Different effects of strength and endurance exercise training on COX-2 and mPGES expression in mouse brain are independent of peripheral inflammation

K. Krüger, J. Bredehöft, F. C. Mooren, and C. Rummel

1Department of Sports Medicine, Justus-Liebig-University Giessen, Giessen, Germany; and 2Institute of Veterinary Physiology and Biochemistry, Justus-Liebig-University Giessen, Giessen, Germany

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Acute and regular exercise training has been shown to modulate memory formation, in particular neuronal plasticity such as synaptogenesis and neurogenesis (39, 42). However, the type of exercise training necessary to ensure these beneficial effects on memory is less clear. While some studies have shown that an increase in cardiovascular fitness is mandatory to achieve (5), others have found that increases in physical activity, by doing strength training, without an increase in cardiovascular fitness also can have beneficial cognitive effects (10). Strength training is accompanied by lower oxygen demands and cardiorespiratory drive than is endurance training and focuses on exercising specific muscle groups. During and after strength training, growth factors and cytokines are released, which might play a role in beneficial regeneration and adaptation processes (11). Moreover, strength training has been shown to be effective in the prevention and treatment of many metabolic and cardiovascular diseases (3). Results obtained from a recent study suggest that a long period of strength training can increase systemic levels of neurotrophic factors, which is accompanied by an improvement of cognitive performance (31). However, no studies have investigated the potential signaling pathways involved in improving cognitive function following strength training.

In general, results obtained from animal studies appear to indicate that an exercise-induced increase in neurotrophic factors, crucial for synaptic efficacy, neuronal connectivity, and use-dependent plasticity, mediate the beneficial effects of exercise (10). In particular, increased levels of brain-derived neurotrophic factor (BDNF) are assumed to induce neurotrophic downstream pathways in different brain regions like the cortex or the hippocampus following exercise training (4). Moreover, it was recently shown that endurance exercise affected cyclooxygenase (COX)-dependent pathways in the brain. COX is widely distributed in central nervous system neurons and thus enables the synthesis of prostaglandins (PGs) from arachidonic acid in the brain. In particular, COX-2 is upregulated in response to external stress signals and inflammatory cytokines (37). Besides mediating inflammatory responses in the brain, COX-2 and microsomal PGE2 synthase (mPGES) also play a role in memory formation (14, 40). In this regard, it was shown that COX inhibitors like ibuprofen inhibit learning-induced increases in PG production. Referring to exercise, it was shown that BDNF and COX interact during use-dependent plasticity, mediate the beneficial effects of exercise (10). In particular, increased levels of brain-derived neurotrophic factor (BDNF) are assumed to induce neurotrophic downstream pathways in different brain regions like the cortex or the hippocampus following exercise training (4). Moreover, it was recently shown that endurance exercise affected cyclooxygenase (COX)-dependent pathways in the brain. COX is widely distributed in central nervous system neurons and thus enables the synthesis of prostaglandins (PGs) from arachidonic acid in the brain. In particular, COX-2 is upregulated in response to external stress signals and inflammatory cytokines (37). Besides mediating inflammatory responses in the brain, COX-2 and microsomal PGE2 synthase (mPGES) also play a role in memory formation (14, 40). In this regard, it was shown that COX inhibitors like ibuprofen inhibit learning-induced increases in PG production.
exercise; how they respond to long-term exercise training or specifically strength training has not been investigated to date. The term “regular exercise training or chronic exercise” describes the process of a repeated series of acute bouts of exercise and regeneration, which leads to a subsequent improvement in baseline physical fitness by inducing functional and structural adaptations (8).

Therefore the aim of the current study was to investigate the effect of a regular strength and endurance training program on cerebral COX-2 expression, inflammatory pathways in the brain, and cerebral cytokines. To identify potential systemic mediators of COX expression, several plasma cytokines were analyzed. We have shown that long-term endurance and resistance training induce COX-2-related pathways independent of peripheral inflammation. Thus the positive effects of this kind of exercise on learning and memory that have previously been suggested might be related to specific changes in the expression of enzymes in the induction of proteaglandins.

