

**Fig. 5** Dynamics of the time of restoration of muscle strength, expressed as the difference between the durations of the first and second attempts. *p < 0.01, differences from control group. *Control* control group, *HH* hypercapnic hypoxia

formation of CPAR was shorter, whereas Δt was longer, compared with the control group.

## Discussion

In the present experimental research carried out on mice, the geroprotective effect of regular hypercapnic-hypoxic exercises was assessed in the context of the average life expectancy and the main criteria of its quality (reproductive function, muscle strength, and behavioral activity).

We assumed that a combination of hypoxia and hypercapnia (hypercapnic hypoxia) could have a significant impact on the organism, serving to increase life span and improve the quality of life. This hypothesis is based, on one hand, on several widely known experimental and clinical observations of the efficiency of hypoxia in increasing resistance and treating diseases and, on the other hand, on the obtained strong evidence of the therapeutic efficiency of hypercapnia. It is worth noting that hypercapnia has a significant positive effect on a number of key geroprotective mechanisms, including angiogenesis (Siafakas et al. 2001), inhibition of apoptosis (Tao

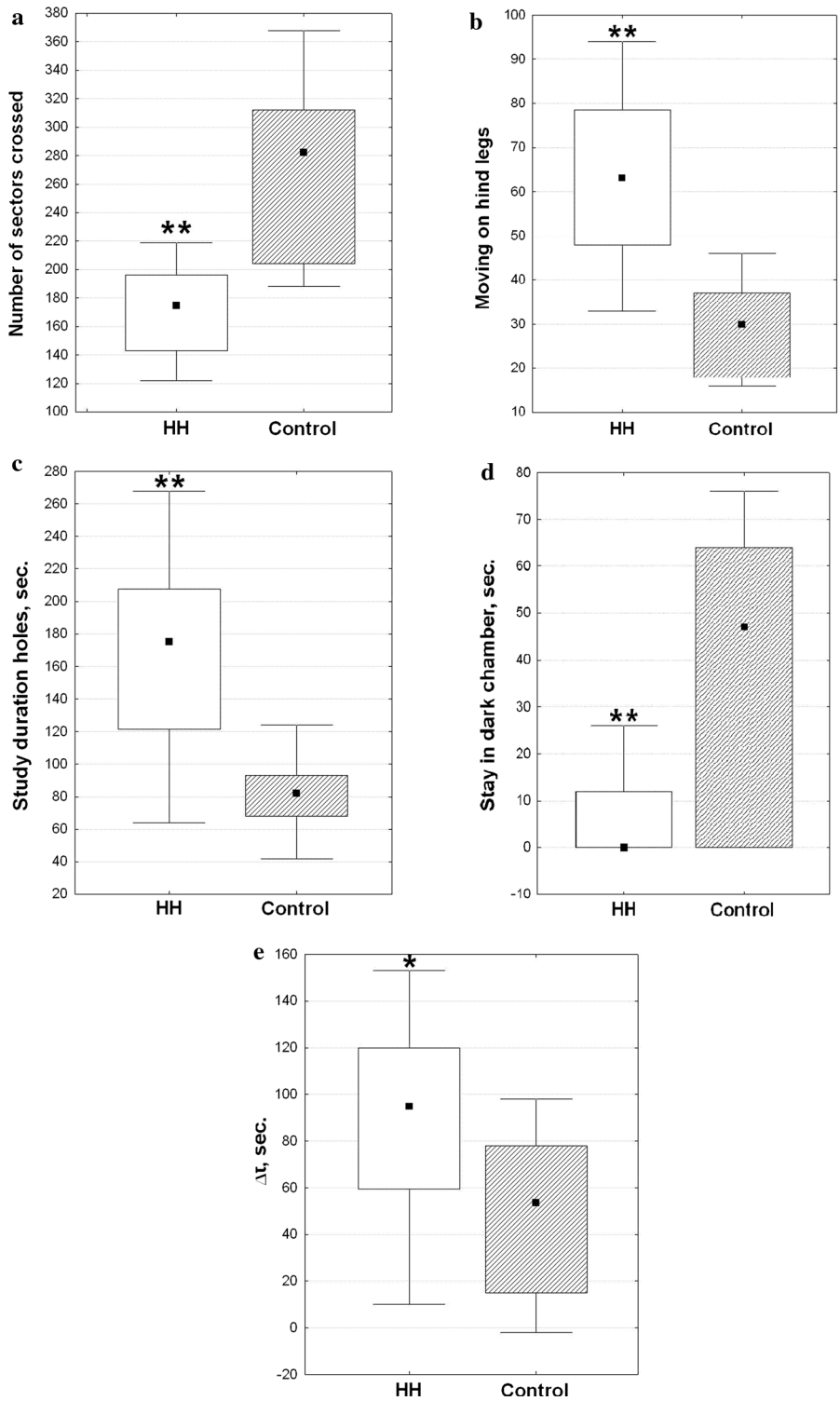


**Fig. 6** Results of the open field and dark/light box behavioral testing. **a** Horizontal motor activity. **b** Vertical motor activity. **c** Exploration of burrows. **d** Time of stay in the dark chamber. **e** Difference between the durations of stay in the dark compartment before the acquisition of CPAR and on its formation (Δt). *$p < 0.05$, differences from control group; **$p < 0.01$, differences from control group. *Control* control group, *HH* hypercapnic hypoxia

et al. 2013), antioxidant activity (Zakynthinos et al. 2007) and chaperones (Bespalov et al. 2014). Therefore, the effects of hypoxia and hypercapnia may be considered as factors, which, in moderate

doses, have a modulating effect on the signaling mechanisms controlled by the vitagene system (heat shock proteins, tyredoxin and the sirtuin protein system). The latter, in turn, is known to play a significant role in protecting against stress and prolonging lifespan (Calabrese et al. 2014). Possibly, this modulating effect can be elicited through epigenetic mechanisms that control expression of genes encoding protective factors.

