## Relationship Between Environmental Factors Including Nutritional Status and Stress of Pregnant Women and Birthweight in Shimane Prefecture

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The relationship among nutritional intake status, stress and infant birthweight was examined using questionnaire-based analyses in pregnant women in Shimane Prefecture, Japan.

In Survey 1, a Food Frequency Questionnaire Based on Food Groups (FFQg) was administered to 26 pregnant women at around 12, 26, and 36 weeks of gestation and the results were analyzed. The group that experienced stress had significantly lighter birthweight and tended to have lower energy intake in early pregnancy and higher energy intake in late pregnancy than the group that felt little or no stress. In Survey 2, a self-administered diet history questionnaire was given to 84 pregnant women at 12 weeks of gestation. The results showed that birthweight was not significantly different between the high and low stress groups, whereas women in the former group consumed significantly more energy with adequate PFC balance. Collectively, PFC balance maintained within recommended range might reduce the influence of stress on infant birthweight.

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## INTRODUCTION

Low birthweight infants have been suggested to be at increased risk of developing non-communicable diseases such as obesity, glucose intolerance, hyperlipidemia, and cardiovascular disorders in the future [1–4]. In Japan, the ratio of low birthweight infants (birthweight less than 2500 g) to total births has increased since 1975 and currently stands at about 10%. This is one of the highest rates among OECD (Organization for Economic Cooperation and Development) countries [5]. The average birthweight in Japan peaked in 1975 and has been decreasing for both males and females, with a national average of 3,000 grams in 2010, about 200 grams less than in 1975 [6]. The "Healthy Parent and Child 21" (2nd phase) policy in Japan has set a goal of reducing the percentage of low birthweight babies [7].

It has been reported that pre-pregnancy thinness (body mass index  $<18.5 \text{ kg/m}^2$ ), poor weight gain during pregnancy, and maternal short stature are risk factors for low birthweight births in Japan [8–10]. In the late 1990s, the percentage of low birthweight



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infants born at full-term (after 37 weeks) increased more than those born preterm infants (less than 37 weeks) in Japan [11].

An analysis of data from World Health Organization (WHO) maternal surveys in 29 countries, including Japan, reported that poor socioeconomic status (which has been associated with poor nutritional status) was associated with a high risk of small for gestational age (SGA) in full-term births [12]. The WHO recommends that nutritional education be given to increase daily energy and protein intake for undernourished pregnant women to reduce the risk of low birthweight births [13]. A study of 136 healthy Japanese pregnant women showed that the average total energy intake was about 1600 kcal/day throughout the three trimesters of pregnancy, and failed to meet the estimated energy requirements throughout the entire pregnancy [14].

In the "Healthy Parent and Child, Shimane Plan," it was reported that, in recent years, the birth rate of low birthweight babies in both preterm and fullterm births has been higher in Shimane Prefecture than in the country of Japan as a whole. In the final evaluation of Healthy Parent and Child 21, the following factors were described as the reasons for the recent increase in the number of low birthweight babies in Japan: (1) thinness among young women, (2) smoking, (3) increase in multiple births due to increased fertility treatment, (4) aging of pregnant women, (5) weight control during pregnancy, (6) reduction of gestational weeks due to the widespread use of cesarean sections, and (7) advances in medical technology [15]. However, no definitive explanation was given for the high percentage of low-birth-weight babies in Shimane Prefecture relative to the rest of Japan [15].

Lifestyle and psychological characteristics of pregnant women that may be related to low birthweight births have been extracted, such as stress during pregnancy and overwhelming workplace conditions [16]. Intriguingly, it has been reported that the risk of SGA is actually reduced by the presence of stress in early pregnancy [8]. It was also reported that a life-events checklist may be an invalid measure of stress in African-American populations [17]. Thus, sometimes disparate results—even results the opposite of those expected—have been reported depending on the survey and evaluation methods, such as the timing and psychological and social contents of stress (for review, see [18]). The situation in Shimane Prefecture is similarly uncertain. In Shimane Prefecture, the labor force participation rate for women of working age (15–64 years old) is 74.6% (national average 67.3%), the highest in Japan, and the labor force participation rate for women of child-rearing age (25–44 years old) is 85.3% (national average 75.6%), also the highest in Japan [19]. It is also necessary to clarify whether these high rates of labor force participation are related to the nutritional intake status of the mother and birthweight of the child.

We previously surveyed the nutritional intake of 29 pregnant women out of 42 participants who were given survey forms during early pregnancy at a birthing center in Shimane Prefecture [20]. The average energy intake was less than the recommended amount, and the intake of many nutrients, including folic acid, was also below the recommended level [20]. In addition, 44.8% of the subjects missed at least one meal per week, and the protein-to-energy ratio was significantly lower in the subjects with missed meals than in the group without missed meals [21]. However, the relationship between nutrient intake and birthweight in early, mid, and late pregnancy is still not clear. Therefore, in Survey 1 of this research, we conducted a longitudinal study on the same group of subjects to clarify the relationship among the nutritional intake status at each stage of pregnancy (early, mid, and late), the presence of stress, and birthweight. Our previous study showed that the energy intake and the intake of many nutrients during early pregnancy did not reach the required levels [20]. However, the number of subjects and the amount of data were limited in that study, so for this report we conducted an additional survey, Survey 2, in which we obtained more detailed information in larger number of samples on the relationship among nutrient intake, stress during pregnancy, employment status during early pregnancy and birthweight to provide basic information for reducing the number of low birthweight babies in Shimane Prefecture.

