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RESEARCH ARTICLE

Metabolic remodelling of mice by hypoxic-hypercapnic environment: imitating the naked mole-rat

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Abstract We hypothesised that hypoxic-hypercapnic environment (HHE) could induce metabolic suppression and associated benefits for health and longevity, as observed in the naked-mole rat (NMR). We developed a model of self-produced HHE (similar to a natural habitat of NMRs), which is simple, reliable and natural, and does not require external sources of gases or complex technical equipment. Here, we showed for the first time that a chronic exposure of mice to HHE could be a unique tool for NMR-like metabolic remodeling, resulting in a long-term and substantial decrease in metabolic rate, body temperature, and food consumption, without significant changes in expression of stress-related genes. Unexpectedly, the HHE accelerated skin wound healing, despite the lower energy expenditure. The self-produced HHE could be considered a model of voluntary calorie restriction. All in all, a chronic exposure to HHE offers a potential of being a lifespan-extending intervention as well as an efficient tool for treating the overweight and associated metabolic disorders.

Keywords Hypometabolism · Hypothermia · Hypoxia · Hypercapnia · Voluntary calorie restriction · Mice · Age

Denis A. Tolstun and Anna Knyazer contributed equally to this work.

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Abbreviations

CR	Calorie restriction
FT3	Free triiodothyronine
FT4	Free thyroxine
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HHE	Hypoxic-hypercapnic environment
HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member
i.p.	Intraperitoneally
MSCs	Mesenchymal stem cells
NMR(s)	Naked mole-rat(s)
qPCR	Quantitative real time polymerase chain reaction

Vo_2	Oxygen consumption
Vco_2	Carbon dioxide production
UCP2	Mitochondrial uncoupling protein 2

Introduction

Energy and temperature are the two all-pervasive factors that could modify virtually all biological processes, aging included. Not surprisingly, the links between aging, energy expenditure and temperature have been objects of close scrutiny since the beginning of the twentieth century (Loeb and Northrop 1916; Pearl 1928). Although many aspects of the relationships remain disputable, it is generally assumed that lower metabolic rate and body temperature could independently or in cooperation with each other contribute to longevity in both poikilotherms and homeotherms (Conti 2008; Flouris and Piantoni 2014; Keil et al. Keil et al. 2015; Lehmann et al. 2008, 2013; Prokopov 2007; Tabarean et al. 2010; Xiao et al. 2015). Lately, the problem acquired additional impetus due to the emergence of a new gerontological model—the naked mole-rat (*Heterocephalus glaber*, NMR), a mouse-sized rodent species which maximum life span (MLS) in captivity exceeds 30 years, i.e. is around 8 times longer than MLS of mice (Buffenstein 2008; Lewis et al. Lewis et al. 2018; Tacutu et al. 2018). Remarkably, metabolic rate and body core temperature of NMR are substantially lower than in similar-sized mice (Goldman et al. 1999; Nathaniel et al. 2012). Other outstanding longevity characteristics of NMRs include (but are not limited to) the absence of the most notorious features of aging—increased mortality and declined reproductive activity (Ruby et al. 2018). Of particular interest is their high resistance to the age-associated pathologies such as cancer (Liang et al. 2010; Seluanov et al. 2018), cardiovascular and pulmonary disorders (Csiszar et al. 2007; Delaney et al. 2013; Grimes et al. 2017), stroke (Nathaniel et al. 2013; Xiao et al. 2017), sarcopenia (Stoll et al. 2016), diabetes (Singer 2011), etc.

