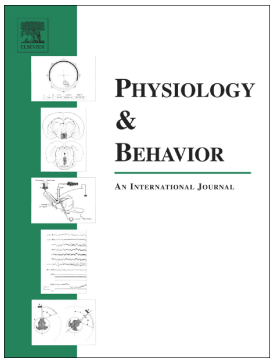

Moderate running exercise prevents excessive immune system activation

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PII: S0031-9384(18)31041-2
DOI: https://doi.org/10.1016/j.physbeh.2019.02.023
Reference: PHB 12461

To appear in: *Physiology & Behavior*

Received date: 21 November 2018
Revised date: 17 February 2019
Accepted date: 18 February 2019

Please cite this article as: D. da Luz Scheffer, K. Ghisoni, A.S.A. Junior, et al., Moderate running exercise prevents excessive immune system activation, Physiology & Behavior, https://doi.org/10.1016/j.physbeh.2019.02.023

Moderate running exercise prevents excessive immune system activation

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Abstract

Benefits of exercise have been documented for many diseases with a chronic progression, including obesity, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, certain types of cancers, and overall mortality. Low-grade systemic inflammation is a key component of these pathologies and it has been demonstrated that can be prevented by performing regularly physical exercise. The aim of this study was to examine the effect of lipopolysaccharide (LPS)-induced inflammation on glucose and insulin tolerance, exercise performance, production of urinary neopterin and striatal neurotransmitters levels in adult male C57BL/6 mice. Increased blood glucose clearance and insulin sensitivity were observed after a single administration of glucose (2 g/kg; p.o.) or insulin (0.5 U/kg, i.p.). However, the repeated injection of LPS (0.33 mg/kg/day, i.p.) decreased glucose tolerance and increase urinary neopterin levels, pointing to systemic inflammation. In parallel to the urinary-increased neopterin, it was observed a significant reduction in the striatal dopamine levels and an increase in the serotonin/dopamine ratio. While a single LPS injection (0.33 mg/kg; i.p) showed impaired performance in the incremental loading test (10 m/min, with 2 m/min increment every 3 min, at 9% grade), a moderate physical exercise protocol (treadmill for three weeks; 5 sessions/week; up to 50 min/day) prevented the exacerbation of

immune system activation and preserved mitochondrial activity in skeletal muscle from mice with continuous LPS infusion (infusion pumps: 0.83 mg/kg/day, i.p.). In conclusion, the peripheral-induced inflammation elicited metabolic alterations that provoked impairment in striatal dopamine metabolism. The moderate exercise prevented the increase of urinary neopterin and preserved mitochondrial activity under LPS-induced inflammatory conditions.

Keywords: neopterin; inflammation; glucose; dopamine; exercise; lipopolysaccharide.

1. Introduction

Physical inactivity and sedentary behavior have become a major public health concern since they represent the second leading single cause of death in the United States [1,2]. A sedentary lifestyle is a major risk factor for many chronic pathologies, including obesity, diabetes, cardiovascular diseases, certain types of cancers, and overall mortality, where the benefits of physical exercise have been extensively documented [3–5].

Animal models have contributed to the understanding and development of current clinical treatments for chronic diseases [6–8]. In addition, basic research is necessary when humans studies have limitations and ethical implications [9]. In this sense, it has been demonstrated that regular exercise brings a number of mental and physical health benefits, including reduced risk of developing diabetes mellitus, obesity, heart disease, metabolic syndromes and cancer [10,11]

Many chronic diseases are associated with persistent and low-grade inflammation [12]. For example, the infiltration of immune cells into white adipose tissue, and therefore inflammation in this tissue, is closely correlated with the development of insulin resistance and type 2 diabetes mellitus (T2DM) [13]. In this context, it is known that patients with T2DM present a higher incidence of development of neurodegenerative diseases [14–17]. T2DM individuals have an increased risk of dementia by 40%, and it has been associated with cognitive decline and reduced hippocampal volume [18]. Several neurodegenerative diseases are also linked to inflammation. Neuroinflammation affects the activation of glial cells and the subsequent release of inflammatory cytokines such as tumor-necrosis factor-α (TNF-α). These cytokines are thought to promote the death of dopamine (DA)-containing neurons in the substantia nigra region of the brain, thereby contributing to the pathology of Parkinson's disease (PD) [19,20]. In addition, it has also been postulated the involvement of the inflammation in the etiology of Alzheimer's disease (AD; for a review see [21]). It should be noted that in addition to the brain inflammation found in many neurodegenerative diseases, systemic inflammation further exacerbates these diseases and promotes the progression to neurodegeneration [22].

Exercise is tightly linked to inflammation and immunity in a complex manner [9,23]. Regular practice of moderate-intensity exercise reduces the level of systemic inflammation [23,24], whereas acute and/or high-intensity exercise promotes an

inflammatory scenario [25,26]. Inflammation has been followed by measuring neopterin in biological fluids. Neopterin is a sensitive biomarker of immune system activation; therefore, a useful tool to follow the anti-inflammatory effect of exercise [26–28]. Neopterin is a byproduct of the *de novo* biosynthesis of tetrahydrobiopterin (BH4) and mainly formed by human monocytes/macrophages and brain cells upon stimulation with the cytokine interferon gamma (INF-γ) (for a review see [29,30]). Elevated neopterin levels have been described in diseases with an inflammatory central and peripheral component and also in high-intensity physical exercise [26–28,31], and it has been used for more than 5 decades as a sensitive biomarker for immune system activation [26,31].

Therefore, herein we investigated the protective effect of moderate intensity-running exercise on immune system activation in mice under bacterial lipopolysaccharide (LPS)-induced inflammation.

2. Materials and methods

2.1 Animals

Male C57BL/6 mice (10-12 weeks, 25-30 g) from the Central Animal House of the Centre for Biological Sciences, Universidade Federal de Santa Catarina (Brazil) were kept in a controlled environment (22 ºC± 1ºC, 12-h light/dark cycle, lights ON 7:00am) with water and food *ad libitum*. The experimental protocols were approved by the Ethics Committee for Animal Research (PP00760/CEUA) of the Universidade Federal de Santa Catarina (Brazil). Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering. Five mice were included per experimental group, unless otherwise stated.

2.2 Physical exercise protocol

Animals were randomly assigned in two groups: *i*) sedentary (Sed) and *ii*) exercised mice (Exe). The physical exercise protocol involved running in a treadmill for 3 weeks. Animals from the exercised group were habituated to the treadmill for 5 days (10 m/min; 10 min). Exercise activity was performed at a speed of 16.5 m/min, which represents 60% of the final speed found in an incremental load test performed after the last day of habituation. Progressive aerobic physical training program consisted of 5

times/week for 3 weeks (time-out on weekends), which was performed in the afternoon (between 5:00 p.m. and 6:00 p.m.). The physical training time had a progressive increase over the 3 weeks (30, 40 and 50 min/day, no inclination) [32].

2.3 Incremental loading test

Incremental test started at a speed of 10 m/min, with 2 m/min increment every 3 min, at 9% treadmill grade until animal exhaustion. Exercise was performed on a horizontal treadmill with twelve individual lanes (26 x 8.5 x 12 cm). No noxious stimulus was used to stimulate running. Time to fatigue (min) and exercise workload (N.m) were considered indexes of exercise performance. Exercise workload (N.m) was calculated as follows: (body mass, kg) x (9.81 m/s^2) x (treadmill speed, m/s) x (time of exercise, min) x (treadmill inclination, %) [33].

2.4 Injections and chronic infusions of LPS

LPS is one of the major components of the outer membrane of gram-negative bacteria. When LPS enters the bloodstream, it activates the Toll 4 receptor (TLR4) located on the surface of immune cells, leading to the release of proinflammatory cytokines and inflammation [34]. Mice were injected intraperitoneally (i.p.) with saline (NaCl 0.9%) or Escherichia coli LPS (lot 3129; serotype 0127:B8; Sigma) 0.33 mg/kg in a volume of 10.0 mL/kg [35]. The treatment provoked higher levels of IL-1β in the brain of LPS-injected mice (up to 100-fold; data not shown), 4 h after the administration. Other group of mice had a mini-osmotic pump (Alzet® Model 2002; Alza, Palo Alto, CA) implanted subcutaneously linked to the peritoneal cavity by a catheter for continue i.p. LPS infusion (0.33 or 0.83 mg/kg/day in a flow of 0.5 μL/h for 2 weeks). For the implantation of the mini-osmotic pumps, animals were anesthetized with ketamine (80 mg/kg) and xylazine (20 mg/kg). The pumps were filled with either saline or LPS [36].

