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Original Research

The Molecular Mechanism of Exercise for Treatment of Patients with Major Depression: A Preliminary Report on the Dynamics of Metabolites of Nitric Oxide and Catecholamines

Atsuko Ikenouchi[1,2,*], Naomichi Okamoto[1,2], Ryohei Igata[2], Tomoya Natsuyama[2], Reiji Yoshimura[2]

[1]Medical Center for Dementia, Hospital of the University of Occupational and Environmental Health, 807-8556 Kitakyushu, Fukuoka, Japan
[2]Department of Psychiatry, School of Medicine, University of Occupational and Environmental Health, 807-8555 Kitakyushu, Fukuoka, Japan
*Correspondence: atsuko-i@med.uoeh-u.ac.jp (Atsuko Ikenouchi)

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Abstract

Background: There has been increasing evidence that exercise therapy is effective in the treatment and prevention of major depression (MD). However, the basic molecular mechanisms underlying the effects of exercise on MD remain unclear. We conducted a preliminary study to clarify the effect of exercise therapy on MD, focusing on the dynamics of nitric oxide (NO) and catecholamine metabolites, which have been found to be associated with MD. **Methods**: Eleven outpatients with mild to moderate MD and 37 healthy controls (HC) were included in the study. The participants' clinical records and questionnaires were screened for their past medical history. For their exercise therapy, the participants were instructed to walk the equivalent of 17.5 kcal/kg/week for 8 weeks. Blood samples were collected from all participants at baseline, 4 weeks, and 8 weeks after the start of exercise therapy, and plasma metabolites of NO (NOx), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) were analyzed. We also assessed the 17-item Hamilton Rating Scale for Depression (HRSD-17) in patients with MD. A mixed-effects regression model was used to compare the mean values by time (baseline, 4, and 8 weeks) for the three corresponding groups (NOx, MHPG, and HVA). **Results**: HRSD-17 scores decreased significantly in the MD group after 8 weeks of exercise therapy. NOx and MHPG increased, but there was no significant change in HVA in the MD group after the exercise therapy. NOx decreased after exercise, and HVA increased significantly from baseline after 4 weeks of exercise but decreased after 8 weeks of exercise in the HC group. **Conclusions**: The effects of exercise on NOx, MHPG, and HVA may differ between MD and HC. The potential mechanisms for the benefits of walking exercise in MD patients will be the subject for future research.

Keywords: exercise; walking; depression; nitric oxide; catecholamine; noradrenaline; 3-methoxy-4-hydroxyphenylglycol; homovanillic acid

1. Introduction

Depression is a common mental disorder, affecting an estimated 3.8% of the population [1]. According to the World Health Organization, approximately 280 million people worldwide have depression [1]. Depression is the leading cause of disability worldwide, accounting for 40.5% of disability-adjusted life years due to mental and substance use disorders [2]. Epidemiological studies have noted that a lack of exercise increases the risk of major depression (MD) and that exercise prevents recurrence [3,4].

The behaviors that promote functional recovery in depressed patients include regular exercise along with sleep hygiene and maintaining a healthy diet [5]. The NICE guideline recommends exercise as one of the first choices for MD of sub-threshold to moderate severity. Antidepressants are not recommended for mild MD in the stepped-care model until simpler treatments have failed because of the low risk-benefit ratio [6]. On the other hand, in clinical practice, antidepressants are the first option in the treatment of depression [7]. In the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) study, the cumulative remission rate up to treatment level 4 was 67% [8]. However, in some cases, the effects of pharmacotherapy alone are insufficient. Walking exercise therapy is effective against depression in treatment-resistant depression [9]. Exercise therapy at a dose of 16.5 kcal/kg/week added to usual medical therapy in hospitalized patients with severe MD is effective in improving depressive symptoms and quality of life [10]. Supervised structured aerobic exercise training is an effective adjunct therapy for treating depressed patients [11]. Exercise is strongly indicated as an effective treatment in patients with depression in a recent meta-analysis of randomized controlled trials (RCTs) [12]. Cochrane reviews have shown that, for MD, exercise therapy has been as effective as medication and psychotherapy [13]. Exercise has been known to have both acute and long-term beneficial effects on MD [14].