Comparison of NMRs and mice revealed relatively small genomic rearrangements after their split from the common murid ancestor (Kim et al. 2011), indicating that striking longevity differences could mainly be associated with their physiology and lifestyle. NMRs are eusocial subterranean rodents which live as relatively big colonies of about 70 individuals in deep and poorly ventilated underground burrows (Goldman et al. 1999; O'Riain and Faulkes 2008) and are extremely resistant to hypoxia (Ilacqua et al. 2017; Larson and Park 2009) and hypercapnia (Branigan et al. 2018). In their habitat, CO_2 content could be increased and O_2 reciprocally decreased, at least temporarily, up to 10% (Bennett and Faulkes 2000; Šumbera 2019). There are reasons to believe that HHE could decrease intensity of metabolic processes and extend life span (Muradian 2013). We hypothesise that such self-generated and balanced HHE could create unique backward loops ensuring physiologically well-tuned decline of body temperature and metabolism. The aim of this research was to clarify whether HHE commonly experienced by NMRs could induce similar metabolic and temperature changes in mice. Specifically, we focused on gross metabolic variables such as O_2 consumption, CO_2 production and thermoregulation as well as food and water consumption upon acute and chronic HHE. We also evaluated whether chronic HHE is stressful for mice and whether it affects such a basic biological process as wound healing.

Materials and methods

Animals

Young (3–4 months), middle-aged (8–12 months) and old (24–26 months) male C57Bl/6 or CBA mice were bred and kept in the standard living conditions in the Animal Facility of the Institute of Gerontology of National Academy of Medical Sciences of Ukraine, Kiev. The animals had free access to water and food. All experiments were approved by the Bioethical Committee of the Institute of Gerontology of National Academy of Medical Sciences of Ukraine (Protocol No. 5 of June 12, 2015).

Acute exposure to self-produced HHE

Young and old CBA mice (10 animals in each age group) were kept individually in open glass jars during 2 h for adaptation to the new environment. Then, the jars were hermetically closed for 3 h, resulting in a gradual increase in CO_2 and a decrease in O_2 content (see "Results" section). Air samples for measurement

of CO_2 and O_2 concentrations were taken every 30 min, simultaneously with registration of the number of moving or sleeping mice. Body surface temperature was measured before and immediately after 3 h of HHE.

Chronic exposure to self-produced HHE

In chronic series of experiments, C57Bl/6 mice were kept in standard cages placed in transparent plastic cuvettes with covers. Slots for air exchange of the cuvettes were adjusted to keep O_2 and CO_2 levels around $10 \pm 2\%$. The content of O_2 and CO_2 in the air was regularly measured by using corresponding blocks of the gas analyzer (Gerb-Minnhardt, Netherland). The cages were cleaned simultaneously with ad libitum food and water replenishment on every other day basis. Food and water consumption and body weight were measured in three age groups (young, middle-aged and old) for 90 days of HHE. Other measurements (Vo_2 and Vco_2, body surface temperature, plasma levels of free triiodothyronine (FT3) and free thyroxine (FT4), expression of stress-related genes (UCP2 and HSP90), and organ weights were performed in young mice at indicated time points (Day 0, 1, 10, 20 and 30 of HHE). Control animals were kept in normoxic atmosphere and underwent the same procedures as experimental animals. The measurements were conducted in morning hours, between 9 and 11 pm.

Food and water consumption

Food and water consumption rates (in percent of the body mass per day) were estimated as differences between the food and water weights before and after the food and water replenishment procedure. The amount of grinded food was separately measured and subtracted when calculating food consumption rate.

Oxygen consumption (Vo_2) and carbon dioxide production (Vco_2)

At indicated time points of chronic exposure to HHE, the cuvettes with mice were hermetically closed for 1 h, then air samples were taken, and the difference between O_2 or CO_2 content before and after the closure was used for calculation of Vo_2 and Vco_2 (in ml g^{-1} h^{-1}), respectively. The content of O_2 and CO_2 in the air was measured using the gas analyzer (Gerb-Minnhardt, Netherland).

Body surface temperature

Body surface temperature was measured by a non-contact infrared thermometer (UNI-T UT912, Austria). The mean value of five consecutive measurements of the temperature at 5–10 cm from the back of freely moving animals was taken as the body surface temperature. In a separate series, we compared the body surface temperature with core (rectal) body temperature in mice. As expected, there was a highly significant positive correlation between the rectal and surface temperatures ($r = 0.81$; $p < 0.001$).

