Effects of skipping breakfast on dietary intake and circulating and urinary nutrient levels during pregnancy

doi: 10.6133/apjcn.201810/PP.0014
Published online: October 2018

Running title: Skipping breakfast during pregnancy

Mie Shiraishi PhD, RNM[1,2], Megumi Haruna PhD, RNM[2], Masayo Matsuzaki PhD, RNM[1,2]

[1] Department of Children and Women's Health, Division of Health Science, Graduate School of Medicine, Osaka University, Osaka, Japan
[2]Department of Midwifery and Women's Health, Division of Health Sciences and Nursing, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Authors' email addresses and contributions:
Mie Shiraishi, mi-shi@sahs.med.osaka-u.ac.jp
Megumi Haruna, mharuna-tky@umin.ac.jp
Masayo Matsuzaki, msumi-tky@umin.ac.jp

All authors planned the study, collected the data, and made substantial contributions to the interpretation of the results. MS drafted the manuscript and analyzed the data.

Corresponding Author: Dr Mie Shiraishi, Department of Children and Women's Health, Division of Health Science, Graduate School of Medicine, Osaka University, 1-7, Yamadaoka, Suita, Osaka 565-0871, Japan. Tel: +81-6-6879-2536. Fax: +81-6-6879-2536. Email: mi-shi@sahs.med.osaka-u.ac.jp

ABSTRACT

Background and Objectives: More than 20% of pregnant Japanese women regularly skip breakfast, thereby resulting in a low intake of several nutrients that are required for fetal development and prevention of pregnancy complications. However, whether skipping breakfast affects circulating levels of these nutrients remains unclear. We investigated whether skipping breakfast during pregnancy was associated with decreases in dietary intake and circulating and urinary levels of several nutrients, including fatty acids and vitamins. **Methods and Study Design:** This cross-sectional study was conducted at a university hospital in Tokyo, Japan, between June and October 2010. Nutrient intakes were assessed using a validated diet history questionnaire. Blood and 24-hour urinary samples were collected for assessing circulating and urinary excretion levels of nutrients. Skipping breakfast was defined as forgoing breakfast including a staple food, such as rice or bread, two or more times per week. Multiple linear regression analyses were used to compare nutrient levels between breakfast skippers and non-skippers after adjusting for confounders. **Results:** Of 97 healthy pregnant women in the second trimester, 37 (38.1%) skipped breakfast two or more times per week. In multiple linear regression analysis, breakfast skippers had significant lower energy-adjusted intakes of protein than non-skippers (p=0.019). In addition, breakfast skippers had significantly lower levels of plasma eicosapentaenoic acid (p=0.008), plasma docosahexaenoic acid (p=0.027), serum β-carotene (p=0.013), urinary urea nitrogen (p=0.027), and urinary potassium (p=0.006), compared to non-skippers. **Conclusions:** Healthcare professionals need to suggest effective strategies for encouraging breakfast skippers to have breakfast regularly and to increase the intake of these nutrients.

Key Words: fatty acids, pregnancy, protein, skipping breakfast, vitamins

INTRODUCTION

Adequate intakes of protein, vitamins, and omega-3 polyunsaturated fatty acids are important for fetal development and prevention of pregnancy.[1-3] Recently, the topic of skipping breakfast during pregnancy has come under new focus due to the fact that this dietary habit may be related to a lack of key nutrients in pregnant women. Previous studies have reported that 20−30% of pregnant Japanese women regularly skip breakfast.[4-7] Shiraishi et al[4] showed that even though breakfast skippers had the same energy intake as the non-skippers, they had lower energy-adjusted intake of protein, omega-3 polyunsaturated fatty acids, calcium, iron, and folate. In non-pregnant women, breakfast skippers were less likely to consume fish, vegetables, and fruits and had low circulating levels of omega-3 polyunsaturated fatty acids, which is a risk factor for coronary heart disease.[8] We hypothesized that skipping breakfast during pregnancy might also adversely affect circulating levels of essential nutrients including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), β-carotene, folate, and vitamin D, because these nutrients are primarily contained in fish, vegetables, and fruits. Such an adverse effect might lead to nutrition-related pregnancy complications including anemia and impaired fetal development.

