Video Article

Supramaximal Intensity Hypoxic Exercise and Vascular Function Assessment in Mice

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Abstract

Exercise training is an important strategy for maintaining health and preventing many chronic diseases. It is the first line of treatment recommended by international guidelines for patients suffering from cardiovascular diseases, more specifically, lower extremity artery diseases, where the patients' walking capacity is considerably altered, affecting their quality of life.

Traditionally, both low continuous exercise and interval training have been used. Recently, supramaximal training has also been shown to improve athletes' performances via vascular adaptations, amongst other mechanisms. The combination of this type of training with hypoxia could bring an additional and/or synergic effect, which could be of interest for certain pathologies. Here, we describe how to perform supramaximal intensity training sessions in hypoxia on healthy mice at 150% of their maximal speed, using a motorized treadmill and a hypoxic box. We also show how to dissect the mouse in order to retrieve organs of interest, particularly the pulmonary artery, the abdominal aorta, and the iliac artery. Finally, we show how to perform ex vivo vascular function assessment on the retrieved vessels, using isometric tension studies.

Introduction

In hypoxia, the decreased inspired fraction of oxygen (O_2) leads to hypoxemia (lowered arterial pressure in hypoxia) and an altered O_2 transport capacity[1]. Acute hypoxia induces an increased sympathetic vasoconstrictor activity directed toward skeletal muscle[2] and an opposed 'compensatory' vasodilatation.

At submaximal intensity in hypoxia, this 'compensatory' vasodilatation, relative to the same level of exercise under normoxic conditions, is well established[3]. This vasodilation is essential to ensure an augmented blood flow and maintenance (or limit the alteration) of oxygen delivery to the active muscles. Adenosine was shown to not have an independent role in this response, while nitric oxide (NO) seems the primary endothelial source since significant blunting of the augmented vasodilatation was reported with nitric oxide synthase (NOS) inhibition during hypoxic exercise[4]. Several other vasoactive substances are likely playing a role in the compensatory vasodilatation during a hypoxic exercise.

This enhanced hypoxic exercise hyperemia is proportional to the hypoxia-induced fall in arterial O_2 content and is larger as the exercise intensity increases, for example during intense incremental exercise in hypoxia.

The NO-mediated component of the compensatory vasodilatation is regulated through different pathways with increasing exercise intensity[3]: if β-adrenergic receptor-stimulated NO component appears paramount during low-intensity hypoxic exercise, the source of NO contributing to compensatory dilatation seems less dependent on β-adrenergic mechanisms as the exercise intensity increases. There are other candidates for stimulating NO release during higher-intensity hypoxic exercise, such as ATP released from erythrocytes and/or endothelial-derived prostaglandins.

Supramaximal exercise in hypoxia (named repeated sprint training in hypoxia [RSH] in the exercise physiology literature) is a recent training method[5] providing performance enhancement in team- or racket-sport players. This method differs from interval training in hypoxia performed at or near maximal speed[6] (V_{max}) since RSH performed at maximal intensity leads to a greater muscle perfusion and oxygenation[7] and specific muscle transcriptional responses[8]. Several mechanisms have been proposed to explain the effectiveness of RSH: during sprints in hypoxia, the compensatory vasodilation and associated higher blood flow would benefit the fast-twitch fibers more than the slow-twitch fibers. Consequently, RSH efficiency is likely to be fiber-type selective and intensity dependent. We speculate that the improved responsiveness of the vascular system is paramount in RSH.

Exercise training has been extensively studied in mice, both in healthy individuals and in pathological mouse models[9,10]. The most common way to train mice is using a rodent treadmill, and the traditionally used regimen is low-intensity training, at 40%-60% of V_{max} (determined using an incremental treadmill test[11]), for 30-60 min[12,13,14,15]. Maximal intensity interval training and its impact on pathologies have been widely studied in

mice[16,17]; thus, interval training running protocols for mice have been developed. Those protocols usually consist of about 10 bouts of running at 80%-100% of V_{max} on a rodent motorized treadmill, for 1-4 min, interspersed with active or passive rest[16,18].

