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Effects of Exercise Intensity on Peripheral Oxidative Stress and Brain 3-Nitrotyrosine and Brain-Derived Neurotrophic Factor Levels in Rats

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Abstract

Oxidative stress plays an important role in both physiological adaptation and the development of various diseases, and exercise intensity is considered a key factor influencing oxidative and neuronal responses. This study investigated the effects of different exercise intensities on peripheral oxidative stress and neuronal factors in the central nervous system. Twelve 8-week-old male Wistar rats were randomly assigned to three groups (n = 4 each): control (CON), moderate-intensity exercise (MOD), and high-intensity exercise (HIGH). Rats in the MOD and HIGH groups performed treadmill running five times per week for 8 weeks at speeds of 15 and 25 m/min, respectively. Blood levels of reactive oxygen metabolites-derived compounds (d-ROMs), biological antioxidant potential (BAP), and the BAP/d-ROMs ratio were measured before the intervention and at Week 4 and Week 8. After the intervention, the frontal cortex, hippocampus, and striatum were collected, and 3-nitrotyrosine (3-NT) and brain-derived neurotrophic factor (BDNF) levels were determined using ELISA. At Week 8, d-ROM levels were significantly higher in the HIGH group than in the CON group. In contrast, BAP levels were significantly lower in the HIGH group than in the MOD group, and the BAP/d-ROMs ratio was significantly lower in the HIGH group than in the CON group. Hippocampal 3-NT levels were significantly lower in the HIGH group than in the MOD group, whereas no significant pairwise differences were observed in the frontal cortex or striatum. BDNF levels in the frontal cortex, hippocampus, and striatum were significantly lower in the HIGH group than in the CON group. These findings indicate that exercise intensity differentially affects oxidative stress and neuronal factors. In particular, prolonged high-intensity exercise was associated with increased peripheral oxidative stress, reduced antioxidant capac-

ity and impaired redox balance, and region-specific alterations in brain 3-NT and BDNF levels. These results suggest that exercise intensity influences physiological responses in both peripheral and central tissues and should be carefully considered when designing exercise interventions.

Keywords

Oxidative Stress, 3-Nitrotyrosine, Brain-Derived Neurotrophic Factor, Exercise Intensity

1. Introduction

Aerobic metabolism is essential for sustaining life and relies on mitochondrial oxygen utilization for energy production. Reactive oxygen species (ROS), generated as byproducts of this process, contribute to oxidative stress, which has been implicated in various pathological conditions [1]. However, oxidative stress also plays an indispensable role in physiological cellular functions, including gene expression and intracellular signaling, and is essential for maintaining cellular homeostasis [2].

The body possesses endogenous antioxidant defense systems including antioxidant enzymes and antioxidants that counteract oxidative stress and maintain redox homeostasis. However, disruption of this balance due to increased oxidative stress or impaired antioxidant capacity results in oxidative damage, contributing to the onset and progression of various diseases [3]. Neurological tissues are particularly vulnerable to oxidative damage because the brain consists of approximately 50% lipids and contains abundant polyunsaturated fatty acids in axons and dendrites, which are highly susceptible to oxidation [4]. Neurodegenerative diseases such as Alzheimer's disease [5] and Parkinson's disease [6] [7], whose prevalence is increasing worldwide with population aging, are closely associated with oxidative stress. Therefore, investigating the mechanisms underlying oxidative stress has become increasingly important.

In addition to ROS, oxidative stress involves reactive nitrogen species (RNS), including nitric oxide (NO), nitrite (NO_2^-), nitrate (NO_3^-), and peroxynitrite ($ONOO^-$), which are produced through the interactions of NO with oxygen, ROS, and various biological molecules. NO is synthesized from L-arginine by nitric oxide synthase (NOS). Under physiological conditions, NO regulates various functions, including blood pressure, neurotransmission, and immune responses. However, when converted into RNS, NO can chemically modify nucleic acids, proteins, and lipids, thereby causing cellular damage [8]. On the other hand, RNS-mediated nitration has been suggested to function not only as a damaging process but also as a signaling mechanism involved in adaptive responses to oxidative stress, highlighting the critical role of NO in redox regulation [9]. Moreover, NO possesses vasodilatory and antithrombotic properties, and its production is enhanced by in-

creased shear stress associated with elevated blood flow during exercise [10]. Consequently, NO has recently attracted attention for its beneficial effects on vascular health and disease prevention.

