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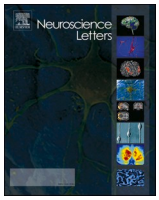

Pre-stroke exercise does not reduce atrophy in healthy young adult mice

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ABSTRACT

Stroke is the main cause of acquired disability in adults. Exercise reduces the risk for stroke and protects against functional loss after stroke. An exercise-induced reduction in key risk factors probably contributes to the protective effect, but direct effects on the brain may also contribute to stroke protection. We previously reported that exercise increases angiogenesis and neurogenesis through activation of the lactate receptor HCA_1. Here we exposed young adult wild-type mice and HCA_1 knockout mice to interval exercise at high or medium intensity, or to intraperitoneal injections of L-lactate or saline for seven weeks before we induced experimental stroke by permanent occlusion of the distal medial cerebral artery (dMCA). The resulting cortical atrophy measured three weeks after stroke was unaffected by exercise or L-lactate pre-treatments, and independent of HCA_1 activation. Our results suggest that the beneficial effect of exercise prior to stroke where no reperfusion occurs is limited in individuals who do not carry risk factors.

1. Introduction

Stroke attacks six million people each year (World Stroke Organization) and nearly 30% of the patients die within six months. Among stroke survivors, 26% end up being dependent on healthcare in their daily life and 46% experience cognitive deficits [1,2], making stroke the most common cause of adult disability.

Risk factors for stroke include advanced age, hypertension, dyslipidemia, diabetes mellitus, smoking, and obesity [2–7]. Reducing these risk factors efficiently decreases stroke incidence and mortality [1,2]. Consequently, a healthy lifestyle with appropriate activity levels and a balanced diet represents the cornerstone of stroke prevention, often in combination with pharmacotherapy. Physical exercise decreases many of the risk factors mentioned above, but also induces direct effects on the brain which may be preventive in stoke. Angiogenesis is one such mechanism [8]. Stroke patients with a higher density of blood vessels appear to have reduced morbidity and survive longer than patients with lower vascular density [9]. This may be explained by a more efficient network of collaterals near the occluded vessel, allowing for supply of blood to -and survival of- the penumbra [10,11].

During exercise L-lactate is released by the active skeletal muscles, accumulates in the blood, and crosses the blood–brain barrier via monocarboxylate transporters [21]. The simultaneous discovery of lactate-induced angiogenesis [22] and the presence of a lactate receptor, hydroxycarboxylic acid receptor 1 (HCA_1; aka HCAR1; GPR81), in the brain [23 24], laid the foundation for our demonstration that HCA_1 activation mediated the angiogenic effects of exercise [18] and induced neurogenesis in the sub-ventricular zone [20]. Theoretically, these effects of HCA_1 activation may underlie exercise-induced neuroprotection in stroke. In the present study we therefore investigate whether HCA_1-dependent mechanisms are important for the neuroprotective effect of pre-stroke exercise.

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2. Material and methods

2.1. Animals

The *in vivo* experiments were approved by the Norwegian Animal Use and Care Committee (FOTS ID 14204 and 12521) and conducted by Federation of Laboratory Animal Science Association (FELASA) certified personnel in strict accordance with the national and regional ethical guidelines. The experiments are reported in compliance with the Animal Research: Reporting in Vivo Experiments (ARRIVE) guidelines, version 2.0. The generation of HCA_1 knockout line has previously been described [18,25]. The mice were housed in GreenLine cages (Sealsafe Plus GM500) up to 8 per cage, at the Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo. The mice had access to food and water ad libitum and were stalled in a 12:12 h light:dark cycle. The cages were enriched with paper for nest-building and a toilet roll core or plastic house. In total, 82 HCA_1 knockout or wild-type mice (4–6 weeks of age) were randomized into four intervention groups: high intensity interval training (HIIT), medium intensity interval training (MIIT), L-lactate injections (LAC), or saline injections (control), yet ensuring an near-equal number of animals and a balanced distribution of males/females in all experimental groups.

