Relationship between the plasma total homocysteine levels and skipping breakfast during pregnancy

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Abstract

Purpose
The frequency of skipping breakfast among reproductive-age women has been increasing in Japan. Skipping breakfast was reported to result in low intake of several nutrients including folate and vitamins required for fetal development and the prevention of pregnancy complications. A high total homocysteine (tHcy) level, which occurs in association with a folate and vitamin B12 deficiency, can lead to adverse perinatal outcomes. The aim of this study was to examine whether skipping breakfast during pregnancy is associated with the folate and vitamin B12 intake, circulating tHcy, folate and vitamin B12 levels.

Methods
Two hundred and fifty-four healthy women with a singleton pregnancy (age: 30.4±4.7, gestational age: 27.5±9.6 weeks) were recruited from a prenatal clinic in metropolitan Tokyo, Japan. The plasma tHcy, serum folate and vitamin B12 levels were measured. The nutrient intake was assessed using a self-administered diet history questionnaire. Information on lifestyle factors relevant to tHcy levels and skipping breakfast was obtained from the questionnaire. We defined skipping breakfast as forgoing breakfast including a staple food, such as rice or bread, more than twice a week during the preceding 1-month period. A multiple regression analysis was performed to determine whether skipping breakfast was associated with plasma tHcy levels, after adjusting for confounding variables.

Results
Thirty percent of the participants skipped breakfast more than twice a week. The rate of primipara was higher than multipara among breakfast skippers (p=0.005). Skipping breakfast was associated with high plasma tHcy levels among the pregnant women after adjusting for confounding factors, including serum folate and vitamin B12 levels (p=0.024). Meanwhile, there were no significant differences in the energy-adjusted intake of folate and vitamin B12 or in serum folate and vitamin B12 levels between the breakfast skippers and the non-skippers.

Conclusion
The relationship between the plasma tHcy levels and skipping breakfast could not be explained by the low levels of serum folate and vitamin B12 among the breakfast skippers, in contrast to our hypothesis. However, our results indicated that any factors relevant to skipping breakfast may affect the tHcy levels during pregnancy. Therefore, health care providers may need to pay closer attention to whether pregnant women have breakfast or not, in order to prevent increased tHcy levels.

Key words: skipping breakfast, homocysteine, pregnancy, folate, vitamin B12
I. Introduction

The frequency of skipping breakfast exceeds 20% among reproductive-age women according to the National Survey on Health/Nutrition in 2008 (Ministry of Health, Labour and Welfare, 2008). Among pregnant women, it has been reported that the rate was between 5 and 30%, although there were differences according to gestational age and the definition of skipping breakfast (Shiraishi, Haruna, Matsuzaki, et al., 2009; Tsuchitori, 2007; Okuno, Iwamoto, Hoyano, et al., 2006; Sakamoto & Miyoshi, 2003). Skipping breakfast results in lower levels of several nutrients, including folate and vitamins (Shiraishi, Haruna, Matsuzaki, et al., 2009; Saitoh & Shimoda, 2007). Folate and vitamins during pregnancy are essential for fetal development and the prevention of pregnancy complications (Tamura & Picciano, 2006; Obeid & Herrmann, 2005; Zhang, Williams, King, et al., 2002).

Homocysteine is the demethylated derivative of methionine, whose metabolism is accelerated by key enzymes such as folate, vitamin B12 (Finkelstein & Martin, 2000). A folate deficiency causes a reduction in 5-methyltetrahydrofolate which is required for remethylation of homocysteine, and a vitamin B12 deficiency causes a loss in methionine synthase activity with downregulation of the remethylation pathway of homocysteine (Carmal, Green, Rosenblatt, et al., 2003; Hague, 2003). In addition, several studies have shown that other lifestyle factors such as smoking, caffeine intake and physical activity might also affect the circulating total homocysteine (tHcy) levels (Refsum, Nurk, Smith, et al., 2006; Ozerol, E., Ozerol, I., Gökdeniz, et al., 2004; Vollset, Refsum, Irgens, et al., 2000).

