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■原著

The effect of prenatal moderate aerobic exercise to inhibit the increase of oxidative stress

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Abstract :

Background : Increase in oxidative stress during pregnancy may lead to deviation from normal pregnancy course. Performing aerobic exercise as a means to reduce oxidative stress during gestation period is an important lifestyle. We evaluated effects of aerobic exercise during gestation period since no study has investigated these using oxidative stress markers.

Objectives : The present study aimed to examine the relationship between aerobic exercise and oxidative stress using physiological indices.

Methods : 17 pregnant women in mid-pregnancy with no abnormalities in the pregnancy progress and no previous mental illness were recruited. Urine samples were collected before commencing aerobic exercise program during the second trimester and after completing 10 weeks of classes（three times per week）during the third trimester. Physiological effects of the exercise were determined by measuring urinary 8-hydroxy-2′-deoxyguanosine and 8-iso-prostaglandin $F_2\alpha$ levels.

Results : Urinary 8-hydroxy-2′-deoxyguanosine level in the third trimester was significantly lower than that in the second trimester. The difference in this level tended to be lower for women who commenced classes at 6 months of pregnancy than for those who commenced at 7 months of pregnancy. However, no significant difference was observed in the correlation between the month of pregnancy and difference in 8-hydroxy-2′-deoxyguanosine level. No significant difference was observed in the correlation between the difference in urinary 8-hydroxy-2′-deoxyguanosine level and exercise intensity during classes. No significant difference in urinary 8-iso-prostaglandin $F_2\alpha$ level was observed between the second and third trimesters.

Conclusions : Performing moderate aerobic exercise three times a week from the second trimester of pregnancy reduced oxidative stress and suppressed 8-hydroxy-2′-deoxyguanosine levels in vivo. In the future, we intend to test the association between aerobic exercise and participant background in a larger population of primipara. Furthermore, comparison of 8-iso-prostaglandin $F_2\alpha$ levels between control and experimental

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groups will be conducted.

Key words : moderate aerobic exercise; oxidative stress; pregnancy; urinary8-hydroxy-2′-deoxyguanosine (8-OHdG); urinary 8-iso-prostaglandin $F_2\alpha$ (8-iso-$PGF_2\alpha$)

Introduction

Oxidative stress is the state in which the balance of the antioxidant defense system breaks down, and the production of oxidative forces such as active oxygen species in the body and free radicals exceeds the

level of antioxidant enzymes and antioxidants in the body. Oxidative stress is implicated in multiple pathological processes associated with human disorders[1]. Excessive generation of active oxygen species causes DNA damage, lipid peroxidation, and protein degeneration; it is also involved in various diseases and their exacerbations. During pregnancy, oxidative stress is enhanced by increased mitochondrial activity due to active energy metabolism in the placenta as well as increased production of active oxygen species[2]. Moreover, DNA that has undergone oxidative damage may affect mitochondrial function and disturb energy supply[3]. Previous studies have shown that elevated levels of the maternal urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, are associated with reduced birth weight and shortened gestation[4),5)]. During pregnancy, the increased excretion of 8-iso-prostaglandin $F_2\alpha$ (8-iso-$PGF_2\alpha$), a marker of endogenous lipid peroxidation results in outcomes related primarily to the mother and secondarily to the fetus (e.g., preeclampsia and medically indicated preterm delivery, respectively)[6]. Both 8-OHdG and 8-iso-$PGF_2\alpha$ are independent biomarkers of oxidative stress during pregnancy and can be used to track different aspects of oxidative stress[5]. On the other hand, the onset and exacerbation of oxidative stress is caused by environmental factors (e.g., radiation and ultraviolet light) as well as lifestyle-related habits including smoking, alcohol consumption, intensive exercise, and mental stress[7),8),9)]. Acute aerobic exercise can create an imbalance between oxidant and antioxidant levels in humans, a state known as oxidative stress, which can damage enzymes, protein receptors, lipid membranes, and DNA[10]. However, regular aerobic exercise can also enhance antioxidant levels and suppress the oxidative stress induced by a single bout of aerobic exercise[11]. In a previous study, the group that performed moderate aerobic exercise (40%–60% of VO_2 max) for 25 minutes three times a week for 10 weeks during the second trimester showed lower weight compared with the control group[12),13),14)]. Moreover, systolic and diastolic blood pressure decreased after the initiation of exercise compared with that before the initiation of exercise[15]. These results indicate that performing moderate aerobic exercise during the second trimester improves excessive weight gain and reduces elevated blood pressure during pregnancy. However, no study has investigated the effects of performing exercise during gestation period using oxidative stress markers. We define it as the aerobic exercise of 40% - 60% of VO_2 max in RPE index 13-15 with the moderate aerobic exercise. We define it as aerobic exercise less than 40% of VO_2 max in RPE index 12 or less with the aerobic exercise.

