Original Article

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Effects of lifestyle factors on urinary oxidative stress and serum antioxidant markers in pregnant Japanese women: A cohort study

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Summary **Oxidative stress plays a major pathological role in pregnancy-related complications. Although oxidative stress is induced by exogenous toxins in association with a poor lifestyle in normal subjects, there is little information on the factors altering oxidative stress and antioxidant levels during pregnancy. The purpose of this study was to determine the relationship between lifestyle factors and oxidative stress/antioxidant levels during each trimester and 1-month postpartum. This prospective cohort study followed 54 healthy women through pregnancy; first, second, and third trimester and 1-month postpartum. Participants were administered a questionnaire on characteristics and lifestyle factors. Morning blood and urine samples were obtained to measure urinary biopyrrins and serum coenzyme Q10 (CoQ10) levels. The levels of urinary biopyrrins and serum CoQ10 increased significantly throughout pregnancy, with peak values registered during the third trimester. Higher biopyrrin levels were significantly associated with non-consumption of morning meal during the first trimester, smoking during the third trimester and 1-month postpartum, alcohol consumption during the third trimester, high food-based polyunsaturated fatty acid intake during the third trimester, and poor mental health scores during the first and third trimesters. Higher CoQ10 levels were significantly associated with no smoking during pregnancy and at 1-month postpartum, and with a high frequency of exercise during the third trimester and 1-month postpartum. Thus, pregnancy represents a state of oxidative stress, which can be counterbalanced by increased levels of antioxidants, such as CoQ10. We speculate that certain lifestyle choices such as avoiding smoking can reduce oxidative stress and increase antioxidant levels during pregnancy.**

Keywords: Biopyrrin, life style, oxidative stress marker, pregnant women, prenatal care

1. Introduction

Various complications of pregnancy, such as preeclampsia (PE), pregnancy-induced hypertension (PIH), and gestational diabetes mellitus (GDM), are associated with oxidative stress (*1-5*). Thus, clinical and experimental evidence indicates that oxidative stress is important in the aetiology of PE, PIH, and GDM. The levels of oxidative stress markers, such as oxidised low-density lipoprotein (LDL) and malondialdehyde, are higher (*6*) and those of antioxidants, such as coenzyme Q10 (CoQ10), α-tocopherol, and vitamin C, are lower (*1,3,7*) in women experiencing PE, PIH, or GDM, compared with healthy pregnant women. Accumulation

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of Reactive oxygen species (ROS) in cells may cause DNA damage (*8*), which may lead to pregnancy-related complications.

Increased oxidative stress and associated DNA damage can be induced by endogenous toxins released during inflammation and by exogenous toxins related to poor lifestyle, such as smoking (*9*), excessive drinking (*10*), and poor mental health (*e.g.*, psychological stress) (*11,12*). On the other hand, the DNA repair process is accelerated by various endogenous and exogenous factors, such as exercise and vitamins, in non-pregnant individuals (*13,14*). Previous studies have reported that the overall health of pregnant women and their infants is influenced by oxidative stress associated with lifestyle factors, such as smoking and vitamin intake (*15-17*). Aycicek and Ipek (*16*) noted high levels of the free radical lipid hydroperoxide (LOOH) and low levels of catalase and total antioxidant capacity (TAC) in the cord blood of pregnant smokers. They also reported a correspondingly higher incidence of low infant body weights in pregnant smokers than in non-smokers (*16*). Another study found that antioxidant supplementation in pregnant women with low levels of superoxide dismutase, an antioxidant, during the first trimester decreased the incidence of PE (*17*). However, there is little or no information on the lifestyle factors that alters oxidative stress during pregnancy. Therefore, the purpose of this study was to *i*) examine oxidative stress levels during normal pregnancy and at 1-month postpartum, and *ii*) identify the lifestyle factors that have an impact on oxidative stress and antioxidants levels at the same time points.

2. Materials and Methods

2.1. *Study design and enrolment*

This prospective cohort study was conducted at an obstetrics and gynaecology clinic in Tokyo between July 2004 and March 2005. Healthy women with singleton pregnancies, who were free of pregnancy complications and chronic illnesses, were asked to participate in this study; the women were followed individually, between the first trimester to 1-month postpartum. Sixty-two of the 75 (82.7%) pregnant women, at 12-13 weeks gestation, who visited the clinic for check-up between July and August 2004 agreed to participate in the study. Demographic data and the results for 8 of the 62 participants were accidentally lost during the study. Therefore, data collected during the first, second, and third trimesters, and at 1-month postpartum from 54 (72.0%) participants were available for analysis. Each woman underwent an ultrasonography examination, prior to enrolment, to determine the accurate gestational age. The study protocol was approved by the ethics review committee of the Graduate School of Medicine, The University of Tokyo, and written informed consent was obtained from each participant.

