Improved Vitamin B-6 Status Is Positively Related to Lymphocyte Proliferation in Young Women Consuming a Controlled Diet\(^1,2\)

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ABSTRACT To examine the effect of increased intake levels of vitamin B-6 (B-6) on lymphocyte proliferation and interleukin 2 (IL-2) concentration, young women (\(n = 7\)) consumed a constant diet containing 1 mg (5.91 \(\mu\)mol) B-6/d for a 7-d adjustment period, followed by three 14-d experimental periods during which the daily B-6 intake was 1.5, 2.1 and 2.7 mg (8.86, 12.41 and 15.95 \(\mu\)mol)/d, respectively. Weekly fasting blood and daily 24-h urine samples were collected. Lymphocyte proliferation and IL-2 production were measured in response to phytohemagglutinin. Vitamin B-6 status improved with increased B-6 intake as measured by plasma pyridoxal 5'-phosphate (PLP) and urinary 4-pyridoxic acid. When subjects consumed 2.1 mg B-6/d for 7 d, lymphocyte proliferation increased by 35% (\(P < 0.05\)) compared with the mean value after consumption of 1.5 mg B-6/d for 14 d. There was no further enhancement after an additional week of 2.1 and 2.7 mg B-6/d for 2 wk. Lymphocyte proliferation was correlated (\(P = 0.01\)) with vitamin B-6 intake (\(r = 0.757\)), plasma PLP (\(r = 0.456\)) and erythrocyte aminotransferase activities (\(r = -0.361\)). Plasma IL-2 concentration and in vitro production did not change throughout the study, although five of seven subjects showed increases with intakes of 2.1 and 2.7 mg B-6/d, respectively, compared with the 1.5 mg/d intake. Concentrations of PLP in peripheral blood mononuclear cells were correlated (\(r = 0.357, P = 0.01\)) with plasma PLP, but not with proliferation. These results show that improving vitamin B-6 status by consuming a B-6 intake higher than the current Recommended Dietary Allowance enhances lymphocyte proliferation.  J. Nutr. 132: 3308–3313, 2002.

KEY WORDS: vitamin B-6 status • young women • peripheral blood mononuclear cells • lymphocyte proliferation • interleukin 2

Pyridoxal 5'-phosphate (PLP)\(^4\) acts as a coenzyme in a number of diverse enzyme reactions (1). Among these, PLP-requiring serine hydroxymethyltransferase plays a critical role in one-carbon metabolism. Vitamin B-6 (B-6) deficiency has been shown to adversely affect nucleic acid synthesis (2,3). Subsequent adverse effects of lowered nucleic acid synthesis on cell multiplication and protein biosynthesis are followed by depletion of protein, RNA and DNA in tissues involved in immune response (4,5). When pyridoxine-deficient rats are compared with pair-fed controls, multiplication of mononuclear cells in immune organs, spleen and thymus is depressed (6).

The effect of B-6 deficiency and supplementation on immune function in humans has been examined in relatively few studies. However, several studies show that B-6 status has a clear relationship with immune response among different groups of people, i.e., uremic patients (7,8), the elderly (9,10) and young (11–13). Lymphocyte mitogenic responsiveness was reduced by dietary B-6 depletion in elderly subjects and was restored by repletion (10) with B-6 intakes above the current Recommended Dietary Allowance (RDA) (14). Daily multivitamin supplementation containing 3 mg B-6 increased the delayed hypersensitivity skin test response (15), and a daily 50 mg B-6 supplement for 8 wk enhanced lymphocyte response to both T- and B-cell mitogens (9) in the elderly. In controlled human studies, low B-6 intakes resulted in decreased lymphocyte mitogenic responsiveness and interleukin 2 (IL-2) production (10), as well as a decreased percentage of helper T cells and serum immunoglobulin (Ig)D concentration (12). However, there are no data that directly quantify a potential dose-response relation between B-6 intake and ex vivo immune response in young adults.

On the basis of data collected in the Third National Health and Nutrition Examination Survey, the mean daily B-6 intake of young women (ages 20–49 y) in the United States is between 1.43 and 1.5 mg (16). This range of intake suggests an adequate B-6 intake in young women compared with the current RDA for adult women [1.3 mg/d (14)]. However, the
RDA of B-6 for adult women was reduced from 1.6 to 1.3 mg/d in the 1998 report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (14) on the basis of a limited number of studies. Evaluating dietary intakes of B-6 that maximize health benefits is important to aid in establishing an adequate recommended intake of B-6 for young women.

