Published in final edited form as:

Brain Behav Immun. 2019 January ; 75: 181–191. doi:10.1016/j.bbi.2018.10.005.

Exercise activates vagal induction of dopamine and attenuates systemic inflammation

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Abstract

Physical exercise is one of the most important factors improving quality of life, but it is not feasible for patients with morbidity or limited mobility. Most previous studies focused on high-intensity or long-term exercise that causes metabolic stress or physiological adaption, respectively. Here, we studied how moderate-intensity swimming affects systemic inflammation in 6–8 week old C57BL/6J male mice during endotoxemia. One-hour swimming prevented hypokalemia, hypocalcemia, attenuated serum levels of inflammatory cytokines, increased anti-inflammatory cytokines but affected neither IL6 nor glycemia before or after the endotoxic challenge. Exercise attenuated serum TNF levels by inhibiting its production in the spleen through a mechanism mediated by the subdiaphragmatic vagus nerve but independent of the splenic nerve. Exercise increased serum levels of dopamine, and adrenalectomy prevented the potential of exercise to induce dopamine and to attenuate serum TNF levels. Dopaminergic agonist type-1, fenoldopam, inhibited TNF production in splenocytes. Conversely, dopaminergic antagonist type-1, butaclamol, attenuated exercise control of serum TNF levels. These results suggest that vagal induction of dopamine may contribute to the anti-inflammatory potential of physical exercise.

Keywords

Exercise; Inflammation; Dopamine; Tumor Necrosis Factor; Endotoxemia; Sepsis

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Financial interests

The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2018.10.005>.

1. Introduction

Inflammation is critical to fight infections, but at the same time, it is a major clinical challenge in modern medicine contributing to multiple diseases including sepsis. Although sepsis is normally originated by an infection, severe sepsis remains a major clinical challenge in modern medicine killing over 250,000 Americans every year despite the efficacy of the new antibiotics (Angus and van der Poll, 2013; Martin et al., 2003; Ulloa, 2005; Ulloa and Tracey, 2005). In addition to the infection, severe sepsis is also characterized by the overzealous production of inflammatory cytokines that cause detrimental systemic inflammation. New antibiotics are more effective in controlling infections, but they do not control deleterious inflammation. Among the inflammatory cytokines, Tumor Necrosis Factor (TNF) is a critical factor regulating the innate immune responses to infection or trauma (Angus, 2007; Hershey and Kahn, 2017; Martin et al., 2003; Sands et al., 1997; Ulloa and Deitch, 2009). However, excessive TNF production becomes more dangerous than the original infection and causes systemic inflammation, cardiovascular shock, and lethal multiple organ failure in sepsis (Cai et al., 2009; Tang et al., 2012; Tracey et al., 1987). Inhibition of TNF production prevents septic shock, organ damage and improves survival in experimental endotoxemia, bacteremia, and sepsis (Feketeova et al., 2018; Remick et al., 1995; Tracey et al., 1987). TNF is not only produced during infections, and it also plays a critical role in multiple aseptic inflammatory disorders such as rheumatoid arthritis (Bassi et al., 2017; Koopman et al., 2016). In addition to TNF, sepsis is also characterized by the production of other inflammatory factors such as interleukin IL6 and interferon $\text{INF-}\gamma$ that contribute to systemic inflammation and multiple organ failure. Thus, recent efforts focus on studying the mechanisms regulating the production of multiple inflammatory factors and their potential clinical translation for the treatment of infectious and inflammatory disorders.

Epidemiological studies show that physical exercise is among the most important factors regulating the immune system and improving quality of life (Gleeson, 2000; Gleeson et al., 2011; Gleeson et al., 2006; Hotamisligil, 2006; Mathis and Shoelson, 2011; Nieman, 2012; Ouchi et al., 2011; Pedersen and Saltin, 2006, 2015; Pradhan et al., 2001). Exercise decreases the risk of multiple conditions including cardiovascular diseases, hypertension, atherosclerosis, metabolic syndrome, diabetes, arthritis, pulmonary disorders, dementia, and various types of cancers (Agarwal et al., 2009; Bagby et al., 1994; Fallon et al., 2001; Khansari et al., 2009; Kim et al., 2014; Masson et al., 2014; Milani et al., 2004; Petersen and Pedersen, 2005). Long-term regular exercise prevents metabolic and immune disorders, but it is not a feasible option for patients with morbidity or limited mobility. Thus, recent studies focus on the mechanisms induced by exercise to control inflammation and their potential clinical translation for treating infectious and inflammatory disorders. Most studies on exercise focus on high-intensity or long-term regular exercise that causes metabolic stress or physiological adaptation (Agarwal et al., 2009; Bagby et al., 1994; Fallon et al., 2001; Khansari et al., 2009; Kim et al., 2014; Masson et al., 2014; Milani et al., 2004; Petersen and Pedersen, 2005). Intense anaerobic exercise induces metabolic stress including hypoglycemia (Gleeson, 2000; Gleeson et al., 2011; Hotamisligil, 2006; Mathis and Shoelson, 2011; Nieman, 2012; Ouchi et al., 2011), whereas long-term training induces