The Japan Maternitybics Association was founded in 1985 (presently known as the Japan Maternity Fitness Association) and has developed a prenatal aerobic exercise program. The aerobic exercise program consisted of 15-min warm-up, 5-min slow training, 25-min moderate-aerobic exercise aerobic, 10-min floor training, and 5-min cool-down patterns.It was found that the effects of the aerobic

exercise program include improved general endurance, increased muscular strength and endurance of various body parts, prevention of weight gain, shortened delivery duration with less blood loss, and a positive effect on one's mental health[16]. However, the relationship between the developed aerobic exercise and oxidative stress remains unclear. Therefore, the purpose of the present study was to evaluate whether aerobic exercise program are effective for pregnant women using two biomarkers as physiological indices.

Materials and methods

Design and setting: We conducted a prospective cohort study. The program developer noted that for effective exercise therapy, moderate- aerobic exercise should be performed for 3–5 times per week and continued for at least approximately 30 min moderate- aerobic exercise of VO_2 max 40%–60% or a subjective rating of perceived exertion (RPE) of 13/15[16]. In addition, the earlier the exercise program are commenced during pregnancy, the greater the efficacy[16]. It has been reported that upon performing the 8-week aerobic exercise program three times per week at 20 min of moderate aerobic exercise, urinary 8-iso-$PGF_2\alpha$ level significantly decreased compared with the pre-exercise baseline values[17]. Therefore, the present study included a 10-week exercise program by all subjects conducted in the morning 3 days per week for 25 min of moderate aerobic exercise, including 35 min of mild- aerobic exercise each day. Exercise was performed in "Japanese" style in an open field while following a dance instructor on stage. Active oxygen species was degraded and eliminated by food-derived antioxidants (e.g., fat-soluble vitamin E and water-soluble vitamin C) as well as antioxidant enzymes (e.g., superoxide dismutase [SOD])[18]. Antioxidant vitamins act as coenzymes of antioxidant enzymes, and antioxidant vitamin supplements have also been reported to be effective[19),20]. Accordingly, the consumption of antioxidant vitamins could have an impact on oxidative stress and/or oxidative defense ability and is considered to be a confounding factor. Therefore, in the present study, during recruitment, the subjects were asked to refrain from taking supplements, including vitamins, for 10 weeks during the study period when measurements were taken.

Ethical considerations: The principal investigator verbally explained the study purpose and methods to the pregnant subjects while presenting the study request form. The subjects were informed that study participation was voluntary and that they could withdraw at any point during the study period. After confirming that the subjects fully understood the study concept, written informed consent was obtained. The attending physicians were promised that their participation, or absence thereof, would not be made public. Furthermore, prior to the start of the exercise program, the subjects were explained that midwives will conduct a medical examination, including the measurement of the fetal heart rate, symptoms of threatened miscarriage, and blood pressure, to confirm that there was no abnormality in the progression of the pregnancy. The subjects were also explained that if their condition became poor during the exercise, the survey will be terminated. Furthermore, the present study was performed with the approval of the ethical review board of Tokyo University (approval number TU-COI13-984).