This study was part of a larger study that evaluated recommended B-6 intake on the basis of assessment of B-6 status (17). In the current study, the relationships among varying levels of B-6 intake, B-6 status indicators and immune response were investigated. The objectives of this study were to determine whether immune response is maximized with a specific B-6 intake, which may be used to evaluate the B-6 requirement for young women, and whether PLP concentration in peripheral blood mononuclear cells (PBMC) reflects plasma PLP concentration and dietary B-6 intake.

SUBJECTS AND METHODS

Subjects. Healthy premenopausal women (n = 8), between the ages of 21 and 37 y, were recruited from the Washington State University community. The study group was comprised of 5 Caucasians and 3 Asians. None were taking vitamin supplements for at least 6 mo before beginning the study, nor any medications that are known to affect B-6 metabolism and/or immune function. Before the study, subjects completed a health history questionnaire, gave a fasting blood sample for chemical evaluation and were administered a xylose absorption test as a measure of intestinal absorption (18). Subjects were asked to keep a 3-d diet record to evaluate their B-6 and nutrient intake before the study began. These diet records were analyzed using Nutritionist IV Diet Analysis (First DataBank, San Bruno, CA). Subjects were instructed to maintain their activity level throughout the study. The study was explained to the subjects and informed consent was obtained. All study procedures were approved by the Washington State University Committee for the Protection of Human Subjects.

Experimental design. The subjects resided in the Human Metabolic Unit of the Department of Food Science and Human Nutrition at Washington State University under supervision of the investigators throughout the 49-d study period. Subjects were asked to record their daily weight and days of their menstrual cycles. The basal diet followed a 14-d menu cycle and provided 1250 kJ [1544 kcal] (5.91 mol) B-6 and 56 g protein daily (17). All meals were prepared in the metabolic kitchen and served in the unit. Reconstituted and cooked egg white powder was added to the basal diet in sufficient amounts for each subject to achieve a total protein intake of 1.2 g/kg bodyweight. Additional energy was supplied to the subjects by hard candies, soft drinks, margarine and cookies (which contributed 22.3% of the total caloric intake). Additional energy was supplied to the subjects by hard candies, soft drinks, margarine, and cookies (which contributed 22.3% of the total caloric intake). Additional energy was supplied to the subjects by hard candies, soft drinks, margarine, and cookies (which contributed 22.3% of the total caloric intake). Additional energy was supplied to the subjects by hard candies, soft drinks, margarine, and cookies (which contributed 22.3% of the total caloric intake).

The experimental design was divided into 4 metabolic periods. The diet for the first 7 d provided 1 mg of B-6/d, and that for the three subsequent 14-d periods provided 1.5 mg (8.86 mol), 2.1 mg (12.41 mol) and 2.7 mg (15.95 mol) of B-6/d, respectively. Additional amounts of B-6 in the last three periods were provided by oral administration of a pyridoxine hydrochloride solution at breakfast.

Sample collection and analysis. Composites of the daily diets were made every other week during the study and analyzed for B-6 by microbiological assay (20). Daily 24-h urine samples were collected under toluene, refrigerated until measured, divided into aliquots and then stored at −20°C until analysis. Urinary creatinine was assessed by an automated procedure (21). Urinary glucose, bilirubin, ketone, blood, pH, protein (Bili-Labstix, Bayer Diagnostics, Elkhart, IN) and pregnancy tests (QuPID, Stanbio Laboratory, San Antonio, TX) were performed weekly.

Blood samples were collected from fasting subjects on d 1, 8, 15, 22, 29, 36, 43 and 50 in heparinized tubes (Becton-Dickinson, Rutherford, NJ). Hemoglobin, hematocrit, white blood cell and lymphocyte numbers were measured in whole blood with a Coulter counter (Coulter T-660, Coulter Electronic, Marietta, GA). Aliquots of each blood sample were centrifuged at 1800 × g at 4°C for 15 min and the plasma stored at −30°C until analyzed. RBC were washed with physiologic saline, and erythrocyte alanine and aspartate aminotransferase (EALT and EAST, respectively) were measured weekly.