The interest in mice exercising at supramaximal intensity (i.e., above the V_{max}) in hypoxia comes from previous results that the microvascular vasodilatory compensation and the intermittent exercise performance are both more increased at supramaximal than at maximal or moderate intensities. However, to our knowledge, there is no previous report of a supramaximal training protocol in mice, either in normoxia or in hypoxia.

The first aim of the present study was to test the feasibility of supramaximal intensity training in mice and the determination of a tolerable and adequate protocol (intensity, sprint duration, recovery, etc.). The second aim was to assess the effects of different training regimen in normoxia and hypoxia on the vascular function. Therefore, we test the hypotheses that (1) mice tolerate well supramaximal exercise in hypoxia, and (2) that this protocol induces a larger improvement in vascular function than exercise in normoxia but also than exercise in hypoxia at lower intensities.

Protocol

The local state's animal care committee (Service de la Consommation et des Affaires Vétérinaires [SCAV], Lausanne, Switzerland) approved all experiments (authorization VD3224; 01.06.2017) and all experiments were carried out in accordance with the relevant guidelines and regulations.

1. Animal housing and preparation

1. House 6- to 8-week-old C57BL/6J male mice in the animal facility for at least 1 week prior to the beginning of the experiments in order for the mice to get used to their new housing conditions. For practical reasons, mice of the same experimental group are usually housed together.
2. Keep the mice in a temperature-controlled room (22 ± 1 °C) with a 12 h light/dark cycle with ad libitum access to food and water.

2. Determination of the maximal speed and standard assessment of performance improvement by treadmill incremental test

NOTE: The following steps are critical to completing the training protocols.

1. Use a motorized treadmill for mice where mice can be on multiple lanes alongside each other, with a 0° inclination and mounted with an electric grid set to 0.2 mA at the back of the lane, in order to encourage the mice to run.
2. Prior to the first test, submit the mice to 4 days of acclimatization to the treadmill, according to the following protocol.
    1. On day 1, have mice run for 10 min at 4.8 m/min.
    2. On day 2, have the mice run for 10 min at 6 m/min.
    3. On day 3, have the mice run for 10 min at 7.2 m/min.
    4. On day 4, have the mice run for 10 min at 8.4 m/min.
3. On day 5, submit the mice to an incremental test to exhaustion, according to the following protocol.
    1. Let the mice warm up for 5 min at 4.8 m/min (at a 0° inclination).
    2. Increase the speed by 1.2 m/min every 3 min (e.g., 5 min at 4.8 m/min, then 3 min at 6 m/min, 3 min at 7.2 m/min, 3 min at 8.4 m/min, etc.) until exhaustion, which is reached when the mouse either spends 3 consecutive seconds on the electric grid or receives 100 shocks (displayed by the apparatus).
    3. Write down the achieved speed (considered as the V_{max}), duration, distance, number of shocks, and total time spent on the grid.
    NOTE: Typically, V_{max} was 28.8 ± 3.7 m/min.
    4. Mid-training, resubmit the mice to this test in order to readjust the speeds of training to the updated V_{max} of the mice (e.g., if the training protocol lasts 8 weeks, then perform a mid-training incremental test at 4 weeks. In that case, replace one of the scheduled trainings by the test), and do so again at the end of the study in order to assess performance improvements.
    5. Implement a 48 h rest period before and after this test.
    NOTE: All the incremental tests were performed in the morning.

3. Hypoxic environment

1. For the training sessions in hypoxia, place the treadmill in the hypoxic box (**Figure 1**) linked to a gas mixer. Use a calibrated oximeter to regularly control the ambient fraction of oxygen (F_iO_2 [i.e., the level of hypoxia]) in the box.
2. Set the gas mixer on 100% of nitrogen (N_2) and use the oximeter to verify the level of hypoxia. Once $F_iO_2 = 0.13$, change the parameter of the gas mixer from 100% N_2 to 13% O_2.
3. In order to avoid prolonged passive exposure to hypoxia, place the mice in a temporary smaller cage with litter and enrichment, and quickly place it in the box once $F_iO_2 = 0.13$ has been reached. Verify that the environment is still at 13% O_2 after putting the cage in; if not, readjust it.
4. Regularly verify the level of O_2 over the course of a training session to make sure that it remains at $F_iO_2 = 0.13 \pm 0.002$.