Elevated tHcy levels negatively affect DNA methylation and cell proliferation (Lentz, 1998). A high tHcy level during pregnancy has recently been identified as a factor contributing to adverse perinatal outcomes including neural tube defects, intrauterine growth retardation, preterm delivery, and preeclampsia (Lindblad, Zaman, Malik, et al., 2005; Scholl & Johnson, 2000; Aubard, Darodes & Cantaloube, 2000; Vollset, Refsum, Irgens, et al., 2000).

Pregnant women with a habit of skipping breakfast might have high plasma tHcy levels, low serum folate levels and low serum vitamin B12 levels due to low intake of folate and vitamin B12. However, little is known about the relationships.

The aim of this study was to investigate the effect of skipping breakfast on the intake of folate and vitamin B12, the circulating tHcy level, and the serum folate and vitamin B12 levels among healthy pregnant women in Japan.

II. Methods

Participants

The present cross-sectional study was conducted at a private obstetric hospital from June to December of 2008 in metropolitan Tokyo, Japan. The study was approved by the Ethical Committee of the Graduate School of Medicine, at the University of Tokyo. Healthy women with a singleton pregnancy and no major complications such as diabetes or pregnancy-induced hypertension were recruited during their first trimester (8 - 12 weeks’ gestation), their second trimester (24 - 27 weeks’ gestation), or their third trimester (34 - 38 weeks’ gestation) at a time when they had routine blood tests. All the women underwent an ultrasound scan at 8 - 12 weeks’ gestation to allow accurate gestational dating. The participants were given detailed information on the study protocol and all participants gave their written informed consent.

Variables and their measurement

Questionnaires were completed by each participant while waiting for a regular pregnancy checkup. Participants, who did not have sufficient time to complete the questionnaires at the hospital, filled out the questionnaires after returning home and then returned them by mail. Any missing or unclear data were resolved by a follow-up telephone interview. All participants completed a questionnaire on their characteristics, including maternal age, gestational age, and prepregnancy body mass index (BMI). Information on lifestyle variables related to plasma tHcy, such as smoking during pregnancy, the use of folic acid supplements and multivitamins, and the frequency of supplement use (regular or irregular) was
also obtained from the questionnaire.

Skipping breakfast was defined as forgoing breakfast including a staple food, such as rice or bread, more than twice a week during the preceding 1-month period, which we determined to be consistent with the definition of the non-pregnant national survey. The regular use of folic acid supplements or multivitamins was defined as using such vitamins four or more times per week.

The women’s dietary intake during the preceding 1-month period was assessed with a validated self-administered diet history questionnaire (DHQ), which measures the daily intake of 150 foods and selected nutrients (Sasaki, Ushio, Amano, et al., 2000; Sasaki, Yanagibori & Amano, 1998). Information on both the frequency and amount of consumption, general dietary behavior and usual cooking methods was collected. Estimates of energy and nutrients were then calculated using an ad hoc computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan (Science and Technology Agency, 2005). The consumption of selected nutrients was energy-adjusted using the density method to minimize the influence of dietary underreporting. Participants who had a severe under- or over-reported energy intake, namely, whose reported energy intake was less than half the energy requirement for the lowest physical activity category, or whose reported energy intake was equal to or more than 1.5 times the energy requirement of moderate physical activity according to the “Dietary Reference Intakes for Japanese” (Ministry of Health, Labour and Welfare of Japan, 2006) were excluded from the present analyses (Sasaki, Katagiri, Tsuji, et al., 2003).

