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Monocarboxylate transporters (MCTs) in skeletal muscle and hypothalamus of less or more physically active mice exposed to aerobic training

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ABSTRACT

Aims: The synthesis of monocarboxylate transporters (MCTs) can be stimulated by aerobic training, but few is known about this effect associated or not with non-voluntary daily activities. We examined the effect of eight weeks of aerobic training in MCTs on the skeletal muscle and hypothalamus of less or more physically active mice, which can be achieved by keeping them in two different housing models, a small cage (SC) and a large cage (LC).

Main methods: Forty male C57BL/6J mice were divided into four groups. In each housing condition, mice were divided into untrained (N) and trained (T). For 8 weeks, the trained animals ran on a treadmill with an intensity equivalent to 80 % of the individual critical velocity (CV), considered aerobic capacity, 40 min/day, 5 times/week. Protein expression of MCTs was determined with fluorescence Western Blot.

Key findings: T groups had higher hypothalamic MCT2 than N groups (ANOVA, $P = 0.032$). Significant correlations were detected between hypothalamic MCT2 and CV. There was a difference between the SC and LC groups in relation to MCT4 in the hypothalamus (LC > SC, $P = 0.044$). Trained mice housed in LC (but not SC-T) exhibited a reduction in MCT4 muscle ($P < 0.001$).

Significance: Our findings indicate that aerobically trained mice increased the expression of MCT2 protein in the hypothalamus, which has been related to the uptake of lactate in neurons. Changes in energy metabolism in physically active mice (kept in LC) may be related to upregulation of hypothalamic MCT4, probably participating in the regulation of satiety.

1. Introduction

It is well known that acid-base balance is regulated chiefly by carriers known as monocarboxylate transporters (MCT). The SLC16 family of MCTs is comprised of 14 members, and all MCT isoforms contain twelve transmembrane (TM) domains, intracellular C and N termini, as well as a large intracellular loop between TMs 6 and 7 [1]. MCTs shows an extensive tissue distribution including the liver, kidney, heart, intestines and the skeletal muscle [1]. MCTs can transport monocarboxylates through the cell membrane, including lactate coupled with a proton, as well as pyruvate and ketone bodies [2,3].

Release of lactate from contracting muscle is a major stimulant of MCTs [4]. For this reason, the skeletal muscle is one of the most studied tissues in the context of exercise and MCTs [5,6]. It has been demonstrated that muscle MCTs (and so lactate transport activity) can be changed when muscle activity is chronically increased by endurance training [7,8], by high-intensity training [9–11], or when muscle activity is blunted by denervation [12] or hindlimb suspension [13]. However, many questions remain such as whether MCTs are upregulated at all levels of muscle activity and whether activities of daily life (prolonged duration of muscle work) can stimulate MCTs.

Lactate released from the muscle is utilized as an energy source and a gluconeogenic precursor in diverse cells, tissues and organs. Lactate is also a signaling molecule functioning in an endocrine manner [14] likely

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acting as a hormone ("lactormone") [15,16]. Many authors have recognized the lactate as a contraction-induced myokine [17], acting as a peripheral signal to the central nervous system [18,19]. Since lactate can pass through the blood-brain barrier [20] and has been shown to have important energy and signaling functions in the brain, analysis of MCTs in brain tissue is important. MCTs have been found in cortex [21], hippocampus [22], cerebellum [23] and in hypothalamus [24,25], however the exact mechanisms regarding influx and efflux functions of MCTs in the brain are still not completely defined. Emerging evidence establishes that glial cells such as astrocytes and tanycytes express MCT4 ($K_m = 35$ mM) which releases lactate to the extracellular space [26,27]. While glial are lactate-producing cells, it is also known that neurons consume lactate [28]. Because of their high affinity ($K_m = 0.7$ mM), it has been believed that MCT2 present in neurons [29] is suited for lactate influx [27,30–32]. Lactate could provide an additional energy source to the neurons [28,33], which are known to be energy-demanding (highly oxidative due to higher density of mitochondria) [27]. The function of MCT2 in neurons (oxidative) and MCT4 in glial cells (glycolytic) in the brain is analogous to the role of MCT1 in oxidative muscle fibers and MCT4 in glycolytic muscle fibers, respectively [27,28].

Considering that lactate has a "widespread reach", it is very important to study how different situations of muscle activation (such as training or active lifestyle) would affect MCTs not only in muscle but also in regions in the brain. There are reports indicating that exercise (including acute and chronic interventions) triggers an upregulation in gene and protein expression of MCTs in skeletal muscle of swimming and running rodents [4,34–36], however studies of the effects of exercise programs on brain MCTs are lacking. Takimoto and Hamada [37] showed that a single bout of exercise in rats was sufficient to increase MCTs in different brain areas. A study by Aveseh, et al. [38], investigated the effects of 8 weeks of exercise training on MCT content via western blotting in the cortex, hippocampus and cerebellum of Wistar rats.

To the best of our knowledge no study evaluated MCTs of trained animals in the hypothalamic tissue, which is known to be of the most important regulator of the endocrine system and energy balance [39–42]. Hypothalamic MCTs are of great importance to be investigated since that lactate mediates the communication between glial cells and neurons [43–51]. It has been proposed that the release of lactate (mainly by the MCT4) from dorsal β1-tanycytes and the uptake of lactate by neurons of arcuate nucleus (through MCT2) are part of the same mechanism by which POMC neurons become excited [27,43]. This supports the view that alterations in hypothalamic MCTs can affect the regulation of satiety. In addition, it is also coherent assume that changes in hypothalamic MCT2 may play a role in lactate clearance [25,27,30,46]. Finally, as hypothalamus is the main controller of various physiological processes, it is likely to suppose that changes in hypothalamic MCTs may affect a variety of behavioral and autonomic functions such as thirst [52,53], SPA [54], thermoregulation [55] as well as sleep and wakefulness [56–58], however such issues remain to be determined.

In rodents, forced-exercise such as motorized treadmill running and/or swimming are the most common models in studies involving MCTs. Other models of physical activity have not yet been tested. No attention has been given to the role of non-voluntary activities of daily living (known as spontaneous physical activity-SPA). It must be stated here that activities within the scope of SPA (e.g. fidgeting, rearing, deambulation and posture maintenance) may be analogous to low-intensity aerobic exercise [59–61]. Increasing SPA can be regarded as a way of promoting a physically active lifestyle. We have previously established a dependable and robust model for discriminating more or less physically active mice. In our model, SPA of mice housed in large cages (LC) is higher than mice housed in small cages (SC), when considering the entire experimental period [62].

This study aimed to investigate the effects of aerobic training on MCT1 and 4 in skeletal muscle as well as MCT1, 2 and 4 in hypothalamic tissues in more or less active mice (based on a model of housing conditions). As enhanced lactate dynamics (achieved through MCTs) can generate a better acidosis control, and therefore an exercise tolerance, it would not be surprising to find interesting associations between MCTs and aerobic and anaerobic capacities, which can be evaluated by critical velocity protocol [63,64]. We hypothesize that aerobic-trained mice who were also housed in a LC will have greater changes in MCTs. Starting from this general hypothesis we formulated the following specific hypotheses. Taking into account that aerobic training is known to enhance lactate clearance [7,65,66], we hypothesize that aerobic training at 80 % of critical velocity (close to maximal lactate steady state intensity in which blood lactate reaches 4 mM), will induce changes in MCTs of greater affinity for lactate such as MCT1 and especially MCT2, which in turn is essential for neurons to metabolize lactate as energy substrate [27,67]. Taking into consideration that muscle contractions while engaging in SPA are not negligible in promoting energy expenditure [59,62,68–74], we think that an enhancement of SPA (housing-induced) may be partly responsible for changes in hypothalamic MCTs, which are involved in feeding regulation [43]. Our research fills a gap in the current literature because it examines hypothalamic MCTs not only in trained animals but also in animals that have undergone different levels of muscle activity.

2. Material and methods

2.1. Animal care

Forty male C57BL/6J mice were obtained from the central animal care facility, at the Multidisciplinary Center for Biological Investigation (CEMIB). They were kept in a room that was maintained on a 12/12 h light–dark cycle (light from 6 am to 6 pm) with a controlled environment (temperature: 22 ± 1 °C, relatively humidity: 45–55 % and noise: smaller than 80 dB). The study followed standards for the conduct of research on animals. All procedures and protocols were approved by an ethical review committee (Comissão de Ética no Uso de Animais- CEUA-UNICAMP, protocol number 4670–1/2017). All procedures were carried out in agreement with guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [75] and the National Research Council Guide for the Care and Use of Laboratory Animals [76].

2.2. Experimental design

Mice aged 150 days were divided into two housing types, small cage (SC) and large cage (LC), to generate animals with less active (SC) or more active (LC) behavior. Rodents were subdivided again into another two groups within each housing: non-trained (N) and trained (T), totaling four experimental groups (SC-N; SC-T; LC-N; LC-T, $n = 10$ per group). Interventions were conducted for 8 weeks, including the application of a critical velocity (CV) protocol at the middle of the experimental design. At the end of the study, all mice were euthanized to determine MCTs in muscle and hypothalamus.

2.3. Housing conditions

The mice of the SC groups were kept in polyethylene cages with dimensions of 40 × 33 × 16 cm (width × length × height, respectively). For the LC groups, animals were housed in a cage with dimensions of 80 × 60 × 33.3 cm. The floor area of the SC and LC groups was 1320 cm^2 and 4800 cm^2, respectively. The floor area for each rodent was 132 and 480 cm^2 for SC and LC, respectively, considering that mice were housed ten per cage. The allocation of mice in different housings conditions was done randomly by raffle to prevent biases.

2.4. Aerobic training

Rodents were progressively allowed to run on a treadmill to let them adapt to the running task. In this adaptation mice ran at intensities of between 8 and 17 m.min^{-1} for a period ranging from 10 to 15 min. Mice performed a warm-up exercise at an intensity of 8 m.min^{-1} for 5 min. The main part of session includes running efforts of 40 min at an intensity equivalent to 80 % of critical velocity (CV), at which intensity it is within the heavy intensity domain [63]. At the end of training session there was a cool-down period (5-min walking at an intensity of 8 m.min^{-1}). Thus, the total time of the exercise session was 50 min (counting warm up and cool down). The training program was performed five times per week. The sessions were conducted at the same time of day (between 6:00 and 10:00 am). Non-trained mice were also manipulated with the intention of mimicking stress to rule out confounding effects on training.