2.5 Glucose Tolerance Test

Mice were fed with glucose (2 g/kg; p.o.) after overnight fasting. Tail blood was drawn at 0, 15, 30, 60, 90 and 120 min following the glucose load. Glucose levels were determined using a glucometer (Optium™Xceed, Abbott, USA). Oral glucose tolerance tests (OGTT) were performed either 6 h after the acute intraperitoneal injection of LPS

or at the end of the sustained LPS intraperitoneal administration. Saline was administered in control mice.

2.6 Insulin Tolerance Test

Fasting mice (6 h) were challenged with insulin (0.5 U/kg Humalog, i.p.) and blood glucose levels were assessed to determine the insulin tolerance test (ITT) (see Section 2.5). Different group of animals (*n=3*) were used for the measurements of OGTT and ITT.

2.7 Neopterin measurement by HPLC

Neopterin levels were measured in urine by high-performance liquid chromatography (HPLC). Urine was collected following these two criteria: *i)* obtain pure urine without contamination with feces or animal feed, (2) collect urine without any direct intervention to avoid stress. The peripheral alteration of urinary neopterin depends on the degree of immune system activation, which is also linked to exercise intensity, duration and training status [11,26]. Urine was collected before (time 0) and 1, 3, 7, 10 and 14 days after the osmotic pump implantation. Samples were collected by the single animal method described elsewhere [37], where a single mouse urinates on cling wraps, outside of the animal cage. The voided urine was then aspirated into microcentrifuge tubes and immediately frozen. On the day of the experiments, samples were thawed and centrifuged at 16,000 × *g* for 10 min at 4ºC. Afterwards, urine was diluted in 10 volumes (v/v) of potassium phosphate buffer 15 mM, containing EDTA 5mM. The HPLC analysis of neopterin was carried out by using a Supercosil LC-18-T 5μm reverse phase column (15 × 4.6 mm), with a flow rate set at 0.7 mL/min and an isocratic elution of 85% potassium phosphate buffer 15 mM, containing 15% acetonitrile, pH 6.4. The temperature of the column compartment was set at 35°C. The identification and quantification of neopterin was performed by a multi-wavelength fluorescence detector (module 2475, Waters, Milford, USA) with excitation of 355 nm and emission 438 nm [26]. The results were expressed as μmol (neopterin)/mol creatinine. Creatinine levels were quantified using a specific kit (Labtest, MG, Brazil).

2.8 Dopamine measurement by HPLC

Dopamine (DA), 3,4-dihydroxyphenyilacetic acid (DOPAC) and serotonin (5-HT) were measured in the striatum by HPLC. Striatum samples were collected at the end the sustained LPS or vehicle infusions for 2 weeks. Five mice from each group were euthanized by decapitation and their brains were removed immediately and the striatum was dissected and immediately frozen and stored at -80°C. The brain tissue was sonicated and centrifuged (16,000 x *g* for 10 min at 4ºC) in chilled 0.1 M perchloric acid. Monoamines and their metabolites present in the supernatants were assessed by HPLC (Alliance e2695, Waters, Milford, USA), with electrochemical detection (Waters 2465, Waters, Milford, USA) with a voltage of +400 mV. The temperature of the column compartment was set at 30 °C. Twenty microliters of supernatant were analyzed in a 150 x 2.0 mm, 4µm, C18 column (Synergi Hydro, California, USA) with a mobile phase containing 90 mM sodium phosphate, 50 mM citric acid, 1.7 mM sodium 1-heptane-sulfonate, 50 µM ethylenediaminetetraacetic acid, 10% acetonitrile, pH 3.0, with a flow of 0.25 mL/min [38]. DA, DOPAC and 5-HT levels in the supernatants were calculated as ng/mg protein.

2.9 Measurement of respiratory chain complex I activity

At the end the sustained LPS or vehicle infusions for 2 weeks, the quadriceps muscles were collected and homogenized in 10 volumes (v/v) of 4.4 mM potassium phosphate buffer, pH 7.4, containing 0.3 M sucrose, 5 mM MOPS, 1 mM EGTA and 0.1% BSA. The homogenates were centrifuged at 3,000 x *g* for 10min at 4ºC. The supernatant was collected and underwent three cycles of freezing and thawing for mitochondrial membrane rupture. Complex I activity was obtained by the rate of NADH-dependent ferricyanide reduction at 420 nm (30ºC, $\varepsilon = 1\ mM^{-1} \times cm^{-1}$) [39].

2.10 Protein determination

Protein content was determined by the method of Lowry [40], using bovine serum albumin as the standard.

3. Statistical analysis

Data are presented as mean ± SEM (standard error of mean). Data were analyzed using one-way or two-way analysis of variance (ANOVA) followed by the post hoc of Bonferroni or Tukey test, when *F* was significant. When comparing two independent groups, Student's *t*-test for independent samples was used. Exercise incremental load test results are presented in percentage of animals that successfully performed the task at specific speeds. In this case, group differences were examined by applying Log-rank (Mantel-Cox). The accepted level of significance for the tests was $P < 0.05$. Statistics and all graphs were performed by using GraphPad Prism 6®. Effect size (ES) was calculated from ANOVA and Student's t-tests studies.

4. Results

4.1 Sustained LPS injections decreased glucose tolerance

Fig. 1 shows the blood glucose and insulin response after a single injection of LPS. LPS increased glucose clearance and decreased AUC, showing a higher nutrient demand at the time of acute inflammation compared to the control group, as shown in Fig. 1A [$F_{(1,5)} = 17.86$, $P < 0.001$] and Fig. 1A'[$t_{(4)} = 8.16$, $P < 0.001$]. In order to verify insulin tolerance, 6 h after a single injection of LPS (2 mg/kg or 0.33 mg/kg), mice were given an injection of insulin and the glycaemia was assessed. LPS acute injection increased insulin sensitivity, as shown in Fig. 1B [$F_{(2,10)} = 35.93$, $P < 0.001$] and Fig. 1B' [$F_{(2,6)} = 13.13$, $P < 0.01$]. Next, we assessed the effect of repeated intraperitoneal LPS administration (0.33 mg/kg/day; 2 weeks) on glucose tolerance (Fig. 1C). In contrast to acute administration (Fig. 1A), the sustained injections of LPS decreased glucose tolerance, as shown in Fig. 1E [$F_{(1,5)} = 56.76$, $P < 0.01$] and Fig. 1E' [$t_{(4)} = 2.96$, $P < 0.05$]. No difference was found in food intake between the groups (Fig. 1D).

4.2 Sustained infusion of LPS for two weeks elicited inflammation and compromised dopamine metabolism

Fig. 2 shows the effect of acute LPS-mediated inflammation on exercise performance, urinary neopterin and monoamines levels in the striatum. Fig. 2A shows the evaluation of the maximum effort capacity through the incremental loading test. The incremental loading test started 4 h or 24 h after a single administration of LPS 0.33 mg/kg (i.p). The exercise performance of the animals decreased after LPS administration, Fig. 2B

[$\chi^2_{(2)}$ = 26.49; P <0.001], as demonstrated by the reduced horizontal [$F_{(2,29)}$ = 11.41; P < 0.001] and vertical [$F_{(2,29)}$ = 12.45; P < 0.001] exercise workloads, Fig. 2C. However, the LPS-induced impairment was more significant during the first stages of the incremental loading test, after 4 h of the inflammatory challenge [$\chi^2_{(2)}$ = 26.49; P <0.001].