Some studies have reported that decreased frequency of meals leads to preterm birth.[9,10] One of the mechanisms is that a deficiency of micronutrients (e.g. vitamin C and calcium) triggers elevated levels of cortisol and oxytoxin, which play a significant role in development of preterm birth.[2] However, we found no studies investigating the association between skipping breakfast and low levels of circulating nutrients during pregnancy. Such research could help to estimate effects of skipping breakfast on maternal health and to establish nutritional recommendations for pregnant women. This study investigated whether skipping breakfast affected dietary intakes, circulating levels of hemoglobin, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), β-carotene, vitamin B-12, folate, vitamin C, vitamin D, and vitamin E, and urinary levels of urea nitrogen (a marker of protein intake), sodium, and potassium among pregnant Japanese women.

MATERIALS AND METHODS

Study participants

This cross-sectional study used secondary data.[11-13] We recruited all healthy pregnant women who visited the outpatient section for their check-up at 15–18 weeks of gestation between June and October 2010. The study was conducted at a university hospital in Tokyo, Japan. The exclusion criteria included diabetes, hypertension, psychological diseases, age less than 20 years, and poor Japanese reading ability. We asked participants to answer questionnaires, to provide non-fasting blood samples, and to undergo a 24-hour urine collection test during an antenatal check-up at 19–22 weeks of gestation.

At the time of enrollment, each participant received written and verbal instructions regarding the 24-hour urine collection method. They were given a 3-L plastic bottle, a 1-L plastic bottle, a 50-mL plastic bottle, 350-mL cups, and a dropper and were instructed to collect their urine for 24 hours anytime during the 5 days preceding their next antenatal checkup (19–22 weeks of gestation). The day before the urine collection, we called the participants and reminded them of the urine collection procedure. We instructed participants to discard the first urine specimen on the day of collection and to collect all urine for the next 24 hours. The total urine volume was marked on the 3-L bottle with a felt-tipped marker. The well-stoppered 3-L bottle was shaken approximately 10 times and an aliquot of pooled urine was placed into a 50-mL plastic bottle. We collected the 50-mL bottle with the urine sample and the empty marked 3-L bottle.

Participants completed a questionnaire while waiting for their antenatal medical check-up at 19–22 weeks of gestation. Women who did not have sufficient time to answer the questionnaire completed it after returning home and submitted them by mail. We drew non-fasting blood samples at the routine blood tests of the antenatal medical check-ups to reduce participant burden. Participants answered the questionnaire either on the same day as their blood sample collection or within a 7-day period after the sample collection.

Variables and their measurement

Each participant completed a questionnaire, which included demographic variables such as maternal age, parity, educational level, and pre-pregnancy weight. Educational level was categorized as either high school/junior college/technical college, or college/university. We calculated pre-pregnancy body mass index (BMI) from the self-reported pre-pregnancy weight and height. Participants were also asked for information on skipping breakfast and personal behaviors related to nutrient levels such as smoking habits and the use of supplements during the preceding 1-month period. On the basis of the definition of the national dietary survey, we defined skipping breakfast as forgoing breakfast including a staple food, such as rice or bread, two or more times per week. Thus, consuming only beverages, fruits, or confectioneries was not regarded as eating breakfast. The regular use of supplements was defined as using four or more times per week.

We used a self-administered diet history questionnaire (DHQ),[14-17] which was validated for Japanese pregnant women,[11-13,18] to assess dietary intake over the previous month. The DHQ is a 22-page structured questionnaire that measures the daily intake of 150 different food and beverage items. It includes questions regarding not only eating frequency and portion sizes but also general dietary behavior and the major cooking methods used. Food and beverage items and their portion sizes were derived from primary data provided by the National Nutrition Survey of Japan and various Japanese recipe books.[15] Nutrient intakes were calculated based on the Japanese Standard of Food Composition Tables.[19] We used energy-adjusted values of food group intakes and nutrient intakes, as the use of energy-adjusted values is recommended in epidemiological studies for reducing intra-individual measurement errors.[20]