Subjects and physical characteristics: We have previously shown decreased 8-OhdG levels in 23 healthy women who performed moderate aerobic exercise[21]. For this study, sample size of 25 pregnant women was considered appropriate taking the dropout rate into account. Participants were recruited as follows. First, we obtained permission from the Head of study cooperation institution and posted an offer poster at the reception. The poster provided email address of a study representative through which subjects could convey their intention to participate. The study representative col-

lected data regarding abnormalities during the course of pregnancy, medical history of the mental disorders to the subject of study candidate who informed me in email on a lesson reservation day on a number, the next time in week of gestation. The sthenia of oxidative stress damages vascular endothelium, leading to hypertension syndrome, which is a common complication during pregnancy[22),23)]. Regarding depression and schizophrenia, it is reported Brief Psychiatric Rating Scale and that the significant plus is associated with Hamilton Depression Rating Scale[24)]. Therefore, subjects with a history of the mental disorders and abnormalities in the course of pregnancy were excluded, and abnormality did a person without the mental disorder to cry with eligibility criteria for pregnancy process. Finally, a total of 17 healthy pregnant women residing in Tokyo were included in the study.

Date collection: During second trimester (pre-commencement), we conducted measurements of body weight, blood pressure, heart rate, and urine sample at rest before aerobic exercise. About weight, the blood pressure, study subjects measure with a scale, the sphygmomanometer in the studio by oneself. A study representative attached Polar FT 1 watch, which measured heart rate, to the participant's chest and arm before starting the exercise. Participants performed urine collection at home 2 hours before arriving and starting the exercise; urine samples were collected in glass vials of predetermined volume. The study representative obtained 5 cc urine in exclusive spitzes. During third trimester (10 weeks post-commencement), we conducted measurements of body weight, blood pressure, heart rate, and urine collection at rest. The question paper has you describe a number i n weight, blood pressure, age, week of gestation before the aerobic exercise. Heart rate was measured as described earlier. Urine samples were collected before the exercise and at 1 hour after the exercise. Urine sampling was performed as described earlier. All subjects performed aerobic exercise program (three times per week). Heart rate monitoring was recorded using a Polar FT1watch.

Correlation was observed between maximum heart rate and maximum oxygen uptake (VO_2 max);herefore, the target heart rate was determined using the Karvonen formula (target heart rate = {(220 – age) – heart rate at rest} × exercise intensity + heart rate at rest), which is often used to set the exercise intensity according to heart rate[25)]. Furthermore, during classes, pregnant women were advised to perform the exercises at an intensity that felt "slightly hard" using RPE, and if the exercise felt "hard," they were not to overdo it but rather move at a slower pace. We made an exclusive spitz freeze up with dry ice, and the study representative conveyed it to the Nikken climbing rope Japan aging control research institute with an exclusive case. We depended on the Nikken climbing rope Japan aging control research institute and analyzed urinary 8-OHdG, urinary 8-iso-$PGF_2\alpha$ by the ELISA method.

Aerobic exercise program: The program consisted of 25 min of moderate aerobic exercise, including 35 min of mild-aerobic exercise (15-min warm-up, 5-min slow training, 10-min floor training, and 5-min cool-down patterns). We define it as the aerobic exercise of 40% - 60% of VO_2 max in RPE index 13-15 with the moderate aerobic exercise. We define it as aerobic exercise less than 40% of VO_2 max in RPE index 12 or less with the aerobic exercise. The aerobic exercise program consisted of A professional dancer led the aerobic exercise program from a stage, and the aerobic exercise program was choreographed to music.