2.2. *Biological sample collection*

At their clinic visits during the first (12 weeks), second (22 weeks), and third (32 weeks) trimester and at 1-month postpartum, participants were asked to complete a food frequency questionnaire and a questionnaire on basic characteristics and lifestyle factors. Morning blood and urine samples were obtained at the time of routine check-ups at the same time when the questionnaires were completed.

After allowing the blood to clot at room temperature, the blood samples were centrifuged at 1,610× g for 10 min, and stored at -50°C. The urine samples were immediately protected from exposure to light and stored at -50°C. All samples were analysed within 6 months.

2.3. *Characteristics and lifestyle data*

Maternal age, gravidity, parity, pre-pregnancy body mass index (kg/m^2), blood pressure (mmHg), and urinary protein levels during pregnancy and at 1-month postpartum, together with data on the newborns, such as gestational weeks at delivery (weeks), birth weights (g), and placental weights (g) were collected from the maternal medical records. Lifestyle variables related to high and low ROS and antioxidant levels, such as current intake of vitamin supplements, alcohol consumption, smoking, and exercise habits, night time sleep (hours), and working status, were obtained from the questionnaire.

2.4. *Mental heath*

Mental health was evaluated by the 12-item General Health Questionnaire (GHQ-12) (*18-20*). The Cronbach's alpha for this study was 0.85. A high GHQ-12 score represents low mental health level. The cut-off point was set at a GHQ-12 score of 4 points (*21*).

2.5. *Dietary data*

The brief, self-administered, diet history questionnaire (BDHQ) was used to obtain data on the intake of various foods during the preceding month (*22*). The BDHQ included 74 questions on the frequency of consumption of 62 specified food items, seasonings, supplements, beverages, and others. Seven or eight frequency responses were used for ranking, from 'never' to 'more than 4 times per day'. Based on the type of consumed foods, the main nutrients consumed by each participant were selected based on the Standard Tables of Food Composition in Japan (5th ed.) (*23*).

2.6. *Measurements of urinary biopyrrin and serum CoQ10 levels*

Biopyrrins are oxidative metabolites of bilirubin and are generated under stress conditions such as poor mental health, infection, ischemia, and surgery (*11,24,25*). We have focused on the biopyrrin level in the clinical setting because it involves noninvasive sampling and reflects lifestyle factors such as psychological status (*11*). Therefore, biopyrrin was adopted as a marker for oxidative stress because it is a predictor of optimal lifestyle during pregnancy. Furthermore, the antioxidant CoQ10 was used to clarify the relationship between oxidative stress and pregnancy complications in previous studies (*1,3*). In addition, CoQ10 has a strong antioxidant effect on the human body; hence, it was considered as a marker for oxidative stress.

2.6.1. *Measurement of urinary biopyrrin*

The biopyrrin levels were measured in duplicate, using a biopyrrin enzyme immunoassay kit that employs an alkaline phosphatase-labelled 24G7 anti-bilirubin monoclonal antibody (Cat. No. B433, Shino-test Corp., Kanagawa, Japan) (*26,27*). The results were expressed relative to urinary creatinine concentrations, which were measured enzymatically (Mitsubishi Chemical Medience, Tokyo, Japan). The biopyrrin immunoassay using 24G7 monoclonal antibody is influenced by hyperbilirubinemia (*26,27*); therefore, the measured urinary biopyrrin level may be influenced by the serum direct bilirubin level. Therefore, the latter was measured by a chemical oxidation-based method (Mitsubishi Chemical Medience, Tokyo, Japan).