Whole blood (15 mL) was layered onto an equal amount of Histopaque-1077 (Sigma Diagnostics) in sterilized 50-mL conical tubes, and centrifuged at 400 × g at room temperature for 30 min. The mononuclear cell layer was removed and washed 3 times with 10 mL Dulbecco’s PBS (Sigma Chemical). The resulting PBMC pellets were dispersed in 2.4 mL PBS. To determine the concentration of cells, an aliquot of cells was treated with Turk’s solution and the number of cells/mL counted using a hemocytometer. Part of the cell suspension was stored at −80°C for PLP analysis. Before analysis, the cell suspension was sonicated for 30 s while immersed in an ice bath and PLP was determined by a tyrosine decarboxylase aepoenzyme/isotopic procedure (25). The interassay CV for PLP of the control sample (n = 6) was 17.5%. All samples from one subject were analyzed in the same assay to minimize the effect of interassay variation. Recovery of PLP added to the control sample averaged 86 ± 12%.

Immunocompetence was assessed by measuring lymphocyte proliferation in response to mitogen stimulation. Immediately after cell counting, the concentration of PBMC was adjusted to 1 × 10^6 cells/L with complete RPMI 10, which was prepared with RPMI 1640 (with l-glutamine and sodium bicarbonate; Sigma Chemical) containing 10% fetal bovine serum (Hyclone, Logan, UT), 50 μmol/L 2-mercaptoethanol, 100,000 U/L penicillin, and 100 μg/mL streptomycin. Before the study, dilutions of phytohemagglutinin (PHA; Sigma Chemical) between 3.125 and 100 μg/mL were prepared in complete RPMI 10 and tested using PBMC from six healthy individuals to determine maximal stimulatory concentration. Concentrations of PHA between 6.25 and 25 μg/mL resulted in maximal lymphocyte proliferation. Thus, three concentrations of PHA (6.25, 12.5 and 25 μg/mL) were tested. For each experimental condition, triplicate wells were prepared. No mitogen was added to control wells to measure background response. Cell suspension (100 μL) and an equal amount of the respective PHA solutions were dispensed into each well of a flat-bottomed 96-well plate (1 × 10^6 cells/well). Cells were incubated 72 h at 37°C with 5% CO₂. 18 h before terminating the culture, 20 μL of 50 μg/L [H] thymidine ([1.0 μCi]; International Chemical Nuclear (ICN), Costa Mesa, CA) was added to each well. Cells were harvested using a semiautomated multwell harvester, which aspirates and lyses cells and transfers DNA onto filter paper. After the filter paper was dried, filter dots containing the total DNA for each well were transferred into liquid scintillation vials and scintillation fluid added. Samples were counted in a Taurus Automatic Liquid Scintillation Counter (ICN Micromedic System, Huntsville, AL).

To determine the PHA concentration resulting in the greatest PBMC IL-2 production, PBMC were collected from five healthy individuals and cultured with PHA titers ranging between 6.25 and 100 μg/mL. Two of five individuals showed a similar PBMC IL-2 production at all concentrations tested.
individuals showed a higher IL-2 production when their cells were incubated with the 100 mg/L concentration. Therefore, the optimal concentration of PHA used to determine PBMC IL-2 production was considered to be 100 mg/L. Cells (1 × 10^6 cells/L) in complete RPMI 10 were incubated with an equal volume of 100 mg/L PHA in 24-well flat-bottomed plates for 48 h. The cell-free supernatant was stored at -70°C until analysis of IL-2 concentration. In vitro PBMC IL-2 concentration after PHA stimulation and plasma IL-2 concentration were determined by enzyme immunoassay kits, Cytokine Direct Human Interleukin 2 and Cytokine Total Human Interleukin 2 (InterGen, Purchase, NY), respectively.

**Statistical analyses.** Statistical analyses were conducted using JMP (SAS Institute, Cary, NC) and SPSS 7.5 (SPSS, Chicago, IL) computer programs. At each time point, means and SD were calculated for immune parameters and status indicators. Urinary 4-PA, repeated-measures ANOVA was used to detect significant differences among baseline, the adjustment period and the end of each subsequent period. For other measures, repeated-measures ANOVA was used to account for day-to-day variation. For urinary 4-PA, repeated-excretion was averaged over the last 3 days of each experimental period to account for day-to-day variation. For urinary 4-PA, repeated-excretion was averaged over the last 3 days of each experimental period.