Statistical analysis: The characteristics of the 17 pregnant women before aerobic exercise program and each variable are represented as the mean + standard deviation or mean. Oxidative stress status variables during the second trimester (pre-commencement), third trimester (10 weeks post-commencement), and their difference (pre-commence-

Table I. Characteristics of 17 females before exercise classes

Variables	Mean, SD (Min–Max)
Age (years)	33.4, 4.0 (27–41)
Weight (kg)	55.5, 6.1 (44.3–66.8)
Body mass index (kg/m^2)	21.5, 2.0 (18.8–25.1)
Resting heart rate (beats per minute)	92.6, 11.3 (74–112)
Systolic blood pressure (mmHg)	108, 9.2 (93–124)
Diastolic blood pressure (mmHg)	58.2, 5.0 (52–67)
Pregnancy in weeks (weeks)	25, 2.3 (21–27)

Table II. 8-OHdG・8-iso-PGF$_2\alpha$ at baseline during the second trimester (pre-commencement), third trimester (after 10 weeks), and their difference (pre-exercise – post-exercise) n = 17

Variables	Second trimester (pre-commencement)	Third trimester (10 post-commencement)	Difference (pre-commencement: 10 weeks after commencement)
	Median (25%, 75%)	Median (25%, 75%)	Median (25%, 75%)
8-OHdG (ng/mgCr)	8.8 (6.2, 9.85)	6.0 (4.3, 6.7)	2.0 (1.2, 3.7)
8-iso-PGF$_2\alpha$ (ng/mgCr)	2.57 (1.19, 4.07)	2.04 (1.75, 2.64)	0.44 (0.635, 1.355)

ment–10 weeks post-commencement) was presented as a median, first quartile (25%), and second quartile (75%). For urinary 8-OHdG and 8-iso-PGF$_2\alpha$ levels, the difference in each index level was calculated by subtracting the third trimester value (10 weeks post-commencement) from the second trimester value (pre-commencement) and was processed for a Wilcoxon signed-rank sum test. Moreover, for the difference in 8-OHdG and exercise class commencement time (at 6 or 7 months of pregnancy), their correlation was examined using a Spearman's rank correlation coefficient. Furthermore, the correlation between the change in 8-OHdG level and exercise intensity during the classes (VO_2 max 10%–30% and 40%–60%) was examined using a Spearman's rank correlation coefficient. All analyses were performed using SPSS Version 20. The level of statistical significance was set at 0.05 for all tests.

Results

The study subjects provided written consent form when an agreement was obtained, and 25 pregnant women were recruited. In total, 8 pregnant women were unable to perform exercise three times a 3week;suffered a fall and were hospitalized, 3 pregnant women whom there was not three times a week of aerobic exercise program continuation, 2 pregnant women who did not get through. The study subjects who were able to continue 10-week aerobic exercise program three times a week were 13 primipara, 4multipara in 17 pregnant women. The characteristics of the subjects are presented in **Table I**. The detailed data for urinary 8-OHdG and 8-iso-PGF$_2\alpha$ as markers of oxidative stress (median, 25% and 75%, respectively) are obtained as follows (**Table II**). During the second trimester (pre-commencement), urinary 8-OHdG level was 8.8 (6.2–9.8) ng/mgCr and urinary 8-iso-PGF$_2\alpha$ level was 2.5 (1.1–4.0) ng/mgCr. During the third trimester (10 weeks post-commencement), urinary 8-OHdG level was 6.0 (4.3–6.7) ng/mgCr and urinary 8-iso-PGF$_2\alpha$ level was 2.0 (1.7–2.6) ng/mgCr. Upon subtracting the post-commencement (10 weeks) values from the second trimester values (pre-commencement), the difference in urinary 8-OHdG level was 2 (1.2–3.7) ng/mgCr. For urinary 8-iso-PGF$_2\alpha$ level, the difference was 0.4 (0.6–1.3) ng/mgCr.