2.2. Exercise regimes

The HIIT regime has been previously described [18,26] and the mice are expected to reach about 90% of VO_{2max}. Briefly, each session consisted of 10 min warm-up at 8 m/min, followed by 10 high intensity intervals of four min each, separated by two min of active rest (5 m/min). Running took place on a treadmill (Columbus Instruments, USA) at 25°incline. The mice were exposed to the HIIT or MIIT for five consecutive days each week, for seven weeks (Fig. 1). The speed setting was adjusted based on the performance of the mice in a maximum running capacity test (MECT) which was performed at the second day of the exercise intervention, and then every other week. The running speed of the HIIT group was about 80% of the MECT result but the speed was increased by 0.2–0.6 m/min per session, depending on the observed performance of the mice (Fig. S1b). The MIIT group exercised at about 60% of their MECT result and the same speed was kept until the next MECT.

The MECT was performed as follows: After a 15-minutes warm up-period at 9.6 m/min, the speed was increased by 1.8 m/min every two min until exhaustion, i.e. when the mice refused to run further. Electric stimuli were given maximally 1–2 times per day/mouse by the intrinsic device of the treadmill (<1.5 mA), but normally a gentle push on the tail was enough to keep the mice running. This exercise regime has been validated extensively [27]. Blood lactate levels of 10 mmol/L have been reported in mice during treadmill exercise at close to VO_{2max} [28] and at close to maximum speed [25].

2.3. Lactate and saline injections

The mice received a daily intraperitoneal (i.p.) injection of sodium L-lactate (2 g/kg body weight; 200 mg/ml dissolved in 0.9% saline; pH 7.4; i.e., 18 mmol/kg), five days a week for seven weeks [18]. The control mice received the same volume (per kg bodyweight) of 0.9% saline (Fig. 1).

2.4. Permanent occlusion of the distal medial cerebral artery

After seven weeks of HIIT, MIIT, lactate injections, or saline injections, stroke was induced by permanent coagulation of the distal middle cerebral artery (dMCA), as previously described [29]. Briefly, the mice were anaesthetized with isoflurane (~70% N_2O, 30% O_2 + 1–4% isoflurane) and given an i.p. injection of buprenorphine 0.3 mg/kg (Temgesic, Indivior, USA). Reflex examination (toe pinch test) was performed to ensure that the mice were deeply anaesthetized. During the surgery, the mice were placed on a heat blanket (35 °C) and anaesthesia (1.5% isoflurane) was administered through a mask. The eyes were covered with Simplex eye ointment (Actavis, Iceland).

The surgical site was disinfected using 0.1% chlorhexidine (Klorhexidin, Fresenius Kabi, Norway). A 1 cm incision was made between the left ear and eye and the temporal muscle was gently detached in its apical and dorsal part by diathermy (VIO 50C, Erbe, Germany) adjusted to 12 W in the bipolar mode. The transparent skull above the MCA M1 branch was thinned using a drill. The last layer of bone was carefully withdrawn with ultra-fine forceps. The diathermy-forceps (7 W; bipolar mode) were placed closely at each side of the dMCA, without touching the vessel. The coagulation was performed downstream of lenticulostriate arteries, proximally and distally on both downstream branches of M1. After 30 s, the artery was gently touched to verify that recanalization did not occur. In case of observed recanalization, electrocoagulation was repeated once. Finally, the temporal muscle was placed back, and the incision was sutured. The surgery procedure took maximally 15–20 min per animal. The mice were then placed in a nursing box at 37 °C for at least 20 min to recover from anaesthesia and returned to their home cage if no signs of injury/pain were seen during this time. Post-operative analgesia (buprenorphine 0.3 mg/kg) was administered i.p. at 24 h after the operation, and then daily for 4 days. The mice were not subjected to exercise or injections during the 3 weeks after stroke.

2.5. Exclusion criteria

The well-being of the animals was monitored closely throughout the experiment. Exclusion criteria, set *a priori* and described in the animal welfare protocol (FOTS 14204), were as follows: at any sign of distress, e.g. weight loss, erratic behaviour, lack of grooming etc, the animal was excluded from the experiment. If an animal performed worse than expected in one interval training session –based on the previous performance of the same individual- it was given additional breaks whenever needed. If the same animal performed worse than expected for two consecutive exercise sessions, the animal was presumed injured or sick and was withdrawn from the study. During surgery, animals who experienced bleeding, or spontaneous recanalization more than once, were excluded. Mice who developed lesions beyond cortical regions, for instance including part of the underlying striatum, were excluded from the analysis. During the exercise, 4 animals (1 wt MIIT, 1 KO MIIT and 2 KO lactate) were excluded because they refused to run and/or