The mean age and height of seven subjects was 28.5 ± 4.6 y and 161 ± 5.2 cm, respectively. Body weights did not change from baseline to the end of the study (59.1 ± 6.1 and 58.1 ± 6.8 kg, respectively). There were no differences in urinary excretion of creatinine, blood hemoglobin concentration and hematocrit among experimental periods (data not shown). Because the hemoglobin concentration was <120 g/L for two subjects, an additional iron supplement (27 mg/d elemental iron, Fergon; Bayer Corporation, Morristown, NJ) was provided to them daily after 1 and 5 wk, respectively.

The range of B-6 intake among subjects before the study was 0.9–2.1 mg/d [mean ± SD: 1.4 ± 0.6 mg (8.27 ± 3.55 μmol/d); 0.020 ± 0.007 mg B-6/g (μ) protein]. Analyzed by microbiological assay, food composites from the three different daily menus of the basal diet contained 0.97 ± 0.04, 1.02 ± 0.04 and 0.97 ± 0.03 mg (5.73 ± 0.24, 6.02 ± 0.24 and 5.73 ± 0.18 μmol) B-6, respectively.

All subjects except one, whose plasma PLP concentration was 27.6 mmol/L and EALT stimulation was 42%, had baseline values indicating adequate B-6 status for plasma PLP, urinary 4-PA, and EALT/EAST activity coefficients (26) at the beginning of the study. At the end of the adjustment period (1 mg B-6/d), plasma PLP concentration and urinary 4-PA excretion decreased 36 and 38%, respectively, compared with baseline (Table 1). Plasma PLP concentration of three subjects and urinary 4-PA excretion of the same three plus one more subject were 24 and 19% lower, respectively, than the suggested values for adequate B-6 status (26) after 7 d of consuming 1 mg B-6/d. However, at this time point, erythrocyte aminotransferase activities of all subjects indicated adequate B-6 status.

As expected, when the B-6 intake increased, urinary 4-PA excretion and plasma PLP concentration increased sequentially throughout the last three experimental periods (Table 1). When comparing PLP concentrations between the 1 mg/d B-6 intake period and the end of subsequent experimental periods, there was a significant increase in the last period with 2.7 mg B-6/d. However, PLP concentration at the end of wk 2 with intakes of 1.5 and 2.1 mg/d did not differ from the value at the end of wk 1 with 1 mg B-6/d. Final mean plasma PLP concentration after 2 wk of 2.7 mg/d was ~60 and 30% higher than that at the end of the periods with intakes of 1.5 mg and 2.1 mg, respectively.

Plasma PLP concentrations were analyzed with B-6 intake (r = 0.558, P = 0.01; Table 2). Activity coefficients of EALT did not change throughout the study (Table 1); however, EAST activity coefficients were significantly lower than baseline at the end of Period 2 and after 7 d of Period 4. Erythro-

**RESULTS**

At d 29, the concentrations of plasma PLP and urinary 4-PA of one subject were 27- and 7-fold higher, respectively, than the mean concentrations of the other 7 subjects. This subject was considered to have not adhered to the study protocol sometime between d 21 and 29. Therefore, the values for this subject were not included in the results.

The mean age and height of seven subjects was 28 ± 5.6 y and 161 ± 3.7 cm, respectively. Body weights did not change from baseline to the end of the study (59.1 ± 6.1 and 58.1 ± 6.8 kg, respectively). There were no differences in urinary excretion of creatinine, blood hemoglobin concentration and hematocrit among experimental periods (data not shown). Because the hemoglobin concentration was <120 g/L for two subjects, an additional iron supplement (27 mg/d elemental iron, Fergon; Bayer Corporation, Morristown, NJ) was provided to them daily after 1 and 5 wk, respectively.

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**TABLE 1**

Vitamin B-6 status measures of women at baseline and after consuming four levels of vitamin B-6 for 7- or 14-d periods

