Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homocysteine in a representative sample of British men and women aged 65 years and over

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Concentrations of pyridoxal phosphate and pyridoxic acid were measured in fasting plasma samples from British men and women aged 65 years and over, participating in a National Diet and Nutrition Survey during 1994–5, selected to be representative of the population of mainland Britain. In this population, the concentration of pyridoxal phosphate declined, whereas pyridoxic acid rose, with increasing age and frailty; however, both status indicators were strongly and directly (with a positive coefficient) correlated with estimates of vitamin B_6 intake. This was little affected by the inclusion of food energy and protein intakes in the model. Forty-eight percent of the participants living in the community and 75% of those living in institutions had plasma pyridoxal phosphate concentrations below a range considered normal from other studies. In a univariate regression model, plasma pyridoxal phosphate concentrations were inversely correlated with plasma homocysteine concentrations, consistent with the hypothesis that vitamin B_6 status may influence plasma homocysteine levels, and hence vascular disease risk. However, this relationship was partly attenuated in a multiple regression model including age, sex, domicile and biochemical status indices, including those of folate and vitamin B_{12}. There was evidence that plasma pyridoxal phosphate was sensitive to metabolic conditions associated with inflammation and the acute-phase reaction, and that plasma pyridoxic acid was sensitive to renal function. Thus, neither index is an ideal predictor of vitamin B_6 status in older people, unless these confounding factors are allowed for. Since poor vitamin B_6 status may have health implications, e.g. for immune function, cognition, and for essential intermediary metabolic pathways in older people, it needs to be investigated as a possible public health problem.

Vitamin B_6: Pyridoxal phosphate: Dietary survey: Elderly

Vitamin B_6 is an essential dietary constituent for man, and a precursor of the enzyme cofactors pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate, which form the active site of a wide range of essential enzymes in living tissues. Biochemical indices of vitamin B_6 status in human subjects have been based on a wide variety of procedures and analytes, and one of the most popular choices at the present time is plasma pyridoxal phosphate concentration (Bates, 1997). Recent advances in the design of high-sensitivity fluorescence detectors for HPLC assay systems have brought this assay within the reach of laboratories which specialize in the measurement of biochemical indices of micronutrient status (Bates *et al*. 1998). Whereas circulating pyridoxal phosphate is a biologically active form of the vitamin which is available for use by the tissues, 4-pyridoxic acid is biologically inactive and represents a major vitamin B_6 catabolite, destined for excretion in the urine. Its relation to vitamin B_6 intake and status may differ from that of pyridoxal phosphate, and it may therefore provide complementary information.

Studies of older people from countries outside Britain (Rose *et al*. 1976; Lowik *et al*. 1989) have indicated that vitamin B_6 biochemical status indices frequently decline towards deficient values with advancing age; however, the implications for health are not clear. Older people who live in institutions may be particularly at risk (Chen & Fan-Chiang, 1981; Lowik *et al*. 1992). It was therefore important to examine a British population, since there are few recent British studies, and to characterize the status of the oldest group (85+ years) and of those living in institutions such as nursing homes. There is a need for clarification of the relationship of the vitamin B_6 status indices with food

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energy and protein intakes, as well as with vitamin B_6 intakes, and of their relationship with sex and with potentially confounding factors such as renal function and acute-phase status. The purpose of the present paper is, therefore, to examine these questions by reporting the results of measurements of pyridoxal 5′-phosphate and of pyridoxic acid in plasma samples from a recent British survey of people aged 65 years and over (Finch *et al*. 1998).

The National Diet and Nutrition Survey series is a rolling series of surveys of different age groups of the British population, commissioned jointly by the Department of Health and the Ministry of Agriculture, Fisheries and Food, which focus on diet, biochemical status, anthropometry and lifestyle, including various risk factors which may impinge on long-term health. This survey of older people living in the UK, and the blood samples thereby generated, has provided an opportunity for appraisal of their vitamin B_6 intakes and status.

One clinically important factor for which vitamin B_6 intakes and status are potentially relevant is the control of plasma homocysteine concentration, which is an independent risk factor for, and index of, vascular diseases (Boushey *et al*. 1995; Graham *et al*. 1997). It may be influenced by several B-vitamins, including vitamin B_6 (Smolin & Benevenga, 1982; Brattstrom *et al*. 1990; Miller *et al*. 1992, 1994; Hu *et al*. 1993; Joosten *et al*. 1993; Selhub *et al*. 1993; Ubbink *et al*. 1993, 1996; Franken *et al*. 1994; Dalery *et al*. 1995; Ellis & McCully, 1995; Naurath *et al*. 1995; Riggs *et al*. 1996; Verhoef *et al*. 1996). Therefore, since plasma homocysteine concentrations were measured on a subset of the survey samples (Bates *et al*. 1997), it was important to explore the relationships between vitamin B_6 status and plasma homocysteine and to ask whether the variation in vitamin B_6 indices contributed independently to inter-subject variations in plasma homocysteine concentration, or whether they represented a proxy for other determinants.

Subjects and methods

The design and execution of the National Diet and Nutrition Survey of people aged 65 years and over has been described (Finch *et al*. 1998); therefore only the main features are summarized here. Permission for the survey procedures was obtained from Local Research Ethics Committees associated with each postcode sector, and the MRC Dunn Nutrition Unit's Ethics Committee. The survey approached 2626 people aged 65 years and over, of whom 2172 (83 %) were living in the community and 454 (17 %) were living in institutions, such as nursing homes. Of these, 1275 who were not in institutions and 412 who were in institutions provided a full 4 d weighed record of food and drink consumed (59 % and 91 % response rates respectively). A total of 986 of those not living in institutions and 290 of those living in institutions provided a blood sample, usually in the early morning, after an overnight fast (45 % and 64 % response rates respectively).

Although it is clearly impossible to eliminate selection bias completely, especially in a survey of frail elderly people, from whom a considerable amount of voluntary cooperation is requested, a number of precautions were taken to try to minimize the potential selection bias problem (Finch *et al*. 1998). Identification of the eligible sample and subsequent selection of those to be approached was performed by strictly random procedures. Use of proxy information-givers where necessary, ensured that even confused elderly people could be, and were, included. Use of a weighting variable, to correct for the effects of disproportionate sampling, was applied both in the survey report (Finch *et al*. 1998) and, where necessary, in the present study. The weighted samples had similar profiles for sex, age and region as those of the population aged 65 years and over from the 1991 census data. If all the participating subjects, including those who achieved only partial participation, were subdivided according to whether they provided both a blood sample and a full 4 d diet record, or one of these, or neither, then those that provided neither were slightly older (by an average of 1·4 years), and they were more likely to be female and to be living alone, than those who provided both. Such biases were corrected, where necessary, by use of the weighting variable.