## MATERIALS AND METHODS

#### Survey 1

## Participants

The Food Frequency Questionnaire Based on Food Groups (FFQg) was administered as a food intake frequency questionnaire at 12, 26, and 36 weeks of gestation to uncomplicated pregnant women undergoing antenatal health checkups at delivery facilities in Shimane Prefecture. This study was approved by the Ethics Committee of The University of Shimane Izumo Campus (approval no. 203).

## Survey items

## 1. Characteristics of participants

Age, height, pre-pregnancy weight, pre-pregnancy BMI, mid-pregnancy weight gain, late pregnancy weight gain, infant birthweight, gender, and early and late pregnancy hemoglobin levels were studied.

#### 2. Nutritional analysis

The Food Frequency Questionnaire Based on Food Groups (FFQg) was used to survey dietary intake. The FFQg is a food intake frequency questionnaire that assesses the content of daily meals through items concerning 29 food groups and 10 cooking methods. The reliability and validity of the questionnaire have been examined, and it has been shown that the total energy and nutrient intakes can be estimated by food group based on the estimated intake and frequency of intake for one week out of the last one to two months using the FFQg [22]. The items about eating habits and health consciousness also allow the examination of eating habits [22].

## 3. Stress

In early, mid, and late pregnancy, the respondents were asked if they felt stressed or tired, and they responded with "often," "sometimes" or "rarely."

## Statistical analysis

Correlations with infant birthweight were assessed by the following steps:

(1) Correlation coefficients were calculated to clarify the relationship between infant birthweight and maternal age, height, pre-pregnancy weight, pre-pregnancy BMI, weight at delivery, and weight gain during pregnancy.

(2) Correlation coefficients were calculated to determine the relationship between infant birthweight and maternal nutrition intake during early, mid, and late pregnancy.

(3) To determine the correlation between stress and infant birthweight/maternal nutrition intake, an unpaired t-test was conducted between two groups of women who answered "often" or "sometimes" and those who answered "rarely" to the question of whether they felt stressed or fatigued during pregnancy.

Statistical processing was performed using IBM SPSS Version 25.0 statistical analysis software. A two-tailed unpaired t-test was used for analysis between two groups, and the significance level was set at 5%.

## Survey 2

## Participants

The data from a previous study [23] were reanalyzed from a different point of view in the present study. In the previous study, the Diet History Questionnaire (DHQ), a self-administered questionnaire on food intake, was administered at 12 weeks of gestation to pregnant women who were undergoing prenatal health checkups at Shimane University Hospital (Study on the Effects of Maternal Nutrition and Lifestyle on the Fetus: Research Control Number: 20160428-6) [23]. The DHQ is a questionnaire developed to quantitatively examine the intake of nutrients and foods [24]. It can calculate the intake of about 40 nutrients and 150 foods, and has been used in a number of nutritional epidemiological studies; its accuracy and reliability are well established [24].

This study was approved by the Ethics Committee of Shimane University (approval number: 20210225-1).

## Survey items

#### 1. Characteristics of participants

Data on the following characteristics were collected for all subjects: age, height, weight in early pregnancy (at 12 weeks), BMI in early pregnancy, weight at delivery, BMI at delivery, weight gain during pregnancy, stress scores, smoking status, employment status, gestational weeks, and infant birthweight.

#### 2. Nutritional analysis

The survey on dietary intake during pregnancy was conducted using the DHQ.

#### 3. Stress

The Japanese version of the Social Readjustment Rating Scale (SRRS) was used for the stress survey [25]. The SRRS consists of 65 items describing life events and lifestyle changes, and the higher the score, the higher the level of stress [25].

### Statistical analysis

Correlations with infant birthweight were assessed by the following steps:

(1) Correlation coefficients were calculated to clarify the relationship between infant birthweight and maternal characteristics, nutrition intake, and stress scores.

(2) To investigate the correlation between maternal nutrient intake and the body size of the child at birth, infants were classified based on the tables of birth size standards by gestational age for Japanese neonates [26] according to their weight and gestational age into the following groups: a light-fordates (LFD) group with birthweight below the 10th percentile weight; an appropriate-for-dates (AFD) group with birthweight between the 10th and 90th percentile weights; and a heavy-for-dates (HFD) group with birthweight above the 90th percentile weights. The 10th and 90th percentile weights were taken from Tables 2 and 3 for boys, and Tables 4 and 5 for girls [26].

(3) The following analysis of variance among the three groups was conducted to examine whether the body size of pregnant women in early pregnancy affects their infant birthweight. The BMI of pregnant women in the first trimester of pregnancy was classified into three groups: underweight (BMI less than 18.5), normal (BMI 18.5 to 25.0), and obese (BMI 25.0 or greater), and analysis of variance among the three groups was conducted.

(4) It has been reported that 30% of those with an SRRS score of less than 150, 50% of those with a score of 150-299, and 80% of those with a score

of 300 or more have the potential for health problems in the following year [27]. In order to examine the effects of stress on infant birthweight and maternal nutrient intake, we classified the pregnant women with SRRS scores of less than 300 points into a low stress group, and those with scores of more than 300 points into a high stress group. We then compared the groups using unpaired t-test.

(5) To examine the correlation between the type of work and infant birthweight/maternal nutrient intake, unpaired t-test was used to compare results between the full-time group and the part-time group.