METHODS

Animals and experimental groups. Eighteen male C57BL/6N mice (aged 10–12 wk) were randomly assigned to an endurance training group (EG), a strength training group (SG), and a control group (CG), which remained sedentary and served as age-matched controls. The initial body weights can be found in Table 1. Four to six mice per cage were housed at 21 ± 1°C in standard cages with free access to food and tap water. The protocol was approved by the Animal Welfare Officer of the Justus-Liebig-University and the Regierungspräsidium Giessen (no. 94/2010).

Exercise training protocols. All animals were housed on a reverse light-dark cycle (lighting on from 2100 to 0900), and exercise training and testing were conducted during the dark cycle. Strength training was undertaken according to the experimental setup and protocol previously described by us (16). Briefly, mice gripped horizontal wires of a metal mesh, placed in a vertical position, with their front and hind paws. The orientation of the mice was such that their head was held upright. A plate placed surrounding the mesh prevented movement away from it (Fig. 1). Strength training was performed five times per week for 3 min and three series for 10 wk. The break between each series was 1 min. Endurance training consisted of mice running on a treadmill for 30 min/day, five times per week for 10 wk. The break between each series was 1 min. Endurance training consisted of mice running on a treadmill for 30 min/day, five times per week for 10 wk. Running speed was 0.26 ± 0.05 m/s, which corresponded to mice running at about 80% of their \( \text{VO}_{2\text{max}} \). The control group (CG) did not perform any strength or endurance training. The time span between the last training session and tissue preparation was ~4 days to prevent any confounding effects of an acute bout of exercise.

Endurance and strength testing. Isometric strength was analyzed by measuring maximum holding time (MHT) in the same experimental setup described in the strength training protocol (15). Briefly, the time taken for mice to release both back paws from the wire was recorded. Endurance capacity was determined by using a treadmill spirometry (custom-made). After 10 min of acclimatization in the treadmill chamber, mice performed a continuous, progressive exercise test until exhaustion to measure \( \text{VO}_{2\text{max}} \). The test started at 0.15 m/s, and the speed was increased by 0.05 m/s every 3 min.

Blood samples and tissue collection. After the 10-wk training period and at least 4 days after the last training session, mice from the three groups were euthanized, and cardiac blood was collected immediately after killing by using a heparinized syringe. Brains of each animal were carefully excised, weighed, and quickly frozen in liquid nitrogen. Frozen brains were cut using a cryostat (2800 Grigocut E; Reichert-Jung, Nümbloch, Germany). Approximately 22 × 80-μm coronal brain sections were mounted in stacks for the entire hypothalamus [between bregma 0.62 and −1.94 mm; Paxinos et al. (26)] on poly-L-lysine-coated glass slides. The coronal stacks were dissected into the hypothalamus and cortex, also encompassing the hippocampus (cortex/hc) for PCR and Western blot analysis (~35 mg for the hypothalamus; −50 mg for the cortex/hc) and stored at −55°C for RNA extraction or protein isolation.

Western blot. Tissue was homogenized on ice for protein isolation in a lysis buffer (50 mM Tris-HCl, 2 mM EDTA, and 1% Nonidet) with a phosphatase inhibitor (cat. P2714, P5726, Sigma-Aldrich, St. Louis, MO). Supernatants were collected and centrifuged for 15 min (6,000 g) and then stored at −55°C. A Bradford protein assay (cat. B6916; Sigma-Aldrich) was used for the determination of the protein concentration.

Samples were mixed (1:1) with 1 × Laemmli sample buffer (cat. 161-0737; Bio-Rad, Munich, Germany), containing 50 μl β-Mercaptoethanol (cat. 161-0710; Bio-Rad) per 1 ml Laemmli buffer and then incubated at 95°C for 5 min. Then, Precision Plus Protein Western C Standards (4 μl, cat. 161-0376; Bio-Rad Laboratories, Hercules, CA), and 50 μg of protein were loaded on a 10% acrylamide gel and electrophoresed at 120 V for 2 h. Thereafter, the protein was transferred (15 V, 3 mA/cm², 60 min) to a nitrocellulose membrane (cat. 162-0150; Bio-Rad), according to the manufacturer’s instructions for semidry transfer and washed with 0.1% Tween 20 in 1 × Tris-buffered saline (cat. 170–6435; Bio-Rad; TBST) three times for 5 min. Membranes were further incubated with blocking solution (10% nonfat dry milk in TBST) for 2 h at room temperature (RT), followed by application of the first antibodies overnight at 4°C [goat-anti-COX-2, 1:300 (cat. sc-1747, Santa Cruz Biotechnology, Dallas, TX); goat-antinuclear factor (NF)αB (p65), 1:400 (cat. sc-372-G, Santa Cruz Biotechnology); rabbit-anti-β-actin, 1:5,000 (cat. 2066; Sigma-Aldrich Chemie, Steinheim, Germany); and for protein ladder detection, streptavidin horseradish peroxidase (HRP; cat. 161-0380, Bio-