Plasma levels of free triiodothyronine (FT3) and free thyroxine (FT4)

After a 6-h fasting, the whole blood was collected by extracting eyeballs under hexobarbital (70 mg/kg, i.p.) anesthesia, and plasma FT3 and FT4 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic systems, Russia).

Quantitative real-time polymerase chain reaction (qPCR) analysis

Total RNA was extracted from the tissue samples using RNA extraction kit Ribozol-A. RNA was reverse-transcribed into cDNA by using RT-kit Reverta-L-100 according to the manufacturer's instructions (AmpliSens, Russia). The PCR primers for all analyzed genes were synthesized by Metabion International AG (Germany). The primer sequences,

Table 1 The list of primer sequences used in this study

Gene	Primer sequence
GAPDH (F)	5′-AGGTCGGTGTGAACGGATTTG-3′
GAPDH (R)	5′-TGTAGACCATGTAGTTGAGGTCA-3′
UCP2 (F)	5′-ATGGTTGGTTTCAAGGCCACA-3′
UCP2 (R)	5′-CGGTATCCAGAGGGAAAGTGAT-3′
HSP90AA1 (F)	5′-TGTTGCGGTACTACACATCTGC-3′
HSP90AA1 (R)	5′-GTCCTTGGTCTCACCTGTGATA-3′

forward (F) and reverse (R), are listed in Table 1. In all experiments, GAPDH was used as a reference gene.

Real-time qPCR amplification was performed using Chromo4 Detection System (Bio-Rad, USA). The reaction mixture contained 5.5 µl of diluted cDNA, 10 pm of each primer, 10 µl of 2.5 × SYBR Green master mixes in a total volume of 25 µl. PCR was conducted at 95 °C for 5 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. Specificity of RT-PCR products was verified by checking the product melting curves. The threshold cycle (Ct) of each target product was determined and the $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change in gene expression compared to the control group (Schmittgen and Livak 2008).

Skin wound healing

Head excision model of skin wound healing was described in detail elsewhere (Yanai et al. 2015, 2016). Briefly, the mice were anesthetized with hexobarbital (70 mg/kg, i.p.), and full-thickness wounds were generated on the crown of the skull, using an 8-mm trephine (Punch Biopsy). The injured tissue was then excised down to the bone with curved sharp scissors. To follow up the wound closure, digital photographs (Cannon IXY, 4 M) of the wound area were taken every day after surgery, from a distance of 25 cm with a ruler aligned next to the wound. To minimize any possible biases, the morphometric analysis of wound closure was performed as a double-blind study. Quantification of the wound area was carried out using the open source NIH ImageJ v1.43 software.

Statistical analysis

Statistical analysis was performed by using Statisitica-6 package of programs (StatSoft, Inc., Tulsa, OK). F-criteria of two-way factorial ANOVA or one-way ANOVA were used to assess the significance of the effects of HHE exposure (F_{HHE}) or age of mice (F_{age}). Pair-wise correlations were estimated by Pearson's coefficient (r) of correlation. The p-values less than 0.05 were considered statistically significant.

Results

Acute exposure to HHE

The atmospheric O_2 content gradually decreased, and CO_2 content gradually increased during a 3-h exposure of mice to self-produced HHE, reaching approximately 14% and 7%, respectively, by the end of HHE exposure. Acute HHE caused a gradual decrease in Vo_2 and Vco_2 of young and old CBA mice, and at the end of the exposure, Vo_2 and Vco_2 were more than twice lower compared with initial values (Fig. 1a). In both age groups, Vco_2 negatively correlated with CO_2 content in atmosphere (Online Resource 1). Factorial ANOVA revealed a highly significant effect of HHE on Vo_2 and Vco_2 ($p < 10^{-25}$), while the effect of age was insignificant. Body surface temperature decreased on average by two centigrade at the end of a 3-hour exposure to HHE (Fig. 1b). This effect of HHE was also highly significant and independent of age (factorial ANOVA: $F_{HHE} = 67.6$, $p < 10^{-9}$ and $F_{age} = 0.1$, $p > 0.9$).