4. Normoxic environment

1. For the training sessions in normoxia, keep the treadmill in the hypoxic box, but remove the gloves so that there is ambient air ($F_iO_2 = 0.21$). The aim is to recreate the same training environment as the mice in hypoxia.

5. Supramaximal intensity training

1. Place the mice on individual lanes in the treadmill (at a 0° inclination) and submit them to the following protocol.
    1. Have the mice warm up for 5 min at 4.8 m/min, followed by 5 min at 9 m/min.
    2. Set the speed of the sprints to 150% of the previously determined V_{max}.
    NOTE: Typically, the sprint velocity was 42.1 ± 5.5 m/min.
    3. Train the mice for four sets of 5x 10 s sprints with 20 s of rest between each sprint. The interset rest is 5 min (**Figure 2**).
    NOTE: Add a cooldown period if the total workload of the training session needs to match that of another training group.

2. Perform this training 3x per week, with preferably 48 h between training sessions.
3. Use cotton swabs as a complementary method to electric shocks to encourage the mice to run. Place a cotton swab in a slit at the top of the lane, between the mouse and the electric grid, and gently nudge the mouse when it reaches the back of the treadmill. This will avoid the delivery of electric shocks and stimulate the mice to run in a softer way.

6. Low-intensity training

1. Place the mice on individual lanes in the treadmill (at a 0° inclination) and submit them to the following protocol.
    1. Have the mice warm up for 5 min at 4.8 m/min, followed by 5 min at 7.2 m/min.
    2. Set the speed of the continuous running session to 40% of the previously determined V_{max}.
    NOTE: Typically, the continuous running velocity was 9.9 m/min.
    3. Train the mice for 40 min.
    4. Perform this training 3x per week with preferably 48 h between training sessions.
    5. Use cotton swabs as a complementary method to electric shocks to encourage the mice to run.

7. Mice euthanasia and organ extraction

1. At the end of the training protocol and at least 24 h after the last incremental test, anesthetize the mouse in an induction chamber using isoflurane (4%-5% in O_2 to induce anesthesia, and 1%-2% in O_2 to maintain anesthesia). Confirm proper anesthetization using the paw retraction reflex (firmly pinch the animal's paw; anesthesia is considered proper when the animal does not react to the stimulus).
2. Using a 25 G needle, perform a percutaneous cardiac puncture, to collect maximum blood volume as previously described[19].
3. Perform a cervical dislocation and remove the skin of the mouse by cutting through the first layer of skin on the abdomen with round-tip scissors and pulling on the two sides of the incision (toward the head and the tail).
4. Cut through the peritoneum under the ribcage on the left side of the mouse with thin-point-tip scissors to reach the spleen and extract it if needed.
NOTE: Dissect out muscles if needed.
5. Dissect out the pulmonary artery.
    1. Using both small scissors and forceps, remove the thoracic cage and clear the heart-lung area.
    2. With "self-closing" tweezers, pinch the heart as close as possible to the apex and pull gently to stretch the base of the aortic arch and the pulmonary artery.
    3. Using the right hand, insert curved tweezers under the pulmonary artery and the aorta, and then move the tweezers back a little to hold only the pulmonary artery (**Figure 3**).
    4. Use the left hand to insert another pair of tweezers to replace the one held with the right hand.
    5. Using sharp straight microscissors in the right hand, dissect the pulmonary artery as close to the heart as possible on one side, and as far away as possible on the other side.
    NOTE:It does not matter which hand holds which instrument, although we have found it easier to cut with the right hand than with the left.
    6. Put it in a 2 mL tube with cold phosphate-buffered saline (PBS) buffer and keep on ice.

