**Supplementary Table S1.** Subject characteristics, vitamin B status and concentrations of one-carbon related metabolites after overnight fast at baseline

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Control  (N = 22) | Folate-rich foods  (N = 21) | Folic acid supplement  (N = 19) | *p* value |
| Age (years) | 22±4 | 23±7 | 21±1 | 0.174 |
| BMI (kg/m2) | 23.7±3 | 24.1±5 | 23.6±3 | 0.938 |
| Energy intake (MJ/d) | 9.4 ±1.5 | 8.8±2.3 | 9.2±1.4 | 0.264 |
| Estimated folate intake (µg/d) | 204±38 | 195±50 | 220±30 | 0.353 |
| Intervention dose (µg/d) | 0 | 250\* | 500 |  |
| Erythrocyte folate (nmol/L) | 694±246 | 630±177 | 614±154 | 0.522 |
| Plasma folate (nmol/L) | 21±5 | 22±3 | 22 ± 3 | 0.559 |
| Plasma cobalamin (pmol/L) | 371±107 | 324±72 | 331 ± 121 | 0.135 |
| Glycine (µmol/L) | 313±54 | 285±18 | 329±122 | 0.192 |
| Alanine (µmol/L) | 485±112 | 505±199 | 474±160 | 0.945 |
| Choline (µmol/L) | 153±290 | 171±146 | 204±339 | 0.159 |
| Leucine (µmol/L) | 186±118 | 171±103 | 166±117 | 0.559 |
| Serine (µmol/L) | 182±46 | 188±82 | 216±56 | 0.214 |
| Lysine (µmol/L) | 197±71 | 214±92 | 274±206 | 0.274 |
| Valine (µmol/L) | 236±37 | 228±57 | 235±50 | 0.695 |
| Threonine (µmol/L) | 130±19 | 127±45 | 153±59 | 0.159 |
| Glutamate (µmol/L) | 282±160 | 266±93 | 263±228 | 0.348 |
| Histidine (µmol/L) | 81±21 | 96±36 | 100±26 | 0.115 |
| Proline (µmol/L) | 259±82 | 256±114 | 296±94 | 0.116 |
| Succinate (µmol/L) | 55±77 | 80±98 | 46±62 | 0.479 |
| Isoleucine (µmol/L) | 77±16 | 66±21 | 81±18 | 0.027 |
| Betaine (µmol/L) | 55±9 | 61±19 | 49±10 | 0.053 |
| Glutamine (µmol/L) | 184±63 | 129±85 | 201±80 | 0.004 |
| 2-Oxoisocaproate (µmol/L) | 4±8 | 5±4 | 4±8 | 0.618 |
| Sarcosine (µmol/L) | 3±2 | 2±1 | 2±1 | 0.995 |
| Aspartate (µmol/L) | 71±47 | 47±22 | 26±14 | 0.049 |

**Supplementary Table S1** continued

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| O-Acetyl-carnitine (µmol/L) | 7±5 | 8±4 | 9±5 | 0.375 |
| Myo-inositol (µmol/L) | 31±9 | 46±17 | 41±23 | 0.016 |
| Propionate (µmol/L) | 30±10 | 44±17 | 41±38 | 0.066 |
| Methionine (µmol/L) | 24±15 | 28±22 | 26±14 | 0.95 |
| Creatinine (µmol/L) | 32±20 | 61±9 | 58±7 | 0.107 |
| Serine/glycine ratio | 0.60±0.16 | 0.71±0.23 | 0.69±0.16 | 0.191 |

Mean energy intake was estimated using a food-frequency questionnaire and Egyptian food composition data.

\*Folate content according to analyses using an in-house HPLC method (1). \*\*According to supplier information.

To define significant metabolites one-way ANOVA on log-transformed data was used. The Bonferroni-adjusted significance level was defined as *p ≤* 0.001. No significant differences (p ≤ 0.001, Bonferroni-adjusted significance level) were observed.