Blood samples were centrifuged for 10 minutes at 3,000 rpm to separate the serum and the plasma within 6 hours of being collected and were then stored at −80 degrees Celsius until the analysis. The samples were analyzed within 6 months of collection. Plasma EPA and DHA levels were assayed using gas chromatography. High performance liquid chromatography was used to measure serum β-carotene levels. Serum vitamin B-12 and folate levels were measured using a chemiluminescent enzyme immunoassay. Serum vitamin E levels were determined using a fluorescence method. We also measured low-density lipoprotein cholesterol (LDL-C) levels by enzymatic analysis because vitamin E levels are affected by LDL levels in the blood.[21] Assays of EPA, DHA, β-carotene, vitamin B-12, folate, vitamin E, and LDL-C were conducted by LSI Medience Corporation, Tokyo, Japan. Serum 25-hydroxyvitamin D (25(OH)D) levels were measured using chemiluminescent immunoassay (LIAISON 25-hydroxy Vitamin D Total Assay; Diasorin, Saluggia, Italy) by Kyowa Medex Co., Ltd, Tokyo, Japan. Circulating 25(OH)D levels are considered to be the best marker for evaluating of vitamin D deficiency, as 25(OH)D is the long-term stable form of 1,25 dihydroxy-vitamin D (the active form of vitamin D).[22] Using colorimetric assays (vitamin C colorimetric assay kit, Immundiagnostik AG, Bensheim, Germany), we measured serum vitamin C levels. In the serum, vitamin C is found as ascorbic acid and dehydro-ascorbate, of which the latter is a fully oxidized form of vitamin C; both forms are biologically active. In the vitamin C assay, oxidation is induced prior to the analysis so that both forms are measured. Optical densities were measured at 492 nm with an automatic enzyme-linked immunosorbent assay plate reader. The concentration level was directly determined from the linear standard curve, in the range of 0.7–30.0 mg/L. Both intra- and inter-assay coefficients of variation for vitamin C were less than 8%. Hemoglobin levels were obtained from medical charts.

We measured the 24-hour total urine volume of each participant using the marked 3-L bottle. Urine samples were stored at room temperature until submission to a researcher and were then stored at −80 degree Celsius until analysis. Urinary urea nitrogen level was determined by the urease-LEDH method using Iatro-LQ UN rate (A) II (LSI Medience Corporation, Tokyo, Japan). This assay was conducted using an automated analyzer (BM6050, JEOL Ltd, Tokyo, Japan). The urea nitrogen level in the 24-hour urine sample can be used for estimating the amount of dietary protein. Urinary levels of sodium and potassium were measured using ion-selective electrodes. The urinary creatinine level was measured by the enzyme method, using the Iatro-LQ CRE (A) II assay (LSI Medience Corporation, Tokyo, Japan). These assays were analyzed by LSI Medience Corporation using an automated analyzer (BM6050, JEOL Ltd, Tokyo, Japan).

Women who failed to collect all urine specimens were excluded from the final analysis. In addition, participants with nonstandard values of creatinine excretion in relation to body weight (creatinine level (mg/day) divided by body weight (kg) of <10.8 or >25.2) were excluded.[23]

Ethical considerations

The research ethics committee approved the study procedures (No. 3197). Each participant received oral and written information about the purpose and protocol of the study, the voluntary nature of their participation, and privacy considerations before giving written informed consent.

Statistical analyses

Differences between breakfast skippers and non-skippers were analyzed using Student's t-test or Mann-Whitney U test for consecutive variables, and using the chi-square test or Fisher's exact test for categorical variables. Multiple linear regression analysis was used to examine the association between skipping breakfast and nutritional levels after adjusting for confounding variables. The variables that fulfilled both of the following conditions were chosen as confounding variables and adjusted for in the multiple regression analyses: variables previously reported to affect nutrient status (e.g. educational levels and pre-pregnancy BMI)[24, 25] and variables significantly associated with skipping breakfast in the univariate analyses. In addition, supplement use was adjusted for in the analyses of circulating levels. The analysis of serum vitamin E levels was adjusted for LDL-C levels in addition to the above variables. All statistical analyses were conducted using the Statistical Package for Social Science (SPSS) software (version 20.0; IBM Corp., Armonk, NY). Differences with a two-tailed p-value of <0.05 were considered statistically significant.

RESULTS

Among 120 pregnant women who met the inclusion criteria, 106 (88.3%) gave written informed consent and answered the questionnaire. Seven were excluded from the analysis because an inadequate amount of blood was drawn and two were excluded due to missing data. Thus, data were analyzed for 97 (80.8%) healthy pregnant women. Of these, 13 others were eliminated from the analysis of urinary levels: ten did not agree to the urine collection, two could not collect all the urine specimens, and one did not meet the creatinine-weight criteria. The final population for the analysis of urine samples comprised 84 women (70.0%).

Table 1 illustrates the characteristics of participants. Thirty-seven women (38.1%) skipped breakfast two or more times per week. There were fewer women with high educational levels among breakfast skippers (p=0.017). All participants did not take medications that would affect the nutrient status, except for oral iron therapy.