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Baseline</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 7</td>
<td>d 7</td>
<td>d 14</td>
<td>d 7</td>
<td>d 14</td>
</tr>
<tr>
<td>Vitamin B-6 intake, mg/d</td>
<td>1.4 ± 0.6</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Urinary 4-PA, μmol/d</td>
<td>4.62 ± 1.39c</td>
<td>2.87 ± 0.93d</td>
<td>4.23 ± 0.61c</td>
<td>6.77 ± 0.80b</td>
<td>9.16 ± 1.21a</td>
</tr>
<tr>
<td>Plasma PLP, nmol/L</td>
<td>46.6 ± 13.94c</td>
<td>29.7 ± 7.1c</td>
<td>35.0 ± 6.7b,c</td>
<td>35.2 ± 6.0b,c</td>
<td>40.2 ± 8.0a,c</td>
</tr>
<tr>
<td>EALT activity coefficient²</td>
<td>1.20 ± 0.12</td>
<td>1.17 ± 0.06</td>
<td>1.17 ± 0.04</td>
<td>1.13 ± 0.04</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>EAST activity coefficient²</td>
<td>1.74 ± 0.25a</td>
<td>1.55 ± 0.08ab</td>
<td>1.66 ± 0.14b</td>
<td>1.58 ± 0.11b</td>
<td>1.61 ± 0.08ab</td>
</tr>
<tr>
<td>PBMC PLP, pmol/10⁶ cells</td>
<td>0.81 ± 0.17</td>
<td>0.78 ± 0.09</td>
<td>0.77 ± 0.11</td>
<td>0.80 ± 0.13</td>
<td>0.83 ± 0.09</td>
</tr>
</tbody>
</table>

1 Values are means ± sd, n = 7. Different letters in a given row denote significant differences, P ≤ 0.05. Abbreviations: 4-PA, 4-pyridoxic acid; EALT, erythrocyte alanine aminotransferase; EAST, erythrocyte aspartate aminotransferase; PBMC, peripheral blood mononuclear cells; PLP, pyridoxal 5'-phosphate.

² Ratio of stimulated:unstimulated activity.
cytochrome P450 activity coefficients were inversely correlated (r = -0.391, P ≤ 0.01) with plasma PLP concentration when values at the eight time points were included (Table 2). Both EALT (r = -0.366, P ≤ 0.01) and EAST (r = -0.272, P ≤ 0.05) activity coefficients were inversely correlated with B-6 intake.

The mean concentration of PBMC PLP did not change significantly during the study (Table 1), although it was higher by the end of the study in six of seven subjects. Concentration of PBMC PLP increased by 23% after 2 wk of 2.7 mg B-6/d compared with that at the end of the 2.1 mg/d intake period. On the basis of all values throughout the study, PBMC PLP concentration was correlated (r = 0.357, P ≤ 0.01) with plasma PLP concentration (Table 2).

The overall change in lymphocyte mitogenic response for the 6.25 mg/L concentration of PHA at each time point is presented in Figure 1. No change was observed in response to PHA with 1.5 mg B-6/d, compared with the response at the end of Period 1 (1 mg B-6/d). However, with 2.1 mg B-6/d, there was a significant increase in response to PHA within 7 d compared with the response after 1.5 mg/d for 2 wk. After an additional week of 2.1 mg B-6/d, no further increase was observed in response to PHA. In addition, there was no difference in response between the end of the 2.1 and 2.7 mg B-6/d intake periods. Compared with the 1 mg B-6/d intake, mitogenic responses to 6.25 mg/L PHA were increased by 47% (P ≤ 0.05) and 50% (P ≤ 0.05) at the end of the experimental periods with 2.1 and 2.7 mg B-6/d, respectively. The magnitude of the stimulation with 6.25, 12.5 and 25 mg/L PHA was similar. Mitogenic responses to each of the three concentrations of PHA were correlated with B-6 intake (r = 0.757, P ≤ 0.01), plasma PLP concentration (r = 0.456, P ≤ 0.01) and erythrocyte aminotransferase activity coefficients (r = -0.342, P ≤ 0.05) (Table 2).

Neither plasma IL-2 nor in vitro PBMC IL-2 concentrations changed significantly during the study. However, excluding two subjects, one whose plasma IL-2 concentrations were twice the mean and one whose concentrations were undetectable (except at the end of the 1-mg intake period), plasma IL-2 concentrations of five subjects were correlated (r = 0.344, P ≤ 0.05) with B-6 intake. Compared with the 1.5 mg B-6/d period, plasma IL-2 concentrations in five of seven subjects increased when they consumed 2.1 and 2.7 mg/d. In vitro PBMC IL-2 production after PHA stimulation was correlated with the number of circulating lymphocytes (r = 0.287, P ≤ 0.05) and the percentage of lymphocytes (r = 0.388, P ≤ 0.01). The number of white blood cells and lymphocytes, and the percentage of lymphocytes in the circulation did not change significantly throughout the study.