physiological adaptation improving resting heart rate, respiratory sinus arrhythmia, cardiac vagal tone and immune regulation (Ekblom et al., 1973; van Lien et al., 2011). Long-term regular training appears to regulate the immune system by inducing metabolic and epigenetic adaptive mechanisms. Regular exercise reduces visceral fat mass and thereby prevents disorders associated with obesity (Hotamisligil, 2006; Mathis and Shoelson, 2011; Nieman, 2012; Ouchi et al., 2011). Thus, long-term training can also prevent inflammation by decreasing the production of adipokines, inflammatory factors produced by adipocytes and fat tissue (Gleeson et al., 2011; Ouchi et al., 2011). Likewise, regular training also induces adaptive epigenetic modifications in immune and non-immune tissue reducing the production of inflammatory factors in monocytes, macrophages (Flynn and McFarlin, 2006; Pedersen and Febbraio, 2008) and skeletal muscle (Pedersen and Febbraio, 2008; Wang et al., 2012; Yeh et al., 2006). Indeed, athletes have significant lower resting levels of inflammatory biomarkers including TNF and IL6 (Nieman et al., 2005). Here, we analyzed whether one session of aerobic, moderate-intensity exercise can regulate inflammation and the innate immune responses to bacterial endotoxin without affecting glycemia or before inducing physiological adaptation. We also analyzed the physiological mechanisms contributing to exercise control of the immune system and whether they are mediated by vagal modulation of the immune system.

2. Material and methods

2.1. Chemicals and reagents

LPS (*E. Coli* 0111:B4), dopamine hydrochloride, fenoldopam, and pergolide were purchased from Sigma-Aldrich® (Saint Louis, MO) and dissolved in sterile, pyrogen-free PBS (Gibco®, Life Technologies, Grand Island, NY). The glucose measuring strips were purchased from PharmaTech Solutions, Inc. (Westlake village, CA). Butaclamol (15 mg/kg body weight i.p.) was given 90 min before and/or after exercise. Pentobarbital sodium was purchased from Diamondback (Scottsdale, AZ); ketamine from Henry Schein animal health (Dublin, OH); xylazine from Akorn animal health (Lake Forest, IL, USA) and enrofloxacin from Bayer Health care (Shawnee Mission, KS, USA).

2.2. Animal experiments

All experimental procedures adhered to *The Guide for the Care and Use of Laboratory Animals* as published by the National Institutes of Health (Copyright © 1996 by the National Academy of Sciences), and were approved by the Institutional Animal Care & Use Committee of the Rutgers New Jersey Medical School. All animal experiments were performed in 6–8 week old ($\approx 25 \pm 5$ g) C57BL/6J male mice from Charles River Laboratories (Wilmington, MA). The animals were maintained at room temperature 70–75F, air humidity 40–70%, 12 h light/dark cycle, with free access to food and water (*ad libitum*) until experimentation. The investigators analyzing the samples were blinded to the experimental treatments. Aerobic exercise protocol. Aerobic moderate-intensity swimming protocol was designed similar as previously described (Evangelista et al., 2003; Trevellin et al., 2014). We adapted a heated bath tank of 50 cm long, 40 cm width and 20 cm height for mice swim. To prevent floating, water bubbling was produced by tubes connected to the air pump system. A heating system kept the water temperature between 30 and 32 °C. Mice

swam one bout for 1 or 2 h, and rested for 1 h before receiving an endotoxin challenge. During training, mice swam for 1 or 2 h every day for 1 (control) or 7 (training) days prior to endotoxemia. Then, mice were injected LPS at 1, 24, 48 or 72 h after exercise, and serum TNF levels were measured at 1.5 h post-LPS. *Endotoxemia* was performed as we previously described in Nat Med (Wang et al., 2004) with the modifications described in Nat Med (Torres-Rosas et al., 2014). Briefly, endotoxin (*E. coli* LPS 0111:B4; Sigma Chemical, Saint Louis, MO) was dissolved in sterile, pyrogen-free PBS (Gibco®: Life Technologies, Grand Island, NY), and sonicated for 20 min immediately before use. Animals received a LD₅₀ dose of LPS (10 mg/kg, i.p.).