Comparison of food group intakes between breakfast skippers and non-skippers are indicated in Table 2. Breakfast skippers had significantly lower intakes of meat and dairy products and higher intake of nuts than non-skippers, after adjusting for educational levels (p=0.033, 0.045 and 0.015, respectively).

Differences in nutrient intakes between breakfast skippers and non-skippers are shown in Table 3. Breakfast skippers had significantly lower energy-adjusted intakes of protein, compared with non-skippers (p=0.019), after adjusting for confounding variables. No differences in other nutrient intakes between the two groups were found.

Table 4 shows the median (interquartile range) circulating and urinary levels of nutrients among breakfast skippers and non-skippers. The results of multiple linear regression analyses confirmed that circulating levels of EPA, DHA and β-carotene, and urinary levels of urea nitrogen and potassium were significantly lower among breakfast skippers than among non-skippers (p=0.008, 0.027, 0.013, 0.027, and 0.006, respectively), after adjusting for educational levels and corresponding supplement use.

DISCUSSION

This study clarified that skipping breakfast during pregnancy was associated with lower circulating levels of EPA, DHA and β-carotene, and lower urinary levels of urea nitrogen and potassium. EPA and DHA are well-known to be essential for fetal brain development and prevention of preterm birth and low birth weight.[26] Low β-carotene status may inhibit fetal development, especially lung development and maturation.[27] Adequate protein intake is also required to build fetal cells.[28] Yılmaz et al[29] reported that the ratio of sodium to potassium is associated with hypertension during pregnancy. Thus, pregnant women are recommended to ensure that they obtain adequate amounts of these nutrients. However, skipping breakfast, which was associated with low circulating levels of these important nutrients, might increase the risk for adverse pregnancy outcomes.

Urinary urea nitrogen levels were significantly lower in breakfast skippers. This was consistent with the result of lower protein intake found among breakfast skippers. Proteins are found mainly in meat, fish, and dairy products. In the present study, meat and dairy product intakes were significantly lower in breakfast skippers, although relationships between fish intake and skipping breakfast were not observed. In particular, in Westernized breakfast dietary pattern, which comprises one of the Japanese dietary patterns, processed meat and dairy products including milk, yogurt, and cheese, are more often consumed for breakfast.[30] Therefore, skipping breakfast would reduce the opportunities of consuming these foods. On the other hand, differences in the intakes of EPA, DHA, β-carotene, and potassium were not found between breakfast skippers and others, although their circulating and urinary levels were significantly lower in breakfast skippers. The contradictory results between reported intakes and biological values might exist because reported intakes were affected by recall bias. In addition, a habit of skipping breakfast tends to reduce the reporting accuracy due to impairment of correctly grasping actual intake, according to a Japanese study in non-pregnant women.[31] In consideration of the circulating and urinary levels results, breakfast skippers are more likely to have lower intake of these nutrients. The best sources of EPA, DHA and β-carotene are fish and vegetables, respectively. Potassium is found in a variety of foods, including vegetables, pulses, meat, fish, and dairy products. Intake of these food groups might be comprehensively low in breakfast skippers, although we did not observe results wherein the intake of each food group was significantly lower in breakfast skippers than in non-skippers. Breakfast skippers might have substantially reduced likelihood for sufficient consumption of these foods, owing to the reduced meal frequency or impaired food consciousness.[32] For better pregnancy outcomes, healthcare professionals would need to support breakfast skippers to make conscious food choices to consume protein, EPA, DHA, β-carotene, and potassium.

It is notable that more than 50% of breakfast skippers had severe vitamin D deficiency (25(OH)D levels <10.0 ng/mL), although the investigation was conducted in the summer season. Irrespective of whether the participants were breakfast skippers or non-skippers, most of them had 25(OH)D levels <20.0 ng/mL, which indicated vitamin D deficiency. Such vitamin D deficiencies would be due to not only low intake of vitamin D, but also deliberate reduction in sunlight exposure and the high prevalence of sunscreen use. Vitamin D intakes among most of our participants were far lower than the adequate level for pregnant Japanese women (7.0 μg/day).[33] Appropriate nutritional counseling, including adequate vitamin D intake and vitamin D production in the skin by sunlight exposure, is imperative for all pregnant Japanese women to obtain optimal 25(OH)D levels.

