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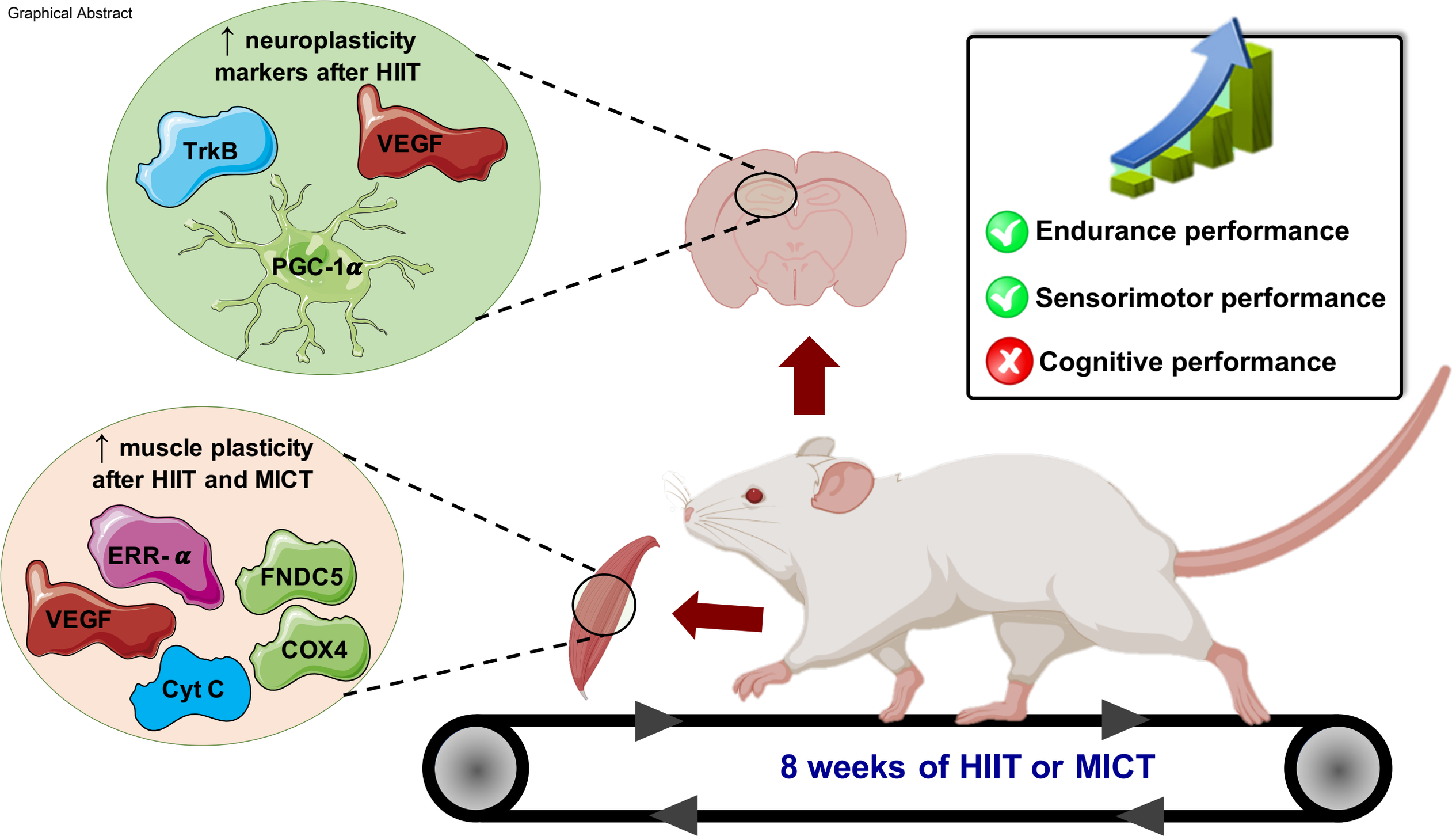
# High *versus* moderate intensity training in rats: Impact on endurance and hippocampal plasticity markers

Annabelle CONSTANS; Caroline PIN-BARRE; Florence MOLINARI; Jean-Jacques TEMPRADO; Thomas BRIOCHE; Christophe PELLEGRINO; Jérôme LAURIN*

# Highlights

- High-intensity training induces higher aerobic fitness than moderate intensity.
- Plasticity is different between regimens in fast- and slow-twitch muscle fibers.
- High-intensity interval training upregulates hippocampal plasticity markers.
- Both training regimens improve sensorimotor functions, but not cognitive functions.

Graphical Abstract



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# High *versus* moderate intensity training in rats: Impact on endurance and hippocampal plasticity markers

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**Non-standard abbreviations:**

HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; $S_{LT}$, speed associated with the lactate threshold; $S_{max}$, maximal speed; $VO_{2max}$, maximal oxygen uptake; ART, adhesive removal test; NOR, novel object recognition test.

# Abstract

This study was designed to compare work-matched high-intensity interval training (HIIT) with moderate-intensity continuous training (MICT) based on lactate threshold on aerobic performance, brain plasticity markers and cognitive functions following 8 weeks in healthy rats. Muscular plasticity and grip strength were also investigated.

Rats performed the incremental exercise test and behavioural tests before (PRE) and after training at day 1 (D1), D15, D29 and D57. Key cerebral markers were assessed by Western blot and quantitative polymerase chain reaction to provide information on brain function related to angiogenesis, aerobic metabolism and neurotrophin activity at D59. Muscular protein levels involved in angiogenesis and aerobic metabolism were measured in both *triceps brachii* and *soleus* muscles.

HIIT induced superior improvement of aerobic fitness compared to work-matched MICT, as indicated by enhancement of speed associated with lactate threshold ($S_{LT}$) and maximal speed ($S_{max}$). In parallel, grip strength increased throughout the HIIT protocol. In the *triceps brachii* muscles, markers of angiogenesis and aerobic activity were upregulated as well as myokine involved in neuroplasticity. Moreover, levels of key brain plasticity markers increased in the hippocampus only following 8 weeks of HIIT, without improving cognitive functions.

These findings might contribute to define physical exercise guidelines for maintaining brain health by highlighting the promising role of HIIT when using $S_{LT}$ for distinguishing low running speed from high running speed. Further studies are required to confirm these brain effects by exploring synaptic plasticity and neurogenesis mechanisms when exercise intensity is standardized and individualized.

**Key words:** Lactate threshold; TrkB; VEGF; PGC-1α; interval training; grip strength.

## 1. Introduction

Beyond its role in performance of athletes, regular endurance training is one of the most effective interventions to improve and/or preserve health over the lifespan. Among endurance exercises, the moderate-intensity continuous training (MICT), which corresponds to 50-70% of maximal heart rate, is most commonly recommended to improve endurance performance as well as cardiovascular, muscular and cerebral functions[1]. However, it has been observed that gains in performance tend to reach a plateau after several weeks of MICT, even though the exercise duration is increased during training programs[2].

To overcome these limitations, it is recommended to vary the exercise intensity by including high-intensity interval training (HIIT), which has been demonstrated to be a safe and time-efficient strategy to improve endurance performance through repeated bouts of high-intensity exercise (*i.e.* above the lactate threshold or at 85–90% of maximal speed or maximal heart rate) separated by recovery periods[2]. However, the respective effects of MICT and HIIT on performance remain unclear in both human and rodent studies. Indeed, previous studies showed superior maximal oxygen uptake ($VO_{2max}$) and/or speed associated with lactate threshold ($S_{LT}$) following HIIT than MICT while others indicated similar effectiveness[3–6]. Also, divergent results have been reported in training-induced changes in aerobic metabolism in fast- and slow-twitch muscle fibres when comparing HIIT and MICT on aerobic and angiogenic markers[3,7].

Among the different effects of MICT and HIIT, those related to brain plasticity and cognition have received little attention in healthy individuals. Yet, endurance training plays a key role in many aspects of life such as academic achievement, job performance, but also for limiting risks of neurological disorders[8]. In healthy rodents, the few studies comparing HIIT and MICT were mainly focused on expression of neurotrophic factors, including brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and vascular-endothelial growth factor (VEGF), in the hippocampus, a brain region showing a considerable degree of synaptic plasticity and adult neurogenesis[9,10]. However, the effects of HIIT and MICT on such neuroplasticity markers remain highly controversial. On the one hand, higher levels of hippocampal BDNF and glial cell line-derived neurotrophic factor (GDNF) expression were promoted by HIIT compared to MICT in rats[5]. On the other hand, some authors demonstrated higher effectiveness of MICT to stimulate hippocampal BDNF, IGF-1, VEGF than a more fatiguing endurance training[11]. After training, the upregulation of neurotrophic factors in the rat hippocampus might be associated with improvements in

cognitive functions[10,11]. Similarly, in trained elderly persons, both greater serum levels of BDNF (or VEGF) and hippocampal volume were correlated with improvements in memory functions[12]. However, a link between neuroplasticity markers and cognitive functions was not systematically observed in both humans and rodents[10,13,14]. A previous meta-analysis indicated that exercise parameters are considered as influential moderators for improving cognitive functions, but very few studies have investigated more than one exercise to define suitable doses to predict cognitive benefits in healthy subjects[13]. Consequently, the optimal exercise duration and intensity required to obtain a cognitive effect remains to be defined[8,11,13,15].

All these inconsistent findings might be primarily explained by differences between training protocols. As an illustration, the type of exercise (*e.g.* treadmill, wheel, swimming) differed between studies as well as exercise intensity and duration. A lack of workload standardization between exercises (= work-matched exercises), intensity progression and training individualization is mainly observed in rodent studies although these parameters are of primary importance to allow comparable exercise doses, as recommended in humans[3,16]. Recently, Gronwald *et al* (2019) suggested that rodent training protocols should be described by including an indicator of the desired exercise intensity, so that they can be reproduced in other studies and extrapolated to humans for clinical/performance purpose[16]. Therefore, we suggest using a submaximal physiological parameter, the $S_{LT}$, to distinguish between high and moderate running speeds *i.e.*, below $S_{LT}$ for low intensity and above for high intensity[6,17]. Despite its major role in exercise intensity prescription and performance assessment, it has received little attention in rodent studies[6,8,16].

Based on these considerations, the main purpose of this study was to determine the respective effects of work-matched HIIT and MICT (based on $S_{LT}$ and $S_{max}$), on endurance performance and to show whether these two training regimens enhanced hippocampal neuroplasticity markers and behavioural (memory) functions in healthy rats. Training protocols lasted 8 weeks, as did previous studies with rodents[4,6]. To the best of our knowledge, it is the first study to compare MICT with HIIT at both brain and muscle levels. The present study is intended to contribute to define exercise guidelines to maintain brain health, which are nowadays inexistent.

