Marginal Vitamin B-6 Deficiency Decreases Plasma (n-3) and (n-6) PUFA Concentrations in Healthy Men and Women^{1–3}

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Abstract

Previous animal studies showed that severe vitamin B-6 deficiency altered fatty acid profiles of tissue lipids, often with an increase of linoleic acid and a decrease of arachidonic acid. However, little is known about the extent to which vitamin B-6 deficiency affects human fatty acid profiles. The aim of this study was to determine the effects of marginal vitamin B-6 deficiency on fatty acid profiles in plasma, erythrocytes, and peripheral blood mononuclear cells (PBMC) of healthy adults fed a 28-d, low-vitamin B-6 diet. Healthy participants ($n = 23$) received a 2-d, controlled, vitamin B-6–adequate diet followed by a 28-d, vitamin B-6–restricted diet to induce a marginal deficiency. Plasma HDL and LDL cholesterol concentrations, FFA concentrations, and erythrocyte and PBMC membrane fatty acid compositions did not significantly change from baseline after the 28-d restriction. Plasma total arachidonic acid, EPA, and DHA concentrations decreased from (mean $\pm$ SD) 548 ± 96 to 490 ± 94 $\mu\text{mol/L}$, 37 ± 13 to 32 ± 13 $\mu\text{mol/L}$, and 121 ± 28 to 109 ± 28 $\mu\text{mol/L}$ [positive false discovery rate (pFDR) adjusted $P < 0.05$], respectively. The total (n-6):(n-3) PUFA ratio in plasma exhibited a minor increase from 15.4 ± 2.8 to 16.6 ± 3.1 (pFDR adjusted $P < 0.05$). These data indicate that short-term vitamin B-6 restriction decreases plasma (n-3) and (n-6) PUFA concentrations and tends to increase the plasma (n-6):(n-3) PUFA ratio. Such changes in blood lipids may be associated with the elevated risk of cardiovascular disease in vitamin B-6 insufficiency. J. Nutr. 142: 1791–1797, 2012.

Introduction

Vitamin B-6 functions as a coenzyme in the form of pyridoxal 5'-phosphate (PLP)¹² in amino acid metabolism, heme biosynthesis, nucleic acid biosynthesis, and gluconeogenesis. Reports of a connection between vitamin B-6 status and lipid metabolism have appeared periodically for more than 80 y, but there is no known coenzyme role of PLP in fatty acid metabolism. Essential fatty acids have an ameliorating effect on the dermatitis induced by vitamin B-6 deficiency in rats (1). Several rat studies showed

that vitamin B-6 deficiency altered the (n-6) fatty acid profiles of tissue lipids, with a decrease of arachidonic acid [20:4(n-6)] and an increase of linoleic acid [18:2(n-6)] (2–5), whereas others reported altered hepatic and renal (n-3) fatty acid profiles of liver and kidney phospholipids in vitamin B-6–deficient rats (6–8). An understanding of the relationships among vitamin B-6 status, blood fatty acid profiles, and human fatty acid metabolism is limited. One study showed that plasma PUFA profiles in vitamin B-6 deficiency did not differ from those in normal vitamin B-6 status. However, the ratios of 20:5(n-3):18:3(n-3) and 22:6(n-3):18:3(n-3) were significantly lower in the vitamin B-6–deficient group (9). Another study showed that 2-y supplementation of folic acid (1000 $\mu\text{g/d}$), vitamin B-6 (10 mg/d), and vitamin B-12 (500 $\mu\text{g/d}$) did not change the fatty acid profiles of plasma phosphatidylcholine among 123 men and women ≥ 65 y of age despite significantly lower plasma homocysteine than the placebo group (10).

PUFA frequently are associated with an immunomodulatory effect in humans. The (n-6) PUFA class can promote inflammation that is mediated by the release of arachidonate-derived proinflammatory metabolites. In contrast, (n-3) PUFA are associated with antiinflammatory effects. Abnormal plasma fatty

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³ Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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¹² Abbreviations used: MANOVA, multivariate ANOVA; PBMC, peripheral blood mononuclear cell; pFDR, positive false discovery rate; PLP, pyridoxal 5'-phosphate.