In this study, 38.1% of the participants regularly skipped breakfast. This represents a larger percentage than that of non-pregnant Japanese women.[34] In the last three decades, the rate of fertile women skipping breakfast in Japan increased from 14.7% (20s of age) and 6.4% (30s of age) in 1985, to 36.4% (20s of age) and 21.4% (30s of age) in 2015.[34] According to a previous Japanese study, the reasons for skipping breakfast were reported to be lack of time, lack of appetite, and long-term lack of breakfast eating habits.35 Changing dietary habits are likely to be difficult, even during pregnancy.[36] Breakfast skippers would also have difficulty in modifying their dietary habits, even though they know that taking a sufficient amount of nutrients is essential for both themselves and their babies. Nevertheless, pregnancy is the best time for women to consider the importance of dietary intake for their own maternal physical and psychological well-being and for proper fetal development. During regular antenatal check-ups, healthcare professionals need to encourage pregnant women to eat breakfast or help them find a realistic and reliable method for taking important nutrients including protein, EPA, DHA, β-carotene, and potassium. Further studies will be required to elucidate which interventional strategies are effective and whether skipping breakfast during pregnancy leads to pregnancy adverse outcomes.

This study had three limitations. First, the number of participants was small due to secondary data, possibly reducing the overall statistical power. Further larger study is needed to confirm the findings of this study. Second, we did not collect fasting blood samples considering the physical effects of fasting during pregnancy, and the burden associated with separating blood sampling from routine blood test for antenatal check-up. Therefore, circulating levels of some nutrients might have been affected by meals consumed before sample collection. In particular, serum β-carotene levels are likely to vary after meals,[37] although EPA and DHA levels were comparatively stable.[38] If non-skippers eat β-carotene-rich foods for breakfast on the day of blood sample collection, the β-carotene levels might increase because of the influence of the breakfast. However, the half-life of β-carotene in serum has been reported to be approximately 1–2 weeks.[39,40] Thus, the β-carotene levels would reflect habitual intake to some extent, not only intake immediately before the blood sample collection. Cautious interpretation is required for the results of serum β-carotene levels. Third, self-reported dietary assessment tools often have measurement errors, such as under-reporting. In pregnant Japanese women, weight-related factors, including low pre-pregnancy BMI and low gestational weight gain, have been reported to correlate with under-reporting.[41] Thus, such measurement errors might affect the results.

Conclusions

This study confirmed that a habit of skipping breakfast during pregnancy should be paid attention to because it leads to low circulating levels of EPA, DHA, and β-carotene, and low urinary levels of urea nitrogen and potassium. Healthcare professionals must inform pregnant women about the possibility of low nutrient levels related to skipping breakfast and suggest effective strategies for encouraging pregnant women to have breakfast regularly and to increase the intake of these nutrients.

ACKNOWLEDGEMENTS

We are deeply grateful to the participants and the hospital staff for their cooperation.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors have no conflicts of interest. This study was supported by Yazuya Food and Health Foundation.

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Table 1. Characteristics of participants

	All participants (n=97) Mean±SD or n (%)	Breakfast skippers (n=37) Mean±SD or n (%)	Non-skippers (n=60) Mean±SD or n (%)	*p*
Age (years)	35.1±4.2	34.8±4.2	35.4±4.2	ns†
Gestational age (weeks)	20.4±1.1	20.5±1.1	20.4±1.1	ns†
Height (cm)	159.5±5.6	158.3±6.5	160.2±4.9	ns†
Pre-pregnancy BMI (kg/m^2)	20.4±2.1	20.7±2.4	20.3±1.9	ns†
Parity: Primigravida	58 (59.8)	22 (59.5)	36 (60.0)	ns‡
Smoking during pregnancy	2 (2.1)	2 (5.4)	0 (0.0)	ns§
Educational level				0.017‡
College / University	49 (50.5)	13 (35.1)	36 (60.0)	
High school / Junior or Technical college	48 (49.5)	24 (64.9)	24 (40.0)	
Working	52 (53.6)	23 (62.2)	29 (48.3)	ns‡
Having nausea during pregnancy	33 (34.0)	13 (35.1)	20 (33.3)	ns‡
Oral iron therapy	12 (12.4)	4 (10.8)	8 (13.3)	ns‡
Regular supplementation				
DHA	3 (3.1)	2 (5.4)	1 (1.7)	ns§
Calcium	17 (17.5)	7 (18.9)	10 (16.7)	ns‡
Iron	21 (21.6)	6 (16.2)	15 (25.0)	ns‡
Vitamin B complex	27 (27.8)	10 (27.0)	17 (28.3)	ns‡
Folic acid	44 (45.4)	14 (37.8)	30 (50.0)	ns‡
Vitamin C	16 (16.7)	5 (17.2)	11 (16.4)	ns‡
Vitamin D	12 (12.4)	2 (5.4)	10 (16.7)	ns§
Vitamin E	7 (7.2)	1 (2.7)	6 (10.0)	ns§