Our recent researches have shown that, compared with the individual use of hypoxia and hypercapnia, sessions of exposure to hypoxia combined with hypercapnia have a maximum effect in increasing resistance to critical oxygen deficiency (Tregub et al. 2013) and brain tolerance to focal cerebral ischemia (Tregub et al. 2015). These findings allow the assumption that the hypothesis of the geroprotective potential of hypercapnic hypoxia has a solid basis, justifies the efforts made to test such hypothesis experimentally.

The results of the study showed that the average life span of mice that had undergone a course of hypercapnic hypoxia was extended by 16% (74 days), which is about 11 human years (Flurkey et al. 2007). Compared with the findings of similar experimental researches on mice, the present results can be regarded as a significant achievement. Thus, while estimating the efficiency of genetically induced telomerase activation, the life span of mice was extended by 18% (Bernardes de Jesus et al. 2012), whereas rapamycin (mTOR-kinase inhibitor, an immunosuppressive drug) fed in the diet of old age mice extended the life span by 13% (Harrison et al. 2009). However, the geroprotective effect obtained in those studies is hardly comparable with our findings, as in the described research mice were subjected to exposures at an old age (1 to 2 years old, telomerase; 600 days old, rapamycin). Whereas our model implied that mice started receiving respiratory exposures when they reached the reproductive age (12 weeks old). At the same time, it is important to note, that the small number of animals in this study, along with overall challenges in translating geroprotective strategies from mouse to human, significantly limit the possibility of formulating direct recommendations on life prolongation. Nevertheless, the results of our study show potential perspectives for application of hypercapnic hypoxia as a means for life extension and improvement of physiological activity at old age.

It is important to note that the drugs used in the above-mentioned studies have side effects, particularly the risk of tumors (Bodnar et al. 1998). At the same time, the effect of hypercapnic hypoxia, as described in the present study, showed a tumor protective tendency against tumors common in mice (Anisimov et al. 2007), thus indicating an advantageous difference for HH compared with the said drugs.

Before the research started, we understood that to characterize the effect of hypercapnic hypoxia on females, it was important to register the parameters of their reproductive function in detail. The results of the experiment showed that hypercapnic hypoxic exposure extended their reproductive youth. This is an important contribution in support of the hypothesis of the geroprotective effect of hypercapnic hypoxia because improved reproductive activity at an old age is one of the most important aspects of healthy longevity (Finch 2009).