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acid profiles are associated with many pathological conditions such as autoimmune disease, cardiovascular disease, diabetes, depression, etc. (11–14), with frequent elevation in the (n-6):(n-3) ratio of plasma PUFA. Elevated plasma FFA concentrations induce insulin resistance and the release of proinflammatory mediators *in vivo* (15,16). Long-chain (n-3) and (n-6) PUFA can be synthesized from dietary essential fatty acids such as linoleic acid and α -linolenic acid. Arachidonic acid, EPA, and DHA serve as precursors of bioactive mediators involved in inflammation response and neuroprotection (17–19), and the PUFA composition of membrane lipids affects cell membrane fluidity and function (20). PG production is directly modulated by the substrate availability of arachidonic acid in cellular membrane phospholipids (21). Thus, perturbation of fatty acid profiles during vitamin B-6 deficiency, even by an indirect mechanism, could have a variety of effects relevant to health.

Clinically severe vitamin B-6 deficiency is rare. However, marginal vitamin B-6 status, as reflected by a plasma PLP concentration between 20 and 30 nmol/L (22) is common in women of reproductive age, especially current and former users of oral contraceptives, male smokers, and the elderly (23–25). Whereas no clinical signs occur in a marginal deficiency, metabolic changes can exist and become more obvious as vitamin B-6 deficiency progresses. Our research group has shown that marginal vitamin B-6 deficiency alters plasma amino acid profiles, with a marked elevation of cystathionine and, to a lesser extent, elevation of glycine (26–29), which confirmed and extended earlier findings (30,31). Tracer kinetic studies have shown that vitamin B-6 restriction yields increased postprandial rates of whole-body cystathionine synthesis and serine production from glycine; there is little or no impact on other aspects of 1-carbon metabolism, including fluxes of cysteine and methionine pools and rates of glycine cleavage and homocysteine remethylation in healthy adults (26–29). However, to our knowledge, fatty acid metabolism has not been previously examined in the context of marginal vitamin B-6 deficiency.

We report here the fatty acid profiles in plasma FFA and total plasma lipids as well as in peripheral blood mononuclear cell (PBMC) and erythrocyte membranes of 23 healthy young adults who participated in 2 recent vitamin B-6 restriction protocols (28,29).

Participants and Methods

Participants. Healthy adult men and nonpregnant women (20–40 y) were recruited and screened by standard clinical measures as previously described (28,29). The characteristics of these individuals are summarized in Table 1. Medical history, dietary habits, and demographic data were assessed by a questionnaire. Inclusion criteria were: no history of gastrointestinal surgery, chronic disease, or chronic drug use; no smoking or alcoholism; no dietary supplementation; no chronic consumption of a high-protein diet; and normal blood tests of hematological, electrolyte, renal, and hepatic function. The 23 eligible participants had adequate nutritional status in terms of serum folate >7 nmol/L, vitamin B-12 >200 pmol/L, plasma homocysteine <12 μ mol/L, plasma PLP >30 nmol/L, and a BMI <28 kg/m². Written informed consent was provided by each participant. The University of Florida Institutional Review Board and the Clinical Research Center Scientific Advisory Committee reviewed and approved this protocol.

Diet. Participants consumed nutritionally adequate meals with standardized composition for 2 d to minimize dietary variations before the study. Thereafter, a continuous, 28-d, dietary intervention with restricted vitamin B-6 content (<0.5 mg/d) was administered to induce marginal vitamin B-6 deficiency (plasma PLP between 20 and 30 nmol/L). During this period, participants came to the University of Florida Institutional Review Board and the Clinical Research Center twice each day to

consume their breakfast and dinner. Lunch was provided to be taken out by the participants and snacks were given to adjust for daily energy needs. The meals were prepared by the CRC Bionutrition Unit (dietary fatty acid and vitamin B-6 intake data are available in Supplemental Table 1). Custom supplements of multivitamins (no vitamin B-6) and minerals were given to the participants during the restriction to ensure adequate intake of other nutrients. Dietary intake data of the 2-d controlled and 28-d vitamin B-6 restricted diet were collected and analyzed using Nutrition Data System for Research Software version 2005/2007 (Nutrition Coordinating Center, University of Minnesota). Compliance in consuming the vitamin B-6–restricted diet was monitored by measuring plasma PLP weekly.