2.3. Ablative surgeries

Animals were anesthetized with intraperitoneal rodent cocktail of 100 mg/kg ketamine and 20 mg/kg xylazine. Anesthesia was confirmed by the absence of withdrawal reflex to toe pinch. Ablative surgeries were performed 3 days before the experimental procedure. Antibiotics (Enrofloxacin 2.5 mg/kg, s.c.; Baytril®, Bayer Health Care™, Swanee Mission, KA) were given only to those animals with ablative surgery started right after surgery and given every 12 h until 24 h before the endotoxic challenge. *Splenectomy*: was performed as we described in J Exp Med (Huston et al., 2006b). Anesthetized animals were subjected to an abdominal incision in the epigastrium and mesogastrium. The spleen was exposed by gentle retraction of the stomach to the side. The three main branches of the spleen artery were stabilized with nylon thread, ligated and cut. The spleen was removed and the wound sutured. *Adrenalectomy*: was performed as we previously described (Vida et al., 2011a). A dorsal incision from the first to the third lumbar vertebrae was performed on anesthetized animals. The latissimus dorsi muscle was dissected and pulled away on both sides until the kidneys were visible and both adrenal glands were removed. *Surgical Cervical Vagotomy* (VGX) was performed as we described (Huston et al., 2006b). Briefly, animals were subjected to an anterior ventral incision on the neck to access the sternocleidomastoid muscle. The sternocleidomastoid muscle was dissected to visualize the carotid artery and the vagus nerve. The right cervical vagus trunk was ligated with size 4–0 silk sutures and sectioned. Sham animals underwent the same procedure but the nerve was not sectioned. *Subdiaphragmatic vagotomy (sVNX)* was performed as previously described in Nat Med (Torres-Rosas et al., 2014). Animals underwent an abdominal incision covering the epigastrium and mesogastrium. The esophagus was exposed at the juncture to the stomach. Subsequently, the two vagal branches on both sides of the dorsal part of the esophagus were exposed by gently pulling down and twisting the stomach and stabilized with nylon thread. *Splenic neurectomy*, was performed as we previously described (Vida et al., 2011a). Briefly, abdominal incision was made to explore and isolate the splenic vessels. After isolation of the splenic vessels, fibers of splenic nerve were observed and sectioned with sharp forceps under aseptic conditions.

2.4. Blood and cytokine analyses

Serum cytokines and blood glucose levels were analyzed at the indicated time points. Serum samples were obtained by clotting the blood for 2 h at room temperature, and centrifuged at 2000g for 15 min at 4 °C. TNF, IL6, IL10, TGFβ1 and IFNγ were analyzed in the endotoxemic mice with the respective ELISA kit (Affymetrix Inc., San Diego, CA). TNF

concentrations in organs were normalized to organ protein concentration as measured using Bradford assay as we described (Huston et al., 2006b). Serum HMGB1 was analyzed using HMGB1 detection ELISA kit (Chondrex Inc., Redmond, WA). Blood catecholamines were determined by ELISA (LDN immunoassays and services, Germany) at 90 min post-stimulation as we described (Vida et al., 2011a; Vida et al., 2011b). Glucose was analyzed from the mouse tail tip blood using the Genstrip (PharmaTech Solutions Inc. Westlake village, CA) and the Onetouch UltraMini glucometer, (LifeScan Inc., Milpitas, CA). The absorbance values (A_{450}) of ELISAs were measured using VERSA max microplate reader (Molecular Devices, Sunnyvale, CA) and analyzed by SoftMax Pro 3.9.1.

2.5. Statistical analyses

All tests were performed using the GraphPad Prism Software® (GraphPad Software, La Jolla, CA). Sample size was determined using standard deviation values and power analyses of our previous studies on the vagus nerve stimulation (Huston et al., 2006b; Vida et al., 2011a). All data in the figures are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using the student's *t*-test (Mann-Whitney *U* test) to compare mean values between two experimental groups. Analyses of three or more groups were performed using the one-way ANOVA with multiple pair-wise comparisons. The time courses and pair-wise comparisons were analyzed with the two-way ANOVA for repeated measures. Normality and homogeneity of variance were confirmed using the Kolmogorov-Smirnov analysis. Statistical analyses of survival were determined using the Logrank (Mantel-Cox) test. $p < 0.05$ were considered statistically significant and represented as: #Student's *t*-test, +One-way ANOVA, *Two-way ANOVA, §Survival Log-rank test.

