frontiers

# *Supplementary Material*

**Supplementary Table S1. Set of reference values used to assess energy and nutrient intakes of the study population.**

| Dietary variables | Dietary reference values (DRVs) | Type of DRV | Source |
|---|---|---|---|
| Energy | 18–29 y: 7.9 (PAL=1.4) – 9 (PAL=1.6) MJ/day<br>30–39 y: 7.6 (PAL=1.4) – 8.7 (PAL=1.6) MJ/day | AR | (EFSA, 2017) |
| **Macronutrients** | | | |
| Total carbohydrates | 45–60 E% | RI | (EFSA, 2017) |
| Sugars (monosaccharides and disaccharides) | <10 E% | RI | (WHO/FAO, 2003) |
| Dietary fiber | 25 g/day | AI | (EFSA, 2017) |
| Protein | 0.66 – 0.83 g per kg BW per day | AR – PRI | (EFSA, 2017) |
| Total fat | 20–35 E% | RI | (EFSA, 2017) |
| Saturated fatty acids (SFA) | <10 E% | RI | (FAO, 2010) |
| Monounsaturated fatty acids (MUFA) | 15–20 E% | RI | (FAO, 2010) |
| Polyunsaturated fatty acids (PUFA) | 6–11 E% | RI | (FAO, 2010) |
| α-linolenic acid (ALA) | 0.5 E% | AI | (EFSA, 2017) |
| Linoleic acid (LA) | 4 E% | AI | (EFSA, 2017) |
| **Minerals** | | | |
| Calcium | 18–24 y: 1000 mg/day<br>≥25 y: 950 mg/day | PRI | (EFSA, 2017) |
| Iron | 16 mg/day | PRI | (EFSA, 2017) |
| Phosphorus | 550 mg/day | AI | (EFSA, 2017) |
| Potassium | 3500 mg/day | AI | (EFSA, 2017) |

| | | | |
|---|---|---|---|
| Sodium | 2 g/day | Safe and adequate intake | (EFSA, 2017) |
| Zinc | LPI 600 mg/day: 9.3 mg/day | PRI | (EFSA, 2017) |
| **Vitamins** | | | |
| Thiamin | 0.1 mg/MJ | PRI | (EFSA, 2017) |
| Riboflavin | 1.6 mg/day | PRI | (EFSA, 2017) |
| Niacin | 1.6 mg NE/MJ | PRI | (EFSA, 2017) |
| Vitamin $B_6$ | 1.6 mg/day | PRI | (EFSA, 2017) |
| Folate | 330 μg DFE/day | PRI | (EFSA, 2017) |
| Vitamin C | 95 mg/day | PRI | (EFSA, 2017) |
| Vitamin A | 650 μg RE/day | PRI | (EFSA, 2017) |
| Vitamin E as α-tocopherol | 11 mg/day | AI | (EFSA, 2017) |
| Vitamin D | 15 μg/day | AI | (EFSA, 2017) |

Abbreviations: AI, adequate intake; AR: average requirement; BW, body weight; DFE, dietary folate equivalents; DRVs, dietary reference values; E%, percentage of energy intake; LPI, level of phytate intake; MJ, megajoule (1 MJ = 238.83 kcal); NE, niacin equivalent; PAL, physical activity level (1.4 low active, sedentary lifestyle; 1.6 moderately active lifestyle); PRI, population reference intake; RE, retinol equivalents; RI, reference intake range for macronutrients; y, years.

References:

EFSA (2017). Dietary reference values for nutrients Summary report. *EFSA Supporting Publications* 14, e15121E.

FAO (2010). Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food and Nutrition Paper* 91, 1-166.

WHO/FAO (2003). *Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation.* Geneva, Switzerland: World Health Organization.

**Supplementary Table S2. Prevalence of underweight, normal weight, overweight, and obesity in the study population based on self-reported pre-pregnancy body weight and height measurements.**

| | **Median BMI (IQR)** $kg/m^2$ | **Underweight** (n) | **Normal weight** (n) | **Overweight** (n) | **Obese** (n) |
|---|---|---|---|---|---|
| Age 20–29 y (n=11) | 22.04 (21.15–22.40) $kg/m^2$ | 1 | 9 | 1 | 0 |
| Age 30–39 y (n=13) | 22.76 (21.64–26.84) $kg/m^2$ | 0 | 8 | 5 | 0 |

Abbreviations: BMI, body mass index; IQR, interquartile range; y, years.