Sample collection and screening measurements. Blood samples were collected after an overnight fast prior to the study, at the end of the 2-d controlled diet, and after the 28-d vitamin B-6 restriction period. Plasma and erythrocytes were separated by centrifuging the whole blood at $1650 \times g$ for 15 min at 4°C within 15 min of collection. PBMC were separated using a commercial kit (Lymphocyte-H, Cedarlane) from the whole blood within 2 h of collection. All blood fractions were stored at –80°C for later analysis. Serum folate and vitamin B-12 concentrations at baseline were measured using a commercial chemiluminescence-based assay (Elecscys, Roche Diagnostics). Plasma PLP and total homocysteine concentrations were determined by reverse-phase HPLC with fluorescence detection (32,33). Plasma TG, total cholesterol, HDL-cholesterol, and LDL-cholesterol were measured according to standard clinical procedures by the Shands Hospital Clinical Laboratories.

Total fatty acid and FFA analysis in plasma. Plasma total lipids were extracted according to Folch et al. (34). Plasma (200 μ L) was added to 4 mL chloroform-methanol (v:v, 2:1) solvent mixture with 0.01% butylated hydroxytoluene. Tricosanoic acid (23:0, 1 g/L) was used as the internal standard. The mixture was vortexed for 30 s and kept at room temperature for 1 h. Proteins were removed by filtration (0.45- μ m filter, Fisher Scientific). Sodium chloride (1 mol/L, 1 mL) was added to the mixture. Phase separation was achieved by centrifugation and the lower chloroform layer was collected and dried under nitrogen gas. Total fatty acids were then transmethylated to form FAME. The 2-mL acetyl chloride-methanol (v:v, 5:95) solvent mixture was added to the reaction vials. The samples were heated at 100°C for 1 h. After cooling, the FAME were extracted by 1 mL isooctane (35) and then washed with 200 μ L NaCl (1 mol/L) and dried over anhydrous sodium sulfate and concentrated to 50–100 μ L under nitrogen gas. FAME were analyzed using a capillary column Omegawax 250 (30 m $\times$ 0.25 mm $\times$ 0.25 μ m; Supelco) on a TraceGC Ultra gas chromatograph with flame ionization detection (Thermo Scientific). The initial column temperature was 130°C holding for 5 min followed by an increase rate of 5°C/min until reaching 250°C and holding for 12 min. The injector and detector temperatures were set as 250 and 270°C, respectively. Injections were performed at a split ratio of 1:50 using helium as the carrier gas. The CV for total plasma fatty acids ranged from 4.2% for stearate to 8.7% for EPA.

Plasma FFA concentrations were analyzed by the Sarah W. Stedman Nutrition and Metabolism Center at Duke University Medical Center as previously described (36). This method involves selective methylation of free fatty acids and stable isotope labeled internal standards using iodomethane followed by quantification using GC-MS. The CV ranged from 3.0% for palmitate to 8.3% for myristate.

Membrane fatty acid analysis in erythrocytes and PBMC. Frozen erythrocytes from a 1-mL pellet were thawed at room temperature and osmotically lysed with 1 mL cold distilled water, centrifuged at $1000 \times g$ for 10 min at 4°C to remove unbroken cells, and then ultracentrifuged at $100,000 \times g$ for 30 min at 4°C using a near-vertical rotor NVT65 (Beckman Coulter). Membrane pellets were dispersed and homogenized by sonication in 300 μ L methanol. A modified lipid analytical method for biological samples with low lipid content was used, combining lipid extraction and fatty acid esterification in one step to minimize sample loss (37). PBMC membrane lipids were similarly analyzed. PBMC were disrupted by sonication for 30 s and were centrifuged at $1000 \times g$ for 10 min at 4°C to remove nuclei and unbroken cells. Total membrane

fractions were collected by ultracentrifugation at $100,000 \times g$ for 30 min at 4°C . The same method was used for GC sample preparation of PBMC membrane FAME (37). FAME derivatized from erythrocyte and PBMC membrane lipids were analyzed by gas chromatograph with flame ionization detection as described earlier. The CV for erythrocytes and PBMC ranged from 2.5% for palmitate to 8.2% for palmitoleate.