![Fig. 1. External view of a mouse during the isometric strength exercise training.]()
Rad) diluted in TBST and 5% nonfat dry milk. Thereafter, membranes were washed (3 times for 5 min) with TBST and incubated for 2 h at RT with the secondary antibodies (HRP-conjugated donkey-anti-rabbit, 1:2,000, cat. 7074, New England Biolabs, Frankfurt, Germany, or HRP-conjugated donkey-anti-goat, 1:2,000, cat. sc-2020, Santa Cruz Biotechnology), which were diluted in TBST and 5% nonfat dry milk. Subsequently, membranes were washed twice in TBST and once in TBS and incubated for 5 min with the Clarity Western ECL Substrate Kit (cat. 170–5060; Bio-Rad). Molecular Imager (Chemi-Doc XRS Imaging System; Bio-Rad) was used for 5–15 min to detect chemiluminescence. A ratio of trace quantity is presented: COX-2/β-Actin or NF-Actin multiplied by 100. Band sizes were 42 kDa for β-Actin, 72 kDa for COX-2, and 69 kDa for NF-β.

**Real-time PCR.** We used Trizol-total RNA extraction (Invitrogen, Carlsbad, CA) for the hypothalamic and cortical/hippocampus (hc) tissue according to the manufacturer’s protocol. One microgram of RNA was employed for reverse transcription followed by quantitative real-time PCR using TaqMan Universal PCR Master Mix with pre-optimized primer/probe mixture (StepOnePlus Real-Time PCR; TaqMan Gene Expression Assay; Applied Biosystems) as previously described (7). Assay IDs for analyzed genes were as follows: GAPDH, 4352339E-1009032; COX-2, Mm00478374_m1; mPGEs, Mm00452105_m1; 1αB3, Mm00477798_m1. In a previous study, GAPDH was chosen as the best housekeeping gene for normalization of cDNA quantities out of the 12 most commonly used housekeeping genes for the same type of mouse brain tissue (15) using the Primer Design Perfect Probe Norm 12 gene kit mouse and the GeNorm software (cat. ge-PP-12-mo; Primer Design, Southampton, UK). Samples were run in duplicates, and values were calculated using the comparative cycle threshold (ΔΔCT) method as x-fold difference from a control sample [sample from control group PBS, value determined as 1].

**Analysis of plasma cytokines.** Serum was tested for apolipoprotein A-I (Apo A-I), CD40 (CD40), CD40 ligand (CD40-L), C-reactive protein (CRP), endothelin-1 (ET-1), eotaxin, epidermal growth factor (EGF) mouse, factor VII, fibrinogen, fibroblast growth factor 9 (FGF-9), fibroblast growth factor basic (FGF-basic), granulocyte chemotactic protein-2 (GCP-2 mouse), growth-regulated alpha protein (GRO), haptoglobin, immunoglobulin A (IgA), interferon gamma (IFN-gamma), interferon gamma-induced protein 10 (IP-10), interleukin-1 alpha (IL-1 alpha), interleukin-1 beta (IL-1 beta), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-18 (IL-18), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), leukemia inhibitory factor (LIF), lymphotactin, macrophage colony-stimulating factor-1 (M-CSF-1), macrophage inflammatory protein-1 alpha (MIP-1 alpha), macrophage inflammatory protein-1 beta (MIP-1 beta), macrophage inflammatory protein-1 gamma (MIP-1 gamma), macrophage inflammatory protein-2 (MIP-2), macrophage inflammatory protein-3 beta (MIP-3 beta), macrophage-derived chemokine (MDC), matrix metalloproteinase-9 (MMP-9), monocyte chemotactic protein 1 (MCP-1), monocyte chemotactic protein 3 (MCP-3), monocyte chemotactic protein-5 (MCP-5), myeloperoxidase (MPO), myoglobin, oncostatin-M (OSM), stem cell factor (SCF), T-cell-specific protein, RANTES, thrombopoietin (TPO), tissue factor (TF), tissue inhibitor of metalloproteinases 1 mouse (TIMP-1 mouse), tumor necrosis factor alpha (TNF-alpha), vascular cellular adhesion molecule-1 (VCAM-1), vascular endothelial growth factor A (VEGF-A), and von Willebrand factor (vWF) by a multiplexed fluorescent bead-based immunoassay (Luminex, Myriad RBM, Austin, TX). Briefly, this method operates with capture antibodies, which are attached to fluorescent microspheres and then measured by flow cytometry. All assays have been validated in a multiplex, based on immunoassay principles and tested for the parameters of the least detectable dose, precision, cross-reactivity, linearity, spiked recovery, correlation to an alternative method, dynamic range, and matrix interferences.