2.5. Critical velocity protocol

Aerobic and anaerobic capacities (CV and ARC, respectively) were determined by the CV protocol previously applied to rodent models following model requirements [62,77]. Here, this protocol was composed of four randomized and individualized continuous runs on a calibrated treadmill (at intensities varying from 17 to 29 m.min^{-1}), with a 24 h of interval between them. Before the start and at the end (after 8 weeks) of the interventions, CV and ARC were accessed. CV protocol was reapplied at the middle of study for training intensity adjustment. The time to exhaustion (tlim) was recorded for different intensities. Exhaustion was considered when the mice were unable to run consistently on the treadmill. The experiments were conducted under the following conditions: (a) tests were applied without electrical stimulus; (b) exercise intensities were individually selected so that the tlim was not >15 min and not <1 min, following protocol recommendations [77]; (c) each test started with a warm-up and ended with a cool-down walk. Finally, the CV and ARC were calculated from the slope (angular coefficient) and y-intercept (linear coefficient), respectively, of the linear regression fit (distance vs. time model), according to the eq. Distance = CV * tlim + ARC.

2.6. Measurement of SPA and food intake

SPA was measured by the gravimetric principle using load cells in accordance with previously established procedures [62,74,78,79]. We measured SPA on a per-cage basis without removing animals from their home cage. All major and sensitive movements such as ambulation, grooming, rearing and fidgeting (also understood as non-voluntary activities of daily living). SPA was recorded throughout the entire study (over the course of 8 weeks) at an acquisition frequency of 200 Hz for 19 continuous hours per day (from 11 am to 6 am). Food intake (over the entire 8-wk period) by weighing the amount of chow put into the feeders and weighing the remaining chow pellets 24 h later. Food intake was normalized by the body mass of mice of a cage. The mice received commercial standard chow (Nuvilab® CR-1; Nuvital, BR). Mice were fed ad libitum.

2.7. Collection of biological material

The animals were fasted overnight and euthanized by cervical dislocation without anesthesia (to preclude effects of anesthetic on neurochemistry). A well-trained executor performed this technique. To study the chronic effects, euthanasia was performed 48 h after the last exercise bout. The harvest of tissues (hypothalamus and soleus) was performed and tissues were rapidly frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.8. Western blot analyses

Tissue was crushed on ice with plastic pestles, and tissues were homogenized in RIPA buffer containing Tris HCl (50 mM), NaCl (150 mM), EDTA (1 mM), IGEPAL® CA-630 (1 %), Deoxycholate (0.5 %), SDS (0.1 %) and 1 % of protease inhibitors (Protease Inhibitor Cocktail, cat# P8340, Sigma-Aldrich®) and 1 % of phosphatase inhibitors (Phosphatase Inhibitor Cocktail Set II, cat# US1524625-1SET, Calbiochem®). For better homogenization, the samples were sonicated for 3 s using a sonicator (Q55, Qsonica®, USA). Total protein concentration was measured using the Bradford method with bovine serum albumin as the standard (Bio-Rad Protein Assay Dye Reagent Concentrate, cat# 5000006, Bio-Rad Laboratories). Samples (40 μg of total protein) were mixed with loading buffer containing lithium dodecyl sulfate (4× Bolt™ LDS Sample Buffer, cat# B0007, Invitrogen) and Mercaptoethanol-1 % (cat# M6250, Sigma-Aldrich), and samples were denatured at 94 °C for 10 min and briefly cooled on ice. Proteins were separated by SDS-PAGE on a 10 % gel (Mini-PROTEAN® TGX™ Precast Gel, cat#4561036, cat#4561034, Bio-Rad Laboratories) and then transferred to PVDF membrane (Invitrogen™, iBlot™ 2 Transfer Stacks, cat# IB24002) using the iBlot™ 2 Gel Transfer Device (20 V for 7 min, cat#IB21001). Membranes were stained with a total protein stain (REVERT™ Total Protein Stain, cat# 926–11010, LI-COR) for quantification of protein loading in each lane. Membranes were blocked with 1 % NFDM (Nonfat dry milk, cat# 9999, Cell Signaling Technology®) in PBS solution for 1 h at room temperature. The incubation with primary antibodies was performed for 1 h at room temperature (for MCT4 cat#BS-2698R, Bioss and MCT2 cat#BS-3995R, Bioss) or overnight at 4 °C (for MCT1 BS-10249R, Bioss) with 5 % NFDM in PBS-0.1 % Tween 20. The membranes were incubated with the secondary antibody (Goat anti-Rabbit IgG H&L IRDye 800CW preadsorbed, cat# ab216773) at a 1:20,000 dilution in 5 % NFDM in in PBS-0.1 % Tween 20 for 1 h at room temperature. Fluorescence signal was detected at 800 nm with the Odyssey Fc System (LI-COR Biosciences, Lincoln, NE, USA).

2.9. Statistical analysis

Results were displayed as box plots using GraphPad Prism software. Values are medians with edges representing 25th–75th percentiles and whiskers representing minimum and maximum values. Anaerobic running capacity (ARC) data were presented as mean and standard error of the mean (SEM). Normality was tested using Shapiro–Wilk. Aerobic and anaerobic capacity results were analyzed by a two-way ANOVA, comparing the effects of training (non-trained vs. trained) and housing space (small vs. large cage) within same time period (pre-intervention, 4th and 8th week). Moreover, ANOVA for repeated measures was carried out to determine intragroup differences over time of aerobic and anaerobic capacity data. Protein expressions (MCTs) at the end of the experiment were analyzed using a two-way ANOVA to compare the training and housing space effects and its interactions. Fischer post hoc test was used to indicate differences among groups. For exploratory reasons, we investigated the relationship between MCTs and the critical velocity outcomes at the eighth week. We used Pearson product-moment correlations. In all cases, the significance level was set at $P \leq 0.05$.

3. Results

Western blot and ANOVA results of MCTs in muscle are also shown in Fig. 1. The most interesting finding was that trained mice housed in LC exhibited the lowest MCT4 protein levels in the muscle. With regard MCT1 in muscle, there was no significant effect for training and housing.

ANOVA results of hypothalamic MCTs protein contents can be found in Fig. 2. Regarding MCT1, there was no significant effect of training and housing. Training increased hypothalamic MCT4 only in mice housed in LC, but not in mice housed in SC. Trained mice exhibited higher MCT2 than non-trained groups, as detected by the effect of training.

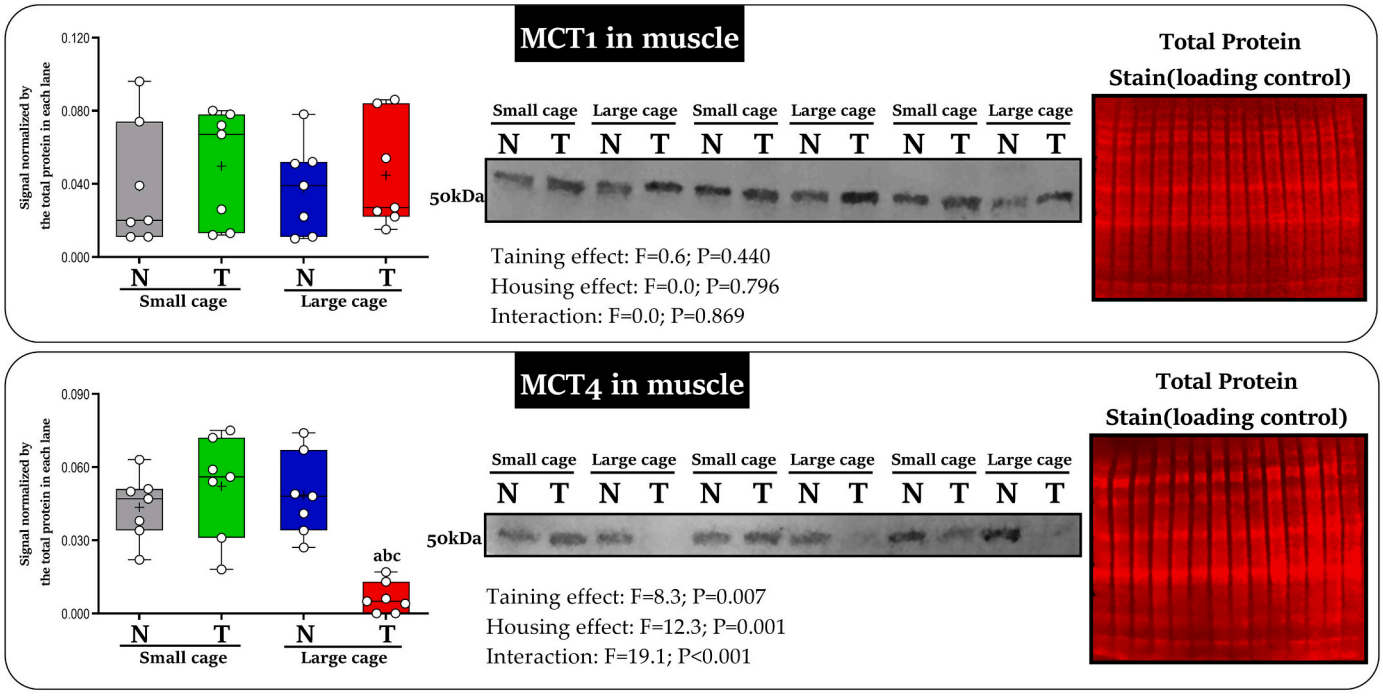

Fig. 1. Protein content of MCT1 and 4 in the soleus muscle for non-trained (N) and trained (T) groups kept in small cage (SC) and large cage (LC). A representative blot image with protein bands is shown. All membranes were stained for total protein to normalize for inter-lane differences in protein loading. Results are presented in Box plots showing individual data points and median value (line in the box) with box edges representing 25th–75th percentiles and whiskers showing range of values (min to max) ($n = 7$ per group). Statistical analysis: a, b, c indicate significant differences ($P \leq 0.05$) in relation to SC–N, SC–T and LC–N, respectively.