6. Perform a whole-body perfusion.
    1. At the top of the right lower limb of the mouse, use tweezers to clear out the external-internal right iliac artery down to the right femoral artery (under the inguinal ligament). Using sharp straight microscissors, make a full cut in the femoral artery.
    2. Insert a 5 mL 25 G syringe filled with cold PBS in the left ventricle of the heart and gently inject the cold PBS to remove the remaining blood from the vessels.
    NOTE: Due to the extraction of the pulmonary artery, it is possible that PBS does not circulate all the way to the incision.

7. Using tweezers, remove the soft tissue surrounding the aorta from the left and right inguinal ligaments to the heart as thoroughly as possible.
NOTE: The heart can be extracted for further analysis if necessary.
8. Using both tweezers and microscissors, dissect out the heart up to the lowest point of the external iliac artery (in both left and right limbs) and place the entirely dissected-out section in a 10 cm-diameter dish with cold PBS.
9. Using tweezers and/or microscissors, finish cleaning the remaining fat around the aorta and arteries by gently pulling or cutting it away from the vessels.
10. Using microscissors, cut the left iliac artery at the left-right iliac artery bifurcation and store it for further analysis.
11. Using microscissors, cut the abdominal aorta under the left renal artery, and place the extracted vessel in cold PBS buffer on ice (**Figure 4**).
12. Keep the remaining cleaned vessel, from the aortic arch to right above the left renal artery, in storage for further analysis.

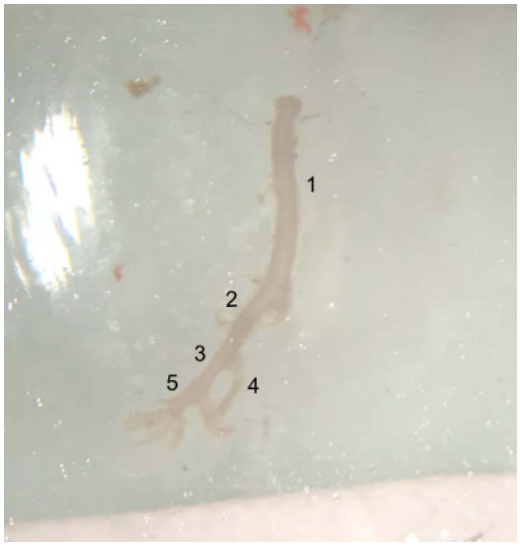

Figure 4: Picture of the dissected vessels. Extracted vessel from the top of the abdominal aorta (underneath the left renal artery) to the end of the right iliac artery, ready to be placed in cold PBS buffer on ice. (**1**) Abdominal aorta. (**2**) Right common iliac artery. (**3**) External iliac artery. (**4**) Internal iliac artery. (**5**) Femoral artery. Please click here to view a larger version of this figure.

8. Ex vivo vascular function assessment

NOTE: A wash corresponds to the emptying and refilling of the chambers with Krebs.

