An appraisal of vitamin B_6 status indices and associated confounders, in young people aged 4–18 years and in people aged 65 years and over, in two national British surveys

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Abstract

Objective: To compare vitamin B_6 status indices with each other and with potential confounding factors, in the datasets from two national British surveys and associated studies.

Design: Vitamin B_6 status was measured by plasma pyridoxal phosphate (PLP) and plasma pyridoxic acid (PA) in both surveys, and also by erythrocyte aspartate aminotransferase activation coefficient (EAATAC) in one of the surveys. Plasma α_1-antichymotrypsin was measured as an index of acute phase status; plasma creatinine was measured as an index of renal function; and plasma total alkaline phosphatase activity was measured as a proxy for PLP hydrolase activity.

Setting: The survey of people aged 65 years and over was carried out in 80 postcode sectors across mainland Britain during 1994–95 and the survey of young people was carried out in 132 postcode sectors across mainland Britain during 1997.

Subjects: Blood samples from *c.* 1000 subjects of both sexes in each survey permitted measurements of plasma PLP and PA. There were also measurements of EAATAC in the young people's survey.

Results: According to published limits of normality, only 5% or less of the young people had unacceptable vitamin B_6 status as measured by plasma PLP. About half had apparently unacceptable status by EAATAC, but this observation is difficult to interpret. The young people had considerably higher plasma concentrations of PLP and lower concentrations of PA than the older people. In both surveys, plasma PLP was strongly correlated with plasma PA and in the young persons' survey it was also correlated, although much less strongly, with the basal activity and activation coefficient of aspartate aminotransferase. Both plasma PLP and EAATAC (but not PA nor basal aspartate aminotransferase activity) were influenced by acute phase status in young people, as indicated by significant correlations with α_1-antichymotrypsin. In people aged 65 years and over, PA (but not PLP) was correlated with renal function, as indicated by its relation with plasma creatinine; however PLP (but not PA) was correlated with plasma alkaline phosphatase activity.

Conclusions: Several potential confounders – acute phase reaction, kidney malfunction and hydrolase activity – may influence vitamin B_6 status indices, although differently for different indices and different age groups. Since older people have relatively poor vitamin B_6 status, which may have important health implications for them, more reliable vitamin B_6 status indices are needed.

Keywords
Vitamin B_6 status
Pyridoxal phosphate
Pyridoxic acid
Aspartate aminotransferase
Confounders
British surveys

The measurement of vitamin B_6 status is a complex problem, illustrated by the fact that many different indices are used, but none are universally accepted[1,2]. Plasma PLP is often preferred, because it is correlated with vitamin B_6 intake and increases during supplementation[3,4]. Recent studies in western countries have shown that older people are especially vulnerable to vitamin B_6 deficiency[4–6], indicating the need for further studies, especially of this age group. There are relatively few studies of the vitamin B_6 status of people living in the UK.

An opportunity to compare and contrast some of the vitamin B_6 status indices, and to examine their relationships with each other and with potentially confounding factors, arose during two recent nationally representative British cross-sectional diet and nutrition surveys, which focused respectively on older adults and on young people aged 4–18 years[7,8]. Vitamin B_6

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intakes and status of the older subjects, including diet–status comparisons, are reported elsewhere[9]. The influence, in older people, of potential confounders such as acute phase status as measured by α_1-antichymotrypsin, renal function as measured by plasma creatinine, and PLP phosphatase activity, for which plasma alkaline phosphatase may act as a proxy index, were also discussed[9]. The purposes of the present study were to examine the differences in magnitude of the vitamin B_6 status indices between the two age groups, and to compare the relationships between the B_6 indices and the confounders in the two age groups.

Subjects and methods

The design and execution of the National Diet and Nutrition Survey (NDNS) of people aged 65 years and over has been described[7] and the NDNS of young people aged 4–18 years[8] was essentially similar. In the older adults' survey, people living both in the community and in institutions such as nursing homes were studied, but the present study is limited to those living in the community, who are generally in better health than those in institutions.

The survey of older people during 1994–95 was subdivided into three age groups (65–74 years, 75–84 years and 85+ years), and participants were randomly sampled from 80 postcode sectors across mainland Britain. Of those not living in institutions, 45% provided a blood sample, usually in the early morning after an overnight fast, of which 919 samples were sufficient in volume for the vitamin B_6 (PLP and PA) analyses. The survey of young people was subdivided into four age groups (4–6 years, 7–10 years, 11–14 years and 15–18 years), randomly sampled from 132 postcode sectors in mainland Britain during 1997. A blood sample, usually in the early morning after an overnight fast, was taken from 44% of the young people, and 1006 of these became available for vitamin B_6 analyses: PLP, PA, EAATAC and its basal activity (EAATbasal). The duration of fieldwork in both surveys was one calendar year, which enabled seasonal variations in status to be studied, and ensured that any seasonal variations would not compromise the validity of the surveys.