**Supplementary Table S3. Pre-pregnancy median intakes of energy, nutrients, and alcohol in the study population and their comparison to the reference values (as reported in Supplementary Table S1).** Significant differences with reference values are marked in bold.

| Dietary variables | Value † | *P*-value ‡ |
|---|---|---|
| Energy | 8.05 (6.73–9.72) MJ/day | 0.6900 |
| Total carbohydrates | 47.14 (41.18–51.82) E% | **<0.001** |
| Sugars | 21.00 (16.93–23.04) E% | **<0.001** |
| Dietary fiber | 17.98 (13.79–22.16) g/day | **<0.001** |
| Protein | 75.04 (68.94–83.31) g/day | **<0.001** |
| Total fat | 37.75 (35.12–40.47) E% | **<0.001** |
| SFA | 11.53 (10.34–12.35) E% | **<0.001** |
| MUFA | 17.86 (15.53–18.75) E% | 0.7400 |
| PUFA | 5.37 (4.46–6.17) E% | **<0.0001** |
| ALA | 0.45 (0.40–0.52) E% | **0.0469** |
| LA | 4.10 (3.53–5.04) E% | 0.2864 |
| Calcium | 710.25 (575.26–832.43) mg/day | **0.0002** |
| Iron | 10.21 (8.52–11.35) mg/day | **<0.0001** |
| Phosphorus | 1226.31 (1100.95–1346.06) mg/day | **<0.0001** |
| Potassium | 3146.39 (2401.40–3296.50) mg/day | **<0.001** |
| Sodium | 2088.30 (1894.28–2372.93) mg/day | 0.3600 |
| Zinc | 9.86 (8.71–10.84) mg/day | 0.4056 |
| Thiamin | 0.93 (0.83–1.24) mg/day | **0.0255** |
| Riboflavin | 1.51 (1.34–1.65) mg/day | 0.1400 |
| Niacin | 20.39 (16.19–25.35) mg NE/day | **<0.0001** |
| Vitamin $B_6$ | 1.92 (1.52–2.24) mg/day | **0.0027** |
| Folate | 262.18 (209.69–297.40) µg DFE/day | **<0.001** |
| Vitamin C | 113.42 (86.81–142.21) mg/day | **0.0300** |
| Vitamin A | 794.96 (628.47–920.25) µg RE/day | **0.006** |
| Vitamin E | 12.43 (10.53–15.80) mg/day | **0.0269** |
| Vitamin D | 2.66 (2.34–3.90) µg/day | **<0.001** |
| Alcohol | 3.64 (0.95–9.94) g/day | **0.0366** |

Abbreviations: ALA, α-linolenic acid; DFE, dietary folate equivalents; E%, percentage of energy intake; LA, linoleic acid; MUFA, monounsaturated fatty acids; NE, niacin equivalent; PUFA, polyunsaturated fatty acids; RE, retinol equivalents; SFA, saturated fatty acids.

† median with interquartile range (Q1 to Q3).

‡ p values from Student's t-test for normally distributed variables (energy, total carbohydrates, sugars, dietary fiber, protein, total fat, SFA, MUFA, ALA, iron, potassium, sodium, zinc, thiamin, niacin, vitamin $B_6$, folate, vitamin C, vitamin D) and Mann-Whitney U test for non-normally distributed variables (PUFA, LA, calcium, phosphorus, riboflavin, vitamin A, vitamin E, alcohol).

**Supplementary Table S4. Pre-pregnancy adequacy of dietary intake of the study population in comparison to the reference values.**

