Vitamin B intake and status in healthy Havanan men, 2 years after the Cuban neuropathy epidemic

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A prospective epidemiological study was carried out over 1 year to evaluate vitamin B complex dietary intake and status in Cuba, 2 years after the Cuban neuropathy epidemic of 1993. Of the 199 healthy middle-aged men selected, 141 completed the study. Volunteers were followed up every 3 months for 1 year. Dietary intake and status of thiamin, riboflavin, vitamin B6, folate and vitamin B12 were assessed each time. The dietary intake of vitamin B complex was low, particularly in June and July (folate), and October (thiamin). A deficient status was observed for vitamin B complex, except for vitamin B6. Vitamin B complex intake and status varied over the year. However, dietary intake and status were poorly related. The results prove that healthy Cuban men represent a vulnerable population in terms of vitamin B complex status and stress the necessity to both promote preventive multivitamin supplementation and produce local food rich in vitamin B complex.

Vitamin B complex: Seasonal variation in intake: Blood indices

From 1991 to 1993, 50 862 cases (461.4/100 000 inhabitants) of optic and peripheral neuropathies were reported in Cuba (Tucker & Hedges, 1993; Pérez-Cristià & Fleites-Mestre, 1994; Roman, 1994; Bowman et al. 1996; Macias-Matos et al. 1996). Food shortage, depletion in micronutrients (particularly in vitamin B complex) and smoking habits were major risk factors of the Cuban neuropathy epidemic (Bowman et al. 1996; Macias-Matos et al. 1996). In tropical countries similar optic and peripheral neuropathies have been attributed to thiamin, folate or vitamin B12 deficiencies (Roman, 1994; Bowman et al. 1996; Sainte-Foie et al. 1997; Dolin et al. 1998). However, during the Cuban neuropathy epidemic, the severity of vitamin B complex deficiencies was similar in both patients with neuropathy and control subjects (Tucker & Hedges, 1993; Macias-Matos et al. 1996). Nevertheless, the number of new cases decreased and most patients suffering from neuropathy (99.9%) improved significantly after the May 1993 nationwide distribution of multivitamin supplement (containing (mg): thiamin 2.5, riboflavin 1.6,
biological study. However, because of insufficient available blood samples, complete biological data for vitamin B complex status were obtained in only 127 volunteers. The dietary and life-habit questionnaires were completed every time by 106 volunteers. The reasons for drop-out were illness, moving or a different job site at the time of clinical, nutritional and biological assessment. However, those volunteers who completed the study and those who did not were similar in terms of age, BMI, race, smoking habits, vitamin supplement use, years of education and employment (Arnaud et al. 2001).

The study protocol was approved by the Cuban Ministry of Public Health, and all volunteers gave their informed written consent. Procedures followed were in accordance with Anon (1994).

Assessment of dietary intake

Food and beverage consumption was quantified by volunteers (household ware) in all periods for seven consecutive days. At the end of each day, a trained dietitian checked by interview information on the food and beverage record for composition and quantity accuracy, completeness and clarity. Nutrient intake was calculated using the Cuban NUTRISIS food composition database (Rodriguez et al. 1992) which contains the nutrient contents of the most commonly consumed food and beverages (n = 628) in Cuba. Nutritional density was calculated as mg/g vitamin/5 MJ.

Blood collection, storage and transport

Blood was collected by venepuncture after overnight fasting in a heparinised vacuum tube and a vacuum tube containing no additive (Becton Dickinson, Pont de Clax, France). The tubes were protected from light and stored on ice immediately after collection. The blood samples were delivered to the Cuban laboratory within 30 min of venepuncture. The tubes were centrifuged at 1700 g for 10 min at 4°C to separate serum or plasma. The buffy coat was then removed from the heparin-treated tube and the remaining erythrocytes were subjected to vortexing in order to obtain a homogeneous cellular suspension before cell sampling. Aliquots of serum (for folate and vitamin B12 determinations) and erythrocytes (for erythrocyte transketolase activation coefficient (ETK-AC), erythrocytes glutathione reductase activation coefficient (EGR-AC) and erythrocyte aspartate aminotransferase activation coefficient (EAST-AC) determinations) were frozen and stored at −20°C within 2 h of sampling. Samples were kept frozen until analysed and thawed only once before assay. Determinations were performed within 1 month.