From the eighty randomly-selected postcode sectors from mainland Britain, the participating subjects were stratified into six subgroups, by age and sex from each of (a) free-living and (b) institution-living (generally nursing home) groups, thus yielding twelve subgroups altogether. The sample of people in institutions was drawn by selecting residential and nursing homes for elderly people in the same postal sectors as the non-institution sample and then randomly selecting three residents from each institution. A fieldworker invited participation, comprising an interview about health and lifestyle, a 4 d weighed dietary record, anthropometric, blood pressure and grip strength measurements, and providing a urine sample and an early morning blood sample, usually fasting. Fieldwork took place from October 1994 to September 1995 in four 3-month waves, in order to take account of possible seasonal variations in food availability and biochemical status.

Participants living in their own homes (or their carers, where proxy information was obtained), completed 4 d weighed records under the supervision of trained fieldworkers. They kept written, structured diary records of all items of food and drink, including snacks, with weights of containers plus food and containers with leftovers, to allow for plate waste. The fieldworkers verified the records with the participants (or carers), checking for information quality, consistency, and agreement with a qualitative diet recall on the subsequent day. For participants living in institutions, it was necessary to modify the dietary assessment method, as they did not prepare or serve their own meals. Records were kept by carers and meals were, where necessary, weighed by the fieldworkers. Compared with the non-institution sample, it is less likely that any under-recording due to omission of items of food or drink would have occurred, but it is possible that plate waste may have been under-recorded, resulting in over-estimation of intakes in some instances. Because of the methodological differences, comparisons of nutrient intakes between subjects in institutions and those not living in institutions should be treated with caution. Diet records were coded at Social and Community Planning Research, and were used to calculate individual daily nutrient intakes using a specially developed nutrient databank, based

on standard food composition tables. Estimates of additional vitamin intakes from vitamin supplements (tablets, syrups, etc.) were included; these were used during the 4 d dietary recording period by about 20 % of participants not living in institutions and by about 14 % of those in institutions. These estimates are lower than those for 'non-prescribed dietary supplements' in the survey report (Finch *et al.* 1998), partly because the latter included supplements that did not provide vitamins or minerals, and partly because they included supplements which were not taken during the 4 d diet estimate, because they were taken less frequently.

A blood sample was taken by a nurse in the subject's home and was subdivided for measurements of haematological indices, clotting factors and biochemical indices. A heparinized subsample was taken in a cool-box to a local hospital laboratory, usually within 4 h, and plasma was separated and stored frozen for periods of up to 3 years, at –80° for the majority of this storage period, before the analyses of pyridoxal phosphate and pyridoxic acid.

In order to investigate possible differences in vitamin B_6 status between young people and older adults, sixty-six samples of heparinized plasma from the feasibility study for the National Diet and Nutrition Survey of Young People Aged 4–18 Years (S Lowe, unpublished results) were analysed. The average age of these young participants was 12·5 years, and the samples were analysed by exactly the same procedures, so as to provide a direct status-comparison between the young and old age groups.

Analyses of plasma pyridoxal phosphate were performed by the procedure of Bates *et al.* (1998). Briefly, 120 μl samples of plasma were thawed in a semi-darkened room, and were mixed with 60 μl water, followed by 200 μl TCA (100 g/l) to give a final TCA concentration of 50 g/l. The mixture was maintained at 50° for 5 min in the dark; it was centrifuged, and the supernatant fraction was then mixed with potassium cyanide in a buffered alkaline solution containing K_2HPO_4. After 25 min at 50° in the dark, the solution was reacidified with phosphoric acid, and 100 μl was injected into the HPLC system. This was based on a Waters Symmetry Shield RP8 (Waters Ltd, Watford, Herts., UK), 5 μ, 4·6 × 250 mm column, with isocratic eluent flowing at 1·5 ml/min. The eluent contained semicarbazide, 75 mmol/l, and KH_2PO_4, 50 mmol/l, pH 2·85 in water. The fluorescent derivative of pyridoxal phosphate (i.e. pyridoxic acid phosphate) eluted after approximately 4·7 min, and the unchanged fluorescent pyridoxic acid eluted after 8·5 min. Both were detected with a Waters 474 Scanning Fluorescence Detector (Waters Ltd), excitation wavelength 325 nm; emission wavelength 418 nm; emission bandwidth 40 nm; flow cell volume 16 μl. The run time was 10 min per sample and the throughput was about forty samples or standards per 8 h day. Calibration was by external pyridoxal phosphate and pyridoxic acid standards in water (5–100 nmol/l), and quality control was by a pooled normal heparinized sample, subdivided into small portions, and stored below –80°. The between-run CV of the estimate for this quality assurance control (11·9 μmol/l pyridoxal phosphate) was 3·5 %, and the CV of between-run duplicates for unknowns was 2–4 % at medium to high values. Similar between-run imprecision was found for pyridoxic acid.

The plasma total homocysteine assay procedure has been described previously (Mansoor *et al.* 1992). Briefly, the plasma samples were treated with sodium borohydride to reduce disulfide bonds, with sulfosalicyclic acid to remove protein, with monobromobimane to yield a fluorescent product, followed by HPLC with fluorescence detection. This assay also quantitated plasma cysteine and cysteinylglycine in the same run.

Data reduction was performed with 'Excel' (version 5.0, Microsoft Corp., USA) and 'DataDesk' (Data Descriptions Inc., Ithaca, NY, USA) computer programmes. Because the pyridoxal phosphate and pyridoxic acid concentrations, the vitamin B_6 intakes and many of the other indices were not normally distributed, logarithmic transformation was used for most of the calculations. ANOVA, Student's *t* test, and univariate and multivariate linear regression were used. $P < 0{\cdot}05$ was taken as the criterion of statistical significance.