2.6.2. *Measurement of serum CoQ10*

Serum CoQ10 levels were measured by high-performance liquid chromatography (HPLC) with an electrochemical detector system (Nanospace S1-2, Shiseido Co. Ltd., Tokyo, Japan) according to the modified method used for the assay of saliva CoQ10 levels, reported by Sekine *et al*. (*28*). The standard CoQ10 sample was kindly provided by Kaneka Co. (Osaka, Japan) through Shiseido Co., Ltd. Other chemicals required for the measurement included HPLC-grade isopropanol (catalog #29128-31, Nacalai Tesque Inc., Kyoto, Japan), methanol (#21929-23, Nacalai Tesque Inc.), and sodium periodate (catalog #410241-100G, Sigma-Aldrich, St Louis, MO, USA). The HPLC system consisted of a concentration column (Capcell Pak C8, 4 × 10 mm, 5 μm), a reduction column (Shiseido CQ, 2 × 20 mm), a separation column (Capcell Pak C18 AQ, 2 × 75 mm, 3 μm), and an electrochemical detector (650 mV) (Nanospace S1-2 3016, Shiseido Co., Ltd). When the samples were loaded, a mobile phase of 50 mM $NaClO_4$ in methanol/distilled water (95/5, v/v) was used. Next, using a column-switching system, serum CoQ10 was eluted from the concentrating column by mobile phase 2 (50 mM NaClO4 in methanol/distilled water (95/5, v/v)). The column oven temperature was set at 40°C. The calibration range of the HPLC system was 1.57-200 ng/mL.

2.7. *Statistical analysis*

Data are expressed as means ± S.D. The generalised linear mixed model, χ^2 test, or Exact test was used for the analysis of lifestyle factors. The relationship between lifestyle factors and biopyrrin or CoQ10 levels at each trimester and 1-month postpartum was tested by multiple regression analyses. The independent variables with clinically relevant factors were selected for multivariate regression analyses. The levels of urinary biopyrrins and serum CoQ10 at the 4 assessment points were tested using a generalised linear mixed model, with the time during pregnancy as the fixed effect and the individuals as random effects. Bonferroni's post-hoc comparisons were used to identify significant differences among the 4 assessment time points. A p-value < 0.05 (two-sided test) was considered significant. All data were analyzed using the Statistical Package for Social Sciences for Windows, version 18.0 (SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1. *Participants*

Table 1 summarises the characteristics and pregnancy outcomes of the participants. The mean age of the 54 women was 31.0 years; 19 women (35.2%) were primigravida, and none developed complications during pregnancy. Although delivery occurred at full-term in all women (between 37 and 42 weeks), 3 newborns (5.6%) were of low birth weight (< 2,500 g), whereas one (1.9%) experienced macrosomia (≥ 4,000 g).

Table 1. Characteristics of pregnant women (n = 54)

Age (years)[1)]	31.0 (22.0 - 39.0)
Parity	1.0 (0 - 4.0)
0	19 (35.2)
1	27 (50.0)
2	7 (13.0)
3	0
4	1 (1.8)
Pre-pregnancy BMI (kg/m^2)[2)]	20.3 (16.1 - 34.7)
Blood pressure ≥ 140/90mmHg[3)]	0
Urinary Protein ≥ 1+[3,4)]	14 (25.9)
Newborn	
Gestational week at delivery (weeks)	39.0 (37.0 - 41.0)
Birth weight (g)	3092.5 (2290.0 - 4300.0)
Low birth weight: < 2500 (g)	3 (5.6)
High birth weight: ≥ 4000 (g)	1 (1.9)
Placenta weight (g)	570.0 (370.0 - 810.0)

Data are median (range; minimum - maximum) or n (%). [1)] Age at study entry. [2)] Body Mass Index measured before a pregnancy. [3)] Data obtained during pregnancy and at one month postpartum.[4)] Dipstick with trace protein was used to detect urine protein. Protein 1+: 30 mg/dl.

3.2. *Biomarker levels*

The mean serum direct bilirubin level was stable during the study (first trimester: 0.24 mg/dL, second trimester: 0.15 mg/dL, third trimester: 0.24 mg/dL, 1-month postpartum: 0.16 mg/dL). The mean urinary biopyrrin level during the first trimester was 2.90 μmol/g Cre, but subsequently increased to 4.95 μmol/g Cre at the second trimester ($p < 0.001$), with a further increase of 1.3-fold in the third trimester (6.27 μmol/g Cre, $p = 0.004$); however, the mean urinary biopyrrin level decreased at 1-month postpartum to 3.52 μmol/g Cre, relative to the level at the third trimester ($p < 0.001$) (Figure 1). Further comparisons showed that the mean urinary biopyrrin level during the third trimester was significantly higher than that during the first trimester ($p < 0.001$), but there was no significant difference between the levels during the first trimester and 1-month postpartum ($p = 0.569$).