Blood analysis

Thiamin, riboflavin and pyridoxal phosphate status were evaluated respectively by ETK-AC EGR-AC and EAST-AC according to previously published methods (Nicholaud et al. 1974; Bayoumi & Rosalki, 1976) at the Département de Biologie Intégrée. Enzyme activity was determined with and without added coenzymes (thiamin pyrophosphate, FAD and pyridoxal-5-phosphate respectively). The activity
ratio (with/without coenzymes) provided the erythrocyte activation coefficients. The variability of measures was controlled by using pools of erythrocytes stored at −20°C in small aliquots. The performance of a new pool of erythrocytes was compared with that of the previous pool. These samples were stable for at least 5 weeks for ETK-AC, 7 weeks for EGR-AC and 4 weeks for EAST-AC. The between-day CV was 3-0 % for ETK-AC, 4-8 % for EGR-AC and 4-0 % for EAST-AC. The values obtained in a French blood-donor population (National Research Council, 1989) are indicated in Table 2. Significant period effects were observed for thiamin, vitamin B6 and folate intake by period.

Statistics

Statistical analysis was performed using SAS software (SAS Institute, Cary, NC, USA). Individual measures of vitamin intake and status and of food consumption were normalised by logarithmic or square root transformation when necessary. For each variable, means, medians, and standard deviations were calculated for the four periods using data from all the volunteers with a complete set of measures. Response trends over time were assessed by variance-covariance analyses on repeated measures using a covariance structure, referring to variance at individual times (variation between volunteers) and a correlation between measures at different times on the same subject (covariation within volunteers), characterises the data. McNemar’s test was performed to evaluate the changes in the frequency of vitamin deficiencies according to periods. McNemar’s test assesses the significance of the difference between two independent samples when the variable of interest is dichotomous. It uses chi-square distribution applied to pair samples (Lei et al. 1998). Spearman correlations were performed between the standardised values of absolute intake or nutrient densities and the standardised values of biological status for individual components of the vitamin B complex. Adjusted Spearman correlations were also performed (Tomassone et al. 1993). Factors known to influence micronutrient status (i.e. age, years of education, BMI, number of cigarettes smoked per day, number of vitamin tablets per week, energy and alcohol intake) were selected as adjustment variables. All tests were considered significant at \( P < 0-05 \).

Results

Vitamin B complex intake by period is indicated in Table 1. The percentage of volunteers with values below two-thirds of the recommended dietary allowances (National Research Council, 1989) is indicated in Table 2. Significant period effects were observed for thiamin, vitamin B6 and folate daily dietary intake (\( P = 0-001 \) in all cases). Thiamin daily dietary intake decreased regularly from March 1995 to October 1995 and increased between October 1995 and February 1996. Vitamin B6 daily dietary intake decreased...
Significance of probability of a difference between periods.

Period 1, March and April 1995; period 2, June and July 1995; period 3, October 1995; period 4, January and February 1996; Periods 2 and 3, rainy season.

Mean value (g/d) was significantly higher in period 1 than in period 4: *

Mean value was significantly lower in period 1 than in period 4: †

Vitamin status is indicated in Table 4 and the percentage of volunteers assessed under the cut-off values of vitamin deficiencies proposed by a European group of experts (Van den Berg et al.1993) is indicated in Table 5. Statistically significant differences between periods were observed for vitamin status. An improvement in both thiamin and pyridoxal status occurred between March 1995 and July 1995. A significant decline in riboflavin status was observed in January and February 1996, compared with the three other periods (P = 0.040). Serum folate concentrations indicated that folate status was lowest in June and July 1995 and highest in October 1995. Serum vitamin B12 concentrations and the percentage of values under 100 pmol/l indicated an improvement in vitamin B12 status in periods 3 and 4 compared with periods 1 and 2. Serum folate and vitamin B12 concentrations were extremely low. As indicated in Table 5, >20 % of volunteers were deficient in thiamin and riboflavin, and more than 60 % of volunteers were folate deficient. The percentage of volunteers suffering from vitamin B12 deficiency varied between March 1995 and July 1995 and remained similar from July 1995 to February 1996. Folate daily dietary intake was lower during the rainy season (periods 2 and 3) than during the dry season (periods 1 and 4). As indicated in Table 2, more than 60 % of the volunteers had daily dietary intakes below two-thirds of the recommended dietary allowances (National Research Council, 1989) for thiamin, riboflavin and vitamin B6 for all the periods studied. For folate dietary intake, these percentages varied from 24.5 to 47.2, depending on the periods.

