Evaluation of Vitamin B₉ Status of Adult Saudis in Al-Qassim Region-Saudi Arabia

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Abstract: The aim of this study was to evaluate vitamin B₉ status of adult Saudis in Al-Qassim region-Saudi Arabia. A sample of 239 (127 males and 112 females) healthy Saudi adults was recruited. Vitamin B₉ status was evaluated by dietary intake and biochemical measurements. Results of dietary intake measurements showed that men of vitamin B₉ intake and intake of vitamin B₉ to protein ratio for males and females were 1.97±0.58 mg/day, 0.021±0.003 and 1.89±0.61 mg/day, 0.020±0.04, respectively. Results of biochemical measurements showed that means of plasma pyridoxal phosphate (PLP) concentration and average urinary 4-Pyridoxic Acid (4-PA) excretion for males and females were 29.6±9.6 nmol L⁻¹ and 7.0±2.4 μmol day⁻¹ and 31.7±10.4 nmol L⁻¹ and 6.8±2.5 μmol day⁻¹, respectively. The study also investigated and discussed other health indicators that are related to vitamin B₉ status including Body Mass Index (BMI), plasma albumin concentration and alkaline phosphatase activity. Results of dietary intakes and biochemical measurements indicated an adequate status of vitamin B₉. There were no significant differences between males and females in any of the previous measurements. This study indicated an adequate status of vitamin B₉ among healthy adult Saudis in Al-Qassim region, however due to the numerous functions and the importance of the vitamin, the study suggested a further evaluation in various groups of the Saudi population in other regions of the country.

Key words: Vitamin B₉ intake, protein intakes, plasma PLP, red blood cell PLP, urinary 4-PA, plasma albumin, plasma alkaline phosphatase, adult Saudi

INTRODUCTION
The term vitamin B₉ refers to three primary forms of 3-hydroxy-5-hydroxymethyl-2-methylpyridine and their phosphorylated forms. The primary forms are Pyridoxal (PL), Pyridoxine (PN) and Pyridoxamine (PM). Their phosphorylated forms are PLP, PNP and PMP (Vasilaki et al., 2008). An additional form of vitamin B₉ that is found only in plant foods is glycosylated pyridoxine. Bioavailability of vitamin B₉ from animal food is quite high and could reach 100% (Reynolds, 1988); whereas the bioavailability of the vitamin from plant foods is lower compared to animal foods due to the presence of glycosylated pyridoxine as well as dietary fiber (Hansen et al., 1996). The phosphorylated forms of the vitamin must be dephosphorylated in the intestine by alkaline phosphatase prior to absorption. The forms of the vitamin are converted to their phosphorylated forms in the human liver and then to the physiological active form, Pyridoxal-5-Phosphate (PLP), which is released to blood bound to albumin (Leklem, 1999). PLP participates as a coenzyme in more than 100 enzymatic reactions. The majority of these reactions are involved in amino acid metabolism (Vasilaki et al., 2008; Leklem, 2001). Thus, the requirement for vitamin B₉ increases with an increase intake of protein. A ratio of >0.016 mg vitamin B₉/g protein is required for adequate status of the vitamin (Hansen et al., 1997). Several studies have reported the high consumption of protein by Saudis. Al-Shoshan (1992) and Miladi (1998) reported a high consumption of red meat and poultry by Saudis. Al-Assaf and Al-Numair (2007) reported high consumption of protein by urban and rural adult Saudi males in Riyadh region. The study illustrated that although the intakes of protein were within the Acceptable Macronutrient Distribution Ranges (AMDRs) of Dietary Reference Intakes (DRI, 2005), they were high due to the high intakes of calories. Two studies have investigated protein and vitamin B₉ content of fourteen popular meat-based Saudi dishes (Sawaya et al., 1988) and six popular cereals and legume-based Saudi dishes (Al-Jebn et al., 1985). The two studies found that vitamin B₉ content, compared to protein, was relatively low. Since high protein consumption among Saudis has been reported and based on the last two mentioned studies, an inadequate status of vitamin B₉ might be expected among Saudi population. Until present, there is no available study that has investigated the status of vitamin B₉ in any group of the Saudi population. Thus, the aim of this study was to evaluate vitamin B₉ status among adult Saudi males and females in Al-Qassim region by dietary and biochemical measurements. The dietary measurements included dietary intake of vitamin B₉ protein, vitamin B₉ protein ratio and % of vitamin B₉ from animal sources. The biochemical measurements included PLP concentrations in plasma and Red Blood Cells (RBC) and urinary 4-Pyridoxic Acid (4-PA). In addition, the study
evaluated two biochemical factors that affect plasma PLP, which are concentration of plasma albumin and plasma alkaline phosphatase activity.