Figure 2 depicts serum CoQ10 levels during pregnancy and after delivery. The mean CoQ10 level increased significantly from 631 ng/mL during the first trimester, to 1,080 ng/mL during the second trimester ($p < 0.001$), and further to 1,639 ng/mL during the third trimester ($p < 0.001$); however, the mean CoQ10 level decreased after delivery to 919 ng/mL, relative to the level at the third trimester ($p < 0.001$). Further comparisons indicated a significant difference between the levels during the first and third trimesters ($p < 0.001$) and between the levels during the first trimester and 1-month postpartum ($p < 0.001$).

3.3. *Relationship between urinary biopyrrin and serum CoQ10 levels*

Spearman's correlation analysis between urinary biopyrrin and serum CoQ10 levels was performed after adjusting for the serum direct bilirubin level and the mean age during each trimester and 1-month postpartum. The Spearman's correlation coefficients were –0.04 for the first trimester and –0.21 for the third trimester (no significant difference), and –0.32 for the second trimester and –0.31 for 1-month postpartum (both, $p < 0.05$).

In addition, multiple linear regression analysis was performed to examine the relationship between the area under the curve (AUC) of the urinary biopyrrin and serum CoQ10 levels measured during the first, second, and third trimesters, and 1-month postpartum, after adjusting for age and serum direct bilirubin levels measured at the same time points. The standardised partial regression coefficient (β) of the relationship was –0.303 ($p < 0.001$), indicating a significant negative correlation between the levels of the 2 indicators. The goodness of fit of the statistical model (adjusted R^2) was 0.33 ($p < 0.001$).

3.4. *Relationship between oxidative stress, antioxidants, and lifestyle factors*

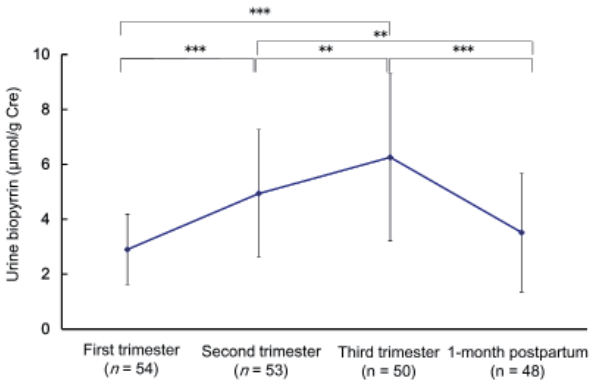

Figure 1. Urinary biopyrrin levels during pregnancy and at 1-month postpartum. Generalised linear mixed models involving the 4 assessment points as fixed effects and the subject as a random effect were established. Bonferroni's post-hoc comparisons were used to identify significant differences among the 4 assessment time points. Data are mean ± S.D. ** $p < 0.01$, *** $p < 0.001$

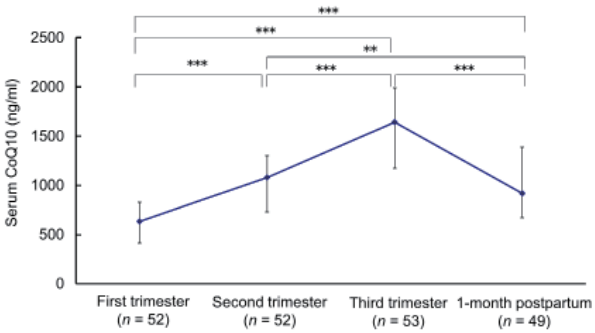

Figure 2. Serum Coenzyme Q 10 levels uring pregnancy and at 1-month postpartum. Generalised linear mixed models involving the 4 assessment points as fixed effects and the subject as a random effect were established. Bonferroni's post-hoc comparisons were used to identify significant differences among the 4 assessment time points. Data are mean ± S.D. ** $p < 0.01$, *** $p < 0.001$.

As shown in Table 2, the proportion of women who exercised (defined as more than 30 minutes, twice per week) was significantly lower during the first trimester (7.4%) than during the second and third trimesters (20% and 20%, respectively), but similar to that at 1-month postpartum (2.0%, $p = 0.007$). Interestingly, the proportion of women who worked during pregnancy changed significantly from 31.7% during the first trimester to 27.8% during the second trimester, 9.3% during the third trimester, and 2.0% during the first postpartum month ($p < 0.001$). Of these women, eight retired, six went on maternity leave, and two stopped working for another reason.