Table 3 shows the mean daily consumption of energy, macronutrients and various food or food groups known to have an effect on vitamin B complex intake and biological status. Vegetable and fruit intake was significantly lower in the rainy season (periods 2 and 3) than in the dry season (periods 1 and 4; P = 0.0001). Between March 1995 and July 1995, intakes of bread, cereals, beans and tubers decreased along with carbohydrate intake, whereas seafood consumption increased. Finally, egg consumption was decreased along with carbohydrate intake, whereas seafood intake increased. Finally, egg consumption was decreased along with carbohydrate intake, whereas seafood intake increased. Finally, egg consumption was decreased along with carbohydrate intake, whereas seafood intake increased.

Table 4. Blood vitamin B status indices in healthy Havanan male volunteers measured at 3-month intervals over 1 year

<table>
<thead>
<tr>
<th>Period</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETK-AC</td>
<td>127</td>
<td>1.23</td>
<td>0.14</td>
<td>0.020</td>
<td>1.19</td>
<td>0.13</td>
<td></td>
<td>1.21</td>
<td>0.12</td>
<td></td>
<td>1.24</td>
<td>0.17</td>
<td>0.010</td>
</tr>
<tr>
<td>EGR-AC</td>
<td>134</td>
<td>1.57</td>
<td>0.32</td>
<td></td>
<td>1.59</td>
<td>0.35</td>
<td></td>
<td>1.61</td>
<td>0.34</td>
<td>0.004</td>
<td>1.61</td>
<td>0.33</td>
<td>0.040</td>
</tr>
<tr>
<td>EAST-AC</td>
<td>134</td>
<td>1.77</td>
<td>0.28</td>
<td>0.001</td>
<td>1.61</td>
<td>0.24</td>
<td></td>
<td>1.69</td>
<td>0.32</td>
<td></td>
<td>1.64</td>
<td>0.34</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum folate (nmol/l)</td>
<td>128</td>
<td>4.7</td>
<td>2.7</td>
<td>0.006</td>
<td>4.1</td>
<td>2.4</td>
<td>0.001</td>
<td>6.7</td>
<td>4.4</td>
<td>0.001</td>
<td>4.7</td>
<td>2.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l)</td>
<td>153</td>
<td>100</td>
<td>55</td>
<td>0.001</td>
<td>106</td>
<td>53</td>
<td>0.001</td>
<td>156</td>
<td>72</td>
<td>0.030</td>
<td>145</td>
<td>68</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mean value (g/d) was significantly higher in period 1 than in period 4: * P = 0.004.

Mean value was significantly lower in period 1 than in period 4: † P = 0.020.

For details of subjects and procedures, see p. 742.

Significance of probability of a difference between periods.

Significance of probability of period effect.

Table 3. Daily intakes of various food and food groups, energy, proteins and carbohydrates in healthy Havanan male volunteers (n 106) evaluated at 3-month intervals over 1 year