Fig. 3 shows the CV results for all groups at three different time points (pre-intervention, 4th and 8th week). Linear regression between distance and time to exhaustion resulted in R^2 around 0.99 (without differences among groups). All groups started with the same physical fitness condition before the start of study (pre-intervention) since no significant differences were found between groups within the same experimental time (pre-intervention) according to post hoc comparisons. There was no found effect of housing on critical velocity at the fourth week ($F = 2.5$; $P = 0.115$), but an effect of training was observed ($F = 7.6$; $P = 0.008$), demonstrating that trained groups (SC-T and LC-T) exhibited higher CV than non-trained groups (SC-N and LC-N). We found no significant interaction between effects of training and housing space on critical velocity at 4th week ($F = 0.0$; $P = 0.802$). At the end of experiment (8th week), trained groups had significantly higher aerobic capacity than non-trained groups, as detected by the significant effect of training on critical velocity ($F = 35.9$; $P < 0.001$). No effect of housing was observed ($F = 2.4$; $P = 0.124$). We found no significant interaction between effects of training and housing space on critical velocity at 8th week ($F = 3.4$; $P = 0.072$).

All mice tolerated the exercise intensity and completed training without adverse events. The training volume that should be carried out (2400 s, 40 min per session) was fulfilled. Fig. 4 exhibits the daily records of SPA and food intake over the entire 8-wk period. LC-mice exhibited higher SPA than SC-mice ($F = 130.7$; $P < 0.001$), demonstrating the consistency of our housing model. Trained mice exhibited lower SPA than non-trained groups, as detected by the effect of training ($F = 51.3$; $P < 0.001$). It was found a significant interaction between training and housing on SPA ($F = 46.2$; $P < 0.001$). No significant effect of training ($F = 0.8$; $P = 0.364$) and interaction ($F = 3.7$; $P = 0.054$) on food intake was detected by two-way ANOVA. An effect of housing was observed ($F = 325.1$; $P < 0.001$), demonstrating that LC-mice exhibited higher food intake than SC-mice.

Table 1 exhibits the results of anaerobic running capacity (ARC). No significant differences were found between groups within the same experimental time (pre-intervention). No significant effect of training ($F = 0.4$; $P = 0.526$) and housing space ($F = 0.0$; $P = 0.762$) on ARC was detected by two-way ANOVA. We found no significant interaction between effects of training and housing space on ARC at pre-intervention ($F = 0.0$; $P = 0.962$).

At the fourth week, we found a significant effect of aerobic training on ARC ($F = 4.9$; $P = 0.032$; T > N), however, there was no effect of housing ($F = 2.8$; $P = 0.097$). There is no significant interaction between effects on ARC at fourth week ($F = 1.0$; $P = 0.176$). With respect to ARC at the end of the 8th week, there was no effect of training ($F = 0.1$; $P = 0.747$) and housing space ($F = 2.2$; $P = 0.140$) or significant interaction ($F = 2.3$; $P = 0.137$).

Regarding associations between critical velocity and MCTs, correlations coefficients including *P*-values and r are shown in Table 2. For SC-T group, a significant negative association between the critical velocity and the levels of hypothalamic MCT1 was found (Fig. 5A). For LC-T group, we found a significant inverse correlation between the critical velocity and the levels of hypothalamic MCT4 (Fig. 5B). When considering the pooled data from all groups, there was a significant correlation between the critical velocity and the MCT2 in hypothalamus, demonstrating that high-aerobic capacity mice tend to have higher protein expression of MCT2 (Fig. 5C).

Associations between anaerobic running capacity (ARC) and MCTs, correlations coefficients including P-values and r are shown in Table 3. No correlation was found between the ARC and the levels of hypothalamic MCT2 (for SC-T group and pooled data), but it was closed to statistical significance.

4. Discussion

We found that aerobic training increases the aerobic capacity and MCT2 in hypothalamus of mice, regardless of housing. Mice housed in LC exhibited higher MCT4 in hypothalamus than SC groups, as detected by the effect of housing. Trained mice housed in LC (but not trained mice housed in SC) exhibited increased MCT4 in hypothalamus and reduced MCT4 in muscle. These findings suggest that aerobic training

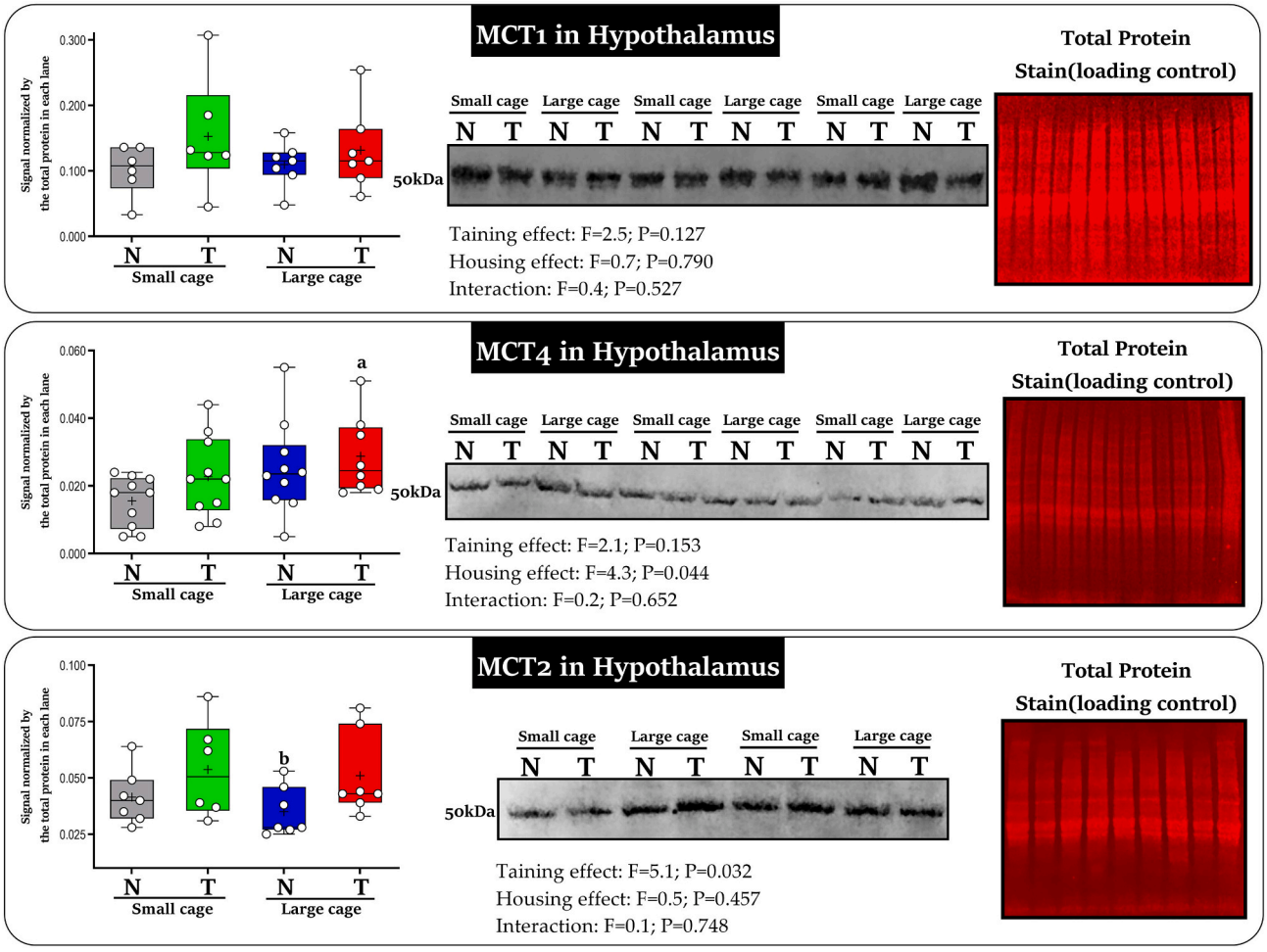

Fig. 2. Protein content of MCT1, 2 and 4 in the hypothalamus for non-trained (N) and trained (T) groups kept in small cage (SC) and large cage (LC). A representative blot image with protein bands is shown. All membranes were stained for total protein to normalize for inter-lane differences in protein loading. Box plots depict median value (line in the box) with box edges representing 25th–75th percentiles and whiskers showing range of values (min to max) (n = 6–10 per group). Statistical analysis: a, b indicate significant differences ($P \leq 0.05$) in relation to SC–N and SC–T, respectively.

(performed at 80 % of critical velocity - within the heavy intensity domain) associated with a more physically active lifestyle exerts hitherto unknown changes in MCTs. Taken together, our findings clearly demonstrate the complexity and responsiveness of the hypothalamus in the context of physical activity demands. Future experiments will be aimed at understanding how muscle activity modulates pathways involving MCTs and also lactate dynamics in different brain areas. As lactate seems to have a dual role as an intercellular messenger and metabolic fuel for neurons [80], it is clear that further analyses are essential to determine the crosstalk between muscle (by means of contraction-induced myokines) and hypothalamus.

The first aspect to be discussed refers to the role of lactate as a signaling molecule (as an intercellular messenger) which helps in the regulation of feeding and energy balance. To contextualize this, it is necessary to provide a brief discussion about tanycytes, which are glia cells that cover the walls of the 3rd ventricle of hypothalamus. The hypothalamic tanycytes have privileged access to hormones (i.e. insulin) and plasma metabolites (i.e. glucose and lactate) since are in contact with blood or cerebrospinal fluid [30]. Tanycytes respond to increases in extracellular glucose levels, which induces a glycolysis-induced lactate production [43,81]. MCT4 exports lactate result from high glycolytic activity in the dorsal β1-tanycytes [82]. The lactate released by tanycytes may be captured through MCT2 in an anorexigenic zone of arcuate nucleus. There is a growing body evidence suggesting that, in hypothalamus, lactate is a signaling molecule that can induce satiety by stimulating POMC neurons [27,30,47,83]. Lactate is able to depolarize POMC neurons through the closure of K_{ATP} channels [83]. This is in consistency with the results of Lam, et al. [84] who showed central administration (i.c.v.) of lactate lowers food intake. In line with this, McCarthy, et al. [85] pointed out that lactate acts as a signal for appetite suppression.