(6) To examine the correlation between smoking status and infant birthweight, unpaired t-test was used to compare results between smokers and non-smokers.

Statistical processing was performed using IBM SPSS ver. 22.0 statistical software. Unpaired t-test was used for analysis between two groups, and one-way analysis of variance (ANOVA) was used for analysis among three groups, with a two-tailed significance level of 5%.

## RESULTS

All values were expressed as the mean  $\pm$  standard deviation (SD).

#### Survey 1

## Flow chart of participant selection (Fig. 1)

The survey forms were distributed to 42 uncomplicated pregnant women receiving antenatal checkups at delivery handling facilities in Shimane Prefecture from July 2017 to August 2018, and 26 who continued to respond to the survey were included in the analysis.

Maternal characteristics of participants (Table 1) Maternal characteristics were as follows: age was 29.8  $\pm$  4.6 years, height was 155.7  $\pm$  5.2 cm, pre-pregnancy weight was 49.8  $\pm$  6.5 kg, pre-pregnancy BMI was 20.6  $\pm$  2.7 kg/m<sup>2</sup>, and weight gain was 10.4  $\pm$  3.2 kg.

In terms of pre-pregnancy BMI, 4 participants (15.4%) were underweight (BMI less than 18.5), 21 (80.8%) were normal weight (BMI between 18.5 and 25.0), and 1 (3.8%) was obese (BMI



Fig. 1. Flow chart of participant selection (Survey1)

greater than 25.0).

Newborn characteristics were as follows. There were 15 males (57.5%) and 11 females (42.3%). The number of gestational weeks was  $39.1 \pm 1.0$  weeks, and the infant birthweight was  $3035.4 \pm 344.2$  g overall, or  $3112.7 \pm 346.1$  g in males and  $2929.9 \pm 327.5$  g in females. The number of low birthweight infants was 1 (3.8%).

### Relationship with infant birthweight

## (1) Correlation between infant birthweight and the maternal characteristics

In the analysis of the correlation between the infant birthweight and the maternal characteristics, gestational weeks showed a weak correlation at r = 0.446. There was no significant correlation between infant birthweight and any of the following characteristics: maternal height, pre-pregnancy weight/BMI, weight gain, or BMI/weight at delivery.

## (2) Nutrient intakes in early, mid, and late pregnancy, and correlation with infant birthweight

The energy intake was  $1518 \pm 400.6$  kcal in early pregnancy,  $1677.7 \pm 300.5$  kcal in mid pregnancy, and  $1730.0 \pm 376.8$  kcal in late pregnancy.

Protein intake was  $50.7 \pm 16.3$  g in early,  $58.9 \pm 11.0$  g in mid, and  $59.4 \pm 12.9$  g in late pregnancy, meeting the estimated average requirement of 40 g/ day (mid pregnancy + 5 g/day, late pregnancy + 20 g). The protein–energy ratio was within the recommended range for pregnant women (13%–20% in early pregnancy and mid pregnancy, 15%–20%

Table 1. Characteristics of participants

	$\Pi = 20$
Variables	Mean ± SD
Maternal characteristics	
Age (years)	$29.8 \pm 4.6$
Height (cm)	$155.7 \pm 5.2$
Pre-pregnancy weight (kg)	$49.8 \pm 6.5$
Pre-pregnancy BMI (kg/m <sup>2</sup> )	$20.6 \pm 2.7$
Middle weight gain (kg)	$5.3 \pm 2.3$
Late weight gain (kg)	$10.4 \pm 3.2$
Hemoglobin : Early (g/dl)	$12.5 \pm 0.6$
Late (g/dl)	$11.2 \pm 0.7$
Newborn characteristics	
Gestational weeks (wk)	$39.1 \pm 1.0$
Birthweight (g)	$3035.4 \pm 344.2$
Male birthweight (g)	$3112.7 \pm 346.1$
Female birthweight (g)	$2929.9 \pm 327.5$

in late pregnancy) in early pregnancy and mid pregnancy, but less than the recommended range in late pregnancy. Fats were  $51.9 \pm 18.3$  g in early pregnancy,  $62.7 \pm 15.8$  g in mid pregnancy, and  $61.2 \pm 15.9$  g in late pregnancy, and the fat energy ratio was higher than the recommended range (20%-30%) in mid and late pregnancy. Carbohydrates were  $207.3 \pm 48.9$  g in early,  $213.6 \pm 42.3$ g in mid, and  $229.8 \pm 51.1$  g in late pregnancy, and the carbohydrate energy ratio was within the recommended range (50%-65%) in each period.

Folic acid intake was  $188.7 \pm 66.8 \ \mu g$  in early,  $210.6 \pm 56.5 \ \mu g$  in mid, and  $221.2 \pm 62.0 \ \mu g$  in late pregnancy, which was less than the estimated average requirement of 200  $\ \mu g/day$  (pregnant women + 200  $\ \mu g/day$ ). Nine respondents (34.6%) in early, 7 (26.9%) in mid, and 5 (19.2%) in late pregnancy responded that they took supplements.

The results of a correlation study of the relationship between infant birthweight and maternal nutrient intake during early, middle, and late pregnancy showed no significant correlation.