Thus, the acute exposure of mice to self-produced HHE resulted in a significant decrease in metabolic rate (Vo_2 and Vco_2) and body temperature, and the patterns of these HHE-induced responses were similar in young and old mice.

Chronic exposure to HHE

Metabolic rate and body temperature

The next series of experiments were carried out on young mice, mostly in order to examine proof-of-concept as such—the possibility to reach a stable hypometabolic state by HHE. As seen in Fig. 2a, b, after an initial decrease (see also Fig. 1), Vo_2 and Vco_2 stabilized at the level of some 40–50% lower than in mice of the control group (one-way ANOVA, $p < 10^{-5}$), and remained at this level until the end of a one-month exposure to HHE. Body surface temperature decreased by around 2.5–3.5 °C (Fig. 2c), and this effect of HHE was highly significant (one-way ANOVA, $p < 10^{-9}$). The HHE-induced decrease in body temperature occurred promptly after HHE initiation (see also Fig. 1), and the achieved level was held during the entire period of HHE.

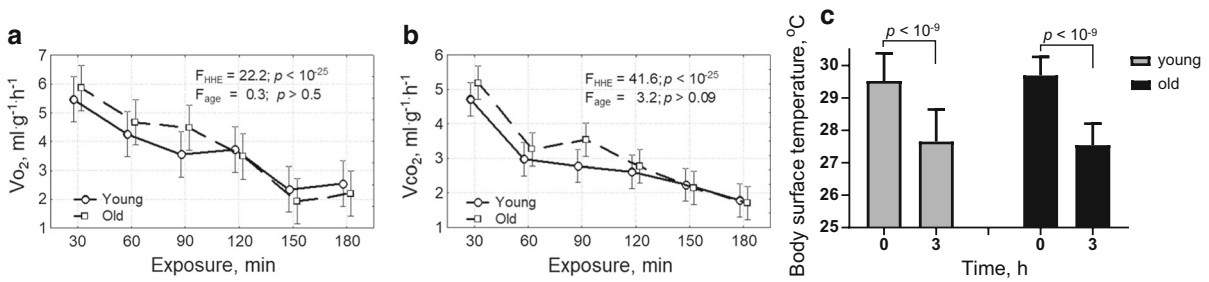

Fig. 1 Effect of acute exposure to self-produced HHE on metabolic rate estimated as **a** Vo_2 and **b** Vco_2, and **c** body surface temperature in young (n = 10) and old (n = 10) male CBA mice. The results are presented as mean ± SD

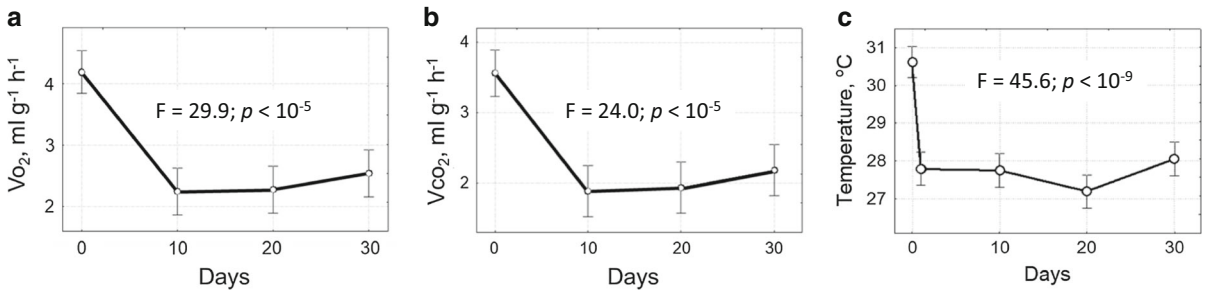

Fig. 2 **a** Oxygen consumption (Vo_2), **b** carbon dioxide production (Vco_2), and **c** body surface temperature in young male C57Bl/6 mice (n = 10) upon chronic exposure to self-produced HHE. The results are presented as mean ± SD