The continuous LPS infusion (0.33 mg/kg/day, i.p.; Fig. 2D), reduced the body weight of the animals, especially in the first two days, as shown in Fig. 2E ($F_{(1,6)}$ = 142.3; P < 0.01). Fig. 2F depicts that sustained LPS-mediated inflammation increased the urinary neopterin levels up to three days after starting the infusion of LPS, and on the seventh day the levels returned to baseline levels [$F_{(1,5)}$ = 4.53; P < 0.05]. Finally, DA levels (Fig. 2G, [$t_{(7)}$ = 2.40, P < 0.05]) and its metabolite DOPAC (Fig. 2H, [$t_{(7)}$ = 2.72, P < 0.05]) were markedly reduced in the striatum of mice receiving the continuous LPS infusion. No difference was observed in the 5-HT levels (Fig. 2I); however, the striatal 5-HT/DA ratio was significantly increased by LPS, Fig. 2J [$t_{(16)}$ = 2.63, P < 0.05].

4.3 Exercise prevented exacerbation of immune system activation and preserved mitochondrial activity

The effect of exercise on neopterin production and activity of mitochondrial complex I in skeletal muscle were assessed in mice receiving sustained infusion of LPS and submitted to physical exercise. Animals performed a moderate training program for 3 weeks followed by continuous infusion of LPS (0.83 mg/kg/day, i.p; mini-osmotic pumps for 2 weeks; Fig. 3A). Fig. 3B shows increased urinary neopterin levels in the Sed LPS group, which were prevented by physical exercise (Exe LPS group; *effect of LPS* [$F_{(3,12)}$ = 28.99; P < 0.001], *interaction* [$F_{(15,60)}$ = 4.38; P < 0.001]). In addition, the exercise *per se* (Exe control group) moderately increased the neopterin production (increased neopterin in a non-inflammatory context). Furthermore, the exercise prevented the reduction on mitochondrial complex I activity observed in skeletal muscle of mice submitted to the LPS administration, Fig. 3C [$F_{(3,9)}$ = 4.98; P < 0.05].

5. Discussion

Acute inflammation is a protective and coordinated response to cellular stress that results from the communication among different types of immune cells and tissues [11].

Low-grade chronic systemic inflammation, characterized by increasing circulating proinflammatory mediators, is a key component of the pathogenesis of many chronic diseases, including obesity and diabetes, and also of physical inactivity [12,41]. Consequently, a reduction in systemic inflammation is observed in individuals who regularly perform physical exercise [10,12]. However, approximately one in three adults and four in five adolescents do not achieve the recommended quantity and quality of daily exercise worldwide [42]. In this scenario, the physical activity guidelines for Americans from the US Department of Health and Human Services recommends that adults with chronic conditions, including obesity, should do at least 150-300 min/week of moderate-intensity, or 75-150 min/week of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate- and vigorous-intensity aerobic activity for health benefits [43]. Additionally, they should perform muscle-strengthening activities of moderate or greater intensity and that involve all major muscle groups on 2 or more days a week, as these activities provide additional health benefits [43].

We showed here that sustained inflammation compromised glucose and insulin tolerance, as it is seen in chronic metabolic diseases and increased neopterin levels, which was prevented by exercise. However, acute inflammation increased tissue insulin sensitivity and glucose tolerance, which is believed to be at least partly attributed to an inhibitory action of LPS on liver glucose production [36,44]. Previous reports have linked insulin resistance and LPS-mediated sustained inflammation [36,45], and several studies have also demonstrated TLR4 expression to be increased in conventional insulin resistance target tissues like skeletal muscle and adipose tissue of T2DM subjects [46,47]. LPS is a strong stimulatory of the release of several cytokines that are key inducers of insulin resistance [45]. When LPS enters the bloodstream, it activates TLR4, leading to the activation of transcription factors such as nuclear factor-κB (NF-κB) and interferon regulatory factor. These transcription factors control the induction of inflammatory mediators, including IL-1β, IL-6, TNF-α ,TNF-β, INF-α, INF-β, INF-γ [48]. Inflammation of adipose tissue is a crucial step in the development of peripheral insulin resistance and obesity [12,41]. The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signaling leads to insulin resistance [41,49]. Exposure of cells to TNF-α or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of insulin receptor substrate 1 (IRS-1), leading to impairment in the ability of IRS-1 to activate

downstream phosphatidylinositol 3-kinase-dependent pathways; and therefore, compromising the insulin signaling [41,49,50].

The nervous system and the brain maintain control of homeostasis through bidirectional communication with peripheral physiological system [51]. Peripheral immune cells are prevented from central nervous system (CNS) entry by the presence of the blood-brain barrier and a local tissue environment that penalizes bone marrow-derived blood cells [52,53]. Subtle changes in the microenvironment of the CNS either due to local alterations (changes of pH, metabolic disturbances or microbleedings, among others) or peripheral changes in the blood circulation (bacterial or viral infection) or in other organs (impaired function, dysbiosis or inflammation) can have a major impact on CNS function, resulting in changes in cognitive function, mood and behavior [53].

Our results also showed that peripheral inflammation, depicted by increased urinary neopterin levels, impacted on the CNS activity, compromising DA metabolism. The role of inflammation in the development of different diseases of the CNS, including AD and PD, is a research area under extensive investigation [20,54]. Persistent hyperglycemia accompanied by hypertriglyceridemia, a hallmark of some chronic metabolic diseases, as diabetes and obesity, promotes epigenetic changes in the CNS that convey in higher susceptibility to neurodegeneration, impairing learning and memory [17]. In fact, our group has demonstrated that sustained hyperglycemia promotes peripheral and central inflammation with cognitive impairment, reduced levels of BDNF, and early signs or neurodegeneration in the hippocampus [17].

Under physiologic conditions, cytokines such as TNF- α and IL-1β have been shown to be involved in a number of essential brain processes such as synaptic remodeling, neurogenesis and long-term potentiation [55]. However, in excess, inflammatory cytokines can affect monoamine neurotransmitter systems and behavior, and evidences indicate that DA function in the basal ganglia may be a primary target of peripheral inflammation [56,57]. The effect of inflammatory cytokines on basal ganglia DA levels may be especially relevant to depression and fatigue, that is also linked to poor exercise performance [58,59].

The striatum is the input module to the basal ganglia, a neuronal circuit necessary for voluntary movement control [60]. Striatal neurons signal activities related to the preparation, initiation and execution of movements [60,61]. Moreover, diseases or drug-induced losses in brain DA lead to movement disorders, where the affected

individuals or experimental animals have difficulties with motor activities. A pilot study of four patients with early-stage Parkinson's disease, striatal D2/D3 dopamine receptor binding was increased in the two patients who engaged in treadmill exercise but not in the two patients who did not, showing the relevance of DA neurotransmission in exercise [62]. In addition, our group also demonstrated improved dopaminergic neurotransmission and enhanced cognition in a preclinical animal model of PD by submitting the animals to the exercise protocol employed in the present work [32]. In agreement, our results showed reduced DA metabolism and impaired exercise performance in an inflammatory scenario induced by LPS. In addition, higher 5-HT/DA ratio favors decreased performance and has been associated with central fatigue [63,64]. In this way, the regular practice of moderate intensity exercise can lead to increased levels of neurotrophic factors, such as GDNF and BDNF, as well as, changes in the levels of different cytokines and altered microglial functions in different parts of the brain that could be beneficial for patients with neurodegenerative diseases or moderate depression [9,39,65].

The effects of exercise on immune system was assessed by measuring urinary neopterin levels [11,29]. Neopterin is a byproduct of the BH4 *de novo* pathway, which requires Mg^{2+}, Zn^{2+} and NADPH as cofactors. Under inflammatory conditions, GTPCH (rate-limiting enzyme of the *de novo* pathway) is upregulated through positive feedback, overpassing the PTPS (second enzyme of the BH4 pathway) catalytic capacity and leading to the accumulation of 7,8-dihydroneopterin triphosphate, which will then form neopterin through a non-enzymatic conversion [67,68]. The peripheral alteration of this parameter depends on the degree of immune system activation, which also is linked to exercise intensity, duration and training status [11,26]. Thus, an increase in neopterin levels in biological fluids has been recognized a sensitive biomarker for immune system activation [26,31].