Statistical analysis. All data are presented as mean $\pm$ SD. Differences in baseline characteristics between men and women were assessed using a 2-sample *t* test. For all other statistical testing comparing data before and after vitamin B-6 restriction, the data were log2-transformed for meeting the Gaussian distributional requirement and evaluated in 3 stages: 1) multivariate ANOVA (MANOVA) was used to evaluate the overall effects of vitamin B-6 restriction on fatty acid profiles of various blood fractions; 2) step-down MANOVA analyses were performed to test the overall effects of restriction on (n-3), (n-6), and (n-9) fatty acid pools, respectively; and 3) paired 1-sample *t* tests were used to evaluate the influence of restriction on individual fatty acid metabolites and ratios of product to precursor fatty acids. For the first 2 steps, significance was determined at the 0.05 level of Type I error rate. For the third step, significance was determined after adjusting for multiple comparisons by controlling the positive false discovery rate (pFDR) at the 0.05 level (38). To evaluate the effect of vitamin B-6 restriction with an account of the effects of dietary fatty acid intake and gender, a linear regression model was fitted, separately, for each fatty acid with available dietary measurements (i.e., regress change in plasma concentration on gender, change in dietary B-6 intake, and change in fatty acid intake). The significance of each effect was determined by controlling the pFDR at the 0.05 level. Data were analyzed using the statistical software SAS 9.3.

Results

Participant characteristics. Table 1 shows the characteristics of all 23 participants at baseline with normal nutritional status for vitamin B-6, folate, and vitamin B-12. There were no significant differences in baseline characteristics between men and women except that female participants had lower plasma PLP at baseline ($P = 0.033$) (Table 1).

Effects of marginal vitamin B-6 deficiency on plasma lipid profiles, plasma total fatty acid, and FFA concentrations. The plasma PLP concentration decreased to the range of marginal deficiency from 52 ± 14 nmol/L to 21 ± 5 nmol/L ($P \leq 0.001$) during the 28-d vitamin B-6 restriction. There was no significant effect on the plasma total homocysteine concentration (Table 2). After adjusting for the pFDR, total cholesterol, TG, HDL-cholesterol, and LDL-cholesterol did not significantly change (Table 2).

The MANOVA analysis of plasma total fatty acids indicated that vitamin B-6 restriction affected the overall fatty acid profiles ($P = 0.0031$) and, subsequently, those of (n-6) ($P =$

TABLE 1 Baseline characteristics of 23 healthy men and women participating in the study¹

Characteristics	Men	Women	Total
<i>n</i>	12	11	23
Age, y	24 ± 5	25 ± 6	25 ± 5
BMI, kg/m ²	24.5 ± 2.7	23.6 ± 2.3	24.1 ± 2.5
Plasma PLP, nmol/L	59 ± 13	$45 \pm 13^*$	52 ± 14
Serum folate, nmol/L	34 ± 8	30 ± 7	32 ± 7
Serum vitamin B-12, pmol/L	349 ± 115	388 ± 129	368 ± 124
Plasma homocysteine, $\mu\text{mol/L}$	7.5 ± 1.0	6.4 ± 1.3	7.0 ± 1.3

¹ Values are means $\pm$ SD. *Different from men, $P < 0.05$. PLP, pyridoxal 5'-phosphate.

TABLE 2 Plasma lipid fractions of 23 healthy men and women at baseline and after 28-d moderate vitamin B-6 restriction¹

Lipid profiles	Baseline	Restricted
HDL-cholesterol, mmol/L	1.42 ± 0.26	$1.35 \pm 0.28^*$
LDL-cholesterol, mmol/L	2.67 ± 0.93	2.64 ± 0.80
Total cholesterol, mmol/L	4.53 ± 0.93	4.48 ± 0.85
TG, mmol/L	0.89 ± 0.26	1.02 ± 0.37
PLP, nmol/L	52 ± 14	$21 \pm 53^{**}$
Homocysteine, $\mu\text{mol/L}$	7.0 ± 1.3	7.1 ± 1.5

¹ Values are means $\pm$ SD, $n = 23$. Asterisks indicate different from baseline: * $P < 0.05$, ** $P < 0.001$. PLP, pyridoxal 5'-phosphate.