Food consumption and body weight

The decrease in energy metabolism should in some way be associated with food consumption, another gross metabolic variable. We examined this possibility in more detailed manner in mice of three age groups, the young, middle-aged, and old. Also, we extended HHE up to 3 months. As seen in Fig. 3a, food consumption swiftly decreased during the first 10–14 days of HHE and stabilized afterwards at the level around 40–50% lower than the control values, thus highly resembling the response of Vo_2 and Vco_2 to chronic HHE (see Fig. 2). Similar changes were also observed for water consumption (data not shown). Notably, the mice of all three age groups (the young, middle-aged, and old) showed similar patterns in both food and water consumption upon a 3-month exposure to HHE. Apparently, as a result of a decreased food consumption, body mass decreased by some 25–30% in both young and old mice, despite the ad libitum feeding (Fig. 3b).

Blood plasma free triiodothyronine (FT3) and thyroxine (FT4)

The thyroid hormones are one of the main regulators of energy metabolism. Therefore, we further evaluated whether hypometabolic effect of HHE could be

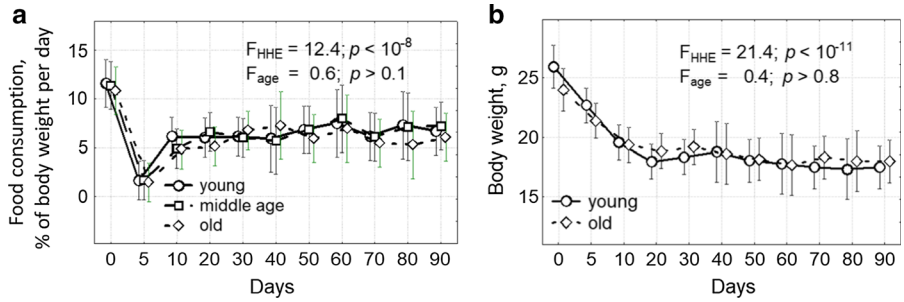

Fig. 3 **a** Food consumption (in percent of the body mass per day) and **b** body weight in young (n = 10), middle-aged (n = 10) and old (n = 10) male C57Bl/6 mice upon chronic exposure to self-produced HHE. The results are presented as mean ± SD

mediated by thyroid hormones. We found only moderate changes in the plasma levels of FT3 and FT4 during chronic HHE. As seen in Fig. 4, FT3 declined during the first 10 days of HHE but normalized afterwards (one-way ANOVA, $p < 0.025$). Unlike FT3, after an initial decrease, FT4 stabilized by Day 10 at 85–90% of the control levels (one-way ANOVA, $p < 0.03$).

Expression of UCP2 and HSP90 genes

To evaluate whether chronic HHE is stressful for mice, we determined the expression of two stress-related genes, UCP2 and HSP90, which transcription activity consistently increases in response to stress (Cadenas 2018; Yavelsky et al. 2004). As seen in Fig. 5, the mRNA levels of UCP2 in the ventromedial hypothalamus and HSP90 in the heart did not change significantly during the course of chronic HHE ($p > 0.5$ after Benjamini correction).

The rate of skin wound healing

The hypometabolic state may affect the basic biological processes, such as tissue wound healing (WH). With this in mind, we evaluated the impact of chronic HHE on the rate of skin WH in young C57Bl/6 mice. Surprisingly, despite the lower energy expenditure, HHE significantly accelerated the closure of head excisional wounds (Fig. 6; Online Resource 2). A 50%-closure of skin wounds occurred by the day 19.7 ± 3.3 and 25 ± 2.4 ($p = 0.03$), and full closure occurred by day 24 ± 3.8 and 29.2 ± 1.6 ($p = 0.02$) after surgery, for HHE-exposed and control mice, respectively.