Our group has also demonstrated that excessive neopterin levels are produced in individuals who performed an ultra-endurance race (alternating off-road running, mountain biking and kayaking; 90km). Post-race biomarkers of muscle damage, oxidative metabolism and immune system activation, including increased neopterin levels, were observed in plasma from these athletes [26]. A significant increase in the urinary neopterin levels was also demonstrated in ultra-marathon runners and rugby players, which both were associated with increased oxidative stress, inflammatory response, muscle damage and fatigue [27,69]. In addition, neopterin is secreted in

human primary neurons and mouse CNS cells under LPS inflammatory stimulus [30]. However, under a non-inflammatory scenario, neopterin has neuroprotective activities, *i.e.* neopterin is a cognitive enhancer, has antioxidant and anti-inflammatory properties, and increases mitochondrial brain activity [11,29,35,70,71]. It is widely known, that all these cytoprotective mechanisms are also induced by the regular practice of moderate intensity physical exercise. Therefore, our group recently proposed that the anti-depressant effect of exercise might be related to increased brain or CSF neopterin levels [11]. Moreover, we also demonstrated that human primary neurons and astrocytes pre-exposed to neopterin are more resistant to oxidative stress and to inflammasome activation under an inflammatory stimulus [11,71]. Mechanistically, neopterin would activate the master transcription factor Nrf2, a key regulator of the cellular antioxidant response, which will further enhance the cellular antioxidant defenses, mitochondrial activity and the anti-inflammatory environment. Increased Nrf2 expression and content, as well as, increased downstream antioxidant proteins have been describe by our group to be increased in the brain after neopterin treatment [29,35,70,71]. Furthermore, the increased expression of Nrf2 was also demonstrated by our group in brain from exercised rodents [32]. According our results and based on the literature, we suggest that moderate intensity physical exercise: (*i*) prevents the exacerbated increase of neopterin in an inflammatory state and (*ii*) physical exercise *per se* increases the neopterin levels as a mechanism to prevent oxidative stress and inflammation.

Several studies have demonstrated that programs of physical exercise of moderate intensity have anti-inflammatory and muscle energetic metabolism effects [10,32,72–74]. Our group has previously observed an increase in the activity of the respiratory chain complex I in mice after physical exercise on treadmill for six weeks [32]. However, here, our data demonstrate that the adaptive effect of exercise on mitochondrial metabolism did not remain after two weeks of recovery, when the biochemical measurements were performed. However, the physical exercise prevented the reduction in the activity of complex induced by the LPS administration, being in agreement with other studies that also demonstrated a protective effect of the exercise on the oxidative mitochondrial metabolism [32,39,73,75]. Therefore, healthy lifestyle that involves the regular practice of physical exercise is a good strategy for the prevention of inflammation and chronic diseases, such as obesity, T2DM, depression and neurodegenerative diseases.

6. Conclusions

In summary, we demonstrated here that sustained peripheral inflammation compromised striatal DA metabolism. In addition, we also demonstrated that 3 weeks of moderate exercise prevented the increase of urinary neopterin and preserves the mitochondria activity of complex I under LPS-induced inflammatory conditions. Therefore, physical exercise shows to be a good preventive strategy to prevent neurodegenerative diseases, and consequently, increase the time and quality of life.

Acknowledgements and funding

This work was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil). Aguiar AS Jr and Latini A are CNPq fellows.

Declarations of interest: none

References

[1] A.H. Mokdad, J.S. Marks, D.F. Stroup, J.L. Gerberding, Actual Causes of Death in the United States, 2000, J. Am. Med. Assoc. (2004). doi:10.1001/jama.291.10.1238.

[2] K.A. Sluka, L. Frey-Law, M. Hoeger Bement, Exercise-induced pain and analgesia? Underlying mechanisms and clinical translation., Pain. 159 Suppl (2018) S91–S97. doi:10.1097/j.pain.0000000000001235.

[3] R.R. Pate, M. Pratt, S.N. Blair, W.L. Haskell, C.A. Macera, C. Bouchard, D. Buchner, W. Ettinger, G.W. Heath, A.C. King, et al., Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine, JAMA. 273 (1995) 402–407.

[4] D.S. Lauderdale, Adiposity and physical activity as predictors of mortality., N. Engl. J. Med. (2005). doi:10.1056/NEJM200503313521322.

[5] P.T. Katzmarzyk, T.S. Church, C.L. Craig, C. Bouchard, Sitting time and mortality from all causes, cardiovascular disease, and cancer, Med. Sci. Sports Exerc. (2009). doi:10.1249/MSS.0b013e3181930355.

[6] S.A. Jagmag, N. Tripathi, S.D. Shukla, S. Maiti, S. Khurana, Evaluation of models of Parkinson's disease, Front. Neurosci. (2016). doi:10.3389/fnins.2015.00503.

[7] W. Stummer, K. Weber, B. Tranmer, A. Baethmann, O. Kempski, Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia, Stroke. (1994). doi:10.1161/01.STR.25.9.1862.

[8] B.L.P. Coelho, L.G.C. Rocha, K.S. Scarabelot, D.L. Scheffer, M.M. Ronsani, P.C.L. Silveira, L. a Silva, C.T. Souza, R. a Pinho, Physical exercise prevents the exacerbation of oxidative stress parameters in chronic kidney disease., J. Ren. Nutr. (2010). doi:10.1053/j.jrn.2009.10.007.

[9] M. Svensson, J. Lexell, T. Deierborg, Effects of Physical Exercise on

Neuroinflammation, Neuroplasticity, Neurodegeneration, and Behavior, Neurorehabil. Neural Repair. (2015). doi:10.1177/1545968314562108.

[10] M. Gleeson, N.C. Bishop, D.J. Stensel, M.R. Lindley, S.S. Mastana, M.A. Nimmo, The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease, Nat. Rev. Immunol. (2011). doi:10.1038/nri3041.

[11] R. de Paula Martins, C. K Lim, K. Ghisoni, A. Staats, K. Dallagnol, A. Solano, G. J. Guillemin, A. Silva Aguiar Jr, A. Latini, Treating depression with exercise: The inflammasome inhibition perspective, J. Syst. Integr. Neurosci. (2016). doi:10.15761/JSIN.1000147.

[12] C. Handschin, B.M. Spiegelman, The role of exercise and PGC1alpha in inflammation and chronic disease, Nature. 454 (2008) 463–469. doi:10.1038/nature07206.

[13] G.S. Hotamisligil, Inflammation and metabolic disorders, Nature. (2006). doi:10.1038/nature05485.

[14] N. Palacios, X. Gao, M.L. McCullough, E.J. Jacobs, A. V Patel, T. Mayo, M.A. Schwarzschild, A. Ascherio, Obesity, diabetes, and risk of Parkinson's disease., Mov. Disord. (2011). doi:10.1002/mds.23855.

[15] J.C. Pressley, E.D. Louis, M.X. Tang, L. Cote, P.D. Cohen, S. Glied, R. Mayeux, The impact of comorbid disease and injuries on resource use and expenditures in parkinsonism, Neurology. (2003). doi:10.1212/WNL.60.1.87.

[16] W. Xu, C. Qiu, M. Gatz, N.L. Pedersen, B. Johansson, L. Fratiglioni, Mid- And late-life diabetes in relation to the risk of dementia: A population-based twin study, Diabetes. (2009). doi:10.2337/db08-0586.

[17] A.P. Remor, R.A. da Silva, F.J. de Matos, V. Glaser, R. de Paula Martins, K. Ghisoni, D. da Luz Scheffer, D.C. Andia, D. Portinho, A.P. de Souza, P.A. de Oliveira, R.D. Prediger, A.I. Torres, R.M.M. Linhares, R. Walz, M.F. Ronsoni, A. Hohl, A. Rafacho, A.S. Aguiar, A.L. de Paul, A. Latini, Chronic Metabolic Derangement-Induced Cognitive Deficits and Neurotoxicity Are Associated with REST Inactivation, Mol. Neurobiol. (2018). doi:10.1007/s12035-018-1175-9.

[18] P.K. Crane, R. Walker, R.A. Hubbard, G. Li, D.M. Nathan, H. Zheng, S. Haneuse, S. Craft, T.J. Montine, S.E. Kahn, W. McCormick, S.M. McCurry, J.D. Bowen, E.B. Larson, Glucose Levels and Risk of Dementia, N. Engl. J. Med. (2013). doi:10.1056/NEJMoa1215740.

[19] M.G. Tansey, T.C. Frank-Cannon, M.K. McCoy, J.K. Lee, T.N. Martinez, F.E. McAlpine, K.A. Ruhn, T.A. Tran, Neuroinflammation in Parkinson's disease: is there sufficient evidence for mechanism-based interventional therapy?, Front. Biosci. (2008). doi:10.2741/2713.