The venous heparinized blood samples, which were obtained by a phlebotomist in the subjects' homes, were taken in a coolbox to a local hospital laboratory within 4 hours and were immediately separated into plasma and red cells. For the aspartate aminotransferase measurements, the red cell pellets were washed with normal saline solution and the buffy coat was removed. All samples were stored for short periods (up to 3 months) at or below −40°C and then at −80°C. The assay used for EAATAC was that of Vuilleumier *et al.*[10]. This measures the rate of oxidation of pyridine nucleotide coenzyme, coupled to the aminotransferase reaction, and it was performed on a Roche Cobas Bio centrifugal analyser[10]. Measurements with and without added cofactor (PLP) permitted calculation of the activation coefficient (EAATAC) which is the ratio of these two enzyme activities. It was found to be necessary to avoid freeze–thaw cycles and prolonged storage to eliminate storage-related changes. Quality assurance samples measured interassay CVs (3.7% for basal activity; 1.8% for the activation coefficient) and maximum drift (0.7% for basal activity; 0.7% for the activation coefficient).

The aminotransferase assays were performed within 6 months, in previously unthawed samples; the PLP and PA assays were performed within 0.75–2 years. It was shown that small changes in PLP and PA, possibly attributable to storage might occur during similar storage periods: thus in a subset of 22 samples which were reanalysed 2–3 years after the initial analyses, the PLP concentration had declined by *c.* 20% ($P=0.06$) and PA had increased by *c.* 20% ($P=0.09$). The pre- and post-storage results for both analytes were, however, strongly correlated with each other ($P<0.0001$). All of the assays were carried out in the same laboratory, with precautions to minimize assay drift.

The assay used for PLP and PA is described elsewhere[11]. Briefly, it involves removal of protein by trichloroacetic acid, conversion of PLP to 4-pyridoxic acid phosphate with cyanide in alkaline medium, acidification, separation by high performance liquid chromatography (HPLC) and quantitation by a sensitive fluorescence detector. The between-run CVs for PLP and PA were 4–5% ($n=10$) and spike recoveries ranged between 97.6% and 102.8% ($n=10$) for both analytes. Further validation was achieved by inter-laboratory sample exchange[11]. Unfortunately, free pyridoxal cannot be detected by this assay.

The procedures used for the measurement of plasma α_1-antichymotrypsin (by turbidometric immunoassay), of total plasma alkaline phosphatase activity (by colourimetric rate assay, using *p*-nitrophenylphosphate) and of plasma creatinine (by the Jaffe reaction), are described elsewhere[7].

Statistical comparisons between groups were performed by analysis of variance, using natural log-transformed data where necessary to achieve normal distributions. These calculations were performed with the DataDesk (Data Descriptions Inc.) computer program. Ethical permission for the survey procedures was obtained from the local research ethics committees that were responsible for each of the postcode sectors used, and from the MRC Dunn Nutrition Unit's Ethics Committee.

Results

Geometric mean values, by age band and by gender, for plasma PLP, plasma PA and EAATAC and its basal activity, in the young people, are shown in Table 1. On the basis of plasma PLP levels, very few of the young people had poor vitamin B_6 status: only 0.5% on the basis of a 20 nmol l^{-1} cut-off[12] or 6% on the basis of a 30 nmol l^{-1} cut-off[1,2]. In contrast, around 54% had poor status on the basis of a cut-off of 1.80 for EAATAC[1,2]. However, the cut-off point for the latter assay is likely to be very method-dependent, so that the published figure may not be appropriate for our study, and this particular observation is therefore difficult to interpret. In males, of the four status indices measured, only EAATAC and EAATbasal activity exhibited a significant age-related gradient towards more deficient values (higher EAATAC, lower EAATbasal) with increasing age, albeit remaining within the normal range. In contrast, in females, both plasma PLP and PA (but not EAATAC nor EAAT-basal activity) exhibited a significant trend towards more deficient values with increasing age. For plasma PLP and PA and EAATAC, the geometric mean values were significantly higher (i.e. better for PLP and PA; worse for EAATAC) in males than in females in the oldest age band, whereas for the basal EAAT activity, the values were higher (i.e. better) for females than for males. This implies that the gender differences in the vitamin B_6 status indices are not consistent between the different indices and different blood compartments.