3. Results

3.1. One session of aerobic, moderate exercise attenuated systemic inflammation in endotoxemia

In order to analyze the direct effects of physical exercise on the immune system, we selected aerobic, moderate-intensity exercise that minimizes physiological metabolic stress (Grimonprez et al., 2015; Krahl et al., 2004). Thus, we first analyzed whether a single session of swimming modulates the innate immune responses to bacterial endotoxin. Swimming for 1 or 2 h before endotoxemia attenuated serum TNF levels by around 30–40% at 1.5 h post-LPS as compared to mice without exercise (Fig. 1a). As we selected moderate exercise to avoid metabolic stress, we analyzed whether this exercise affected glycemia. Swimming for 1 h affected neither glycemia before or after the septic challenge (Fig. 1b). Time course experiments showed that exercise significantly attenuated serum TNF levels over time and did not merely delay its production (Fig. 1c). By contrast, exercise did not affect serum IL6 levels (Fig. 1d). Exercise also attenuated the production of other inflammatory factors such as IFN γ and HMGB1, which are 'late' factors produced at 4–6 h and 18–24 h post-LPS, respectively (Fig. 1e, f). Swimming for 1 h also increased the production of anti-inflammatory cytokines such as TGF β 1 and IL10 for up to 6 h post-LPS (Fig. 1g, h). These results showed that a single session of swimming for 1 h modulated the innate immune responses to bacterial endotoxin without affecting glycemia before or after the septic challenge.

3.2. Exercise improved blood chemistry and survival in endotoxemia

Next, we analyzed whether exercise improves blood chemistry and mice survival in endotoxemia. Blood chemistry analyses showed that mice with or without exercise had similar anion gap, natremia, and chloremia (Figs. 2a and S1a, b). Swimming for 1 h did not prevent early hypokalemia at 3 h but significantly improved potassium levels at 24 h post-LPS (Fig. 2b). Exercise prevented blood hypocalcemia, ionized calcium, and total carbon dioxide levels both at 3 h and 24 h post-LPS (Fig. 2c, d). Exercise did not prevent early uremia at 3 h but significantly improved blood urea nitrogen levels at 24 h post-LPS (Fig. 2e). Animals with exercise had slightly but not significant higher levels of creatinine at 24 h post-LPS (Fig. 2f). The ratio blood urea nitrogen to creatinine may suggest an overall beneficial effect of exercise in kidney function at 24 h post-LPS (Fig. 2g). Next, we analyzed whether exercise improves survival in endotoxemia. One single session of 1 h swimming prior endotoxemia did not improve mice survival (Fig. 3a). Given that most of the animals died around 2 days post-LPS, we analyzed whether exercising every day for 7 days prior endotoxemia can further decrease inflammation and improve survival. Mice swam for 1 h or 2 h every day for 1 or 7 days prior endotoxemia. Swimming for 1 or 2 h every day during 7 days inhibited serum TNF levels by around 40% similar to that of a single bout of exercise for one day (Fig. 3b, c). Next, we analyzed whether swimming every day for 7 days induces a more lasting anti-inflammatory mechanism. Mice swam for 1 h a day during 1 or 7 days. Then, mice were injected LPS at 1, 24, 48 or 72 h after the last bout of exercise, and serum TNF levels were measured at 1.5 h post-LPS. Swimming one time for 1 h attenuated TNF production even when the LPS was injected at 24 h after the exercise (Fig. 3d). Swimming 1 h every day for 7 days induced a more lasting effect, and attenuated TNF production even when the LPS was injected at 72 h after the last bout of exercise. Training for 7 days also improved mice survival in experimental endotoxemia (Fig. 3e). Survival was recorded for two weeks and no late deaths were observed, suggesting that training improved survival and did not merely delay mortality.

3.3. Exercise inhibited splenic TNF production through subdiaphragmatic vagus nerve

In order to study how exercise attenuates serum TNF levels, we analyzed organ TNF levels. Bacterial endotoxin induced TNF production in all the organs but the highest TNF concentrations were found in the spleen (Fig. 4a). Mice with exercise have similar TNF levels in all the other organs, but around 40% lower splenic TNF levels than control endotoxemic mice without exercise (Fig. 4a). These results suggested that exercise attenuated serum TNF levels by inhibiting its production in the spleen. Thus, we analyzed whether splenectomy can affect exercise control of inflammation. Exercise attenuated serum TNF levels by 40% in sham but not in splenectomized mice, showing that splenectomy prevented exercise control of serum TNF levels (Fig. 4b). Given that exercise can increase the vagal tone (Bonaz et al., 2017; Carnevali and Sgoifo, 2014) and that the vagus nerve can inhibit splenic TNF production (Borovikova et al., 2000; Huston et al., 2006a; Huston et al., 2006b), we analyzed whether the vagus nerve contributes to the effects of exercise. Unilateral right cervical vagotomy did not prevent the anti-inflammatory potential of exercise (Fig. S2). By contrast, bilateral cervical vagotomy abrogated the potential of exercise to attenuate serum TNF levels during endotoxemia (Fig. 4c). We further studied the role of the vagus nerve by performing subdiaphragmatic vagotomy. Total subdiaphragmatic