1. According to a previously described protocol[20], cut the isolated pulmonary artery, abdominal aorta, and right iliac artery segments into vascular rings of 1.5-2.0 mm long and 0.5-1.0 mm in diameter, and mount each ring on two 0.10 mm-diameter stirrups passed through the lumen.
2. Suspend the vessel rings in vertical organ chambers filled with 10 mL of modified Krebs-Ringer bicarbonate solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 11.1 mM glucose) maintained at 37 °C and aerated with 95% O_2-5% CO_2 (pH 7.4). One stirrup is anchored to the bottom of the organ chamber and the other one is connected to a strain gauge for the measurement of isometric force in grams.
3. Bring the vessels to their optimal resting tension: stretch the rings to 0.5 g for the pulmonary artery, 1.5 g for the iliac artery, and 2 g for the abdominal aorta, and wash them after a 20 min period of equilibration. Repeat the stretch-equilibration-wash steps 1x.
4. To test the viability of the vessels, contract the rings with 235 µL of KCl (10^{-1} M) for 10 min, wash them for another 10 min, and contract again with 235 µL of KCl (10^{-1} M) for about 20 min until reaching a plateau.
5. Wash the vessels again for 10 min and add 58.4 µL of indomethacin (10^{-5} M) (an inhibitor of cyclooxygenase activity) for at least 20 min in order to avoid possible interference of endogenous prostanoids.
6. Add cumulative doses of phenylephrine (Phe) from 10^{-9} (10 µL) to 10^{-4} M (or 10^{-9} to 10^{-5} M for the pulmonary artery; 9 µL for all concentrations above 10^{-9} M) to contract the vessels.
7. After the last dose of Phe, wait for about 1 h until the vessels reach a relatively stable contraction state (plateau).
8. Add cumulative doses of the endothelium-dependent vasodilator acetylcholine (ACh), from 10^{-9} (58.5 µL) to 10^{-4} M (40 µL for all concentrations above 10^{-9} M), to induce NO-mediated relaxation.
9. At the end of the relaxation curve, wash the vessels for 10 min, and add 58.4 µL of indomethacin (10^{-5} M), as well as 184 µL of NG-nitro-L-arginine (NLA, 10^{-4} M), which is an inhibitor of the NOS, for at least 20 min.
10. Contract the vessels again with a unique dose of 10 µL of Phe (10^{-5} and 10^{-4} M for the pulmonary artery and 10^{-4} M for the abdominal aorta and the iliac artery) for 1 h, to induce a relatively stable contraction.
11. Add a unique dose of 40 µL of ACh (10^{-4} M) until reaching a plateau.
12. Wash the vessels again for 10 min, before adding 58.4 µL of indomethacin (10^{-5} M) and 184 µL of NLA (10^{-4} M) for 20 min.
13. Contract the vessels with 10 µL of Phe (10^{-5} and 10^{-4} M) for 1 h.
14. Add cumulative doses (10^{-9} [58.4 µL] to 10^{-4} M [40 µL for all concentrations above 10^{-9} M]) of the NO donor diethylamine (DEA)/NO, in order to assess the endothelium-independent NO-induced relaxation.
15. At the end of the experiment, store the vessels in liquid nitrogen for future analyses if needed.

Representative Results

To our knowledge, the present study is the first to describe a program of supramaximal intensity training in normoxia and in hypoxia for mice. In this protocol, mice ran four sets of five 10 s sprints with a 20 s recovery in between each sprint. The sets were interspersed with 5 min of recovery periods. It was unknown whether the mice would be capable of sustaining such a protocol and complete it properly. However, according to **Figure 5**, the body weight gain of the mice undergoing the supramaximal intensity training was similar to that of the mice undergoing the low-intensity training, both in normoxia and in hypoxia.

The wellness of the animals was monitored twice a week, using score sheets, based on the following criteria: appearance, natural behavior, and body weight. Each of those criteria was graded up to a score of 3, and a mouse with a score of 3 in any of those criteria was considered in pain and/or distress due to the sustained protocol and had to be euthanized. No mouse ever reached a score of 3 over the course of any of the training regimens (**Table 1**).

As presented in the introduction, it has been hypothesized that supramaximal training, in particular when combined with hypoxia, would induce a compensatory vasodilation. This phenomenon aims at providing sufficient O_2 to the contracting muscles, thereby compensating for the imbalance between O_2 supply and demand that is enhanced by the combination of supramaximal intensity training and hypoxia. In order to investigate this hypothesis, we used the second technique presented here, the ex vivo vascular function assessment, on the pulmonary artery, the abdominal aorta, and the right iliac artery. **Figure 6** shows the dose-response curves obtained at the end of the protocol, on the abdominal aorta of a mouse from the group training at supramaximal intensity in hypoxia. This graph shows the whole process of contraction-relaxation observed after the addition of different pharmacological agents (KCL, Phe, ACh, NLA, and [DEA]/NO) in the organ baths.

Figure 7 shows the dose-response relaxation curve for the right iliac artery to increasing concentrations of ACh. The two represented groups are the supramaximal-intensity-in-normoxia group (SupraN) and the supramaximal-intensity-in-hypoxia group (SupraH). The preliminary results show that SupraH tended to improve ACh-induced relaxation compared to SupraN, with significant differences at 10^{-5} M and 10^{-4} M.