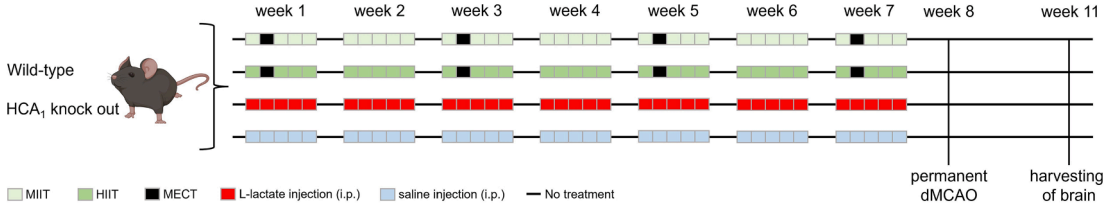

Fig. 1. Timeline. WT and HCA1 KO mice were subjected to medium intensity interval training (MIIT; light green), high intensity interval training (HIIT; dark green), lactate injections (red) or saline injections (blue), 5 days a week for 7 weeks. Maximum endurance capacity test (MECT) was performed every second week (black). Thereafter, all mice received permanent distal medial cerebral artery occlusion (dMCAO). The brains were harvested 3 weeks after stroke and analyzed.

performed worse than expected for two consecutive days. During the surgery and the first post-operative day, 5 mice met the exclusion criteria or died (1 wt HIIT, 1 wt MIIT, 2 wt lactate, 1 KO MIIT), giving a mortality of 6% for the stroke surgeries. Five animals were excluded from the analyses as they showed atrophy beyond the neocortex (1 wt HIIT, 1 wt MIIT, 1 KO MIIT, 2 KO saline).

2.6. Fixation

Three weeks after the dMCAO induction, the mice were anaesthetized with an i.p. injection of zolazepam 3.3 mg/ml, tiletamin 3.3 mg/ml, xylazine 0.5 mg/ml, and fentanyl 2,6 μg/ml), 0.1 ml/g bodyweight. After 5–15 min, reflex examination was performed. The deeply anesthetized mice were then transcardially perfused with 4% formaldehyde (freshly made and filtered) in 0.1 M sodium phosphate (NaPi) buffer; pH 7.4. A cannula, attached to the peristaltic pump, was inserted in the left ventricle and the fixative was pump into the circulation at a rate of 5 ml/min. The right auricle was perforated to avoid increased pressure in the circulation. The perfusion was maintained for 8 min. The brain was gently removed from the skull and stored in a 4% PFA solution at + 4 °C over night. The following day, the brain was transferred to 0.4% PFA and kept at + 4 °C.

2.7. Cryosection

Before sectioning, the brains were allowed to saturate overnight in 30% sucrose for cryoprotection. Serial sections (20 μm) were produced at −20 °C using a Thermo Scientific™ HM 450 Sliding Microtome. The sections were transferred chronological to the wells of tissue cultures plates (VWR® Tissue culture plates) filled with 5 ml 0.1 M NaPi with 0.05%.

2.8. Staining with cresyl violet

Every 6th section was mounted on glass slides (Superfrost Plus™, Thermo Scientific) and stained with cresyl violet (CV) as follows: EtOH 95% (15 min); 70% (1 min); 50% (1 min) in phosphate buffered saline (PBS). Then the sections were rinsed twice in PBS (2 min + 1 min) before incubation with filtered CV (1 g/L) at 60 °C; 8 min and rinsed again for 2x2 minutes in PBS. The sections were then dehydrated in 95% EtOH for 1 min and exposed to 1% glacial acetic acid in 95% EtOH for 3 s (differentiation). After a brief rinse in 95% EtOH (5 s), the slides were visually examined, and any over-stained sections were differentiated again until a desirable result. Finally, the sections were immersed in 100% ethanol, transferred to Neo-Clear (Merck, Germany) for 1 min, mounted with Neo-Mount (Merck, Germany) and cover slipped.