For the difference in urinary 8-OHdG level, a Wilcoxon signed-rank sum test was performed. As a result, urinary 8-OHdG level significantly de-

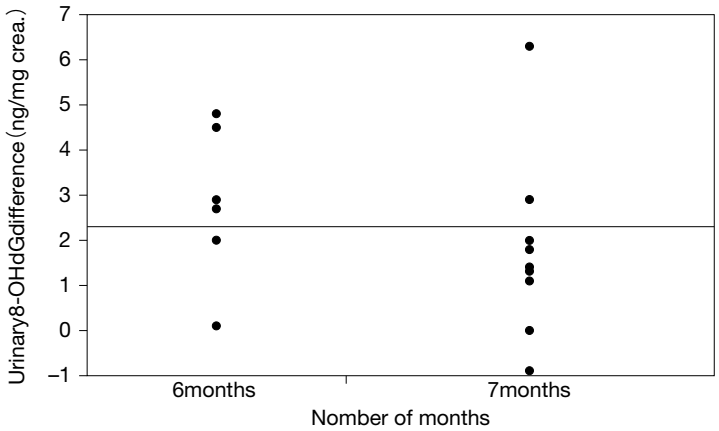

Figure 1. Correlation between the difference in urinary 8-OHdG level and the bivariant month of pregnancy; X axis: number of months.

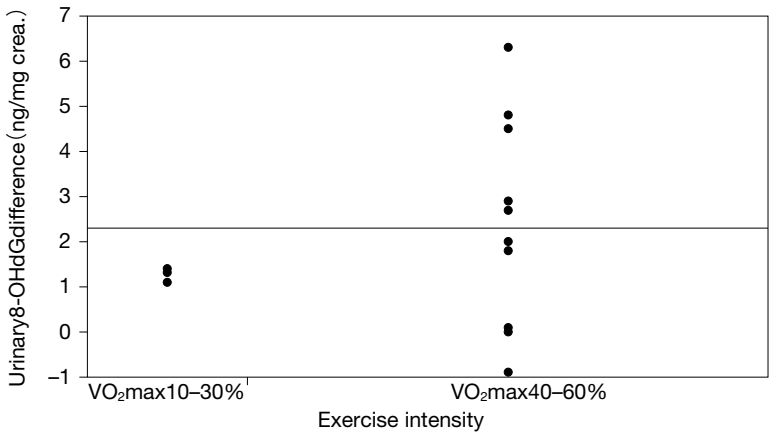

Figure 2. Correlation between the difference in urinary 8-OHdG level and bivariant VO_2 max; X axis: exercise intensity.

creased during the third trimester (10 weeks post-commencement) compared with that during the second trimester (pre-commencement) (n = 17; p =0.0001).Furthermore, difference in urinary8-OHdG levels at different aerobic exercise program commencement times (n = 7 at 6 months of pregnancy; n = 10 at 7 months of pregnancy) were examined using Wilcoxon test. We found that urinary 8-OHdG levels were not significantly different between aerobic exercise program commencement times (p =0.0869) (**Figure 1**). Furthermore, the correlation between the change in urinary 8-OHdG level and exercise intensity during classes (n = 4 at VO_2 max 10%–30%; n = 13 at VO_2 max 40%–60%) was examined using Spearman's rank correlation coefficient. Upon examining whether the correlation coefficient was 0, no significant difference was observed in the correlation between exercise intensity and difference in 8-OHdG level (**Figure 2**). Wilcoxon signed-rank sum test was performed to examine the difference in urinary 8-iso-$PGF_2\alpha$ level. As a result, no significant difference was ob-

served for the two time-points (i.e., during the second trimester [pre-commencement of classes] and third trimester [10 weeks post-commencement]).