The number of pregnant women who missed a meal at least once a week was 10 (38.5%) in the early, 8 (30.8%) in the mid, and 6 (23.1%) in the late pregnancy group. The most common responses to the question "What do you think about missing meals?" were "No appetite" (5 respondents) in the early pregnancy group, "I want to avoid missing meals" (3 respondents) in the mid pregnancy group, and "No appetite" (3 respondents) in the late pregnancy group.

n = 26

Table 2. Corre	ble 2. Correlation between stress and infant birthweight / maternal energy intake Mean ± SD					n = 26	
	Birthweight (g)	Gestational weeks (wk)	$\begin{array}{c} \text{Pre-pregnancy}\\ \text{BMI}\\ (\text{kg/m}^2) \end{array}$	Weight gain (kg)	Early	Energy Mid (kcal)	Late
Stress group $n = 14$	2909.2±289.0	39.1±1.0	19.8±2.1	10.9±3.4	1432.9±377.2	1669.8±290.1	1769.0±362.2
Little or no stress group n = 12	3182.6±355.9	39.1±1.1	21.4±3.1	9.8±3.0	1617.2±420.3	1687.0±325.0	1684.6±404.3
<i>p</i> -value	0.041*	0.887	0.132	0.380	0.250	0.888	0.580

Table 2. Correlation between stress and infant birthweight / maternal energy intake

\*P < 0.05 by unpaired t-test.

## (3) Correlation between stress and infant birthweight/maternal energy intake (Table 2)

A comparison of infant birthweight and gestational weeks between 14 (53.8%) women who reported feeling stressed or tired during pregnancy (stress group) and 12 (46.2%) women who reported feeling little or no stress (little or no stress group) showed no significant difference in weeks of delivery. The infant birthweight of the stress group was  $2909.2 \pm 289.0$  g, and that of the little or no stress group was  $3182.6 \pm 355.9$  g. The infant birthweight of the stress group lower than that of the little or no stress group. Energy intake tended to be lower during early pregnancy in the stress group.

### Survey 2

#### Flow chart of participant selection (Fig. 2)

Of the 108 women whose nutritional status could be assessed, 84 who met the eligibility criteria (pregnant women with singleton pregnancies) and did not meet any of the exclusion criteria (pregnant women with maternal perinatal complications, complications in the newborn, or very preterm births of less than 28 weeks) were analyzed. The association of nutritional intake, smoking, stress, and type of work with the infant birthweight was examined.

## Characteristics of participants (Table 3)

Maternal characteristics were as follows: primiparae were 39 (46.4%); age was  $32.3 \pm 4.4$  years; height was  $157.9 \pm 5.4$  cm; early pregnancy weight was  $52.8 \pm 8.8$  kg; early pregnancy BMI was  $21.2 \pm 3.4$ kg/m<sup>2</sup>; weight at delivery was  $62.4 \pm 8.9$  kg; and weight gain was  $9.6 \pm 3.3$  kg.

In terms of BMI at 12 weeks of gestation, 17



Fig. 2. Flow chart of participant selection (Survey2)

Table 3. Maternal and newborn characteristics

	11 - 64
Variables	Mean $\pm$ SD
Maternal characteristics	
Age (years)	$32.3 \pm 4.4$
Height (cm)	$157.9 \pm 5.4$
Early pregnancy weight (kg)	$52.8 \pm 8.8$
Early pregnancy BMI $(kg/m^2)$	$21.2 \pm 3.4$
Weight at delivery (kg)	$62.4 \pm 8.9$
BMI at delivery $(kg/m^2)$	$25.0 \pm 3.1$
Weight gain (kg)	$9.6 \pm 3.3$
Newborn characteristics	
Gestational weeks (wk)	$38.8 \pm 1.1$
Birthweight (g)	$3017.0 \pm 364.9$
Male birthweight (g)	$3083.9 \pm 361.9$
Female birthweight (g)	$2950.1 \pm 359.7$
	n = 75
Stress	$275.3 \pm 145.7$

n = 84

participants (20.2%) were underweight (BMI less than 18.5), 55 (65.5%) were normal weight (BMI between 18.5 and 25.0), and 12 (14.3%) were obese (BMI greater than 25.0).

Newborn characteristics were as follows: males were 42 (50.0%), females were 42 (50.0%), gestational weeks were  $38.8 \pm 1.1$  weeks, and infant birthweight was  $3017.0 \pm 364.9$  g overall,  $3083.9 \pm 361.9$  g for males, and  $2950.1 \pm 359.7$  g for females. The number of low birthweight infants was 5 (6.0%).

Of the 84 participants, stress scores could be assessed in 75 participants. The stress score was  $275.3 \pm 145.7$ .

Of the subjects whose employment details were known, 32 worked full-time, 11 worked part-time, 1 was in management, and 5 worked at night; employment details were unknown in 35 subjects. Regarding smoking, 57 (67.9%) of the subjects did not smoke, 4 (4.8%) smoked 1-9 cigarettes/day, 15 (17.9%) smoked 10-19 cigarettes/day, and smoking status was unknown in 8. In terms of physical activity level (PAL), 43 (51.2%) had low PAL (PAL level I: most time spent in a seated position and mainly engaged in static activities), 40 (47.6%)had normal PAL (level II: most work sedentary, but with some movement around the workplace, work in a standing position, serving of customers, etc., or engagement in commuting, shopping, housework, light sports, etc.), and 1 (1.2%) had high PAL (level III: jobs requiring a lot of moving or standing).