Exercise-induced physiological changes have been shown to influence the production of both ROS and RNS [11]. Continuous exercise performed at moderate aerobic intensity has been reported to activate antioxidant defense systems and suppress oxidative stress [12]. In contrast, anaerobic or high-intensity exercise has been shown to increase RNS levels in blood and skeletal muscle [13]. However, previous studies have reported conflicting results. Some studies have reported that exhaustive exercise does not alter the oxidative stress status in the brain [14], whereas others have demonstrated that exercise decreases RNS levels [15]. Therefore, the mechanisms underlying exercise-induced ROS and RNS production remain unclear. Although exercise intensity appears to influence oxidative and nitrosative responses [16], the evidence remains inconclusive. Therefore, the present study aimed to investigate the effects of different exercise intensities on oxidative stress and neuronal factors, including 3-nitrotyrosine (3-NT) and brain-derived neurotrophic factor (BDNF), in both peripheral tissues and the central nervous system.

2. Materials and Methods

2.1. Ethical Approval

All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Experiment Committee of Takasaki University of Health and Welfare (Protocol No. 2011).

2.2. Animals

Twelve male Wistar rats (8 weeks old) were used in this study. After 2 weeks of treadmill familiarization followed by 1 week of rest, the animals were randomly assigned to one of three groups: control (CON, n = 4), moderate-intensity exercise (MOD, n = 4), and high-intensity exercise (HIGH, n = 4). All animals were housed individually in standard cages under a 12-h light/12-h dark cycle with free access to food and water.

2.3. Exercise Protocol

Rats in the MOD and HIGH groups underwent treadmill running using a small-animal treadmill (KN-73; Natsume Seisakusho Co., Ltd., Tokyo, Japan) five times per week for 8 weeks. Each exercise session lasted 30 min. Based on previous studies [17] [18], the running speeds were set at 15 m/min for the MOD group to represent aerobic exercise and 25 m/min for the HIGH group to represent anaerobic exercise. Rats in the CON group were placed on the treadmill for 30 min without running (0 m/min).

2.4. Measurements

2.4.1. General Condition

Before each exercise session, body weight was measured as an indicator of excessive exercise load and stress. Data obtained before the intervention and at the end of Weeks 4 and 8 were used for the analysis.

2.4.2. Biochemical Analyses

1) 3-NT

On the day after completion of the 8-week intervention, in the late afternoon and at least 24 h after the final exercise session, the rats were deeply anesthetized with sevoflurane and decapitated after the absence of the toe-pinch reflex was confirmed. The frontal cortex, hippocampus, and striatum were dissected according to the rat brain atlas [19], rapidly frozen, and stored at −80˚C until analysis.

Each tissue sample (1 mg) was homogenized in 10 μL of CelLytic™ MT Cell Lysis Reagent (Sigma-Aldrich, USA) containing protease inhibitor. The homogenates were centrifuged at 12,000 ×g for 10 min at 4˚C, and the supernatants were collected for analysis. Levels of 3-NT were measured using an OxiSelect™ Nitrotyrosine ELISA Kit (Cell Biolabs, Inc., San Diego, CA, USA).

2) BDNF

BDNF samples were prepared using the same procedure as described for 3-NT. BDNF concentrations were measured using a Mature BDNF Rapid™ ELISA Kit (Biosensis Pty. Ltd., Australia).

3) Reactive Oxygen Metabolites-Derived Compounds (d-ROMs) and Biological Antioxidant Potential (BAP)

Blood samples were collected before the intervention and at Week 4 and Week 8. Under awake conditions and with gentle restraint, 0.3 mL of blood was collected from the tail vein before exercise. Plasma samples were analyzed using a FREE Carrio Duo analyzer (Wismerll Co., Ltd., Tokyo, Japan).

The d-ROMs test evaluates oxidative stress by measuring hydroperoxides. Plasma samples were mixed with an acidic buffer, and hydroperoxides were converted into radicals in the presence of iron ions. The generated radicals reacted with N,N-diethyl-para-phenylenediamine, and the resulting color intensity was measured.

The BAP test evaluates antioxidant capacity by assessing the ability of plasma antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in decolorization. The degree of decolorization was quantified spectrophotometrically and used as an index of antioxidant capacity.

Additionally, the BAP/d-ROMs ratio was calculated and used as an index of redox balance.

2.5. Statistical Analysis

Statistical analyses were performed using JASP software (version 0.95.2). Given the small sample size (n = 4 per group) and deviations from normality, nonparametric statistical methods were used. For longitudinal measurements, including

body weight, d-ROMs, BAP, and the BAP/d-ROMs ratio, changes over time within each group were analyzed using the Friedman test. When a significant difference was detected, post hoc pairwise comparisons were performed using the Conover test with Holm adjustment for multiple comparisons. Between-group differences at each time point were analyzed using the Kruskal-Wallis test, followed by Dunn's post hoc test with Holm adjustment for multiple comparisons when a significant difference was detected. For single time-point measurements of 3-NT and BDNF in the frontal cortex, hippocampus, and striatum, between-group differences were analyzed using the Kruskal-Wallis test, followed by Dunn's post hoc test with Holm adjustment for multiple comparisons when appropriate. Statistical significance was set at $p < 0.05$. Data are presented as median [25th-75th percentile].