An interesting finding was that LC-groups which exhibited an up-regulation of MCT4 in hypothalamus also had the highest levels of food intake when considering the entire experimental time. There are evidences to believe that increased content of MCT4 in hypothalamus may favor anorexigenic signaling, in order to limit appetite [43,83]. Although we do not have any clear answer, we speculate that in our experimental model, the POMC/CART pathway is more activated in LC-mice, most likely in response to high postprandial glucose (due to high food intake of LC-mice) [30]. Supporting this it has been demonstrated that brain MCTs are positively regulated in hyperglycemic rats [86–88]. With regard the relationships, we found a significant inverse relationship between critical velocity and hypothalamic MCTs. Mice who exhibited higher levels of CV also had the lowest amount of MCT4 (only for the LC–T group) and MCT1 (only for the SC–T group). From a speculative point of view, it may be hypothesized that satiety signaling is switched to inhibition for trained mice who high CV. Improved tissue glucose uptake in high-aerobic capacity mice [89,90] could be responsible for a down-regulation of hypothalamic MCT1 and 4 (since hypothalamus would be receiving less glucose from bloodstream). At the

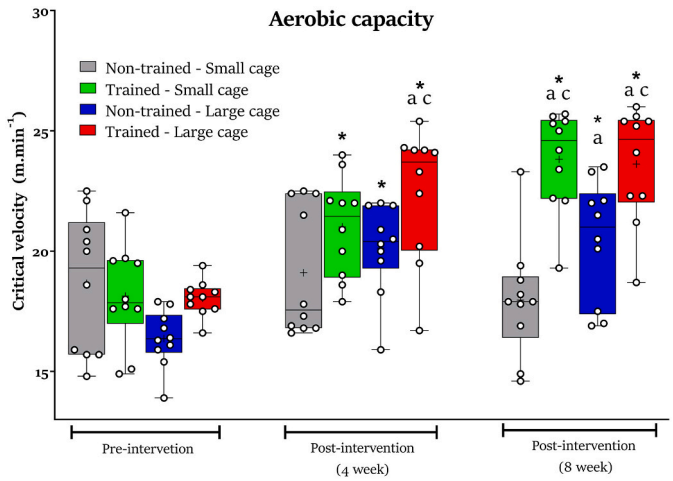

Fig. 3. Critical velocity (m.min^{-1}) over experiment for non-trained (N) and trained (T) groups kept in small cage (SC) and large cage (LC). Box plots depict median value (line in the box) with box edges representing 25th–75th percentiles and whiskers showing range of values (min to max) ($n = 10$ per group). Symbols of statistical significance (within same week $P \leq 0.05$): a, c indicate significant differences in relation to SC–N and LC–N, respectively. * indicate significant differences from initial condition (pre-intervention) with regard to intragroup differences over time tests by ANOVA for repeated measures.

moment we have no data to elucidate these relationships. Future in-depth studies will need to be conducted in order to understand mechanisms involving aerobic capacity, glycidic metabolism, MCTs and feeding behavior in an integrated perspective. In fact, more questions than certainties remained for anyone studying the hypothalamic MCTs, which can have divergent roles on feeding. For example, MCT4 knockdown in rats decreased their food intake [91], which is opposite to that reported in MCT1 knockdown rats [92]. These findings are intriguing and emphasizes that feeding behavior is a complex process, which cannot be explained solely by the hypothalamic MCTs, but further depends on the coordinated action of anorectic (POMC/CART) and orexigenic neuropeptides (NPY/AgRP) [93].

In addition to a paracrine role of lactate (from tanycytes to neurons), there is a possibility that lactate coming from the blood stream (from contracting muscles) can act directly on neurons. With respect to endocrine properties of lactate, accumulating evidence supports the notion that lactate can mediate brain adaptations in several respects

Table 2
Pearson's correlations between MCTs and the critical velocity at the eighth week for each isolated group and considering the entire data. Asterisks indicate significant correlations by Pearson test. HYP: hypothalamus and MUS: Skeletal muscle.

	Critical velocity				
	Non-trained (small cage)	Trained (small cage)	Non-trained (large cage)	Trained-(large cage)	Pooled data from all mice
MCT1 HYP	$r = 0.18$, $P = 0.725$	$r = -0.83$, $P = 0.040$*	$r = -0.09$, $P = 0.838$	$r = -0.62$, $P = 0.133$	$r = 0.02$, $P = 0.911$
MCT4 HYP	$r = 0.22$, $P = 0.539$	$r = 0.24$, $P = 0.499$	r = 0.22, $P = 0.537$	$r = -0.70$, $P = 0.049$*	r = 0.22, $P = 0.174$
MCT2 HYP	$r = 0.36$, $P = 0.425$	$r = -0.25$, $P = 0.679$	$r = 0.19$, $P = 0.675$	$r = 0.55$, $P = 0.198$	$r = 0.40$, P = 0.040*
MCT1 MUS	$r = 0.41$, $P = 0.356$	$r = 0.16$, $P = 0.730$	$r = 0.32$, $P = 0.481$	$r = 0.14$, $P = 0.752$	$r = 0.28$, P = 0.137
MCT4 MUS	$r = 0.10$, $P = 0.817$	r = 0.18, $P = 0.699$	$r = 0.48$, $P = 0.270$	$r = 0.56$, $P = 0.185$	$r = -0.03$, $P = 0.864$

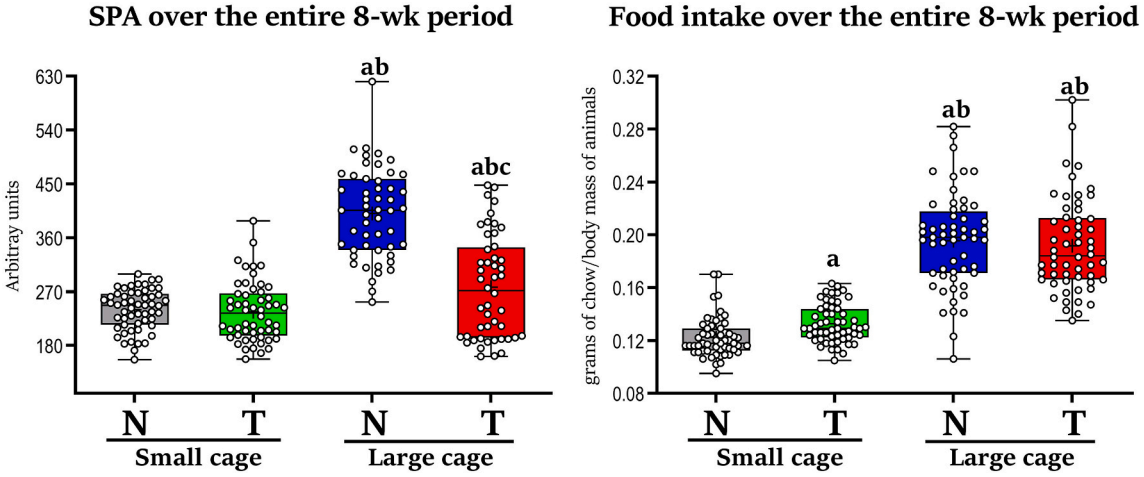

Fig. 4. Daily records of SPA and food intake for non-trained (N) and trained (T) groups kept in small cage (SC) and large cage (LC) over the course of study. This analysis considers all data obtained over the entire 8-wk period ($N = 52$ experimental days). Data are exhibited as box plots, considering median value (line in the box) with box edges representing 25th–75th percentiles and whiskers showing range of values (min to max). Statistical analysis: a, b, c indicate significant differences ($P \leq 0.05$) in relation to SC–N, SC–T and LC–N, respectively.

Table 1
Anaerobic running capacity (ARC) for non-trained (N) and trained (T) groups kept in small cage (SC) and large cage (LC) at three experimental times.

	Pre-intervention				4th week				8th week			
	Small cage		Large cage		Small cage		Large cage		Small cage		Large cage	
	N	T	N	T	N	T	N	T	N	T	N	T
ARC (m)	7.8 ± 0.7	7.2 ± 1.2	7.5 ± 1.1	6.8 ± 1.2	6.9 ± 1.0^{b}	18.1 ± 6.0*	5.9 ± 1.3^{b}	8.5 ± 2.2^{b}	8.4 ± 0.9	10.3 ± 2.6	8.4 ± 1.6	5.4 ± 0.6^{b}

Data are in the mean ± SEM (n = 10 per group). Two-way ANOVA was used within same experimental time. Statistical analysis: b indicates significant differences ($P \leq 0.05$) in relation to SC–T. * indicates significant differences in relation to baseline status (pre-intervention) according to ANOVA for repeated measures.