vagotomy prevented exercise control of serum TNF levels (Fig. 4d). Given that subdiaphragmatic vagus nerve can activate the splenic nerve to inhibit TNF production in the spleen (Peña et al., 2011; Rosas-Ballina et al., 2008; Rosas-Ballina et al., 2011; Vida et al., 2011a), we analyzed the role of the splenic nerve by performing surgical neurectomy. Neurectomy of the splenic nerve did not significantly prevent exercise control of serum TNF levels (Fig. 4e). We reasoned that subdiaphragmatic vagus nerve may modulate TNF production in the spleen by inducing soluble factors in another organ. Thus, we isolated primary culture of splenocytes from naïve mice (without exercise) and we incubated them with serum from mice with or without exercise. Then, the splenocytes were challenged *in vitro* with endotoxin, and TNF production was analyzed in the conditioned culture media. The serum from mice with exercise attenuated TNF production in primary culture of splenocytes from naïve mice without exercise (Fig. 4f). Conversely, we isolated primary culture of splenocytes from mice with or without exercise and challenged them *in vitro* with endotoxin. Primary culture of splenocytes from mice with or without exercise produced similar TNF levels (Fig. 4g). These results showed that exercise attenuated splenic TNF production through serum factors induced by the subdiaphragmatic vagus nerve.

3.4. Dopaminergic D1-antagonist prevented exercise control of serum TNF levels

Given that exercise improved renal function and that the subdiaphragmatic vagus nerve innervates the adrenal glands (Torres-Rosas et al., 2014; Ulloa et al., 2017), we analyzed their role in exercise by performing surgical adrenalectomy. Adrenalectomy prevented the potential of exercise to attenuate serum TNF levels (Fig. 5a). In line with these results, previous studies reported that vagal stimulation can induce the production of dopamine in the adrenal glands (Torres-Rosas et al., 2014; Ulloa et al., 2017), so we analyzed whether exercise induces dopamine. Exercise increased dopamine serum levels by 2-fold for up to 3 h (fig. 5b). We also observed that adrenalectomy prevented the potential of exercise to increase serum dopamine levels (Fig. 5c). These results suggested that dopamine may contribute to the potential of exercise to attenuate splenic TNF production in endotoxemia. Thus, we analyzed whether dopamine inhibits TNF production in splenocytes. Dopamine inhibited LPS-induced TNF production of primary culture of splenocytes in a concentration dependent manner (Fig. 5d). Given that dopamine signals through either D1-like (D1R, D5R) or D2-like (D2R, D3R, D4R) dopaminergic receptors, we analyzed specific dopaminergic agonists including fenoldopam (a highly selective agonist for D1-like receptors) and pergolide (a selective agonist for D2-like receptors) (Gingrich and Caron, 1993; Grenader and Healy, 1991; Weber et al., 1998). Fenoldopam and pergolide are well-characterized stable and specific dopaminergic agonist with about 100-fold greater affinity for D1-like or D2-like receptors, respectively (Denef et al., 1980; Gorissen and Laduron, 1979; Grenader and Healy, 1991; Tiberi and Caron, 1994; Torres-Rosas et al., 2014; Weber et al., 1998). Fenoldopam was more effective than dopamine and pergolide at inhibiting LPS-induced TNF production in primary culture of splenocytes (Fig. 5d). The role of dopaminergic D1-like receptors in exercise was also confirmed *in vivo* by using butaclamol, a well-characterized D1-receptor antagonist (Denef et al., 1980; Gorissen and Laduron, 1979). Administration of butaclamol before the exercise prevented the potential of exercise to attenuate serum TNF levels in endotoxemia (Fig. 5e). As a control, administration of butaclamol after the exercise did not affect serum TNF levels (Fig. 5f). Together, these

results suggested that exercise induced the production of dopamine, which attenuates TNF production in splenocytes via dopaminergic D1-like receptors.