[20] P.S. Whitton, Inflammation as a causative factor in the aetiology of Parkinson's disease, Br. J. Pharmacol. (2007). doi:10.1038/sj.bjp.0707167.

[21] F. Zipp, O. Aktas, The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases, Trends Neurosci. (2006). doi:10.1016/j.tins.2006.07.006.

[22] V.H. Perry, C. Cunningham, C. Holmes, Systemic infections and inflammation affect chronic neurodegeneration, Nat. Rev. Immunol. (2007). doi:10.1038/nri2015.

[23] A.M.W. Petersen, B.K. Pedersen, The anti-inflammatory effect of exercise., J. Appl. Physiol. (2005). doi:10.1152/japplphysiol.00164.2004.

[24] M. Gleeson, Immune function in sport and exercise., J. Appl. Physiol. (2007). doi:10.1152/japplphysiol.00008.2007.

[25] R. a Pinho, L. a Silva, C. a Pinho, D.L. Scheffer, C.T. Souza, M. Benetti, T. Carvalho, F. Dal-Pizzol, Oxidative stress and inflammatory parameters after an Ironman race., Clin. J. Sport Med. (2010). doi:10.1097/JSM.0b013e3181e413df.

[26] R. Dantas de Lucas, F. Caputo, K. Mendes de Souza, A.R. Sigwalt, K. Ghisoni, P.C. Lock Silveira, A.P. Remor, D. da Luz Scheffer, L.G. Antonacci Guglielmo, A. Latini, Increased platelet oxidative metabolism, blood oxidative stress and neopterin levels after ultra-endurance exercise, J. Sports Sci. 32 (2014). doi:10.1080/02640414.2013.797098.

[27] A. Lindsay, J. Lewis, C. Scarrott, N. Draper, S.P. Gieseg, Changes in acute biochemical markers of inflammatory and structural stress in rugby union, J. Sports Sci. (2015). doi:10.1080/02640414.2014.971047.

[28] D. Fuchs, G. Granditsch, A. Hausen, G. Reibnegger, H. Wachter, URINARY NEOPTERIN EXCRETION IN COELIAC DISEASE, Lancet. (1983). doi:10.1016/S0140-6736(83)90437-3.

[29] K. Ghisoni, R. de P.R.D.P. Martins, L. Barbeito, A. Latini, Neopterin as a potential cytoprotective brain molecule, 2015. doi:10.1016/j.jpsychires.2015.10.003.

[30] R. de Paula Martins, K. Ghisoni, C.K. Lim, A.S. Aguiar, G.J. Guillemin, A. Latini, Neopterin preconditioning prevents inflammasome activation in mammalian astrocytes, Free Radic. Biol. Med. 115 (2018) 371–382. doi:10.1016/j.freeradbiomed.2017.11.022.

[31] B. Widner, F. Leblhuber, D. Fuchs, Increased neopterin production and tryptophan degradation in advanced Parkinson's disease, J Neural Transm. 109 (2002) 181–189.

[32] A.S. Aguiar, M. Duzzioni, A.P. Remor, F.S.M. Tristão, F.C. Matheus, R. Raisman-Vozari, A. Latini, R.D. Prediger, Moderate-Intensity Physical Exercise Protects Against Experimental 6-Hydroxydopamine-Induced Hemiparkinsonism Through Nrf2-Antioxidant Response Element Pathway., Neurochem. Res. 41 (2016) 64–72. doi:10.1007/s11064-015-1709-8.

[33] A.S. Aguiar, A.E. Speck, I.M. Amaral, P.M. Canas, R.A. Cunha, The exercise sex gap and the impact of the estrous cycle on exercise performance in mice, Sci. Rep. 8 (2018) 10742. doi:10.1038/s41598-018-29050-0.

[34] S. Spulber, K. Edoff, L. Hong, S. Morisawa, S. Shirahata, S. Ceccatelli, Molecular hydrogen reduces lps-induced neuroinflammation and promotes recovery from sickness behaviour in mice, PLoS One. (2012). doi:10.1371/journal.pone.0042078.

[35] K. Ghisoni, A.S. Aguiar, P.A. de Oliveira, F.C. Matheus, L. Gabach, M. Perez, V.P. Carlini, L. Barbeito, R. Mongeau, L. Lanfumey, R.D. Prediger, A. Latini, Neopterin acts as an endogenous cognitive enhancer, Brain. Behav. Immun. 56 (2016) 156–164. doi:10.1016/j.bbi.2016.02.019.

[36] A.T. Nguyen, S. Mandard, C. Dray, V. Deckert, P. Valet, P. Besnard, D.J. Drucker, L. Lagrost, J. Grober, Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: Involvement of the GLP-1 pathway, Diabetes. (2014). doi:10.2337/db13-0903.

[37] B.T. Kurien, R. Hal Scofield, Mouse urine collection using clear plastic wrap, Lab. Anim. (1999). doi:10.1258/002367799780578525.

[38] A.A. Castro, K. Ghisoni, A. Latini, J. Quevedo, C.I.C.I. Tasca, R.D.S.R.D.S. Prediger, Lithium and valproate prevent olfactory discrimination and short-term memory impairments in the intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of Parkinson's disease, Behav. Brain Res.

229 (2012) 208–215. doi:10.1016/j.bbr.2012.01.016.
[39] A.S. Aguiar, E. Stragier, D. da Luz Scheffer, A.P. Remor, P.A. Oliveira, R.D. Prediger, A. Latini, R. Raisman-Vozari, R. Mongeau, L. Lanfumey, Effects of exercise on mitochondrial function, neuroplasticity and anxio-depressive behavior of mice, Neuroscience. 271 (2014). doi:10.1016/j.neuroscience.2014.04.027.
[40] R. Lowry, OH, Rosebrough, NJ, Farr AL, Randall, Protein measurment with the folin phenol reagent, J. Biol. Chem. (1951). doi:10.1016/0304-3894(92)87011-4.
[41] K.E. Wellen, G.S. Hotamisligil, Inflammation, stress, and diabetes, J. Clin. Invest. (2005). doi:10.1172/JCI200525102.
[42] P.C. Hallal, L.B. Andersen, F.C. Bull, R. Guthold, W. Haskell, U. Ekelund, J.R. Alkandari, A.E. Bauman, S.N. Blair, R.C. Brownson, C.L. Craig, S. Goenka, G.W. Heath, S. Inoue, S. Kahlmeier, P.T. Katzmarzyk, H.W. Kohl, E.V. Lambert, I.M. Lee, G. Leetongin, F. Lobelo, R.J.F. Loos, B. Marcus, B.W. Martin, N. Owen, D.C. Parra, M. Pratt, P. Puska, D. Ogilvie, R.S. Reis, J.F. Sallis, O.L. Sarmiento, J.C. Wells, Global physical activity levels: Surveillance progress, pitfalls, and prospects, Lancet. (2012). doi:10.1016/S0140-6736(12)60646-1.
[43] U.S. Department of Health and Human Services., Physical Activity Guidelines for Americans, Washington, D.C., 2018.
[44] J.R. Stevens, R.P. McMillan, J.T. Resendes, S.K. Lloyd, M.M. Ali, M.I. Frisard, S. Hargett, S.R. Keller, M.W. Hulver, Acute low-dose endotoxin treatment results in improved whole-body glucose homeostasis in mice, Metabolism. (2017). doi:10.1016/j.metabol.2016.12.008.
[45] P.D. Cani, J. Amar, M.A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A.M. Neyrinck, F. Fava, K.M. Tuohy, C. Chabo, A. Waget, E. Delmée, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrières, J.F. Tanti, G.R. Gibson, L. Casteilla, N.M. Delzenne, M.C. Alessi, R. Burcelin, Metabolic endotoxemia initiates obesity and insulin resistance, Diabetes. (2007). doi:10.2337/db06-1491.
[46] S.J. Creely, P.G. McTernan, C.M. Kusminski, ff. M. Fisher, N.F. Da Silva, M. Khanolkar, M. Evans, A.L. Harte, S. Kumar, Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes, AJP Endocrinol. Metab. (2006). doi:10.1152/ajpendo.00302.2006.
[47] M.R. Dasu, S. Devaraj, S. Park, I. Jialal, Increased Toll-Like Receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects, Diabetes Care. (2010). doi:10.2337/dc09-1799.
[48] R. Medzhitov, C.A. Janeway, Decoding the patterns of self and nonself by the innate immune system, Science (80-.). (2002). doi:10.1126/science.1068883.
[49] G.S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M.F. White, B.M. Spiegelman, IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance., Science. (1996). doi:10.1126/science.271.5249.665.
[50] Z. Gao, D. Hwang, F. Bataille, M. Lefevre, D. York, M.J. Quon, J. Ye, Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex, J. Biol. Chem. (2002). doi:10.1074/jbc.M209459200.
[51] V.A. Pavlov, K.J. Tracey, Neural regulation of immunity: Molecular mechanisms and clinical translation, Nat. Neurosci. (2017). doi:10.1038/nn.4477.
[52] B. Obermeier, R. Daneman, R.M. Ransohoff, Development, maintenance and disruption of the blood-brain barrier, Nat. Med. (2013). doi:10.1038/nm.3407.
[53] M. Prinz, J. Priller, The role of peripheral immune cells in the CNS in steady