Exercise has been shown to have antidepressant-like and anxiolytic effects in wild-type mice, as it improves performance on classical tests measuring depression-like behavior (learned helplessness, forced swimming, tail

suspension paradigm) and anxiety (elevated cross maze, open field) [15]. Chronic exercise was shown to reduce depressive-like behavior in rats exposed to uncontrollable stress [16]. Thus, there is growing evidence that exercise is effective against depression in humans and animals.

Nitric oxide (NO) is a gaseous signaling factor that exerts neuroprotective or neurotoxic effects depending on its concentration and cellular environment [17]. NO is formed by NO synthase (NOS) from L-arginine during its enzymatic conversion. The three major isoforms of NOS are neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). nNOS and eNOS are Ca^{2+}-calmodulin-dependent enzymes that are constitutively expressed mainly in neurons and endothelial cells [18]. Endothelial dysfunction, as quantified by flow-mediated dilatation, is associated with clinically relevant symptoms of depression [19,20]. The endothelial dysfunction decreases the NO produced by the endothelium. There are numerous reports of peripheral NO metabolism in depression, with mixed results. Some reports suggest that eNOS activity and metabolites of NO (NOx) in the blood are increased in depression, while others suggest that they are decreased [21–26]. Physical activity and NO are related, and physical activity enhances the production of NO [27]. Several *in vivo* studies have demonstrated that NO can modulate the level of catecholamines in the central nervous system [28,29].

Catecholamines are adaptive and maladaptive stress hormones that activate behavioral and physiological processes that facilitate the levels of stress [30]. Endogenous catecholamines include dopamine, norepinephrine, and epinephrine [30]. Catecholamines are produced and released by the sympathetic nervous system, brain, and adrenal medulla, and exert effects on multiple organ systems [30,31]. In the 1960s, it was discovered that inhibiting neuronal uptake of norepinephrine, a major target of tricyclic antidepressants, alleviated depressive symptoms, leading to the hypothesis and subsequent demonstration that deficiencies in catecholamine transmission are responsible for depression [32]. Imbalance of noradrenaline, serotonin, and dopamine, which play important roles as neurotransmitters, are known to occur in the brains of depressed patients, leading to the emergence of depressive symptoms. Therefore, the monoamine hypothesis has been proposed as a target for pharmacotherapy [33]. Regular exercise increases plasticity in several neurotransmitter systems, including dopamine and norepinephrine [34].

It has been reported that plasma concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of norepinephrine, reflect the tone of norepinephrinergic neurons and that homovanillic acid (HVA), a major metabolite of dopamine, reflect the tone of dopaminergic neurons [35]. The level of MHPG and HVA in plasma varies widely among patients because depression is a heterogeneous disease [36]. We have previously reported the relationship between depressive symptoms and the response to antidepressants and the concentration of MHPG, as well as the relationship between psychotic MD and the concentration of HVA [36–38].

There is a well-established association of exercise interventions on depression. However, the biological mechanisms for these effects have not been fully elucidated. It has been postulated that catecholamines and NO are involved in the effects of exercise therapy for MD on depression, but this has not yet been clarified. The purpose of this study is to clarify the kinetics of catecholamine and NO metabolites during exercise therapy for patients with MD, and to conduct a preliminary investigation to elucidate the mechanism for their beneficial effects on exercise therapy.