**DISCUSSION**

This study suggests that a higher level of B-6 intake than the current RDA of 1.3 mg/d for young women (14) is necessary to obtain the maximum ex vivo lymphocyte mitogenic response. B-6 intake at a level 0.8 mg higher than the RDA for 1 wk improved ex vivo peripheral blood lymphocyte response to the T-cell mitogen, PHA. This improvement was maintained with an additional 0.6 mg of B-6 for 2 wk. However, this improvement was not achieved with an intake close to the currently recommended level of B-6 (14). Concentrations of PBMC PLP tended to increase after 2 wk of a B-6 intake that was double the current RDA. The mitogenic response of lymphocytes at baseline was not expected to be as high as the

![Figure 1](https://example.com/figure1.png)
response observed in the 2.1 mg B-6/d period because the estimated mean B-6 intake before the study was 1.4 ± 0.6 mg/d, although plasma PLP concentrations at baseline and the end of the 2.1 mg B-6/d period were similar. In fact, the mean response to PHA at baseline was significantly lower than that with 2.1 mg/d, and remained so with 1 and 1.5 mg B-6/d.

Lymphocyte proliferation has been used in human studies to demonstrate the effect of B-6 intake on immune function, including a change in lymphocyte mitogenesis in young women (13) and elderly men and women (9,10). The findings in young women (13) are in agreement with an increased lymphocyte response to PHA with 2.1 and 2.7 mg B-6/d for ≥7 d in the present study. In concert with the increase in lymphocyte response, there was a 20% increase in plasma PLP concentration in the study by Wang (13), and a mean increase of 24% in the current study. Conversely, a decreased plasma PLP concentration was found to correlate with decreased lymphocyte proliferation in elderly subjects (10). Meydani et al. (10) showed that impaired lymphocyte response to T- and B-cell mitogens after B-6 depletion in the elderly returned to normal with 2.1 mg/d, and remained so with 1 and 1.5 mg B-6/d.

In addition to the beneficial effect of certain levels of B-6 intake on improvement of immune parameters, there are a number of studies that have determined the association of B-6 deficiency with impaired immune response (7,8,10–12,27). A marginal B-6 deficiency induced by 11 wk of B-6 intake at 30 (11) and 50% (12) of the current RDA was accompanied by a lower percentage of T-helper cells and decreased concentration of IgD.

To our knowledge, the current study is the first to determine levels of B-6 that maximize immune parameters of young women in a well-controlled metabolic setting. The findings from the present study, along with previous findings, support a positive relationship between immune response and B-6 status. In addition, the current study suggests that intakes between 1.5 and 2.1 mg B-6/d, levels of B-6 that can be achieved by diet alone, may result in maximizing immune response in young women.

B-6 deficiency in both animals (28,29) and humans (10,27) has been shown to be associated with impairment of immune response. The basic derangement appears to be caused by the decreased rate of production of one-carbon units necessary for synthesis of nucleic acids (3). Interleukin 2 production is also adversely affected by in vitro B-6 deficiency induced by 4-deoxypyridoxine (30) due to lowered activity of PLP-dependent serine hydroxymethyltransferase. In controlled human studies, investigators found significant changes in PBMC IL-2 production with B-6 depletion (10) or supplementation (13). Interleukin 2 production was significantly lower when plasma PLP concentration was <30 nmol/L, compared with baseline when plasma PLP concentration was >40 nmol/L (10). In addition, a significant increase in IL-2 production was shown when the plasma PLP concentration was increased from 35 to 54 nmol/L (13). A significant change in PBMC IL-2 production after PHA stimulation was not found in the present study.

Plasma PLP is the most commonly used index for determining B-6 status (26), but there remains a lack of consensus among investigators as to which biochemical measurement is the best indicator of B-6 status. At least two measures are presently recommended to properly assess B-6 status (26,31). Functional indices have been used, but are currently limited to neurologic and immunologic measurements (32). With respect to the measurements of immunologic function related to B-6 status, the current study and earlier studies (10,13) have shown a consistent relationship between biochemical indices of B-6 status and immunologic measurements. Because the immunologic measurements used in B-6 metabolic studies discussed earlier are involved mainly with lymphocyte function, measuring PLP concentration in lymphocytes may contribute to understanding the relationship between lymphocyte function and B-6 status. In addition, because PLP functions as an intracellular coenzyme, determination of the intracellular concentration of PLP may be more pertinent than the extracellular concentration. However, the extent to which PLP concentration in isolated lymphocytes reflects the plasma concentration, and the degree to which it is correlated with cellular function as reflected by immune response, has not been clearly elucidated.