4. Discussion

Previous studies on exercise focused on high-intensity or long-term regular exercise that causes metabolic stress or physiological adaptation, respectively (Agarwal et al., 2009; Bagby et al., 1994; Fallon et al., 2001; Khansari et al., 2009; Kim et al., 2014; Masson et al., 2014; Milani et al., 2004; Petersen and Pedersen, 2005). Original studies showed that exercise can reduce inflammation chronically (Bradley et al., 2008; Pond, 2002; Vieira et al., 2009) and acutely (Castellani et al., 2014) and increases survival following an immune challenge (Keylock et al., 2008). In order to analyze the direct effects of exercise on the immune system, we selected moderate-intensity aerobic swimming that minimizes metabolic stress. One of the principal physiological responses to physiological stress is hepatic glucogenesis and hyperglycemia. Our results show that one session of 1 h swimming reduced serum TNF levels without affecting glycemia before or after the endotoxemic challenge. Our results concur with previous studies showing that moderate bouts of swimming minimize metabolic stress (Grimonprez et al., 2015; Krahel et al., 2004). However, physiological stress during swimming can cause vagal activation as previously reported in the polyvagal theory (Bassi et al., 2018; Kolacz and Porges, 2018; Porges, 1992). Our results show that one bout of moderate-intensity aerobic swimming induced different effects than that reported in intense or long-term exercise. Intense exercise induced hypoglycemia and increased serum IL6 levels by inducing its production in skeletal muscle (Keller et al., 2001; Starkie et al., 2003). Long-term regular training for four-weeks reduced serum levels of TNF and IL6 in Wistar-Kyoto endotoxemic rats (Chen et al., 2007). By contrast, we show that aerobic swimming for 1 h attenuated serum TNF levels without affecting glycemia or serum IL6 levels in endotoxemic mice. The time course experiments showed that exercise attenuated serum levels of TNF, IFN γ and HMGB1 and did not merely delay the inflammatory responses to bacterial endotoxin. Although ‘early’ inhibition of TNF production could attenuate the production of other cytokines such as HMGB1, several results suggest that exercise can attenuate these late inflammatory cytokines through a TNF-independent mechanism. Indeed, endotoxemic TNF KO-mice have the same cytokine levels as wild-type mice with the exception of lower colony-stimulating factor activity (Marino et al., 1997). Furthermore, exercise actually increased the production of TGF β 1 and IL10, even after the attenuated production of TNF. These results indicate that moderate aerobic exercise attenuated serum TNF levels without affecting glycemia and before the production of anti-inflammatory cytokines.

Exercise also attenuated the production of ‘late’ inflammatory factors such as HMGB1. HMGB1 is a typical example of damage-associated molecular pattern (DAMP), and it is secreted by both activated macrophages and necrotic cells around 15–20 h after the endotoxemic challenge. Thus, inhibition of HMGB1 serum levels revealed the potential of exercise to prevent both macrophage activation and tissue damage. Previous studies on organ function were only performed after long-term training, and four-week daily treadmill training attenuated hepatic and pulmonary injury in endotoxemic rats (Chen et al., 2007). Likewise, eight-week daily treadmill training reduced creatine kinase, thiobarbituric acid

reactive species and carbonyl levels in male Wistar endotoxemic rats (Coelho et al., 2013). In these studies, animals underwent several weeks daily training, which induces cardiovascular adaptation reducing basal heart rate and arterial blood pressure (Chen et al., 2007; Coelho et al., 2013). Thus, it is unknown whether the organ protection was due to these adaptive mechanisms. Our present study shows that a single bout of swimming attenuated organ damage including hypokalemia, hypocalcemia, and improved the ratio blood urea nitrogen to creatinine suggesting kidney protection. However, these effects did not improve mice survival in endotoxemia. Given that most of the animals died around 2 days post-LPS, we reasoned that a single bout of swimming may induce a short-term temporal protection, whereas training for several days may induce a more lasting effect. One-hour swimming attenuated serum TNF levels even when the LPS was injected at 24 h after the exercise. Swimming every day for 7 days, attenuated TNF production, even when the LPS was injected at 72 h after the last exercise, and improved mice survival in endotoxemia. Thus, swimming for 1 h attenuated inflammation and organ dysfunction, but adaptive mechanisms during regular exercise induce lasting effects and improve survival in endotoxemia. Although one bout of exercise is not likely to induce a physiological adaption, future studies with daily training shall consider the cardiovascular and metabolic effects and the effects of exercise (both acute and chronically) on body weight, adiposity and food intake.