#### Correlations with infant birthweight

(1) Correlation between infant birthweight and characteristics, nutrition intake status, and stress scores

 Correlations between infant birthweight and maternal characteristics were examined as a whole, and for mothers of boys (hereinafter referred to as "boys") and mothers of girls (hereinafter referred to as "girls").

Correlation coefficients with the overall gestational weeks were as follows: whole, r = 0.389; boys, r = 0.403; and girls, r = 0.396. Correlation coefficients with the height of pregnant women were as follows: whole, r = 0.315; boys, r = 0.422. Correlation coefficients with the early pregnancy weight were as follows: whole, r = 0.256.

- Correlation coefficients with the weight at delivery were as follows: whole, r = 0.385; boys, r = 0.333; girls, r = 0.431. Correlation coefficients with weight gain were as follows: overall, r = 0.361; girls, r = 0.432. BMI at delivery was weakly correlated with the whole group at r = 0.272; girls, r = 0.366. No correlations were found between infant birthweight and either age or early pregnancy BMI.
- 2) Weak correlation coefficients between infant birthweight and nutrition intake in early pregnancy were as follows. For the overall subject group: vitamin B12, r = 0.244; seeds and nuts, r = -0.237. For boys: vitamin A, r = 0.403; vitamin B2, r = 0.361; vitamin C, r = 0.316; vitamin B12, r = 0.432; folic acid, r = 0.333: manganese, r = 0.372; seeds and nuts, r = -0.338; sugar, r = 0.358; green and yellow vegetables, r = 0.365; other beverages, r = 0.350; and seafood, r = 0.343. All these results showed weak correlations. In girls, there was no correlation greater than r = 0.3 between infant birthweight and nutritional intake status.
- 3) The correlation between infant birthweight and the stress score of the pregnant women was r = -0.017.

(2) Comparison by body size at birth (Table 4)

When participants were classified into three groups according to the body size at birth gestational age [26], 11 (13.1%) were in the LFD (below the 10th percentile) group, 60 (71.4%) were in the AFD (between the 10th and 90th percentile) group, and 13 (15.5%) were in the HFD (above the 90th percentile) group.

1) Characteristics of the subjects (Table 4)

The maternal height and BMI at delivery were significantly lower in the LFD group than in the HFD group (p = 0.019, p = 0.045), and the maternal weight in early pregnancy (p = 0.021), and weight at delivery were significantly lighter in the LFD group than in the HFD group (p = 0.002).

2) Nutritional intake status

The energy intake of the LFD group was

		Mean ± SD						
		Birthweight	Gestational weeks	Height	Early pregnancy BMI	Weight at delivery	Weightgain	Energy
		$(\mathbf{g})$	$(\mathbf{wk})$	(cm)	$(kg/m^2)$	(kg)	(kg)	(kcal)
LFD n=11		$2572.6 \pm 162.2$	$39.2 \pm 1.1$	$155.6 \pm 4.2$	$20.4 \pm 3.1$	58.1±8.0	$8.7 \pm 2.3$	$1568.8 \pm 452.6$
AFD n=60		$2976.6 \pm 245.6$	38.7±1.0	$157.5 \pm 5.3$	21.1 ± 3.3	61.5±8.2	9.4±3.2	1467.3±546.3
HFD n=13		3579.6±265.2	39.2±1.2	$161.5 \pm 5.4$	22.6±3.8	$70.2 \pm 9.4$	$11.4 \pm 3.9$	$1808.8 \pm 485.4$
	<u>†1</u>	< 0.001*	0.185	0.015*	0.218	0.001*	0.093	0.110
m 1	<u>†2</u>	< 0.001*	0.341	0.526	0.860	0.413	0.806	0.827
<i>p</i> -value	<u>†3</u>	< 0.001*	0.333	0.034*	0.287	0.003*	0.121	0.092
	<u>†4</u>	< 0.001*	0.998	0.019*	0.237	0.002*	0.122	0.510

Table 4. Correlation between subject characteristics and infant body size at birth

\*P<0.05 by one-way analysis of variance.

p-values: †1 for LFD/AFD/HFD, †2 for LFD/AFD, †3 for AFD/HFD, and †4 for LFD/HFD.

Table 5. Infant birthweight and maternal weight gain by BMI in early pregnancy

		Mean ± SD		
Variable	Underweight	Normal	Obese	<i>p</i> -value
	n = 17	n = 55	n = 12	
Birthweight (g)	$3041.2 \pm 403.3$	$2986.3 \pm 331.4$	3123.3 ± 459.5	0.482
Gestational weeks (wk)	$38.9 \pm 1.3$	$38.7 \pm 1.0$	$39.2 \pm 1.0$	0.373
Weight gain (g)	$11.1 \pm 3.1$	$9.5 \pm 3.2$	$8.3 \pm 3.6$	0.070

\*P<0.05 by one-way analysis of variance.

 $1568.8 \pm 452.6$  kcal in early pregnancy, which was below the estimated requirement.

Protein intake was  $47.5 \pm 14.4$  g for the LFD group, which was within the estimated mean requirement and above the recommended 50 g for the HFD group.

There was no significant difference in energy intake, protein, fat, or carbohydrate intake among the three groups. Vitamin B12 and potassium intakes were significantly lower in the LFD group than in the HFD group, and vitamin B6, vitamin B12, folic acid, potassium, magnesium, and dietary fiber intakes were significantly lower in the AFD group than in the HFD group (data not shown).

In the comparison of PFC balance, which is the composition of the ratio of caloric value supplied by the three macronutrients, i.e., protein, fat, and carbohydrate, there was no significant difference among the three groups (data not shown).