Table 2 depicts the geometric mean concentrations of plasma PLP and PA in the two major age groups (young people and people aged 65 and over). Plasma PLP levels were much lower, and PA levels were much higher, in the older people. These differences were not an artefact of different periods of sample storage. As noted previously[10], 48% of older people not living in institutions had plasma PLP levels below 30 nmol l^{-1}, and 75% of those living in institutions had PLP levels below this value. Correlations between the vitamin B_6 status indices in the young persons' survey and the correlation between plasma PLP and PA in older people are shown in Table 3. There were very strong correlations, in both age groups, between plasma PLP and plasma PA and, in the young people, between EAATAC and EAATbasal. However, the correlations between the plasma and the erythrocyte indices, though significant, were weaker.

Relationships between the vitamin B_6 status indices and plasma α_1-antichymotrypsin, exploring the influence of acute phase status on the vitamin B_6 status indices, are depicted in Table 4. Although there were few very high values of antichymotrypsin – above the upper limit (0.65 g l^{-1}) of the normal range – there was a strong inverse relation with PLP in both age groups, and with EAATAC in the young people (Table 4). However, PA was not correlated with antichymotrypsin in either age group (Table 4), and it therefore seems unlikely that acute phase status influences plasma PA.

Table 4 also examines the relationships of the vitamin B_6 indices with plasma creatinine (an index of renal function) and with plasma alkaline phosphatase (a proxy for PLP hydrolase). In young people, neither PLP, PA nor the two erythrocyte indices were correlated with plasma creatinine, but in the older people, PA was strongly and positively correlated with plasma creatinine. The relation between each of the status indices and plasma alkaline phosphatase was very weak in young people; therefore the rate of hydrolysis of PLP by this route was probably slow. There was, however, a strong, inverse relation between PLP and plasma alkaline phosphatase in the older people.

Older people are, of course, much more dependent on medications than young people, and this, too, might influence the vitamin B_6 status indices[1,2]. Overall, there was a significant inverse relationship between the total number of drugs used by each individual and their plasma PLP (with adjustment for age and gender: regression coefficient = −0.41, SE = 0.13, $P = 0.002$), which was not eliminated – indeed it was somewhat strengthened (coefficient = −0.41, SE = 0.12, $P = 0.0005$) – by the inclusion of vitamin B_6 intake and use of vitamin B_6 supplements in the model. However, there was no significant relationship between the number of drugs used and plasma PA (coefficient = 0.047, SE = 0.11, $P = 0.7$). Of 14 classes of medicines, each tested separately and each of which was being used by at least some members of the older population sample, a significantly lower plasma PLP level was associated only with use of medications for respiratory diseases (bronchodilators, antihistamines, etc.) ($P = 0.002$ by multivariate regression, corrected for age and gender). Paradoxically, those older people who used beta-blockers or antiplatelet drugs other than aspirin for cardiovascular problems had significantly *increased* levels of both PLP and PA ($P < 0.02$) and those taking angiotensin-converting enzyme inhibitors also had significantly *raised* levels of PA ($P = 0.005$). Some, but not all, of these relationships were eliminated by the inclusion of vitamin B_6 intakes, or vitamin B_6 supplement use, in the model.

Discussion

Previous studies of vitamin B_6 status in young people are sparse. Results obtained for plasma PLP in the present survey of young people were similar to those reported for 10–11-year-old Dutch boys (52.9 ± 19.3 SD nmol l^{-1})[13], for adolescent American girls (42.1–48.1 nmol l^{-1})[14], and for 13–14-year-old English adolescents (40–58 nmol l^{-1})[15]. The lower values for

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Table 1 Plasma concentrations of pyridoxal 5′-phosphate (PLP), 4-pyridoxic acid (PA), erythrocyte aspartate aminotransferase activation coefficient (EAATAC) and basal activity (EAAT basal) in young people aged 4–18 years