0.0004), (n-3) ($P = 0.0182$), and (n-9) ($P = 0.0013$) fatty acid classes in healthy adults. The concentrations of γ -linolenic acid and arachidonic acid both decreased (adjusted $P < 0.05$ and $P < 0.001$, respectively), as did plasma total EPA (adjusted $P = 0.018$) and DHA (adjusted $P = 0.018$). There were no changes in linoleic acid and α -linolenic acid. The total (n-6) PUFA concentration also did not change after restriction, but the total (n-3) PUFA concentration decreased ($P = 0.01$). Consequently, the plasma (n-6):(n-3) PUFA ratio increased modestly ($P = 0.035$). The concentrations of plasma FFA, which are often viewed as indicators of inflammation, exhibited no significant changes from the 28-d restriction (Table 3).

A summary of dietary fatty acids and other major diet constituents of the 2-d controlled diet and 28-d vitamin B-6-restricted diet are listed in Supplemental Table 1. Based on the dietary intake data of fatty acids in the protocol, we built linear regression models (not shown) to evaluate the effects of a low-vitamin B-6 diet on changes of plasma fatty acid profiles after adjusting for gender and dietary intake of fatty acids (one model per fatty acid). The low-vitamin B-6 diet was correlated to the changes in plasma palmitic acid, linoleic acid, and DHA concentrations (pFDR adjusted $P < 0.05$) (Supplemental Table 2). Changes in dietary intake of palmitic acid, linoleic acid, and DHA were associated with changes in the plasma concentrations of these respective fatty acids in total plasma lipids (pFDR adjusted $P < 0.05$). The gender of participants did not contribute to any changes in the plasma fatty acid profiles after adjusting for dietary vitamin B-6 and fatty acid intake. A similar regression analysis on plasma FFA and RBC fatty acids indicated that dietary fatty acid intake and gender were not significant. These regression models provided us with an alternative perspective to examine the interactions among vitamin B-6, dietary fatty acids, and fatty acid profiles in human blood and supported the conclusions derived from direct statistical analysis of fatty acid patterns and ratios.

We calculated the ratios of precursor:product fatty acids in PUFA metabolism (Table 4) to provide a measure of relative fatty acid pool sizes, which would be reflective of relative rates of fatty acid interconversion and turnover in vivo. The ratio of plasma total 20:4(n-6):18:2(n-6) decreased (adjusted $P \leq 0.01$) after vitamin B-6 restriction (Table 4). MANOVA analysis also indicated that vitamin B-6 restriction affected the overall ratios of long-chain (n-6) PUFA:18:2(n-6) ($P = 0.001$).

Effects of marginal vitamin B-6 deficiency on membrane fatty acid composition in erythrocytes and PBMC. No changes of fatty acid composition in erythrocyte and PBMC membrane lipids after vitamin B-6 restriction were observed (Table 4; Supplemental Table 3). The slight increase in oleic acid [18:1(n-9)] in PBMC membrane (raw $P = 0.025$) was not

TABLE 3 Plasma total fatty acid and FFA concentrations in 23 healthy men and women at baseline and after 28-d moderate vitamin B-6 restriction¹