To obtain information on caffeine intake as a variable related to tHcy levels, a part of the DHQ was modified to calculate the total caffeine intake from coffee, green tea, oolong tea, black tea, cocoa, cola and chocolate, with the permission of the author of the DHQ. The caffeine content of beverages was determined as a reference of the observed caffeine levels in the beverages Japanese women had consumed in a previous cohort study (Yamada, Sasaki, Murakami, et al., 2010). The following conversion factors were used to estimate the amount of caffeine intake: the study estimated 105 mg of caffeine for 150 ml of coffee, 72 mg for green tea and oolong tea, 42 mg for black tea, and 13 mg for cocoa. In addition, the caffeine content was defined as 29 mg for 250 ml of cola, and 51 mg for 100 g of chocolate.

The amount of physical activity was measured using a modified Japanese version of the pregnancy physical activity questionnaire (PPAQ) that is the only validated instrument designed specifically to assess physical activity during pregnancy in Japan (Chasan-Taber, Schmidt, Roberts, et al., 2004). The Japanese version of the PPAQ is a semi-quantitative questionnaire that tries to determine the time spent participating in 33 activities including household/caregiving (13 activities), occupational (5 activities), sports/exercise (8 activities), transportation (4 activities), and inactivity (3 activities). For each activity, participants were asked to select the category that best approximates that amount of time spent in that activity per day or week during the preceding 1-month period. Activities of light or greater intensity were added together to calculate the average MET-hours per day of physical activity.

Non-fasting blood samples were drawn at the clinic on the day when the participants answered the questionnaires. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum and the plasma and then were stored at -80 degrees Celsius until the analysis. The plasma tHcy levels were measured using high performance liquid chromatography (HPLC). The serum folate and vitamin B₁₂ levels were measured using a chemiluminescent enzyme immunoassay (CLEIA). These assays were conducted by SRL, Inc. Tokyo, Japan.

Statistical Analysis

The differences in the characteristics between breakfast skippers and non-skippers were compared using the chi-square test, Student’s t-test, or the Mann-Whitney U test after normality was tested by the Shapiro-Wilk test. If the distribution of a variable was normal, the level was shown as the mean ± SD. Otherwise, when the distribution of a variable was not normal, the level was shown by median (interquartile range).

To determine which variables were associated with plasma tHcy levels, a multiple regression analysis was used, entering a habit of skipping breakfast and the bio-
logical markers as independent variables. In addition, gestational age, smoking during pregnancy, caffeine intake, and level of physical activity were adjusted as potential confounding variables relevant to \( \text{tHcy} \) levels. These variables were checked for multicollinearity. A multiple regression analysis was conducted after the logarithmic transformation of the plasma \( \text{tHcy} \) levels.

Statistical analyses were carried out using the SPSS software package for Windows, version 15.0 (SPSS Japan Inc.). All statistical tests were two-sided; a \( p \) value of less than 0.05 was considered to be statistically significant.

**III. Results**

A total of 321 pregnant women were recruited; of these, 292 (91.0%) gave their written informed consent, answered the questionnaire, and had blood samples drawn. Thirty-eight of the 292 participants were excluded from the analyses: 17 had missing data, 13 provided an inadequate amount of blood sample, 4 had pregnancy complications, and 4 had a severe under- or over-reported energy intake. The data from 254 pregnant women (79.1%) were analyzed; 38 were in the first trimester, 104 were in the second trimester and 112 were in the third trimester.

The characteristics of the participants are shown in Table 1. Seventy-six pregnant women (30%) skipped breakfast more than twice a week. The gestational age at the recruitment of the breakfast skippers was significantly lower than that of the non-skippers. The rate of breakfast skippers among primiparous participants was higher than of multiparous participants.

Table 2 shows the median levels of biological markers, dietary intake and physical activity. The plasma \( \text{tHcy} \) levels of the breakfast skippers were significantly higher than that of the non-skippers (\( p = 0.006 \)). Meanwhile, there were no significant differences in serum folate and vitamin \( \text{B}_{12} \) levels between the breakfast skippers and the non-skippers. These results were similar after excluding the women who took folic acid supplements and multivitamins (data not shown).