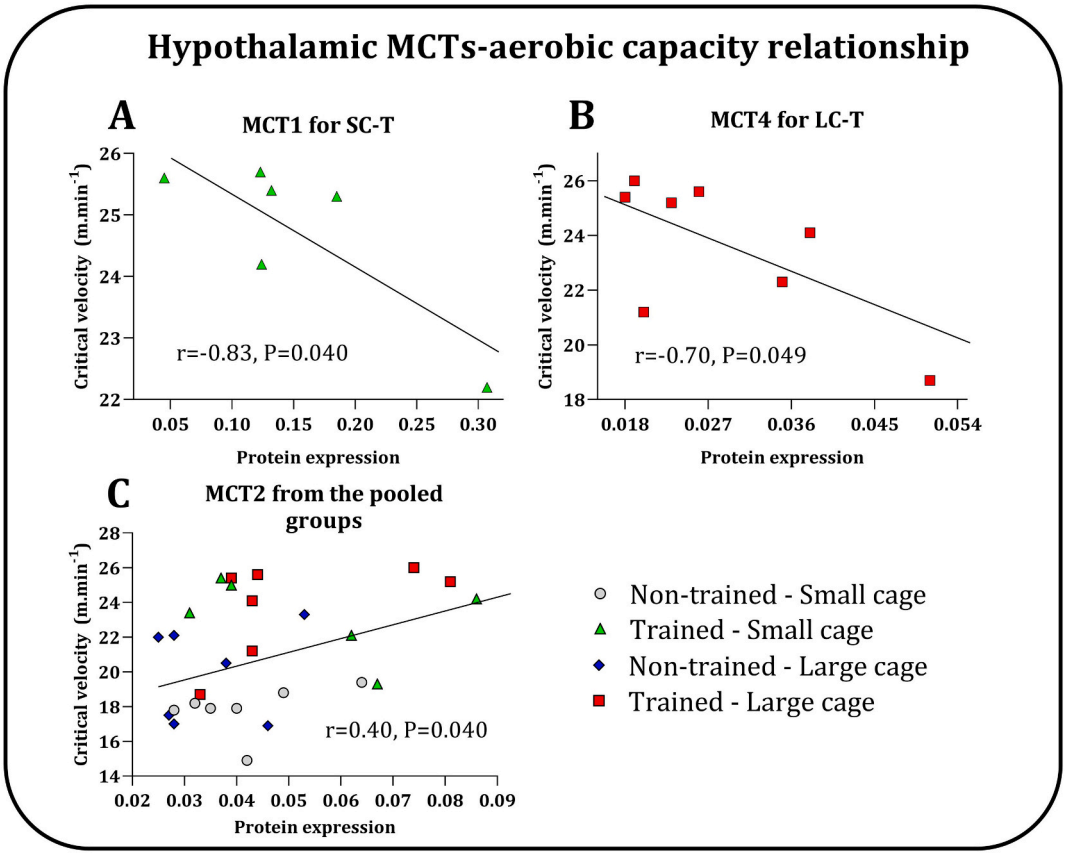

Fig. 5. Relationships between critical velocity (aerobic capacity) with hypothalamic MCT1 (Panel A) and with hypothalamic MCT4 (Panel B) for trained (T) groups kept in small cage (SC) and large cage (LC), respectively. The panel C shows the hypothalamic MCT2-aerobic capacity relationship from the pooled groups.

Table 3
Pearson's correlations between MCTs and the anaerobic running capacity (ARC) at the eighth week for each isolated group and considering the entire data. Asterisks indicate significant correlations by Pearson test. HYP: hypothalamus and MUS: Skeletal muscle.

	Anaerobic running capacity				
	Non-trained (small cage)	Trained (small cage)	Non-trained (large cage)	Trained-(large cage)	Pooled data from all mice
MCT1 HYP	r = −0.25, P = 0.624	r = 0.13, P = 0.801	r = −0.62, P = 0.131	r = −0.45, P = 0.306	r = −0.06, P = 0.767
MCT4 HYP	r = −0.11, P = 0.755	r = 0.42, P = 0.266	r = −0.00, P = 0.987	r = 0.01, P = 0.970	r = 0.08, P = 0.608
MCT2 HYP	r = 0.08, P = 0.857	r = 0.84, P = 0.073	r = −0.15, P = 0.748	r = 0.22, P = 0.625	r = 0.36, P = 0.068
MCT1 MUS	r = −0.65, P = 0.112	r = 0.03, P = 0.937	r = −0.53, P = 0.219	r = 0.73, P = 0.058	r = −0.09, P = 0.626
MCT4 MUS	r = 0.07, P = 0.872	r = −0.16, P = 0.721	r = −0.63, P = 0.123	r = 0.29, P = 0.518	r = 0.11, P = 0.560

including neurogenesis, cognitive function, neuroplasticity and cerebrovascular plasticity [17,94,95]. Higher blood lactate concentrations results in elevated BDNF plasma concentrations [96]. Further, it has been found that prolonged exposure to L-lactate can promote neurogenesis [97]. Although we have no measurement of lactatemia (from mice in their respective home-cages), activities within the scope of SPA are expected to produce not >2 mM. In terms of comparison, SPA would be analogous to a low-intensity aerobic exercise (something below the first lactate threshold) [98]. Such activities could, in theory, stimulate hypothalamic MCT2 (K_m = 0.7 mM) which are suited for lactate uptake at low substrate concentrations [80]. Our findings reinforces the idea that even low-to-moderate lactate producing activities is able to sensitize hypothalamic MCTs. Even if release of large amounts of lactate into the bloodstream does not happen in non-voluntary activities of daily living (housing in LC), it does not mean that these activities are without consequence (for hypothalamic MCT stimulation).

It is important to discuss the efficiency of our aerobic training in promoting adaptations in aerobic capacity (critical velocity - CV) in mice housed either in SC or LC. Increase of aerobic capacity in response to aerobic training may be attributed to the time of exposure to the exercise stimulus. The main part of the aerobic training session lasts 40 min, and this portion of time may be considered adequate if compared to a common endurance training. Further, mice performed running at an intensity equivalent to 80 % of CV, and this intensity was purposely chosen because it is within the heavy intensity domain [63]. Taking into account that CV overestimate by ~10 % the maximal lactate steady state [77,99], we recognized that our training program is based on aerobic exercise with intensities close to maximal lactate steady state. Training at 80 % of CV, it is expected to observe stable blood lactate concentrations around 4 mM (not <2 mM and not >5 mM) [63,98]. With this training profile, it would be possible to stimulate MCTs with the highest affinity for lactate, as is the case of MCT2 (the lowest Michaelis constant K_m = 0.7 mM) [29,43]. Our findings reinforces the idea that training performed at heavy intensity domain is able to sensitize hypothalamic MCT2 (but this effect was not observed for MCT1), supporting the idea that hypothalamic MCTs may respond differently to exercise. Taking into account that MCT1 K_m (7 mM) (in astroglial-rich primary cultures) had a 2-fold lower affinity for lactate as compared with MCT1 K_m (3.5 mM), which, in turn, is conventionally adopted for several tissues [2,29,43,100,101], we predicted that a more intense training (>7 mM of blood lactate) would be necessary to stimulate hypothalamic MCT1. This may help to explain why MCT1 in hypothalamus has not changed

after an aerobic training.

Previous investigations have shown that lactate accumulation in blood and skeletal muscle is reduced in endurance-trained rats [7,66,102]. This enhanced efficiency for lactate clearance could potentially be achieved by: (a) improved buffering in active and less active skeletal muscles because this tissue is also a consumer of lactate [5,6,103]; (b) greater conversion of lactate to glucose - gluconeogenesis [65]; and (c) enhanced clearance in other organs such as the heart, liver and kidney [16]. In all these above possibilities, the transport function of MCTs should inevitably be necessary. Although their role is less well understood, brain MCT2 appears to be essential for lactate clearance. It has been believed that lactate is used during increased neuronal activity, providing an energy substrate for mitochondrial oxidation [104–107]. In the present study, trained groups have a significantly higher MCT2 levels in hypothalamus compared to non-trained groups. We believed that an enhanced MCT2 in hypothalamus could further contribute to the clearance of lactate, which would explain the increase in aerobic capacity, as reinforced by a significant correlation between MCT2 and critical velocity. The results in the present study are not sufficient to demonstrate a causal effect of MCT2 and lactate clearance.

We believe that not only the training strategies but also the non-voluntary activities of daily life should be more widely appreciated by exercise physiologists. Our results show that mice kept in LC (in the absence of exercise training) exhibited higher aerobic capacity than groups mice kept in SC. Our results showing increase of aerobic capacity indicate the great potential of active lifestyle (by a large cage) in improving health and performance. As a large cage can generate a prolonged duration of muscle work, it would not be surprising to find an upregulation of MCTs in mice housed in LC, however, our MCTs data do not support this view in full. It must be remembered that only MCT4 in hypothalamus was increased in mice kept in LC than mice kept in SC.

The decreased of MCT4 in muscle of LC-T mice deserve discussion. An explanation for this finding is that there is an inhibitory mechanism by which MCT4 is repressed. It is reasonable to consider that MCT4 in oxidative muscle (soleus) is clearly affected by the combination between aerobic training and an active lifestyle. Data presented here seem to support the view that MCT4 (in an oxidative muscle) is downregulated in situations where aerobic metabolism is in higher demand. In support of this line of thinking, it has been found that an inhibition of MCT4 downregulates glycolysis and increases the flux of Krebs tricarboxylic acid cycle (TCA cycle) [108]. Although do not have data to support this, we speculate that skeletal muscle (as it happens in nucleus pulposus cells) might oxidize the excess lactate into pyruvate, which then enters the TCA cycle and increases the TCA cycle flux [108,109]. This adaptation in LC-T mice would indicate a great advantage for a well-trained aerobic organism.

The final aspect to be discussed is about the anaerobic running capacity (ARC), which has been attributed to the total work underpinned only by anaerobic metabolism [110]. In coherence with this construct, Fukuda, et al. [111] showed that creatine supplementation (related to alactic anaerobic metabolism) improves ARC in men. In practical terms, ARC gives an estimated distance (in meters) that an individual can run solely on stored anaerobic energy sources [111,112]. Based on this concept, it has been believed that efforts performed below or at CV intensity can be sustained for longer with no anaerobic energy reserve depletion [112]. Despite of this apparent consistency, it is necessary to mention that ARC is still not well understood. Some researchers have argued that ARC does not provide an measure of the amount of work or distance that can be performed with anaerobic energy stores alone [113]. It is clear that more investigations are needed to define the physiological meaning of ARC. To our knowledge, there are no available data about the relationship between ARC and MCTs. In SC-T group, ARC was close ($r = 0.84$, $P = 0.073$) to a significant correlation with hypotalamic MCT2. Curiously, aerobic-trained mice (housed in SC) exhibited a pronounced increase of ARC at fourth week. This is a contradictory finding since high-intensity interval training has been reported to enhance anaerobic work capacity (analogue of ARC) in untrained male students [114]. Although ARC have been primarily assigned to stored anaerobic energy sources, it seems coherent to speculate that ARC could also be influenced by the tolerance for lactate accumulation (resulted from the own anaerobic process [115]). An improved functioning of MCTs (in whole-body tissues) could delay systemic acidosis and, consequently, to allow greater maintenance of exercise (likely increasing ARC). This should be further addressed in future studies, especialy using different strategies such as lactic tolerance workouts (within the severe-intensity domain).