2.9. Imaging and atrophy volume measurements

Images of the CV-stained coronal sections were obtained at 20x magnification using an automated slide scanner system (Axio Scan Z1, Carl Zeiss Microscopy, Germany). The images were analysed using FIJI (Image J, version: 2.0.0-rc-69/1.52i) by observers who were blinded to the treatments and genotypes. The ipsylesional and contralesional cortex was outlined according to The Allen Brain Atlas (https://mouse.brain-map.org/experiment/thumbnails/100048576?image_type = atlas). The lesion area in each section was calculated by subtracting the area of the ipsilateral cortex (excluding any visibly damages and/or scared tissue) from the area of the contralateral cortex. This was done for every 6th section (31–39 sections per animal) between 1.645 mm rostral and 2.355 mm caudal of bregma and multiplied by the inter-section distance of 120 μm to reveal the atrophy volume.

2.10. Immunohistochemistry

From each animal, one 20 μm coronal brain section was mounted on Superfrost Plus slides. The sections were roughly at bregma -0.245, where most animals had maximum atrophy. The sections were incubated in pepsin (10 mg/ml, in 0.2 M HCl) at 37 °C for 20 min for antigen-retrieval and rinsed 3x10 min in PBS. The sections were then exposed to blocking solution (10% fetal calf serum and 0.5% triton x100 in PBS) for 2 h prior to incubation with the primary rabbit anti-collagen IV antibodies (Abcam ab6586 diluted 1:500 in blocking solution) overnight. The following day, the sections were rinsed 6x10 min in PBS and incubated for 2 h with anti-rabbit Alexa Fluor 488 (IgG, catalogue #A21206, diluted 1:500 in blocking solution). The sections were rinsed in PBS 3x10 min before incubation with DAPI (4′,6-diamidino-2-phenylindole, D9542, Sigma-Aldrich, St. Louis, MO, USA; stock solution 1 mg/ml diluted 1:5000 in PBS) at room temperature for 15 min. Finally, the sections were rinsed in PBS 3x10 min and cover slipped with ProLong Gold (Thermo Fisher Scientific, USA).

Z-stack images (20 μm optical thickness) of the whole coronal brain sections were obtained at 20x magnification using an automated slide scanner system (Axio Scan Z1, Carl Zeiss Microscopy, Germany). Images were analysed using Fiji (version 2.0.0-rc-69/1.52p; Java 1.8.0_172). The ipsilateral region of interest (ROI) was defined as the cortical area surrounding the infarct core by 500 μm; in case where no obvious lesion was visible, the cortical area in the assumed lesion centre was measured, as outlined in Fig. 2A. Similarly, the contralesional ROI was defined as the symmetrical position to the stroke lesion, constituting an area of 500 μm^2. Capillaries were outlined using a semi-automated method, adapted with the Trainable Weka Segmentation (TWS) plug-in [30] and vessels above 10 μm in diameter where excluded from the ROI. Capillary density was given as the percentage of the ROI covered by collagen IV-stained capillaries. All measurements were limited to the cortex and performed by an observer who was blinded to the genotypes and treatments.

2.11. Statistics

Test of Homogeneity of Variances (IBM SPSS vs26) gave a p-value ≫ 0.05 for all analysis. Therefore, the groups were compared using one-way ANOVA with Tukey post-hoc (IBM SPSS vs26). The significance level was set at 0.05 for all tests. The underlying data material are available from the corresponding authors upon request.

3. Results

3.1. Exercise or lactate injections did not affect the lesion size

At three weeks after dMCAO, the neocortex was visibly thinner in the ipsylesional left hemisphere in comparison to the contralesional side (Fig. 2A). In addition, some of the sections showed remains of damaged tissue, either appearing as lighter areas containing smaller and more densely packed nuclei, or as darker-appearing scared tissue. As expected with the permanent dMCAO model, we found the largest lesion area around bregma −1 mm (±1mm). The healthy-appearing cortical tissue of the left hemisphere was subtracted from the cortical area of the right hemisphere in every sixth coronal section, summed, and multiplied by the distance between the sections to calculate the lesion volume. The resulting lesion volumes were unaffected by exercise −both HIIT and MIIT− and by lactate injections in both genotypes ($p = 0.984$; one-way ANOVA) (Fig. 2B).

As a measure of reproducibility in the measurements, the infarct area in 72 sections were measured twice. The second measurements identified an area of healthy cortical tissue that was 99% ± 4.5% (average ± SD) and 97% ± 7.1% (average ± SD) of the first measurement in the contralateral and ipsilateral cortices, respectively.