BMI: body mass index; DHA: docosahexaenoic acid; ns: not significant.
Statistical analyses were performed to compare the differences in characteristics between breakfast skippers and non-skippers. †Student's t-test, ‡The chi-square test, §Fisher's exact test.

Table 2. Comparison of energy-adjusted food group intakes between breakfast skippers and non-skippers

Food (g/1,000 kcal)	Breakfast skippers (n=37)	Non-skippers (n=60)	Mann-Whitney *U* test	Multiple linear regression analyses	
	Median (Interquartile range)	Median (Interquartile range)	*p*	β	*p*
Cereals	194 (158–241)	203 (161–236)	ns	-0.057	ns
Nuts	0.41 (0.00–1.20)	0.15 (0.00–0.46)	ns	0.352	0.015
Pulses	26.8 (13.3–39.8)	30.9 (20.3–39.4)	ns	-0.163	ns
Potatoes	16.4 (11.0–24.7)	14.1 (9.8–20.9)	ns	0.138	ns
Sugar	5.69 (3.91–7.09)	5.73 (4.39–7.43)	ns	-0.076	ns
Confectioneries	45.6 (29.6–85.2)	40.0 (25.9–54.9)	ns	0.169	ns
Fat	0.49 (0.19–1.00)	0.44 (0.14–1.18)	ns	-0.065	ns
Oil	11.1 (7.7–14.3)	11.1 (7.7–14.3)	ns	-0.066	ns
Fruits	77.7 (46.5–142.4)	76.3 (46.2–99.9)	ns	-0.017	ns
Green and yellow vegetables	50.6 (31.1–70.3)	61.0 (33.3–78.8)	ns	-0.108	ns
Other vegetables	43.2 (30.5–65.1)	50.8 (34.1–70.3)	ns	-0.074	ns
Mushrooms	7.39 (3.59–12.78)	6.35 (2.37–9.93)	ns	0.128	ns
Seaweeds	4.46 (2.58–9.56)	4.48 (2.23–8.18)	ns	0.023	ns
Non-alcoholic beverages	376 (160–615)	322 (218–579)	ns	-0.049	ns
Fish and shellfish	25.3 (17.5–34.4)	25.1 (17.6–35.5)	ns	0.040	ns
Meat	31.9 (20.7–42.3)	37.3 (30.0–52.1)	0.026	-0.224	0.033
Eggs	12.7 (4.8–18.0)	14.4 (6.3–22.0)	ns	-0.179	ns
Dairy products	76.0 (31.0–102.4)	100.4 (67.2–141.1)	0.008	-0.211	0.045

Data are median (interquartile range). ns, not significant.
Multiple linear regression analyses were used to examine whether skipping breakfast was associated with energy-adjusted food group intakes after adjusting for educational level.
Skipping breakfast was entered as a dummy variable (yes=1, no=0).
Each dependent variable (food group intakes) was entered in the models after log-transformation

Table 3. Comparison of energy-adjusted nutrient intakes between breakfast skippers and non-skippers