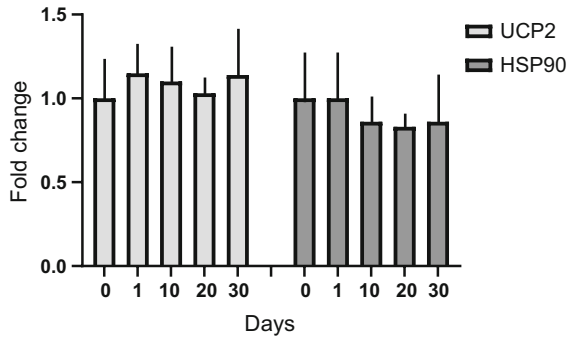

Fig. 5 Expression of **a** the hypothalamic UCP2 and **b** heart HSP90 in young male C57Bl/6 mice (n = 5 for each time point) upon chronic exposure to self-produced HHE. The mRNA levels were assessed by qPCR and presented as fold change (mean ± SEM) compared to the control group (see "Materials and methods" section). The changes in UCP2 or HSP90 expression were insignificant ($p > 0.05$)

Discussion

Pharmacological or physical means for a short-term decrease in metabolism and/or body temperature have been well known (Cuddy 2004; Johansen et al. 2014; Joseph et al. 2012; Sandu et al. 2016a) and are used in a wide variety of areas ranging from the complex surgical operations (hypothermia) to routine treatments at home. In striking contrast, a long-term (chronic) decrease in energy expenditure and/or body temperature in homeotherms appears to be a much more difficult issue and remains practically unsolved up to now. For example, life-long calorie restriction or genetic modifications usually induce moderate or disputable decrease in body temperature and metabolic rate (Conti et al. 2006; Hunter et al. 1999; Roth et al. 2002). Even "heavy" invasions, like destruction of the hypothalamic centers and/or removal of

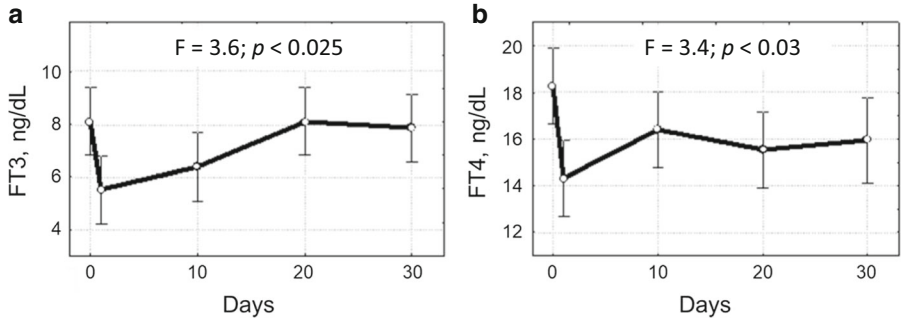

Fig. 4 Blood plasma levels of the thyroid hormones **a** FT3 and **b** FT4 in young male C57Bl/6 mice (n = 5 for each time point) upon chronic exposure to self-produced HHE. The results are presented as mean ± SD

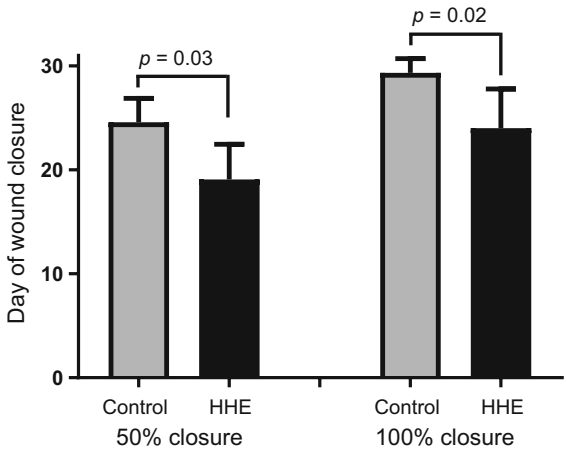

Fig. 6 Skin wound healing rate in young male C57Bl/6 mice (n = 6 in each group) upon chronic exposure to self-produced HHE. Round full-thickness wounds were administered to mice and the measurements of the wound area were made on a daily basis. Time of 50% and full closure are presented as mean ± SD

peripheral subordinate tissues, usually ensured only transient effects, because within a week or two, new centers of regulation emerge and recover the impaired "thermostat" (reviewed by Frolkis and Muradian 1991).