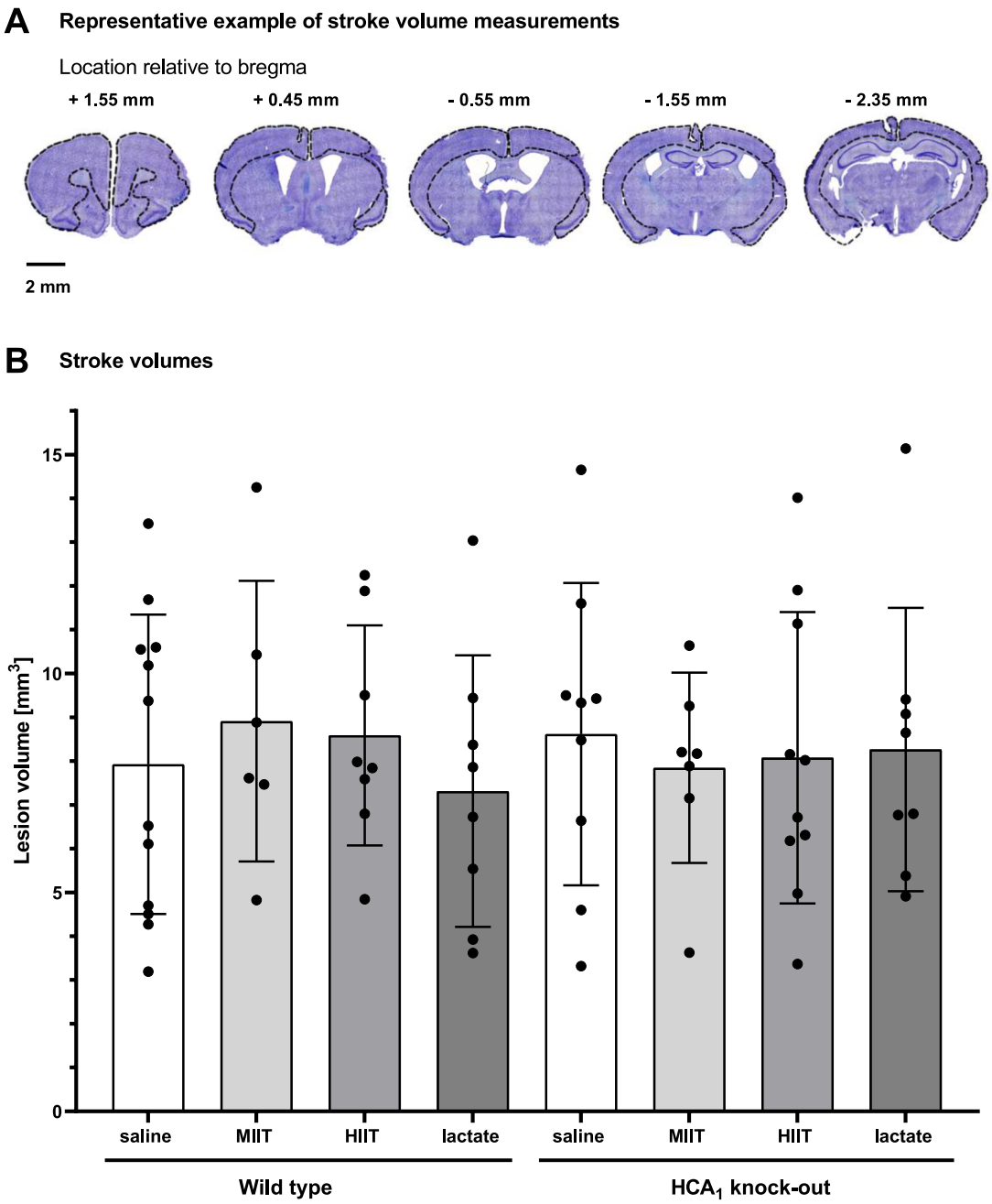

Fig. 2. Stroke volumes. MIIT, HIIT or lactate injections did not affect atrophy volumes in neither WT nor HCA_1 KO mice. **A)** Five representative cresyl violet-stained sections (out of > 31 sections per animal) with the contralateral and ipsilateral region of interest (ROI) indicated by dotted lines. The rostral-caudal location relative to bregma is given above each section. Scale bar: 2 mm. **B)** Atrophy volumes (mean ± SD): WT saline 7.93 ± 3.42 mm^3 (n = 12) and KO saline 9.28 ± 3.02 mm^3 (n = 8), WT MIIT 8.91 ± 3.20 mm^3 (n = 6), KO MIIT 7.85 ± 2.17 mm^3 (n = 7), WT HIIT 8.59 ± 2.51 mm^3 (n = 8), KO HIIT 8.08 ± 3.33 mm^3 (n = 10), WT lactate 7.32 ± 3.10 mm^3 (n = 8), KO lactate 8.27 ± 3.24 mm^3 (n = 8). No differences were found between the groups (One-way ANOVA; p = 0.984, SPSS).