## 2. Materials and Methods

### 2.1 Ethics approval statement

All experimental protocols were performed in accordance with relevant guidelines and were approved by the Animal Care Committees of Marseille n°14 (approval number: APAFiS #11558). Also, all animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The 3Rs principle was applied: i) Replacement – No substitution *in vitro* model exists to mimic endurance training effects on muscle and brain functions at the same time; ii) Reduction – The same animals performed training, incremental and behavioural tests to reduce the number of animals; iii) Refinement – Experiments were either non-invasive or without awakening. Signs of animal suffering and welfare were monitored. Indeed, animals were excluded when one of the following points was observed: 1) animals unable to run on treadmill during the familiarization or during the training protocol, 2) clinical signs of pain and discomfort (prostrated animals with absence of movement in the cage and/or hunched posture, ungroomed appearance, weight loss higher than 20%, dehydration, decreased urine/fecal output, piloerection, chronic porphyrin staining around eyes, nose or forelimbs and rapid respirations) and 3) absence of social interaction in the cage and decrease of food and/or water intake. Rats were housed by 3-4 per cage in order to provide sufficient space for essential aspects of rat behaviour and preserve social interaction. Food and water were given ad libitum and rats maintained at 22°C with a 12-h light/dark cycle. Enriched environment was also provided for all animals.

### 2.2 Animals

Thirty adult male Sprague-Dawley rats (**Fig. 1A** for time course of body weight**;** JANVIER®, France) were randomly assigned to one of the following groups:

1) Control (n=10), in which rats did not perform training program,

2) HIIT (n=10) in which rats performed the 8-week HIIT program,

3) MICT (n=10), in which rats performed the 8-week MICT program in a work-matched manner (see below).

All animals were subjected to incremental exercise tests on treadmill and behavioural tests at PRE, day (D) 15, D29, D43 and D57. Molecular measurements were performed on the

hippocampus and *triceps brachii/soleus* muscles at D59 **(Fig. A.1)**. All acquisitions and analyses were performed in a single-blind manner.

### 2.3 Incremental exercise test on treadmill: $S_{LT}$ and $S_{max}$

All rats were familiarized before the experiment with running on a treadmill (5 min per day for 5 days, at low speeds, 10-15 $m.min^{-1}$) to limit stress of rats during training. After a 5-min warm-up at 9 $m.min^{-1}$, the speed increment was 3 $m.min^{-1}$ every 3 min with a starting running speed set at 12 $m.min^{-1}$. At the end of each speed level, a blood sample was collected from the tail vein during a 15-20 s rest period to measure lactate concentration ($mmol.L^{-1}$) with a portable device (Lactate Scout+, EKF diagnostics®, France). Tail incision method is considered stress-free and allows intra-individual comparisons[18]. The lactate threshold was determined when 1) the inflection point of blood lactate concentration was observed and/or 2) an increase of 1 $mmol.L^{-1}$ between two blood lactate values was measured[19]. The speed corresponding to lactate threshold was $S_{LT}$. The test was stopped when the rat could not maintain the imposed running speed. The last completed speed level was considered as the maximal speed ($S_{max}$).

### 2.4 Work-matched HIIT and MICT programs on treadmill

For the first two weeks, running speed was determined according to $S_{LT}$ and $S_{max}$ obtained during the incremental exercise test at PRE. Then, running speeds were adjusted from $S_{LT}$ and $S_{max}$ obtained at D15 for the following weeks. Training sessions were performed 5 days/week. Both training protocols started with a 5-min warm-up at speed 30% below $S_{LT}$.

***2.4.1 HIIT***. Training sessions were composed of 4x4 min of high intensity exercise (running speed fixed at 80-100% of the variation between $S_{LT}$ and $S_{max}$) interrupted by a 3-min active recovery (30% below $S_{LT}$). Thus, one session lasted 28 min (exercise + active recovery). It ensured that running speed remained above $S_{LT}$ and below $S_{max}$. Indeed, HIIT intensity need to be higher than lactate threshold to be considered as HIIT regimen[3]. Running speed progressively increased over the 8 weeks **(Table B.1)**.

***2.4.2 MICT.*** Running speed was fixed at 20% below $S_{LT}$ to avoid fatigue accumulation. Session duration (sustained exercise without recovery period) depended on HIIT workload (exercise + active recovery) to match the total amount energy expenditure (isocaloric sessions) between groups as follows: *Mass (kg) x Intensity ($m.min^{-1}$) x Duration (min) x*

*Treadmill inclination (°) x 9.8 (J/kg.m)*[20]. The total workload was the same between groups because energy expenditure needed to be equivalent between exercise regimens. Therefore, experimenters trained each rat individually.

**2.5 Sensorimotor and cognitive assessments**

These behavioural tests were randomly conducted (with a 10-min rest period between each test) before incremental exercise tests to both avoid fatigue effects on results and reduce stress placed on the animals. Moreover, stress could be also reduced by other methodological procedures:

(i) Each experiment was performed by the same researcher throughout protocol for each behavioural test. It also allows to limit result variability.

(ii) The researcher and the rat were alone, in a quiet room, during the test procedure, especially for cognitive assessments.

(iii) Rats were familiarized with this arena before starting protocol of behavioural tests (5 days, 5 min/day), as for treadmill habituation.

***2.5.1 Forelimb grip force.*** The grip force exerted by both forelimbs was quantified by using a grip force tester (Grip Strength Tester bio-GT3, Bioseb®, Vitrolles, France)[19]. To summarize, when rat forelimbs grasped the middle of bar, the experimenter pulled it following the axis of sensor until grasp was released. The time interval between each trial was fixed to 1 min. Five to ten trials were performed per session. The top 2 maximal forces (in grams) were averaged and normalized by animal weight (grip strength ratio). For instance, the grip strength measured at PRE was normalized by the body weight measured at PRE and the D29 grip strength was normalized by the weight measured at D29.

***2.5.2 Adhesive removal test (ART).*** This test was used to independently assess tactile sensitivity and motor coordination of the right forepaw[21]. Before each testing session, the rat was placed into a box (67x37x30) during 90 seconds for a new familiarization period. Thereafter, a round adhesive tape (Ø 8 mm) was alternatively applied two times under left and right forepaws. The order of placing adhesive tape was randomized. The time to detect and to remove the adhesive tape for each forepaw was measured in second (s) and averaged between the 2 trials per session. The maximal recording time was fixed to 120 s.

***2.5.3 Novel object recognition test (NOR).*** The NOR is used for studying memory functions in rodents[22]. This test was performed in an open-field box (62.5x34.5x32 cm). Two trials were performed per session. The first trial (familiarization phase) consisted in exhibiting two identical objects in two opposite and symmetrical corners of the box for 5 min. Then, rats returned to their home cage for a 60-min inter-trial to assess short-term memory. During the second trial (test phase), a new object with different size, colour, shape and material replaced one of the objects. Location of objects remained the same for the two trials to avoid any influence of spatial memory[23]. Box and objects were cleaned in order to remove any scent. The interaction time with the object (e.g., touching, climbing and sniffing with nose at a 2-cm distance) was recorded. Recognition index (R.I.) of memory was calculated by the formula: time to investigation of new object/time to investigation of both previous objects.

***2.5.4 Y-maze alternation.*** It is used to assess spatial memory avoiding the need for extensive training and conventional reinforcers[24]. Alternation is an instinctive exploratory behaviour for rats, which induces alternative choice between Y-maze arms. The maze design was composed of 3 arms (49x12x25), labelled A, B and C joined at 120° angle from a central point. Rats were placed at the end of one arm and moved through the Y-maze during 5 min without reinforcement such as food or hand stimulation. An alternation was defined as consecutive entry into 3 arms of the 4 paws. Total number and sequence of arm entries were manually recorded. Spontaneous alternation was quantified by the formula: number of consecutive entrance/number of entrances into each arm-2. If the number of entrances into each arm was fewer than 8 times, data was not considered as a reliable recording. After each test, the maze was totally cleaned with alcohol hydrogel to avoid olfactory cues.

**2.6 Muscle and brain analyses at the molecular level**

Brain plasticity was first assessed by quantifying two neurotrophin receptors, the tyrosine kinase TrkB (high affinity with BDNF) and neurotrophin receptor p75 ($p75^{NTR}$, low affinity with BDNF), providing information on hippocampal neurotrophin activity. BDNF is a major neurotrophin, highly sensitive to endurance training and was only measured by qPCR as previously observed[25]. VEGF and peroxisome proliferator-activated receptor γ 1-α (PGC-1α) reflected angiogenesis, neurogenesis and aerobic metabolism[26]. Myokines known to have beneficial effect on brain function, such as Fibronectin Type III Domain Containing 5 (FNDC5) and Cathepsin B (CTSB) were also measured into both the

hippocampus and the *triceps brachii* and *soleus* muscles[25]. Together with brain measurements, we also assessed the protein contents involved in mitochondrial biogenesis and angiogenesis in muscles. PGC-1α is a key transcriptional coactivator that regulates genes involved in energy metabolism and mitochondrial biogenesis in both brain and muscles by interacting with transcriptional factors such as ERR-α. Moreover, PGC-1α activity is known to be influenced by SIRT1. Aerobic muscular plasticity could also be observed by changes in mitochondrial enzyme activity reflected among others by Cytochrome C (Cyt C) and COX4.

To assess the influence of training regimens on these markers, rats were anaesthetized with intraperitoneal ketamine/xylazine injection (90mg/kg-10mg/kg) 48h after the last incremental exercise test (at D59). *Soleus* and *triceps brachii* muscles were first removed. After decapitation, hippocampi were collected. Tissues were flash-frozen in liquid nitrogen and stored at -80°C until analyses.

### 2.7 RT-PCR and quantitative PCR (qPCR) analysis in the hippocampus

Total RNA was isolated from hippocampus using RNeasy Plus Mini kit and complementary DNA (cDNA) was synthesized using Quantitect Reverse Transcription kit, according to the manufacturer's protocol (QIAGEN®). qPCR was performed on a Light cycler 480 using SYBR-Green chemistry (Roche®) and specific primers for *Rattus norvegicus Rpl13a* (QT00425873) as control. qPCR was performed with 5 µL of diluted cDNA template, specific primers (0.6 µM) and SYBR Green I Master Mix (7.5 µL) at a final volume of 15 µL. Specific primers included Ppargc1a (QT00189196), Bdnf (QT00375998), Fndc5 (QT00382102) and Ntrk2 (QT00181923). Each reaction was performed at an annealing temperature of 60°C and for 50 cycles. Reactions were performed in duplicate and melting-curve analysis was performed to assess the specificity of each amplification. A standard curve was performed for each gene with a control cDNA diluted at different concentrations and repeated for each plate used. Relative expression was assessed with the calculated concentration in respect to standard.

### 2.8 Western blot analysis

***2.8.1 Hippocampus.*** Equal amount of proteins (40 µg) was separated in Bolt Bis-Tris Plus gel (4-12%; Invitrogen gel, ThermoFischer®) and transferred to nitrocellulose membrane (7 min). Membranes were blocked in Tris-buffered saline with Tween 20 (TBS-T) containing 5% of bovine serum albumin (BSA) for 1h. Membranes were exposed overnight to a 4°C temperature with the primary antibodies diluted in blocking solution (TBS-T/2.5% BSA)

indicated in **Table B.2**. Then, membranes were washed in TBST-T and incubated with the corresponding secondary antibody (1:500, Alexa Fluor 543 anti-rabbit, A-32732; Alexa Fluor 488 anti-mouse, A-11001; ThermoFisher®) 2h at room temperature before fluorescence detection. Signal intensities were measured with the image analysis software G box (Syngene®) and normalized to α-tubulin protein levels. Quantification was performed using Gel Plot Analysis plugin (ImageJ®). Intensity values were expressed relative to Control group.