Skipping breakfast was associated with high \( \text{tHcy} \) levels during pregnancy after adjusting for confounding factors, including serum folate and vitamin \( \text{B}_{12} \) levels (\( p = 0.024 \), Table 3).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of participants</th>
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<tr>
<td><strong>All participants</strong> (n=254)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Gestational age at recruitment (weeks)</td>
</tr>
<tr>
<td>first trimester</td>
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<tr>
<td>second trimester</td>
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<tr>
<td>third trimester</td>
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<td>Height (cm)</td>
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<td>Prepregnancy body mass index (kg/m²)</td>
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<td>Parity</td>
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<td>Primipara</td>
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<td>Multipara</td>
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<td>Smoking during pregnancy</td>
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<tr>
<td>Smoker</td>
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<tr>
<td>Non-smoker</td>
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<tr>
<td>Education level</td>
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<tr>
<td>High school and below</td>
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<tr>
<td>Junior college/Technical college</td>
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<tr>
<td>College/University</td>
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Data are mean ± SD or n (%). ns: not significant. Student’s \( t \)-test or the chi-square test was conducted.
IV. Discussion

This is the first investigation to demonstrate an association between the plasma tHcy levels and breakfast skipping during pregnancy.

The definition of skipping breakfast as used by previous studies of pregnant women was inconsistent, because the perception of whether eating occasions are viewed as meals or snacks is a somewhat subjective interpretation. Therefore, to correlate with the definition of non-pregnant national survey, we defined breakfast skipping as forgoing breakfast with a staple food, such as rice or bread, more than twice a week during the preceding 1-month period. Thirty percent of the participants had a habit of breakfast skipping, which was a percentage similar to that of non-pregnant women (Ministry of Health, Labour and Welfare, 2008). Having breakfast everyday in the first trimester might be difficult because nausea related to pregnancy may likely affect the frequency of eating, the timing of eating, and also what the women eat. However, it should be noted that more than 20% of our participants skipped breakfast habitually, even in the second and third trimester, during which fewer people suffered from nausea. A previous study showed that the dietary patterns prior to pregnancy continued throughout pregnancy (Crozier, Robinson, Godfrey, et al, 2009). Although skipping breakfast was not mentioned in this study, it was reported that the dietary pattern scores and frequency of consumption of foods changed little before and throughout pregnancy. It might therefore be difficult for pregnant women to modify their prepregnancy dietary habits by themselves, even though they know that consuming a sufficient amount of nutrients, which are required especially during pregnancy, is important for both themselves and their babies. In the present study, we were unable to assess whether a habit of skipping breakfast among pregnant women continued based on prepregnancy habits. Further longitudinal studies from prepregnancy to birth will therefore be needed to elucidate whether interventional strategies.
against skipping breakfast are required.

The rate of the primiparous participants among breakfast skippers was higher than the multiparous participants, as reported by a previous study (Shiraishi, Haruna, Matsuzaki, et al., 2009). Multipara might be likely to change dietary habits in accordance with their children’s lifecycle.

We hypothesized that serum folate and vitamin B_{12} levels are low among breakfast skippers due to low intakes of folate and vitamin B_{12}. However, the energy-adjusted intakes of folate and vitamin B_{12} were not significantly different between the breakfast skippers and non-skippers, although previous studies have indicated that a relationship between skipping breakfast and low energy-adjusted intake of several nutrients (Shiraishi, Haruna, Matsuzaki, et al., 2009; Saitoh & Shimoda, 2007). In addition, there were also no significant differences in the serum folate and vitamin B_{12} levels between breakfast skippers and non-skippers, although the absolute intake of folate and vitamin B_{12} was lower among the breakfast skippers than non-skippers. Meanwhile, the plasma tHcy levels were higher among breakfast skippers compared to non-skippers. This indicates that these nutrient variables alone do not influence plasma tHcy levels. For instance, skipping breakfast was associated with having other unfavorable lifestyles, including frequent snacking, a late supper, a low amount of physical activity, and smoking, according to the previous studies (Nishiyama, Muto, Minakawa, et al., 2009; Saitoh & Shimoda, 2007; Okuno, Iwamoto, Hoyano, et al., 2006; Keski-Rahkonen, Kaprio, Rissanen, et al., 2003). In addition, it has been reported that non-pregnant breakfast skippers had higher total cholesterol and glucose levels, and high glucose levels have been reported to be maintained for longer periods after lunch (Qin, Yokoyama, Naruse, et al., 2003; Sakata, Matsumura, Yoshimura, et al., 2001). These related factors might overlap and affect tHcy levels, although these relationships in pregnant women are unclear. Further studies are required to clarify the factors that are relevant to skipping breakfast during pregnancy and other effects of skipping breakfast on the maternal body during pregnancy.