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread, cereals, beans and tubers: g/d</td>
<td>719</td>
<td>21</td>
<td>0.005</td>
<td>644</td>
<td>18</td>
<td>0.001</td>
<td>621</td>
<td>16</td>
<td></td>
<td>588</td>
<td>18</td>
<td>0.0001</td>
</tr>
<tr>
<td>% derived from polished rice</td>
<td>35.7*</td>
<td>1.4</td>
<td></td>
<td>35.5</td>
<td>1.1</td>
<td></td>
<td>36.7</td>
<td>1.3</td>
<td></td>
<td>35.9</td>
<td>1.4</td>
<td>0.0041</td>
</tr>
<tr>
<td>Vegetables, fruit and fruit juice, (g/d)</td>
<td>153</td>
<td>12</td>
<td>0.001</td>
<td>92</td>
<td>7</td>
<td></td>
<td>89</td>
<td>5</td>
<td>0.0001</td>
<td>202</td>
<td>12</td>
<td>0.0001</td>
</tr>
<tr>
<td>Milk and dairy products (g/d)</td>
<td>91</td>
<td>8</td>
<td></td>
<td>123</td>
<td>11</td>
<td></td>
<td>111</td>
<td>10</td>
<td></td>
<td>108</td>
<td>12</td>
<td>0.0670</td>
</tr>
<tr>
<td>Meat (g/d)</td>
<td>51†</td>
<td>3</td>
<td></td>
<td>57</td>
<td>4</td>
<td></td>
<td>58</td>
<td>4</td>
<td></td>
<td>67</td>
<td>4</td>
<td>0.0235</td>
</tr>
<tr>
<td>Eggs (g/d)</td>
<td>34</td>
<td>2</td>
<td>0.001</td>
<td>19</td>
<td>2</td>
<td>0.007</td>
<td>36</td>
<td>2</td>
<td></td>
<td>37</td>
<td>2</td>
<td>0.0013</td>
</tr>
<tr>
<td>Fish, shellfish and crustacea (g/d)</td>
<td>31</td>
<td>2</td>
<td>0.0075</td>
<td>42</td>
<td>3</td>
<td></td>
<td>36</td>
<td>2</td>
<td></td>
<td>37</td>
<td>2</td>
<td>0.0013</td>
</tr>
<tr>
<td>Alcoholic beverages (g/d)</td>
<td>47</td>
<td>11</td>
<td></td>
<td>59</td>
<td>10</td>
<td></td>
<td>58</td>
<td>12</td>
<td></td>
<td>59</td>
<td>9</td>
<td>0.4890</td>
</tr>
<tr>
<td>Other type of food (g/d)</td>
<td>215</td>
<td>17</td>
<td></td>
<td>182</td>
<td>13</td>
<td>0.036</td>
<td>143</td>
<td>12</td>
<td></td>
<td>152</td>
<td>14</td>
<td>0.0040</td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td>7.0</td>
<td>2.0</td>
<td></td>
<td>6.9</td>
<td>1.9</td>
<td></td>
<td>6.7</td>
<td>1.8</td>
<td></td>
<td>6.6</td>
<td>1.8</td>
<td>0.1050</td>
</tr>
<tr>
<td>Proteins (g/d)</td>
<td>61</td>
<td>16</td>
<td></td>
<td>64</td>
<td>16</td>
<td></td>
<td>62</td>
<td>16</td>
<td></td>
<td>62</td>
<td>16</td>
<td>0.7320</td>
</tr>
<tr>
<td>Carbohydrates, (g/d)</td>
<td>258</td>
<td>74</td>
<td>0.007</td>
<td>236</td>
<td>66</td>
<td>0.0100</td>
<td>237</td>
<td>67</td>
<td></td>
<td>235</td>
<td>70</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

Mean value (g/d) was significantly higher in period 1 than in period 4: * P = 0.004. Mean value was significantly lower in period 1 than in period 4: † P = 0.020.
between 20% (periods 3 and 4) and 50% (periods 1 and 2). Despite low vitamin B6 intake, the corresponding status remained adequate.

Spearman correlation coefficients are reported in Table 6. No significant correlation was observed between thiamin intake and status. Correlation between riboflavin, vitamin B6, or folate intake and the corresponding biological status depended on periods, intake expression (absolute intake or nutritional densities) and adjustment. No significant correlation was observed between absolute dietary intake and biological status, except for riboflavin in January–February 1996 (r = 0.194, n = 129, P = 0.027) and for folate in March–April 1995 (r = 0.187, n = 181, P = 0.012). The three models presented a significant relationship between riboflavin intake and status in January and February 1996.

### Discussion

In Havanan men, vitamin B complex dietary intake was low compared with reported values for adult men in developed countries (Costa de Carvalho et al. 1996; Brants et al. 1997; Alberti-Fidanza et al. 1998; Bell et al. 1998). As far as we know, few studies have been conducted in developing countries, particularly in adult males. Compared with the dietary intakes observed in Cuba at the beginning of the neuropathy epidemic in 1992, the energy and folate daily dietary intakes reported in the present study were lower (Pérez-Cristià & Fleites-Mestre, 1994). In contrast, thiamin and protein daily dietary intakes were higher than those in 1992 (Pérez-Cristià & Fleites-Mestre, 1994). In addition, vitamin B6 and riboflavin dietary intakes were similar to those reported in 1992 (Pérez-Cristià & Fleites-Mestre, 1994). The low energy intake probably makes it difficult to meet vitamin B complex requirements, and therefore contributes to the fact that a high percentage of Cuban volunteers present with thiamin, riboflavin and vitamin B6 daily dietary intakes below two-thirds of the recommended dietary allowances (National Research Council, 1989). The low vitamin intake in the Cuban population studied suggests that this population is at risk for vitamin B complex deficiencies and thus for neuropathy, although when the present study was done few cases of neuropathy had been reported. This low incidence of neuropathy contrasts with the very severe economic crisis, the low dietary intake of vitamin B complex and the low consumption of multivitamin supplements. Nevertheless, when a risk factor is very common in a population, its prevalence may reflect a risk even if its presence in an individual is not predictive of disease (Bowman et al. 1996).