Results

Table 1 shows the arithmetic and geometric mean concentrations of the two forms of vitamin B_6 in plasma, the participants having been subdivided by age, sex and type of domicile. The overall significance of the inter-group differences (twelve groups) for log(plasma pyridoxal phosphate), by ANOVA, was F 13·6, $P < 0{\cdot}0001$. The relationship of log(pyridoxal phosphate) with age (sexes combined) was significant and inverse by linear regression (t −4·1, $P < 0{\cdot}0001$) for the non-institution group, but it was non-significant (t +0·3) for the institution group. There was no significant sex difference, by regression. For log(plasma pyridoxic acid), the overall significance of the inter-group differences (twelve groups) by ANOVA was F 4·3, $P < 0{\cdot}0001$. The relationship with age (sexes combined) was significant and direct by linear regression (t +6·6, $P < 0{\cdot}0001$ for the non-institution group and t +2·5, $P = 0{\cdot}01$ for the institution group). Again, there were no significant sex differences. After adjustment for age and sex, by multiple regression, people living in institutions had significantly lower values of log(pyridoxal phosphate) than those living in the community (t 9·2; $P < 0{\cdot}0001$), but log(pyridoxic acid) did not differ between the two types of domicile (t 1·6; $P = 0{\cdot}1$). Examination of interaction effects revealed the following significant interactions in a multivariate regression model: for pyridoxal phosphate, age × sex (inverse, $P = 0{\cdot}0004$); sex × domicile (inverse, $P = 0{\cdot}0005$); age × domicile (inverse, $P < 0{\cdot}0001$); age × sex × domicile (direct, $P = 0{\cdot}0002$); and for pyridoxic acid, age × domicile (inverse, $P = 0{\cdot}0002$).

It is clear that the pyridoxal phosphate concentrations were higher in the non-institution than the institution group and that there was a significant trend towards lower values with increasing age. Sex alone, however, exhibited no significant relationship with pyridoxal phosphate. For pyridoxic acid, there was a marked upward trend with age, but there were no significant differences associated with sex or with domicile group. There were no significant differences in either of the vitamin B_6 indices between people who were living on their own, and people who were living with others, in the non-institution group (results not shown). A weighting-factor adjustment for differences in age, sex, domicile

Table 1. Plasma concentrations of pyridoxal phosphate and pyridoxic acid, by age, sex and domicile categories, in people aged 65 years and over*

(Arithmetic mean values with standard deviations, and geometric mean values)

		Plasma pyridoxal phosphate (nmol/l)			Plasma pyridoxic acid (nmol/l)		
Category	*n*	Arithmetic mean	SD	Antilog of $\log_{10}$ mean	Arithmetic mean	SD	Antilog of $\log_{10}$ mean
		UW	UW	UW (W)	UW	UW	UW (W)
Non-institution, males							
65–74 years	211	42·3	28·7	36·4 (36·6)	15·8	9·7	14·0 (14·1)
75–84 years	193	39·1	25·3	33·3 (33·4)	17·8	9·8	15·8 (16·0)
85+ years	68	35·7	23·4	29·3 (23·9)	23·2	12·9	20·6 (20·7)
Non-institution, females							
65–74 years	184	46·5	46·7	36·9 (37·7)	18·4	32·3	14·0 (14·3)
75–84 years	154	45·1	40·7	34·8 (34·5)	19·9	17·8	16·3 (16·1)
85+ years	109	34·9	29·0	28·6 (28·8)	20·3	16·8	16·9 (17·1)
Institution, males							
65–74 years	26	26·7	22·8	22·4 (21·3)	18·1	18·1	13·7 (12·8)
75–84 years	58	23·2	14·9	19·1 (19·0)	16·2	9·7	14·1 (13·9)
85+ years	53	23·4	18·1	20·3 (19·2)	20·6	12·4	17·8 (17·9)
Institution, females							
65–74 years	10	23·3	11·3	21·2 (21·5)	15·4	6·4	14·2 (12·7)
75–84 years	47	24·3	20·8	19·7 (18·9)	16·8	15·9	13·7 (13·7)
85+ years	62	31·9	43·4	24·0 (24·9)	20·0	28·6	14·2 (14·7)

UW, unweighted data; W, data adjusted by a weighting factor, designed to eliminate biases in age, sex, domicile etc. (see p. 192).
* All subjects from whom the biochemical status measurements were obtained were included in this analysis.

and geographical distribution between the selected sample and the entire UK population aged 65 years or over, made little difference to the calculated values (Table 1).

Of the respondents, 157 (13·4 % of those with vitamin B_6 status assays) had eaten or drunk something since the evening before their blood sample was taken, but there was no significant difference between the log mean pyridoxal phosphate or pyridoxic acid values for these non-fasting samples and the corresponding values from the 86·6 % who had not eaten or drunk anything during the same period (results not shown).

Table 2 shows vitamin B_6 intake estimates, together with food energy and protein intake estimates, for the twelve groups of participants shown in Table 1. Energy and protein intakes were normally distributed, but vitamin B_6 intakes were positively skewed and were therefore log-transformed. Because nutrient density may be more important in determining vitamin B_6 status than total daily vitamin B_6 intake,

Table 2. Intakes of food energy, protein and vitamin B_6, by age, sex and domicile categories, in people aged 65 years and over*

		Food energy (MJ/d)		Protein intake (g/d)		Antilog of log(vitamin B_6 intake)† (mg/d)	Adjusted antilog of log(vitamin B_6 intake)‡ (mg/d)	
Category	*n*	Arithmetic mean	SD	Arithmetic mean	SD	Mean	Mean	95 % CI
Non-institution, males								
65–74 years	207	8·27	2·02	74·7	16·9	2·20	1·87	1·07, 3·25
75–84 years	188	7·90	1·80	69·4	16·5	1·95	1·77	0·96, 3·24
85+ years	61	7·22	1·85	62·7	16·6	2·00	1·99	0·54, 7·29
Non-institution, females								
65–74 years	181	6·18	1·37	58·7	13·1	1·80	1·95	0·77, 4·92
75–84 years	147	5·94	1·37	54·8	13·5	1·60	1·82	0·63, 5·21
85+ years	100	5·82	1·47	50·1	15·1	1·38	1·65	0·38, 7·18
Institution, males								
65–74 years	26	7·95	1·98	68·6	14·9	1·83	1·67	0·94, 2·99
75–84 years	58	8·02	2·11	65·5	17·5	1·74	1·64	0·91, 2·93
85+ years	53	8·09	2·16	65·1	17·9	1·77	1·66	0·81, 3·43
Institution, females								
65–74 years	10	7·59	2·31	65·6	16·6	1·60	1·53	0·88, 2·65
75–84 years	44	7·05	1·64	59·1	13·5	1·54	1·60	0·84, 3·05
85+ years	61	7·01	1·46	55·7	14·0	1·52	1·64	0·81, 3·29