***2.8.2 Soleus and triceps brachii muscles***. The Western blot for muscles and brain was performed in different laboratories. Methods were thus different. Nevertheless, it was not considered as a limitation in this study because there was no comparison between muscles and hippocampal proteins. Equal amount of proteins (60 µg) was separated on Criterion TGX Stain Free Gels (4-20%, Bio-Rad®, Hercules, CA, USA). Gels were imaged after activation using Bio-Rad ChemiDoc™ Touch Imaging System and transferred to nitrocellulose membranes using Bio-Rad Trans-Blot® Turbo™ Transfer System (7 min). Membranes were blocked for 1h at room temperature in TBS-T containing 5% of skimmed milk. They were then incubated overnight at 4°C with the primary antibodies indicated in **Table B.3**. Membranes were washed in TBS-T and incubated for 1h with a peroxidase-conjugated secondary antibody diluted in the blocking solution. Membranes were washed in TBS-T, revealed by using a Pierce ECL kit (32106; Thermo Scientific®) and imaged for chemiluminescence using ChemiDoc Touch Imaging System (Bio-Rad). Image analysis was done using Bio-Rad Image Lab™ software v5.1. Signals were normalized to total proteins as measured on the stain-free gel image. Intensity values were expressed relative to Control group. Each membrane contained protein samples of each group for comparison.

### 2.9 Statistical analysis

Results of grip strength and cognitive tests, $S_{max}$ and $S_{LT}$ were compared by means of two-way ANOVA (training regimens x time). Results of molecular measurements were compared by means of one-way ANOVA (training regimens). Post-hoc comparisons were performed with Tukey multiple post-test comparisons. Pearson's correlation coefficient with two-tailed significance was used to analyse the relationship between brain/muscle proteins and aerobic parameters, as well as the relationship between muscle FNDC5 and brain proteins. Results were considered significant when $p<0.05$. All data were expressed in mean ± SD.

## 3. Results

No difference was observed between Control, HIIT and MICT groups at PRE for sensorimotor, cognitive and incremental exercise tests.

### 3.1 Characteristics of endurance training protocols

Running speed during MICT was lower than HIIT from the 1st to 8th training weeks (-39%; -41%; -36%; -37%; -41%; -42%; -43% and -44%, respectively). Session duration of MICT was longer than HIIT form the 1st to 8th weeks (+36%; +37%; +33%; +29%; +50%; +45%; +47%; +47%, respectively). All included animals were able to perform each session of MICT and HIIT protocols. Progression of exercise intensity, duration and energy expenditure over the 8 weeks of training was described for each group in **Table 1**. No difference was observed between groups for gains in body weight over training protocols (**Fig. 1A**).

### 3.2 Incremental exercise test

$S_{LT}$ and $S_{max}$ increased from PRE to D15 for the 2 trained groups. However, these parameters continued to be enhanced by HIIT from D15 to D57 whereas an early plateau of performance was observed in the MICT group. Finally, HIIT induced superior improvement of aerobic fitness compared to work-matched MICT following 8 weeks of training.

***3.2.1 $S_{LT}$.*** Two-way ANOVA analysis showed significant difference between training regimens ($F_{(2,108)}$ = 52.5), times ($F_{(3,108)}$ = 22.32) and training regimens x times ($F_{(6,108)}$ = 11.55) with $p<0.001$ for all. At D15, D29 and D57, $S_{LT}$ of HIIT and MICT groups was higher than Control ($p<0.001$) **(Fig. 1B)**. At D57, $S_{LT}$ of HIIT group was higher than MICT group ($p<0.01$). After HIIT, $S_{LT}$ increased from PRE to D57 (+73±31%; $p<0.001$). Moreover, $S_{LT}$ increased from D15 to D57 ($p<0.05$). After MICT, $S_{LT}$ increased from PRE to D57 (+50±33%; $p<0.001$) without difference between D15 and D57.

***3.2.2 $S_{max}$.*** Two-way ANOVA analysis showed significant difference between training regimens ($F_{(2,108)}$ = 54.69), times ($F_{(3,108)}$ = 22.35) and training regimens x times ($F_{(6,108)}$ = 10. 04) with $p<0.001$ for all. At D15, D29 and D57, $S_{max}$ of HIIT and MICT groups was higher than Control ($p<0.001$) **(Fig. 1C)**. $S_{max}$ of HIIT group at D57 was higher than MICT group ($p<0.01$). After HIIT, $S_{max}$ increased from PRE to D57 (+64±31%; $p<0.001$), but also from D15 to D29 ($p<0.05$) and to D57 ($p<0.01$). After MICT, $S_{max}$ increased from PRE to D57 (+42±32%; $p<0.001$) and from D15 to D29 ($p<0.05$) without difference between D15/D29

and D57 contrary to HIIT. No difference was observed for maximal concentration of blood lactate at the end of incremental exercise test at D57.

Interestingly, the performance varied from a rat to another during the incremental exercise test at PRE. Indeed, $S_{LT}$ varied from 21 to 33 m.min$^{-1}$ and $S_{max}$ from 27 to 48 m.min$^{-1}$ (including animals from HIIT, MICT and Control groups). Given that rats were randomly assigned to the 3 groups, the range of $S_{LT}$ within each group was as follows: from 24 to 30 m.min$^{-1}$ for Control, from 21 to 33 m.min$^{-1}$ for both HIIT and MICT. For $S_{max}$, the range was as follows: from 30 to 36 m.min$^{-1}$ for Control, from 30 to 48 m.min$^{-1}$ for HIIT, from 27 to 39 m.min$^{-1}$ for MICT.

### 3.3 Muscles

***3.3.1 Relative protein content in the triceps brachii muscle at D59.*** One-way analysis showed significant difference between training regimens for VEGF ($F_{(2,25)}$ = 7.53, p<0.01), ERR-α ($F_{(2,24)}$ = 3.84, p<0.05), COX4 ($F_{(2,22)}$ = 4.90, p<0.05) and FNDC5 ($F_{(2,26)}$ = 6.95, p<0.01). VEGF levels were higher in MICT and HIIT groups (p<0.01) than Control **(Fig. 2A)**. The SIRT1 levels tended to be higher in MICT group than Control ($F_{(2,27)}$ = 3.13, p=0.059) **(Fig. 2B)**. The levels of ERR-α in HIIT group were higher than Control (p<0.05) **(Fig. 2C)**. The levels of COX4 were higher in HIIT and MICT groups than Control (p<0.05) **(Fig. 2D)**. Furthermore, the levels of FNDC5 were higher in the HIIT group than Control and MICT groups (p<0.05 and p<0.01 respectively) **(Fig. 2E)**. No difference between groups was observed for the following proteins: PGC-1α, BDNF and CTSB.

***3.3.2 Relative protein content in the soleus muscle at D59.*** One-way analysis showed significant difference between training regimens for VEGF ($F_{(2,20)}$ = 5.00, p<0.05) and Cyt C ($F_{(2,23)}$=6.06, p<0.01). MICT and HIIT groups displayed higher VEGF levels than Control (p<0.01 and p<0.05, respectively) **(Fig. 3A)**. The levels of Cyt C in HIIT group were higher than Control and MICT groups (p<0.05 for both) **(Fig. 3B)**. No difference was observed for PGC-1α, BDNF, ERR-α, CTSB and COX4 (data not shown).

### 3.4 Hippocampus measurements

***3.4.1 qPCR at D59.*** The amount of *Pgc-1α* mRNA and *Ntrk2* mRNA was significantly reduced for HIIT group when compared to other groups (p<0.05; **Fig. 4**). No significant difference was observed for *Bdnf*, *Fndc5* and *p75* expression. However, no significant

difference was found for MICT group for mRNA expression of *Bdnf*, *Pgc-1α, Fndc5, p75* and *Ntrk2* (data not shown).

***3.4.2 Western Blotting at D59.*** One-way analysis showed significant difference between training regimens for VEGF ($F_{(2,21)}$ = 11.10, p<0.001), TrkB ($F_{(2,21)}$ = 4.16, p<0.05) and PGC-1α ($F_{(2,21)}$ = 4.35, p<0.05). The levels of PGC-1α in HIIT group were higher than Control (p<0.01) and showed a tendency to be higher than MICT group (p=0.0574) **(Fig. 5A)**. Similarly, the levels of VEGF were higher in HIIT group than MICT and Control groups (p<0.001 for both) **(Fig. 5B)**. The levels of TrkB were higher in HIIT group than MICT group (p<0.05) **(Fig. 5C)**. No difference was observed after any training for the expression of $p75^{NTR}$, FNDC5 and Cathepsin B (data not shown).

### 3.5 Correlation between aerobic parameters ($S_{LT}$ and $S_{max}$) and muscles and brain proteins

The FNDC5, VEGF and ERRα, but not COX4 and SIRT1, in the *triceps brachii* were positively correlated with $S_{LT}$ (r = 0.46, r = 0.44 and r = 0.45; p<0.05 for all, respectively) and $S_{max}$ (r = 0.5; p<0.01, r = 0.39; p<0.05 and r = 0.48; p<0.01) **(Fig. 6A-F)**. Only the SIRT1 in the *soleus* was negatively correlated with $S_{max}$ (r = -0.42; p<0.05) **(Fig. 6G)**.

The hippocampal PGC-1α was positively correlated with $S_{LT}$ (r = 0.41; p<0.05) and $S_{max}$ (r = 0.4; p<0.05) **(Fig. 7A-B)**. The hippocampal TrkB was positively correlated with $S_{LT}$ (r = 0.56; p<0.01) and $S_{max}$ (r = 0.53; p<0.01) **(Fig. 7C-D)**. Also, the hippocampal TrkB, but not VEGF and PGC-1α, was positively correlated with the FNDC5 in the *triceps brachii* (r = 0.72; p<0.001) **(Fig. 7E)**.

### 3.6 Sensorimotor and cognitive tests

Both endurance training regimens induced higher grip strength exerted by both forelimbs at the end of protocol and decreased the time to detect and to remove the adhesive tape. However, cognitive performances remained stable.

***3.6.1 Forelimb grip strength.*** Two-way ANOVA analysis showed significant difference between times ($F_{(4,110)}$ = 5.24, p<0.001) and Training regimens x times ($F_{(8,110)}$ = 2.87, p<0.01). At D57, the grip strength ratio was higher in HIIT (5.0±0.6) and MICT (4.7±0.6) groups compared to Control (3.8±0.7; p<0.01). The grip strength ratio increased from D15 (3.6±0.6) to D57 in HIIT group (p<0.01). No difference was observed between MICT and

HIIT groups. Interestingly, grip strength was reduced from D29 to D57 in Control ($p<0.01$) **(Fig. 8A)**.

***3.6.2 ART.*** For the time to detect, two-way ANOVA analysis showed significant difference between Training regimens ($F_{(2,110)} = 6.65$, $p<0.01$). The time to detect of the right forepaw is significantly lower in HIIT and MICT groups than Control at D57 ($p<0.001$ for both) **(Fig. 8B)**. In Control group, the time to detect was significantly increased at D57 compared to D28 ($p<0.05$).

For the time to remove, two-way ANOVA analysis showed significant difference between Training regimens ($F_{(4,110)} = 9.62$, $p<0.001$), times ($F_{(4,110)} = 6.67$, $p<0.05$) and Training regimens x times ($F_{(8,110)} = 13.03$, $p<0.01$). The time to remove at D57 was lower in HIIT and MICT groups than Control ($p<0.001$ for both) **(Fig. 8C)**. The time to remove significantly increased at D57 for the right forepaw compared to D14 ($p<0.05$).