### Table 6. Spearman correlation coefficients for correlation between standardised vitamin B complex daily dietary intake and status for healthy Havanan male volunteers

<table>
<thead>
<tr>
<th>Period* Correlation model</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nutritional density†</td>
<td>Adjusted model‡</td>
<td>Nutritional density†</td>
<td>Adjusted model‡</td>
</tr>
<tr>
<td>Thiamin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.050</td>
<td>−0.026</td>
<td>−0.094</td>
<td>−0.117</td>
</tr>
<tr>
<td>P</td>
<td>0.497</td>
<td>0.727</td>
<td>0.292</td>
<td>0.191</td>
</tr>
<tr>
<td>n</td>
<td>188</td>
<td>188</td>
<td>138</td>
<td>135</td>
</tr>
<tr>
<td>Riboflavin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>−0.102</td>
<td>−0.061</td>
<td>−0.143</td>
<td>−0.094</td>
</tr>
<tr>
<td>P</td>
<td>0.164</td>
<td>0.414</td>
<td>0.092</td>
<td>0.287</td>
</tr>
<tr>
<td>n</td>
<td>187</td>
<td>187</td>
<td>140</td>
<td>137</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>−0.148</td>
<td>0.097</td>
<td>−0.181</td>
<td>−0.207</td>
</tr>
<tr>
<td>P</td>
<td>0.043</td>
<td>0.194</td>
<td>0.031</td>
<td>0.018</td>
</tr>
<tr>
<td>n</td>
<td>188</td>
<td>188</td>
<td>141</td>
<td>138</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.164</td>
<td>0.138</td>
<td>0.057</td>
<td>0.103</td>
</tr>
<tr>
<td>P</td>
<td>0.027</td>
<td>0.071</td>
<td>0.510</td>
<td>0.249</td>
</tr>
<tr>
<td>n</td>
<td>181</td>
<td>181</td>
<td>137</td>
<td>134</td>
</tr>
</tbody>
</table>

* Period 1, March and April 1995; period 2, June and July 1995; period 3, October 1995; period 4, January and February 1996; periods 2 and 3, rainy season.
† Spearman correlation between nutritional density (expressed as mg or μg vitamin intake/5 MJ) and biological status (standardised values).
‡ Spearman correlation between absolute daily dietary intake and biological status adjusted by age, years of education, BMI, number of cigarettes smoked per day, number of vitamin tablets per week, energy and alcohol intake (standardised values).
The measure of static and functional status indices gives more precise information on the prevalence of deficiency in vitamin B complex status. However, the results largely depend on the method used for their determination, and there is currently no clearly accepted cut-off values for vitamin B complex deficiencies (Van den Berg et al. 1993; Tuck et al. 2000). The cut-off values used are based on the proposals of a European group of experts (Van den Berg et al. 1993) and the values obtained in well-nourished populations using the same methods. Nevertheless, compared with studies conducted in men from developed countries and the laboratories’ reference values, the Cuban population studied was deficient in thiamin, riboflavin, folate and vitamin B₁₂, whereas pyridoxal status was similar (Van den Berg et al. 1993; Costa de Carvalhal et al. 1996; Benton et al. 1997; Brussaard et al. 1997a,b; Ford & Bowman, 1999; Tuck et al. 2000). Compared with values observed in Cuba at the beginning of the neuropathy epidemic in 1992, serum folate and vitamin B₁₂ concentrations were lower (Pérez-Cristià & Fleites-Mestre, 1994), and thiamin status was more deficient (Macias-Matos et al. 1996). Unfortunately, riboflavin and vitamin B₉ biological status was not measured in 1992. In addition, the pyridoxal status of the Cuban men studied remained within the reference range despite a low vitamin B₉ daily dietary intake. These results may reflect the difficulty of measuring accurately the intake by using diet records and the accurate evaluation of recommended dietary intake; they could also be explained by the low protein intake. Indeed, low protein intake has been reported to increase plasma vitamin B₉ concentrations (Hansen et al. 1996).