In view of the above, an over two-fold decrease in metabolic rate (oxygen consumption, carbon dioxide production), together with a marked decrease in body temperature induced by chronic exposure of mice to self-produced HHE (Fig. 2) are the most important and principal findings of this study. To our best knowledge, HHE is one of a few, if any, interventions capable of causing *sustained, long-lasting and substantial* hypometabolism and hypothermia in homeotherms. Of note, the used model of self-produced HHE is simple, reliable and natural. It was achieved by maintaining the mice in containers with limited ventilation, that is, in conditions similar to a natural habitat of NMRs. In contrast to the flow-type hypoxia or hypercapnia models, HHE does not require external sources of gases and complex technical equipment. The latter is often accompanied by unpredictable side-effects which are especially critical in long-term experiments.

Most likely, the hypothermic effect of HHE is primarily attributed to alterations in mitochondrial metabolism and vascular responses. Hypoxia could limit the electron flax in the electron transport chain, whereas hypercapnia could decrease the rate of energy generation via suppression of the three decarboxylation reactions occurring during utilization of each molecule of pyruvate in the citric acid cycle. Along with attenuated heat production, both hypoxia and hypercapnia induce strong vasodilatory responses, thus promoting heat loss (reviewed by Reglin and Pries 2014; Liu et al. 2019). Although the role of thyroid hormones in regulation of body metabolism and temperature is well established (Fekete and Lechan 2014), it appears that they have only a moderate impact in mediating hypometabolic/hypothermic effects of HHE (Fig. 4). Remarkably, despite the lower energy expenditure, the HHE accelerated wound healing in mice, rather than slowing it (Fig. 6). This could in part be attributed to enhanced mobilization and functional capacity of mesenchymal stem cells in response to HHE, including their wound-healing activity (Hu et al. 2018; Lee et al. 2009; Shojaei et al. 2019). Likewise, HHE could accelerate wound healing by stimulating the cell proliferation (Tsuji et al. 2013). In another study, post-stroke gaseous (hydrogen sulfide, H_2S) hypothermia was shown to stimulate angiogenesis in the damaged brain of aged rats (Sandu et al. 2016b).

An important observation is that after the short initial period of a decrease in metabolic rate, body temperature and food consumption, the inhibitory effects of HHE reached a steady-state, and the levels of these variables remained stable up to the end of the follow-up (Figs. 2, 3). This indirectly points towards a well-regulated *resetting* of energy and thermal homeostases and a swift adaptation of mice to HHE, actually without visible behavioral or stress responses. Indeed, we did not observe any significant elevation in the expression of stress-related genes UCP2 and HSP90 (Fig. 5). Also, we did not detect any visible changes in the gross behavioral responses in HHE-treated mice, such as spontaneous motor activity or sleeping, as well as excessive shivering or cuddling to keep body warmer (data not shown). It seems that the HHE-exposed mice managed optimizing their metabolic expenditures and got used to the hypothermia.

HHE is distinguished by low inertia and reversibility of the effects. Mice could repeatedly enter and exit from the suppressive metabolic state within minutes, without obvious signs of exhaustion (data not shown). In another model of the H_2S-induced metabolic suppression, the animals also recovered within minutes when returned back to normal atmospheric

conditions (Joseph et al. 2012). Of note, NMRs remained relatively active and continued to explore their environment even at 3% O_2 (Ilacqua et al. 2017; Larson and Park 2009).