**Statistical analysis.** All data are expressed as means ± SE. Differences between pretraining and posttraining were compared with a repeated measures ANOVA. If significant main effects were observed, post hoc analysis was conducted by using the Newman-Keuls multiple comparison test. Pearson’s correlation analysis was used to identify any significant relationships between MHT and COX-2 expression or COX-2 expression and IL-6. In all cases, P < 0.05 was accepted as being significant. Data were analyzed using Prism 5 and SPSS 22 statistical analysis program.

**RESULTS**

**Body weight and exercise capacity.** In all groups, body weight increased significantly from the pretraining to the posttraining time point without any significant differences between the groups (Table 1). Regarding exercise capacity, the VO2 max observed in both the cortex/hc and the hypothalamus of mice increased significantly after training (P < 0.05, Fig. 2A), while no significant changes were observed in mice from the SG or the CG. Similarly, MHT increased significantly only in mice from the ST group after training (P < 0.05), whereas no significant changes were observed in the other groups (Fig. 2B).

**Expression of inflammatory mediators in the brain.** COX-2 mRNA expression in the cortex/hc of mice from the EG increased significantly after training compared with CG (P < 0.01). There was no significant difference in the COX-2 mRNA expression in the hypothalamus after training (Fig. 3, A and B). A significant increase in COX-2 protein levels was observed in both the cortex/hc and the hypothalamus of mice from the SG (P < 0.05/P < 0.01, Fig. 3, C and D) compared

![Fig. 2. Exercise capacity pretraining and posttraining from mice from the control group (CG), endurance training group (EG), and strength training group (CG). Change in endurance capacity was analyzed by measuring VO2 max (4), and change in strength capacity was determined by measuring maximum holding time (MHT; B) (n = 6). Data are given as means ± SE, for all figures. #Significant differences with pretraining (P < 0.05). *Significant differences between the groups.](http://jap.physiology.org)
with CG. NFκB protein levels were significantly increased in mice of both exercise groups in the hypothalamus (P < 0.05), while no changes were found in the cortex/hc (Fig. 3, E and F) compared with CG.

Expression of mPGES mRNA, the downstream enzyme of COX-2 required for PGE2 synthesis, was not significantly altered in the cortex/hc, while a significant increase in expression was found in the hypothalamus after both endurance as well as strength training (P < 0.01/P < 0.05, Fig. 4, A and B) compared with CG. In contrast, inhibitor of (1) κBα mRNA expression, a negative regulator of NFκB signaling and a marker of NFκB activation (18, 38), was only significantly increased in the cortex/hc in mice of the EG (P < 0.05), but not in the hypothalamus (Fig. 4, C and D) compared with CG.

Expression of cerebral cytokines, markers of mitochondrial stress and signaling pathways. To analyze the role of potential intracerebral signaling pathways or mediators of COX-2 activation, expression of several cytokines, markers of mitochondrial stress and signaling pathways, were analyzed in the cortex/hc and the hypothalamus. No altered expression levels were found for the cytokines interleukin (IL)-1β, IL-6, IL1 receptor antagonist (ra), the mitochondrial stress markers mitochondrial transcription factor A (TFAM), nuclear respiratory factor 1 (NRF1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), and the signaling molecules suppressor of cytokine signaling (SOSC3) and NF-IL6 after both training regimes (data not shown).