state and disease, Nat. Neurosci. (2017). doi:10.1038/nn.4475.
[54] K.I. Mosher, T. Wyss-Coray, Microglial dysfunction in brain aging and Alzheimer's disease, Biochem. Pharmacol. (2014). doi:10.1016/j.bcp.2014.01.008.
[55] R. Yirmiya, I. Goshen, Immune modulation of learning, memory, neural plasticity and neurogenesis, Brain. Behav. Immun. (2011). doi:10.1016/j.bbi.2010.10.015.
[56] J.C. Felger, A.H. Miller, Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise, Front Neuroendocr. 33 (2012) 315–327. doi:10.1016/j.yfrne.2012.09.003.
[57] B. Liu, Role of Microglia in Inflammation-Mediated Neurodegenerative Diseases: Mechanisms and Strategies for Therapeutic Intervention, J. Pharmacol. Exp. Ther. (2003). doi:10.1124/jpet.102.035048.
[58] J.S. Lou, Physical and mental fatigue in Parkinson's disease: epidemiology, pathophysiology and treatment, Drugs Aging. 26 (2009) 195–208. doi:10.2165/00002512-200926030-00002.
[59] C.E. Garber, J.H. Friedman, Effects of fatigue on physical activity and function in patients with Parkinson's disease, Neurology. 60 (2003) 1119–1124.
[60] R. Báez-Mendoza, W. Schultz, The role of the striatum in social behavior, Front. Neurosci. (2013). doi:10.3389/fnins.2013.00233.
[61] J.R. Hollerman, L. Tremblay, W. Schultz, Involvement of basal ganglia and orbitofrontal cortex in goal-directed behavior, in: Prog. Brain Res., 2000. doi:10.1016/S0079-6123(00)26015-9.
[62] B.E. Fisher, Q. Li, A. Nacca, G.J. Salem, J. Song, J. Yip, J.S. Hui, M.W. Jakowec, G.M. Petzinger, Treadmill exercise elevates striatal dopamine D2 receptor binding potential in patients with early Parkinson's disease, Neuroreport. (2013). doi:10.1097/WNR.0b013e328361dc13.
[63] T.E. Foley, M. Fleshner, Neuroplasticity of dopamine circuits after exercise: implications for central fatigue, Neuromolecular Med. 10 (2008) 67–80. doi:10.1007/s12017-008-8032-3.
[64] S.P. Bailey, J.M. Davis, E.N. Ahlborn, Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue., J. Appl. Physiol. 74 (1993) 3006–12. doi:10.1152/jappl.1993.74.6.3006.
[65] A.R. Sigwalt, H. Budde, I. Helmich, V. Glaser, K. Ghisoni, S. Lanza, E.L. Cadore, F.L.R. Lhullier, A.F. de Bem, A. Hohl, F.J. de Matos, P.A. de Oliveira, R.D. Prediger, L.G.A. Guglielmo, A. Latini, Molecular aspects involved in swimming exercise training reducing anhedonia in a rat model of depression, Neuroscience. 192 (2011). doi:10.1016/j.neuroscience.2011.05.075.
[66] N. Franscini, N. Blau, R.B. Walter, A. Schaffner, G. Schoedon, Critical role of interleukin-1beta for transcriptional regulation of endothelial 6-pyruvoyltetrahydropterin synthase, Arter. Thromb Vasc Biol. 23 (2003) e50-3. doi:10.1161/01.ATV.0000099785.65848.F1.
[67] E.R. Werner, N. Blau, B. Thöny, Tetrahydrobiopterin: biochemistry and pathophysiology, Biochem. J. 438 (2011) 397–414. doi:10.1042/BJ20110293.
[68] B. Thony, G. Auerbach, N. Blau, Tetrahydrobiopterin biosynthesis, regeneration and functions, Biochem J. 347 Pt 1 (2000) 1–16.
[69] S. Mrakic-Sposta, M. Gussoni, S. Moretti, L. Pratali, G. Giardini, P. Tacchini, C. Dellanoce, A. Tonacci, F. Mastorci, A. Borghini, M. Montorsi, A. Vezzoli, Effects of mountain ultra-marathon running on ROS production and oxidative damage by micro-invasive analytic techniques, PLoS One. (2015).

doi:10.1371/journal.pone.0141780.

[70] K. Ghisoni, A. Latini, Kuehne LK, Reiber H, Bechter K, Hagberg L, Fuchs D., Cerebrospinal fluid neopterin is brain-derived and not associated with blood-CSF barrier dysfunction in non-inflammatory affective and schizophrenic spectrum disorders. Journal of Psychiatric Research, , J. Psychiatr. Res. 63 (2015). doi:10.1016/j.jpsychires.2015.02.002.

[71] R. de P. Martins, K. Ghisoni, C.K.C.K. Lim, A.S.A. Jr, G.J.G.J. Guillemin, A. Latini, R. de Paula Martins, K. Ghisoni, C.K.C.K. Lim, A.S. Aguiar, G.J.G.J. Guillemin, A. Latini, Neopterin preconditioning prevents inflammasome activation in mammalian astrocytes, Free Radic. Biol. Med. in press (2018). doi:10.1016/j.freeradbiomed.2017.11.022.

[72] K.M.C. Dallagnol, A.P. Remor, R.A. da Silva, R.D. Prediger, A. Latini, A.S. Aguiar, Running for REST: Physical activity attenuates neuroinflammation in the hippocampus of aged mice, Brain. Behav. Immun. 61 (2016) 31–35. doi:10.1016/j.bbi.2016.07.159.

[73] T. Tuon, P.S. Souza, M.F. Santos, F.T. Pereira, G.S. Pedroso, T.F. Luciano, C.T. De Souza, R.C. Dutra, P.C.L. Silveira, R.A. Pinho, Physical training regulates mitochondrial parameters and neuroinflammatory mechanisms in an experimental model of Parkinson's disease, Oxid. Med. Cell. Longev. (2015). doi:10.1155/2015/261809.

[74] T. Kizaki, T. Maegawa, T. Sakurai, J. etsu Ogasawara, T. Ookawara, S. Oh-ishi, T. Izawa, S. Haga, H. Ohno, Voluntary exercise attenuates obesity-associated inflammation through ghrelin expressed in macrophages, Biochem. Biophys. Res. Commun. (2011). doi:10.1016/j.bbrc.2011.08.117.

[75] L.A. Silva, C.A. Pinho, K.S. Scarabelot, D.B. Fraga, A.M.J. Volpato, C.R. Boeck, C.T. Souza, E.L. Streck, R.A. Pinho, Physical exercise increases mitochondrial function and reduces oxidative damage in skeletal muscle, Eur. J. Appl. Physiol. (2009). doi:10.1007/s00421-008-0971-8.