2. Materials and Methods

2.1 Participants and Procedures

We included 11 patients diagnosed with current mild to moderate MD by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision criteria through a structured interview using the Mini International Neuropsychiatric Interview (M.I.N.I.) by a psychiatrist in the outpatient psychiatry department of Hospital of the University of Occupational and Environmental Health, Japan [39]. All patients had no psychiatric comorbidities other than depression. They were all taking psychiatric medications and had not changed medication (paroxetine, fluvoxamine, sertraline, milnacipran, duloxetine, mirtazapine, amoxapine, sulpiride) for more than 8 weeks. The age of the patients ranged from 28 to 69 years. We recruited 37 healthy controls (HC) from a group of Japanese healthcare professionals using a bulletin board. The age of the HC group ranged from 22 to 59 years. We conducted a structured interview with the HC group using M.I.N.I. to exclude psychiatric comorbidities. We checked medical records and questionnaires in the MD group and questionnaires in the HC group to exclude participants with a history or current symptoms of serious cardiovascular disease (stroke, myocardial infarction, angina pectoris), but none of the participants had such a condition. We defined participants who performed some form of exercise for at least 150 minutes per week as having an exercise habit [40]. We also checked the smoking history, alcohol consumption, and exercise habits of all participants with a self-administered questionnaire. We measured blood pressure and performed biochemical tests to determine the presence of hypertension, hyperlipidemia, and diabetes in all participants.

All participants were instructed to walk the equivalent of 17.5 kcal/kg/week; the public health dose, on at least three different days per week on a self-administered basis [40]. For example, a participant weighing 60 kg had to perform exercise equivalent to 1050 kcal per week; it takes 34 steps to burn 1 kcal [41], then the participant needs to walk 35,700 steps per week. If the participants walk five days a week, their goal is 7140 steps per day. In this way, the number of calories consumed and the required steps would

depend on the participant's body weight.

The participants were also told to wear and record a pedometer that displayed the number of steps taken and calories burned to assess adherence to the prescribed exercise regimen, and their adherence was checked at their biweekly visits. We defined good adherence as those who achieved the target number of steps. We collected blood samples from all participants at baseline (before starting exercise therapy), 4 weeks, and 8 weeks after the start of exercise therapy. We assessed depression using the Zung Self-Rating Depression Scale (SDS) for both groups at baseline and 8 weeks after the start of exercise therapy [42]. The 17-item Hamilton Rating Scale for Depression (HRSD-17) was assessed by attending physician (AI), a well-trained psychiatrist, at baseline, and 4 and 8 weeks after the intervention.

2.2 Blood Samples and Assay Method

All blood tests were done at 9:00 AM, before breakfast and at least 12 h after the last medication. Venous blood was obtained from the participants in the supine position. The plasma samples were quickly separated in a centrifuge and stored at –80 °C until assay.

The NOx levels in the plasma were analyzed using the Griess method with high-performance liquid chromatography ENO-20 (50060403, Eicom, Kyoto, Japan). The same volume of methanol was added to the plasma, and the mixture was vortexed for 10 seconds and centrifuged at 10,000 $\times$ g for 10 minutes at 4 °C . A 10 μL portion of the collected supernatant was injected into ENO-20 for measurement. The analysis was carried out by measuring the absorbance of the azo dye produced by the "diazotization-coupling" reaction. Nitrite and nitrate were determined by comparing the results with those measured using a standard solution (NO-STD, Eicom, Kyoto, Japan). The detection limit was 20 nM/10 μL (0.2 pmol).

Plasma levels of MHPG and HVA were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) [43]. The plasma HVA levels were analyzed by HPLC-ECD according to the method of Yeung *et al*. [44] with slight modification. In brief, each cyanobonded solid-phase extraction cartridge was preconditioned with methanol followed by glass-distilled water. To each cartridge, 0.3 mL of plasma sample or standard and 0.1 mL of working internal standard solution (5 ng of 5-hydroxyindolecarboxylic acid in 0.01 M KH_2PO_4, pH 7.2) were added. The samples were deproteinized with 1mL of acetonitrile. After mixing by vortex and centrifugation (1760 $\times$ g, 4 °C for 10 minutes), an aliquot (5 μL) of supernatant was allowed to pass through the cartridge slowly under a mild vacuum (15 mmHg). The cartridge was washed with 0.2 mL of distilled water and extracted containing 1 mL of ethylacetate, and then the aliquot was evaporated to dryness under nitrogen gas. After dissolution in mobile phase (200 μL), a 10 μL portion of this solution was injected into the HPLC. The detection limit was 0.5 μ/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