Fatty acids	Total fatty acids		FFA	
	Baseline	Restricted	Baseline	Restricted
16:0 (palmitic acid), $\mu\text{mol/L}$	2230 $\pm$ 388	2190 $\pm$ 365	110 $\pm$ 59	110 $\pm$ 39
16:1(n-7) (palmitoleic acid), $\mu\text{mol/L}$	134 $\pm$ 55	112 $\pm$ 41*	15 $\pm$ 9	14 $\pm$ 4
18:0 (stearic acid), $\mu\text{mol/L}$	947 $\pm$ 189	940 $\pm$ 213	33 $\pm$ 15	38 $\pm$ 16
18:1(n-9) (oleic acid), $\mu\text{mol/L}$	1400 $\pm$ 346	1440 $\pm$ 294	168 $\pm$ 92	173 $\pm$ 56
18:2(n-6) (linoleic acid), $\mu\text{mol/L}$	2650 $\pm$ 409	2650 $\pm$ 474	69 $\pm$ 45	66 $\pm$ 27
18:3(n-6) (γ -Linolenic acid), $\mu\text{mol/L}$	42 $\pm$ 20	37 $\pm$ 15*	—	—
20:4(n-6) (arachidonic acid), $\mu\text{mol/L}$	548 $\pm$ 96	490 $\pm$ 94*	3.0 $\pm$ 1.7	2.8 $\pm$ 1.3
18:3(n-3) (α -linolenic acid), $\mu\text{mol/L}$	58 $\pm$ 18	56 $\pm$ 18	6.0 $\pm$ 5.5	5.5 $\pm$ 4.4
20:5(n-3) (EPA), $\mu\text{mol/L}$	37 $\pm$ 13	32 $\pm$ 13*	—	—
22:6(n-3) (DHA), $\mu\text{mol/L}$	121 $\pm$ 29	109 $\pm$ 28*	—	—
Σ SFA, $\mu\text{mol/L}$	3170 $\pm$ 555	3130 $\pm$ 554	143 $\pm$ 73	148 $\pm$ 54
Σ MUFA, $\mu\text{mol/L}$	1530 $\pm$ 391	1550 $\pm$ 322	182 $\pm$ 99	187 $\pm$ 59
Σ (n-6) PUFA, $\mu\text{mol/L}$	3240 $\pm$ 446	3180 $\pm$ 498	72 $\pm$ 46	69 $\pm$ 27
Σ (n-3) PUFA, $\mu\text{mol/L}$	216 $\pm$ 42	197 $\pm$ 46*	6.0 $\pm$ 5.5	5.5 $\pm$ 4.4
(n-6):(n-3) ratio	15.4 $\pm$ 2.8	16.6 $\pm$ 3.1*	13.1 $\pm$ 4.8	15.9 $\pm$ 8.3

¹ Values are means $\pm$ SD, $n = 23$. *Different from baseline, $P < 0.05$ (pFDR adjusted). pFDR, positive false discovery rate.

significant after adjusting for pFDR (Supplemental Table 3). In addition, MANOVA analysis indicated an overall effect of vitamin B-6 restriction on the product:precursor ratios of PBMC membrane total fatty acids ($P < 0.05$), but there was no effect on individual (n-3), (n-6), and (n-9) fatty acid pools in either erythrocyte or PBMC membrane lipids.

Discussion

To our knowledge, this is the first study to investigate whether marginal vitamin B-6 deficiency affects fatty acid profiles in healthy adults. We observed a significant decrease of plasma total arachidonic acid, EPA, and DHA concentrations but no changes in the levels of their precursor fatty acids, linoleic acid and α -linolenic acid, after the 28-d vitamin B-6 restriction. Our regression modeling showed that the changes in plasma 18:2(n-6) and DHA were significantly related both to vitamin B-6 intake and intake of these fatty acids during restriction. Consequently, the changes in plasma total 18:2(n-6) and DHA were not solely due to the vitamin B-6 restriction. However, overall, the changes observed constitute strong evidence for effects of low vitamin B-6

status on PUFA metabolism. For example, the ratio of 20:4(n-6):18:2(n-6) in plasma total lipids decreased, which suggested that the synthesis, turnover, or both of 20:4(n-6) was altered with marginal vitamin B-6 deficiency. These findings are consistent with those reported to occur in vitamin B-6–deficient rats (2–5,7,8). FFA concentrations in plasma were measured in this study, which was not done in previous rat studies. Because FFA usually originate from the mobilization of lipid droplets stored in adipose tissue or from the hydrolysis of circulating phospholipids (12), the absence of a change in the concentration or profile of FFA suggests that marginal vitamin B-6 deficiency did not affect lipid mobilization. We acknowledge that the short (2-d) duration of the prerestriction, vitamin B-6–adequate diet and the lack of a repletion phase with prolonged vitamin B-6 supplementation were limitations of the experimental design. Although these were unavoidable in the overall context of this research, the close agreement between our observations and previous studies with controlled diets in rodents supports our conclusion that the changes in (n-3) and (n-6) fatty acid profiles were attributable to vitamin B-6 restriction.