Levels of systemic cytokines. To analyze a potential role for peripherally released cytokines in COX-2 expression in the brain, a panel of 50 proinflammatory, antiinflammatory, hematopoietic, chemotactic, and coagulative plasma cytokines were analyzed. While many cytokines were not affected in their basal expression levels after training, most inflammatory factors, such as IL-1α, IL-18, MIP1y, MIP-2, IL-2, IP-10, RANTES, FGF-9, MMP9, and TIMP, decreased after training. Other proteins, like IL-7 and myoglobin, decreased in the EG, while they were upregulated in the SG after training. In both exercise groups an upregulation of IL-6 was found in mice from the EG and the SG (Fig. 5).
Association between COX-2 expression, exercise capacity, and cytokines. A positive association was found between MHT and COX-2 expression in the cortex/hc in mice from the ST group ($r = 0.39, P < 0.05$). Furthermore, an association was found between COX-2 mRNA expression in the cortex and plasma IL-6 levels after endurance exercise ($r = 0.37, P < 0.05$).

**DISCUSSION**

We have shown that long-term strength and endurance training increases COX-2 expression in the cortex and hippocampus of mice. In the hypothalamus, strength training affected the COX-2 pathway including inflammatory signaling via NFkB. Both training regimes exhibited mainly antiinflammatory responses in the circulation, with an increase in IL-6 levels after training, suggesting a role for circulating IL-6 in COX-2 induction in the brain.

COX-2 expression in the brain has mostly been investigated in pathological contexts such as fever or inflammatory diseases (36, 38). In this regard, it was shown that COX-2 expression is activated by IL-6 followed by the production of PG and the generation of fever. IL-6 acts to induce PG via an IL-6 receptor mediated activation of the Janus kinase signal transducer and activator of transcription 3 (JAK-STAT3) pathway in brain endothelial cells (9, 35, 37, 44). Recent evidence suggests that acute bouts of exercise also affect the cyclooxygenase pathways in the brain (19). Others have shown that an acute bout of treadmill exercise temporarily increases COX-2 expression in the hippocampus of rats (14). The increase in COX-2 in the hippocampus was positively correlated to the results of an inhibitory avoidance test suggesting a relationship between COX-2 expression and aversive memory after exercise (19). Several other studies supported a general role of COX-2 expression in synaptic plasticity and spatial learning (19, 32, 40, 44) in addition to its function in mediating brain inflammation (18). It was also shown that regular exercise training, in addition to acute exercise, improves cognitive function by increasing brain plasticity because of an increase in neurogenesis and synaptogenesis (10, 42). This knowledge supports the results of the current study demonstrating that COX-2 expression was increased after a period of training. In particular, strength training did increase COX-2 in the cortex/hc and the hypothalamus. The pronounced effects of strength training on COX signaling are supported by the results of Portugal et al. (31), who demonstrated that not only endurance but also strength or resistance training mediates increases in cognitive function.

Interestingly, we found a positive correlation between training and COX-2 expression. Therefore we speculate that the increased COX-2 expression is part of a general adaptive response to the training. While it is known that strength training primarily results in muscular adaptation processes, it might also affect brain signaling. Mechanistically, we assume that a more efficient exercise stimulus induces a stronger increase of growth factors or other potential mediators of adaptation (25). However, since we did not measure improvement of learning ability or brain plasticity, we cannot exclude that the observed changes in brain signaling would impair learning and memory processes. In this regard, it was shown that restraint caused stress-induced alterations of the oxidative status in rat brains, which is accompanied by COX-2 induction and potential damaging results (20), although we did not detect any change in markers of mitochondrial stress in the present study (PGC1α, NRF1, TFAM). Data suggest that the ambivalent role of COX-2 on brain plasticity and learning might be related to the magnitude of COX-2 induction. While a strong stress stimulus might result in a negative physiological response, a controlled moderate stimulus given by exercise might induce a positive physiological response (28). However, since we did not find any significant regulation of NFkB or mPGES in the cortex/hc, we suggest that both long-term exercise regimes represent moderate stress stimuli, which are distinct from acute stress conditions.
from the acute exercise effects. In general, exercise is suggested to be a quantifiable physiological stressor. Thereby, each single bout of acute exercise might induce a moderate inflammatory process that has long-lasting effects on brain signaling (43). Similarly, adaptation processes in other tissues, like muscles, are referred to inflammatory signaling pathways (1, 12).

