**Supplementary materials**

**Maternal dietary intake of vitamin A during pregnancy**

**was inversely associated with congenital diaphragmatic hernia:**

**the Japan Environment and Children’s Study**

A shortened version of the title: Vitamin A and congenital diaphragmatic hernia

Takehiro Michikawa1,2\*, Shin Yamazaki 1, Makiko Sekiyama1, Tatsuo Kuroda3, Shoji F. Nakayama1, Tomohiko Isobe1, Yayoi Kobayashi1, Miyuki Iwai-Shimada1, Eiko Suda1, Toshihiro Kawamoto1, Hiroshi Nitta1 and the Japan Environment and Children’s Study Group4

1*Centre for Health and Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan.*

2*Department of Environmental and Occupational Health, School of Medicine, Toho University, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540, Japan.*

3*Department of Paediatric Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.*

4*The Study Group members are listed in the Appendix*

**Table of contents:**

Supplementary Table 1……....………………………………………………………………………2

Supplementary Table 2……....………………………………………………………………………3

Supplementary Table 3……....………………………………………………………………………4

Supplementary Table 4……....………………………………………………………………………6

Supplementary Table 5……....………………………………………………………………………8

Supplementary Figure 1 .…....……………………………………………………………………...10

**Supplementary Table 1.** Spearman's correlation coefficients for total vitamin A and other intakes in early pregnancy

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 　 | Total vitamin A (retinol activity equivalents) | Retinol  | α-Carotene  | β-Carotene  | β-Crypto xanthin | Total vegetables  | Green and yellow vegetables |
| Total vitamin A (retinol activity equivalents) | 1 |  |  |  |  |  |  |
| Retinol  | 0·68  | 1 |  |  |  |  |  |
| α-Carotene  | 0·59  | 0·06  | 1 |  |  |  |  |
| β-Carotene | 0·68  | 0·08  | 0·89  | 1 |  |  |  |
| β-Cryptoxanthin  | 0·23  | -0·01 | 0·10  | 0·22  | 1 |  |  |
| Total vegetable  | 0·49  | 0·05  | 0·56  | 0·74  | 0·24  | 1 |  |
| Green and yellow vegetables | 0·48  | 0·08  | 0·51  | 0·68  | 0·22  | 0·87  | 1 |

**Supplementary Table 2.** Odds ratios (ORs) and 95% confidence intervals (CIs) for congenital diaphragmatic hernia according to quartile of vitamin A intake (retinol activity equivalents) in early pregnancy, Japan Environment and Children's Study (2011-2014)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Q1 (low) | Q2 (mid-low)  | Q3 (mid-high) | Q4 (high) |
| 　 | 　 | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| Median intake (interquartile range) (μg/day) | 230 (185, 264) | 346 (320, 373) | 468 (433, 509) | 738 (631, 940) |
| No. of participants | 22414  | 22415  | 22414  | 22415  |
| No. of cases | 14  | 8  | 9  | 9  |
| Adjusted model1\* | Reference | 0·6  | (0·2, 1·4) | 0·7  | (0·3, 1·5) | 0·7  | (0·3, 1·5) |
| Adjusted model2† | Reference | 0·6  | (0·2, 1·3) | 0·6  | (0·3, 1·4) | 0·6  | (0·3, 1·5) |

\* Adjusted for maternal age at delivery.

† Adjusted for maternal age at delivery, smoking habits, alcohol consumption, pre-pregnancy body mass index, current history of diabetes or gestational diabetes, and infertility treatment. Participants with missing values for these factors were excluded, which left 89,481 in the adjusted model2.

**Supplementary Table 3.** Baseline characteristics of 89,658 mothers with respect to total vitamin A intake in early pregnancy, Japan Environment and Children's Study (2011-2014)