***3.6.3 Cognitive assessment.*** For both cognitive tests (*i.e.* NOR and Y-maze), no difference was observed between and within the three groups throughout training protocols **(Table 2)**.

## 4. Discussion

### 4.1 Methodological considerations for training protocols in rodents

All animals reached the lactate threshold during incremental exercise. It confirms that, contrary to maximal parameters, this physiological determinant of performance does not depend on motivation and fatigue[17]. The lactate threshold might thus be appropriate for patients to assess endurance capacities and to distinguish the different ranges of exercise intensities, as performed in rats with cerebral ischemia[19]. Given that $S_{LT}$ reflects the threshold at which metabolite accumulation starts to occur, we have distinguished the physiological underpinnings of high running speeds (from 31 $m.min^{-1}$ during the first week to 46 $m.min^{-1}$ during the $8^{th}$ week) and of low running speeds (from 19 $m.min^{-1}$ to 27 $m.min^{-1}$). It strongly differed compared with previous studies using empirical or maximal intensities. For instance, high running speeds could vary from 18 $m.min^{-1}$ to 52 $m.min^{-1}$ while 10-12 $m.min^{-1}$ (lower than warm-up speed in our study) were considered as moderate intensities[27]. In addition, most conclusions about exercise-related brain plasticity come from studies using running wheel[10]. However, the speed cannot be controlled in these conditions, thereby rendering clinical applications rather uncertain[16]. The inter-animal differences of $S_{LT}$ and $S_{max}$ found during the first incremental exercise test suggest that running speed should be systematically individualized by using $S_{LT}$, as previously observed in humans[28]. Nevertheless, our method is more constraining for experimenters because it is time-consuming (individual training for each rat) and involves several incremental exercise tests with repetitive blood lactate measurements. Nevertheless, standardization and individualization of exercise intensity seem essential when protocols are intended to produce an effect on brain health. Interestingly, we observed higher improvement of performance after 15 days for both training regimens thereby suggesting that it could be the appropriate period for accurately reassessing individual running speed from $S_{LT}$ and $S_{max}$ in healthy rodents.

The translational relevance from rodent models to humans exhibits some limitations. First, cognitive tests for rodents were limited to spatial and short-term memory assessment. Possibly, MICT and HIIT target other cognitive functions than those tested in the present experiment. Indeed, in healthy older individuals for instance, MICT was superior to HIIT in enhancing executive functions while HIIT was the most beneficial for improvement in information processing speed[29]. Second, our cognitive tests might be insufficiently sensitive to detect training effects in healthy rats because it is known that healthy subjects exhibit a lower reserve for cognitive gains than people with mild cognitive impairments[13].

Third, it is difficult, if not impossible, to test the different exercise types (rowing and cycling) in rodents that might have major influence on neuroplasticity and behavioural functions[13]. Fourth, treadmill training is suitable for individualized training in rats despite the fact that it is stressful for them, as attested by an increase of both corticosterone levels and blood lactate concentration[30]. Therefore, stress needed to be controlled in every single experiment. It was achieved by submitting rats to daily manipulation by experimenters and to familiarization to the different experimental procedures. Despite these methodological precautions, it is likely that our results were partially affected by stress.

### 4.2 Different kinetic of $S_{LT}$ and $S_{max}$ suggest that HIIT induce superior improvement of aerobic fitness as compared to work-matched MICT in a time-efficient manner.

$S_{LT}$ is a major indicator of endurance performance. Indeed, higher increase of $S_{LT}$ suggests that rats were able to run at higher speeds over long distances in both MICT and HIIT[30]. Our results suggested that HIIT might be more effective than MICT to reduce fatigue accumulation at a given submaximal intensity, as previously demonstrated in professional cyclists[31]. HIIT was also more effective to improve $S_{max}$ than work-matched MICT, even though the duration of HIIT sessions was lower (from -30 to -50%) than MICT sessions. Given that a strong positive correlation has been observed between $VO_{2max}$ and $S_{max}$ in rodents[32], our findings suggest that HIIT was more effective than work-matched MICT to improve $VO_{2max}$. It is in agreement with results observed in a previous study in humans[33]. This assumption needs to be considered with caution because $S_{max}$ depends on various factors, such as capacity of anaerobic metabolism, running economy and motivation.

The endurance performance continued to be improved until the end of HIIT protocol whereas it was stable from the first weeks of MICT. It suggests that HIIT could improve the endurance performance on a longer period than MICT, explaining the observed difference in $S_{LT}$ and $S_{max}$ at 8 weeks. Interestingly, an early plateau of performance after MICT was also observed in humans, limiting gains in performance over time[2]. In rats, a previous study found different gains of performance with time, as compared to results of the present study[6]. However, it is noticeable that in this previous study, MICT and HIIT protocols were insufficient to improve endurance performance following 8 weeks of training. Surprisingly, the duration of HIIT sessions was longer than MICT and the running speeds were similar between these regimens in contrast to our results.

### 4.3 Work-matched HIIT and MICT promote specific muscular plasticity related to mitochondrial biogenesis.

Both training regimens seemed equally effective to enhance VEGF levels after 8 weeks. VEGF is frequently associated with beneficial effects of physical exercise because VEGF plays a critical role in angiogenesis and muscle oxidative capacities by improving $O_2$ supply to active muscles[34]. In our study, the effect of work-matched HIIT and MICT on aerobic metabolism has been observed from different signalling pathways, mainly in the *triceps brachii* muscles. For MICT group, the tendency to increase of SIRT1 levels in the *triceps brachii* might contribute to mitochondrial biogenesis that was reinforced by the increase of COX4 levels, even though PGC1-α levels remained stable[35]. Following HIIT, ERR-α levels were higher in the *triceps brachii*. Also, ERR-α was involved in mitochondrial biogenesis and oxidative phosphorylation in humans even if PGC-1α expression remained stable[36]. The beneficial role of HIIT in mitochondrial biogenesis was presumably reinforced by the increase of COX4 levels. Interestingly, higher Cyt C levels were observed in the *soleus* suggesting a beneficial effect of HIIT in both slow- and fast-twitch muscle fibres. The correlation between the *triceps brachii* protein contents and aerobic parameters indicated that muscle plasticity is associated with endurance performance in healthy rats, as in humans[37]. Additionally, it was established that ERR-α is involved in the FNDC5 gene expression[25]. We have found an increase of FNDC5 levels in the *triceps brachii* after HIIT but not after MICT, which is consistent with results observed in humans[38]. It might indicate a higher effectiveness of HIIT than work-matched MICT to promote FNDC5 in active fast-twitch muscle fibres. Given that FNDC5 stimulates BDNF by passing through the blood-brain barrier, the increase of FNDC5 might contribute to promote neuroplasticity markers after HIIT[25]. Interestingly, such relationship is reinforced in our study by the positive correlation observed between the upregulation of *triceps brachii* FNDC5 and hippocampal TrkB.

### 4.4 Eight weeks of HIIT enhance levels of neuroplasticity markers in the hippocampus.

In healthy rats, HIIT increased levels of VEGF, PGC-1α and TrkB in the hippocampus contrary to work-matched MICT. An increase in these markers is usually associated with synaptic plasticity, neurogenesis and/or angiogenesis suggesting that HIIT might play a potential role in neuroplasticity[9,26]. The increase in hippocampal VEGF levels confirms results of a previous study in mice, in which VEGF and cerebral angiogenesis were related to lactate production during intense exercise[39]. However, it has also been found higher VEGF

levels in the mouse hippocampus after MICT compared with intense training (incremental exercises, not HIIT)[11]. In this previous study, running speeds were not individualized and remained very low relative to those used in the present study for MICT[11]. Interestingly, we have also observed different responses of VEGF between muscle and brain. Indeed, HIIT increased VEGF levels in both the hippocampus and muscles while MICT was only effective to promote it in active muscles.

Presumably, the increase in brain VEGF levels might influence cerebral aerobic metabolism and neuroplasticity processes[26]. The increase in PGC-1α levels in the hippocampus after HIIT, which is a transcriptional co-activator involved in mitochondrial biogenesis, is in accordance to those who found similar results in older mice following 7 weeks of intense training[40]. PGC-1α upregulation is also considered as a viable tool to fight against a wide range of neurodegenerative diseases by interacting with the BDNF/TrkB pathway in the hippocampus[9,25]. Numerous works have demonstrated beneficial effects on neuronal survival and synaptic plasticity exerted by higher TrkB levels[9]. Indeed, the upregulation of TrkB is considered a major cascade of BDNF actions for neuroplasticity. Our results are thus in line with those who found a higher increase of hippocampal BDNF after HIIT compared to MICT in healthy rats[5] while other studies found opposite results by using different ways to compare intense and moderate intensity exercises[4,11]. The correlation between the PGC-1α and TrkB upregulation with improvements in $S_{LT}$ and $S_{max}$ is in line with those showing in humans that the serum BDNF level was related to $VO_{2peak}$ and the first ventilatory threshold[41]. It is thus suggested with caution that aerobic fitness improvements might be associated with an upregulation of neurotrophic activity in the hippocampus. Also, it was found in mice a positive correlation between the BDNF mRNA levels and workload levels[42], suggesting a possible role of exercise intensity in upregulating the hippocampal BDNF/TrkB pathway. Surprisingly, the *Ntrk2* and *Pgc-1α* expression is downregulated following HIIT in parallel with higher TrkB and PGC-1 α protein contents. Such downregulation of gene expression following training remains difficult to explain but it might partially be related to the negative loop feedback induced by these protein contents on their mRNA expression[25]. The absence of changes in both gene expression and protein contents induced by MICT is consistent with this hypothesis.

Despite our comprehensive approach to define the respective effects of each training regimen on neuroplasticity, some limitations need to be highlighted. Indeed, we were unable to determine which neuroplasticity processes (synaptic plasticity and neurogenesis) were specifically stimulated by HIIT when intensity was standardized and individualized from $S_{LT}$

and $S_{max}$. Moreover, although the hippocampus is one of the most responsive brain structures to exercise, other regions are also involved in behavioural functions. Further studies should investigate neuroplasticity effects of work-matched training regimens in different brain structures at different time points.

### 4.5 No effect of exercise intensity and duration was observed on spatial working memory and recognition memory after 8 weeks. Is endurance training sufficient to improve cognitive performance?

Many studies have found a positive relationship between physical activity and cognition, but others have failed to detect a link between them[43]. Our results reinforce previous findings suggesting that endurance training does not systematically improve cognitive functions, even when neurotrophic factors are upregulated[15]. Also, a recent meta-analysis found that exercise intensity has no clear effect on cognitive benefits in healthy humans, but such result should be interpreted with caution due to high risks of methodological bias relative to exercise intensity prescription[13]. Moreover, our results are in line with findings showing that improvements in aerobic fitness is not necessary related to cognitive benefits[13]. However, cognitive benefits were observed in both humans and rodents when endurance training was combined with cognitive tasks[10]. To explain these greater benefits, authors postulated that neuroplasticity might be facilitated by endurance and guided by cognitive training with the formation of specific neuronal pathways[44]. Based on the present findings, cognitive tasks might be effectively combined with HIIT, and not only with MICT, to potentiate or maintain cognitive functions over time in healthy subjects.