2.6. Use of Artificial Intelligence Tools

ChatGPT (OpenAI, San Francisco, CA, USA) was used for English translation and language refinement of the manuscript. The author reviewed and edited all AI-generated text and is fully responsible for the content of this article.

3. Results

Table 1 summarizes the body weight, d-ROMs, BAP, and BAP/d-ROMs ratios measured before the intervention and at Week 4 and Week 8.

Table 1. Body weight, d-ROMs, BAP, and BAP/d-ROMs ratio at baseline, Week 4, and Week 8.

	Body weight (g)			d-ROM (U.CARR)		
	PRE	4W	8W	PRE	4W	8W
CON	304.0 [293.0 - 317.0]	332.5 [328.0 - 340.3]	341.5 [333.8 - 356.5]	406.5 [375.0 - 414.0]	357.0 [342.3 - 377.8]	347.5 [343.8 - 355.5]
MOD	336.0 [325.8 - 337.3]	344.5 [337.8 - 351.0]	356.0 [353.3 - 359.5]	411.0 [401.0 - 418.3]	388.0 [370.3 - 407.3]	382.0 [373.5 - 393.3]
HIGH	337.0 [327.3 - 349.8]	369.5 [354.0 - 381.0]	386.0 [372.8 - 395.5]	377.0 [375.3 - 385.8]	386.0 [366.8 - 408.5]	462.5 [449.8 - 472.3]
	BAP (μmol/L)			BAP/d-ROMs		
	PRE	4W	8W	PRE	4W	8W
CON	2461.5 [2373.8 - 2671.0]	2543.0 [2501.3 - 2568.3]	2476.5 [2396.8 - 2508.0]	6.862 [5.927 - 7.761]	7.125 [6.708 - 7.471]	7.168 [6.754 - 7.337]
MOD	2781.5 [2681.8 - 2919.3]	2558.0 [2470.3 - 2668.5]	2576.0 [2547.5 - 2607.8]	6.767 [6.492 - 7.226]	6.582 [6.200 - 7.111]	6.760 [6.437 - 7.055]
HIGH	2641.0 [2611.3 - 2693.0]	2398.0 [2306.5 - 2472.0]	2144.5 [2074.3 - 2239.0]	7.052 [6.807 - 7.195]	6.113 [5.745 - 6.491]	4.644 [4.595 - 4.768]

Data are presented as median [25th-75th percentile]. PRE: before the intervention; 4W: 4 weeks after the intervention; 8W: 8 weeks after the intervention; CON: control group; MOD: moderate-intensity exercise group; HIGH: high-intensity exercise group.

3.1. Body Weight

Body weight changed significantly over time in all groups (CON: Friedman test, $\chi^2(2) = 8.000$, $p = 0.018$; MOD: $\chi^2(2) = 8.000$, $p = 0.018$; HIGH: $\chi^2(2) = 8.000$, $p = 0.018$). Conover post hoc tests with Holm adjustment showed that body weight significantly increased from PRE to Week 4, from PRE to Week 8, and from Week 4 to Week 8 in all groups (all adjusted $p < 0.001$). Kruskal-Wallis tests showed no significant between-group differences at PRE ($\chi^2(2) = 4.308$, $p = 0.116$), Week 4 ($\chi^2(2) = 3.731$, $p = 0.155$), or Week 8 ($\chi^2(2) = 4.967$, $p = 0.083$) (Figure 1).

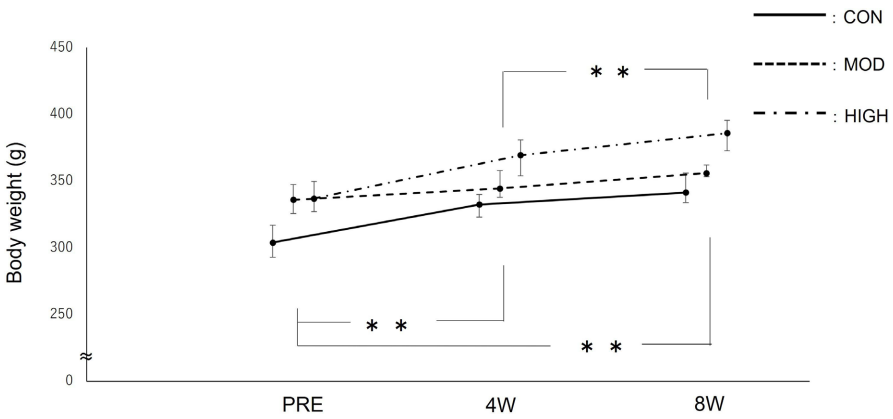

Figure 1. Changes in body weight during the intervention period. Body weight significantly increased over time in all groups. No significant between-group differences were observed at any time point. Data are presented as median [25th-75th percentile]. *p < 0.05, **p < 0.01 for within-group comparisons.