In order to focus on the direct effects of exercise on inflammation, we analyzed how 1 h swimming attenuated serum TNF levels. Exercise attenuated splenic TNF levels by around 40% as compared to control mice without exercise. Then, exercise attenuated serum TNF levels in sham but not in splenectomized mice. Given that previous studies reported that exercise can increase the vagal tone (Bonaz et al., 2017; Carnevali and Sgoifo, 2014) and that vagal stimulation can inhibit splenic TNF production (Borovikova et al., 2000; Huston et al., 2006a; Huston et al., 2006b), we analyzed the role of the vagus nerve in exercise control of inflammation. Both, cervical or subdiaphragmatic vagotomy prevented exercise control of serum TNF levels, showing that exercise control of inflammation is mediated by the vagus nerve. These results concur with previous studies showing that vagal stimulation can attenuate TNF production in arthritis (Bassi et al., 2017; Koopman et al., 2016), ischemia/reperfusion (Altavilla et al., 2006; Bernik et al., 2002; Cai et al., 2009), hemorrhage/resuscitation (Cai et al., 2009; de Souza et al., 2010), post-operative recovery (Grech et al., 2016), pancreatitis (Van Westerloo et al., 2006), endotoxemia (Borovikova et al., 2000; Deitch and Ulloa, 2010; Huston et al., 2006b) and severe sepsis (Ulloa, 2011; van Westerloo et al., 2005; Wang et al., 2004). Our results also concur with previous studies showing that exercise increased the vagal cardiovascular tone (Bonaz et al., 2017; Carnevali and Sgoifo, 2014; Ulloa et al., 1998). But, other study reported that exercise modulates cardiovascular but not through the vagus nerve (Neto et al., 2017). Thus, the effects of exercise on vagal activity are controversial depending on the physical activity. However, the previous studies focused on vagal regulation of the cardiovascular system, whereas our studies focused on vagal regulation of the immune system. Furthermore, our results are independent of the vagal regulation of the cardiovascular system because they were prevented by subdiaphragmatic vagotomy. Exercise control of TNF was also independent on the splenic nerve, suggesting that the subdiaphragmatic vagus nerve modulates splenic TNF

production by inducing soluble factors in another organ. Indeed, the serum from mice with exercise attenuated TNF production in primary culture of splenocytes from naïve mice without exercise. These results suggested that exercise attenuated splenic TNF production through serum factors regulated by subdiaphragmatic vagus nerve.

Given that exercise improved renal function and that the vagus nerve can induce the production of dopamine in the adrenal glands (Torres-Rosas et al., 2014; Ulloa et al., 2017), we analyzed whether exercise induces dopamine. Exercise increased serum dopamine levels and adrenalectomy prevented this effect and the control of serum TNF levels. Similar recent studies showed that moderate exercise can induce dopamine, which contributes to the mesolimbic reward pathway during exercise (Mitchell et al., 2018; Robison et al., 2018; Rosso et al., 2018), enhances cognition by activating D1-dopaminergic receptors (McMorris, 2016a,b), protects dopaminergic neurons against inflammation-induced degeneration (Wu et al., 2011), decreases oxidative stress and inflammation, and restores renal dopamine D1 receptors function in elderly rats (Asghar et al., 2007). Our results show that dopamine inhibited LPS-induced TNF production of primary culture of splenocytes in a concentration dependent manner. D1-agonist, fenoldopam, was more effective than dopamine and pergolide at inhibiting TNF production in splenocytes. The role of dopamine in exercise was also confirmed in *vivo* by using butaclamol, a canonical D1-antagonist. Administration of butaclamol attenuated exercise control of serum TNF levels. Although these results suggest that vagal regulation of dopamine contributes to the effects of exercise, future genetic studies in knockout mice will be required to determine whether other factors from the adrenal glands may also contribute to the effects of exercise. These results concur with our previous studies showing that D1-agonist such a fenoldopam can prevent systemic inflammation, organ dysfunction and mortality in endotoxemia and polymicrobial peritonitis (Torres-Rosas et al., 2014). These results warrant future studies to determine how different type of exercise regulates different neuronal networks and their contribution to the cardiovascular, metabolic and immunological benefits induced by exercise. These studies will allow the design of novel therapeutic strategies for the treatment of infectious and inflammatory disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

GS is supported by the CAPES Brazilian Foundation PDSE - 88881.132328/2016-01. LU is supported by the NIH R01-GM114180, Eastern Scholarship JZ2016010, and NSFC #81774429.

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Fig. 1.

Exercise attenuated systemic inflammation. (a) Control (without exercise) or exercise (Ex: swimming for 1 or 2 h) C57BL/6 mice were challenged with LPS (LPS; i.p. 10 mg/kg) and serum TNF levels were analyzed at 1.5 h post-LPS. ⁺p < 0.05 vs LPS (One-way ANOVA; n=4). (b-h) Control or exercise (Ex) mice were challenged with LPS and serum levels of (b) glucose, (c) TNF, (d) IL6, (e) IFN γ , (f) HMGB1, (g) TGF β 1 and (h) IL10 were analyzed at the indicated time points. (*p < 0.05 vs control, Two-way ANOVA; n=3.)