Figure 1: Hypoxic setup. The treadmill is placed inside the homemade glovebox, which is linked to a gas mixer. Please click here to view a larger version of this figure.

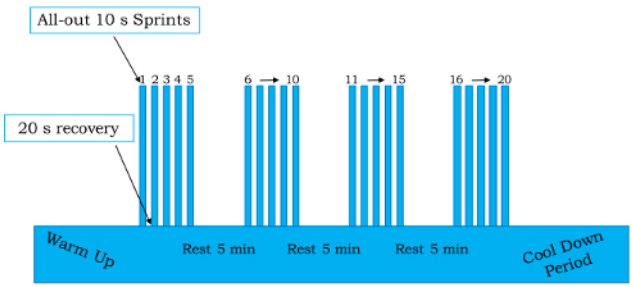

Figure 2: Description of a supramaximal intensity training session. The mice performed four sets of five 10 s sprints, interspersed with 20 s of rest. The interset rest was 5 min. This figure is adapted from Faiss et al.[21]. Please click here to view a larger version of this figure.

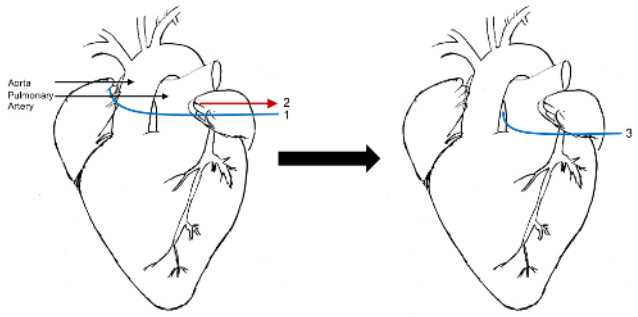

Figure 3: Schematic representation of the technique to retrieve the pulmonary aorta. (**1**) Place the tweezers under both the pulmonary artery and the aorta. (**2**) Pull back the tweezers in the direction of number 2 in order to keep the tweezers under the pulmonary aorta only. (**3**) Final position of the tweezers. Please click here to view a larger version of this figure.

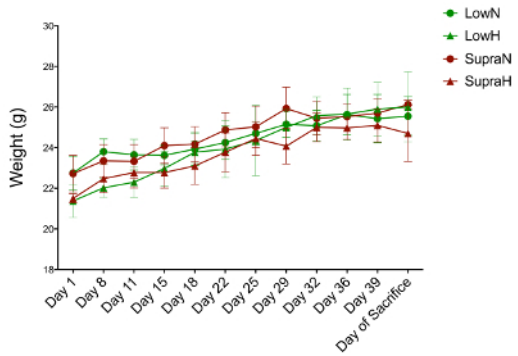

Figure 5: Body weight evolution over the course of the experiment. In green, the low-intensity training groups; in red, the supramaximal intensity training groups. There was no significant difference between any of the groups at any of the time points (*n* = 4 mice per group; the data are presented as mean ± SD). Statistical analysis was performed using a two-way repeated measure ANOVA). Please click here to view a larger version of this figure.

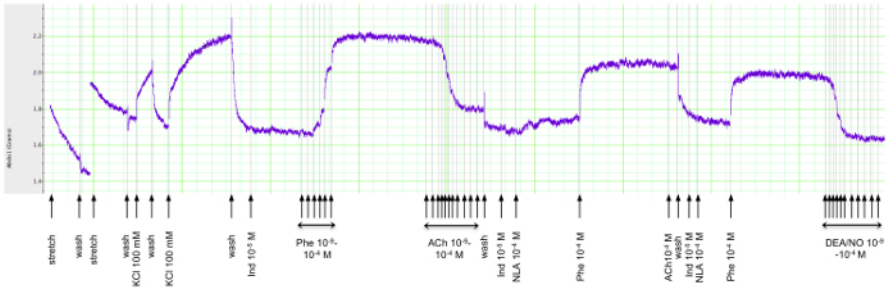

Figure 6: Vascular function assessment curves. Succession of contraction and relaxation phases induced throughout the entire protocol, expressed in grams. Representative recording of the variations in vessel tension in response to the applied substances, in a ring of the abdominal aorta isolated from a mouse trained at supramaximal intensity in hypoxia. Please click here to view a larger version of this figure.