MATERIALS AND METHODS
Recruitment of the sample from Al-Qassim region was done by personal contact and by advertisement. All volunteers received a questionnaire asked for age, weight, height, health status, exercise habits, use of medications and use of dietary supplements. For female volunteers, the questionnaire also asked for pregnancy, lactation and use of oral contraceptives. Users of dietary supplements or drugs that are known to affect vitamin B₃ metabolism, absorption or urinary excretion (Bhagavan, 1985) were excluded. Only healthy and non-users of medications or dietary supplements (non-pregnant, non-lactating and non-users of oral contraceptives for females) were eligible to participate in the study. The study protocol was explained to each participant individually and informed consent was obtained. All participants received written instructions about keeping three consecutive days of food records and urine collection. For each participant, energy requirement (based on weight, height, age and physical activity level) and energy intake (based on his/her food record intake), were determined by the Food Processor (2001). Participant(s) for whom their difference between energy requirement and energy intake exceeded 15% was (were) excluded since this difference indicated incomplete or invalid food records. The study was carried out in 239 healthy Saudi adults, 127 males and 112 females and was performed between May and October 2008. The three consecutive day food records were analyzed by the Food Processor, version 7.8 (2001) (ESHA Research, Salem, OR, USA) to determine the dietary measurements. On the fourth day weight and height were measured and BMI was calculated for each participant. 15 ml of blood sample was collected from each participant in heparinized tubes (Becton Dickinson, NJ, USA) by trained personal. Blood samples were centrifuged at 1800g at 5°C for 20 min. and plasma samples were separated and stored at -70 (±5°C) until analysis. Red blood cells were washed three times using cold 0.9% saline and centrifugation of 1800g for 20 min at room temperature (Miale, 1977). Red blood cells samples were stored at -70 (±5°C) until analysis. The urine samples were collected in brown opaque three-liter polyethylene bottles containing 15 ml of toluene as a preservative. Total volume of each day was measured and a sample of 10 ml was obtained and stored at -25°C until analysis. Plasma and Red Blood Cell (RBC) PLP were analyzed by HPLC with fluorometric detection as described by Kimura et al. (1996). Urinary 4-PA was analyzed by HPLC method as described by Gregory and Kirk (1979). Plasma albumin concentration was determined by colorimetric method as describe by Slater et al. (1975). Plasma alkaline phosphatase activity was measured by colorimetric method as described by Roy (1970). All samples were analyzed in duplicate and the average was reported if the difference did not exceed 8%.

Data analysis: Data analysis was performed using the Statistical Package for the Social Sciences, version 11.0 (SPSS) computer software. Descriptive statistics were adapted to display data in means ± SD and percentages. The statistical method of t-test was used to compare the mean values obtained between the males and females. Person correlation analysis was used to detect association between vitamin B₃ intake and plasma PLP concentration and urinary 4-PA excretion. Differences and correlations were considered significant whenever the p-value was (p≤0.05).

RESULTS
Characteristics of participants are shown in Table 1. Means of height and weight were significantly lower in the female group compared to the male group. However, there was no significant difference between the two groups in the means of BMI. According to the classification of the National Health, Lung and Blood Institute (1998), the study found that in the male group, 63.8% (n = 81) of participants were overweight or obese and 34.8% (n = 44) were normal. In the female group, 66.1% (n = 74) of participants were overweight or obese and 30.4% (n = 34) were normal. Results of dietary intake are shown in Table 2. The female participants consumed less protein and vitamin B₃ compared to males, but the differences were not significant. The

| Table 1: General characteristics among Adult Saudis in Al-Qassim Region-Saudi Arabia* |
|------------------------------------------|-----------------|-----------------|
| Characteristics                         | Males (n = 127) | Females (n = 112) |
| Age (yr)                                 | 28.7±6.86       | 25.6±7.22       |
| Height (cm)                              | 168.3±8.3       | 156.2±5.6       |
| Weight (kg)                              | 76.6±12.9       | 63.2±6.8       |
| BMI (kg/m²)                              | 27.3±3.3        | 26.7±4.6       |