Although we tried to make the best possible choices, our study is not immune to criticism. Regarding the design, our study does not allow us to determine the optimal amount of time in LC or the best training strategy for achieving MCTs changes. In this line, we also have no time course of the assessments, especially in analyzing trained mice kept in LC that returned to SC at later time points (for verifying if MCTs adaptations would disappear). Regarding measurements, we are not able to analyze the MCTs for each hypothalamic nucleus since the entire hypothalamus was dissected and homogenized. We did not examine whether experimental animals could have a better mitochondrial function, which in turn could elicit oxidation of lactate [16,116–118]. Although linked to MCTs (the main variables of our study), we did not perform lactate measurements or kinetics assays of lactate. While this could be considered a limitation, we have adopted a less-invasive approach for avoiding disturbing the normal life of rodents. This was necessary since we are interested in quantifying SPA from animals in their home cage with minimal human influence. Another approach to be highlighted is the use of critical velocity protocol which determines aerobic capacity without any collection of blood samples.

Despite limitations, our study is a first step in understanding whether aerobic training and a physically active lifestyle could affect MCTs in hypothalamus that is of interest in various neurological disciplines. The originality of our study was to investigate, in an animal model, the protein expression of MCTs not only in trained animals but also in animals that have undergone different levels of muscle activity. We investigated whether the effects of aerobic training were dependent in more or less active mice. Our finding that aerobic training increase MCTs especially in the hypothalamus of mice housed in a large cage adds knowledge to the field of exercise physiology. This further strengthen the role of hypothalamic MCTs in response to exercise/physical activity interventions. Considering that hypothalamus regulates several physiological and behavioral processes that are critical for survival, our findings provide new insights in the field of medicine and biology. We believe that lactate is a link between periphery and central nervous system for the regulation of nutrient utilization (hypothalamic MCTs are keys in this network). This could bring us much closer to understanding brain adaptations in animals with different levels of muscle activity. Next steps will be to explore whether adaptations resulted from aerobic training would be attributed to changes of MCT in different brain regions.

5. Conclusion

In summary, our findings suggest that aerobic training associated with a more active lifestyle was more effective in modulating MCTs. Aerobic training increased MCT4 in hypothalamus and decreased MCT4 in muscles of mice housed in LC, but not mice housed in SC. These changes seem to be independent of aerobic capacity given that both trained groups exhibited a similar increase in critical velocity. In addition, our data indicate a strong positive influence of aerobic training, and therefore of aerobic capacity, on MCT2 in hypothalamus.

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Ethics approval

All procedures and protocols were approved by an ethical review committee (Comissão de Ética no Uso de Animais- CEUA-UNICAMP, protocol number 4670-1/2017). All methods were carried out in accordance with relevant guidelines and regulations (Canadian Council on Animal Care guidelines and the Guide for the Care and Use of Laboratory Animals). The authors confirm that they have followed laws and standards for the protection of animals used for scientific purposes.

Credit authorship contribution statement

Scariot PP, Gobatto CA and Manchado-Gobatto FB conceived and designed research. Scariot PP conducted experiments. Scariot PP, Manchado-Gobatto FB, Beck WR, Papoti M, Ginkel PRV and Gobatto CA analyzed data and wrote, reviewed as well as approved the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

There is no financial/personal interest or belief that could affect their objectivity. 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.

All authors have seen and approved the final version of the manuscript being submitted.

References

[1] M.A. Felmlee, R.S. Jones, V. Rodriguez-Cruz, K.E. Follman, M.E. Morris, Monocarboxylate transporters (SLC16): function, regulation, and role in health and disease, Pharmacol. Rev. 72 (2020) 466–485.
[2] A.P. Halestrap, N.T. Price, The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation, Biochem. J. 343 (Pt 2) (1999) 281–299.
[3] C. Juel, A.P. Halestrap, Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter, J. Physiol. 517 (Pt 3) (1999) 633–642.
[4] L. Coles, J. Litt, H. Hatta, A. Bonen, Exercise rapidly increases expression of the monocarboxylate transporters MCT1 and MCT4 in rat muscle, J. Physiol. 561 (2004) 253–261.
[5] C. Thomas, D.J. Bishop, K. Lambert, J. Mercier, G.A. Brooks, Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status, Am. J. Physiol. Regul. Integr. Comp. Physiol. 302 (2012) R1–R14.
[6] H. Pilegaard, G. Terzis, A. Halestrap, C. Juel, Distribution of the lactate/H+ transporter isoforms MCT1 and MCT4 in human skeletal muscle, Am. J. Physiol. 276 (1999) E843–E848.
[7] J.C. McDermott, A. Bonen, Endurance training increases skeletal muscle lactate transport, Acta Physiol. Scand. 147 (1993) 323–327.
[8] H. Dubouchaud, G. Butterfield, E. Wolfel, B.C. Bergman, G.A. Brooks, Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 278 (2000) E571–E579.
[9] A. Bonen, K.J. McCullagh, C.T. Putman, E. Hultman, N.L. Jones, G. J. Heigenhauser, Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate, Am. J. Physiol. 274 (1998) E102–E107.
[10] C. Juel, C. Klarskov, J.J. Nielsen, P. Krustrup, M. Mohr, J. Bangsbo, Effect of high-intensity intermittent training on lactate and H+ release from human skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 286 (2004) E245–E251.
[11] H. Pilegaard, K. Domino, T. Noland, C. Juel, Y. Hellsten, A.P. Halestrap, J. Bangsbo, Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle, Am. J. Physiol. 276 (1999) E255–E261.
[12] K.J. McCullagh, A. Bonen, Reduced lactate transport in denervated rat skeletal muscle, Am. J. Physiol. 268 (1995) R884–R888.
[13] H. Dubouchaud, P. Granier, J. Mercier, C. Le Peuch, C. Prefaut, Lactate uptake by skeletal muscle sarcolemmal vesicles decreases after 4 wk of hindlimb unweighting in rats, J. Appl. Physiol. 80 (1996) (1985) 416–421.
[14] L.B. Gladden, Lactate metabolism: a new paradigm for the third millennium, J. Physiol. 558 (2004) 5–30.
[15] G. Brooks, Lactate shuttles in nature, Biochem. Soc. Trans. 30 (2002) 258–264.
[16] G. Brooks, Lactate as a fulcrum of metabolism, Redox Biol. 35 (2020), 101454.
[17] T. Hashimoto, H. Tsukamoto, S. Ando, S. Ogoh, Effect of exercise on brain health: the potential role of lactate as a myokine, Metabolites 11 (2021) 813.
[18] A. Philp, A.L. Macdonald, P.W. Watt, Lactate – a signal coordinating cell and systemic function, J. Exp. Biol. 208 (2005) 4561–4575.
[19] L.F. Barros, Metabolic signaling by lactate in the brain, Trends Neurosci. 36 (2013) 396–404.
[20] W.H. Oldendorf, Blood brain barrier permeability to lactate, Eur. Neurol. 6 (1971) 49–55.
[21] L. Pellerin, G. Pellegri, J. Martin, P.J. Magistretti, Expression of monocarboxylate transporter mRNAs in mouse brain: Support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3990–3995.
[22] M.T. Tseng, S. Chan, A. Schurr, Ischemia-induced changes in monocarboxylate transporter 1 reactive cells in rat hippocampus, Neurol. Res. 25 (2003) 83–86.
[23] L.H. Bergersen, P.J. Magistretti, L. Pellerin, Selective postsynaptic co-localization of MCT2 with AMPA receptor GluR2/3 subunits at excitatory synapses exhibiting AMPA receptor trafficking, Cerebral Cortex (New York, N.Y. : 1991) 15 (2005) 361–370.
[24] E.K. Ainscow, S. Mirshamsi, T. Tang, M.L.J. Ashford, G.A. Rutter, Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K + channels, J. Physiol. 544 (2002) 429–445.
[25] C. Cortés-Campos, R. Elizondo, P. Llanos, R.M. Uranga, F. Nualart, M.A. García, MCT expression and lactate Influx/Efflux in tanycytes involved in glia-neuron metabolic interaction, PLoS One 6 (2011), e16411.
[26] L.M. Supplie, T. Düking, G. Campbell, F. Diaz, C.T. Moraes, M. Götz, B. Hamprecht, S. Boretius, D. Mahad, K.A. Nave, Respiration-deficient astrocytes survive as glycolytic cells in vivo, J. Neurosci. 37 (2017) 4231–4242.
[27] L.H. Bergersen, Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle, Neuroscience 145 (2007) 11–19.
[28] J.J. Todd, Lactate: valuable for physical performance and maintenance of brain function during exercise, Biosci. Horiz. 7 (2014).
[29] S. Bröer, B. Rahman, G. Pellegri, L. Pellerin, J. Martin, S. Verleysdonk, B. Hamprecht, P.J. Magistretti, Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing Xenopus laevis oocytes. Expression of two different monocarboxylate transporters in astroglial cells and neurons, J. Biol. Chem. 272 (1997) 30096–30102.
[30] C. Cortes-Campos, R. Elizondo, C. Carril, F. Martínez, K. Boric, F. Nualart, M. A. Garcia-Robles, MCT2 expression and lactate influx in anorexigenic and orexigenic neurons of the arcuate nucleus, PLoS One 8 (2013), e62532.
[31] P.J. Magistretti, I. Allaman, Lactate in the brain: from metabolic end-product to signalling molecule, Nat. Rev. Neurosci. 19 (2018) 235–249.
[32] T. Matsui, H. Omuro, Y.F. Liu, M. Soya, T. Shima, B.S. McEwen, H. Soya, Astrocytic glycogen-derived lactate fuels the brain during exhaustive exercise to maintain endurance capacity, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 6358–6363.
[33] B. Quistorff, N.H. Secher, J.J.Van Lieshout, Lactate fuels the human brain during exercise, FASEB J. 22 (2008) 3443–3449.
[34] G.G. de Araujo, C.A. Gobatto, F. de Barros Manchado-Gobatto, L.F. Teixeira, I. G. Dos Reis, L.C. Caperuto, M. Papoti, S. Bordin, C.R. Cavaglieri, R. Verlengia, MCT1 and MCT4 kinetic of mRNA expression in different tissues after aerobic exercise at maximal lactate steady state workload, Physiol. Res. 64 (2015) 513–522.
[35] L.D.M. Forte, N.A. Rodrigues, A.V. Cordeiro, T. de Fante, L.A.P. Simino, A. S. Torsoni, M.A. Torsoni, C.A. Gobatto, F.B. Manchado-Gobatto, Periodized versus non-periodized swimming training with equal total training load: physiological, molecular and performance adaptations in Wistar rats, PLoS One 15 (2020), e0239876.
[36] S.K. Baker, K.J. McCullagh, A. Bonen, Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle, J. Appl. Physiol. 84 (1998) (1985) 987–994.
[37] M. Takimoto, T. Hamada, Acute exercise increases brain region-specific expression of MCT1, MCT2, MCT4, GLUT1, and COX IV proteins, J. Appl. Physiol. 116 (2014) (1985) 1238–1250.
[38] M. Aveseh, R. Nikooie, V. Sheibani, S. Esmaeili-Mahani, Endurance training increases brain lactate uptake during hypoglycemia by up regulation of brain lactate transporters, Mol. Cell. Endocrinol. 394 (2014) 29–36.
[39] V.V. Frolkis, V.V. Bezrukov, Y.K. Duplenko, E.D. Genis, The hypothalamus in aging, Exp. Gerontol. 7 (1972) 169–172.
[40] C.B. Saper, B.B. Lowell, The hypothalamus, Curr. Biol. 24 (2014) R1111–R1116.
[41] S. Hiller-Sturmhöfel, A. Bartke, The endocrine system: an overview, in: Alcohol Health and Research World 22, 1998, pp. 153–164.
[42] E. Roh, M. Kim, Brain regulation of energy metabolism, Endocrinol. Metab. 31 (2016) 519–524.
[43] R. Elizondo-Vega, C. Cortes-Campos, M.J. Barahona, K.A. Oyarce, C.A. Carril, M. A. García-Robles, The role of tanycytes in hypothalamic glucosensing, J. Cell. Mol. Med. 19 (2015) 1471–1482.
[44] L. Pellerin, Lactate as a pivotal element in neuron-glia metabolic cooperation, Neurochem. Int. 43 (2003) 331–338.