During the first trimester, 5 (9.3%) and 4 (7.4%) women took vitamin supplements (*e.g.*, vitamins C, E, B, and/or folic acid), on a regular and irregular basis, respectively. These proportions did not change significantly after delivery ($p = 0.15$). As shown in Table 3, daily food-related intake of vitamins, including folate and vitamins B6, B12, C, and E, did not change significantly from pregnancy to after delivery.

Table 2. Lifestyle Factors during pregnancy and at one month postpartum

Items	First trimester (n = 54)	Second trimester (n = 54)	Third trimester (n = 54)	1- month postpartum (n = 49)	p[1)]
Breakfast habits	43 (79.6)	40 (74.1)	44 (81.5)	40 (81.6)	0.87
Night-time sleep (h)	7.5 (4.5 - 10)	7.0 (5.0 - 9.0)	7.0 (3.5 - 10.0)	5.0 (3.0 - 7.0)	< 0.001***
Day time sleep (min)	30.0 (0 - 120)	37.5 (0 - 120)	30.0 (0 - 180)	30.0 (0 - 120)	0.41
Vitamin supplements intakes					
Regular intakes	5 (9.3)	8 (14.8)	4 (7.4)	0	0.15
Irregular intakes	4 (7.4)	2 (3.7)	3 (5.6)	3 (6.1)	
Alcohol consumption	2 (3.7)	2 (3.7)	3 (5.6)	2 (4.1)	0.96
Smoking habits	5 (9.3)	5 (9.3)	4 (7.4)	3 (6.1)	0.93
Number of cigarettes in a day[2)]	12.5 (5.0 - 20.0)	5.0 (2.0 - 15.0)	10.0 (3.0 - 20.0)	10.0 (8.0 - 10.0)	0.53
Exercise habits[3)]	4 (7.4)	11 (20.4)	11 (20.4)	1 (2.0)	0.007**
Number of exercise per week[4)]	5.3 (2.0 - 7.0)	4.0 (2.0 - 14.0)	5.0 (2.0 - 7.0)	3.0 [5)]	0.997
Current work	17 (31.7)	15 (27.8)	5 (9.3)	1 (2.0)	< 0.001***
Mental Health (GHQ score)[6)]					
0 - 12 points	1.0 (0 - 11)	0.0 (0 - 10)	0.0 (0 - 11)	1.0 (0 - 9)	0.11
≥ 4 points	13 (24.3)	4 (7.4)	8 (14.8)	10 (20.4)	0.14

Data are median (range; minimum - maximum) or n (%). [1)] **: $p < 0.01$, ***: $p < 0.001$. Numerical data: Generalized linear mixed models which contains 4 research points as fixed effect and subject as random effect was conducted. Categorical: χ^2 test or Exact test was conducted. [2)] Number of cigarettes smoked per day. [3)] At least two 30-min session per week. [4)] Number of exercises performed by those who routinely exercise. [5)] Real number in a pregnant woman who have exercise habits. [6)] GHQ: General Health Questionnaire. A high score indicates low mental health. n = 53 in second and n = 48 in 1-month postpartum for missing data.

Table 3. Daily dietary intake from first trimester to 1-month postpartum from BDHQ[1)]

Items	First trimester	Second trimester	Third trimester	1-month postpartum	p[4)]
n	54	54	53[2)]	44[3)]	
Folate (μg/1000kcal)	144.4 (60.7 - 294.7)	139.7 (74.9 - 339.7)	132.9 (80.6 - 311.2)	247.8 (72.8 - 247.8)	0.85
Vitamin B6 (mg/1000kcal)	0.6 (0.2 - 0.8)	0.6 (0.4 - 0.9)	0.6 (0.3 - 0.9)	0.6 (0.2 - 0.8)	0.68
Vitamin B12 (μg/1000kcal)	3.3 (0.2 - 7.5)	3.8 (1.2 - 15.2)	3.6 (0.7 - 9.2)	3.2 (0.6 - 5.6)	0.06
Vitamin C (mg/1000kcal)	51.4 (17.3 - 151.6)	48.3 (18.6 - 111.4)	46.3 (20.0 - 125.9)	51.6 (10.3 - 124.5)	0.71
Vitamin E (mg/1000kcal)	4.6 (2.2 - 7.2)	4.6 (2.4 - 7.7)	4.6 (2.6 - 7.1)	4.2 (2.3 - 6.0)	0.05
Amont of Alcohol consumption (g/1000kcal/day)	0 (0 - 7.0)	0 (0 - 1.1)	0 (0 - 1.2)	0 (0 - 9.7)	0.3
Polyunsaturated fatty acid intake (g/1000kcal/)	6.9 (3.9 - 10.9)	7.3 (4.1 - 12.3)	7.2 (4.4 - 11.1)	7.1 (3.7 - 9.2)	0.3