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  | Total vitamin A (retinol activity equivalents) intake in early pregnancy |
|  |  | No. of women\* | Low (bottom quartile) (%) | High (remaining three quartiles) (%) |
| No. of women | 89658 | 22414 | 67244  |
|  |  |  |  |  |
| Dietary intake in early pregnancy |  |  |  |
|  | Total vitamin A (μg/day), median | 89658 | 230  | 468  |
|  | Energy (kJ/day), median | 89658 | 6686 | 7171  |
|  | Retinol (μg/day), median | 89658 | 100  | 182  |
|  | α-Carotene (μg/day), median | 89658 | 156  | 449  |
|  | β-Carotene (μg/day), median | 89658 | 1061  | 2402  |
|  | β-Cryptoxanthin (μg/day), median | 89658 | 256  | 488  |
|  | Total vegetables (g/day), median | 89658 | 100  | 180  |
|  | Green and yellow vegetables (g/day), median | 89658 | 32  | 81  |
|  | Folate (μg/day), median | 89658 | 186 | 265 |
|  | Vitamin C (mg/day), median | 89658 | 58 | 90 |
| Age at delivery (years) |  |  |  |
|  | < 25 | 8657 | 14·3  | 8·1  |
|  | 25-29 | 24609 | 30·6  | 26·4  |
|  | 30-34 | 31791 | 31·9  | 36·7  |
|  | ≥ 35 | 24601 | 23·2  | 28·8  |
| Smoking habits |  |  |  |
|  | Never smoked | 52225 | 50·8  | 60·9  |
|  | Ex-smokers who quit before pregnancy | 20956 | 23·3  | 23·4  |
|  | Smokers during early pregnancy | 16351 | 25·9  | 15·7  |
| Alcohol consumption |  |  |  |
|  | Never drank | 30848 | 34·8  | 34·3  |
|  | Ex-drinkers who quit before pregnancy | 16731 | 17·6  | 19·0  |
|  | Drinkers during early pregnancy | 42062 | 47·6  | 46·7  |
| Pre-pregnancy body mass index (kg/m2) |  |  |  |
|  | < 18·5  | 14472 | 16·8  | 15.9 |
|  | 18·5-24·9  | 65668 | 70·8  | 74·1  |
|  | ≥ 25·0  | 9480 | 12·4  | 10·0  |
| Current history of diabetes or gestational diabetes |  |  |
|  | No | 86832 | 96·7  | 96·9  |
|  | Yes | 2826 | 3·3  | 3·1  |
| Infertility treatment |  |  |  |
|  | No | 83592 | 94·5  | 92·8  |
|  | Ovulation stimulation / artificial insemination by sperm from husband | 3295 | 3·2  | 3·9  |
|  | Assisted reproductive technology | 2741 | 2·3  | 3·3  |
| Educational background (years) |  |  |  |
|  | < 13 | 31688  | 47·6  | 32·0  |
|  | ≥ 13 | 56704  | 52·4  | 68·0  |
| Household income (million Japanese yen/year) |  |
|  | < 6 | 60358  | 78·4  | 71·3  |
|  | ≥ 6 | 22281  | 21·6  | 28·7  |
| Occupation in early pregnancy |  |  |  |
|  | Administrative, managerial, professional, and engineering | 20649 | 20·0  | 24·2  |
|  | Clerical  | 15282 | 17·4  | 17·1  |
|  | Sales and service  | 19543 | 27·3  | 20·2  |
|  | Homemaker | 24797 | 23·0  | 29·5  |
|  | Others | 8713 | 12·3  | 9·0  |
| Use of multi-vitamin supplement in early pregnancy |  |  |
|  | No | 84413 | 95·6  | 94·2  |
|  | Yes | 4863  | 4·4  | 5·8  |
| Routine use of folic acid supplement |  |  |  |
|  | No (< 4 times/week) | 64503  | 76·3  | 71·7  |
|  | Yes (≥ 4 times/week) | 24025  | 23·7  | 28·3  |
| Morning sickness |  |  |  |
|  | No or not severe | 78739 | 88·8  | 89·1  |
|  | Severe | 9694 | 11·2  | 10·9  |
| Week of pregnancy at delivery |  |  |  |
|  | < 37 weeks (preterm) | 4183 | 5·2  | 4·5  |
|  | ≥ 37 weeks | 85475 | 94·8  | 95·5  |
| Parity |  |  |  |
|  | 0 | 39091 | 49·1  | 42·0  |
|  | ≥ 1 | 50261 | 50·9  | 58·0  |
| Infant sex |  |  |  |
|  | Boys | 46032 | 51·4  | 51·3  |
|  | Girls | 43618 | 48·6  | 48·7  |

\* Subgroup totals do not equal the overall number because of missing data.

**Supplementary Table 4**. Odds ratios (ORs) and 95% confidence intervals (CIs) for congenital diaphragmatic hernia, for vitamin A and other intakes in mid-late pregnancy, Japan Environment and Children's Study (2011-2014)\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | Low (bottom quartile) | High (remaining three quartiles) |
| 　 | 　 | 　 | 　 | OR | 95% CI |
| Total vitamin A (retinol activity equivalents) | 　 | 　 |
|  |  | Median intake (μg/day) | 227  | 466  |
|  |  | No. of participants | 22156 | 66486 |
|  |  | No. of cases | 10 | 26 |
|  |  | Adjusted model1† | Reference | 0·9  | (0·4, 1·9) |
|  |  | Adjusted model2‡ | Reference | 0·9  | (0·4, 1·8) |
|  | Retinol |  |  |  |
|  |  | Median intake (μg/day) | 83  | 189  |
|  |  | No. of participants | 22149 | 66493 |
|  |  | No. of cases | 10 | 26 |
|  |  | Adjusted model1† | Reference | 0·9  | (0·4, 1·8) |
|  |  | Adjusted model2‡ | Reference | 0·9  | (0·4, 1·8) |
|  | α-Carotene |  |  |
|  |  | Median intake (μg/day) | 106  | 451  |
|  |  | No. of participants | 22158 | 66484 |
|  |  | No. of cases | 15 | 21 |
|  |  | Adjusted model1† | Reference | 0·5  | (0·2, 0·9) |
|  |  | Adjusted model2‡ | Reference | 0·5  | (0·2, 0·9) |
|  | β-Carotene |  |  |
|  |  | Median intake (μg/day) | 848  | 2352  |
|  |  | No. of participants | 22159 | 66483 |
|  |  | No. of cases | 9 | 27 |
|  |  | Adjusted model1† | Reference | 1·0  | (0·5, 2·2) |
|  |  | Adjusted model2‡ | Reference | 1·0  | (0·5, 2·2) |
|  | β-Cryptoxanthin |  |  |
|  |  | Median intake (μg/day) | 28  | 532  |
|  |  | No. of participants | 22155 | 66487 |
|  |  | No. of cases | 9 | 27 |
|  |  | Adjusted model1† | Reference | 1·0  | (0·5, 2·1) |
|  |  | Adjusted model2‡ | Reference | 1·0  | (0·5, 2·1) |
| Total vegetables |  |  |
|  |  | Median intake (g/day) | 69  | 175  |
|  |  | No. of participants | 22157 | 66485 |
|  |  | No. of cases | 10 | 26 |
|  |  | Adjusted model1† | Reference | 0·9  | (0·4, 1·9) |
|  |  | Adjusted model2‡ | Reference | 0·9  | (0·4, 1·8) |
|  | Green and yellow vegetables |  |  |
|  |  | Median intake (g/day) | 22  | 83  |
|  |  | No. of participants | 22157 | 66485 |
|  |  | No. of cases | 9 | 27 |
|  |  | Adjusted model1† | Reference | 1·0  | (0·5, 2·2) |
| 　 | 　 | Adjusted model2‡ | Reference | 1·0  | (0·5, 2·2) |