In terms of intake by food group, seafood intake was significantly lower in the LFD group than in the HFD group, and confectionery and other vegetable intakes were significantly lower in the AFD group than in the HFD group (data not shown).

(3) Comparison by BMI in early pregnancy (Table 5) Participants were classified into three groups by early pregnancy BMI. There were 17 participants (20.2%) in the underweight group, 55 (65.5%) in the normal group, and 12 (14.3%) in the obese group.

1) Characteristics of the subjects (Table 5)

When infant birthweight was compared, there was no significant difference among the three groups. Weight gain was  $11.1 \pm 3.1$  kg in the underweight group,  $9.5 \pm 3.2$  kg in the normal group, and  $8.3 \pm 3.6$  kg in the obese group, with a trend toward weight gain in the underweight group (p = 0.070).

2) Nutrient intake

There was no significant difference in nutrient intake among the three groups.

	Mean		
Variable	<b>Low stress</b> (less than 300)	High stress (over 300)	<i>p</i> -value
	n = 46	n = 29	
Birthweight (g)	$3033.5 \pm 379.7$	$2997.1 \pm 360.8$	0.681
Gestational weeks (wk)	$38.7 \pm 1.1$	$38.9 \pm 1.0$	0.627
Early pregnancy BMI (kg/m <sup>2</sup> )	$21.5 \pm 3.1$	$20.4 \pm 3.4$	0.160
Weight gain (g)	$9.8 \pm 3.2$	$9.7 \pm 3.7$	0.899

Table 6. Correlation between stress and infant birthweight / maternal weight gain

\*P < 0.05 by unpaired t-test.

Table 7. Correlation between stress and maternal nutrient intake

	Mean	Mean $\pm$ SD		
Variable	Low stress (less than 300)	High stress (over 300)	<i>p</i> -value	
	n = 46	n = 29		
Energy (kcal)	$1409.2 \pm 451.7$	$1663.1 \pm 641.1$	0.048*	
Protein (g)	$45.1 \pm 15.0$	$54.9 \pm 22.2$	0.025*	
Dietary fats (g)	$46.3 \pm 22.4$	$54.0 \pm 25.9$	0.182	
Carbohydrate (g)	$199.2 \pm 58.1$	$235.2 \pm 94.5$	0.044*	
Protein energy ratio (%)	$12.7 \pm 2.0$	$13.3 \pm 2.0$	0.262	
Fat energy ratio (%)	$28.4 \pm 6.9$	$28.9 \pm 6.6$	0.775	
Carbohydrate energy ratio (%)	$57.9 \pm 8.0$	$56.8 \pm 7.5$	0.559	
Vitamin $B_1$ (mg)	$0.6 \pm 0.2$	$0.7 \pm 0.3$	0.039*	
Vitamin $B_2$ (mg)	$1.0 \pm 0.3$	$1.1 \pm 0.6$	0.186	
Vitamin $B_6$ (mg)	$0.7 \pm 0.2$	$0.9 \pm 0.4$	0.018*	
Vitamin $B_{12}$ (µg)	$3.3 \pm 1.8$	$4.1 \pm 2.6$	0.135	
Folic acid (µg)	$189.2 \pm 59.5$	$239.9 \pm 117.2$	0.037*	

\*P < 0.05 by unpaired t-test.

With respect to intake by food group, the intake of milk was significantly lower in the underweight group than in the normal group.

## (4) Correlation between stress and infant birthweight/maternal nutrient intake (Tables 6,7)

1) Duration of pregnancy, infant birthweight and maternal weight gain (Table 6)

The results of the comparison between the low stress group (n = 46 participants; 61.3%) and the high stress group (n = 29; 38.7%) showed no significant differences in the duration of pregnancy or infant birthweight.

## 2) Nutrient intake (Table 7)

Comparison of the differences in nutrient intake between the high stress group and low stress group showed that the energy intake was  $1409.2 \pm 451.7$  kcal/day for the low stress group and  $1663.1 \pm 641.1$  kcal/day for the high stress group, with the high stress group consuming significantly more energy (p = 0.048). The high stress group consumed significantly more protein, n-3, carbohydrates, vitamin K, vitamin B1, niacin, vitamin B6, folic acid, pantothenic acid, sodium, potassium, magnesium, phosphorus, iron, zinc, copper, dietary fiber and vitamin A (Table 7, and data not shown).

There were no significant differences in PFC balance between the low stress and high stress groups, and the protein energy ratio, fat energy ratio, and carbohydrate energy ratio were all within the recommended range.

In terms of intake by food group, the highstress group consumed significantly more cereals, green and yellow vegetables, and other vegetables.

# (5) Correlation between type of work and infant birthweight/maternal nutrient intake (Tables 8, 9)

 Infant birthweight and maternal weight gain (Table 8)

Mean $\pm$ SD				
Variable	Full-time	Part-time	<i>p</i> -value	
	n = 32	n = 11		
Birthweight (g)	$2965.4 \pm 400.5$	$3000.1 \pm 339.9$	0.799	
Gestational weeks (wk)	$39.0 \pm 1.1$	$38.5 \pm 0.9$	0.189	
Early pregnancy BMI $(kg/m^2)$	$20.7 \pm 3.3$	$22.3 \pm 3.3$	0.169	
Weight gain (g)	$10.1 \pm 2.6$	$7.8 \pm 4.3$	0.122	

Table 8. Correlation between type of work infant birthweight / maternal weight gain

\*P < 0.05 by unpaired t-test.