	Males				Females			
Age	PLP (nmol l^{-1}): G mean* (ln mean, SD)(*n*)	PA (nmol l^{-1}): G mean* (ln mean, SD)	EAATAC: G mean* (ln mean, SD)(*n*)	EAATbasal: G mean* (ln mean, SD)	PLP (nmol l^{-1}): G mean* (ln mean, SD)(*n*)	PA (nmol l^{-1}): G mean* (ln mean, SD)	EAATAC: G mean* (ln mean, SD)(*n*)	EAATbasal: G mean* (ln mean, SD)
4–6 years	56.9 (4.04, 0.38)(66)	11.3 (2.42, 0.37)	1.76 (0.56, 0.090)(73)	13.5 (2.61, 0.18)	60.1 (4.10, 0.37)(67)	10.8 (2.38, 0.39)	1.75 (0.56, 0.095)(78)	13.3 (2.59, 0.20)
7–10 years	60.8 (4.11, 0.39)(156)	11.5 (2.44, 0.40)	1.76 (0.57, 0.086)(167)	13.1 (2.57, 0.19)	58.9 (4.08, 0.43)(119)	10.5 (2.35, 0.47)	1.78 (0.58, 0.087)(133)	13.2 (2.58, 0.23)
11–14 years	56.7 (4.04, 0.46)(162)	10.9 (2.39, 0.45)	1.80 (0.59, 0.091)(178)	13.3 (2.58, 0.23)	55.7 (4.01, 0.41)(150)	9.6 (2.26, 0.39)	1.83 (0.61, 0.086)(163)	12.5 (2.53, 0.22)
15–18 years	58.5 (4.07, 0.41)(137)	11.5 (2.45, 0.42)	1.81 (0.59, 0.096)(154)	11.8 (2.47, 0.34)	49.3 (3.88, 0.44)(148)	9.2 (2.22, 0.46)	1.77 (0.57, 0.089)(163)	13.2 (2.58, 0.28)
Linear trend vs. age (*P*)								
	0.7	0.9	0.008	<0.0001	<0.0001	0.004	0.7	0.8
Significance of gender differences (*P*)								
4–6 years	0.41	0.49	0.74	0.64				
7–10 years	0.51	0.07	0.28	0.66				
11–14 years	0.63	0.01	0.12	0.02				
15–18 years	0.0003	<0.0001	0.04	0.002				

*Geometric (G) means obtained by analysis of $\log_e$-transformed data. The SD of the $\log_e$ mean can be considered as a coefficient of variation. The numbers of subjects for PA are the same as those for PLP, and the numbers for EAATbasal are the same as for EAATAC.

Table 2 Comparison between young and older people for plasma pyridoxal 5′-phosphate (PLP) and 4-pyridoxic acid (PA)

Category	*n*	PLP (nmol l^{-1}): G mean (95%CI)	PA (nmol l^{-1}): G mean (95%CI)
Young people (4–18 years)	1006	56.5 (54.5–58.5)	10.6 (8.6–12.6)
Older people (65 years and over)	919	34.0 (32.0–36.0)	15.5 (13.5–17.5)
Student's *t*-test between age groups		$t=13.0$; $P<0.0001$	$t=10.1$; $P<0.0001$

PLP in the older girls than in older boys is in accord with the lower values reported for adult women than for men[16]. In the present study, whereas PLP and PA both declined with increasing age in girls, and older girls had lower concentrations than older boys, this pattern was not observed for EAATAC; indeed vitamin B_6 status according to EAATAC did not change with age in girls, but it deteriorated slightly with increasing age in boys. The data in Table 2 confirm that young people had considerably higher plasma PLP levels than older adults, whereas their plasma PA levels were lower[9]. In view of the potential health implications of poor vitamin B_6 status[17–21], public health intervention may be advisable for older people, but more research on its functional implications is needed.

The strong correlations between plasma PLP and PA in both age groups indicate that these compounds are metabolically closely related. We suggest that the overall supply of vitamin B_6 probably determines the strength of this relationship, independently of the observed age-related changes in the ratio between the two metabolites. The weaker relationship between the plasma indices on the one hand and the red cell enzyme indices on the other, suggests that each compartment may be independently controlled. Driskell and Moak[14] observed a significant correlation between plasma PLP and aminotransferase in American adolescent girls, but van Poppel *et al.*[13] failed to confirm this in Dutch boys. It is likely that the red cell indices reflect status over a longer period of time than the plasma indices, but it cannot be assumed that the red cell indices are therefore necessarily better indicators of body stores.