*Values are expressed as mean ± SD.  

| Table 2: Intake of protein, vitamin B₃, vitamin B₆; protein ratio and percentage of vitamin B₆ from animal sources among Adult Saudis in Al-Qassim Region-Saudi Arabia* |
|------------------------------------------|-----------------|-----------------|
| Nutrient and source                      | Males (n = 127) | Females (n = 112) |
| Protein (g)                              | 102.3±19.7      | 96.6±20.6       |
| Vitamin B₃ (mg)                          | 1.57±0.56       | 1.89±0.61       |
| B₆ protein ratio (mg g⁻¹)                | 0.021±0.003     | 0.020±0.04      |
| % of B₆ from animal sources              | 56.3±9.7        | 38.9±10.4       |

*Values are expressed as mean ± SD.  

*Different letters in a given row denote a significant differences; P-value ≤0.05.
percentage of vitamin B₉ from animal source was significantly lower in female group compared to male group. This indicates that males compared to females, consumed more meats and poultry and less cereals, legumes and vegetables. Major source of vitamin B₉ of both groups were mutton, chicken, banana and watermelon. Mean intake of protein for both groups as well as for all participants from both groups were within the range of the AMDRs for protein (10-35% of energy), which was established in the DRI (2005). The mean intake of vitamin B₆ was adequate for both male and female groups, based on the recommended value of DRI (1998) (1.3 mg/day for adult male and female). None of the participants from either group had an average intake of vitamin B₉ below 1.3 mg/day. The biochemical indices of vitamin B₉ status are shown in Table 3. The means concentration of plasma PLP and means urinary 4-PA excretion of both group indicated an adequate status according to the suggested values for adequate status. These suggested values were ≥20 nmol L⁻¹ (Lui et al., 1985) for plasma PLP and ≥0.3 μmol day⁻¹ for urinary 4-PA excretion (Leklem, 2001). None of the participants of the two groups had plasma PLP concentration or urinary 4-PA excretion below the suggested values of adequacy. Comparison of males and females groups showed no significant difference in any of vitamin B₉ biochemical indices. Vitamin B₉ intake was significantly correlated with both plasma PLP concentration and urinary 4-PA excretion (r = 0.37, p≤0.02) (r = 0.36, p≤0.02), respectively. The mean albumin concentrations for both groups were within normal range (38-55 g L⁻¹ for males and 35-52.5 g L⁻¹ for females) (Gibson, 2005) with no significant difference between the two groups. Similarly, the means of plasma alkaline phosphatase activity for both groups were within the normal range (13-39 UL) (Lee and Nieman, 2003).