### 4.6 HIIT and MICT could prevent the decline in the force, motor coordination and tactile sensitivity over time.

The increase of grip strength in HIIT group from PRE to D57 likely explains the difference with Control at D57. However, the difference between MICT and Control seems to be mainly related to the grip strength decline in Control rather than an effect of MICT. Our results were in line with previous findings in healthy rats indicating a greater grip strength following HIIT compared with MICT due to higher muscle activation and/or muscle hypertrophy during HIIT sessions[45,46]. Similarly, both training regimens seemed to maintain motor coordination and tactile sensitivity by avoiding a decrease of mechanical sensitivity as observed in Control.

## 5. Conclusions

By using $S_{LT}$ to separate the high from low running speeds, such training protocol can be easily reproduced in future preclinical experiments and extrapolated to humans for clinical/performance purpose. Indeed, $S_{LT}$ can be investigated in preclinical studies because it is an important indicator of quality of life. For instance, this parameter can be reached by rats with severe cerebral ischemia allowing planning endurance training depending on aerobic abilities of each animal[19,47]. Such method is also suitable to define the optimal exercise parameters of endurance programs for patients with cardiovascular, metabolic or neurological disorders who cannot reach their maximal aerobic capacities. Therefore, the use of $S_{LT}$ can increase the complementary between human and animal studies for a given pathology by developing exercise programs from similar physiological parameters.

The beneficial effects of HIIT on endurance performance as well as on the prevention of sensorimotor decline over time are relevant for many diseased individuals. Moreover, this time-efficient strategy enables to reduce the duration of patient care while maximizing beneficial effects of endurance training. Given that HIIT also appears promising to enhance neuroplasticity markers in the hippocampus, we recommend incorporating HIIT into physical exercise guidelines for maintaining brain plasticity.

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**Conflict of Interest**

Authors report no conflict of interest

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**Author Contributions:**

AC performed research / analysed data / wrote the paper (behaviour, muscle and brain parts).

CPB performed research / analysed data / revised the paper (behaviour, muscle and brain parts).

FM performed research / analysed data for qPCR.

JJT designed research / revised the paper.

TB designed research (muscle part).

CP performed research (brain part) / revised the paper.

JL designed research (behaviour, muscle and brain parts) / performed research / wrote the paper.

| | | Running speed ($m.min^{-1}$) | Recovery speed ($m.min^{-1}$) | Session duration (min) | Total workload (Joules) |
|---|---|---|---|---|---|
| **HIIT** | Week 1 | 31.4±2.5 | 19.6±4.7 | 28 | 1423±50 |
| | Week 2 | 32.6±2.5 | 19.6±4.7 | | 1721±106 |
| | Week 3 | 41.8±5.1 | 27.8±2.1 | | 2646±34 |
| | Week 4 | 42.4±5.1 | 27.8±2.1 | | 2997±105 |
| | Week 5 | 45.0±3.7 | 30.8±7.3 | | 3561±197 |
| | Week 6 | 45.5±3.7 | 30.8±7.3 | | 3647±197 |
| | Week 7 | 45.3±2.8 | 26.4±1.7 | | 3696±246 |
| | Week 8 | 45.7±2.8 | 26.4±1.7 | | 3986±48 |
| **MICT** | Week 1 | 19.2±2.0 | - | 38.0±0.2 | 1423±50 |
| | Week 2 | 19.2±2.0 | | 38.3±0.4 | 1721±106 |
| | Week 3 | 26.7±2.2 | | 37.3±1.1 | 2646±34 |
| | Week 4 | 26.7±2.2 | | 36.3±2.2 | 2997±105 |
| | Week 5 | 26.4±3.9 | | 42.1±0.2 | 3561±197 |
| | Week 6 | 26.4±3.9 | | 40.4±2.2 | 3647±197 |
| | Week 7 | 25.7±2.7 | | 41.1±0.5 | 3696±246 |
| | Week 8 | 25.7±2.7 | | 41.0±1.5 | 3986±48 |

**Table 1: Exercise intensity, duration and energy expenditure during HIIT and MICT.** Session duration was indicated without the 5-min warm-up. No recovery period for MICT because it was a continuous exercise. Running and recovery speeds were indicated for each week of training in $m.min^{-1}$. These speeds were maintained during each session of a given week. Session duration was expressed in minutes (min). Energy expenditure was indicated in Joules. All data were expressed in mean ± SD.

| | | NOR test | | | | | Y-Maze test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **PRE** | **D15** | **D29** | **D43** | **D57** | **PRE** | **D15** | **D29** | **D43** | **D57** |
| **Control** | Mean | 0.61 | 0.52 | 0.62 | 0.59 | 0.46 | 0.65 | 0.66 | 0.68 | 0.70 | 0.74 |
| | SD | 0.18 | 0.13 | 0.16 | 0.30 | 0.26 | 0.15 | 0.13 | 0.16 | 0.09 | 0.08 |
| **HIIT** | Mean | 0.59 | 0.57 | 0.57 | 0.56 | 0.63 | 0.70 | 0.75 | 0.64 | 0.66 | 0.64 |
| | SD | 0.14 | 0.22 | 0.17 | 0.09 | 0.20 | 0.18 | 0.16 | 0.14 | 0.20 | 0.12 |
| **MICT** | Mean | 0.53 | 0.61 | 0.49 | 0.51 | 0.54 | 0.63 | 0.68 | 0.70 | 0.60 | 0.70 |
| | SD | 0.18 | 0.16 | 0.21 | 0.16 | 0.09 | 0.12 | 0.11 | 0.11 | 0.12 | 0.10 |

**Table 2: NOR and Y-maze tests.** These tests were performed before the training protocol (PRE) and 15, 29, 43 days of training (D15, D29 and D43) as well as after the end of training at day 57 (D57). For NOR, the recognition index (R.I.) of memory was calculated by the formula: time to investigation of new object/time to investigation of both previous objects. For Y-maze, spontaneous alternation was quantified by the formula: number of consecutive entrance/number of entrances into each arm-2. All data were expressed in mean ± SD.

## Figure Legends

**Fig. 1. Time course of body weight and endurance performance throughout the 8 weeks of training**. **(A) Body weight.** Body weight (in grams) was measured each day of training for workload measurement, but we only indicated in the present figure the weight at the end of each week (in grams). W= week. **Time course of endurance performance expressed in % of PRE value: (B) $S_{LT}$ and (C) $S_{max}$.** *Higher $S_{LT}$ and $S_{max}$ in HIIT and MICT groups compared to Control from D15 to D57. $^{\$}$Increase of $S_{LT}$ and $S_{max}$ in HIIT and MICT groups compared to PRE. $^{+}$Increase of $S_{LT}$ between D15 and D57 and increase of $S_{max}$ between D15 and D29/D57 in HIIT group. ‡Increase of $S_{LT}$ between D15 and D29 for $S_{max}$ in MICT group. $^{\#}$Higher $S_{LT}$ and $S_{max}$ in HIIT group compared to MICT group at D57. All data were expressed in mean ± SD.

**Fig. 2. Impact of exercise training on protein relative levels within the *triceps brachii* at D59 measured by Western blot.** Ratio protein/stain free was calculated and expressed relative to Control group. Top of the figure represented the relative levels of **(A)** VEGF, **(B)** SIRT1, **(C)** ERR-α, **(D)** COX4 and **(E)** FNDC5. Representative cropped immunoblots from the same membrane are shown at the bottom. *Increase of protein levels between groups. All data were expressed in mean ± SD.

**Fig. 3. Impact of exercise training on protein relative levels within the *soleus* at D59 measured by Western blot.** Ratio protein/stain free was calculated and expressed relative to Control group. Top of the figure represented the relative levels of **(A)** VEGF and **(B)** Cyt C. Representative cropped immunoblots from the same membrane are shown at the bottom. *Increase of protein levels between groups. All data were expressed in mean ± SD.

**Fig. 4. Impact of exercise training on *PGC-1α* and *Ntrk2* mRNA within the hippocampus at D59 measured by qPCR.** Relative expression of **(A)** *PGC-1α* and **(B)** *Ntrk2*. *Increase of protein levels between groups. All data were expressed in mean ± SD.

**Fig. 5. Impact of exercise training on protein relative levels within the hippocampus at D59 measured by Western blot.** Ratio protein/α-tubulin was calculated and expressed relative to Control group. Top of the figure represented the relative levels of **(A)** PGC-1α, **(B)** VEGF and **(C)** TrkB. Representative cropped immunoblots from the same membrane are

shown at the bottom. *Increase of protein levels between groups. All data were expressed in mean ± SD.

**Fig. 6. Correlation between aerobic parameters ($S_{LT}$ and $S_{max}$) and muscles proteins.** (A-B) FNDC5, (C-D) VEGF, (E-F) ERRα in the *triceps brachii* were positively correlated with both $S_{LT}$ and $S_{max}$, respectively. (G) SIRT1 in the *soleus* was negatively correlated with $S_{max}$. Pearson's correlation with two-tailed significance was conducted (n=25-30, pooled samples of Control, MICT and HIIT groups).

**Fig. 7. Correlation between aerobic parameters ($S_{LT}$ and $S_{max}$) and brain proteins.** The hippocampal (A-B) PGC-1α and (C-D) TrkB were positively correlated with $S_{LT}$ and $S_{max}$ respectively. (E) The hippocampal TrkB was positively correlated with FNDC5 in the *triceps brachii*. Pearson's correlation with two-tailed significance was conducted (n=25-30, pooled samples of Control, MICT and HIIT groups).

**Fig. 8. Sensorimotor tests throughout 8 weeks of MICT or HIIT. A) Grip strength.** The grip strength was normalized to body weight. For instance, the grip strength measured at PRE (in grams) was normalized by body weight measured at PRE and the D29 grip strength was normalized by body weight measured at D29. Data are expressed in percentage of PRE value. #Increase from D15 to D57 in HIIT group. *Higher grip strength in HIIT and MICT groups compared to Control at D57. +Decrease of grip strength between D29 and D57 for Control. **ART. B) Time to detect. C) Time to remove.** *Reduced time to detect and time to remove in HIIT and MICT groups compared to Control at D57. Time is expressed in seconds (s). +Increase of time to detect and time to remove at D57 compared to D28 or D14 for Control, respectively. All data were expressed in mean ± SD.