\* We included 88,642 women who had valid data on food-frequency questionnaire during the second/third trimester and delivered their infants > 28 weeks of gestation.

† Adjusted for maternal age at delivery.

‡ Adjusted for maternal age at delivery, smoking habits, alcohol consumption, pre-pregnancy body mass index, current history of diabetes or gestational diabetes, and infertility treatment. Participants with missing values for these factors were excluded, which left 88,481 in the adjusted model2.

**Supplementary Table 5.** Association between vitamin A and other intakes in mid-late pregnancy, and congenital diaphragmatic hernia among women with adequate-weight (18·5 ≤ pre-pregnancy body mass index < 25·0 kg/m2)\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | Low (bottom quartile) | High (remaining three quartiles) |
| 　 | 　 | 　 | 　 | OR | 95% CI |
| Total vitamin A (retinol activity equivalents) | 　 | 　 |
|  |  | No. of participants | 15700 | 49254 |
|  |  | No. of cases | 9 | 19 |
|  |  | Adjusted model1† | Reference | 0·7  | (0·3, 1·6) |
|  |  | Adjusted model2‡ | Reference | 0·7  | (0·3, 1·6) |
|  | Retinol |  |  |  |
|  |  | No. of participants | 15646 | 49308 |
|  |  | No. of cases | 7 | 21 |
|  |  | Adjusted model1† | Reference | 1·0  | (0·4, 2·3) |
|  |  | Adjusted model2‡ | Reference | 1·0  | (0·4, 2·3) |
|  | α-Carotene |  |  |
|  |  | No. of participants | 15884 | 49070 |
|  |  | No. of cases | 13 | 15 |
|  |  | Adjusted model1† | Reference | 0·4  | (0·2, 0·8) |
|  |  | Adjusted model2‡ | Reference | 0·4  | (0·2, 0·8) |
|  | β-Carotene |  |  |
|  |  | No. of participants | 15847 | 49107 |
|  |  | No. of cases | 8 | 20 |
|  |  | Adjusted model1† | Reference | 0·9  | (0·4, 2·0) |
|  |  | Adjusted model2‡ | Reference | 0·9  | (0·4, 2·0) |
|  | β-Cryptoxanthin |  |  |
|  |  | No. of participants | 15958 | 48996 |
|  |  | No. of cases | 7 | 21 |
|  |  | Adjusted model1† | Reference | 1·0  | (0·4, 2·3) |
|  |  | Adjusted model2‡ | Reference | 1·0  | (0·4, 2·3) |
| Total vegetables |  |  |  |  |  |
|  |  | No. of participants | 15908 | 49046 |
|  |  | No. of cases | 10 | 18 |
|  |  | Adjusted model1† | Reference | 0·6  | (0·3, 1·3) |
|  |  | Adjusted model2‡ | Reference | 0·6  | (0·3, 1·3) |
|  | Green and yellow vegetables |  |  |  |  |  |
|  |  | No. of participants | 15803 | 49151 |
|  |  | No. of cases | 9 | 19 |
|  |  | Adjusted model1† | Reference | 0·7  | (0·3, 1·6) |
| 　 | 　 | Adjusted model2‡ | Reference | 0·7  | (0·3, 1·6) |

\* We included 64,954 women who had valid data on food-frequency questionnaire during the second/third trimester and delivered their infants > 28 weeks of gestation.

† Adjusted for maternal age at delivery.

‡ Adjusted for maternal age at delivery, smoking habits, alcohol consumption, current history of diabetes or gestational diabetes, and infertility treatment. Participants with missing values for these factors were excluded, which left 64,862 in the adjusted model2.

**Supplementary Figure 1.** Participants’ flow chart