Fig. 2. Exercise improved blood chemistry. Control or exercise (Ex) mice were challenged with LPS and serum levels of (a) anion gap (AnGap), (b) potassium (K), (c) ionized calcium (iCa), (d) total carbon dioxide (TCO2), (e) urea nitrogen (BUN), (f) creatinine (CR), or (g) ratio of blood urea nitrogen to creatinine (BUN/CR) were analyzed at 3 or 24 h post-LPS. * $p < 0.05$ vs control (two-way ANOVA; $n=4$).

Fig. 3.

Training exercise improved survival in endotoxemia. (a) Kaplan-Meier survival analyses of control or exercise (Ex: swimming for 1 h) mice and challenged with LPS (i.p. 10 mg/Kg; n=10). (b, c) Mice swam for 1 h (b) or 2 h (c) a day for 1 (1 d) or 7 (7 d) days, and then mice were challenged with LPS at 1 h after the last bout of exercise. ⁺p < 0.05 vs LPS (One-way ANOVA; n=4). (d) Mice swam for 1 h a day during 1 or 7 days, and were challenged with LPS at 1, 24, 48 or 72 h after the last bout of exercise. Serum TNF levels were measured at 1.5 h post-LPS. (*p < 0.05 vs control, Two-way ANOVA; n=4). (e) Kaplan-Meier survival analyses of control (without exercise) or exercise (Ex: swimming for 1 h every day for 7 days) mice before the LPS challenge (i.p. 10 mg/kg). §p < 0.05 vs control (Survival Log-rank test; n=18).

Fig. 4.

Exercise attenuated survival in endotoxemia. (a) Control or exercise (Ex: swimming for 1 h) mice were challenged with LPS (i.p. 10 mg/kg, n=4) and TNF concentrations in the indicated organs were analyzed at 1.5 h post-LPS. #p < 0.05 vs control (Student's *t*-test; n=3). (b) Mice underwent sham or surgical splenectomy (SPX) 3 days before LPS. (c–e) Control or exercise (Ex) mice underwent sham or surgical (c) bilateral cervical (cVGX) or (d) total subdiaphragmatic (sVGX) vagotomy or (e) surgical neurectomy of the splenic nerve (SNX), 2 days before the LPS. +p < 0.05 vs LPS (One-way ANOVA; n=4). (f) Primary

culture of splenocytes (SPLN) from naïve mice (without exercise) were incubated *in vitro* with serum from control or exercise (Ex) mice for 3 h prior the LPS challenge *in vitro*. TNF levels were analyzed in the conditioned culture media at 3 h post-LPS. (* $p < 0.05$ vs control, Two-way ANOVA; $n=4$). (g) Primary culture of splenocytes from control (without exercise) or exercise (Ex) mice were challenged *in vitro* with LPS.

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Fig. 5.

Dopaminergic D1-antagonist prevented exercise control of TNF. (a) Mice underwent sham or surgical adrenalectomy (ADX) 3 days before LPS. Control or exercise (Ex: swimming for 1 h) mice were challenged with LPS and serum TNF concentrations were analyzed at 1.5 h post-LPS. $^+p < 0.05$ vs LPS (One-way ANOVA; $n=4$). (b) Serum levels of dopamine (DA) were analyzed in control or exercise (Ex) mice at the indicated time points after exercise. ($^*p < 0.05$ vs control, Twoway ANOVA; $n=3$). (c) Mice underwent sham or surgical adrenalectomy (ADX) 3 days before LPS. Control or exercise (Ex) mice were challenged with LPS and serum dopamine (DA) concentrations were analyzed at 1 h post-LPS. $^+p < 0.05$ vs LPS (One-way ANOVA; $n=4$). (d) Primary culture of splenocytes from control mice

were treated with dopamine (DA), fenoldopam (FE) or pergolide (PE) for 1 h before LPS challenge. TNF levels were analyzed in the conditioned culture media at 3 h post-LPS. (* $p < 0.05$ vs control, Two-way ANOVA; $n=3$.) (e, f) Mice were treated with butaclamol at 2 h (e) before or (f) right after exercise. Control or exercise (Ex) mice rested for 1 h and then they were challenged with vehicle or LPS (i.p. 10 mg/kg; $n=4$). Serum TNF concentrations were analyzed at 1.5 h post-LPS. ⁺ $p < 0.05$ vs LPS (One-way ANOVA; $n=4$).

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