Discussion

During pregnancy, the production of active oxygen species increases because of elevated oxidative metabolism[26]. Over the course of each pregnancy trimester, the maternal body exhibits marked changes in metabolism and in various hormone levels to prepare for fetal development and childbirth. The placenta synthesizes cholesterol and fatty acids and produces hormones such as chorionic gonadotropin, placental lactogen, and estrogen, which are necessary for embryonic/fetal development[27]. The production of such hormones is the greatest during the third trimester. Mitochondria are found in cells of the placenta that are necessary for hormone production, and they release active oxygen species corresponding to 2%–3% of oxygen consumption. Furthermore, basal metabolism during the third trimester increases by 10%–20% compared with that during the first trimester[28]. Moreover, the amount of oxygen consumption in the maternal body increases along with the rise in basal metabolism[29]. The difference in urinary 8-OHdG level during pregnancy exhibited no significant difference among healthy individuals at 3.88 ± 1.07 ng/mgCr, during the first trimester at 4.23 ± 1.35 ng/mgCr, and during the second trimester at 5.34 ± 2.01 ng/mgCr[30]. However, the urinary 8-OHdG level during the third trimester was found to be significantly higher than the aforementioned three values with a value of 28.73 ± 16.82 ng/mgCr[30]. This suggests that active oxygen species in the body of pregnant women is more abundant during the third trimester than during the second trimester. However, in the present study, when examining urinary 8-OHdG in the second trimester (pre-commencement) and third trimester (10 weeks post-commencement), we observed significant levels during the third trimester compared with those during the second trimester (n = 17; p =0.0001). Increase, ischemia of the oxygen intake to occur at aerobic exercise, a re-return current, injury of the activity muscle have an influence on the oxidative stress[31]. In particular, with increase in oxygen intake during aerobic exercise, generation of reactive oxygen species is elevated[32]. However, it is reported that reactive oxygen species do not increase with the exercise intensity of less than 70% of VO_2 max[33]. 8-OHdG levels were 0.88 ± 0.55 ng/mgCr before the initiation of excursive and 1.45 ± 0.96 ng/mgCr after 10 weeks of exercise. A previous study including elderly people after cancer therapy has reported that 8-OHdG levels in the experimental group significantly decreased compared with that in the control group[34]. According to the 2011 guideline of the prescription by ACSM, the exercise intensity necessary for the improvement of health and physical strength is moderate aerobic exercise of 40%–60% of VO_2 max[35]. Moderate aerobic exercise of this intensity can decrease of the oxidative. In this study, as for the age average of the pregnant woman, the heart rate average during a 33.4 (SD4.0) age, aerobic exercise enforcement was 137.1 ± 14.3bpm. It is heart rate an average of 148 (SD4.6bpm) at aerobic exercise 60%VO_2max, heart rate an average of 139 (5.6bpm) at 50%VO_2max, heart rate an average of 130 (SD6.6bpm) at 40% of VO_2 max, heart rate an average of 120 (SD4.6bpm) at 30%VO_2 max, heart rate an average of 111 (SD8.9bpm) at 20%VO_2 max, heart rate an average of 101 (SD10.1bpm) at 10% VO_2 max when we calculate exercise intensity using the Karvonen formula. In total, 13 women performed moderate aerobic exercise for 25 minutes (3 at 60% VO_2 max, 2 at 50% VO_2 max, and 2 at 40% VO_2 max). In this study, 8-OHdG levels decreased as approximately 80% of the subjects performed 40% of the aerobic exercise at an exercise intensity of 60% VO_2 max, which is similar to previous study. Moderate -aerobic exercise is continued on a habitual basis, it will give rise to various defense and adap-

tive mechanisms, including the reduction of active oxygen species generation and induction of antioxidant enzymes[10]. In rats, 10 weeks of moderate -aerobic exercise increased SOD production[10]. In rats, exercise of moderate intensity were shown to greatly increase SOD[36]. The oxidative stress response in the body causes a disruption in the balance between oxidation reaction and antioxidant activity[18]. In the present study, aerobic exercise program were performed three times per week for 10 consecutive weeks, and it was suggested that SOD increased and oxidative stress decreased during the third trimester.