# Appendices

## Appendix A: supplementary figure in materials and methods

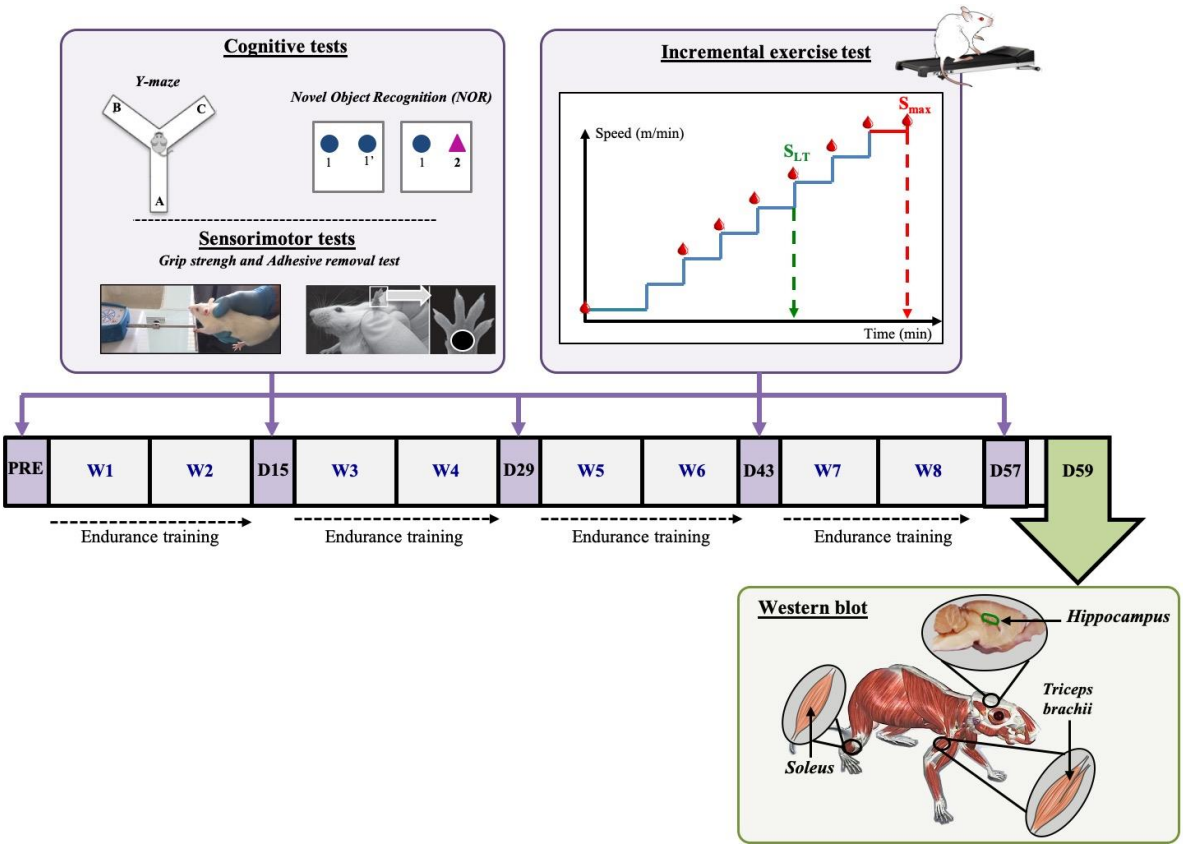



**Fig. A.1: Experimental protocol during 8 weeks of endurance training**. W = week, D = day. Endurance training refers either to MICT or HIIT. Cognitive, sensorimotor and incremental exercise tests were performed at PRE, D15, D29, D43 and D57. Cognitive and sensorimotor tests always preceded incremental exercise tests on treadmill to avoid fatigue accumulation and stress. Behavioral tests were randomly conducted with a 10-min rest interval. Running speed associated with lactate threshold ($S_{LT}$) and maximal running speed (speed to exhaustion or $S_{max}$) were obtained during incremental exercise test and expressed in $m.min^{-1}$. The NOR and Y-maze tasks enabled to detect potential improvement in memory functions. Grip strength of forelimbs was also tested. The NOR, Y-maze and grip strength test were randomly performed by healthy rats. At D59, the hippocampus and cortex, but also *soleus* and *triceps brachii* muscles were removed for Western blot analyses.

## Appendix B: supplementary tables in materials and methods

| Weeks of Training | Sessions | Intensity % of $S_{max}$-$S_{LT}$ | |
|---|---|---|---|
| 1 | $1^{st}$ – $4^{th}$ | 80 | After incremental exercise test at PRE |
| 2 | $5^{th}$ – $9^{th}$ | 95 | |
| 3 | $10^{th}$ – $13^{th}$ | 80 | After incremental exercise test at D15 |
| 4 | $14^{th}$ – $18^{th}$ | 85 | |
| 5 | $19^{th}$ – $23^{th}$ | 90 | |
| 6 | $24^{th}$ – $28^{th}$ | 95 | |
| 7 | $29^{th}$ – $33^{th}$ | 100 | |
| 8 | $34^{th}$ – $38^{th}$ | 100 | |

**Table B.1: Determination of running speed from $S_{LT}$ and $S_{max}$ over the 8 weeks of HIIT.** The running speed was individualized from the $S_{LT}$ and $S_{max}$ obtained at PRE and D15 (incremental exercise tests). It progressively increased depending on the % of the difference between $S_{max}$ and $S_{LT}$ ($S_{max}$-$S_{LT}$). During active recovery, running speed was fixed at 30% below $S_{LT}$. For instance, if $S_{LT}$ was observed at 36 $m.min^{-1}$ and $S_{max}$ at 45 $m.min^{-1}$ after the first incremental exercise test, speed for the first week of training was 43.2 $m.min^{-1}$ according to the following formula: ((45-36)*0.8)+36.

| Antibody | Species | Company | Dilution | Molecular weight (kDa) |
|---|---|---|---|---|
| CTSB | mouse | Abcam (Ab58802) | 1/500 | 31 - 43 |
| FNDC5 | rabbit | Adipogen (AG-25B-0027) | 1/1000 | 12-27-37 |
| PGC-1α | rabbit | Abcam (Ab54481) | 1/1000 | 92 - 105 |
| $p75^{NTR}$ | mouse | Santa Cruz (Sc-53631) | 1/1000 | 75 |
| TrkB | mouse | Santa Cruz (Sc-377218) | 1/500 | 95 - 145 |
| VEGF | mouse | Santa Cruz (Sc-7269) | 1/500 | 42 |
| α-tubulin | mouse | Invitrogen (#62204) | 1/10 000 | 55 |

**Table B.2: Antibodies used in Western blotting of hippocampal lysates.** Abbreviations: Tropomyosin-related kinase B (TrkB), Neurotrophin receptor p75 ($p75^{NTR}$), peroxisome proliferator-activated receptor γ 1-α (PGC-1α), vascular-endothelial growth factor (VEGF), irisin/FNDC5 and Cathepsin B (CTSB).

| Antibody | Species | Company | Dilution | Molecular weight (kDa) |
|---|---|---|---|---|
| BDNF | rabbit | Abcam (Ab108319) | 1/1 000 | 15 |
| CTSB | mouse | Abcam (Ab58802) | 1/500 | 31 - 43 |
| COX4 | mouse | Santa Cruz (Sc-69360) | 1/500 | 17 |
| Cyt C | mouse | Santa Cruz (Sc-13560) | 1/500 | 15 |
| ERR-α | mouse | Santa Cruz (Sc-65720) | 1/500 | 53 |
| FNDC5 | rabbit | Adipogen (AG-25B-0027) | 1/1 000 | 12 |
| PGC-1α | rabbit | Millipore (Ab3242) | 1/1 000 | 105 |
| SIRT1 | mouse | Cell Signaling (3931s) | 1/1 000 | 110 |
| VEGF | mouse | Santa Cruz (Sc-7269) | 1/500 | 25 |
| Peroxidase-conjugated | mouse | Santa Cruz (sc-2004) | 1/5 000 | |
| | rabbit | Santa Cruz (sc-2005) | 1/5 000 | |

**Table B.3: Antibodies used in Western blotting of muscle tissue lysates.** Abbreviations: brain-derived neurotrophic factor (BDNF), vascular-endothelial growth factor (VEGF), peroxisome proliferator-activated receptor γ 1-α (PGC-1α), irisin/FNDC5, Cathepsin B (CTSB), Cytochrome c oxidase subunit 4 (COX4), Sirtuine 1 (SIRT1) and Estrogen Related Receptor-alpha (ERRα).

Figure 1

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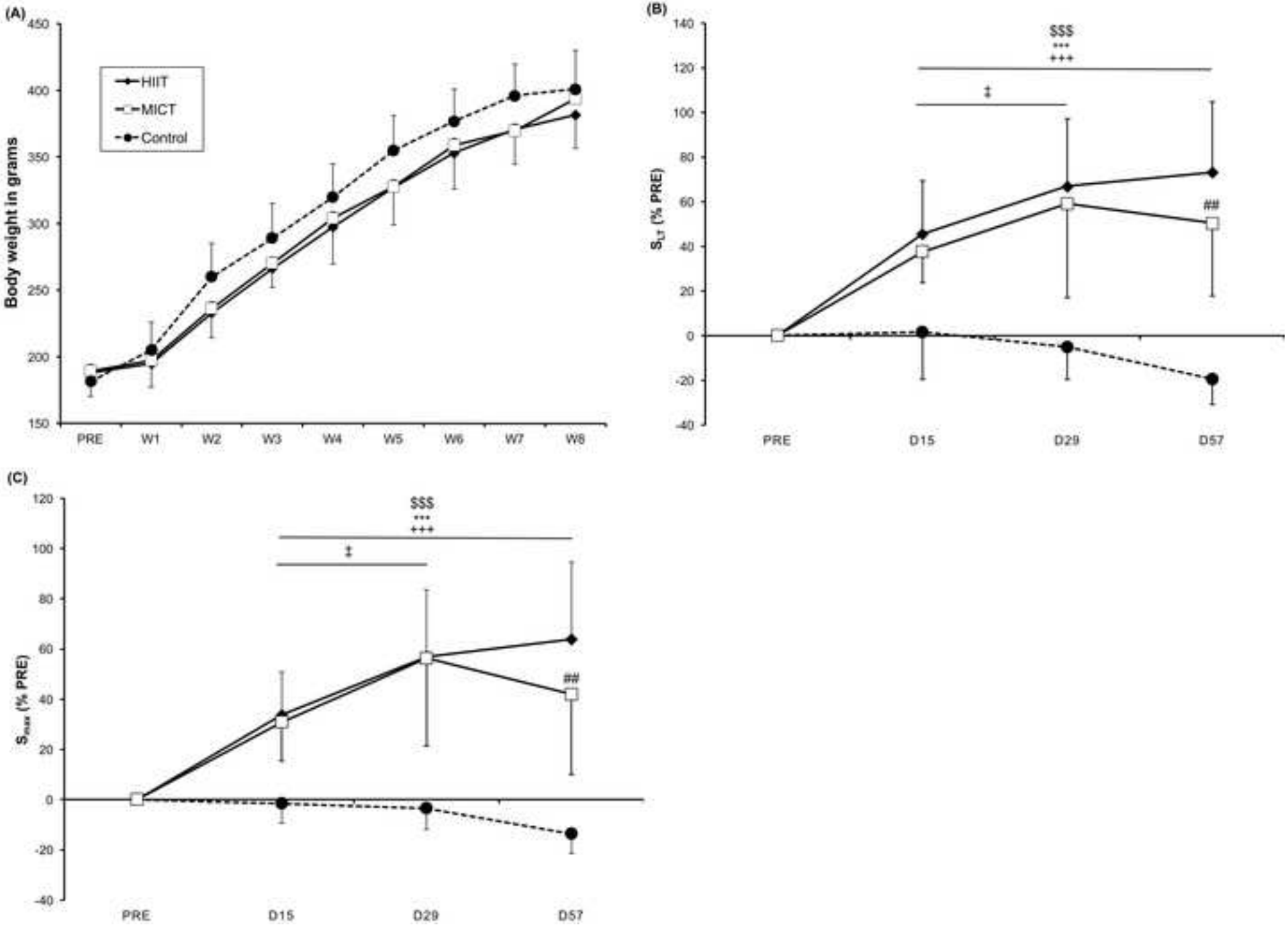