3.2. d-ROMs

No significant changes in d-ROM levels over time were observed in the CON (Friedman test, $\chi^2(2) = 0.500$, $p = 0.779$), MOD ($\chi^2(2) = 1.733$, $p = 0.420$), or HIGH group ($\chi^2(2) = 6.000$, $p = 0.050$). Kruskal-Wallis tests showed no significant between-group differences at PRE ($\chi^2(2) = 2.848$, $p = 0.241$) or Week 4 ($\chi^2(2) = 1.423$, $p = 0.491$). However, a significant between-group difference was observed at Week 8 ($\chi^2(2) = 9.269$, $p = 0.010$). Dunn's post hoc test with Holm adjustment showed that d-ROM levels were significantly higher in the HIGH group than in the CON group at Week 8 (adjusted $p = 0.007$), whereas no significant differences were observed between the other groups (Figure 2).

3.3. BAP

No significant changes in BAP levels over time were observed in the CON (Friedman test, $\chi^2(2) = 2.000$, $p = 0.368$) or MOD group ($\chi^2(2) = 3.500$, $p = 0.174$). In contrast, BAP levels changed significantly over time in the HIGH group ($\chi^2(2) = 8.000$, $p = 0.018$). Conover post hoc tests with Holm adjustment showed that BAP levels significantly decreased from PRE to Week 4, from PRE to Week 8, and from Week 4 to Week 8 in the HIGH group (all adjusted $p < 0.001$). Kruskal-Wallis tests showed no significant between-group differences at PRE ($\chi^2(2) = 2.192$, $p =$

0.334) or Week 4 ($\chi^2(2) = 2.808$, p = 0.246). However, a significant between-group difference was observed at Week 8 ($\chi^2(2) = 8.115$, p = 0.017). Dunn's post hoc test with Holm adjustment showed that BAP levels were significantly lower in the HIGH group than in the MOD group at Week 8 (adjusted p = 0.013), whereas no significant differences were observed between the other groups (Figure 3).

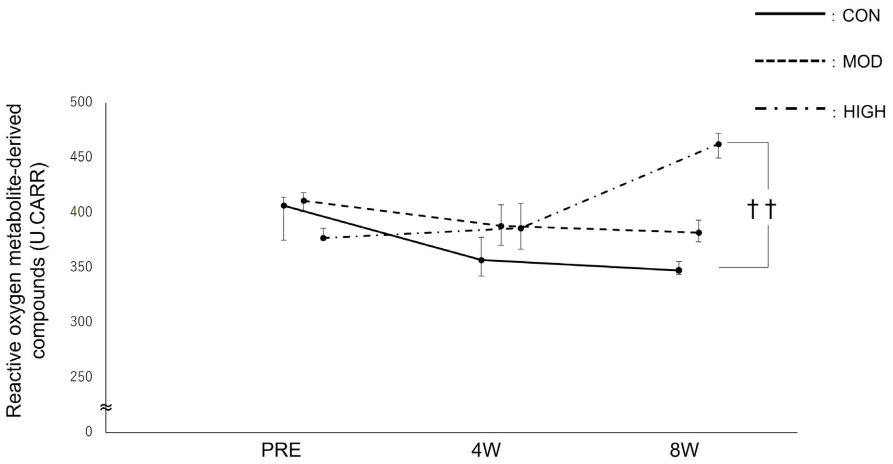

Figure 2. Changes in d-ROMs levels during the intervention period. No significant changes in d-ROM levels over time were observed in any group. At Week 8, d-ROM levels were significantly higher in the HIGH group than in the CON group. Data are presented as median [25th-75th percentile]. †p < 0.05, ††p < 0.01 for between-group comparisons.