The geroprotective effect of the combined effects of moderate doses of hypoxia and hypercapnia found in our study can be viewed promisingly from the prospective of the hormesis paradigm (Leak et al. 2018). The main reason for this is the fact that both the lack of oxygen and carbon dioxide as well as their excess in the body causes serious damage. Therefore, the positive effects, demonstrated for the intermittent effects of moderate doses of hypoxia and hypercapnia, could be achieved only with a certain therapeutic range of gas concentrations, duration of sessions, and frequency of courses. In addition, it is important to emphasize that intermittent hypercapnia and hypoxia have already been considered as hormetic stimuli (Pruimboom and Muskiet 2018).

A similar positive tendency regarding muscle strength and physical fatigability was noted in old-age mice after hypercapnic hypoxic exposures. Aging is well known to diminish strength and increase fatigability (Anisimov et al. 2007). Consequently, the positive changes in those variables observed in our study indicate the geroprotective potential of hypercapnic hypoxia. It is important to point out that physical capacity at all the stages of the research was tested in all the females irrespective of the phase of their reproductive cycle (absence of pregnancy, pregnancy, breast-feeding, and menopause). Such step was taken in order to average the obtained results as much as possible and to avoid receiving incomplete data in

each group as they would impede statistical objective analysis.

In the present research, regular hypercapnic hypoxic exposures increased the stress resistance of mice, whereas the increased vertical motor activity and time of exploration of burrows can be interpreted as reduced generalized excitation with activated exploratory behavior (Gould et al. 2010). Besides, the formation of a conditioned passive avoidance reflex required less time. These improvements in stress resistance and cognitive functions in the animals can also be regarded as the indices of healthy longevity (Jafari 2015).

It is important to remember that the prevalence of positive or negative effects of hypercapnia is determined, to a great extent, by $CO_2$ concentration, exposure time, and multiplicity. For example, exposure to pronounced continuous hypercapnia (10% $CO_2$) for 3, 7, 14, and 21 days causes skeletal muscle atrophy in mice (Jaitovich et al. 2015), whereas a 24-h exposure to 7% $CO_2$ environment forms an immunosuppressive effect in *Drosophila* (Helenius et al. 2009). At the same time, exposure to 19% $CO_2$ environment for 1, 6, or 72 h extends the lifespan of *Caenorhabditis elegans*, exerting a negative influence on motor activity and reproductive function (Sharabi et al. 2009).

## Conclusion

Repeated respiratory exercises with hypercapnic hypoxia result in increased life span in laboratory mice, their improved reproductive and cognitive functions, increased motor and search activity, as well as physical stamina in old age.

**Funding** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Compliance with ethical standards**

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

**Human and animal rights** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (EU Directive 2010/63/EU for animal experiments).

## References

Agadzhanian NA, Radysh IV, Severin AE, Ermakova NV (1995) Ecology, adaptation and biorhythms. Aviakosm Ekolog Med 29(3):16–19

Anisimov VN, Popovich IG, Zabezhinski MA (2007) Methods of evaluating the effect of of pharmacological drugs on aging and life span in mice biological aging: methods and protocols. In: Tollefsbol TO (ed) Methods in molecular biology, vol 371. Humana Press, Totowa, pp 227–236

Bespalov AG, Tregub PP, Kulikov VP, Pijanzin AI, Belousov AA (2014) The role of VEGF, HSP-70 and protein S-100B in the potentiation effect of the neuroprotective effect of hypercapnic hypoxia. Patol Fiziol Eksp Ter 2:24–27

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279:349–352

Boretto JM, Cabezas-Cartes F, Ibargüengoytía NR (2018) Slow life histories in lizards living in the highlands of the Andes Mountains. J Comp = Physiol B 188:491–503

Bourin M, Hascoët M (2003) The mouse light/dark box test. Eur J Pharmacol 463(1–3):55–65