**DISCUSSION**

The results of BMI showed prevalence of overweight and obesity among male and female participants. This is in agreement with several previous studies conducted in the Saudi Arabia population (Al-Nuaim et al., 1987; El-Hazmi and Warsy, 1997; Madani et al., 2000; Al-Assaf and Al-Numair, 2007). The aim of this study was not to evaluate obesity, but the importance of the BMI data is to indicate that the majority of participants were in the state of well or over nutrition. Thus, vitamin B₉ deficiency is not expected since signs of its deficiency due to dietary deficiency are rarely seen (Leklem, 2001; Dakshinamurti and Dakshinamurti, 2007). Dietary intake data showed high mean protein intake for both groups although they were within the AMDRs. This was due to the high intake of calories, which is in agreement with the data of the BMI mentioned earlier. Intake of protein and vitamin B₉ were adequate for both groups, which is in agreement with previous data (Al-Assaf and Al-Numair, 2007). The mean vitamin B₉ to protein ratio (Table 2) indicated an adequate vitamin B₉ status for both groups based on the suggested ratio reported by Leklem (1990) (≥0.020). The mean % of vitamin B₉ from animal sources was significantly lower in females group compared to males group, which suggests lower bioavailability of the vitamin for females group compared to males group. Also, females group compared to males group had lower mean intake of vitamin B₉ and lower vitamin B₉; protein ratio (not significant). However, both plasma and RBC PLP concentrations were higher (not significant) in females group compared to males group. There are two explanations for this conflict. First, males compared to females consumed more protein, which may lower plasma PLP concentration. This relation was reported in previous studies (Miller et al., 1985; Hansen et al., 1996; Hansen et al., 1997). The second explanation is the prevalence of cigarette smoking among Saudi males as reported in several previous studies (Jarallah et al., 1999; Siddiquie et al., 2001; Al-Assaf, 2007). Several studies reported the adverse effect of cigarette smoking on plasma PLP concentration, which is in agreement with results of this study (Serfontein and Ubbink, 1988; Giraud et al., 1995; Giraud and Driskell, 1994). However, all of the indices of vitamin B₉ status indicated an adequate status for both groups. The mean plasma PLP concentration was significantly correlated with vitamin B₉ intake. This correlation is in agreement with previous reports (Chang et al., 2007; Kretsch et al., 1991; Huang et al., 1998). Also, the mean urinary 4-PA excretion was significantly correlated with vitamin B₉ intake, which is in agreement with previous data (Shultz and Leklem, 1981). There is no available suggested value of vitamin B₉ adequacy base on RBC PLP concentration. However, RBC PLP concentrations found in this study were lower than the concentrations reported by Reynolds et al.

**Table 3: Concentration of plasma PLP, Red Blood Cell (RBC) PLP and urinary 4-PA among Adult Saudis in Al-Qassim Region- Saudi Arabia**

<table>
<thead>
<tr>
<th>Biochemical Indices</th>
<th>Males (n = 127)</th>
<th>Females (n = 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PLP (nmol L⁻¹)</td>
<td>29.6±5.6²</td>
<td>31.7±10.4²</td>
</tr>
<tr>
<td>RBC PLP (nmol L⁻¹)</td>
<td>26.8±9.8⁴</td>
<td>27.6±8⁴</td>
</tr>
<tr>
<td>Urinary 4-PA (μmol day⁻¹)</td>
<td>7.0±2.4⁴</td>
<td>6.8±2.5⁴</td>
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</table>

*Values are expressed as mean ± SD. **Averages of three consecutive days, ***Different letters in a given row denote a significant differences, P-value ≤0.05.

**Table 4: Concentration of plasma albumin and alkaline phosphatase activity among Adult Saudis in Al-Qassim Region-Saudi Arabia**

<table>
<thead>
<tr>
<th>Biochemical measurements</th>
<th>Males (n = 127)</th>
<th>Females (n = 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma albumin (g L⁻¹)</td>
<td>48.7±1.9⁴</td>
<td>49.1±2.0⁴</td>
</tr>
<tr>
<td>Plasma alkaline phosphatase (UL)</td>
<td>26.3±4.7⁴</td>
<td>24.2±3.8⁴</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD. ***Different letters in a given row denote a significant differences, P-value ≤0.05.
(1988). The last mentioned study reported that a high intake of vitamin B₉ (2.11±0.12 mg/day) resulted in RBC PLP concentration of 85±6 nmol L⁻¹. Comparison of the results of this study with other studies conducted in Saudi population is not possible since this is the first study investigated vitamin B₉ status among Saudi people. The normal mean of plasma alkaline phosphatase activity and plasma albumin concentration found in this study of both groups suggested no adverse affect on plasma PLP, thus no adverse affect on vitamin B₉ status as indicated by plasma PLP concentration. Results of the last two mentioned biochemical measurements are in agreement with normal ranges of adult Saudis reported by El-Hazmi et al. (1982) and Scott (1982).

Conclusion: In conclusion, results of this study showed that adult Saudi living in Al-Qassim region-Saudi Arabia had adequate status of vitamin B₉. This adequacy was evaluated by dietary measurements including vitamin B₉ intake, protein intake and vitamin B₉ to protein ratio. The adequacy was also measured by biochemical indices including plasma PLP concentration and urinary 4-PA excretion. This study suggests that, due to the numerous functions of vitamin B₉, further evaluations of the vitamin status are needed in various groups of Saudi population in other regions of the country.

REFERENCES