The plasma MHPG levels were also analyzed by HPLC-ECD according to the method of Minegishi and Ishizaki [45]. In brief, the plasma was separated by centrifugation at 600 $\times$ g at 4 °C . Extraction was performed under a vacuum using Bond-Elut columns (Varian, Palo-Alyto, CA, USA) prepacked with 100 mg of C18-bonded silica (40 μm) in a 1-mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an aspirator, were prepared by washing with 1 mL methanol followed by 1 mL of water. After the addition of 50 μL of a solution of vanillyl alchol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, the samples were passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and easily eluted hydrophilic compounds. The adsorbed materials were eluted with 20 μL of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40:60, v/v). A 20 μL portion of this solution was injected into the HPLC. The detection limit was 0.5 ng/mL, and the calibration curve was linear up to 40 ng/mL. The intra- and interassay coefficients of variation were 6% and 8%, respectively. The recovery rate was more than 80%.

2.3 Statistical Analyses

All statistical analyses were performed using the EZR (ver. 1.50, Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R. The clinical and biochemical data of the study participants are expressed as mean (standard deviation). We used standard errors for the statistical analyses. The normality of the distribution of HRSD-17 and SDS score was checked by histogram, but since it could not clearly determine normal distribution, a nonparametric test was conducted. We compared HRSD-17 score at baseline and after 8 weeks using a Wilcoxon signed-rank sum test in the MD group. We used the Mann-Whitney U test to compare the SDS at baseline between the MD and HC groups. We compared SDS at baseline and after 8 weeks using a Wilcoxon signed-rank sum test in MD and HC group respectively.

To compare the mean values by time (baseline, 4, and 8 weeks) for the three corresponding groups (NOx, MHPG, and HVA), the changes between baseline–4 weeks and between baseline–8 weeks were compared using a mixed-effects regression model, with blood data as a dependent variable and age, sex, and time as covariates. Random effects were used to set up intercepts, and time was treated as a nominal variable at baseline, 4, and 8 weeks. The interaction between the MD and HC groups was also examined using a mixed-effects regression model. Interaction was assessed by comparing the slope of change in each blood data over the baseline–4 and baseline–8-week period be-

tween the two groups. Therefore as a stratified analysis, we created three models each for the temporal changes of NOx, MHPG and HVA in the MD group, and three models each for the temporal changes of NOx, MHPG and HVA in the HC group. As an analysis of interaction, MD and HC were simultaneously assigned as a group, and three models each for NOx, MHPG and HVA were created; A total of nine models were developed. Multiple regression analysis, incorporating age and sex as covariates, was used to correlate the amount of change in HDRS-17 and SDS with the amount of change in metabolites such as NOx, HVA, and MHPG before and after exercise. Similarly, multiple regression analysis, which is adjusted for age and sex, was used to examine the relationship between metabolites, change and actual exercise dose, and between SDS change and actual exercise dose in the MD and HC group.

All tests were two-trial and statistical significance was set at $p < 0.05$. We analyzed residuals to determine the mixed-effects regression model fit and confirmed normal distribution. In a mixed-effects regression model, analysis of variance was used for correcting multiple testing (global test) and only the items that showed a statistically significant difference were included for the next set of individual tests.