In contrast, the high prevalence of thiamin, riboflavin, folate and vitamin B₁₂ deficiencies previously observed in developing countries results from inadequate intake, low income, type of food consumed, smoking habits and possibly in vivo photolysis (Brand & Eaton, 1978; Tuck & Hedges, 1993; Bates et al. 1994; Sainte-foie et al. 1997; Bovet et al. 2000). The typical Cuban diet is rich in rice and beans. The high daily dietary carbohydrate intake (approximately 60% daily dietary energy intake) as well as the high consumption of polished rice (approximately 18% total food intake) observed in the present study could contribute to the marginal thiamin status, as previously observed in other tropical countries (Bovet et al. 1998). In addition, the relatively low consumption of food rich in riboflavin and vitamin B₁₂ (i.e. meat and dairy products) in Cuba could partially account for the deficient riboflavin and vitamin B₁₂ status (Bates et al. 1994; Costa de Carvalhal et al. 1996). The dietary folate status could be attributed to the low consumption of green vegetables, liver and yeast compared with previous studies (Guillan et al. 1986; Brants et al. 1997; Alberti-Fidanza et al. 1998; Ford & Bowman, 1999), but also to the method used. Indeed, using the cut-off value proposed by the manufacturer (3.4 nmol/l), the percentage of deficient volunteers varied from 21% in October 1995 to 50% in June–July 1995. In contrast, in the present study alcohol consumption was probably not an important contributing factor to vitamin B complex status. Alcoholism is recognized as having a negative effect on vitamin B complex status (Costa de Carvalhal et al. 1996; Benton et al. 1997; Brussaard et al. 1997a,b; Alberti-Fidanza et al. 1998; Bovet et al. 1998; Ford & Bowman, 1999), but vitamin B complex status is generally not modified in moderate drinkers such as those in the Cuban population studied (Benton et al. 1997; Brussaard et al. 1997b). Indeed, <6% of the volunteers presented with γ-glutamyl transferase activity >75 IU/l (results not shown) and the average alcohol consumption was 9.5 g/d. Tobacco use could also contribute to riboflavin deficiency (Benton et al. 1997), but its effect on thiamin, folate and vitamin B₁₂ deficiencies remains unclear (Macias-Matos et al. 1996; Benton et al. 1997; Brussaard et al. 1997b; Bovet et al. 1998; Ford & Bowman, 1999). Moreover, as reported elsewhere (Barnoun et al. 2000), a more deficient riboflavin status was observed in Cuban smokers than in Cuban non-smokers, whereas thiamin, folate and vitamin B₁₂ status were similar in smokers and non-smokers. The low serum folate and vitamin B₁₂ concentrations suggest the need for the determination of more sensitive and specific markers of vitamin B₁₂ deficiency (i.e. serum methylmalonic acid; Stabler et al. 1996) and folate deficiency (i.e. erythrocyte folate; Van den Berg et al. 1993). Nevertheless, these very low serum folate and vitamin B₁₂ concentrations could contribute to the rather high serum homocysteine concentrations (mean 18.4 (range 8.6–77.9 μmol/l) observed in the La Lisa volunteers in April 2000 (V Ducros, P Fleites-Mestre, R Perez-Christia and J Barnoun, unpublished results), compared with the reference values of the laboratory (Ducros et al. 1999). Finally, the deficient vitamin B complex status in the Cuban population studied suggests that clinical deficiencies were likely to develop if conditions worsened.