[45] M.K. Jha, B.M. Morrison, Glia-neuron energy metabolism in health and diseases: new insights into the role of nervous system metabolic transporters, Exp. Neurol. 309 (2018) 23–31.
[46] L. Bergersen, O. Waerhaug, J. Helm, M. Thomas, P. Laake, A.J. Davies, M. C. Wilson, A.P. Halestrap, O.P. Ottersen, A novel postsynaptic density protein: the monocarboxylate transporter MCT2 is co-localized with delta-glutamate receptors in postsynaptic densities of parallel fiber-purkinje cell synapses, Exp. Brain Res. 136 (2001) 523–534.
[47] L. Pellerin, G. Pellegri, P.G. Bittar, Y. Charnay, C. Bouras, J.L. Martin, N. Stella, P. J. Magistretti, Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle, Dev. Neurosci. 20 (1998) 291–299.
[48] P.J. Magistretti, L. Pellerin, D.L. Rothman, R.G. Shulman, Energy on demand, Science 283 (1999) 496–497.
[49] S. Mangia, I.A. Simpson, S.J. Vannucci, A. Carruthers, The in vivo neuron-to-astrocyte lactate shuttle in human brain: evidence from modeling of measured lactate levels during visual stimulation, J. Neurochem. 109 (Suppl 1) (2009) 55–62.
[50] G.A. Dienel, N.F. Cruz, Nutrition during brain activation: does cell-to-cell lactate shuttling contribute significantly to sweet and sour food for thought? Neurochem. Int. 45 (2004) 321–351.
[51] L. Pellerin, P.J. Magistretti, Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10625–10629.
[52] A.K. Johnson, J. Buggy, Periventricular preoptic-hypothalamus is vital for thirst and normal water economy, Am. J. Physiol. 234 (1978) R122–R129.
[53] F. Fernandes-Costa, A.F. de Lima Flôr, M.S. França-Silva, C. de Moura Balarini, J. L. de Brito Alves, V. de Andrade Braga, J.Campos Cruz, Central interaction between nitric oxide, lactate and glial cells to modulate water and sodium intake in rats, Brain Res. Bulletin 186 (2022) 1–7.
[54] C.M. Kotz, C. Wang, J.A. Teske, A.J. Thorpe, C.M. Novak, K. Kiwaki, J.A. Levine, Orexin a mediation of time spent moving in rats: neural mechanisms, Neuroscience 142 (2006) 29–36.
[55] J.A. DiMicco, D.V. Zaretsky, The dorsomedial hypothalamus: a new player in thermoregulation, Am. J. Physiol. Regul. Integr. Comp. Physiol. 292 (2007) R47–R63.
[56] C.B. Saper, T.C. Chou, T.E. Scammell, The sleep switch: hypothalamic control of sleep and wakefulness, Trends Neurosci. 24 (2001) 726–731.
[57] P.G. Haydon, Astrocytes and the modulation of sleep, Curr. Opin. Neurobiol. 44 (2017) 28–33.
[58] M.P. Parsons, M. Hirasawa, ATP-sensitive potassium channel-mediated lactate effect on orexin neurons: implications for brain energetics during arousal, the journal of neuroscience : the official journal of the society for, Neuroscience 30 (2010) 8061–8070.
[59] T. Garland, H. Schutz, M.A. Chappell, B.K. Keeney, T.H. Meek, L.E. Copes, W. Acosta, C. Drenowatz, R.C. Maciel, G. van Dijk, C.M. Kotz, J.C. Eisenmann, The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives, J. Exp. Biol. 214 (2011) 206–229.
[60] E.E.C. Polisel, W.R. Beck, P.P.M. Scariot, T.M.M. Pejon, C.A. Gobatto, F. B. Manchado-Gobatto, Effects of high-intensity interval training in more or less active mice on biomechanical, biophysical and biochemical bone parameters, Sci. Rep. 11 (2021) 6414.
[61] P.P.M. Scariot, C.A. Gobatto, E.E.C. Polisel, A.E.C. Gomes, W.R. Beck, F. B. Manchado-Gobatto, Early-life mice housed in standard stocking density reduce the spontaneous physical activity and increase visceral fat deposition before reaching adulthood, Lab. Anim. 21 (236772211065915) (2022) 1–12.
[62] P.P.M. Scariot, F.B. Manchado-Gobatto, T.A. Prolla, I.G. Masselli Dos Reis, C. A. Gobatto, Housing conditions modulate spontaneous physical activity, feeding behavior, aerobic running capacity and adiposity in C57BL/6J mice, Horm. Behav. 115 (2019), 104556.
[63] G.A. Gaesser, D.C. Poole, The slow component of oxygen uptake kinetics in humans, Exerc. Sport Sci. Rev. 24 (1996) 35–71.
[64] A.M. Jones, A. Vanhatalo, M. Burnley, R.H. Morton, D.C. Poole, Critical power: implications for determination of VO2max and exercise tolerance, Med. Sci. Sports Exerc. 42 (2010) 1876–1890.
[65] C.M. Donovan, G.A. Brooks, Endurance training affects lactate clearance, not lactate production, Am. J. Physiol. 244 (1983) E83–E92.
[66] C.M. Donovan, M.J. Pagliassotti, Enhanced efficiency of lactate removal after endurance training, J. Appl. Physiol. 68 (1990) (1985) 1053–1058.
[67] M.T. Wyss, R. Jolivet, A. Buck, P.J. Magistretti, B. Weber, *In Vivo* Evidence for lactate as a neuronal energy source, J. Neurosci. 31 (2011) 7477–7485.
[68] C.K. Gavini, S. Mukherjee, C. Shukla, S.L. Britton, L.G. Koch, H. Shi, C.M. Novak, Leanness and heightened nonresting energy expenditure: role of skeletal muscle activity thermogenesis, american journal of physiology, Endocrinol. Metab. 306 (2014) E635–E647.
[69] B.E. Levin, Spontaneous motor activity during the development and maintenance of diet-induced obesity in the rat, Physiol. Behav. 50 (1991) 573–581.
[70] J.A. Levine, N.L. Eberhardt, M.D. Jensen, Role of nonexercise activity thermogenesis in resistance to fat gain in humans, Science 283 (1999) 212–214.
[71] C.M. Novak, C. Escande, P.R. Burghardt, M. Zhang, M.T. Barbosa, E.N. Chini, S. L. Britton, L.G. Koch, H. Akil, J.A. Levine, Spontaneous activity, economy of activity, and resistance to diet-induced obesity in rats bred for high intrinsic aerobic capacity, Horm. Behav. 58 (2010) 355–367.
[72] C.E. Perez-Leighton, K. Boland, C.J. Billington, C.M. Kotz, High and low activity rats: elevated intrinsic physical activity drives resistance to diet-induced obesity in non-bred rats, Obesity (Silver Spring) 21 (2013) 353–360.
[73] J.A. Teske, C.J. Billington, M.A. Kuskowski, C.M. Kotz, Spontaneous physical activity protects against fat mass gain, Int. J. Obes. 36 (2012) 603–613.
[74] P.P.M. Scariot, F.B. Manchado-Gobatto, P.R. Van Ginkel, T.A. Prolla, C. A. Gobatto, Aerobic training associated with an active lifestyle exerts a protective effect against oxidative damage in hypothalamus and liver: the involvement of energy metabolism, Brain Res. Bull. 175 (2021) 116–129.
[75] European convention for the protection of vertebrate animals used for experimental and other scientific purposes, Guidelines for Accommodation and Care of Animals, 2006.
[76] Guide for the Care and Use of Laboratory Animals, Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research, 2011.
[77] F.B. Manchado-Gobatto, C.A. Gobatto, R.L. Contarteze, M. Papoti, G.G. Araujo, M.A.R. Mello, Determination of critical velocity and anaerobic capacity of running rats, J. Exerc. Physiol. Online 13 (2010) 40–49.
[78] B.J. Biesiadecki, P.H. Brand, L.G. Koch, S.L. Britton, A gravimetric method for the measurement of total spontaneous activity in rats, Proc. Soc. Exp. Biol. Med. 222 (1999) 65–69.
[79] B. Chausse, C. Solon, C.C. Caldeira da Silva, I.G. Masselli Dos Reis, F. B. Manchado-Gobatto, C.A. Gobatto, L.A. Velloso, A.J. Kowaltowski, Intermittent fasting induces hypothalamic modifications resulting in low feeding efficiency, low body mass and overeating, Endocrinology 155 (2014) 2456–2466.
[80] P. Proia, C.M. Di Liegro, G. Schiera, A. Fricano, I. Di Liegro, Lactate as a metabolite and a regulator in the central nervous system, Int. J. Mol. Sci. 17 (2016) 1450.
[81] B. Matei, L. Fanny, What is the physiological role of hypothalamic tanycytes in metabolism? Am. J. Physiol. Regul. Integr. Comp. Physiol 320 (2021) R994–R1003.
[82] C. Blouet, G.J. Schwartz, Hypothalamic nutrient sensing in the control of energy homeostasis, Behav. Brain Res. 209 (2010) 1–12.
[83] P. Órdenes, P.S. Villar, E. Tarifeño-Saldivia, M. Salgado, R. Elizondo-Vega, R. C. Araneda, M.A. García-Robles, Lactate activates hypothalamic POMC neurons by intercellular signaling, Sci. Rep. 11 (2021) 21644.
[84] C.K.L. Lam, M. Chari, P.Y.T. Wang, T.K.T. Lam, Central lactate metabolism regulates food intake, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E491–E496.
[85] S.F. McCarthy, H. Islam, T.J. Hazell, The emerging role of lactate as a mediator of exercise-induced appetite suppression, Am. J. Physiol. Endocrinol. Metab. 319 (2020) E814–E819.
[86] C. Allard, L. Carneiro, S.C. Collins, C. Chrétien, S. Grall, L. Pénicaud, C. Leloup, Alteration of hypothalamic glucose and lactate sensing in 48h hyperglycemic rats, Neurosci. Lett. 534 (2013) 75–79.
[87] K. Pierre, A. Parent, P.Y. Jayet, A.P. Halestrap, U. Scherrer, L. Pellerin, Enhanced expression of three monocarboxylate transporter isoforms in the brain of obese mice, J. Physiol. 583 (2007) 469–486.
[88] M. Canis, M.H. Maurer, W. Kuschinsky, L. Duembgen, R. Duelli, Increased densities of monocarboxylate transporter MCT1 after chronic hyperglycemia in rat brain, Brain Res. 1257 (2009) 32–39.
[89] C.E. Mondon, C.B. Dolkas, G.M. Reaven, Site of enhanced insulin sensitivity in exercise-trained rats at rest, Am. J. Physiol. 239 (1980) E169–E177.
[90] J.L. Ivy, J.C. Young, J.A. McLane, R.D. Fell, J.O. Holloszy, Exercise training and glucose uptake by skeletal muscle in rats, J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 55 (1983) 1393–1396.
[91] R. Elizondo-Vega, K. Oyarce, M. Salgado, M.J. Barahona, A. Recabal, P. Ordenes, S. López, R. Pincheira, P. Luz-Crawford, M.A. García-Robles, Inhibition of hypothalamic MCT4 and MCT1-MCT4 expressions affects food intake and alters orexigenic and anorexigenic neuropeptide expressions, Mol. Neurobiol. 57 (2020) 896–909.
[92] R. Elizondo-Vega, C. Cortés-Campos, M.J. Barahona, C. Carril, P. Ordenes, M. Salgado, K. Oyarce, M.L. García-Robles, Inhibition of hypothalamic MCT1 expression increases food intake and alters orexigenic and anorexigenic neuropeptide expression, Sci. Rep. 6 (2016) 33606.
[93] J.J.G. Hillebrand, D. de Wied, R.A.H. Adan, Neuropeptides, food intake and body weight regulation: a hypothalamic focus, Peptides 23 (2002) 2283–2306.
[94] Z. Huang, Y. Zhang, R. Zhou, L. Yang, H. Pan, Lactate as potential mediators for exercise-induced positive effects on neuroplasticity and cerebrovascular plasticity, Front. Physiol. 12 (2021).
[95] P. Müller, Y. Duderstadt, V. Lessmann, N.G. Müller, Lactate and BDNF: key mediators of exercise induced neuroplasticity? J. Clin. Med. 9 (2020) 1136.
[96] T. Schiffer, S. Schulte, B. Sperlich, S. Achtzehn, H. Fricke, H.K. Strüder, Lactate infusion at rest increases BDNF blood concentration in humans, Neurosci. Lett. 488 (2011) 234–237.
[97] Y. Lev-Vachnish, S. Cadury, A. Rotter-Maskowitz, N. Feldman, A. Roichman, T. Illouz, A. Varvak, R. Nicola, R. Madar, E. Okun, L-lactate promotes adult hippocampal neurogenesis, Front. Neurosci. 13 (2019) 403.
[98] N.A. Jamnick, R.W. Pettitt, C. Granata, D.B. Pyne, D.J. Bishop, An examination and critique of current methods to determine exercise intensity, Sports Med. 50 (2020) 1729–1756.
[99] P.V. Mezzaroba, M. Papoti, F.A. Machado, Comparison between lactate minimum and critical speed throughout childhood and adolescence in swimmers, Pediatr. Exerc. Sci. 26 (2014) 274–280.
[100] S. Bröer, H.P. Schneider, A. Bröer, B. Rahman, B. Hamprecht, J.W. Deitmer, Characterization of the monocarboxylate transporter 1 expressed in Xenopus laevis oocytes by changes in cytosolic pH, Biochem. J. 333 (Pt 1) (1998) 167–174.
[101] S. Bröer, A. Bröer, H.P. Schneider, C. Stegen, A.P. Halestrap, J.W. Deitmer, Characterization of the high-affinity monocarboxylate transporter MCT2 in Xenopus laevis oocytes, Biochem. J. 341 (Pt 3) (1999) 529–535.