3.2. Capillary density was unaffected by exercise, lactate injections and the presence of HCA_1

We have previously reported that HCA_1-activation was responsible for exercise-induced angiogenesis in the cortex and the hippocampal formation [18]. Since increased capillary density may induce a protective effect in stroke, we investigated whether an enhanced density of capillaries could be seen in response to exercise or lactate injections in the HCA_1 WT mice three weeks after the end of the exercise intervention. In the present study, we did not find significantly increased capillarization in response to exercise or lactate, neither in the ipsilateral nor contralateral side (Fig. 3A-C), which is in line with the lack of effect of exercise on stroke volume. Furthermore, there was no difference in capillarization between WT mice and HCA_1 KO mice (Fig. 3B and C).

3.3. The running performance increased during the exercise intervention

All animals exposed to HIIT increased their running performance every 14 days (figure S1), as previously reported for this exercise regime [18], and no difference in running capacity was observed between the genotypes. Since the running speed of the intervals were set based on the MECT results, and WT and KO were exercised together, there was no difference in running speed or exercise intensity between HCA_1 KO and WT animals. The steady increase in MECT performance, and the

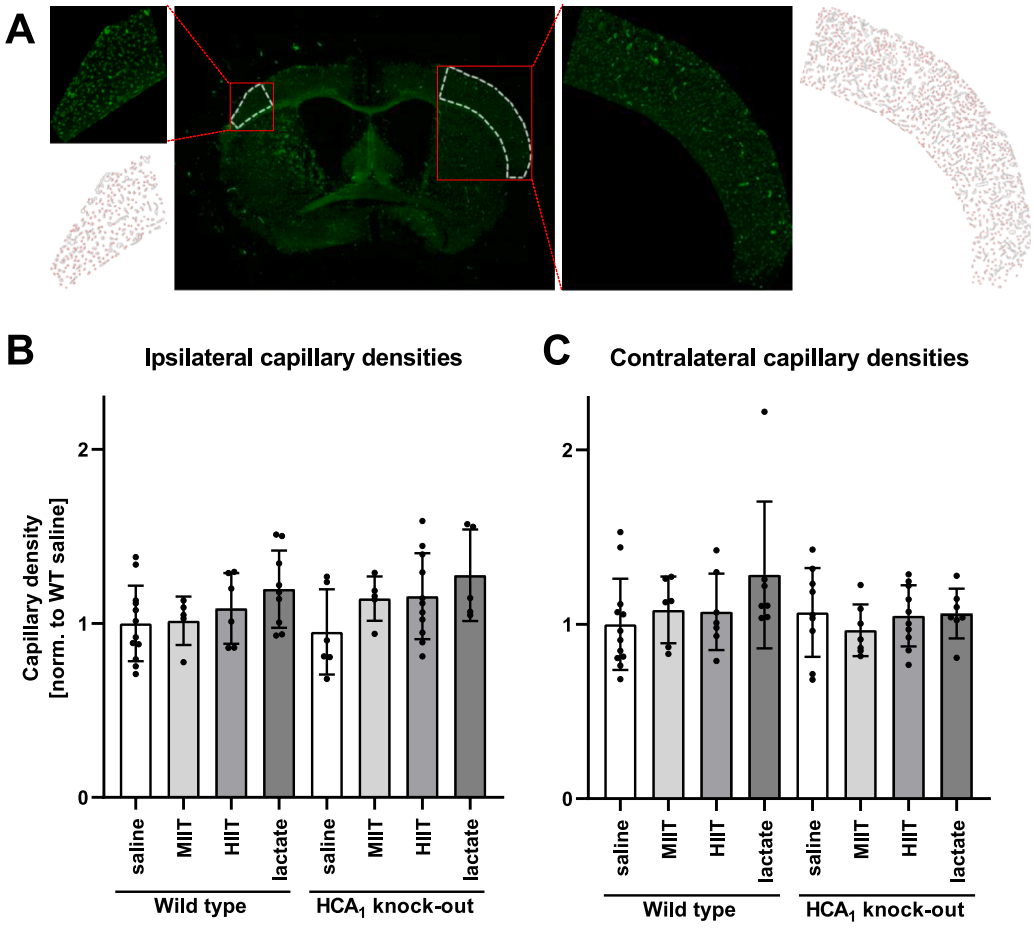

Fig. 3. Capillary density. MIIT, HIIT or lactate injections did not affect the capillary density in neither WT nor HCA_1 KO mice. **A)** Representative contra- and ipsilateral ROI, respectively (dotted line), the capillaries within that ROI, and the mask used to calculate the capillary surface. **B)** Capillary density on the ipsilateral side (mean%±SD): WT saline 1.0 ± 0.22, n = 12; n = 6; WT MIIT 1.02 ± 0.14n = 5; WT HIIT 1.08 ± 0.2; WT lactate 1.2 ± 0.22, n = 9. KO saline 0.95 ± 0.24, n = 6; KO KO MIIT 1.14 ± 0.13n = 5; HIIT 1.16 ± 0.25, n = 11; KO lactate 1.28 ± 0.26, n = 5. No differences were found between the groups (one-way ANOVA; p = 0.127). **C) Capillary density in the contralesional side (mean% ±SD, normalised to WT Saline): WT saline 1.0 ± 0.26, n = 12; WT MIIT 1.08 ± 0.18n = 6; WT HIIT 1.07 ± 0.22, n = 7; WT lactate 1.28 ± 0.42, n = 7. KO saline 1.07 ± 0.25, n = 9; KO MIIT 0.97 ± 0.15n = 7; KO HIIT 1.05 ± 0.18, n = 10; KO lactate 1.06 ± 0.14, n = 9. No differences were found between the groups (one-way ANOVA; p = 0.365).**

independency of HCA_1 on running capacity was also seen in the mice exposed to MIIT (figure S1). As expected, the mice exposed to HIIT increased their performance in the MECT more than the mice exposed to MIIT.