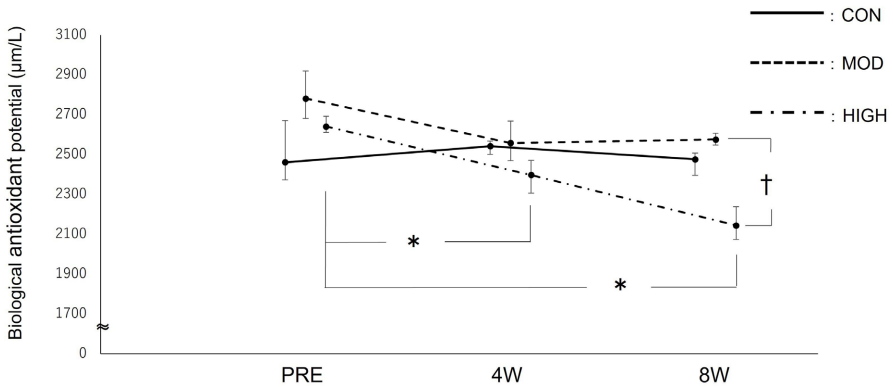

Figure 3. Changes in BAP levels during the intervention period. In the HIGH group, BAP levels significantly decreased over time. At Week 8, BAP levels were significantly lower in the HIGH group than in the MOD group. Data are presented as median [25th-75th percentile]. *p < 0.05, **p < 0.01 for within-group comparisons; †p < 0.05, ††p < 0.01 for between-group comparisons.

3.4. Redox Balance (BAP/d-ROMs Ratio)

No significant changes in the BAP/d-ROMs ratio over time were observed in the CON (Friedman test, $\chi^2(2) = 0.000$, p = 1.000) or MOD group ($\chi^2(2) = 3.500$, p = 0.174). In contrast, the BAP/d-ROMs ratio changed significantly over time in the HIGH group ($\chi^2(2) = 6.500$, p = 0.039). Conover post hoc tests with Holm adjustment showed that the BAP/d-ROMs ratio at Week 8 was significantly lower than

that at PRE (adjusted p = 0.008) and Week 4 (adjusted p = 0.025), whereas no significant difference was observed between PRE and Week 4. Kruskal-Wallis tests showed no significant between-group differences at PRE ($\chi^2(2) = 0.115$, p = 0.944) or Week 4 ($\chi^2(2) = 1.885$, p = 0.390). However, a significant between-group difference was observed at Week 8 ($\chi^2(2) = 7.731$, p = 0.021). Dunn's post hoc test with Holm adjustment showed that the BAP/d-ROMs ratio was significantly lower in the HIGH group than in the CON group at Week 8 (adjusted p = 0.024), whereas no significant differences were observed between the other groups (Figure 4).

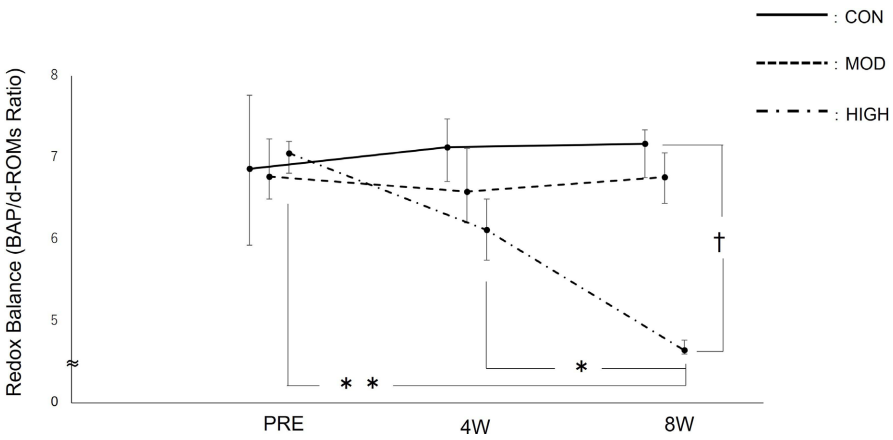

Figure 4. Changes in the BAP/d-ROMs ratio during the intervention period. In the HIGH group, the BAP/d-ROMs ratio at Week 8 was significantly lower than at PRE and Week 4. At Week 8, the BAP/d-ROMs ratio was significantly lower in the HIGH group than in the CON group. Data are presented as median [25th-75th percentile]. *p < 0.05, **p < 0.01 for within-group comparisons; †p < 0.05, ††p < 0.01 for between-group comparisons.

3.5. 3-NT

3.5.1. Frontal Cortex

The 3-NT levels in the frontal cortex were 6.001 [4.861 - 6.600] nM in the CON group, 5.401 [4.204 - 6.547] nM in the MOD group, and 2.152 [1.989 - 2.239] nM in the HIGH group. No significant between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 5.692$, p = 0.058) (Figure 5(a)).

3.5.2. Hippocampus

The 3-NT levels in the hippocampus were 5.325 [3.627 - 6.829] nM in the CON group, 7.400 [7.043 - 7.707] nM in the MOD group, and 2.113 [1.853 - 2.558] nM in the HIGH group. A significant between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 8.654$, p = 0.013). Dunn's post hoc test with Holm adjustment showed that 3-NT levels were significantly lower in the HIGH group than in the MOD group (adjusted p = 0.010), whereas no significant differences were observed between the other groups (Figure 5(b)).