Figure 2

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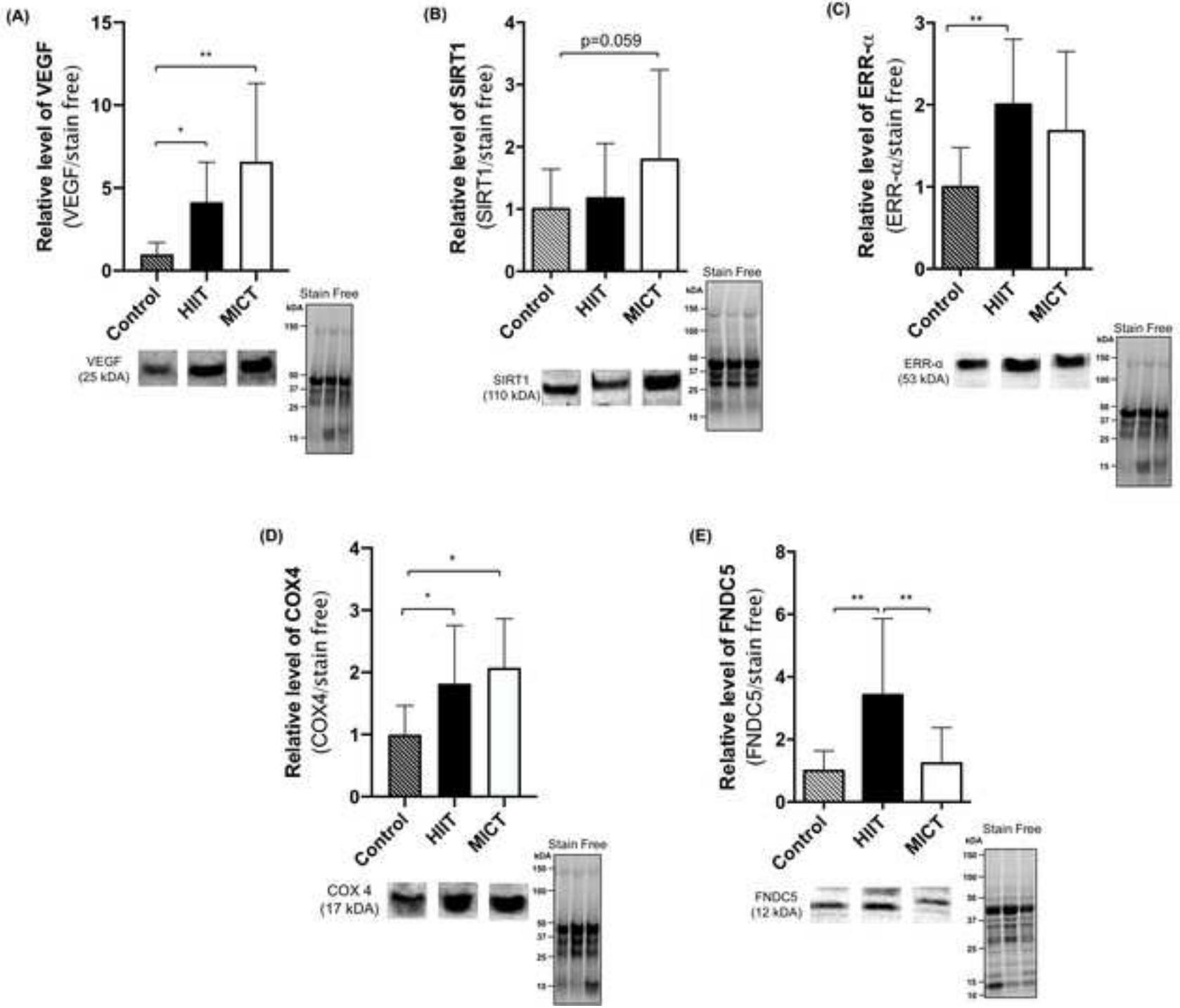


Figure 3

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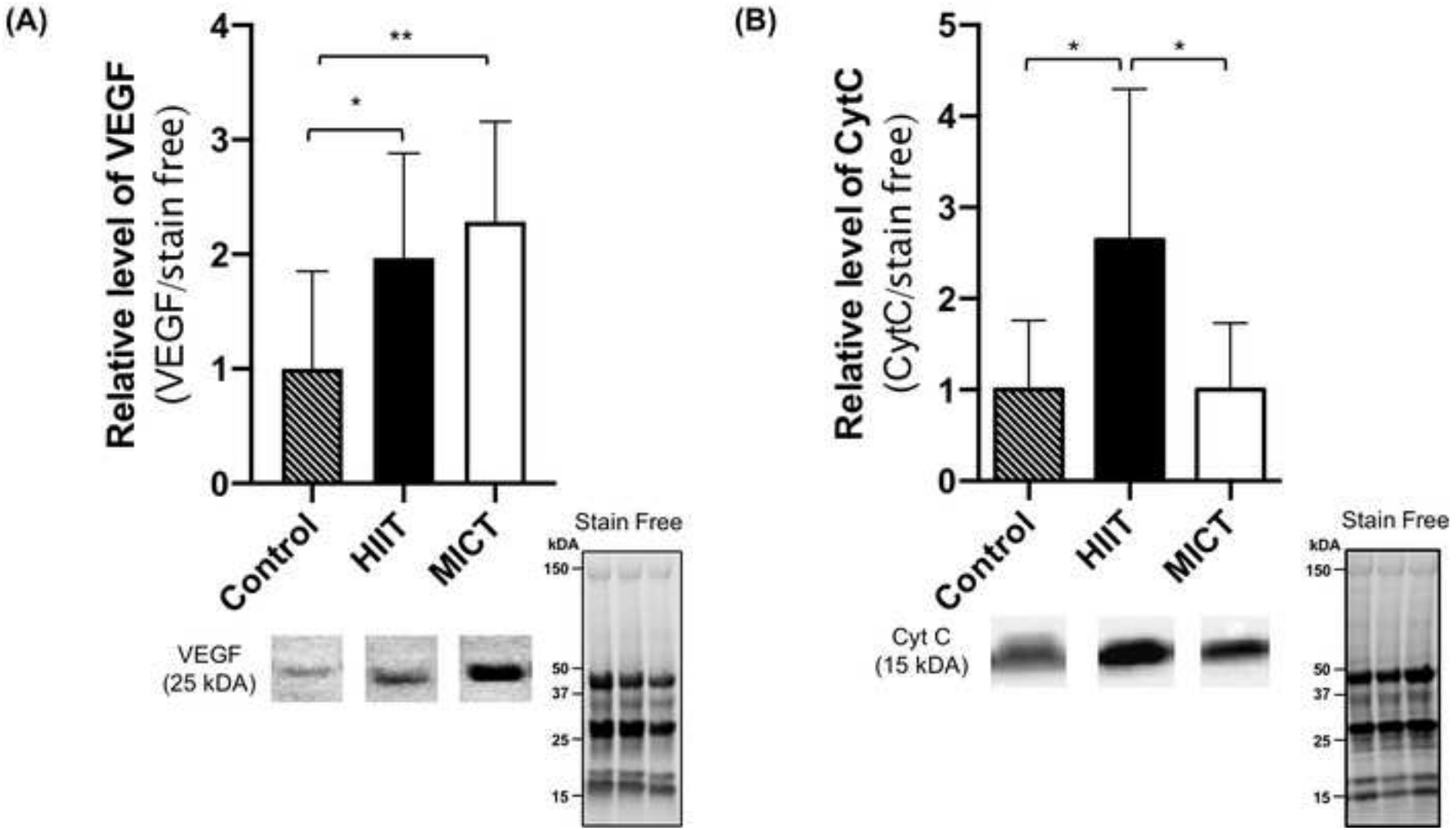


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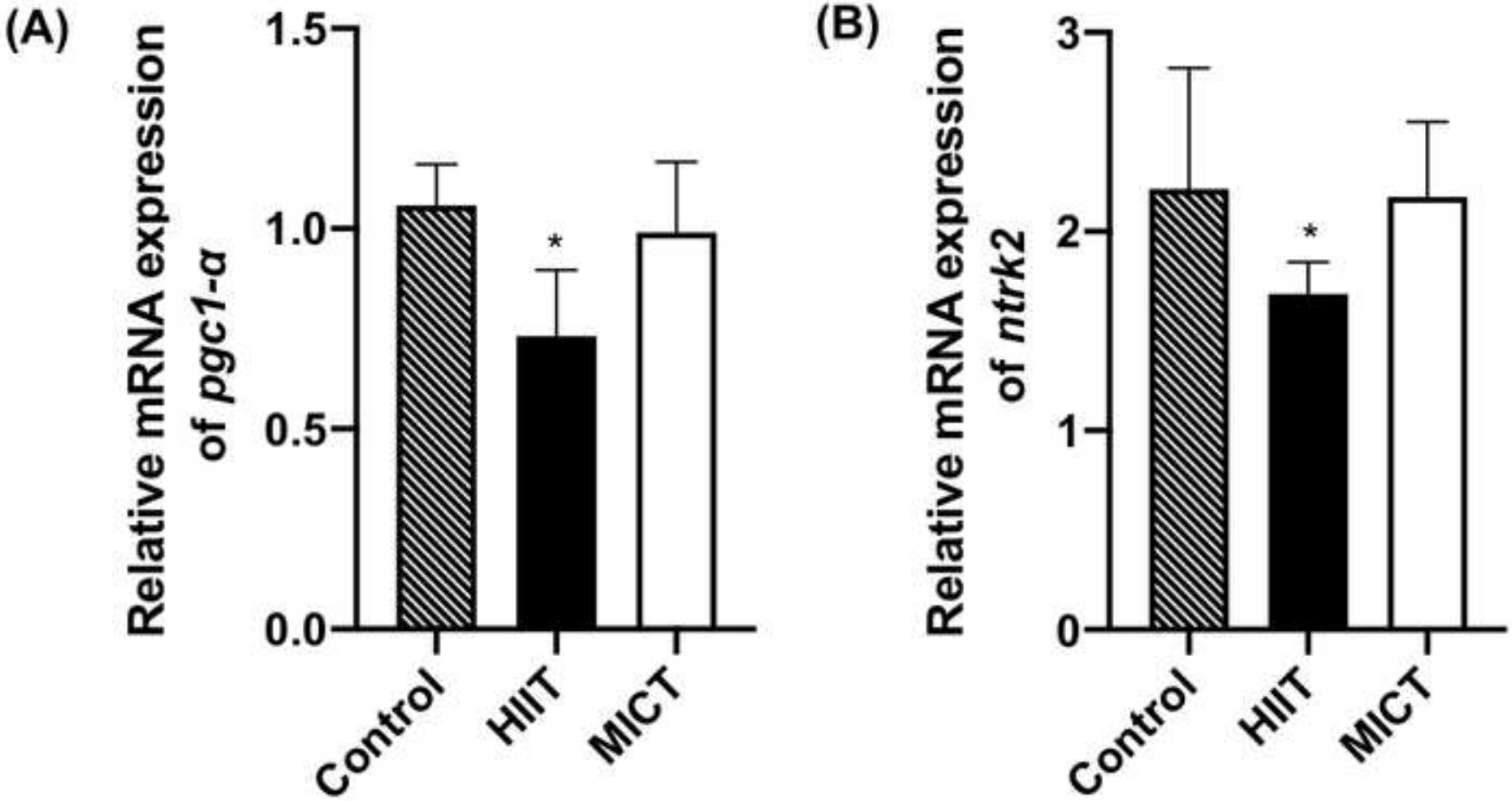