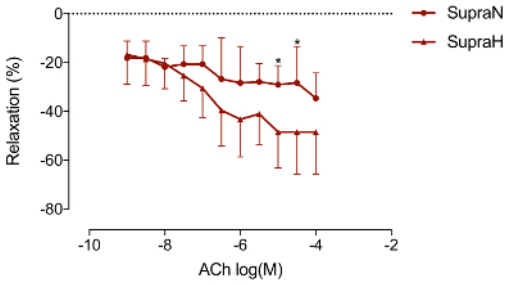

Figure 7: Pharmacological responses of an isolated iliac artery preconstructed with phenylephrine (Phe) to acetylcholine (ACh). Cumulative dose-response relaxation curve of the right iliac artery to increasing concentrations of ACh (10^{-9} to 10^{-4} M). The results are expressed as mean ± SD of the percentage of change in the tension induced by the vasodilator, with n = 3 in SupraN and n = 4 in SupraH. Statistical analysis was performed using a two-way ANOVA for repeated measures test. *p < 0.05 vs. SupraN. Please click here to view a larger version of this figure.

Animal Welfare Monitoring Sheet
Authorization n°
Experimentators:

		Baseline					MidExp						End of Exp
Body weight baseline:	**Date**	24/07	31/07	03/08	07/08	10/08	14/08	17/08	21/08	24/08	28/08	31/08	04/09
ANIMAL ID:	**Day**	01	08	12	15	19	22	25	29	32	36	39	43
Types of interventions:	**Score**	0	0	0	0	0	1	0	1	1	1	1	1
		/	/	/	(1)	/	/	/	/	/	/	/	(3)
APPEARANCE													X
Normal	0	X	X	X	X	X	X	X	X	X	X	X	X
General lack of grooming	1												
Piloerection	2												
Hunched posture	3												
NATURAL BEHAVIOUR													
Normal	0	X	X	X	X	X	X	X	X	X	X	X	X
Less mobile and/or isolated	2												
Vocalization, self mutilation, restless or still	3												
BODY WEIGHT													
% of body weight change compared to mean body weight of the control group													
0-5% weight loss	0	X	X	X	X	X		X					
6-10% weight loss	1						X		X	X	X	X	X
11-15% weight loss	2												
>15% weight loss	3												
TOTAL SCORE		0	0	0	0	0	1	0	1	1	1	1	1
Signature (initials)													

CRITERIA FOR MICE EUTHANASIA
Any mice with a total combined score ≥5
Any mice with one or more individual maximum score of 3

Type of interventions:
(1) Tail vein blood collection
(2) Forced treadmill running test
(3) Cardiac puncture blood collection

Table 1: Typical score sheet of a mouse training at supramaximal intensity in hypoxia. We used score sheets to monitor the welfare of the mice. A score of 3 in any of the criteria indicated (appearance, natural behavior, and body weight) or a total score of 5 (by addition of the score of each category) meant the animal was suffering and had to be euthanized.

Discussion

The first objective of this study was to assess the feasibility of hypoxic high-intensity training in mice and to determine the adequate characteristics of the protocol that would be well tolerated by mice. Purposely, since there is no data using supramaximal (i.e., more than V_{max}) intensity training in mice, we had to perform trials based on previous protocols developed with athletes, which consisted of four to five sets of five all-out sprints (about 200% of V_{max}), interspersed with 20 s active recoveries, with an interset active recovery of 5 min[21,22]. Therefore, the initial protocol consisted of six sets of six 10 s sprints at 200% of V_{max}, interspersed with 20 s of passive recovery and with an interset passive recovery of 3 min, performed five times per week. After a few try-out runs at 200% of the V_{max}, considering the mice had trouble sustaining such a high intensity, we decided to lower the speed to 150% of the V_{max}. With that exercise intensity, we tried to run the mice over the length of a full protocol and adjusted both the number of sprints within each set and the number of sets per session. Finally, we increased the recovery time between sets and decreased the frequency of the training sessions. Following a trial-and-error method, we established a final optimal protocol that is very similar to the one used on athletes and made possible for mice to tolerate this supramaximal intensity test.