3. Results

The characteristics of the study population are summarized in Table 1. There was an approximately 20-year difference in mean age between the MD and HC groups, but there were no obvious differences in sex ratio. There were 78.3% participants with good adherence in the HC group and 63.6% in the MD group. The SDS scores at baseline were significantly higher in the MD group than in the HC group ($p < 0.001$). After 8 weeks of exercise therapy, SDS showed a decreasing trend in the MD group ($p = 0.057$) and no obvious change in the HC group ($p = 0.58$). The HRSD-17 scores of the MD group decreased significantly from baseline to 8 weeks after the start of exercise ($p = 0.012$). The metabolites data showed statistically significant differences in the global test, the comparison at each time point was examined, and statistically significant differences were found in NOx between 0 and 4 weeks ($p = 0.022$) and MHPG between 0 and 8 weeks ($p < 0.001$) in the MD group. We also found statistically significant differences in NOx levels between 0 and 8 weeks ($p = 0.037$) and HVA between 0 and 4 weeks ($p < 0.001$) in the HC group (Table 2). A statistically significant difference was observed between the MD and HC groups at 4 weeks ($p = 0.004$) and 8 weeks ($p < 0.001$) in NOx, at 8 weeks in MHPG ($p = 0.004$) and 4 weeks in HVA ($p = 0.005$) (Table 3) (Figs. 1,2,3). In the MD and HC groups, there was no correlation between the amount of change in HDRS-17 or SDS before and after exercise and the amount of change in metabolites such as NOx, HVA, and MHPG. There was no correlation between actual exercise dose and changes in metabolites such as NOx, HVA, and MHPG in both groups. There was an association between improvement in SDS and actual exercise dose in the HC group (partial regression coefficient: 0.185, 95% CI: 0.048~0.321, $p = 0.009$), but not in the MD group (partial regression coefficient: 0.340, 95% CI: –0.727~1.400, $p = 0.48$).

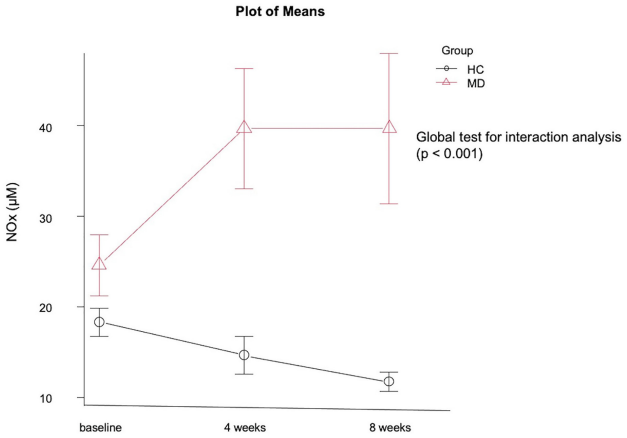

Fig. 1. Course of NOx after starting exercise therapy. Statistically significant difference in the effect of walking on blood NOx data in the MD and HC groups at 4 weeks ($p = 0.004$) and 8 weeks ($p < 0.001$) after the start of exercise therapy. The bars denote the standard error. NOx, nitric oxide; MD, major depression; HC, healthy control; baseline, before starting exercise therapy.

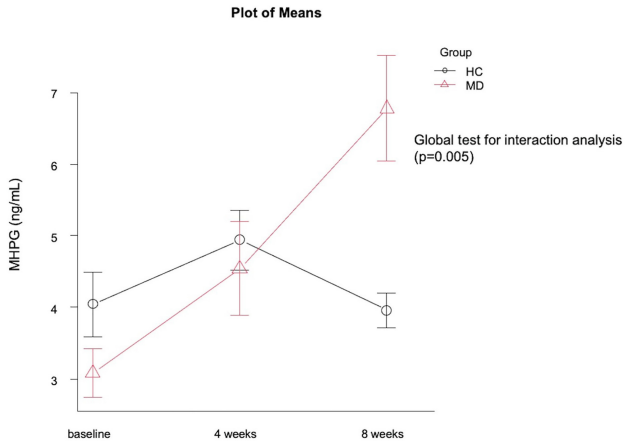

Fig. 2. Course of MHPG after starting exercise therapy. Statistically significant difference in the effect of walking on blood MHPG data in the MD and HC groups at 8 weeks ($p = 0.004$) after the start of exercise therapy. The bars denote the standard error. MHPG, 3-Methoxy-4-hydroxyphenylglycol; MD, major depression; HC, healthy control; baseline, before starting exercise therapy.

Table 1. The demographics of the participants.