Figure 1: **Effect of acute and sustained bacterial lipopolysaccharide (LPS) administration on glucose and insulin tolerance**. **A**) Oral glucose tolerance test (OGTT, 2 g/kg, p.o) performed 6 h after acute intraperitoneal injection of vehicle (saline, *n*=3/group) or LPS (2 mg/kg, *n*=3/group; repeated measure one-way ANOVA followed by the Bonferroni's test (d = 1.14). **A'**) AUC of glucose response; Student's *t*-test. **B**) Insulin tolerance test (ITT, 0.5 U/kg, i.p.) performed 6 h after injection of vehicle (saline, *n*=3) or LPS (2 mg/kg or 0.33 mg/kg, *n*=3/group); repeated measure one-way ANOVA followed by the Bonferroni's test ($\eta^2 = 0.108$). **B'**) AUC of insulin response; one-way ANOVA followed by the Bonferroni's test. **C**) Experimental design 1. C57BL/6 3-month-old mice, received a single LPS (0.33 mg/kg, i.p) or vehicle (saline) injection per day, for 2 weeks. After fasting overnight, the OGTT test was performed. **D**) Food intake during 13 days with repeated daily injection of vehicle (saline, *n*=3) or LPS (0.33 mg/kg/day, i.p, *n*=3/group); Student's *t*-test. **E**) OGTT performed at the end of the repeated daily injection of vehicle (saline, *n*=3/group) or LPS (0.33 mg/kg, i.p, *n*=3/group); repeated measure one-way ANOVA followed by the Bonferroni's test (d = -0.62). **E'**) AUC of glucose response after repeated LPS injections; Student's *t*-test. Values are mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 LPS 2 mg/kg or LPS 0.33 mg/kg vs. control group.

Figure 2: Effect of bacterial lipopolysaccharide (LPS) administration on exercise performance, urinary neopterin and monoamines levels in the striatum. A) Experimental design 2. The animals were adapted to the treadmill for 5 consecutive days at a speed of 10 m/min for 10 min**.** The incremental loading test was performed 4 h and 24 h after a single dose of LPS injection (0.33 mg/kg, i.p). **B**) Mouse physical performance on treadmill incremental loading test (*n*=11/group); Log-rank (Mantel-Cox). **C**) Vertical and horizontal workloads during incremental loading test; one-way ANOVA followed by the Bonferroni test. **D**) Experimental design 3. Sustained administration of LPS (0.33 mg/kg/day) by mini-osmotic pumps connected to an intraperitoneal catheter (Alzet® model 2002) for 2 weeks. **E**) Body weight and **F**) urinary neopterin production (*n*=5/group); repeated measure one-way ANOVA followed by the Bonferroni's test. **G**) Dopamine (DA), **H**) 3,4-dihydroxyphenyilacetic acid (DOPAC), **I**) Serotonin (5-HT) levels and **J**) 5-HT/DA ratio (*n*=5/group); Student's *t*-test. Values are mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 LPS 0.33 mg/kg vs. control group.

Figure 3: Effect of exercise on the inflammatory response. **A**) Experimental design 4. The animals submitted to physical exercise were adapted to a treadmill for 5 consecutive days at a speed of 10 m/min for 10 min**,** followed by 3 weeks of moderate exercise on the treadmill. Animals ran at a speed of 16.5 m/min with a progressive increase in time over the 3 following weeks (30, 40 and 50 min/day). Afterwards, mini-osmotic pumps containing LPS 0.83 mg/kg/day were implanted and LPS was infused for 2 weeks. **B**) Neopterin urinary levels; repeated measure two-way ANOVA followed by the Bonferroni's test. **C**) Complex I activity in skeletal muscle (*n*=5/group); two-way ANOVA followed by the Tukey's test. Values are mean ± SEM. B) **P<0.001 Sed LPS 0.83 mg/kg/day vs. Sed control group, **P<0.001 Exe control vs. Sed control, *P<0.05 Ex control vs. Exe LPS 0.83 mg/kg/day group, # P<0.001 Sed LPS 0.83 mg/kg/day vs. Exe LPS 0.83 mg/kg/day group; C) *P<0.05 Sed LPS 0.83 mg/kg/day vs. Sed control.

Highlights

- Repeated LPS injections decreased glucose tolerance;
- Continuous LPS infusion increased immune system activation;
- Continuous LPS infusion decreased striatal dopamine levels;
- Running exercise prevented exacerbation of immune system activation;
- Running exercise preserved muscle mitochondrial activity.

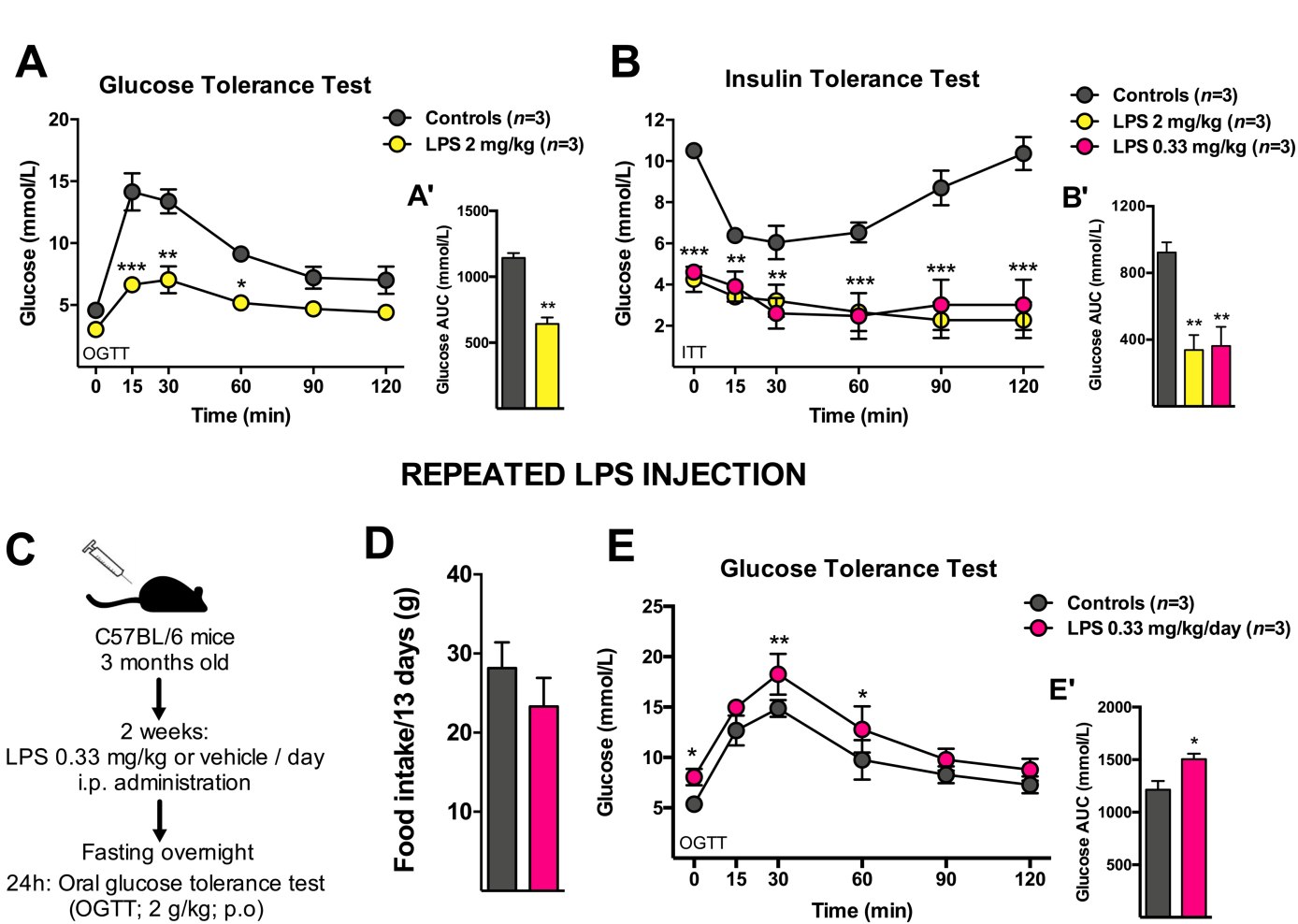
ACUTE LPS INJECTION
A
Glucose Tolerance Test
Controls (n=3)
LPS 2 mg/kg (n=3)
Glucose (mmol/L)
OGTT
Time (min)
A'
Glucose AUC (mmol/L)
B
Insulin Tolerance Test
Controls (n=3)
LPS 2 mg/kg (n=3)
LPS 0.33 mg/kg (n=3)
Glucose (mmol/L)
ITT
Time (min)
B'
Glucose AUC (mmol/L)
REPEATED LPS INJECTION
C
C57BL/6 mice
3 months old
2 weeks:
LPS 0.33 mg/kg or vehicle / day
i.p. administration
Fasting overnight
24h: Oral glucose tolerance test
(OGTT; 2 g/kg; p.o)
D
Food intake/13 days (g)
E
Glucose Tolerance Test
Controls (n=3)
LPS 0.33 mg/kg/day (n=3)
Glucose (mmol/L)
OGTT
Time (min)
E'
Glucose AUC (mmol/L)