Data are shown at the Median (Range; Minimum - Maximum) or n (%). [1)] BDHQ; brief, self-administered, diet history questionnaire. [2)] Missing data for 1 out of 54 participants. [3)] Missing data for 5 of 49 participants. [4)] The generalized linear mixed model (which included the four time points as fixed effect and subject as random effect) was used.

Table 4. Lifestyle factors relating to levels of Biopyrrin by trimester of pregnancy

Items	First trimester (n = 54)		Second trimester (n = 52)[1)]		Third trimester (n = 49)[2)]		1- month postpartum (n = 42)[3)]	
	β	p	β	p	β	p	β	p
Breakfast habits (0: no, 1: yes)	- 0.33	0.04*	0.11	0.49	- 0.12	0.39	0.02	0.89
Smoking habits (0: no, 1: yes)	- 0.19	0.23	- 0.03	0.87	0.29	0.03*	0.66	< 0.001***
Amont of Alcohol consumption (g/1000kcal/day)	- 0.04	0.79	- 0.02	0.87	0.45	< 0.001***	- 0.02	0.89
Polyunsaturated fatty acid intake (g/1000kcal/day)	- 0.11	0.44	- 0.06	0.68	- 0.44	0.002**	0.12	0.41
Number of exercise per week (times/week)	0.22	0.14	- 0.24	0.11	0.09	0.49	- 0.09	0.53
GHQ Score: 0 - 12 points[4)]	0.29	0.045*	- 0.08	0.57	0.29	0.02*	- 0.06	0.65
Adjusted R^2	0.08	0.12	- 0.06	0.78	0.34	0.001**	0.31	0.003**

β: standardized partial regression coefficient. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ by multiple regression analysis. [1)] n = 52 in the second trimester with missing data for biopyrrin level and the GHQ. [2)] n = 49 in the third trimester with missing data for biopyrrin level (n = 4) and dietary status. [3)] n = 42 in the second trimester with missing data for biopyrrin level (n = 1), the GHQ (n = 1), and dietary status (n = 5). [4)] GHQ (General Health Questionnaire): the higher the score indicate the lower the mental health.

Tables 4 and 5 indicate the relationship between various lifestyle factors during each trimester and urinary biopyrrin levels at same points. Higher biopyrrin levels were significantly associated with non-consumption of morning meals during the first trimester ($p < 0.05$), smoking habits during the third trimester (p

Table 5. Lifestyle factors relating to levels of Coenzyme Q10 by trimester of pregnancy

Items	First trimester ($n = 52$)[1]		Second trimester ($n = 51$)[2]		Third trimester ($n = 52$)[3]		1- month postpartum ($n = 43$)[4]	
	β	p	β	p	β	p	β	p
Breakfast habits (0: no, 1: yes)	- 0.28	0.11	- 0.28	0.060	- 0.29	0.051	- 0.21	0.16
Smoking habits (0: no, 1: yes)	- 0.37	0.031*	- 0.37	0.014*	- 0.38	0.013*	- 0.55	0.001**
Amont of Alcohol consumption (g/1000kcal/day))	0.02	0.87	- 0.07	0.59	0.08	0.54	- 0.003	0.98
Polyunsaturated fatty acid intake (g/1000kcal/day)	- 0.08	0.61	0.13	0.34	- 0.18	0.20	- 0.28	0.06
Number of exercise per week (times/week)	0.24	0.11	0.24	0.09	0.36	0.012*	0.43	0.004**
GHQ Score: 0 - 12 points[5]	- 0.03	0.83	0.06	0.67	0.04	0.76	- 0.04	0.8
Adjusted R^2	0.05	0.22	0.11	0.07	0.16	0.03*	0.29	0.005**

β: standardized partial regression coefficient. *: $p < 0.05$, **: $p < 0.01$ by multiple regression analysis. [1] $n = 52$ in the first trimester with missing data for CoQ10 level. [2] $n = 51$ in the second trimester with missing data for CoQ10 ($n = 2$) and the GHQ ($n = 1$). [3] $n = 52$ in the third trimester with missing data for CoQ10 ($n = 1$) and dietary status ($n = 1$). [4] $n = 43$ in third trimester for missing data of GHQ ($n = 1$) and dietary data ($n = 5$). [5] GHQ (General Health Questionnaire): the higher the score indicate the lower the mental health.