	MD group	HC group
Participants, n	11	37
Sex, male/female	4/7	15/22
Age, years; mean (sd)	54.5 (12.7)	34.4 (9.6)
Smoking, %	0	18.9
Drinking, %	36.3	16.2
Exercise habites, %	54.5	62.2
Comorbidity		
Hypertention (≥140/90 mmHg), %	0	8.1
Hyperlipidemia (LDL ≥140 mg/dL or TG ≥150 mg/dL or HDL <40 mg/dL), %	54.5	37.8
Diabetes (HbA1c ≥6.5%), %	0	0
Number of required steps/8 week, mean (sd)	267814.9 (67142.4)	295968.9 (72933.2)
Number of actual steps/8 week, mean (sd)	285416.7 (150411.7)	461065.6 (154047.8)
SDS at baseline, mean (sd)	52.2 (7.4)	32.9 (8.4)
SDS at 8 week, mean (sd)	49.8 (8.2)	33.6 (8.3)
HSRD-17 at baseline, mean (sd)	9.5 (1.0)	-
HSRD-17 at week 4, mean (sd)	8.9 (0.8)	-
HSRD-17 at week 8, mean (sd)	8.5 (0.8)	-

SDS, Zung Self-rating Depression Scale; HSRD-17, 17-item Hamilton Rating Scale for depression; sd, standard deviation; baseline, before starting exercise therapy.

Table 2. The comparison of biochemical data at each time point.

	Partial regression coefficient	95% CI	Standard error	t value	*p* value
MD group					
NOx change (baseline and 4 weeks)	0.0000168	0.00000312∼0.0000314	0.00000728	2.31	0.022
MHPG change (baseline and 8 weeks)	2.98	1.51∼4.43	0.744	4.00	<0.001
HC group					
NOx change (baseline and 8 weeks)	–0.00000680	–0.0000110∼–0.00000254	0.00000217	–3.13	0.037
HVA change (baseline and 4 weeks)	3.29	1.97∼4.63	0.679	4.85	<0.001

All *p* value was adjusted for age and sex. MD, major depression; HC, healthy control; CI, confidence interval; baseline, before starting exercise therapy.

Table 3. Effect modification of biochemical data between HC and MD group (interaction analysis).

	Partial regression coefficient	95% CI	Standard error	t value	*p* value
NOx					
Group* baseline and 4 weeks	0.0000198	0.00000906∼0.0000307	0.00000560	3.54	<0.001
Group* baseline and 8 weeks	0.0000198	0.00000917∼0.0000304	0.00000548	3.61	<0.001
MHPG					
Group* baseline and 8 weeks	3.02	1.03∼5.02	1.03	2.92	0.004
HVA					
Group* baseline and 4 weeks	–3.93	–6.56∼–1.32	1.35	–2.89	0.005

All *p* value was adjusted for age and sex. CI, confidence interval; Asterisk (*) means interaction analysis; baseline, before starting exercise therapy.

4. Discussion

In this study, objective symptoms of MD assessed by HRSD-17 were significantly improved after the walking exercise intervention in the MD group. A clinically meaningful reduction of flare in SDS was observed, though it did not reach statistical significance due to the small sample size. There was a positive correlation between actual exercise dose and improvement in SDS in the HC group. Adherence to exercise therapy in previous studies was about 50–90% [9,40,46,47]. Adherence in the present study was also in this range. The observed variations in range may be due to differences in the method and duration of exercise therapy.

The link between exercise and MD has been well established. Decreased physical activity is associated with an

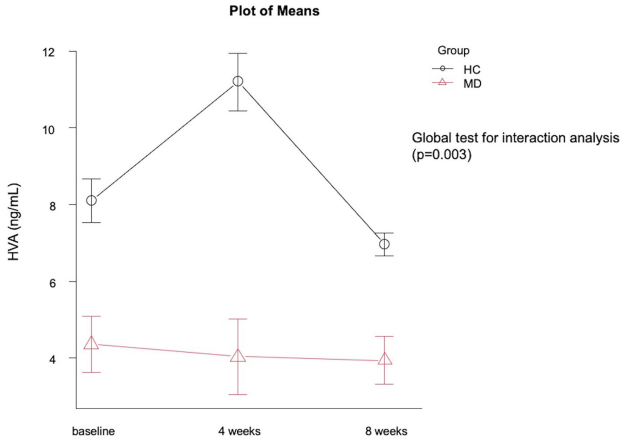

Fig. 3. Course of HVA after starting exercise therapy. Statistically significant difference in the effect of walking on blood HVA data in the MD and HC groups at 4 weeks ($p = 0.005$) after the start of exercise therapy. The bars denote the standard error. HVA, homovanillic acid; MD, major depression; HC, healthy control; baseline, before starting exercise therapy.