3.5.3. Striatum

The 3-NT levels in the striatum were 6.739 [5.808 - 8.986] nM in the CON group,

6.361 [5.037 - 7.849] nM in the MOD group, and 2.742 [2.224 - 3.033] nM in the HIGH group. A significant overall between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 7.385$, $p = 0.025$). However, Dunn's post hoc tests with Holm adjustment showed no significant pairwise differences between groups (Figure 5(c)).

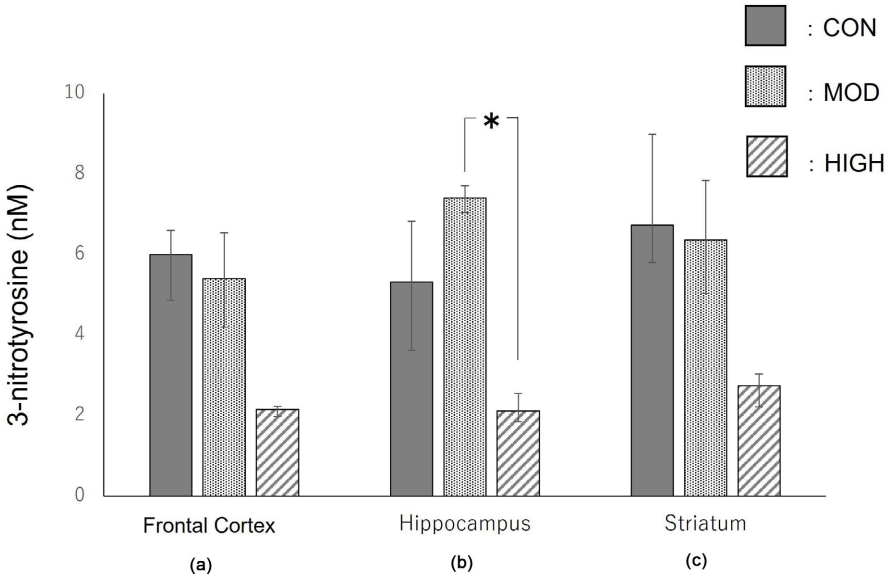

Figure 5. 3-Nitrotyrosine levels in the frontal cortex (a), hippocampus (b), and striatum (c) after 8 weeks of intervention. In the hippocampus, 3-NT levels were significantly lower in the HIGH group than in the MOD group. No significant pairwise differences were observed in the frontal cortex or striatum. Data are presented as median [25th-75th percentile]. *$p < 0.05$, **$p < 0.01$.

3.6. BDNF

3.6.1. Frontal Cortex

The BDNF levels in the frontal cortex were 6.321 [5.280 - 7.044] pg/mL in the CON group, 3.590 [3.217 - 4.161] pg/mL in the MOD group, and 1.374 [1.221 - 1.591] pg/mL in the HIGH group. A significant between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 8.769$, $p = 0.012$). Dunn's post hoc test with Holm adjustment showed that BDNF levels were significantly lower in the HIGH group than in the CON group (adjusted $p = 0.010$), whereas no significant differences were observed between the other groups (Figure 6(a)).

3.6.2. Hippocampus

The BDNF levels in the hippocampus were 14.687 [11.133 - 21.171] pg/mL in the CON group, 10.802 [9.193 - 12.594] pg/mL in the MOD group, and 2.485 [2.135 - 3.131] pg/mL in the HIGH group. A significant between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 8.000$, $p = 0.018$). Dunn's post hoc test with Holm adjustment showed that BDNF levels were significantly lower in the HIGH group than in the CON group (adjusted $p = 0.018$), whereas no significant differences were observed between the other groups (Figure 6(b)).

3.6.3. Striatum

The BDNF levels in the striatum were 8.145 [5.846 - 10.927] pg/mL in the CON group, 3.835 [3.254 - 4.501] pg/mL in the MOD group, and 1.640 [1.289 - 1.985] pg/mL in the HIGH group. A significant between-group difference was observed (Kruskal-Wallis test, $\chi^2(2) = 9.846$, $p = 0.007$). Dunn's post hoc test with Holm adjustment showed that BDNF levels were significantly lower in the HIGH group than in the CON group (adjusted $p = 0.005$), whereas no significant differences were observed between the other groups (Figure 6(c)).