In the hypothalamus, levels of inflammatory signals seem to be higher in response to strength training since, here, upregulations of COX-2, NFκB, and mPGES were found. The hypothalamus represents an autoregulatory region of the brain, which is involved in the cardiorespiratory response to exercise (21). Circulating mediators like cytokines can gain access to the hypothalamus via a leaky blood brain barrier in the so-called circumventricular organs (CVOs). Some of the CVOs are located in the hypothalamus surrounding the third ventricle (34, 38). During acute exercise, the autonomic nervous system reacts and participates in the maintenance of homeostasis. After regular exercise training, the sympathetic nervous system adapts and decreases its activity (23). However, whether these functional changes are mirrored by the signaling changes observed in our study remains speculative. The inflammatory changes in the hypothalamus may simply be a result of repeated challenges of homeostasis and circulating mediators.

Changes in several plasma cytokines and growth factors were analyzed to determine the systemic inflammatory response and potential mediators of COX-2 regulation following exercise training. Interestingly, levels of most inflammatory cytokines were not affected by strength or endurance training. In line with previous studies, both exercise modes induced mainly anti-inflammatory effects indicated by a decrease of proinflammatory cytokines like IL-1α, IL-18, or chemokines like RANTES (22, 30, 33).

Some other cytokines, like IL-7 and myoglobin, are differently regulated between the strength and endurance exercise groups. Endurance training significantly downregulated these proteins, while strength training significantly upregulated their expression. It is suggested that the increase of myoglobin is a result of exercise-induced muscle damage known to occur following strength training or eccentric exercise training (25, 27). Since muscle damage was not accompanied by a systemic inflammatory process, it is unlikely that it affects brain signaling. The upregulation of IL-7 after strength training is suggested to be part of the release of growth factors, which is regularly reported after strength or resistance training (12). Our finding that both types of exercise upregulated IL-6 and COX-2 and a positive association was found between systemic IL-6 and COX-2 expression indicates that circulating IL-6 is possibly involved in the regulation of COX-2 in the brain following exercise training. An increased expression of IL-6 in response to acute and chronic exposure to exercise has been shown by others (30). It is suggested that the contracting skeletal muscle releases IL-6 into the circulation (27, 29). On the basis of previous data (16) it is assumed that both training regimes might affect body composition by increasing the relative proportion of muscle tissue. Therefore the IL-6 release might be amplified in the training groups. In contrast to the release of IL-6 during inflammatory conditions, it is suggested that the IL-6 released during exercise exhibits primarily antiinflammatory effects (29). This finding might explain why most systemic inflammatory factors were downregulated after exercise training.

The connection between systemic IL-6 and brain-COX-2 expression was investigated during experimental conditions of fever (9, 37, 44). Here, it was shown that circulating IL-6 acts on endothelial cells in the brain followed by an increased mPGES and COX-2 induction. mPGES-1 is known to be an enzyme for PGE2 induction downstream of COX-2 (36). Therefore IL-6 might also be a potential mediator of COX-2 and mPGES expression after exercise training. However, since our findings are mainly descriptive and we did not find any significant change in SOCS3-mRNA expression, a potential activation marker of the IL-6-stimulated JAK-STAT3-signaling pathway (18, 38), these connections remain speculative.

Taken together, our findings demonstrate that strength training and endurance training induce COX-2 expression in the brain. The induction of COX-2 expression in the cortex/HC and hypothalamus appears to be more pronounced following strength training. The induction of these signals seems to be independent from systemic inflammatory processes because most inflammatory cytokines were not upregulated after training. A potential mediator of COX-2 expression after training might be the systemically released IL-6. However, further research is necessary to elucidate the role of inflammatory pathways on brain plasticity after endurance and strength exercise training.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

K.K., F.C.M., and C.R. conception and design of research; K.K. and J.B. performed experiments; K.K., J.B., and C.R. analyzed data; K.K., F.C.M., and C.R. interpreted results of experiments; K.K. and C.R. prepared figures; K.K. drafted manuscript; K.K., F.C.M., and C.R. edited and revised manuscript; K.K., F.C.M., and C.R. approved final version of manuscript.

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