* The estimates were confined to those subjects who also provided a blood sample for the biochemical indices, and all were adjusted by a population weighting factor, as described on p. 192.
† Without adjustment for energy and protein intakes.
‡ Adjusted for energy and protein intakes.

when comparing individuals with each other, an estimate which was adjusted for energy and protein intakes is also included in Table 2. In a multiple regression model, age, sex and domicile were all significantly ($P < 0{\cdot}0001$) related to energy intake, which decreased with age, was higher in males than females, and was higher in the institution than in non-institution groups. For protein intake, there were similar relationships with age and sex, but there was no significant difference between institution and non-institution domicile. Log(vitamin B_6 intake), on a total daily intake basis, declined significantly with age ($P < 0{\cdot}0001$) and was higher in men than women ($P < 0{\cdot}0001$), but there were no significant differences between institution and non-institution groups. However, when protein and energy intakes were included in the regression model, the relationships between vitamin B_6 intake and age or sex became non-significant. It appears that the intergroup differences in vitamin B_6 intake were not attributable to differences in the vitamin B_6 : protein ratio.

Both vitamin B_6 status indices (plasma pyridoxal phosphate and pyridoxic acid) were strongly correlated with vitamin B_6 intake (Table 3). The effects of adjustment for

Table 3. Linear regression analysis showing the interdependence of the biochemical status indices and vitamin B_6 intakes*

Independent (x) variables and model variations according to the inclusion of energy and/or protein	Variables included in the model					
	Log(vitamin B_6 intake) (mg/d)	Energy intake (kJ/d)	Protein intake (g/d)	Age (years)	Sex (1, 2)	Domicile (1, 2)
(a) Log(plasma pyridoxal phosphate) as the dependent (y) variable						
Neither protein nor energy included:						
Coefficient	0·620	–	–	$-1{\cdot}56\times10^{-3}$	0·067	–0·171
SE (coefficient)	0·038	–	–	$1{\cdot}00\times10^{-3}$	0·015	0·018
P	< 0·0001	–	–	0·11	< 0·0001	< 0·0001
Model adjusted R^2 (%)	27·5					
Energy included:						
Coefficient	0·666	$-1{\cdot}13\times10^{-5}$	–	$-1{\cdot}74\times10^{-3}$	0·052	–0·163
SE (coefficient)	0·042	$0{\cdot}45\times10^{-5}$	–	$1{\cdot}0\times10^{-3}$	0·016	0·018
P	< 0·0001	0·012	–	0·07	0·001	< 0·0001
Model adjusted R^2 (%)	27·9					
Protein included:						
Coefficient	0·642	–	$-5{\cdot}09\times10^{-4}$	$-1{\cdot}73\times10^{-3}$	0·063	–0·170
SE (coefficient)	0·044	–	$5{\cdot}3\times10^{-4}$	$1{\cdot}0\times10^{-3}$	0·015	0·018
P	< 0·0001	–	0·33	0·08	< 0·0001	< 0·0001
Model adjusted R^2 (%)	27·5					
Both protein and energy included:						
Coefficient	0·656	$-1{\cdot}37\times10^{-5}$	$4{\cdot}78\times10^{-4}$	$1{\cdot}63\times10^{-3}$	0·053	–0·162
SE (coefficient)	0·045	$0{\cdot}56\times10^{-5}$	$6{\cdot}6\times10^{-4}$	$1{\cdot}0\times10^{-3}$	0·016	0·019
P	< 0·0001	0·015	0·47	0·10	0·0009	< 0·0001
Model adjusted R^2 (%)	27·8					
(b) Log(plasma pyridoxic acid) as the dependent (y) variable						
Neither protein nor energy included:						
Coefficient	0·512	–	–	$8{\cdot}15\times10^{-3}$	0·022	–0·055
SE (coefficient)	0·034	–	–	$0{\cdot}90\times10^{-3}$	0·013	0·016
P	< 0·0001	–	–	< 0·0001	0·083	0·0007
Model adjusted R^2 (%)	20·1					
Energy included:						
Coefficient	0·613	$-2{\cdot}45\times10^{-5}$	–	$7{\cdot}76\times10^{-3}$	–0·011	–0·037
SE (coefficient)	0·037	$0{\cdot}38\times10^{-5}$	–	$0{\cdot}79\times10^{-3}$	0·014	0·016
P	< 0·0001	< 0·0001	–	< 0·0001	0·42	0·023
Model adjusted R^2 (%)	22·8					
Protein included:						
Coefficient	0·593	–	$-18{\cdot}7\times10^{-4}$	$7{\cdot}55\times10^{-3}$	$5{\cdot}54\times10^{-3}$	–0·051
SE (coefficient)	0·039	–	$-4{\cdot}6\times10^{-4}$	$0{\cdot}90\times10^{-3}$	$13{\cdot}4\times10^{-3}$	0·016
P	< 0·0001	–	< 0·0001	< 0·0001	0·88	0·002
Model adjusted R^2 (%)	21·2					
Both protein and energy included:						
Coefficient	0·616	$-2{\cdot}36\times10^{-5}$	$-1{\cdot}75\times10^{-4}$	$7{\cdot}72\times10^{-3}$	–0·011	–0·037
SE (coefficient)	0·039	$0{\cdot}49\times10^{-5}$	$5{\cdot}8\times10^{-4}$	$0{\cdot}90\times10^{-3}$	0·014	0·016
P	< 0·0001	< 0·0001	0·76	< 0·0001	0·40	0·023
Model adjusted R^2 (%)	22·7					

* The analysis involved 1136 sets of data. For details, see p. 192.