To our knowledge, few studies have reported seasonal variations in vitamin B complex intake and status, especially in tropical countries. Moreover, the seasonal variations observed in Cuba were difficult to compare with those of previous studies, which were performed in countries where income, seasonal changes in diet and sunlight exposure (Brand & Eaton, 1978) are completely different. In addition, biochemical determinations must be performed a short while after sampling because of changes during storage. However, precautions were taken to limit between-run differences. Variation in thiamin intake was different from that observed in France (Guillan et al. 1986). In French men thiamin intake has been reported to be lower in spring and summer than in autumn and winter. With regard to EGR-AC variation, our results did not completely match the riboflavin status improvement from July to October, and the riboflavin status decline from December to March reported in Gambian women (Bates et al. 1994). Riboflavin intake remained similar throughout the year in Havana, as previously observed in French men (Guillan et al. 1986), but in contrast with the paradoxical decrease observed in Gambian women during the rainy season (Bates et al. 1994). The similar consumption of dairy products over the year could partly contribute to the similar daily dietary intake of riboflavin throughout the year. As vitamin B₉ is widely distributed in food (Benton et al. 1997), variation in vitamin B₉ intake and status throughout the year was not expected. Moreover, in French men vitamin B₉ intake has been reported to remain similar...
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throughout the year (Guillard et al. 1986). For folate, the daily dietary intake of Havanan men and serum concentrations were the lowest in June and July, which contrasts with the improvement in folate intake and erythrocyte folate concentration in June and July observed in Gambian women (Bates et al. 1994), and with the increase in serum folate concentration during the summer observed in elderly British men (Clarke et al. 1998). However, in the present study, fruit and vegetable consumption and folate intake variation followed those described in previous studies (Costa de Carvalho et al. 1996; Bell et al. 1998; Ford & Bowman, 1999). For vitamin B₁₂, the improvement in serum concentrations observed between June 1995 and October 1995 in the Havanan population studied contrasts with the stability observed throughout the year in elderly British men (Clarke et al. 1998). Further work is needed to better evaluate seasonal variation using a higher frequency of repeated measures for each volunteer.

Thiamin intake and status were not correlated, in agreement with previous studies carried out in developed and developing countries (Costa de Carvalho et al. 1996; Alberti-Fidanza et al. 1998; Bovet et al. 1998). The significance of Spearman correlations between riboflavin, vitamin B₉, and folate intake and status depended on period, intake expression (absolute intake or nutritional density) and adjustment. Significant correlations $P < 0.05$ between riboflavin and vitamin B₉ intake and biological status have been reported by Hereberg et al. (1994), but not by other researchers (Costa de Carvalho et al. 1996; Brussaard et al. 1997a; Alberti-Fidanza et al. 1998). Contrary to previous observations (Costa de Carvalho et al. 1996; Brussaard et al. 1997b), serum folate concentrations were not strongly influenced by recent folate dietary intake. However, the folate and vitamin B₉ nutritional density related more to the corresponding biological status, suggesting that the type of consumed food is an important confounding factor. Moreover, several determined and underdetermined factors could influence the relationship between intake and corresponding biological status. First, the estimation of daily dietary intake using food composition tables does not adequately take into account the modifications of vitamin content during cooking (Costa de Carvalho et al. 1996; Alberti-Fidanza et al. 1998; Bovet et al. 1998) and could contribute to the lack of correlation between thiamin intake and status.

Second, vitamin B complex bioavailability depends on the type of food consumed (Costa de Carvalho et al. 1996; Brants et al. 1997; Brussaard et al. 1997a; Alberti-Fidanza et al. 1998) and could explain the period effect on the significance of correlation. Third, changes in intake are counterbalanced by modifications in metabolism (i.e. absorption, tissue mobilisation and excretion) and could also contribute to the period effect on the significance of the correlation. Finally, vitamin B complex status can be affected by nutritive and non-nutritive variables (Van den Berg et al. 1993), as suggested by modifications in the correlation models. Adjustment for variables known to affect vitamin B complex status, including age, years of education, BMI, smoking habits, vitamin supplement use, energy and alcohol intake, modified the correlation between folate intake and status in periods 1, 3 and 4, and between vitamin B₉ intake and status in periods 1 and 2. These results suggest that the folate and vitamin B₉ diet–blood associations are confounded by one or more of these variables and that they should be adjusted in studies that assess these associations (Tomassone et al. 1993).

In conclusion, our results clearly demonstrate that the apparently healthy Cuban men selected in the present study represent a group potentially vulnerable to developing clinical vitamin B complex deficiencies. Under these conditions, the subject’s health would deteriorate, particularly in the presence of increased requirements relating to illness, intensive training or food shortage resulting from hurricane or plagues. Thus, the development of a policy to promote the production and supply of food rich in vitamin B complex must be encouraged, and multivitamin supplementation programmes must be maintained in the meantime. Further work is nevertheless needed to better investigate the dysfunction generated by folate and vitamin B₁₂ deficiencies and to better identify the relationship between vitamin B complex intake, seasonal food consumption and status.

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