| Dietary variables | Adequate intake n (%) | Low intake n (%) | Excessive intake n (%) |
|---|---|---|---|
| Energy | 6 (25%) | 10 (42%) | 8 (33%) |
| Total carbohydrates | 17 (71%) | 7 (29%) | 0 (0%) |
| Sugars | 0 (0%) | -- | 24 (100%) |
| Dietary fiber | 3 (13%) | 21 (88%) | -- |
| Protein | 3 (13%) | 0 (0%) | 21 (88%) |
| Total fat | 6 (25%) | 0 (0%) | 18 (75%) |
| SFA | 5 (21%) | 0 (0%) | 19 (79%) |
| MUFA | 17 (71%) | 5 (21%) | 2 (8%) |
| PUFA | 7 (29%) | 17 (71%) | 0 (0%) |
| ALA | 8 (33%) | 16 (67%) | 0 (0%) |
| LA | 13 (54%) | 11 (46%) | 0 (0%) |
| Calcium | 2 (8%) | 22 (92%) | -- |
| Iron | 0 (0%) | 24 (100%) | -- |
| Phosphorus | 24 (100%) | 0 (0%) | -- |
| Potassium | 1 (4%) | 23 (96%) | -- |
| Sodium | 10 (42%) | -- | 14 (58%) |
| Zinc | 14 (58%) | 10 (42%) | -- |
| Thiamin | 15 (63%) | 9 (38%) | -- |
| Riboflavin | 8 (33%) | 16 (67%) | -- |
| Niacin | 24 (100%) | 0 (0%) | -- |
| Vitamin $B_6$ | 16 (67%) | 8 (33%) | -- |
| Folate | 3 (13%) | 21 (88%) | -- |
| Vitamin C | 17 (71%) | 7 (29%) | -- |
| Vitamin A | 17 (71%) | 7 (29%) | -- |
| Vitamin E | 15 (63%) | 9 (38%) | -- |
| Vitamin D | 0 (0%) | 24 (100%) | -- |
| Alcohol | 19 (79%) | -- | 5 (21%) |

**Supplementary Table S5. Prevalence of low, moderate, and high adherence to a Mediterranean-type diet before pregnancy in the study population.**

| | **Median MEDI-LITE score (IQR)** | **Low adherence** (n) † | **Moderate adherence** (n) † | **High adherence** (n) † |
|---|---|---|---|---|
| Age 20–29 y (n=11) | 10.00 (9.50–11.50) | 2 | 8 | 1 |
| Age 30–39 y (n=13) | 10.00 (8.00–13.00) | 0 | 9 | 4 |

Abbreviations: IQR, interquartile range; y, years.

† Low adherence range 0–6; moderate adherence range 7–12; high adherence range 13–18.

**Supplementary Table S6. List of the main pre-pregnancy anthropometric and dietary data for which a correlation with vaginal health status (Nugent score) during pregnancy was investigated.** Correlations were searched by calculating Spearman correlation coefficient (r) after correction for multiple comparisons (i.e., Bonferroni-Holm correction). Significant differences with reference values are marked in bold.

| **Parameter** | **r** | ***P-value*** |
|---|---|---|
| **BMI** | **0.43** | **0.034** |
| **Animal protein** | **0.42** | **0.039** |
| **Total carbohydrates** | **-0.42** | **0.041** |
| **Sugars** | **-0.41** | **0.046** |
| Alcohol | 0.40 | 0.055 |
| Calcium | 0.38 | 0.068 |
| Riboflavin | 0.36 | 0.083 |
| Phosphorus | 0.34 | 0.105 |
| MUFA | 0.32 | 0.133 |
| Total fat | 0.30 | 0.160 |
| Carotene | 0.28 | 0.188 |
| Total protein | 0.26 | 0.215 |
| PUFA | -0.26 | 0.221 |
| LA | -0.26 | 0.226 |
| Zinc | 0.23 | 0.279 |
| Vitamin D | -0.18 | 0.402 |
| Potassium | 0.16 | 0.452 |
| Vitamin $B_6$ | 0.16 | 0.457 |
| Oleic acid | 0.16 | 0.464 |
| Cholesterol | -0.15 | 0.484 |
| Folate | 0.14 | 0.507 |
| Niacin | 0.14 | 0.508 |
| Dietary fiber | 0.14 | 0.525 |
| Iron | 0.14 | 0.525 |
| Vitamin C | 0.12 | 0.580 |
| ALA | 0.10 | 0.630 |
| SFA | 0.09 | 0.676 |
| Vitamin A | 0.08 | 0.693 |
| Thiamin | 0.08 | 0.694 |
| Vitamin E | 0.05 | 0.822 |
| Sodium | 0.04 | 0.851 |
| Plant protein | 0.04 | 0.861 |
| Energy | 0.02 | 0.937 |
| MEDI-LITE score | -0.01 | 0.952 |

**Supplementary Figure S1. Portions of $^{1}$H-NMR spectra, representative of all the spectra obtained in this study. The name of each molecule appears over the signal employed to quantify it. To optimize the visualization of each portion, a different spectrum with a convenient signal-to-noise ratio was selected.**