However, in the present study, the regards to the correlation between exercise intensity during prenatal exercise（n = 4 at VO_2 max 10%–30%; n = 13 at VO_2 max 40%–60%）and difference in urinary 8-OHdG level, there was no significant difference observed . In this study, pregnant women who performed aerobic exercise at an intensity of 10%–30% of VO_2 max included 3 of the 4 multipara and 13 primipara. Multipara played a greater role within households; thus, they may have performed considerably more exercise during their daily life compared with primipara[30]. Primipara may present with changes in hormone production and body shape as well as expectations from those around places heavy pressure on them, which causes emotional stress[37]. This load of emotional stress leads to increased cortisol secretion, which is an antistress hormone that activates the sympathetic nerve system, thereby producing abundant active oxygen species. Furthermore, smoking increases the production of active oxygen species within the body[38]. It is necessary to consider everyday life active mass, psychological stress, smoking as confounders in future studies.

Regarding the biological antioxidant potential（BAP）having antioxidant activity, it has been reported that on comparing 16–18 weeks and 28–29 weeks, BAP was 2,004.0 ± 342.0 μM during the second trimester and 1,775.6 ± 310.7 μM during the third trimester[30]. These results suggest that as pregnancy progresses, oxidative stress increases and antioxidant stress decreases. Furthermore, differences in urinary8-OHdG levels at aerobic exercise program commencement times（n = 7 at 6 months of pregnancy; n = 10 at 7 months of pregnancy）, as assessed using Wilcoxon test, were not significant（p =0.0869）. In the present study, because the subject sample was small with only 17 subjects, the statistical power was insufficient; thus, no significant difference was observed. To examine whether exercise prevents increase in oxidative stress, a large-scale survey with a larger subject sample is required. Furthermore, the relationship between the time of class commencement and SOD as well as the relationship between exercise intensity and SOD should be examined. However, no significant difference was found in the urinary 8-iso-$PGF_2\alpha$ level between the second trimester prior to commencing the aerobic exercise program and the third trimester after commencement. There was no significant difference observed in the urinary 8-iso-$PGF_2\alpha$ level between the three time-points（i.e., in healthy individuals at 64.95 ± 16.43 ng/mgCr, during the first trimester at 65.28 ± 26.87 ng/mgCr, and during the second trimester at 70.08 ± 28.37 ng/mgCr）[30]. During the third trimester, urinary 8-iso-$PGF_2\alpha$ level was 398.29 ± 228.99 ng/mgCr, indicating a significant increase compared with the aforementioned three times[30]. The metabolism of pregnant women greatly changes with the physiological changes due to pregnancy. In particular, during the third trimester, the maternal energy source changes from glucose to lipids, and as a result, serum-free fatty acid and phospholipid concentrations increase[39]. The neutral fat in the body of pregnant women is converted to fatty acids and is used as the maternal energy source. Moreover, fatty acid oxidation occurs and it produces active oxygen species. During the third trimester, a significant amount of active oxygen species is produced in the pregnant moth-

er's body, and it is suggested that in particular, abundant lipid peroxidation products are produced. As physiological changes occur during pregnancy, lipid peroxidation products are produced in abundance in pregnant women's body in the third trimester. It must be noted that as a pilot study, this work was performed using a small group of middle-aged healthy pregnant Japanese women, who were very interested and participated in this study, without the inclusion of a control group.

No previous study has investigated the effect of aerobic exercise in the pregnant woman using the oxidative stress markers as a physiological index. If oxidative stress markers can demonstrate maximum oxygen intake, frequency, duration of the aerobic exercise to decrease stress, a midwife can conduct health education sessions about concrete contents of aerobic exercise for pregnant women. Therefore, our findings regarding the concrete contents of aerobic exercise are important for the development of health education programs for pregnant women, which is imperative to ensure a normal course of pregnancy course. Our findings are novel evidence for midwives to practice the health education for pregnant women; may be discovered.

Conclusions

Performing moderate aerobic exercise three times a week during second to third trimesters of pregnancy is effective for reducing 8-OHdG levels in vivo. Future studies with more number of primipara and greater sampler size are imperative to test associations between participant backgrounds, such as psychological stress and smoking habits. Particularly, considerable increase in 8-iso-$PGF_2\alpha$ levels late during pregnancy needs comparative assessment with a control group.

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