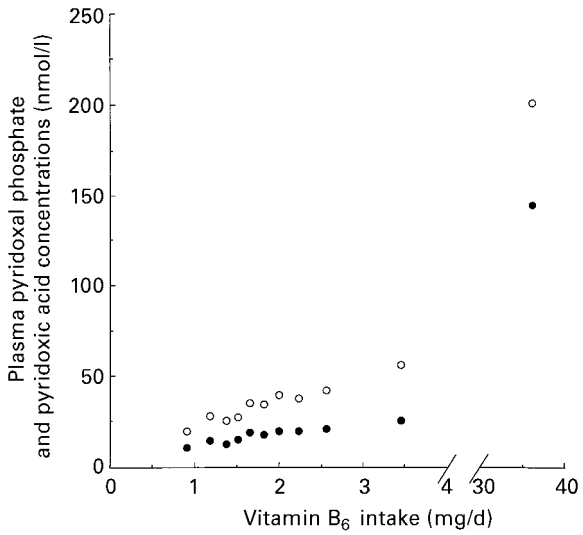

Fig. 1. Relationship of plasma pyridoxal phosphate (○) and pyridoxic acid (●) to vitamin B_6 intake in subjects from the National Diet and Nutrition Survey: People Aged 65 Years and Over. Vitamin B_6 intakes between 0·135 mg/d (lowest) and 6·0 mg/d were divided into ten equal segments. Not all of the participants provided a blood sample for the plasma assays; there were between 105 and 124 subjects with plasma index measurements in each segment. There were seven subjects with higher intakes, between 6·0 and 101·8 mg/d, whose mean values are shown to the right of the figure. For each segment, the mean plasma concentrations of pyridoxal phosphate and pyridoxic acid were calculated, with adjustments (by linear regression) for age, sex, type of domicile, and intakes of energy and protein.

energy or protein intake, or both, and the effects of including age and sex on these relationships, were minimal. Therefore, either the total daily intake of the vitamin, or its ratio to food energy or protein, can predict the status indices.

Fig. 1 shows the relationships between the two biochemical status indices, plasma pyridoxal phosphate and plasma pyridoxic acid as *y* variables, and adjusted vitamin B_6 intake as the *x* variable. Subjects with mean daily vitamin B_6 intakes below 4·0 mg were divided into ten groups with increasing intakes, and the seven remaining subjects with higher intakes (above 4·0 mg/d) formed a separate, eleventh group. Fig. 1 shows that both indices increased progressively with increasing intakes, although pyridoxal phosphate increased more steeply and consistently than pyridoxic acid, especially over the lower intake range.

Since there was a significant downward trend in plasma pyridoxal phosphate with increasing age, and an upward trend in plasma pyridoxic acid with increasing age (Table 1), it was of interest to compare these indices between people aged 65 years or over, and a smaller group of young people, average age 12·5 years. The result of this comparison is shown in Table 4. The young people had considerably higher plasma pyridoxal phosphate concentrations, and lower pyridoxic acid concentrations, than the older age group. These age differences remained highly significant even after removal of those subjects (in the older group) who had some evidence of poor renal function, i.e. plasma urea concentrations >10 mmol/l (results not shown).

Table 5 explores the relationship between the biochemical indices of vitamin B_6 status and the concentration of homocysteine in the same plasma samples (see Bates *et al.* 1997). In a univariate linear regression, with no other determinants, the inverse relationship between plasma pyridoxal phosphate and homocysteine concentrations was strong (t −11·0, $P < 0·0001$), but it became progressively weaker as other determinants were added, in a multiple regression model. With age, sex, domicile, serum and erythrocyte folate and serum vitamin B_{12} included, it was still significant at t −2·4, $P = 0·019$. There were moderately strong direct relationships between plasma pyridoxal phosphate (or pyridoxic acid) and other B-vitamin status indices such as serum and erythrocyte folate or serum vitamin B_{12} (t ratios from 6·1 to 13·4; R^2 from 4 to 14 % of the variance explained, in a simple univariate regression model). This covariance of B-vitamin status indices may be one of the reasons for the reduced significance of plasma pyridoxal phosphate in the full multivariate model. For pyridoxic acid, the relationship with plasma homocysteine paradoxically became a direct positive relationship with some of the combinations of determinants. Two other S-containing compounds in plasma, cysteine and cysteinylglycine, were less strongly related to the vitamin B_6 status indices (results not shown). Cysteinylglycine exhibited a moderately strong direct relationship with both pyridoxal phosphate and pyridoxic acid; cysteine was weakly related to pyridoxic acid but not at all to pyridoxal phosphate (results not shown).

Table 4. Comparison between people aged 65 years and over and young people, average age 12·5 years, with respect to their plasma pyridoxal phosphate and pyridoxic acid concentrations*

(Log mean values and standard deviations; antilog of log mean and 95 % confidence intervals)

	Plasma pyridoxal phosphate				Plasma pyridoxic acid			
Category	Log mean	SD	Antilog of log mean	95 % CI	Log mean	SD	Antilog of log mean	95 % CI
People aged 65 years or over	1·504	0·285	31·9	8·6, 118·6	1·172	0·243	14·9	4·8, 45·5
Young people aged 4–18 years	1·753	0·164	56·6	26·6, 120·5	1·088	0·155	12·2	6·0, 25·0
Student's *t* test between age groups	*t* 11·4				*t* 4·4			
P for difference	< 0·0001				< 0·0001			

* The group of people aged 65 years and over comprised all subjects who yielded vitamin B_6 status analyses in this survey, adjusted by a weighting factor as described on p. 192. The group of young people was not a random sample of the British population, but was typical of their age group.

Table 5. Linear regression analysis of log(total plasma homocysteine) *v.* log(plasma pyridoxal phosphate) or log(plasma pyridoxic acid), with adjustment for other determinants

	Log(total plasma homocysteine)											
	Analysis for log(plasma pyridoxal phosphate)						Analysis for log(plasma pyridoxic acid)					
Multivariate regression model containing:	df	*t*	Coefficient	SE (coefficient)	Adjusted R^2	*P*	df	*t*	Coefficient	SE (coefficient)	Adjusted R^2	*P*
No other determinants	931	–11	–0·202	0·018	11·4	< 0·0001	930	–2·4	–0·056	0·023	0·5	0·017
Serum and erythrocyte folates	902	–5·3	–0·101	0·019	24·1	< 0·0001	901	+3·8	+0·085	0·023	22·9	0·0002
Serum and erythrocyte folates and serum vitamin B_{12}	895	–4·5	–0·084	0·019	27·3	< 0·0001	894	+4·5	+0·100	0·022	27·3	< 0·0001
Age, sex, domicile, serum and erythrocyte folates and serum vitamin B_{12}	892	–2·4	–0·045	0·019	32·1	0·019	891	+3·3	+0·072	0·022	32·4	0·001

* The *t* values were obtained in a multivariate forward stepwise linear regression model, with log(total plasma homocysteine) as the dependent (*y*) variable, introducing as independent (*x*) variables, the vitamin B_6 status determinant, followed by each of the other independently-significant, log-transformed biochemical determinants of total homocysteine which had been previously identified (Bates *et al.* 1997). The adjusted R^2 estimates are the percentage of the variance explained.