Calabrese V, Scapagnini G, Davinelli S, Koverech G, Koverech A, De Pasquale C, Salinaro AT, Scuto M, Calabrese EJ, Genazzani AR (2014) Sex hormonal regulation and hormesis in aging and longevity: role of vitagenes. J Cell Commun Signal 8(4):369–384

Chen WJ, Chen HW, Yu SL (2005) Gene expression profiles in hypoxic preconditioning using cDNA microarray analysis: altered expression of an angiogenic factor, carcinoembryonic antigen-related cell adhesion molecule 1. Shock 24:124–131

de Jesus BB, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA (2012) Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Mol Med 4(8):691–704

Fierstra J, Sobczyk O, Battisti-Charbonney J, Mandell DM, Poublanc J, Crawley AP, Mikulis DJ, Duffin J, Fisher JA (2013) Measuring cerebrovascular reactivity: what stimulus to use? J Physiol 591(23):5809–5821

Finch CE (2009) Update on slow aging and negligible senescence—a mini-review. Gerontology 55:307–313

Flurkey K, Currer JM, Harrison DE (2007) The mouse in aging research. In: Fox JG et al (eds) The mouse in biomedical research second III. Academic Press, Cambridge, pp 637–672

Gould TD, Dao DT, Kovacsics CE (2010) The open field test in mood and anxiety related phenotypes in mice. Neuromethods 42:1–20

Gustin SE, Western PS, McClive PJ, Harley VR, Koopman PA, Sinclair AH (2008) Testis development, fertility, and survival in ethanolamine kinase 2-deficient mice. Endocrinology 149(12):6176–6186

Hall CS (1936) Emotional behavior in the rat. The relationship between emotionality and ambulatory activity. J Comp Physiol Psychol 22:345–352

Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA (2009) Rapamycin

fed late in life extends lifespan in genetically heterogeneous mice. Nature 460(7253):392–395

Helenius IT, Krupinski T, Turnbull DW, Gruenbaum Y, Silverman N, Johnson EA, Sporn PH, Sznajder JI, Beitel GJ (2009) Elevated CO2 suppresses specific Drosophila innate immune responses and resistance to bacterial infection. Proc Natl Acad Sci USA 106(44):18710–18715

Jackson HF, Broadhurst PL (1982) The effects of parachlorophenylalanine and stimulus intensity on open-field test measures in rats. Neuropharmacology 21(12):1279–1282

Jafari M (2015) Healthspan pharmacology. Rejuvenation Res 18(6):573–580

Jaitovich A, Angulo M, Lecuona E, Dada LA, Welch LC, Cheng Y, Gusarova G, Ceco E, Liu C, Shigemura M, Barreiro E, Patterson C, Nader GA, Sznajder JI (2015) High CO2 levels cause skeletal muscle atrophy via AMP-activated kinase (AMPK), FoxO3a protein, and muscle-specific Ring finger protein 1 (MuRF1). J Biol Chem 290(14):9183–9194

Krivoruchko A, Storey KB (2010) Forever young: mechanisms of natural anoxia tolerance and potential links to longevity. Oxid Med Cell Longev 3(3):186–198

Kulikov VP, Bespalov AG, Yakushev NN (2009) The state of cerebral hemodynamics in conditions of prolonged adaptation to hypercapnic hypoxia. Neurosci Behav Physiol 39(3):269–273

Kulikov VP, Osipov IS, Tregub PP (2015) Optimal hypercapnic hypoxia conditions for increasing resistance to acute hypoxia. Aviakosm Ekolog Med 49(5):25–28

Laffey JG, Tanaka M, Engelberts D, Luo X, Yiang S, Tanswell TK, Post M, Lindsay T, Kavanagh BP (2000) Therapeutic hypercapnia reduces pulmonary and systemic injury following in vivo lung reperfusion. Am J Respir Crit Care Med 162:2287–2294

Leak RK, Calabrese EJ, Kozumbo WJ, Gidday JM, Johnson TE, Mitchell JR, Ozaki CK, Wetzker R, Bast A, Belz RG, Bøtker HE, Koch S, Mattson MP, Simon RP, Jirtle RL, Andersen ME (2018) Enhancing and extending biological performance and resilience. Dose Response 16(3):1559325818784501