This study had several limitations. First, the number of participants was small, and this may have reduced the overall statistical power. Second, as noted above, other factors relevant to breakfast skippers are likely to affect the result. Third, some misestimating of the nutrient intakes and physical activity levels could have occurred because such information was collected using self-administered questionnaire methods. Fourth, we did not collect fasting blood samples in consideration that the subjects were pregnant women, although the tHcy, folate and vitamin B_{12} levels might have been affected by contents of meals that had been recently consumed prior to the collection of samples.

In conclusion, skipping breakfast during pregnancy might require increased attention in the future due to the fact that it appears to induce high tHcy levels. The duration of pregnancy is an enormous opportunity during which health care providers have frequent contact with pregnant women. For pregnant women, the pregnancy period is a good opportunity to reconsider their own nutritional status and change any unfavorable dietary habits. Health care providers must inform pregnant women about the possible adverse effects related to skipping breakfast and suggest corrective strategies to encourage pregnant women to regularly have breakfast, so that they may more easily change such adverse dietary habits.

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妊娠中の血漿総ホモシステイン値と朝食欠食の関連

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抄録

目的
妊娠期の朝食欠食は、胎児栄養や妊娠合併症予防に必要とされている葉酸やビタミン類の摂取不足を導く可能性が高いことが指摘されている。主に葉酸やビタミンB12の不足によって生じるホモシステイン高値は、子宮内胎児発育遅延や妊娠高血圧症候群の発症に関連しており、ホモシステイン値を増加させない生活習慣を持つことは重要である。本研究は、妊娠中の朝食欠食が栄養素摂取量や血液中の総ホモシステイン（tHcy）値、葉酸値、ビタミンB12値に関連しているかを明らかにすることを目的とした。

方法
埼玉県の産婦人科クリニックで妊娠検診を受診する妊娠初期から末期の254名を対象とした調査を2008年6月から12月に実施した。栄養素摂取量は、自動式食事歴法質問票を用いて評価し、各栄養素の測定誤差を排除するために密度法によって示した。質問紙調査と同時に血液を採取し、血漿tHcy値、血清葉酸値、血清ビタミンB12値を測定した。本研究では、朝食欠食を「週に2回以上、主食を含む朝食を摂取しないこと」と定義した。

結果
30％の妊婦が朝食欠食習慣を有していた。初産婦は、経産婦に比べ朝食欠食習慣を持つ者が多かった（p = 0.005）。交差要因調整後も、朝食欠食者では有意に血漿tHcy高値に関連していた（p = 0.024）。しかし、血清葉酸値やビタミンB12値、エネルギー調整した葉酸・ビタミンB12摂取量には、朝食摂取の有無による有意差は見られなかった。

結論
本研究では、朝食欠食に関連する要因が血漿tHcy値の上昇を導く一因となっている可能性が示された。しかしながら、仮説に反して、血漿tHcy高値と朝食欠食との関係を血清葉酸や血清ビタミンB12の低値によって説明することはできなかった。妊娠合併症の発症に関連する血漿tHcy高値を予防するためには、朝食を習慣的に摂取するための工夫が妊娠薬とともに考え、朝食の摂取を推奨する必要があるかもしれない。

キーワード：朝食欠食、ホモシステイン、妊娠、葉酸、ビタミンB12