Plasma pyridoxal phosphate was not strongly correlated with the indices of renal function (plasma urea and creatinine), whereas pyridoxic acid was positively and strongly related to these indices. Conversely, pyridoxic acid was not strongly correlated with indices of acute-phase status (e.g. plasma α_1-antichymotrypsin; Cu; blood leucocyte count), whereas pyridoxal phosphate was inversely correlated with these indices. Nearly all of these correlations remained highly significant after adjustment for age, sex, domicile and vitamin B_6 intakes.

There was evidence of a moderate seasonal variation in plasma pyridoxal phosphate concentrations (Table 6) with the lowest values occurring in the winter season (January to March) and the highest in the summer (July to September). This characteristic pattern of seasonal variation was not observed, however, for plasma pyridoxic acid or for vitamin B_6 intakes.

Table 7 illustrates the variation of each of the two indices of vitamin B_6 status and vitamin B_6 intake, with the geographical regions of mainland Britain. It is clear that

Table 6. Seasonal variation of vitamin B_6 status indices and intake in subjects from the National Diet and Nutrition Survey: People Aged 65 Years and Over*

Index	Wave 1 (October–December)	Wave 2 (January–March)	Wave 3 (April–June)	Wave 4 (July–September)
Plasma pyridoxal phosphate (nmol/l)				
Mean	38·0 (39·9)	33·4 (35·8)	38·2 (40·6)	42·5 (45·2)
Antilog of log(mean)	31·1 (31·4)	27·9 (30·5)	29·2 (30·4)	33·7 (35·2)
Plasma pyridoxic acid (nmol/l)				
Mean	18·4 (19·5)	17·5 (16·8)	19·8 (20·1)	18·1 (17·8)
Antilog of log(mean)	15·2 (14·6)	14·7 (14·2)	16·2 (16·1)	15·1 (14·7)
Vitamin B_6 intake (mg/d)				
Mean	2·4 (3·1)	1·9 (1·9)	1·9 (2·0)	2·0 (2·2)
Antilog of log(mean)	1·9 (1·9)	1·8 (1·8)	1·7 (1·8)	1·8 (1·8)

* All subjects with vitamin B_6 status measurements were included in this analysis. The numbers in parentheses were adjusted by the population weighting factor, see p. 192. Significance of seasonal variation by ANOVA: log(pyridoxal phosphate): F 4·8, $P < 0·0001$; log(pyridoxic acid): F 1·7, $P = 0·16$; log(vitamin B_6 intake): F 1·2, $P = 0·32$.

Table 7. Log-mean values of vitamin B_6 status indicators by geographical regions of mainland Britain in subjects from the National Diet and Nutrition Survey: People Aged 65 Years and Over*

		Antilog of $\log_{10}$(plasma pyridoxal phosphate)			Antilog of $\log_{10}$(plasma pyridoxic acid)			Antilog of $\log_{10}$(vitamin B_6 intake)		
Region	*n*	A	B	C	A	B	C	A	B	C
Scotland	90–95	28·3	22·3	22·9	13·9	13·6	14·0	1·62	1·56	1·62
Northern England	145–148	27·7	24·1	24·6	12·9	12·5	12·9	1·65	1·59	1·65
Manchester/Liverpool	111–120	26·6	22·1	22·5	14·1	13·5	13·8	1·77	1·66	1·72
South Midlands	197–199	29·2	24·7	25·2	14·0	13·5	13·8	1·71	1·62	1·67
Wales	64	32·8	25·1	25·4	14·3	13·7	14·0	1·81	1·75	1·79
East Anglia	109–111	35·4	28·4	28·8	15·1	14·7	15·0	1·83	1·71	1·76
London and South East	203–214	37·2	29·4	28·5	16·6	15·8	15·2	1·98	1·87	1·77
Central Southern and South West	214–224	35·2	28·2	28·7	16·0	15·2	15·6	1·90	1·76	1·82
ANOVA F ratio (7 df)		3·6	4·9	4·2	3·7	3·5	2·6	3·3	3·4	2·4
P		0·0007	< 0·0001	0·0002	0·0006	0·0009	0·011	0·002	0·001	0·02

* The estimates were all adjusted by a population weighting factor, see p. 192. Series A were not further adjusted; series B were adjusted for age, sex, domicile, self-reported health and energy intake; series C were adjusted for all of these factors and also for regional differences in the use of vitamin B_6 supplements.

the vitamin B_6 intakes and both of the indices of B_6 status exhibited a significant north-south gradient, with the lower intakes and status indices occurring in the north of the country. The significance of this regional variation was not diminished by the inclusion of age, sex, domicile, energy intake and self-reported health (four categories) in the model. Examination of food choices indicated that people living in the south of Britain had greater intakes of several kinds of vitamin B_6-providing foods, including potatoes, green vegetables and salads, certain types of fruit, poultry, cheese, and fortified breakfast cereals (results not shown).

Discussion

Although a number of studies have indicated poor vitamin B_6 status and low dietary vitamin B_6 intakes in older people (Rose *et al*. 1976; Kant *et al*. 1988; Lowik *et al*. 1989, 1990, 1994; Manore *et al*. 1989; Euronut SENECA Investigators, 1991; Ribaya-Mercado *et al*. 1991; Russell & Suter, 1993; Ferroli & Trumbo, 1994; Pannemans *et al*. 1994; Riggs *et al*. 1996; Bailey *et al*. 1997), there remains considerable uncertainty about the functional significance and public health importance of this problem. One of the reasons for this uncertainty is the paucity of information about the relative usefulness of the different biochemical indices that have been used to define vitamin B_6 status (Leklem, 1990; Driskell, 1994; Kretsch *et al*. 1995). Plasma pyridoxal phosphate and urinary excretion of pyridoxic acid have both been proposed as status indicators, but there remains a need for clarification of the types of both nutritional and non-nutritional factors which can affect them.