Lin HJ, Wang CT, Niu KC, Gao C, Li Z, Lin MT, Chang CP (2011) Hypobaric hypoxia preconditioning attenuates acute lung injury during high-altitude exposure in rats via up-regulating heat-shock protein 70. Clin Sci (Lond) 121(5):223–231

Lukyanova LD, Germanova EL, Kopaladze RA (2009) Development of resistance of an organism under various conditions of hypoxic preconditioning: role of the hypoxic period and reoxygenation. Bull Exp Biol Med 147:400–404

Matsubayashi K, Okumiya K (2012) Field medicine: a new paradigm of geriatric medicine. Geriatr Gerontol Int 12(1):5–15

Muradian K (2013) "Pull and push back" concepts of longevity and life span extension. Biogerontology 14(6):687–691

Neckár J, Papousek F, Nováková O, Ost'ádal B, Kolár F (2002) Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. Basic Res Cardiol 97(2):161–167

Obrenovitch TP (2008) Molecular physiology of preconditioning-induced brain tolerance to ischemia. Physiol Rev 88(1):211–247

Pruimboom L, Muskiet FAJ (2018) Intermittent living; the use of ancient challenges as a vaccine against the deleterious effects of modern life—a hypothesis. Med Hypotheses 120:28–42

Serebrovskaya TV, Xi L (2016) Intermittent hypoxia training as non-pharmacologic therapy for cardiovascular diseases: practical analysis on methods and equipment. Exp Biol Med (Maywood) 241(15):1708–1723

Sharabi K, Hurwitz A, Simon AJ, Beitel GJ, Morimoto RI, Rechavi G, Sznajder JI, Gruenbaum Y (2009) Elevated CO2 levels affect development, motility, and fertility and extend life span in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 106(10):4024–4029

Sharp FR, Ran R, Lu A, Tang Y, Strauss KI, Glass T, Ardizzone T, Bernaudin M (2004) Hypoxic preconditioning protects against ischemic brain injury. NeuroRx 1(1):26–35

Shatilo VB, Korkushko OV, Ischuk VA, Downey HF, Serebrovskaya TV (2008) Effects of intermittent hypoxia training on exercise performance, hemodynamics, and ventilation in healthy senior men. High Alt Med Biol 9(1):43–52

Siafakas NM, Jordan M, Wagner H, Breen EC, Benoit H, Wagner PD (2001) Diaphragmatic angiogenic growth factor mRNA responses to increased ventilation caused by hypoxia and hypercapnia. Eur Respir J 17:681–687

Tao T, Liu Y, Zhang J, Xu Y, Li W, Zhao M (2013) Therapeutic hypercapnia improves functional recovery and attenuates injury via antiapoptotic mechanisms in a rat focal cerebral ischemia/reperfusion model. Brain Res 1533:52–62

Tregub P, Kulikov V, Bespalov A (2013) Tolerance to acute hypoxia maximally increases in case of joint effect of normobaric hypoxia and permissive hypercapnia in rats. Pathophysiology 20(3):165–170

Tregub P, Kulikov V, Motin Y, Bespalov A, Osipov I (2015) Combined exposure to hypercapnia and hypoxia provides its maximum neuroprotective effect during focal ischemic injury in the brain. J Stroke Cerebrovasc Dis 24(2):381–387

Van Zutphen LF, Baumans V, Beynen AC (2001) Principles of laboratory animal science. Elsevier, New York

Yang CC, Lin LC, Wu MS (2009) Repetitive hypoxic preconditioning attenuates renal ischemia/reperfusion induced oxidative injury via upregulating HIF-1 alpha-dependent bcl-2 signaling. Transplantation 88:1251–1260

Zakynthinos S, Katsaounou P, Karatza MH, Roussos C, Vassilakopoulos T (2007) Antioxidants increase the ventilatory response to hyperoxic hypercapnia. Am J Respir Crit Care Med 175(1):62–68

Zhou Q, Cao B, Niu L, Cui X, Yu H, Liu J, Li H, Li W (2010) Effects of permissive hypercapnia on transient global cerebral ischemia–reperfusion injury in rats. Anesthesiology 112:288–297