[102] P.P.M. Scariot, F.B. Manchado-Gobatto, A.S. Torsoni, I.G.M. dos Reis, W.R. Beck, C.A. Gobatto, Continuous aerobic training in individualized intensity avoids spontaneous physical activity decline and improves MCT1 expression in oxidative muscle of swimming rats, Front. Physiol. 7 (2016).
[103] Y. Tanaka, T. Inagaki, D.C. Poole, Y. Kano, pH buffering of single rat skeletal muscle fibers in the in vivo environment, Am. J. Physiol. Regul. Integr. Comp. Physiol. 310 (2016) R926–R933.
[104] A. Karagiannis, T. Gallopin, A. Lacroix, F. Plaisier, J. Piquet, H. Geoffroy, R. Hepp, J. Naudé, B.Le Gac, R. Egger, B. Lambolez, D. Li, J. Rossier, J.F. Staiger, H. Imamura, S. Seino, J. Roeper, B. Cauli, Lactate is an energy substrate for rodent cortical neurons and enhances their firing activity, eLife 10 (2021), e71424.
[105] D. Smith, A. Pernet, W.A. Hallett, E. Bingham, P.K. Marsden, S.A. Amiel, Lactate: a preferred fuel for human brain metabolism in vivo, J. Cereb. Blood Flow Metab. 23 (2003) 658–664.
[106] L.F. Barros, J.W. Deitmer, Glucose and lactate supply to the synapse, Brain Res. Rev. 63 (2010) 149–159.
[107] S. Baltan, Can lactate serve as an energy substrate for axons in good times and in bad, in sickness and in health? Metab. Brain Dis. 30 (2015) 25–30.
[108] E.S. Silagi, E. Schipani, I.M. Shapiro, M.V. Risbud, The role of HIF proteins in maintaining the metabolic health of the intervertebral disc, Nat. Rev. Rheumatol. 17 (2021) 426–439.
[109] E.S. Silagi, E.J. Novais, S. Bisetto, A.G. Telonis, J. Snuggs, C.L. Le Maitre, Y. Qiu, I. J. Kurland, I.M. Shapiro, N.J. Philp, M.V. Risbud, Lactate efflux from intervertebral disc cells is required for maintenance of spine health, J. Bone Miner. Res. 35 (2020) 550–570.
[110] S. Green, Measurement of anaerobic work capacities in humans, Sports Med. 19 (1995) 32–42.
[111] D.H. Fukuda, A.E. Smith, K.L. Kendall, T.R. Dwyer, C.M. Kerksick, T.W. Beck, J. T. Cramer, J.R. Stout, The effects of creatine loading and gender on anaerobic running capacity, J. Strength Cond. Res. 24 (2010) 1826–1833.
[112] F.Y. Nakamura, G. Pereira, D.W. Hill, S. Berthoin, E. Kokubun, There is no anaerobic work capacity replenishment at critical power intensity: an indirect evidence, Sci. Sports 23 (2008) 244–247.
[113] L. Bosquet, P.R. Delhors, A. Duchene, G. Dupont, L. Leger, Anaerobic running capacity determined from a 3-parameter systems model: relationship with other anaerobic indices and with running performance in the 800 m-run, Int. J. Sports Med. 28 (2007) 495–500.
[114] D.G. Jenkins, B.M. Quigley, The influence of high-intensity exercise training on the wlim-tlim relationship, Med. Sci. Sports Exerc. 25 (1993) 275–282.
[115] V.J. Clemente-Suárez, J.J. Arroyo-Toledo, Use of biotechnology devices to analyse fatigue process in swimming training, J. Med. Syst. 41 (2017) 94.
[116] D.A. Kane, Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work, Front. Neurosci. 8 (2014).
[117] B. Glancy, D.A. Kane, A.N. Kavazis, M.L. Goodwin, W.T. Willis, L.B. Gladden, Mitochondrial lactate metabolism: history and implications for exercise and disease, J. Physiol. 599 (2021) 863–888.
[118] A. Young, C. Oldford, R.J. Mailloux, Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues, Redox Biol. 28 (2020), 101339.