3.4. Animal welfare

Throughout the experiment, the mice showed signs of good health and normal behavior, e.g. shiny fur, nest building, and grooming. Despite small week-to-week variations in the bodyweight of individual mice, all the mice increased their weight throughout the intervention period (figure S2). During the three weeks after the operation, most animals continued to increase in bodyweight, but for some animals the bodyweight did not change, or even decreased slightly. Only four mice in total decreased in weight during this period; none lost>10% of their bodyweight (2 g). Furthermore, we found no differences in weight at the start of the experiment, or in weight development during the experiment, between treatment groups or genotypes. The lactate injected mice showed minor wounds or swellings at the injection site, but these healed within 1–2 days or less.

4. Discussion

Here we demonstrate that the lesion volume resulting from permanent dMCAO was unaffected by seven weeks of pre-treatment with exercise, either HIIT or MIIT, or lactate injections. Furthermore, no difference on capillary density in the cerebral cortex was observed between the groups.

The effect of physical activity in the prevention and treatment of stroke is multifactorial. Human stroke cases are often associated with risk factors [1–3] like advanced age [4,5], sex [31,32], hypertension [6,33], obesity [7,34], diabetes mellitus [35], and cardiac diseases [36]. The international INTERSTROKE study identified 11 factors that collectively accounted for 88% of the stroke risk [37], including the factors listed above. Exercise alleviates most of the these risk factors [38–41] and may reduce stroke risk and improve stroke outcome [42–44]. The animals used in the present study, however, were healthy young adults who did not possess any of the major risk factors mentioned above.

The lack of effect of both exercise regimes used in this study, raises the question of whether protective effects of exercise in stroke is mainly mediated through a reduction in the burden of risk factors, and not by direct effect in the brain. Previous studies reporting effects of exercise as a preventive strategy in stroke, have focused on pre-stroke exercise habits in humans [45,46] and, hence, do not separate between effect mediated though reduction of risk factors and effect of exercise on the brain *per se*. A prospective study following 21,794 men over 20 years is in line with our findings, as they report that the level of physical activity before stroke did not affect the functional outcomes after stroke when the men initially had a low level of risk factors [47].