< 0.05) and 1-month postpartum ($p < 0.001$), alcohol consumption during the third trimester ($p < 0.001$), high food-based polyunsaturated fatty acid intake during the third trimester ($p < 0.01$), and higher GHQ-12 scores during the first and third trimesters ($p < 0.05$, both trimesters). Higher CoQ10 levels were significantly associated with no smoking habits during all trimesters ($p < 0.05$) and 1-month postpartum ($p < 0.001$) and higher frequencies of exercise during the third trimester ($p < 0.05$) and 1-month postpartum ($p < 0.01$).

4. Discussion

The present study investigated changes in urinary biopyrrin levels during pregnancy and the immediate postpartum period in healthy Japanese women. The relationships between various lifestyle factors and markers of oxidative stress and antioxidant levels during each trimester and 1-month postpartum were analysed. The results showed significant increases in the levels of urinary biopyrrins (a marker of oxidative stress) and CoQ10 (an antioxidant) during pregnancy, with peak levels achieved during the third trimester. These results, in normal pregnant women, indicate the usefulness of urinary biopyrrins as markers of oxidative stress during pregnancy. These findings also add support to previously published results that demonstrated increased levels of oxidative products in healthy pregnant women, including lipid hydroperoxides (*29*), oxidised LDL (*30*), and malondialdehyde (*9*) in longitudinal studies as well as increased levels of urinary biopyrrins in cross-sectional studies (*31*). Furthermore, the findings of significant increases in antioxidant levels during pregnancy matched those reported for levels of vitamin E (*30*), TAC, uric acid (*29*), and CoQ10 (*32,33*).

Urinary biopyrrins and CoQ10 levels decreased significantly after delivery, relative to those recorded during the second and third trimesters. Previous studies reported that pregnancy is associated with a state of oxidative stress due to increased placental mitochondrial activity and production of ROS, primarily the superoxide anion (*34*). The placenta also produces other ROS such as nitric oxide and peroxynitrite, which have pronounced effects on placental function, including trophoblast proliferation and differentiation and vascular reactivity (*34*). We also previously reported that the mean level of urinary biopyrrins was 1.7 ± 0.9 μmol/g Cre in non-pregnant women (*31*), which is markedly lower than that found in pregnant women in the present study. Thus, the above studies and the present findings suggest that the reason for the decrease in the levels of both markers after delivery is related to the termination of pregnancy and the tissue that produced the ROS (i.e., the placenta).

The standardised partial regression coefficient (β) for the relationship between urinary biopyrrins and serum CoQ10 from the first trimester to 1-month postpartum was –0.303 ($p < 0.001$). In other words, there was a significant negative correlation between urinary biopyrrins and serum CoQ10 levels. We have also reported that the mean level of serum CoQ10 in non-pregnant women was 623 ± 237 ng/mL (*35*), which is markedly lower than that found in pregnant women in the present study. The elevated levels of antioxidants (*i.e.*, serum CoQ10) during pregnancy seem to counterbalance the heightened state of oxidative stress that also exists during pregnancy.

Urinary biopyrrin concentration was negatively correlated with the serum CoQ10 level. On the other hand, both urinary biopyrrin and serum CoQ10 levels increased until the third trimester and decreased at 1-month postpartum. Regardless of the negative correlation between the AUCs of both markers, the standard deviation of biopyrrin was greater than that of CoQ10. This indicates that participants with higher CoQ10 AUCs had lower biopyrrin AUCs throughout pregnancy and at 1-month postpartum. Our results showed that the levels of oxidative stress markers and

antioxidants were the highest at the third trimester. In this regard, Shimomura *et al*. (*36*) examined men and non-pregnant women during normal psychological changes and concluded that the maximum attainable increase in urinary biopyrrin levels was twofold that of the normal value. However, our results showed that the levels of urinary biopyrrins can increase by more than twofold during pregnancy. A comparison of the results from other studies also indicated that the third trimester was associated with increased oxidative stress, represented by the level of urinary biopyrrins during that period of pregnancy, than that reported following ultramarathons (4.05 μmol/g Cre) (*37*) and in patients with depression (4.7 μmol/g Cre) (*11*).