	Breakfast skippers (n=37)	Non-skippers (n=60)	Mann-Whitney *U* test	Multiple linear regression analyses	
	Median (Interquartile range)	Median (Interquartile range)	*p*	β	*p*
Energy (kcal/day)	1609 (1312–2231)	1771 (1533–2045)	ns	-0.045	ns
Protein (% of energy)	13.3 (12.0–14.5)	14.3 (13.5–15.3)	0.005	-0.246	0.019
Fat (% of energy)	29.5 (26.5–32.7)	30.2 (25.9–33.4)	ns	-0.056	ns
n-3 PUFA (% of energy)	1.10 (0.99–1.23)	1.29 (1.15–1.46)	ns	-0.067	ns
EPA (% of energy)	0.08 (0.06–0.11)	0.08 (0.05–0.12)	ns	0.060	ns
DHA (% of energy)	0.14 (0.10–0.18)	0.14 (0.10–0.19)	ns	0.037	ns
Carbohydrate (% of energy)	57.2 (52.4–61.8)	54.6 (51.2–59.6)	ns	0.118	ns
β-carotene (μg /1000kcal)	1383 (859–1934)	1493 (1009–2140)	ns	-0.132	ns
Vitamin B-12 (μg/1000kcal)	2.92 (2.45–3.73)	3.05 (2.49–4.00)	ns	-0.041	ns
Folate (μg/1000kcal)	145 (109–195)	161 (130–198)	ns	-0.149	ns
Vitamin C (mg /1000kcal)	50.2 (36.7–73.6)	51.8 (37.7–67.2)	ns	0.005	ns
Vitamin D (μg/1000kcal)	3.07 (2.40–3.87)	3.33 (2.64–4.17)	ns	0.015	ns
α-tocopherol (mg /1000kcal)	4.21 (3.44–5.23)	4.32 (3.84–5.19)	ns	-0.063	ns
Sodium (mg/1000kcal)	2208 (1873–2421)	2212 (2025–2471)	ns	-0.173	ns
Potassium (mg /1000kcal)	1181 (996–1363)	1226 (1096–1391)	ns	-0.119	ns
Calcium (mg /1000kcal)	287 (231–349)	320 (260–366)	ns	-0.108	ns
Iron (mg /1000kcal)	3.55 (3.09–4.51)	3.83 (3.58–4.37)	ns	-0.158	ns

PUFA: polyunsaturated fatty acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ns: not significant.
Data are median (interquartile range).
Multiple linear regression analyses were used to examine whether skipping breakfast was associated with nutrient intakes after adjusting for educational levels.
Skipping breakfast was entered as a dummy variable (yes=1, no=0).
Each dependent variable (nutrient intakes) was entered in the models after log-transformation, except for energy, protein, fat, and carbohydrate

Table 4. Differences in circulating and urinary levels of nutrients between breakfast skippers and non-skippers

	Breakfast skippers (n=37)	Non-skippers (n=60)	Mann-Whitney *U* test	Multiple linear regression analyses	
	Median (Interquartile range)	Median (Interquartile range)	*p*	β	*p*
Blood					
Hemoglobin (mg/dL)	11.3 (10.6–11.8)	11.4 (10.7–11.9)	ns	0.033	ns
EPA (μg/mL)	19.0 (15.0–30.5)	29.0 (21.3–36.0)	0.003	-0.279	0.008
DHA (μg/mL)	114 (96–136)	128 (112–147)	0.039	-0.231	0.027
β-carotene (μg/dL)	30.7 (21.5–40.5)	39.7 (28.5–62.5)	0.005	-0.261	0.013
Vitamin B-12 (pg/dL)	321 (261–406)	326 (293–424)	ns	-0.062	ns
Folate (ng/mL)	9.2 (6.2–13.0)	10.2 (7.2–14.2)	ns	-0.005	ns
Vitamin C (mg/L)	9.6 (8.2-11.3)	10.8 (8.9-12.4)	ns	0.021	ns
25(OH)D (ng/mL)	9.9 (6.4–14.3)	12.4 (9.4–16.2)	ns	-0.177	ns
Vitamin E (mg/dL)	1.52 (1.35–1.59)	1.41 (1.26–1.63)	ns	0.110	ns
Urine†					
Urea nitrogen (g/day)	6.0 (5.0–7.2)	7.0 (6.0–7.7)	0.034	-0.252	0.027
Sodium (mg/day)	3242 (2722-3595)	3192 (2484–4222)	ns	-0.043	ns
Potassium (mg/day)	1554 (1185-1851)	1834 (1452–2377)	0.006	-0.313	0.006

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ns: not significant; 25(OH)D: 25-hydroxyvitamin D.
Multiple linear regression analyses were used to examine whether skipping breakfast was associated with circulating nutrient levels after adjusting for confounding variables (educational levels and supplement use). The analysis of serum vitamin E levels was adjusted for low-density lipoprotein cholesterol (LDL-C) levels in addition to the above confounding variables (Spearman's correlation coefficient between serum vitamin E levels and LDL-C levels was 0.458, $p<0.001$) .Skipping breakfast was entered as a dummy variable (yes=1, no=0). The dependent variables (nutrient levels) were entered in the models after log-transformation.
†Urine samples were obtained from 84 women (Breakfast skippers: n=32, Non-skippers; n=52).