Folate (vitamin B9), is commonly found in dark green leafy vegetables, beans, peas, and nuts. Folate and folic acid are sometimes used interchangeably; however, folate is the vitamin found in nature, while folic acid is a synthetic oxidized, water-soluble form that is used for therapeutics [1]. The prevalence of folate deficiency in healthy people with normal dietary intake has declined progressively in countries that have implemented routine folic acid supplementation of foods. This practice began in the late 1990s in many parts of the world [2], although a national program of folic acid fortification does not yet exist in Israel.

Overt folate deficiency may cause megaloblastic anemia [3] and may increase the risk of several cancers [4] and Alzheimer's disease [5,6]. A common practice today is to prescribe folic acid supplementation to all pregnant females to decrease the incidence of neural tube defects [7].

Folate and vitamin B12 have important roles in the methionine homocysteine cycle. Homocysteine is normally metabolized by two divergent pathways: transsulfuration and methylation. In the transsulfuration pathway, homocysteine is converted to cysteine by cystathionine-β-synthase and by cystathionine-γ-lyase where vitamin B6 is a cofactor. In the methylation pathway, homocysteine is re-methylated to methionine by either methionine synthase, where vitamin B12 and folate are cofactors, or by betaine-homocysteine methyltransferase. Methionine is transmethylated back to homocysteine through the production of S-adenosyl methionine and S-adenosyl-L-homocysteine [Figure 1]. Low levels of folate may be associated with elevated levels of homocysteine [8] which in itself is a known risk factor for the development of atherosclerosis [9,10] and chronic kidney disease (CKD) [11].

In a large cohort study, we showed that males have significantly higher homocysteine plasma levels than females [12]. This difference was attributed to lower levels of folate in males. Potential explanations for sex differences in folate levels are: sex differences in vegetarianism, the fact that females are urged to consume folic acid while pregnant, and genetic polymorphism in folate metabolism.

The aim of the current study was to assess in detail the sex differences in folate levels and specifically their relation to plasma homocysteine levels.

The study population consisted of a cross-sectional sample of males and (non-pregnant) females aged 20–80 years, who were referred by their employers for routine medical screening at a...
tertiary medical center in Israel between the years 2000 and 2014. None of the patients was hospitalized at the time. Screening consisted of a thorough medical history and a complete physical examination, a broad series of blood and urine tests, a chest X-ray, an electrocardiogram, an exercise stress test, a respiratory function test, and a full ophthalmology examination. For the purpose of this study, we used the data from the most recent visit.

Data on smoking habits were collected from direct questioning on the day of examination at the screening center. Patients receiving vitamin B12 or folate supplements were excluded from the analysis.

The various blood tests were performed after an overnight 12-hour fast. Between May 2000 and May 2011 serum vitamin B12 and folate levels were measured using the Immulite® Assay (Siemens Healthcare GmbH, Erlangen Germany). From May 2011 until the end of the study, they were measured using the Architect® assay (Abbott, Wiesbaden, Germany). In both assays, measuring methods of plasma homocysteine were similar and reference levels were 5.0–15 µmol/L. Homocysteine concentrations above 15 µmol/L are considered to be elevated [13].

The definition of low or deficient folate levels differs between laboratories. For the purpose of this study we used two cutoff levels: a liberal cutoff of low levels of folate (levels below 12.2 nmol/L) [14] and a restricted definition of deficient folate levels (levels below 7 nmol/L). This level is the lower limit of the normal range of folate at our laboratory.

A computer program was created to transfer all data from each visit to an Excel database file (Excel spreadsheet, Microsoft Excel, Version 97-2003, Microsoft Corp, Richmond, CA, USA). The study was approved by the Helsinki Ethics Committee of Rabin Medical Center.

STATISTICAL ANALYSIS
Baseline characteristics were compared between males and females using Student’s t-test for continuous variables and the Chi-square test for categorical variables. The odds ratios (ORs) and 95% confidence interval (95%CI) of having low levels of
folate in relation to sex were assessed by using logistic regression. Univariate analyses were performed in Model 1. Model 2 was adjusted for age alone and Model 3 was adjusted for age, smoking status, body mass index, kidney function (estimated glomerular filtration rate), and albumin and triglycerides levels. As sex differences are at least partially affected by hormonal changes, all analyses were repeated stratifying two age groups, one before and the other after the age of 55 (by that age menopause is assumed for the female population).

Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). A P value < 0.05 was considered statistically significant.

RESULTS

The cross-sectional analysis included 13,550 patients; 32% were female. The clinical and laboratory characteristics of these participants are presented in Table 1. The mean ± standard deviation (SD) age of the males was 48.4 ± 9.5 years and 47.6 ± 9.4 years for females.

The average folate levels were 19.2 ± 8.6 and 22.4 ± 10.3 nmol/L in males and females, respectively (P < 0.001). The prevalence of folate levels below 12.2 nmol/L was 19.5% in males vs. 11.6% in females (P < 0.001). The prevalence of folate levels below 7.0 nmol/L was 1.7% in males vs. 0.9% in females (P < 0.001).