The present results showed that the higher biopyrrin and lower CoQ10 levels during each trimester were significantly associated with smoking habits during the third trimester and during the first month postpartum for biopyrrin, and during each trimester and the first month postpartum for CoQ10. A high frequency of exercise during the third trimester and the first month postpartum was significantly associated with higher CoQ10 levels. Higher biopyrrin levels during each trimester were also significantly associated with non-consumption of the morning meal during the first trimester, alcohol consumption during the third trimester, high food-based polyunsaturated fatty acid intake during the third trimester, and higher GHQ-12 scores during the first and third trimesters.

These results are in agreement with those of previous studies, which showed significantly higher plasma concentrations of malondialdehyde, a marker of oxidative stress ($p < 0.001$), and significantly lower levels of ascorbic acid, an antioxidant ($p < 0.001$), in men with high alcohol consumption than in men with low alcohol consumption (*10*). Furthermore, smoking during pregnancy has been reported to be associated with high levels of LOOH, malondialdehyde, and free radicals, and with low levels of catalase, TAC, vitamin E, and β-carotene in cord or maternal blood (*9*). High consumption of vitamin C-rich fruits and vegetables was reported to be independently and significantly associated with reduced oxidised LDL levels and with increased TAC and glutathione peroxidase activity in healthy young adults (*14*). The combination of balanced food and exercise has also been stressed. For example, a previous review concluded that regular exercise results in upregulation of antioxidant defence mechanisms, which help minimise oxidative stress (*13*).

The current study reported that the GHQ-12 total score decreased between the first and third trimesters; however, the decrease was not significant ($p = 0.11$; from the first trimester to 1-month postpartum). This result was consistent with our cross-sectional study of 594 pregnant women between their first and third trimesters. Therefore, it is feasible to assess the neurologic manifestations by the GHQ-12 questionnaire. Anan *et al*. also reported that only somatic symptoms in 4 subscales of the GHQ-28 decreased significantly from early (12-16 weeks) to late (32-26 weeks) gestation among healthy, pregnant, Japanese women; their mental health, including 'anxiety and insomnia' and 'severe depression', did not decrease (*38*). However, somatic symptoms would be expected to decline throughout pregnancy as pregnant women experience dramatic changes to their bodies. Moreover, previous studies have also reported that biopyrrin levels increase under stress conditions, such as poor mental health, in non-pregnant women or men (*11,12*). Our results showed that biopyrrin levels increase in association with lower mental health scores during pregnancy.

Although previous studies have also examined the relationship between lifestyle and oxidative stress or antioxidant capacity, the present study demonstrated that lifestyle factors during each trimester correlated with oxidative stress and antioxidant levels. These findings may be useful for future interventional studies aimed at preventing pregnancy-related complications in women and their newborns.

The present study has some limitations. First, we cannot generalize the results to the larger Japanese population because the participants were recruited from a single clinic in a Tokyo suburb with a small sample size. Therefore, many independent variables could not be analyzed by multiple linear regression. Second, self-reporting bias might affect the values of lifestyle parameters such as smoking, alcohol consumption, and dietary intake. Third, we could not explain why, in the first and second trimesters, urinary biopyrrin level showed no significant association with smoking habits. This result might indicate the presence of factors that were not analyzed, such as morning sickness or rapid placental and fetal growth in the first and second trimesters. Fourth, we used the total CoQ10 level to represent the antioxidant status of pregnant women without measuring levels of ubiquinone-10 and ubiquinol-10 separately because approximately 95% of total CoQ10 exists in the human circulation as ubiquinol-10 (*39*). However, we may have achieved greater accuracy in our results if we had measured the levels of ubiquinone-10 and ubiquinol-10 separately.

5. Conclusion

The present study confirmed that pregnancy represents a state of oxidative stress and elevated antioxidant levels, based on the observed biopyrrin and counterbalancing CoQ10 levels. We also speculate that certain lifestyle choices can reduce oxidative stress and increase antioxidant levels, such as the avoidance of alcohol consumption, smoking cessation, the maintenance of stable mental health, and regular exercise during pregnancy and the early postpartum periods.

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