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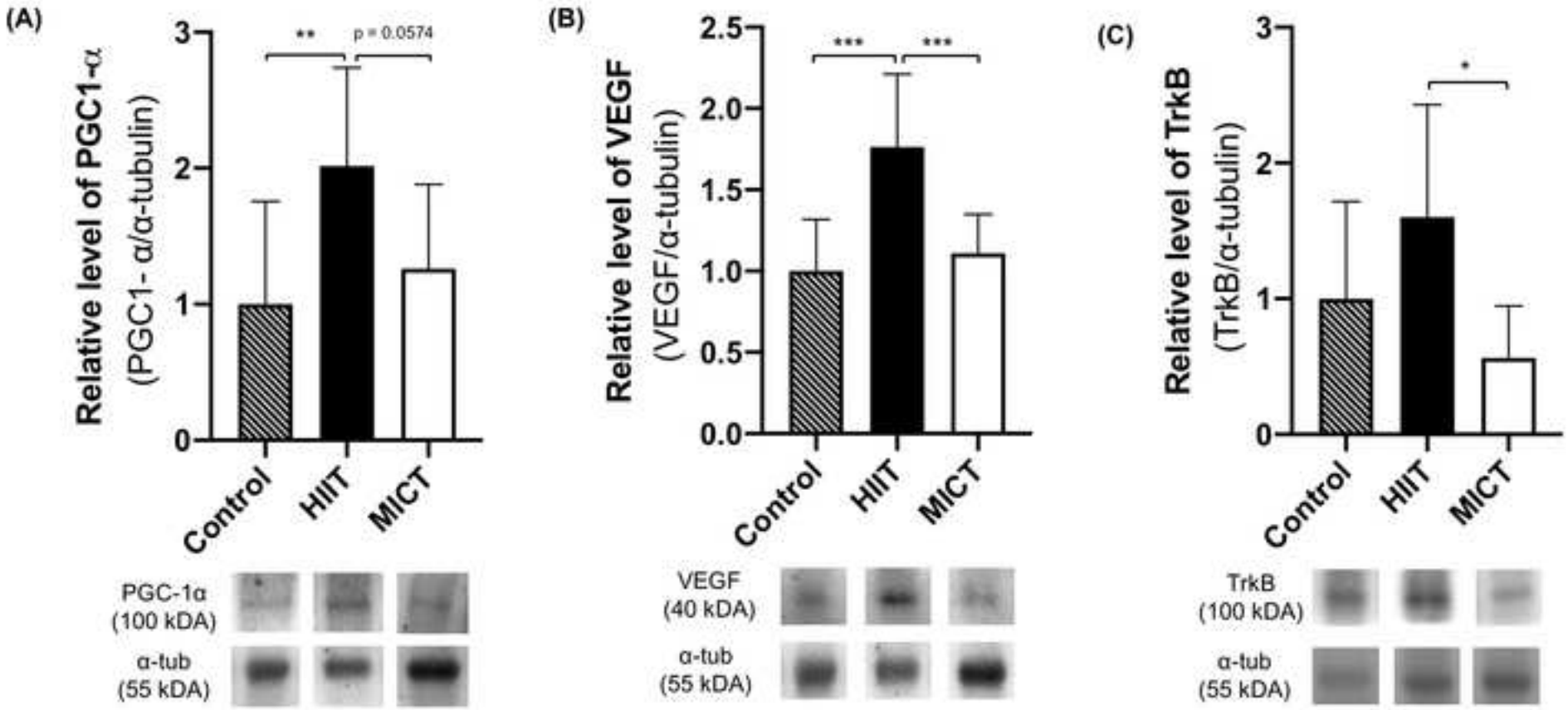


Figure 6

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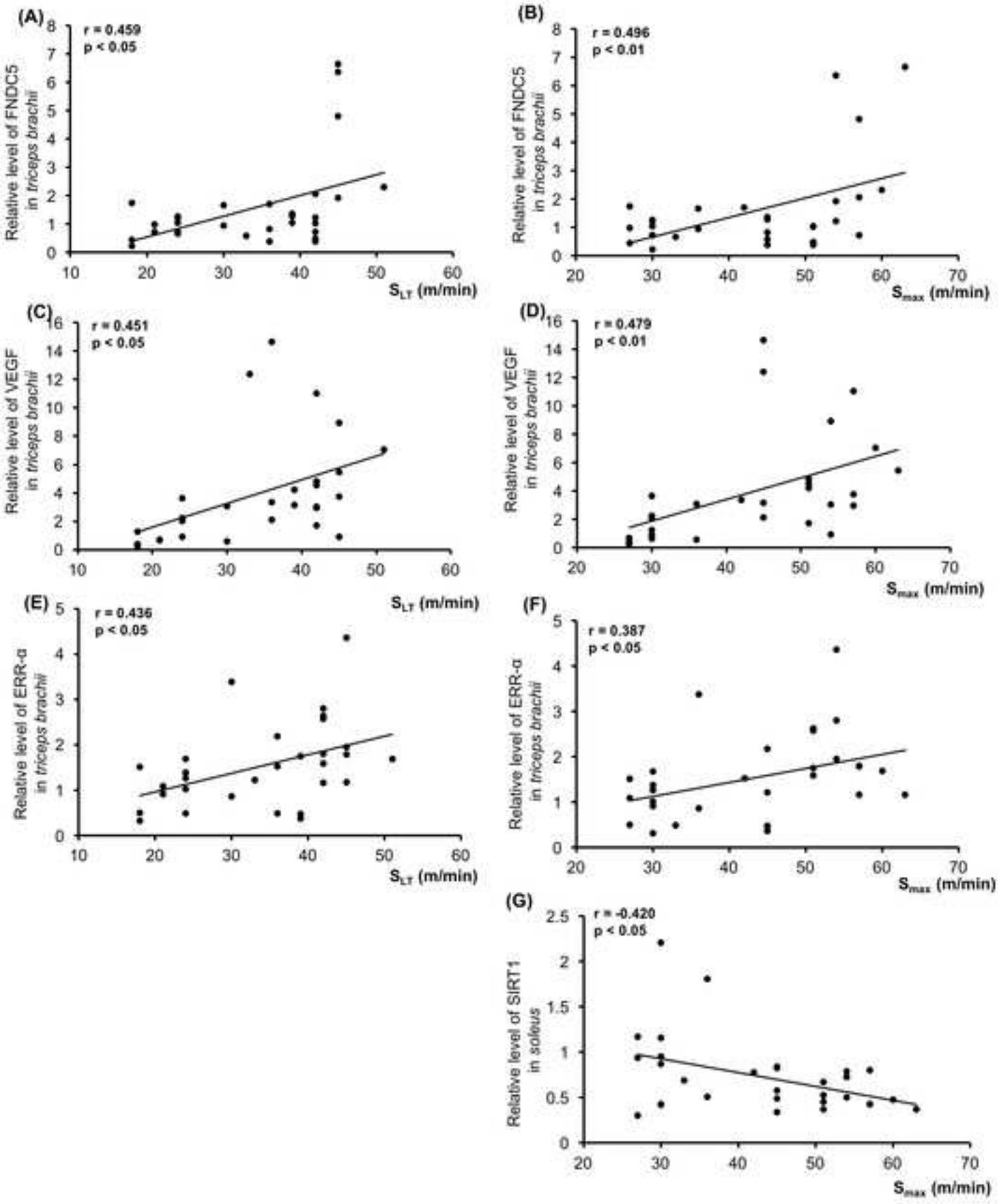


Figure 7

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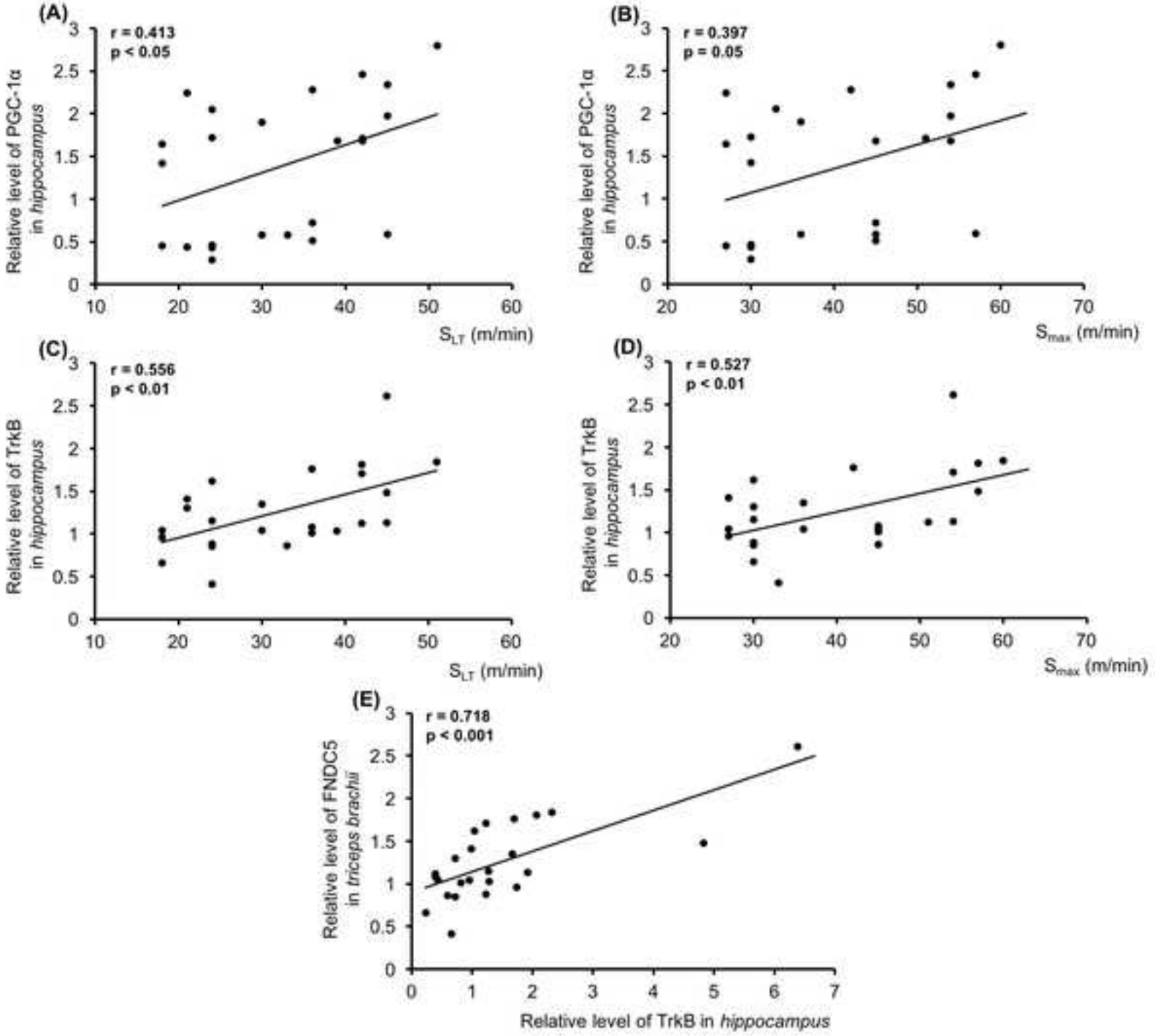


Figure 8

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