increased risk of MD [3]. A longitudinal retrospective study of students showed that individuals who are physically active have a lower incidence of MD [48]. A meta-analysis of prospective cohort studies has shown that physical activity reduces the risk of MD [49]. Furthermore, we have previously reported that an exercise intervention of walking significantly reduced depressive feelings among workers with no exercise habits [4]. Studies in the US have shown that aerobic exercise doses as per the public health recommendations (7 kcal/kg/week, five times a week or 17.5 kcal/kg/week, three times a week) are effective treatments for mild to moderate depression, while lower doses (7 kcal/kg/week, three times per week) are shown to be equivalent to placebo [40]. A home exercise program of 30–45 minutes/day of walking 5 days a week for 12 weeks for treatment-resistant MD patients has been reported to improve MD and contribute to remission in 26% of these patients [9].

In the present study, the effects of exercise intervention on NOx differed between the HC group and MD group. Plasma NOx levels increased in the MD group and decreased in the HC group after exercise therapy. Exercise decreases the production of reactive oxygen species (ROS) in the vascular endothelium and increases the bioavailability of NO by increasing blood flow and shear stress [50]. Laminar shear stress in blood vessels increases during exercise and is associated with a rapid increase in gene and protein expression of eNOS [51]. During high-intensity exercise, the production of ROS is greater than that of NO, and endothelial function is reduced [52,53]. It has been suggested that the shear stress-mediated effects, NO production, and biological activity vary qualitatively and quantitatively depending on the exercise involved [54]. Six months of exercise training in hypertensive women increases NOx and decreases arterial blood pressure [55]. Physical training is associated with increased expiratory NO content [56], although some studies suggest a decrease in expiratory NO content after physical exercise [57–59].

There are controversial reports of both increased [22,60] and decreased [25,26,61,62] NOx levels in MD. In MD, plasma NOx levels are high, but normalize after 8 weeks of treatment with sertraline, citalopram, fluoxetine, and fluvoxamine [22,60]. In contrast, it has been reported that MD results in decreased eNOS activity and NOx in platelets [25], and decreased NOx in polymorphonuclear leukocytes [61]. We have reported that plasma NOx levels are significantly decreased in depression and inversely correlated with the severity of MD, and that NOx are not affected by paroxetine, but they are significantly increased by serotonin and norepinephrine reuptake inhibitor (SNRI) such as milnacipran [26,62]. It has been reported that exercise increases eNOS activity by stimulating the noradrenergic nervous system and regulating eNOS phosphorylation via β3-adrenergic receptors [63]. Increased noradrenaline stimulates β-receptors, activates A-kinase, and inhibits the RhoA/ROCK system, resulting in increased eNOS activity and NO levels, and activation of β1-adrenergic receptors releases NO from the endothelium of mesenteric resistance arteries [54,64]. In summary, SNRI and exercise enhance noradrenaline neurons, which may affect plasma NO predominantly through noradrenergic signaling. SNRIs and exercise for depressed patients may have potentiated noradrenergic neurons and affected plasma NO mainly through noradrenergic signaling. The reason for the decrease in NOx with exercise intervention for HC is unclear. It may be natural due to the complexity of NO exchange and the multisystem nature of physiological responses to physical exercise [65].