Table 9. Correlation between type of work and maternal nutrient intake

	Mean		
Variable	<b>Full-time</b>	Part-time	<i>p</i> -value
	n = 32	n = 11	
Energy (kcal)	$1606.2 \pm 549.7$	$1277.7 \pm 449.4$	0.082
Protein (g)	$50.8 \pm 16.9$	$41.1 \pm 12.1$	0.086
Dietary fats (g)	$53.0 \pm 25.1$	$39.7 \pm 17.6$	0.111
Carbohydrate (g)	$226.1 \pm 78.4$	$185.3 \pm 73.5$	0.138
Protein energy ratio (%)	$12.6 \pm 2.3$	$13.0 \pm 1.5$	0.588
Fat energy ratio (%)	$28.7 \pm 6.8$	$27.8 \pm 8.2$	0.706
Carbohydrate energy ratio (%)	$57.4 \pm 8.3$	$58.0 \pm 8.3$	0.840

\*P < 0.05 by unpaired t-test.

Table 10. Correlation between smoking status and infant birthweight / maternal weight gain

Mean ± SD				
Variable	No smoking	Smoking	<i>p</i> -value	
	n = 57	n = 19		
Birthweight (g)	$3059.5 \pm 373.2$	$2900.9 \pm 333.4$	0.104	
Gestational weeks (wk)	$38.9 \pm 1.1$	$38.6 \pm 0.9$	0.420	
Early pregnancy BMI $(kg/m^2)$	$20.8 \pm 3.2$	$21.7 \pm 3.2$	0.291	
Weight gain (g)	9.4 ± 3.5	$10.8 \pm 2.7$	0.136	

\*P < 0.05 by unpaired t-test.

The results of the comparison between the two working-status groups, i.e., the full-time group (n = 32, 74.4%) and part-time group (n = 11, 25.6%), the weight gain was  $10.1 \pm 2.6$  kg in the full-time group and  $7.8 \pm 4.3$  kg in the part-time group. There was no significant difference in the duration of pregnancy or infant birthweight between the two groups.

#### 2) Nutrient intake (Table 9)

A comparison of nutritional intake by work status showed that the mean energy intake of nutrients was  $1606.2 \pm 549.7$  kcal/day for the fulltime group and  $1277.7 \pm 449.4$  kcal/day for the part-time group, n-3, vitamins B2 and B6, folic acid, pantothenic acid, potassium, iron, zinc, copper and cholesterol were also significantly higher in the full-time group (Table 9, data not shown). By food group, the intake of potatoes, beans, and eggs was significantly higher in the full-time group.

## (6) Correlation between smoking status and infant birthweight/maternal weight gain (Table 10)

Comparison between the no smoking group and the smoking group showed no significant difference in the characteristics and the duration of pregnancy, but the infant birthweight tended to be lighter in the smoking group (p = 0.104).

## DISCUSSION

## Relationship between the subject characteristics

## and infant birthweight

In the examination of the relationship between the infant birthweight and the gestational weeks, a weak correlation was observed in both Survey 1 and Survey 2. In Survey 2, weak correlations were found between birthweight and maternal height, weight in early pregnancy, weight at delivery, and BMI at delivery, weight gain. In terms of maternal characteristics compared by birthweight, LFDs had shorter maternal height and lower early pregnancy weight and delivery weight than HFDs. A previous study demonstrated that the observed association between maternal height and fetal growth measures (i.e., birth length and birth weight) is mainly defined by fetal genetics [28]. In previous studies [8–10], thinness (body mass index  $<18.5 \text{ kg/m}^2$ ) before pregnancy and poor weight gain during pregnancy were reported as risk factors for low birthweight and undue weight gain, suggesting that the maternal body shape affects the infant birthweight. In 2019, the percentage of thin women in their 20s in Japan was 20.7% [29], and a survey in Shimane Prefecture in 2016 reported that approximately 20% of women in their 20s and 30s were thin, and the proportion of thin people was higher among women in their 30s and 40s than in the rest of the country [30]. Since 15% of the subjects in Survey 1 and 20.2% of the subjects in Survey 2 were emaciated, education about body shape is necessary even before pregnancy.

The highest weight gain during pregnancy was observed in the underweight group (BMI less than 18.5) in early pregnancy  $(11.1 \pm 3.1 \text{ kg})$ . In 2006, the Ministry of Health, Labour and Welfare published recommended levels of weight gain for pregnant mothers in consideration of their pre-pregnancy body shape (optimal weight gain). These levels were revised in March 2021 to the following: 12-15 kg (9-12 kg before the revision) for low body weight (BMI less than 18.5) and 10-13 kg (7-12 kg before revision) for normal body weight (BMI between 18.5 and 25.0), and 7-10 kg (individualized before revision) for obesity degree 1 (BMI 25.0 to 30) [31]. Average weight gains of the underweight and normal groups in the present study were slightly less than the lower limit of the revised desired weight gain. This may be due to the

fact that the new guidance tailors the diet and nutritional intake to the mother's specific body type.