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Table 1. Patients characteristics by sex

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>48.4 ± 9.5</td>
<td>47.6 ± 9.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m2), mean ± SD</td>
<td>27.5 ± 4.0</td>
<td>25.7 ± 4.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>16.1</td>
<td>17.0</td>
<td>0.171</td>
</tr>
<tr>
<td>eGFR (CKD-EPI) ml/min/1.73 m2, mean ± SD</td>
<td>95.0 ± 13.8</td>
<td>99.8 ± 13.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.9 ± 1.0</td>
<td>13.1 ± 1.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum glucose concentration (mg/dl), mean ± SD</td>
<td>100.6 ± 20.1</td>
<td>94.2 ± 15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum albumin concentration (g/dl), mean ± SD</td>
<td>4.5 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl), mean ± SD</td>
<td>196.3 ± 36.6</td>
<td>199.3 ± 37.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl), mean ± SD</td>
<td>138.4 ± 88.8</td>
<td>106.1 ± 61.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl), mean ± SD</td>
<td>47.5 ± 10.4</td>
<td>60.3 ± 13.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum folate concentrations (nmol/L), mean ± SD</td>
<td>19.2 ± 8.6</td>
<td>22.4 ± 10.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum vitamin B12 concentrations (pmol/L), mean ± SD</td>
<td>295.0 ± 125.1</td>
<td>320.4 ± 141.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Plasma homocysteine concentrations (µmol/L), mean ± SD</td>
<td>12.6 ± 5.9</td>
<td>9.6 ± 3.1</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

BMI = body mass index, CKD-EPI = chronic kidney disease epidemiology collaboration, eGFR = estimated glomerular filtration rate, HDL = high-density lipoproteins, SD = standard deviation

In patients with folate levels below 12.2 nmol/L and normal B12 levels, homocysteine levels above 15 µmol/L were found in 32.4% of males and 11.4% of females (P < 0.001) and similarly, in patients with folate levels below 7.0 nmol/L, homocysteine levels above 15 µmol/L were found in 56.4% of males and 41.7% of females (P = 0.192). Logistic regression models of male sex as a determining factor for low folate levels are presented in Table 3. A subgroup analysis comparing patients younger and older than 55 years was performed to eliminate the estrogen effect found in younger females. Compared to females, males had a significantly higher OR (95%CI) of having folate levels below 12.2 nmol/L. Using the non-adjusted model, OR = 1.84 (95%CI 1.66–2.05) and using the adjusted model for age, smoking status, body mass index, kidney function, and albumin and triglyceride levels OR = 2.02 (95%CI 1.82–2.27). With a cutoff of folate levels lower than 7 nmol/L, a slightly higher OR was observed in favor of females. The subgroup analysis did not reveal any difference between the two age groups, thus eliminating estrogen as a factor in the sex differences in folate levels.
In this large cohort of 13,550 patients we found that low levels of folate are relatively common with a prevalence of 19.5% and 11.6% in males and females, respectively. Since the state of Israel does not have a national program of folic acid fortification, those results are not surprising. However, the sex differences are somewhat surprising with a two-fold greater risk for folate deficiency among males. Another important sex difference was the effect of low folate levels on homocysteine plasma levels. We show that in males with low folate and normal vitamin B12 levels, there were significantly higher homocysteine plasma levels compared to females. Furthermore, the subgroup analysis comparing subsets younger and older than 55 years of age did not reveal any effect on sex differences in folate levels, thus most probably eliminating estrogen as a factor in the sex differences in folate levels.

Several factors could explain why females have higher folate levels than males. Females of child-bearing age are urged to consume folic acid supplements before, and especially during, pregnancy, to avoid the possibility of the fetus developing neural tube defects [7]. The second factor may be dietary behavior with a higher rate of vegetarians found among females. In a comprehensive review of vegetarianism, Ruby [15] highlighted this sex aspect of vegetarianism. He pointed out that meat has long stood as a symbol of a person’s strength and dominance over the natural world. The idea that meat is primarily a human’s food is found across many cultures. Some studies have shown that males associate meat with masculinity. Sex differences also emerge in the tendency to view vegetarianism through the lens of ethics, where females will endorse the belief that a vegetarian diet is less harmful to the environment and prevents cruelty to farm animals. It is thus unsurprising that vegetarianism is found more commonly in females and may account for the differences in folate levels. Last, genetic factors cannot be excluded as a contributing factor in the sex difference.