MHPG levels also differed in dynamics between the MD group and HC group. MHPG levels began to increase in the fourth week and continued to increase in the eighth week in MD. In previous studies, plasma MHPG levels may tend to be higher in depressed patients than in controls, but there is no significant difference between patients and controls [66]. There are reports of higher plasma MHPG levels in depressed patients than in controls, suggesting that there may be subgroups of patients with higher and lower levels as seen in urinary MHPG excretion [67]. Although depression is thought to be associated with decreased norepinephrine activity [68], several studies in depressed patients have also reported increased MHPG levels, suggesting an association between anxiety states and increased central norepinephrine activity [66,69]. Samson *et al.* [70] found that psychomotor retardation was linearly correlated with MHPG levels in patients with depression. Treatment of depressed patients with SSRIs such as fluoxetine, fluvoxamine, paroxetine, and sertraline has been reported to decrease plasma MHPG concentrations with improvement

in MD [36,37,71,72]. In addition, treatment of MD with SNRIs such as milnacipran and duloxetine has been reported to improve MD and increase plasma MHPG levels [36,73]. It was reported that urinary MHPG levels were elevated in depressed teenage women after a 6-week, three-times-a-week pool-walking exercise program [74]. In animal studies, treadmill running has been shown to temporarily increase noradrenergic and dopaminergic activity in the striatum and hypothalamus [75,76]. There is evidence to suggest that even low-intensity exercise can cause an increase in norepinephrine concentrations, as long as the exercise is of adequate duration [77]. The present study suggests that plasma MHPG levels increased after exercise therapy in MD, suggesting that the norepinephrine nervous system may be activated after exercise.

In our study, HVA also differed in dynamics between the HC group and MD group. HVA increased significantly in the HC group during the first 4 weeks of exercise, but it subsequently decreased at 8 weeks. It has been reported that dopamine metabolism increases during exercise through an increase in brain calcium levels, which promotes dopamine synthesis and affects brain function [78]. However, at the same time, serotonin levels are increased, which has been shown to inhibit the increase in dopamine [79]. This may be one of the reasons why the increase in HVA was only temporary.

Our study has several limitations. First, the sample size was small, therefore the statistical power was limited. Caution should be used in interpreting the results of the interaction analysis. Second, although the analysis was statistically adjusted, the sex, age, and number of participants in the HC and MD groups differed. Third, we cannot conclude that the exercise intervention itself caused changes in metabolites or changes in HRSD-17 in MD, since the non-exercising subject group was not established. Fourth, the influence of medications in the MD group and the original exercise habits in both groups cannot be ruled out. Fifth, patients with mild to moderate MD were recruited, but only very mildly depressed patients with low HRSD-17 scores consented to the study. Sixth, we cannot exclude the possibility that other imperceptible confounding factors may have influenced the results. It is unclear why the metabolite changes in the HC and MD groups differed after the exercise therapy in the current study. Therefore, further studies are needed need to confirm and expand on these results.

5. Conclusions

Changes in NOx, MHPG, and HVA due to walking exercise intervention differ between the MD group and HC group. This study does not allow us to conclude that these metabolites are involved in the mechanism of the exercise effect on MD, but it does provide a foundation for future studies to elucidate how these metabolic changes contribute to the beneficial effects of walking exercise in MD patients.

Abbreviations

CBT, cognitive behavioral therapy; MD, major depression; RCTs, randomized controlled trials; NO, nitric oxide; NOS, NO synthase; nNOS, neuronal NOS; eNOS, endothelial NOS; iNOS, inducible NOS; NOx, metabolites of NO; MHPG, 3-Methoxy-4-hydroxyphenylglycol; HVA, homovanillic acid; M.I.N.I., Mini International Neuropsychiatric Interview; HC, healthy controls; SDS, Zung Self-rating Depression Scale; HRSD-17, 17-item Hamilton Rating Scale for depression; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; ROS; reactive oxygen species; SNRI, serotonin and norepinephrine reuptake inhibitor.

Author Contributions

AI and RY investigated and drafted the manuscript. NO, RI and TN analyzed the data. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was performed in compliance with the 1975 Declaration of Helsinki (revised in 2008). All procedures involving human participants were approved by the Ethics Committee of the University of Occupational and Environmental Health, Japan (Approval No. 10-076), and written informed consent was obtained from all the participants.

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Conflict of Interest

The authors declare no conflict of interest.

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