Other investigators have measured PLP concentrations in leukocytes (33–35), mononuclear cells (13,36) and lymphocytes (35). In general, PLP concentrations in leukocytes have been reported to be 20–40% higher (33,34) than the PBMC PLP concentration measured in the current study when the respective plasma PLP concentration is comparable. This difference may be explained in part by the large proportion of granulocytes in the leukocyte fraction (33) and the higher protein content in the mixed leukocytes than in lymphocytes (~70% higher) (35). Concentrations of PBMC PLP reported by Shephard et al. (36) agreed in part with values obtained in the current study. As reported by Wang (13), baseline PBMC PLP concentration was only one third that at baseline in the present study; after supplementation with 1.5 mg B-6/d, PBMC PLP concentration tended to increase to values somewhat lower than those observed in our study. Supplementation with 50 mg B-6/d for 3 d significantly increased PBMC PLP concentration compared with baseline. This suggests that the change in PBMC PLP concentration in response to diet is slower than that seen in plasma.

Although PBMC PLP concentration responded slowly to dietary B-6, plasma and PBMC PLP concentrations were correlated (r = 0.357, P ≤ 0.01; Table 2) in the current study. Similarly, this relationship was also demonstrated by a parallel increase in PLP concentration in plasma and PBMC in the study by Wang (13). However, no correlation was observed between PBMC PLP concentration and lymphocyte proliferation in the present study. This could be due to small sample size or length of the experimental periods. However, Wang (13) observed a significant correlation between these two measures in 10 free-living women consuming a self-selected diet plus a B-6 supplement. Although B-6 intake varied from 1 mg to 2.7 mg/d in the current study, PBMC tended to maintain their PLP concentration at baseline levels. In an in vitro study by Trakatellis et al. (30), B-6 deficiency induced by 4-deoxypyridoxine was shown to decrease lymphocyte proliferation significantly. Therefore, when there is sufficient PLP present in PBMC, PLP concentration in PBMC may not be the primary factor controlling lymphocyte proliferation.

Van den Berg et al. (12) showed that a marginal B-6 deficiency resulting from an intake of ~50% of the current RDA did not elicit a significant change in the number and percentage of total lymphocytes in young men. However, the percentage of T-helper cells (IL-2 producing cells) was significantly lowered by this marginal B-6 deficiency (12). Furthermore, a positive influence of pyridoxine supplementation on the percentage of T3+ and T4+ cells was demonstrated in a
study by Talbott et al. (9). Due to an alteration in the helper T-cell population by changes in B-6 intake, there may be an increased lymphocyte response to PHA and a tendency for an increase in plasma IL-2 concentration as seen in the present study. However, the range of B-6 intake examined in the current study was both lower and higher than those used in the studies by Talbott et al. (9) and van den Berg et al. (12), respectively. Therefore, further studies are warranted to determine the effect of dietary B-6 intake on T-cell subpopulations and maturation as well as T-cell function, thereby providing further insight regarding the B-6 intake that optimizes immune function.

In conclusion, improving B-6 status in young women by consuming a B-6 intake > 1.5 mg/d may have a positive effect on immune response as reflected by a significant enhancement of lymphocyte proliferation. PLP is required to maintain normal lymphocyte function related to serine hydroxymethyltransferase activity (2). However, a change in proliferative activity does not appear to relate directly to PBMC PLP concentration at the levels of B-6 intake tested in the current study. The most important observation in the current study is that there is a B-6 intake, or a range of intakes, that may provide an optimal immune response in humans, as indicated by lymphocyte proliferation. Although lymphocyte proliferation was the measurement found to be enhanced in this study, it is noteworthy that this immune index improved as the B-6 status increased during an intake higher than the current RDA. There is a need for further investigation of other measures of immune function related to B-6 status to aid in determining the level of B-6 intake required to attain the most favorable immune response in humans. Additionally, the relationship between plasma and PBMC PLP concentrations warrants further investigation with either a larger sample size and/or a longer period of study. Examining both in vivo and in vitro immune response in the same study, under conditions of graded intakes of B-6, would provide insights into the use of immune response as an indicator of B-6 requirement/status.

**LITERATURE CITED**