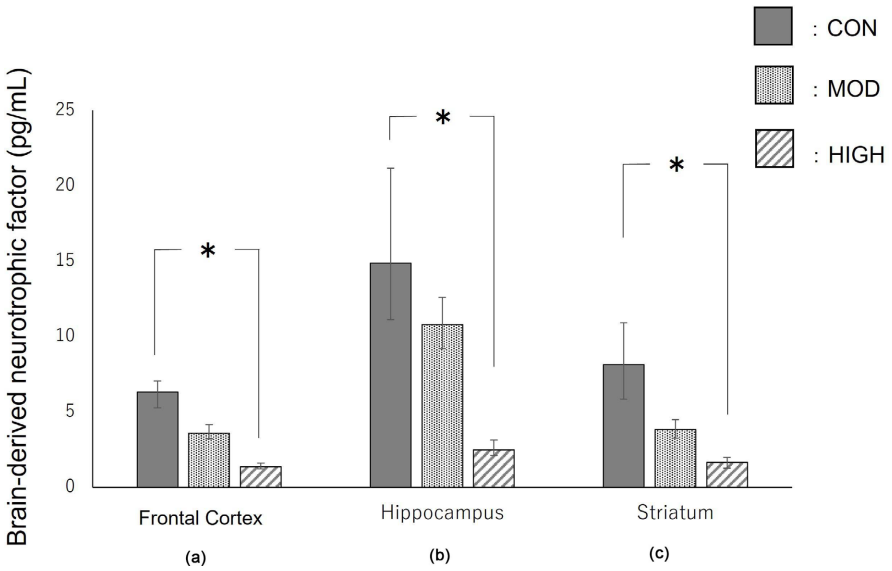

Figure 6. Brain-Derived neurotrophic factor (BDNF) levels in the frontal cortex (a), hippocampus (b), and striatum (c) after 8 weeks of intervention. BDNF levels were significantly lower in the HIGH group than in the CON group in the frontal cortex, hippocampus, and striatum. No significant differences were observed between the other groups. Data are presented as median [25th-75th percentile]. *$p < 0.05$, **$p < 0.01$.

4. Discussion

The present study investigated the effects of different exercise intensities on oxidative stress and neuronal factors in the peripheral tissues and central nervous system. The principal findings were that prolonged high-intensity exercise was associated with increased peripheral oxidative stress, reduced antioxidant capacity, and impaired redox balance. In the brain, the effects differed among regions and markers: hippocampal 3-NT levels were significantly lower in the HIGH group than in the MOD group, whereas BDNF levels in the frontal cortex, hippocampus, and striatum were significantly lower in the HIGH group than in the CON group.

The d-ROM test results indicated that high-intensity exercise increased peripheral oxidative stress. This finding is consistent with previous studies reporting enhanced ROS production following high-intensity exercise [12]. At Week 8, d-ROM levels were significantly higher in the HIGH group than in the CON group,

whereas no significant between-group differences were observed at PRE or Week 4. Although no significant changes in d-ROM levels over time were detected within any group, these findings suggest that prolonged high-intensity exercise may be associated with increased peripheral oxidative stress. Additionally, BAP levels decreased significantly over time in the HIGH group, and at Week 8, BAP levels were significantly lower in the HIGH group than in the MOD group. These findings suggest that sustained high-intensity exercise may be associated not only with increased oxidative stress but also with reduced antioxidant capacity [20].

Consistent with these findings, the BAP/d-ROMs ratio changed significantly over time in the HIGH group. At Week 8, the ratio was significantly lower than at PRE and Week 4 and was also significantly lower in the HIGH group than in the CON group. The lower BAP/d-ROMs ratio observed in the HIGH group at Week 8 suggests that prolonged high-intensity exercise may gradually shift systemic redox balance toward oxidative stress. Previous studies have shown that long-term moderate exercise enhances antioxidant defenses [21]. In contrast, sustained high-intensity exercise may place a greater oxidative burden on the organism and impair the balance between oxidative stress and antioxidant defenses [22]. Nevertheless, because of the small sample size in the present study, these findings should be interpreted cautiously.

The brain 3-NT results showed a different pattern from the peripheral oxidative stress markers. In the hippocampus, 3-NT levels were significantly lower in the HIGH group than in the MOD group. In the frontal cortex, no significant between-group difference was observed. In the striatum, the overall Kruskal-Wallis test was significant, but post hoc pairwise comparisons did not reveal significant differences between groups. Therefore, the present results do not support a generalized reduction in brain 3-NT levels following high-intensity exercise but instead suggest a region-specific response, particularly in the hippocampus. In general, increased oxidative stress is associated with elevated 3-NT levels [23]. However, the present findings differed from this conventional view. 3-NT is a marker of protein nitration induced by peroxynitrite generated from NO and superoxide, and its levels are influenced not only by oxidative stress but also by NO production and peroxynitrite formation [24]. Therefore, the lower hippocampal 3-NT level observed in the HIGH group may reflect alterations in nitrosative stress, NO production [25] [26], or neuronal activity [24], rather than a simple reduction in oxidative stress. Because neither NO nor NOS activity was directly measured in the present study, these possible explanations remain hypothetical and cannot be confirmed from the present findings.