Changes in the pattern of circulating fatty acids can be associated with changes in membrane fatty acid composition by

TABLE 4 Ratios of product to precursor fatty acids for plasma total lipids, erythrocytes, and PBMC membrane lipids in 23 healthy men and women at baseline and after 28-d moderate vitamin B-6 restriction¹

Ratios	Plasma		Erythrocytes		PBMC	
	Baseline	Restricted	Baseline	Restricted	Baseline	Restricted
(n-9) Pathway						
16:1(n-7):16:0	0.06 $\pm$ 0.02	0.05 $\pm$ 0.02	0.01 $\pm$ 0.01	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
18:1(n-9):18:0	1.52 $\pm$ 0.47	1.59 $\pm$ 0.43	0.67 $\pm$ 0.06	0.69 $\pm$ 0.07	0.67 $\pm$ 0.05	0.70 $\pm$ 0.05
(n-6) Pathway						
18:3(n-6):18:2(n-6)	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	—	—	—	—
20:4(n-6):18:2(n-6)	0.21 $\pm$ 0.04	0.19 $\pm$ 0.04*	1.47 $\pm$ 0.26	1.45 $\pm$ 0.26	2.12 $\pm$ 0.34	2.20 $\pm$ 0.37
(n-3) Pathway						
20:5(n-3):18:3(n-3)	0.70 $\pm$ 0.35	0.62 $\pm$ 0.24	—	—	—	—
22:6(n-3):18:3(n-3)	2.28 $\pm$ 0.90	2.15 $\pm$ 0.80	—	—	—	—

¹ All values are means $\pm$ SD, $n = 23$. Significantly different from baseline, $P \leq 0.01$. PBMC, peripheral blood mononuclear cell.

incorporation into the membrane lipid bilayer or exchange with membrane fatty acids. The mechanisms of this exchange of fatty acids from plasma to blood cells include: transfer of albumin-bound FFA by fatty acid binding proteins, transfer of phosphatidylcholine from plasma lipoproteins to cell membranes, and transfer of nonesterified fatty acids from the hydrolysis of TG in lipoproteins (39,40). The changes of plasma (n-6) and (n-3) PUFA observed in the present study apparently were not of sufficient magnitude or duration to alter the cellular fatty acid profiles in this 28-d vitamin B-6 restriction in which steady state likely would not have been attained. The small and nonsignificant change in PBMC oleic acid reported here is similar to the response of erythrocyte membrane lipids reported in rats fed a vitamin B-6-free diet for 20 wk, which is a much more severe deficiency (41). In that study, liver oleyl and arachidonyl CoA-lysophospholipid acyltransferase activities were significantly higher in vitamin B-6-deficient rats than in pair-fed controls, suggesting that more arachidonate and oleate were incorporated into membrane phospholipids.

A potential mechanism responsible for the observed lower plasma long-chain PUFA with vitamin B-6 deficiency is impairment of PUFA interconversion. Hepatic $\Delta 6$ desaturase activity has been reported to be lower in vitamin B-6-deficient rats (3,4). Another hypothesis to account for our findings is that changes in fatty acid profiles are due to the decrease of phosphatidylcholine synthesis from phosphatidylethanolamine methylation (5). Severe vitamin B-6 deficiency has been shown to decrease phospholipid methylation by elevating homocysteine and lowering the phosphatidylethanolamine methyltransferase cosubstrate, S-adenosylmethionine, concentration while elevating the phosphatidylethanolamine methyltransferase inhibitory metabolite, S-adenosylhomocysteine, in rat liver microsomes (42,43). However, in our study, no significant change was found in the plasma homocysteine concentration (26,28), global DNA methylation (28), or in whole-body transmethylation flux (29). One may hypothesize that this extent of vitamin B-6 deficiency causes transient postprandial hyperhomocysteinemia and S-adenosylhomocysteine elevation, as would occur in a methionine load test during more severe vitamin B-6 deficiency (44). However, we believe that this phenomenon was minimal in view of our recent report (29) that postprandial plasma homocysteine was only marginally increased in participants of the present study (plasma total homocysteine after vitamin B-6 restriction: preprandial, $7.5 \pm 0.5 \mu\text{mol/L}$ vs. postprandial, $9.2 \pm 0.7 \mu\text{mol/L}$; $P < 0.001$; means $\pm$ SEM, $n = 10$). These findings strongly suggest that cellular methylation reactions, including phospholipid methylation, were not impaired at this level of vitamin B-6 deficiency.