It is worthwhile to mention that O_2 and CO_2 contents in the earth atmosphere underwent dramatic changes, and the modern atmosphere with a high O_2 and low CO_2 is a relatively recent acquisition (Berner 2003; Mills et al. 2014; Planavsky et al. 2014). The major period of life evolution occurred at extraordinary low O_2 and high CO_2, thus suggesting that the basic life- and longevity-supporting systems remained hypoxic/hypercapnic by their essence. To survive in a high O_2 atmosphere, successful species had to modify the ancient or invent new adaptive and defense systems, apparently associated with elevated complexity and expenditures. According to the "nostalgia concept", living systems somehow "remember" and are striving to return to the primordial and metabolically relaxed conditions (Muradian 2015). If so, maintenance of animals in HHE (which corresponds to the balanced atmospheric O_2 and CO_2 at the interface of the Proterozoic and Phanerozoic eons, around 500–600 million years ago) could help bypassing or decreasing functional loadings of the evolutionary later invented mechanisms. This, in turn, may ensure a higher metabolic stability, one of the main determinants of mammalian longevity (Olshansky and Rattan 2005; Lehmann et al. 2013). Yet, whether HHE could enhance metabolic stability needs experimental validation. In view of a strong association between longevity and lower metabolic rate and/or body temperature in both poikilotherms and homeotherms (Frolkis and Muradian 1991; Conti 2008; Prokopov 2007; Tabarean et al. 2010; Lehmann et al. 2008, 2013; Flouris and Piantoni 2014; Keil et al. 2015; Xiao et al. 2015; Yanai et al. 2017), a chronic exposure to HHE offers a potential of being a lifespan-extending intervention. Indeed, in cold-blooded organisms, hypoxia and/or hypercapnia more than often resulted in a lower metabolic rate and longevity-promoting effect (Timchenko et al. 2008; Sharabi et al. 2009; Leiser et al. 2013; Tasaki et al. 2018). A growing body of evidence indicates that hypoxia/hypercapnia could have beneficial health outcomes in mammals as well (Kulikov et al. 2019 and references therein). Recently, Kulikov et al. (2019) showed that even transient periodic exposures of mice to hypercapnic hypoxia (a daily 30-min exposure for 21 days, with a 2-month interval between sessions) increased their fitness and extended the average life span by 16%. In another important study (Tyshkovskiy et al. 2019), the mice were exposed to chronic hypoxia of 11.8% O_2 in the air for 32 days, i.e. quite close to the regimen used in our study. The authors found a high similarity between gene expression signatures in response to hypoxia and several recognized longevity-promoting interventions. Based on this finding, they suggested that chronic hypoxia could extend a mouse lifespan (Tyshkovskiy et al. 2019).

Further supporting this notion is HHE-induced reduction in food consumption (Fig. 3). In fact, HHE-treatment could be regarded as a model of "voluntary" calorie restriction (CR), because experimental animals reduced food consumption of their own free will at ad libitum feeding regimes. It is noteworthy that HHE stabilized food consumption at the same level (by around 40% lower control values) as in the most efficient conventional CR models with longevity-promoting effect. However, in conventional (forced) CR, animals usually overate during relatively short periods of food availability and then starve until the next feeding. The HHE-treatment lacks such perturbations (as well as other well-known disadvantages of forced CR), and from this point of view, the HHE-treated mice resemble more the transgenic long-lived αMUPA mice (Miskin et al. 2005). Whatever the differences, it is important to note that the HHE-treated mice exhibited a stronger decrease in energy expenditure and body temperature than mice in conventional CR models.

Body mass is a relatively easy assessed index. Despite its significance is often ignored, body weight is a highly informative and reliable index highlighting integral effects of all anabolic and catabolic processes. The HHE-induced changes in body weight coincide well with food consumption and metabolic rate (Vo_2 and Vco_2). The observed decrease in body mass should mostly be the result of utilization of the lipid deposits as the weight of most internal organs did not change significantly (data not shown). This indicates that HHE could be an efficient tool for treating the overweight and associated metabolic disorders. In support of this notion could be the results of our pilot experiments on the Streptozotocin model of type 1 diabetes, which showed a significant improvement of glucose metabolism in HHE-treated mice (data not shown).

All in all, a chronic exposure of mice to HHE could promote NMR-like metabolic remodeling, resulting in a long-term and substantial decrease in metabolic rate, body temperature, and food consumption—conditions that are strongly associated with lifespan extension. Though many aspects of this association are still debatable, the proposed "4H" approach (Hypoxia + Hypercapnia → Hypometabolism + Hypothermia) definitely warrants further investigation and appears to be easily translatable to other species, humans included.

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Compliance with ethical standards

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

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