BDNF levels also differed according to exercise intensity. In the frontal cortex, hippocampus, and striatum, BDNF levels were significantly lower in the HIGH group than in the CON group. In contrast, no significant differences were detected between the MOD and CON groups. Although moderate exercise has been shown to promote BDNF expression [27], the present findings do not demonstrate a significant increase in BDNF following moderate-intensity exercise. Rather, the con-

sistently lower BDNF levels observed in the HIGH group across all three brain regions suggest that prolonged high-intensity exercise may adversely affect factors associated with neuroplasticity. Excessive exercise may exert inhibitory effects on neuroplasticity [28]. One possible explanation for the lower BDNF levels observed in the HIGH group may involve chronic stress responses [29] and endocrine alterations [30] associated with repeated high-intensity exercise. However, because stress-related hormones were not measured in the present study, this possibility remains speculative and cannot be confirmed from the present findings. In addition, although brain tissues were collected at least 24 h after the final exercise session, residual effects of the final exercise bout cannot be completely excluded. Therefore, the observed 3-NT and BDNF levels may not exclusively reflect chronic adaptations to the 8-week exercise intervention. Differences in the timing of tissue collection relative to exercise could also be one factor contributing to the discrepancy between the present findings and previous studies.

Collectively, the present findings suggest that prolonged high-intensity exercise is associated with increased peripheral oxidative stress, reduced antioxidant capacity, and impaired systemic redox balance. In the central nervous system, the responses were marker- and region-specific: hippocampal 3-NT levels were lower in the HIGH group than in the MOD group, while BDNF levels were lower in the HIGH group than in the CON group across the frontal cortex, hippocampus, and striatum. These findings indicate that peripheral and central biochemical responses to exercise intensity may not occur in parallel. However, given the small sample size and the exploratory nature of the present study, these findings should be interpreted cautiously and require confirmation in larger studies.

Limitations

This study had several limitations. The sample size was relatively small, with only four animals in each group, which may have limited the statistical power and generalizability of the findings. Given the small sample size and deviations from normality, nonparametric statistical methods were used; nevertheless, the results should be interpreted cautiously. Therefore, future studies with larger sample sizes are warranted.

Another limitation is that the relationship between peripheral blood marker levels and brain biochemical changes remains unclear. Because peripheral oxidative stress markers and brain 3-NT and BDNF levels were assessed in different tissues, the present study cannot establish a direct relationship between systemic redox status and biochemical changes in the brain.

In addition, NO production and NOS activity were not measured. Therefore, the mechanisms underlying the lower hippocampal 3-NT levels observed in the HIGH group cannot be determined from the present findings. In particular, lower 3-NT levels should not be interpreted simply as indicating reduced oxidative stress.

Furthermore, stress-related hormones, such as corticosterone, were not measured. Although there was no substantial variation in the timing of brain tissue

collection among animals, the potential influence of circadian variation in corticosterone on BDNF levels cannot be completely excluded. Therefore, the possibility that repeated high-intensity treadmill exercise induced chronic stress or endocrine alterations that contributed to the lower brain BDNF levels remains speculative. Although blood collection procedures were identical across all groups, repeated restraint and tail-vein sampling may have induced handling-related stress, and the possibility of differential stress responsiveness among groups cannot be completely excluded. Future studies should include stress-related biomarkers to examine this potential mechanism.

Exercise intensity was also defined according to treadmill running speed, and physiological indices of exercise intensity, such as oxygen consumption or blood lactate levels, were not directly measured. Therefore, the actual physiological intensity experienced by individual animals could not be confirmed. Future studies incorporating physiological measures of exercise intensity are needed to more precisely determine the relationship between exercise intensity and peripheral and central biochemical responses.

5. Conclusion

The present study suggests that prolonged high-intensity exercise is associated with increased peripheral oxidative stress, reduced antioxidant capacity, and impaired redox balance. In the brain, hippocampal 3-NT levels were lower in the HIGH group than in the MOD group, whereas BDNF levels in the frontal cortex, hippocampus, and striatum were lower in the HIGH group than in the CON group. These findings suggest that exercise intensity may differentially influence peripheral redox status and biochemical responses in the brain. However, given the small sample size, these findings should be interpreted cautiously and require confirmation in future studies with larger sample sizes.

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Author Contributions

Yoichi Ohno conceived and designed the study, performed the experiments, analyzed the data, interpreted the results, prepared the manuscript, and approved the final version.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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