## Relationship between maternal nutritional intake and infant birthweight

The average energy intake in early, mid, and late pregnancy in Survey 1, and in the early pregnancy in Survey 2 were both lower than the estimated requirements, and the average energy intake in early pregnancy was less than 1600 kcal, as in the previous study [14]. The estimated energy requirement for 30–49 year-old non-pregnant women is 1,750 kcal for physical activity level I (2,050 kcal for level II), and 1,800–2,100 kcal when 50 kcal for early pregnancy is added [32]. According to the National Health and Nutrition Survey of 2019 Japan, the energy intake of women was 1600 kcal in their 20s and 1673 kcal in their 30s [29], and energy intake tends to be low in women who are not pregnant.

Although energy intake was also low in the present study, the PFC balance was within the recommended range. This may have contributed to the lack of association between nutrient intake and infant birthweight.

In Survey 2, there was no significant difference in nutrient intake between the LFD and AFD groups, but the LFD group tended to consume more energy, fat, n-6, and carbohydrates on average. Intakes of vitamin B12 and potassium were significantly lower in the LFD group. Intake of vitamin B12 also has an effect on anemia. Although hemoglobin levels were not investigated in this study, the fact that in both the LFD and AFD groups intake of vitamin B12 was below the estimated average requirement suggests that intake of this vitamin should be promoted.

The intake of seafood was lower in the LFD group than in the HFD group, whereas there was no significant difference in the energy intake between the two groups. Seafood is low in calories and contains high quality animal protein. It also contains many nutrients, such as vitamins (D, E, B12), essential minerals (potassium, calcium, magnesium, etc.), and a variety of functional components such as highly unsaturated fatty acids (DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid) [33]. It

is therefore necessary to take advantage of the local dietary habits, which in Japan often include seafood, and provide specific guidance for intake of seafood and other regional staples.

In Survey 1, the number of pregnant women who missed a meal at least once a week was 10 (38.5%) in the early, 8 (30.8%) in the mid, and 6 (23.1%) in the late pregnancy group. The most common responses to the question "What do you think about missing meals?" were "No appetite" (5) in the early, "I want to avoid missing meals" (3) in the mid, and "No appetite" (3) in the late pregnancy group. In the early stages of pregnancy, morning sickness may be a factor, but as a characteristic of the diet in Shimane Prefecture, it has been reported that about one in three women in their 20s are not in the habit of eating breakfast every day [30], and nationally, about 20% of women in their 20s tend to be thin and to have low energy intake, and the rate of missing breakfast has been reported to be 30% for Japanese women in their 20s. Therefore, it is necessary to educate women about their body shape and dietary intake not only during pregnancy but also before pregnancy, since their pre-pregnancy eating habits may continue after pregnancy.

### Relationship between stress and infant birthweight

In the Survey 2 on early pregnancy, there was no significant difference in the infant birthweight or weight gain during pregnancy between the high stress group and the low stress group. The high stress group consumed significantly more energy and other nutrients, and by food group, more green and yellow vegetables and other vegetables, than the low stress group. Further, in Survey 2, PFC balance was within the recommended range for both the high and low stress groups. On the other hand, in Survey 1 covering the entire period of pregnancy, comparing the stress group with the little or no stress group, the stress group had a higher weight gain during pregnancy, but significantly lower infant birthweight. In terms of nutritional intake during the pregnancy, the stress group tended to have a lower energy intake in early pregnancy and a higher energy intake in mid and late pregnancy than the little or no stress group. This discrepancy between the results of surveys may be related to the different methods used to investigate stress.

An international systematic review reported that a healthy dietary pattern characterized by a high intake of vegetables, fruits, whole grains, low-fat dairy products, and fat-free protein foods tends to be associated with a lower risk of SGA [34]. Healthy diet with an adequate PFC balance may reduce the influence of stress on infant birthweight.

## Association between employment status and infant birthweight

In the comparison of nutrient intake, the part-time group tended to consume less of many nutrients, but there was no significant difference in the infant birthweight between the two groups. There was also no significant difference between the two groups in the PFC balance. It is possible that well-balanced nutrition may have been responsible for the lack of difference in birthweight between the two groups in this study.

Shimane Prefecture ranks first in Japan in both the labor force participation rate of women and the labor rate of the child-rearing generation, and the percentage of regular employees and staff is 52.1% [19]. Since 50% of working women are non-regular employees, it is necessary to monitor their dietary intake and provide them with lifestyle guidance in light of their working conditions.

## CONCLUSIONS

The infant birthweight was weakly correlated with the gestational weeks in both Survey 1 and Survey 2, and with maternal height, weight in early pregnancy, weight at delivery, and weight gain during pregnancy in Survey 2. The average energy intake for early, mid, and late pregnancy in Survey 1 and early pregnancy in Survey 2 were all below the estimated requirements, but the PFC balance was within the recommended range. Intake of vitamin B12 was significantly lower in the LFD group. These findings suggest that pre-pregnancy body shape and nutritional status during pregnancy may affect the infant birthweight. Therefore, education about nutritional intake, including pre-pregnancy body shape, energy intake, vitamin intake, and nutritional balance, is necessary not only during but also before pregnancy. In Survey 1 covering the entire period of pregnancy, the stress group had a higher weight gain during pregnancy, but significantly lower infant birthweight. In Survey 2, there was no significant difference in infant birthweight between the high and low stress groups in early pregnancy. Energy intake was significantly higher with adequate PFC balance in the high stress group. Discrepant results between the surveys may be related to the different methods used to investigate stress, and PFC balance within the recommended range might reduce the influence of stress on infant birthweight.

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All authors have nothing to disclose related to the content of this study.

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