The *National Diet and Nutrition Survey: People Aged 65 Years and Over* (Finch *et al*. 1998), together with our linked study of plasma total homocysteine (Bates *et al*. 1997), has provided an opportunity to re-examine these questions, in the context of a nationally-representative sample of older people living in mainland Britain. Although the task of obtaining a representative sample of older people is not easy, partly because those who are most frail are least likely to agree to participate, several specific precautions were taken to try to ensure that the sample was as representative as possible of the entire (census) population.

The comparisons shown in Tables 1 and 3 show that, whereas plasma pyridoxal phosphate concentrations tend to decline with age and increasing frailty (except for people living in institutions), those of plasma pyridoxic acid clearly increase with age. There were significant interactions between age, sex and type of domicile, particularly with respect to plasma pyridoxal phosphate, indicating that these three factors may affect vitamin B_6 status in a complex, interactive manner. An inverse relationship between age and plasma pyridoxal phosphate concentration has previously been reported (Rose *et al*. 1976; Joosten *et al*. 1993; Pannemans *et al*. 1994; Driskell, 1994). Possible reasons for this age trend (Kant *et al*. 1988; Lowik *et al*. 1989; Russell & Suter, 1993) might include: (a) lower vitamin B_6 intakes in older people; (b) less efficient retention of the vitamin; (c) increased B_6 catabolism. Clearly, total vitamin B_6 intake and the vitamin B_6 : energy value tend to decline with age, although the vitamin B_6 : protein value does not, and this may, in turn, affect plasma pyridoxal phosphate concentrations. Increased catabolism appears also to be supported by our data. The pyridoxic acid : pyridoxal phosphate value was only 0·22 in young people (Table 4) whereas it was 0·5 in people aged 65 years or over, and rose as high as 0·88 in one subgroup (Table 1). This was not entirely a function of renal impairment (see later discussion), because even those older people with low blood concentrations of urea and creatinine had higher pyridoxic acid : pyridoxal phosphate values than young people. The reason for the absence of a decline in plasma pyridoxal phosphate with age in people in institutions is not clear; however, it is likely that people who require institutional care at a relatively early age are more frail than their counterparts living in the community, and that this factor may dominate their status picture.

In contrast to the conclusions of Manore *et al*. (1989), of the Euronut SENECA Investigators (1991) and of Bailey *et al*. (1997), the data in Table 3 suggest that plasma pyridoxal phosphate provided a good reflection of dietary intakes of vitamin B_6 between individuals. The same was true for pyridoxic acid, which reflected vitamin B_6 intakes somewhat more closely at high than at low intakes, and, being a catabolic product, probably reflects the removal of amounts of absorbed vitamin B_6 that are greater than normal tissue requirements. The relationships between vitamin B_6 intake and its status indices were scarcely affected by the introduction of food energy or protein intakes into the model (Table 3), even though vitamin B_6 intake (and to a lesser extent plasma pyridoxal phosphate) was directly correlated with energy and with protein intakes. It is clear from Table 3 that energy (but not protein) intake could independently modulate the relationships between vitamin B_6 intake and the status indices, plasma pyridoxal phosphate and plasma pyridoxic acid. The lower the energy intake for a given vitamin B_6 intake, the higher the values of these blood status indices. The absence of an independent effect of protein intake on the vitamin B_6 status indices is in accord with observations by van der Wielen *et al*. (1996) who found that in elderly Europeans, the intake of animal protein lost its significance as a predictor of plasma pyridoxal phosphate concentration when vitamin B_6 intake was also included in their regression model. Thus, in elderly people, protein intake apparently may act as a weak proxy for vitamin B_6 intake if vitamin B_6 is not included in the model, but it does not exhibit any inverse relationship with the biochemical indices, which might occur if a high protein intake exerted a metabolic stress on the vitamin B_6-dependent pathways of amino acid turnover. Presumably the intake of protein is not great enough for that to occur. The contribution of vitamin B_6 from alcoholic drinks and the effect of alcoholic drinks on vitamin B_6 status of people participating in the UK survey, is addressed elsewhere (Walmusley *et al*. 1999). There is a significant increase in vitamin B_6 status with the inclusion of moderate amounts of alcoholic drinks in the diet.

A common problem with dietary estimation is that of under-reporting, and an approximate assessment of the extent of this problem, and a prediction of which individuals may be most affected, may be made on the basis of a comparison between calculated BMR (from ages and body weights) and estimates of energy intakes (Department of Health, 1992). However, many older people do lose weight,

so that a low estimate of energy intake may be perfectly genuine, and not the result of under-reporting. If the lowest third of subjects in the present survey with respect to their estimated energy intake : BMR value, i.e. those with a calculated BMR $<1{\cdot}06\times$ estimated energy intake (Goldberg *et al*. 1991), were omitted from the calculations, the estimated vitamin B_6 intake rose by 6 %; the vitamin B_6 : protein value rose by 2·1 %; plasma pyridoxal phosphate rose by 3·0 % and pyridoxic acid fell by 1·1 % (results not shown). Thus the vitamin B_6 intake and status indices were not greatly affected by this exclusion procedure, and under-reporting was not considered to be a major issue, with respect to the questions addressed by this study.

With regard to functional significance, many recent studies have focused on plasma homocysteine, as a powerful index and probable cause of increased risk of vascular disease, whose plasma and tissue concentrations respond favourably to improvements in folate, vitamin B_{12} and vitamin B_6 status (Brattstrom *et al*. 1990; Miller *et al*. 1992, 1994; Hu *et al*. 1993; Joosten *et al*. 1993; Selhub *et al*. 1993; Ubbink *et al*. 1993, 1996; Franken *et al*. 1994; Dalery *et al*. 1995; Ellis & McCully, 1995; Naurath *et al*. 1995; Robinson *et al*. 1995; Riggs *et al*. 1996; Verhoef *et al*. 1996). Vitamin B_6 supplements were observed to reduce the raised concentrations of homocysteine seen in patients with cystathionine β-synthase (*EC* 4.2.1.22) deficiency, but the role of this vitamin in the commoner forms of mild hyperhomocysteinaemia is less clear. The data in Table 5 show that in a univariate regression model, plasma pyridoxal phosphate level was very strongly correlated with plasma total homocysteine level, but that as other important B-vitamin determinants of homocysteine were added, in a multivariate linear regression model, pyridoxal phosphate lost some of its power of prediction and was therefore less robust as an independent predictor of homocysteine concentrations. The problem of covariance of B-vitamin status indices has complicated the picture; however, the conclusions of the present study are entirely compatible with those of Miller *et al*. (1992), Selhub *et al*. (1993), Franken *et al*. (1994), Ubbink *et al*. (1996) and Verhoef *et al*. (1996). The plasma samples used in the present study were nearly all from fasting subjects. The weight of evidence suggests that vitamin B_6 status is more important as a determinant of plasma homocysteine in non-fasting subjects, and especially following a methionine load (Brattstrom *et al*. 1990; Miller *et al*. 1992, 1994; Franken *et al*. 1994; Ubbink *et al*. 1996; Mansoor, 1997). The relationship of pyridoxic acid with homocysteine may be confounded by its strong relationship with renal function in older people.