The third National Health and Nutrition Examination Survey (NHANES III) conducted in the United States between 1988 and 1994 shed some light on the genetic aspect of folate levels [16]. Using the DNA bank from that survey, Yang et al. [17] concluded that the methylenetetrahydrofolate reductase (MTHFR)
The 677C→T polymorphism was associated with significant differences in serum folate and homocysteine concentration before folic acid fortification. The MTHFR 677C→T variant leads to an alanine to valine substitution in the folate binding site of the enzyme. In another study Russo and colleagues [18] showed that patients with a TT genotype had significantly lower folate levels (14.3 nmol/L in the CC genotype vs. 12.3 nmol/L in the TT genotype, \( P < 0.0001 \)). Moreover, a sex difference was found in the prevalence of the TT genotype, which was higher in males compared to females (15.2% versus 13.5%). This genetic sex difference may add to the possible explanations why males have lower folate levels.

How does our data on sex differences in folate levels compare to other studies? Two studies carried out in the United States, before the mandatory folic acid fortification of flour in 1998, showed males to have lower levels of folate compared to females. Data from the NHANES III collected from 3433 Caucasian Americans showed folate levels of 16.9 and 18.4 nmol/L in males and females respectively [19]. Similarly, the Framingham Offspring Study Cohort of 1829 patients found folate levels of 12.3 and 14.1 nmol/L in males and females, respectively (\( P < 0.001 \)). In that study, folate levels below 12.5 nmol/L were found in 52.7% of males and 46.9% of females \( P = 0.02 \) [18]. The HELENA study, conducted on a random sample of 3516 European adolescents between the ages of 12.5 and 17.5 years, did not find significant differences in folate levels between males and females, with 15.9 and 16.01 nmol/L, respectively [20]. In a survey of 2422 Chinese adults, a country without a national fortification system, levels of folate were low and sex differences were also noted. The folate levels in males and females were 10.0 nmol/L in males and 13.8 nmol/L in females (\( P < 0.050 \)) [21].

The main strength of our study is the inclusion of a large cohort (13,550 males and females) with documented homocysteine concentrations, together with a complete datasets of clinical and laboratory findings. In the current study, apart from showing sex differences in folate levels, we also demonstrated the association of low folate and homocysteine levels. In a previous study, we showed significantly lower levels of plasma homocysteine levels in females [12]. Many factors could account for this sex difference. The current study of folate status in males and females adds to the possible sex differences in homocysteine levels, as low folate levels may lead to elevated levels of homocysteine [8].

Hyperhomocysteinemia is associated with atherosclerosis and cardiovascular disease [9,10] as well as with CKD [11]. However, it should be noted that despite convincing evidence associating hyperhomocysteinemia with coronary artery disease, clinical trials attempting to lower high homocysteine plasma concentrations by giving folate and vitamin B12 to these patients, have not shown a conclusive positive effect [22-24]. For CKD, in a study from China, a country with no fortification of folate, it was shown that adding folic acid delayed CKD progression [25].

**LIMITATIONS**

The study group was not drawn from a population sample but from those attending an examination center. This sampling limits the generalizability of the findings. We did not have data regarding the MTHFR genotype of the patients and levels of B6 were not measured.

**CONCLUSIONS**

Folate levels were found to be lower in males compared to females. This finding may contribute to the higher homocysteine levels found in males, and therefore be a factor in their increased risk of developing atherosclerosis and coronary artery disease. A national fortification system with folate acid could end this sex difference.

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**References**

The two most common monogenic diseases, transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) result from mutations in the hemoglobin β-subunit gene (*HBB*), which is an essential element of adult hemoglobin A (a2b2). Current therapies for TDT and SCD are limited and do not address the underlying causes. Frangoul and colleagues reported the treatment of two patients (one with TDT and the other with SCD) using gene therapy. After myeloablation, the patients were infused with their own hematopoietic stem and progenitor cells subjected to CRISPR-Cas9 gene editing of the erythroid-specific enhancer region of BCL11A. This transcription factor represses the expression of γ-globin, a component of a2γ2 fetal hemoglobin that is known to ameliorate the severity of these disorders. More than a year later, both patients showed sustained engraftment of edited cells in the bone marrow and increased fetal hemoglobin expression, which relieved symptoms and obviated the need for transfusions.

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**Etan Israel**

**Capsule**

**Oscillations around memory**

Hippocampal oscillations in the theta range have been hypothesized to play a central role in organizing neuronal ensembles to link together item and contextual representations. Experimental evidence in rodents shows the importance of theta oscillations for associative memory. However, the role of hippocampal theta oscillations in human memory is not as well understood. Kota et al. administered an associative recognition memory task to epilepsy patients who happened to have electroencephalogram electrodes implanted for other medical reasons. Theta oscillatory power increase in the 2- to 5-Hz range and phase reset in the hippocampus reflected processes supporting recollection, rather than familiarity, during encoding and retrieval. These observations link theta-range activity to associative memory encoding and retrieval in humans.

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**Etan Israel**

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**The nicest thing in the world you can do for anybody is let them help you.**

John Steinbeck (1902-1968), American author of 27 books, winner of the 1962 Nobel Prize in Literature

**People who demand neutrality in any situation are usually not neutral but in favor of the status quo.**

Max Eastman (1883-1969), American writer on literature, philosophy and society; a poet and a prominent political activist