High dietary folic acid intake is associated with genomic instability in peripheral lymphocytes of healthy adults.

Khadijah I. Alnabbat1,2, †, Ali M. Fardous1†, Aiman Ismail1, Diane C. Cabelof1, and Ahmad R. Heydari1,3,\*

1 Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48202, USA;
kalnabbat@kfu.edu.sa (K.A.); ali.fardous@wayne.edu (A.M.F.); diane.cress@wayne.edu (D.C.C.)

2 Department of Food and Nutrition Sciences, King Faisal University, Al Hufūf 31982, Saudi Arabia

3 Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI 48202, USA

**\*** Correspondence: ahmad.heydari@wayne.edu (A.R.H); Tel.: +1-313-5772459; Fax: +1-313-5778616

† These authors contributed equally to this work.

**Supplemental Table S1:** Food Survey



**Supplemental Table s2: General participants characteristics.** 

**N**, number of participants; **SD**, standard deviation; **M**, males; **F**, Females. **BMI**: body mass index, **WHR**: waist to hip ratio. p-value refer to comparison between M &F.

**Supplemental Table S3**: Mean systemic markers.

|  |  |  |
| --- | --- | --- |
| Systemic Markers  | Mean (SD) | Normal Rang |
| Serum folate (µg/L) a | 14.8 (5.12) | 2 - 20 |
| RBC folate (µg/L) a | 608.32 (168.4) | 140 - 628 |
| Homocysteine (µmol/L)  | 21.41 (7.82) | 4 -15 |
| Serum B12 (pg/ml) | 459.5 (186.5) | 200 - 950 |
| MMA (nmol/L)  | 125.7 (49.1) | 87 - 318 |
| Plasma B6 (µg/L) | 18.9 (15.4) | 5 - 50 |
| Plasma B2 (µg/L) | 20.21 (23.6) | 4 - 24 |
| HGB (g/dL) | 14.92 (1.81) | **M**: 13.5 -17.5 **F:** 12 - 15.5 |
| HCT (%) | 48.1 (5.7) | **M**: 42 - 54 **F:** 38 - 46 |
| RBC (106/L) | 5.10 (0.6) | **M**: 4.7 - 6.1 **F:** 4.2 - 5.4 |
| WBC (109/L) | 6.26 (1.68) | 4.5 - 11 |
| Neut (%) | 51.6 (10.7) | 45 - 75 |
| Lymph (%) | 36.4 (8.9) | 20 - 40 |
| Mono (%) | 8.1 (2.3) | 2 – 8  |
| MCV (fL) | 94.6 (8.7) | 80 - 96 |
| MCH (pg) | 29.3 (2.1) | 23 - 31 |
| MCHC (g/dL) | 31.1 (1.74) | 32 - 36 |
| RDW-SD (fL) | 46.6 (5.6) | 39 - 46 |
| PLT (103/µl) | 264.3 (63.93) | 150 - 400 |
| MPV (fL) | 11.19 (0.83) | 7 – 11 |

**Supplemental Table S4: Nutrient intake.**

|  |  |  |
| --- | --- | --- |
| *Nutrient Intake*  | *Mean (SD)* | *RDA or AI c* |
| Protein (g)  | 89.3 (37.2) | 0.8 g/kg/day |
| Choline (mg) | 306.5 (130.5) | M: 550 mg/ day F: 425 mg/day  |
| Iron (mg) | 14.9 (6.8) | M: 8 mg/ day F: 18 mg/day |
| Vitamin B1 (mg) | 1.31 (0.54) | M: 1.2 mg/ day F: 1.1 mg/day |
| Vitamin B2 (mg) | 1.22 (0.81) | M: 1.3 mg/ day F: 1.1 mg/day |
| Vitamin B3 (mg)a | 21.2 (10.2) | M: 16 mg/ day F: 14 mg/day |
| Vitamin B6 (mg) | 1.54 (0.92) | M: 1.3 mg/ day F: 1.3 mg/day |
| Vitamin B12 (mcg) | 4.94 (12.2) | M: 2.4 mcg/ day F: 2.4 mcg/day |
| Folate (mcg) | 401.76 (230.9) | M: 400 mcg/ day F: 400 mcg/day |
| Folate (mcg DFE) b | 495.9 (303.8) |

a recommendation is expressed as niacin equivalent (NE)

b recommendation is expressed as dietary folate equivalent (DFE)

C recommendations are expressed as Recommended Dietary allowance (RDA) or Adequate Intake (AI) for group ages (19-50 yr). n=33