Total cholesterol and LDL concentrations have been reported to be increased in rats with subnormal vitamin B-6 status, but the plasma HDL concentration did not differ from the pair-fed controls (45). An increase in plasma TG and cholesterol also was reported in severely vitamin B-6-deficient rats relative to vitamin B-6-adequate controls (46). In our study, we observed a decrease of plasma HDL-cholesterol and an increase of TG after vitamin B-6 restriction (raw $P < 0.05$); however, there were no significant changes occurring in plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG after adjusting for pFDR. Thus, changes in plasma cholesterol and lipoprotein distribution do not appear to be responsible for the association of a low plasma PLP concentration with an increased risk of cardiovascular disease (47–51). Based on these findings, we postulate that prolonged changes in (n-3) and (n-6) fatty acid profiles, rather than lipoprotein profiles per se, may be an important factor in the linkage between marginal vitamin B-6 status and cardiovascular risk.

Erythrocytes lack the capacity of long-chain PUFA biosynthesis and are thus a good reflection of long-term dietary intake of fatty acids (52). It generally takes 4–6 wk to equilibrate erythrocyte membrane fatty acid composition with diet (53). In our 4-wk study, the lack of changes in erythrocyte fatty acid composition suggests that changes in dietary fatty acid intake during the study had little influence on membrane composition, although steady state would not have been attained. We think that the observed changes in plasma (n-3) and (n-6) PUFA profiles in this study were primarily due to an effect of the low vitamin B-6 intake and not from the change of dietary fatty acid intake in our vitamin B-6-restricted diet compared with the participants' prestudy diets. This conclusion was supported by the regression model in which vitamin B-6 intake, rather than dietary fatty acid intake, was the primary determinant of the plasma fatty acid profiles.

Plasma PLP concentration has been shown to be inversely related to inflammation (54), although the implications remain unclear. We observed that the (n-6):(n-3) PUFA ratio significantly increased after vitamin B-6 restriction. An elevated (n-6):(n-3) PUFA ratio is generally associated with the increased risk of inflammation (11–14). However, the significant but relatively modest changes in arachidonate, EPA, and DHA in plasma observed in this study may modestly influence eicosanoid synthesis and may modulate the onset of inflammation. A low plasma PLP concentration is associated with an increase in the inflammation marker C-reactive protein that is independent of plasma homocysteine concentration (51,55,56). This association has been hypothesized to be associated with an upregulation of tryptophan catabolism in inflammation that may contribute to a decline in plasma PLP (57). It is important to recognize, however, that vitamin B-6 restriction in the absence of other inflammatory conditions did not cause an elevation of C-reactive protein in a previous study with this vitamin B-6 restriction protocol (27).

In conclusion, short-term, marginal, vitamin B-6 deficiency decreased plasma long-chain (n-6) and (n-3) PUFA concentrations. These changes may have important implications regarding the pathobiology of cardiovascular disease and other disorders in which the metabolism of long-chain fatty acids is perturbed.

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J.F.G. and P.W.S. designed the study; Y.L. and B.S.C. coordinated the study; M.Z. conducted fatty acid analysis, analyzed the data, and wrote the paper with J.F.G.; M.A.R. provided technical and analytical support; M.N.S. designed the diets and calculated diet composition; Y.C. and K.E.M. performed statistical analysis and helped with data interpretation; J.R.B. and C.B.N. analyzed plasma FFA profiles; and J.F.G. had primary responsibility for the final content. All authors read and approved the final manuscript.

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