Figure 1

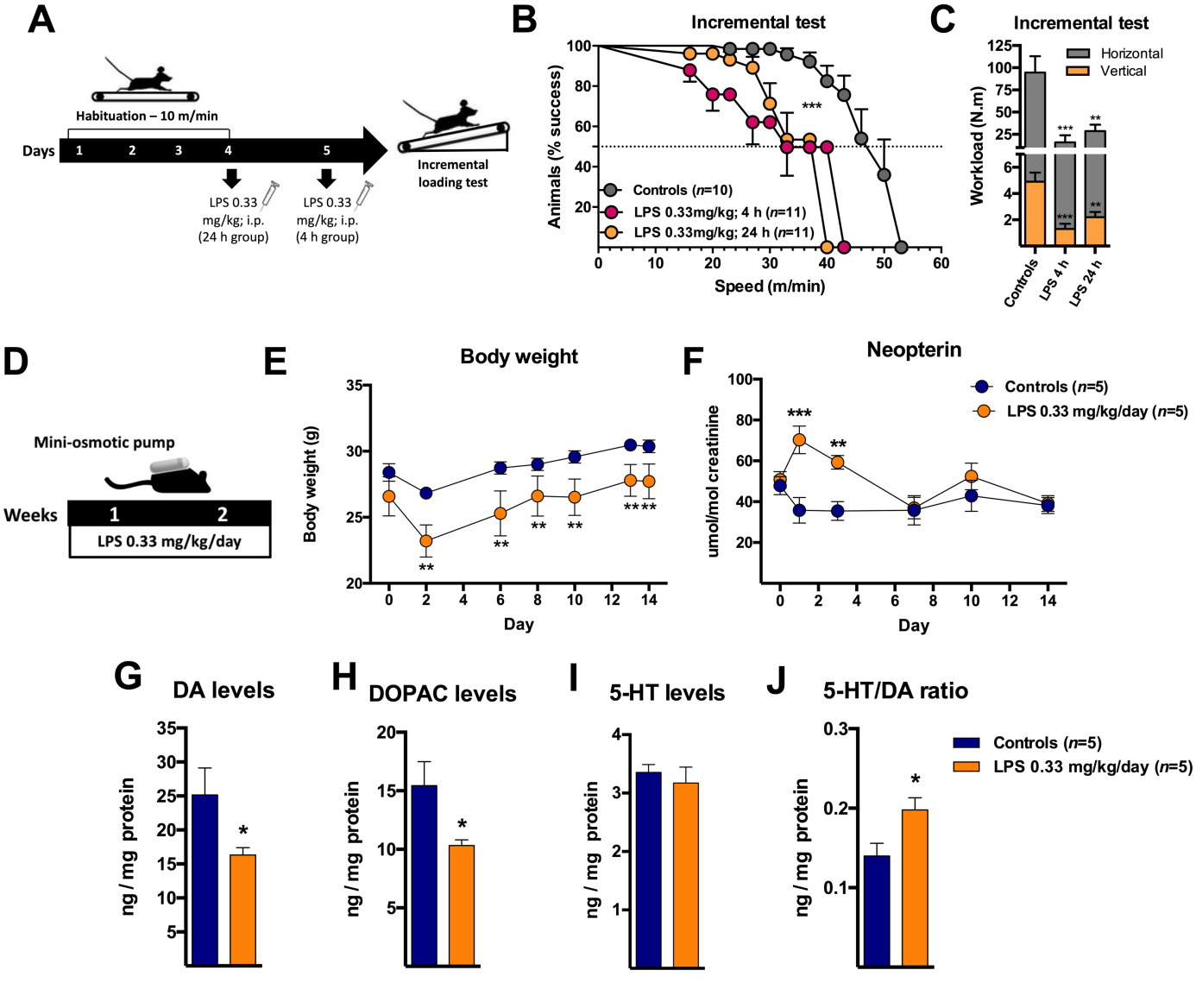
A
Habituation – 10 m/min
Days
1
2
3
4
5
LPS 0.33 mg/kg; i.p. (24 h group)
LPS 0.33 mg/kg; i.p. (4 h group)
Incremental loading test
B
Incremental test
Animals (% success)
Speed (m/min)
Controls (n=10)
LPS 0.33mg/kg; 4 h (n=11)
LPS 0.33mg/kg; 24 h (n=11)
***
C
Incremental test
Workload (N.m)
Horizontal
Vertical
Controls
LPS 4 h
LPS 24 h
D
Mini-osmotic pump
Weeks
1
2
LPS 0.33 mg/kg/day
E
Body weight
Body weight (g)
Day
F
Neopterin
umol/mol creatinine
Controls (n=5)
LPS 0.33 mg/kg/day (n=5)
Day
G
DA levels
ng / mg protein
H
DOPAC levels
ng / mg protein
I
5-HT levels
ng / mg protein
J
5-HT/DA ratio
ng / mg protein
Controls (n=5)
LPS 0.33 mg/kg/day (n=5)

Figure 2

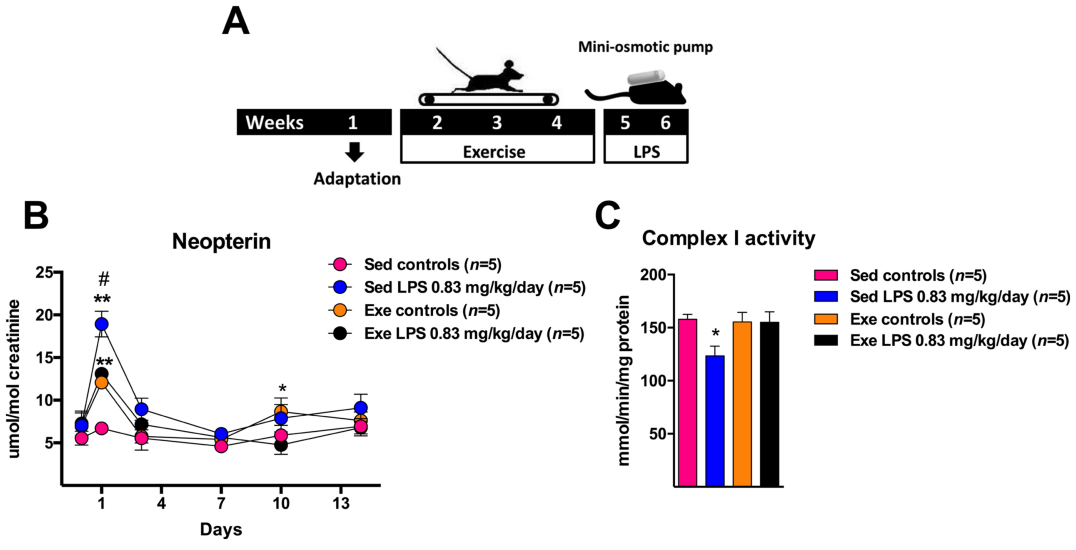
A
Mini-osmotic pump
Weeks 1
2 3 4
5 6
Exercise
LPS
Adaptation
B
Neopterin
Sed controls (n=5)
Sed LPS 0.83 mg/kg/day (n=5)
Exe controls (n=5)
Exe LPS 0.83 mg/kg/day (n=5)
umol/mol creatinine
Days
C
Complex I activity
mmol/min/mg protein
Sed controls (n=5)
Sed LPS 0.83 mg/kg/day (n=5)
Exe controls (n=5)
Exe LPS 0.83 mg/kg/day (n=5)

Figure 3