The HIIT regime used in the present study has previously been shown to induce angiogenesis in the neocortex and the hippocampus [18], and neurogenesis in the subgranular and subventricular zones [20], excluding the possibility that the lack of effect in the present study was caused by the use of an inadequate exercise regime. One point to consider, however, is the three-weeks period after stroke induction, during which our mice did not exercise. During such periods of detraining, a reduction in neurotrophic factors [50] and inflammatory resistance [51] have been observed. Bed-rest represent an extreme form of detraining; in humans, bed-rest after exercise induces a decline in

exercise-induced adaptive gene responses and oxygen-uptake capacity [52–54]. The three weeks of detraining may therefore explain why the capillary density was not increased in the present study, despite the previous report of HCA_1-dependent angiogenesis in response to exercise [18]. An alternative interpretation could be that the induction of the stroke *per se* reduced angiogenesis. The lack of exercise-induced angiogenesis even in the contralateral cortex, however, suggest that detraining rather that the stroke itself explain why no angiogenetic effect of HIIT -via HCA_1- was found in the present study.

A myriad of rodent stroke models exist and preclinical intervention studies show great variability when it comes to effectiveness. Variability may reflect the use of different species or strains, and whether (and which) genetic alterations have been induced. Furthermore, the method by which stroke is induced, including whether reperfusion occurs or not may differ, as well as the timing, dosage, and the rout of administration of the intervention. All these factors may influence whether neuroprotection is observed or not. Hence, choosing an appropriate preclinical model is essential for the translational value of pre-clinical studies of stroke prevention or therapy. In the current study, we use the permanent dMCAO model. The lesion produced by this model almost exclusively affects the neocortex and encompasses about 10–15% of the affected hemisphere [29,55]. The model thereby mimics the majority of human strokes affecting the MCA territory [29,55,56] where no spontaneous or treatment-induced recanalization occurs. We cannot exclude that pre-treatment with HIIT, MIIT −or lactate treatment for that matter− could have protected against ischemia–reperfusion injury and, hence, be beneficial in a transient ischemic stroke model. Except one publication [63], most studies reporting a protective effect of exercise employ the transient stroke model [64–66]. Nevertheless, only a few stroke patients obtain recanalization. In the US, only 3.7–9% of all large-vessel strokes obtain recanalization [69], and therefore the permanent MCAO is a good model for the remaining > 90% of the patients. and has been recommended in the standards regarding preclinical neuroprotective and restorative drug development, including Stroke (STAIR) [70], and an increasing portion of stroke researchers [69,71].

Another point to consider, is the recovery time after stroke induction. Often, lesion volumes are measured 2–7 days after stroke in mice [72–77]. In the current study, we aim to investigate the long-term effects on stroke outcomes. We therefore used a recovery phase of three weeks, investigating atrophy at a timepoint where necrosis, apoptosis and regenerative processes were assumed to be reaching a steady state. We cannot exclude the possibility that measuring lesion volumes at an earlier time point could have led to a different result. Hence, the possibility that pre-stroke exercise or lactate treatment accelerates recovery −HCA_1 dependently or not− remains. We believe, however, that measuring the outcome in the chronic phase after stroke is a more relevant outcome measure for the long-term effects in human patients. Theoretically, more sensitive outcome-measures could have revealed small changes in lesion volumes, neural survival, glial activation and/or behavioural consequences of the stroke. Considering that no tendency was observed towards a smaller lesion volume in animals exposed to exercise, we consider it unlikely that using other measures would have altered the conclusion of the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Author contribution statement

CM designed the project. CM and SG planned the experiment. SG, KDP, CB and TDN performed the exercise intervention and lactate/saline treatments (supervised by CM). SG performed all the surgeries (trained by A.L). KDP, CB and TDN mounted the sections and performed cresyl violet staining. KDP, CB, TDN, HLD and CLC measured the brain atrophy (supervised by SG and CM). SG assembled all the data and performed the statistics. KDP and CB performed the collagen IV labeling and KDP measured the capillary densities. CT performed analysis of behavioral outcomes, including algorithm development and data extraction (data inconclusive and not included in this paper). All the authors discussed the data and their interpretations. SG, KDP and CM made the figures. CM drafted the manuscript with important contributions from SG and KDP. CM and SG finalized the manuscript based on input from all the other authors. All authors read and approved the final version of the manuscript.

Appendix A. Supplementary material

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