**Supplemental Table S5: Proposed comparison structures.**

**Structure 1, serum folate tertiles**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tertiles (n) | T1(11) | T2(11) | T3(11) | p- value  |
| Serum Folate (ng/ml) | 9.3 (1.9) | 14.4 (1.3) | 20.6 (2.8) | < 0.0001 |
| RBC Folate (ng/ml) | 563.5 (144.7) | 571.6 (119.5) | 689.9 (210.9) | 0.14 |
| Homocysteine (µmol/L) | 27.4 (7.2) | 20.0 (6.2) | 16.8 (6.3) | 0.002 |
| Total folate Intake (µg DFE)  | 576.0 (339.4) | 626.3 (336.4) | 484.8 (209.4) | ns |
| Folic acid intake (µg DFE)  | 204.2 (204.8) | 233.7 (169.1) | 187.1 (89.6) | <0.0001 |
| FAR | 0.31 (0.01) | 0.36 (0.01) | 0.42 (0.02) | ns |

**Structure 2, total folate intake tertiles**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tertiles (n) | T1(11) | T2(11) | T3(11) | p- value  |
| Total folate intake (µg DFE)  | 289.8 (73.4) | 490.5 (59.4)  | 906.9 (237.9) | < 0.0001 |
| Folic acid intake (µg DFE)  | 98.6 (53.6) | 152.2 (39.1) | 374.2 (168.3) | < 0.0001 |
| FAR | 0.36 (0.03) | 0.32 (0.01) | 0.41 (0.01) | ns |
| Serum Folate (ng/ml) | 13.9 (4.4) | 15.4 (4.8) | 15.0 (6.3) | ns |
| RBC Folate (ng/ml) | 637.2 (215.7) | 571.3 (145.1) | 616.5 (143.6) | ns |
| Homocysteine (µmol/L) | 25.5 (7.7) | 19.0 (4.8) | 19.6 (9.2) | 0.09 |

**Structure 3, folic acid intake tertiles**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tertiles (n) | T1(11) | T2(11) | T3(11) | p- value  |
| Folic acid (µg DFE)  | 85.6 (35.6) | 155.9 (18.5) | 383.4 (157.8) | <0.0001 |
| Folate (µg DFE)  | 362.4 (124.2) | 503.9 (240.9) | 820.7 (298.1) | <0.0001 |
| FAR | 0.24 (0.01) | 0.38 (0.02) | 0.46 (0.01) | 0.003 |
| Serum Folate (ng/ml) | 13.9 (4.5) | 14.5 (5.2) | 15.9 (5.1) | ns |
| RBC Folate (ng/ml) | 583.7 (210) | 605.6 (146.2) | 635.7 (153.9) | ns |
| Homocysteine (µmol/L) | 24.1 (7.1) | 21.4 (8.3) | 18.8 (7.8) | ns |

**Structure 4, folic acid:total folate intake ratio tertiles (FAR).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tertiles (n) | T1(11) | T2(11) | T3(11) | p- value  |
| FAR  | 0.2 (0.06) | 0.35 (0.04) | 0.54 (0.02) | < 0.0001 |
| Folic acid (µg DFE)  | 99.1 (51) | 180.7 (92.5) | 345.1 (184.5) | < 0.0001 |
| Folate (µg DFE)  | 492.0 (253.9) | 510.2 (240.4) | 684.9 (369.1) | ns |
| Serum Folate (ng/ml) | 13.6 (4.8) | 14.5 (5.2) | 16.2 (5.5) | ns |
| RBC Folate (ng/ml) | 581.7 (205.8) | 625.9 (144.2) | 617.3 (168.4) | ns |
| Homocysteine (µmol/L) | 23.2 (7.0) | 20.5 (7.8) | 20.4 (8.9) | ns |

FAR; folic acid; total folate intake ration, DFE; (dietary folate equivalent), Data presented as mean (± SD), n=33. P values denotes significant difference between tertiles.

**Supplementary Table S6:** Qiagen- RT2 qPCR Primers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene Symbol | Gene name  | NM\_Number  | Band Size  | Reference position  | Catalog number  |
| MLH1 | mutL homolog 1 | NM\_000249 | 102 | 1822 | PPH00196F-200 |
| ACTB | Beta Actin | NM\_001101 | 174 | 730 | PPH00073G-200 |
| UNG | Uracil-DNA glycosylase  | NM\_003362 | 84 | 2068 | PPH01727E-200 |
| MGMT | O-6-methylguanine-DNA methyltransferase | NM\_002412 | 87 | 160 | PPH01519F-200 |
| MTHFR | Methylenetetrahydrofolate reductase (NAD(P)H) | NM\_005957 | 103 | 2107 | PPH00027F-200 |
| MTR | 5-methyltetrahydrofolate-homocysteine methyltransferase | NM\_000254 | 128 | 3883 | PPH10237A-200 |
| HPRT1 | Hypoxanthine phosphoribosyltransferase 1 | NM\_000194 | 57 | 332 | PPH01018C-200 |