Correlations between the vitamin B_6 status indicators and indicators of renal function, namely plasma urea and creatinine, were significant for pyridoxic acid but not for pyridoxal phosphate. Pyridoxic acid is likely to be retained in the blood if renal function is impaired, and vice versa, its excretion is likely to be enhanced if diuretics are used. Correlations which were significant for pyridoxal phosphate but not pyridoxic acid included inverse relationships with plasma α_1-antichymotrypsin, alkaline phosphatase (*EC* 3.1.3.1), and leucocyte count, and direct relationships with plasma Zn, albumin, Fe, cholesterol and blood haemoglobin. This constellation of factors may constitute a measure of frailty, and of activation of the acute-phase reaction, since the inverse correlation with α_1-antichymotrypsin was particularly strong. The strong inverse correlation with alkaline phosphatase may imply an increased rate of degradation of pyridoxal phosphate to free pyridoxal, in people with a high activity of this enzyme (Kant *et al*. 1988). Factors such as these, and the critical relationship between the timing of dietary estimates and that of phlebotomy for biochemical measurements, may account for some of the discrepancies recorded between previous studies, with respect to the correlations observed between intake estimates and the biochemical picture.

The seasonal variation in plasma pyridoxal phosphate concentrations in the present study, which was not matched by any detectable variation in pyridoxic acid or in vitamin B_6 intakes (Table 6), may be linked to winter illness. As noted earlier, plasma pyridoxal phosphate, like plasma retinol, Fe, Zn, Cu, Se and vitamin C, appears to reflect acute-phase status, in addition to specific micronutrient adequacy. Because pyridoxic acid does not appear to share this sensitivity to acute-phase status, it may be able to provide some information about vitamin B_6 adequacy which is complementary to that of pyridoxal phosphate. It may, of course, be better to measure pyridoxic acid in urine rather than in plasma, and to express it as the rate of urinary excretion per 24 h, or per unit of creatinine. However, this requires additional fieldwork procedures and additional sample analyses, which increase costs and workload. The strength of the present protocol was that all the biochemical information on vitamin B_6 status from each subject was obtained in a single chromatographic run, and from a single biological sample.

Table 7 shows that pyridoxal phosphate, pyridoxic acid, and vitamin B_6 intakes all exhibited a significant north-south geographical gradient, consistent with evidence (Bates *et al*. 1997; Finch *et al*. 1998) that B-vitamin-rich foods are less frequently used by people living in the north than in the south of Britain.

With the previously-proposed lower cut-off limit for normal vitamin B_6 status of 30 nmol pyridoxal phosphate/l plasma (Leklem, 1990; Driskell, 1994), $561/1175 = 47{\cdot}7$ % of the survey participants were found to have subnormal status (see Bailey *et al*. 1997). For those living in institutions, this proportion was even higher at $190/255 = 75{\cdot}5$ %. With the more conservative cut-off value of 20 nmol/l, used by the Euronut SENECA Investigators (1991), the proportion of biochemically deficient values was 24·5 % overall and 46 % of those in institutions. Of the 24·5 % with plasma pyridoxal phosphate concentrations below 20 nmol/l, 72 % also had pyridoxic acid concentrations below the median value of 15 nmol/l. This estimate of 24·5 % biochemical deficiency is very close to the estimate of 23·3 % for older people living in other European countries (Euronut SENECA Investigators, 1991). Low vitamin B_6 status in elderly Americans living in institutions has also been reported previously (Russell & Suter, 1993).

Ribaya-Mercado *et al*. (1991) found, in a metabolic ward-based study with controlled intakes, that healthy elderly people required a daily intake of about 1·90–1·96 mg vitamin B_6 to achieve adequate status. If the calculations in the previous paragraph are applied to those National Diet

and Nutrition Survey participants (*n* 525) whose estimated vitamin B_6 intakes were 1·96 mg/d or greater, 27 % had plasma pyridoxal phosphate concentrations below 30 nmol/l and 11 % had concentrations below 20 nmol/l. Of the eighty people in institutions with intakes of 1·96 mg vitamin B_6/d or greater, 65 % had plasma pyridoxal phosphate concentrations below 30 nmol/l and 32 % had concentrations below 20 nmol/l. This might suggest that an intake that has been deemed to be adequate does not always result in adequate status. However, the individual vitamin B_6 intake estimates in the survey may not have been truly representative of long-term intakes, and comparisons with metabolic ward studies are, thus, not always easy to interpret.

In contrast to the biochemical picture, which seems to indicate a high prevalence of vitamin B_6 deficiency in older British people, the estimated vitamin B_6 intakes in this study population did not appear to be low in relation to the dietary reference values (Department of Health, 1991), which are calculated as a ratio to protein intakes. The mean intake of vitamin B_6 overall was 152 % of the reference nutrient intake in men and 134 % in women. Only 2 % of men and 3 % of woman had estimated vitamin B_6 intakes below the lower reference nutrient intake (Finch *et al*. 1998). A very similar picture was observed by Bailey *et al*. (1997). We now need to ask: are the dietary reference values set too low for older people?; is the ratio to protein entirely appropriate for people with low protein intakes?; is the biochemical index cut-off limit set too high?, or are there complex metabolic reasons why the biochemical index may not accurately reflect vitamin B_6 status in older people? Similar questions also need to be asked for many other micronutrients, in older people.

Since vitamin B_6 status probably affects immune function (Talbott *et al*. 1987) and cognitive performance (Riggs *et al*. 1996) in older people, such questions about vitamin B_6 adequacy seem highly relevant for the definition of public health nutrition policy and priorities.

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