**Supplementary Table S2.** Concentration (µmol/L, mean ± STD) of plasma metabolites1 in the control, folate-rich foods and folic acid supplement groupswhich show differences at 12 weeks of intervention

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Metabolite  (µmol/L) | Control  group | Folate-rich foods group | Folic acid supplement group | VIP  (95% CI) | *p*  value |
| Metabolites with significant differences after Bonferroni correction and VIP > 1 | | | | | |
| Glycine | 589±298B | 357±191C | 806±223A | 3.3 (2.9) | <0.001 |
| Choline | 127±87B | 60±61C | 221±70A | 2.0 (1.7) | <0.001 |
| Threonine | 178±60A.B | 138±37B | 222±66A | 1.5 (1.0) | 0.001 |
| Histidine | 110±28 B | 94±19 B | 132±28 A | 1.0 (0.9) | 0.001 |
| Metabolites with VIP>1 | | | | | |
| Alanine | 743±339 A.B | 506±222B | 861±365 A | 2.9 (2.2) | 0.008 |
| Leucine | 195±58A.B | 161±43B | 232±57A | 1.4 (1.2) | 0.002 |
| Serine | 278±89A.B | 217±59B | 318±107A | 1.5 (1.2) | 0.014 |
| Lysine | 232±84A.B | 197±5B | 286±70A | 1.4 (1.1) | 0.005 |
| Proline | 373±155A.B | 285±52 B | 415±142A | 1.7 (0.9) | 0.009 |
| Glutamate | 209±79A.B | 171±52B | 256±59A | 1.4 (1.0) | 0.003 |
| Valine | 285±64A.B | 248±54B | 321±65A | 1.3 (1.1) | 0.006 |
| Succinate | 78±46A.B | 47±32B | 107±53A | 1.2 (0.8) | 0.002 |
| Isoleucine | 105±29A.B | 90±23B | 128±35A | 1.0 (0.8) | 0.003 |
| Metabolites with significance in univariate analyses (*P* ≤ 0.05) but VIP < 1 | | | | | |
| 2-Oxoiso-caproate | 7±5A | 3±2B | 8±4A |  | <0.001 |
| Betaine | 66±18B | 61±15B | 85±18A |  | 0.001 |
| Formate | 51±18A | 39±11B | 57±13 A |  | 0.001 |
| Propionate | 17±8A,B | 12±5 B | 21±9 A |  | 0.001 |
| Phenyl-alanine | 79±26 A,B | 63±23 B | 95±26 A |  | 0.002 |
| Methionine | 46±14 A | 34±13 B | 53±19 A |  | 0.004 |
| Tyrosine | 93±29 A,B | 79±24 B | 112±32 A |  | 0.008 |
| 3-Methyl-2-oxovalerate | 15±2 A | 8±3 B | 15±11 A |  | 0.013 |
| Dimethyl sulfone | 8±2 B | 8±3 B | 10±2 A |  | 0.014 |
| O-Acetyl-carnitine | 12±6 A,B | 9±4 B | 14±5 A |  | 0.023 |
| Creatinine | 66±7 A,B | 61±12 B | 70±10 A |  | 0.041 |

1Only metabolites with significant differences between groups are listed. To define significant metabolites one-way ANOVA on log-transformed data (*p* ≤ 0.05) was used. The Bonferroni-adjusted significance level was defined as *p ≤* 0.001. Different superscripts within same row represent significant differences. OPLS and OPLS-DA were used for discrimination of the intervention groups. VIP >1 was used to define discriminative metabolites.

**(A)**

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**(B)**



**Supplementary Fig. S1.**

(A) Orthogonal partial least squares (OPLS) analysis of plasma metabolite profiles after the 12-weeks intervention showing the separation along the horizontal component of the folic acid supplement group (stars) from the folate-rich foods group (square); component 1 (R2[x]) = 67.2%; cv ANOVA P > 0.05. Codes next to symbols refer with D3 to the last day of intervention, with C, B and FA to control, folate-rich foods and folic acid supplement group and subsequent numbers to subject code. The x-axis depicts the number of volunteers, the y-axis depicts separation of the predictive compound.

(B) Variable Influence of Projection (VIP) plots from orthogonal partial least squares-discriminate analysis (OPLS-DA) of the overall plasma metabolites after the 12-weeks intervention. Metabolites with VIP 95% confidence interval (CI) > 1 were designated discriminative variables.