There is a slight possibility that the performance of the mice might be severely underestimated, as observed from large differences between previous studies utilizing animal exercise protocols[23,24]. However, in the present study, based on pre-experiment values, it would have been impossible to impose a higher relative intensity on the animals considering the need to complete the entire repeated sprint session. Moreover, the V_{max} values reported in this study (28.8 ± 3.7 m/min) seem to be in the range of values previously reported in the same C57BL/6J strain[25,26,27,28]. For example, Lightfoot et al.[25] reported values of ~28 m/min and Muller et al.[27] values of 28.3 m/min. Therefore, we are confident that the supramaximal intensity corresponds to sprint training intensity in these mice.

Although critical speed (CS) has been shown (1) to be a valuable mean for prescribing exercise intensity in healthy humans and patients[29] and (2) to be perfectly determined in mice[23,24,30], the exercise intensity prescription based on the determination of V_{max} remains relevant. It is known that, in mice, the determined VO_{2peak} and VO_{2max} depend on the protocol, and, as with humans, VO_{2max} can be determined with a ramp exercise protocol[11]. Since the aim of the present study was to determine the feasibility of supramaximal repeated sprint in mice, and despite the relevance of CS, we do not believe that using V_{max} would be a flaw regarding the objectives of this study.

While observing mice behavior, it became clear that the electric grid at the rear of the treadmill admittedly encouraged mice to run; however, it also seemed to contribute to their fatigue. Indeed, the grid being slightly shifted from the running band, the mice had to generate an extra effort to get back on the lane. We decided to complement this stimulation with another, softer, one, namely the cotton swab stimulation, which decreased the number of shocks received by the animals and prevented them from having to get back from the grid to the lane. Despite the recommendation by Kregel et al.[31], it remains unclear whether stress is reduced using the air puff stimulation compared to the electric grid[32].

As far as we know, only one study has used "sprint interval training"[33]. However, since the highest intensity in that study corresponded to 75%-80% of V_{max} and the sprint duration was 1.5 min, that protocol was very different from the present one (i.e., 150% of V_{max}; 10 s). It was unknown whether supramaximal intensity would be tolerated by the mice. In the present study, we provide results showing that the animals did perform very well in this supramaximal intensity training, both in hypoxia and normoxia. For instance, **Figure 5** shows an increase in body weight over the training period similar to that observed in the low-intensity groups. Similarly, **Table 1** reflects the level of welfare with a score lower than 3 in all groups. Altogether, those physiological parameters indicate that both hypoxia and supramaximal intensity training were very well tolerated by mice.

The second objective of the present study was to assess the vascular function of the pulmonary artery, the abdominal aorta, and the iliac artery, using isometric vessel tension studies[20]. This technique allows determining whether the intervention of interest impacted the ability of the vessels to contract and relax in response to pharmacological drugs. As shown in **Figure 7**, the iliac artery was relaxed using increasing concentrations of ACh. The observed curves reflect a progressive increase in the relaxation of the vessels, more marked for the SupraH group. If any of the observed curves had been completely flat and around 0% of relaxation, it could mean that the drug was not delivered to the organ chamber, or that the vessels had been damaged during the dissection or the mounting on the stirrups, or that one of the drugs was not administered at the optimal dose or for long enough.

The supramaximal intensity training in hypoxia is now transferred to mice and could potentially be used on pathological models in order to improve various parameters, including vascular function, which can be assessed using isometric vessel tension studies.

Disclosures

The authors have nothing to disclose.

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