The fact that plasma PLP is inversely correlated with α_1-antichymotrypsin, an index of acute phase status[22] in both age groups, suggests that acute phase status may be a major confounding factor, affecting plasma PLP at all ages. Since this is true even for plasma antichymotrypsin levels below $0.65\,g\,l^{-1}$, the effect seems not to be confined to severely abnormal acute phase status. Plasma PA was much less affected by acute phase status in both age groups, and by medicine use in the older sample; paradoxically, EAATAC was inversely correlated with the acute phase marker in the young people. It is not known whether the relationship between the vitamin B_6 indices and α_1-antichymotrypsin is transient, or whether it reflects a long-term metabolic characteristic. Although PA is usually measured in urine rather than plasma, the results of our studies suggest that plasma PA, which is easily included in the plasma PLP assay, may be worth considering as a complementary index of vitamin B_6 status, with a different sensitivity to confounding factors than PLP[9].

Kidney function, measured by plasma creatinine, was not a major determinant of plasma PLP in either survey, but it was an important determinant of PA in the

Table 3 Between-index linear regression significance, for vitamin B_6 status indices, in young people aged 4–18 years and in people aged 65 years and over

		Regression of $\log_e$-transformed indices		
y	*x*	*r*	*P*	Degrees of freedom
Young people (4–18 years)				
PLP	PA	+0.60	<0.0001	989
PLP	EAATAC	−0.16	<0.0001	988
PLP	EAATbasal	+0.12	0.0002	978
PA	EAATAC	−0.16	<0.0001	988
PA	EAATbasal	+0.11	0.0004	978
EAATAC	EAATbasal	+0.92	<0.0001	1105
Older people (65 years and over)				
PLP	PA	+0.56	<0.0001	915

EAATAC, erythrocyte aspartate aminotransferase activation coefficient; EAATbasal, basal (unstimulated) activity of erythrocyte aspartate aminotransferase; PA, plasma pyridoxic acid; PLP, plasma pyridoxal phosphate.

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Table 4 Linear regression significance between vitamin B_6 status indices and potential confounding indices, in young people aged 4–18 years and in people aged 65 years and over

		Regression of $\log_e$-transformed indices		
y	*x*	*r*	*P*	Degrees of freedom
Young people (4–18 years)				
PLP	ACT	−0.15	< 0.0001	842
PA	ACT	+0.007	0.85	842
EAATAC	ACT	−0.11	0.0008	924
EAATbasal	ACT	+0.04	0.23	917
PLP	AlkP	−0.02	0.63	569
PA	AlkP	+0.08	0.04	569
EAATAC	AlkP	+0.08	0.04	614
EAATbasal	AlkP	−0.008	0.87	609
PLP	P-Creat	+0.00	0.99	520
PA	P-Creat	+0.04	0.39	520
EAATAC	P-Creat	−0.02	0.60	562
EAATbasal	P-Creat	+0.025	0.54	557
Older people (65 years and over)				
PLP	ACT	−0.26	< 0.0001	887
PA	ACT	+0.02	0.63	887
PLP	AlkP	−0.34	< 0.0001	878
PA	AlkP	−0.06	0.09	878
PLP	P-Creat	−0.03	0.32	879
PA	P-Creat	+0.23	< 0.0001	879

ACT, plasma α_1-antichymotrypsin; AlkP, plasma (total) alkaline phosphatase activity; EAATAC, erythrocyte aspartate aminotransferase activation coefficient; EAATbasal, basal (unstimulated) activity of erythrocyte aspartate aminotransferase; PA, plasma pyridoxic acid; P-Creat, plasma creatinine; PLP, plasma pyridoxal phosphate.

older people, some of whom had very high plasma creatinine concentrations[9]. Therefore, in populations where renal malfunction is common, the use of plasma PA as an index of vitamin B_6 status may prove unsatisfactory. In most previous studies of PA, its' concentration has been measured in urine, since it is an excretory product. Nevertheless, there could be some practical advantages in measuring it in plasma.

The relationship between plasma PLP and plasma alkaline phosphatase[23,24] was non-significant in young people but was significant in older people, even though the total observed range of plasma alkaline phosphatase activities was similar in both age groups. Plasma alkaline phosphatase may, therefore, be an effective proxy indicator of tissue PLP phosphatase activity in older people, but not in young people. Alternatively, the conversion of free pyridoxal to PLP may proceed more efficiently in young than in older people, in which case the hydrolytic activity of tissue or plasma phosphatase enzymes may be rapidly reversed. Like plasma antichymotrypsin, plasma alkaline phosphatase activity was only relevant to the relationship between plasma PLP and vitamin B_6 body stores in the older, but not the young, people.

In conclusion, this study has helped to identify several markers for confounding factors, and some important differences between young and older people. There remains an important need for further functional information, and more appropriate markers, to help interpret the vitamin B_6 indices and to determine whether dietary intervention is needed, especially for older people[25,26].

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