Original Article

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2012.02570.x

Vitamin B6 status and interferon-γ-mediated immune activation in primary hyperparathyroidism

M. H. E. Christensen[1,2], E. K. R. Pedersen[1,3], Y. Nordbø[4], J. E. Varhaug[4,5], Ø. Midttun[6], P. M. Ueland[1,7], O. K. Nygård[1,3], G. Mellgren[1,2] & E. A. Lien[1,2]

From the [1]Institute of Medicine, University of Bergen; [2]Hormone Laboratory, Haukeland University Hospital; [3]Department of Heart Disease, Haukeland University Hospital; [4]Department of Surgery, Haukeland University Hospital; [5]Institute of Surgical Science, University of Bergen; [6]Bevital A/S; and [7]Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway

Abstract. Christensen MHE, Pedersen EKR, Nordbø Y, Varhaug JE, Midttun Ø, Ueland PM, Nygård OK, Mellgren G, Lien EA (Institute of Medicine, University of Bergen, Bergen; Hormone Laboratory, Haukeland University Hospital, Bergen; Department of Heart Disease, Haukeland University Hospital, Bergen; Department of Surgery, Haukeland University Hospital, Bergen; Institute of Surgical Science, University of Bergen, Bergen; Bevital A/S, Bergen; Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway). Vitamin B6 status and interferon-γ-mediated immune activation in primary hyperparathyroidism. *J Intern Med* 2012; **272**: 583–591.

Objectives. Primary hyperparathyroidism (PHPT) has been associated with low-grade inflammation and elevated risk of cardiovascular disease (CVD). In inflammatory conditions, interferon-γ (IFN-γ) activity is enhanced and a decreased circulating concentration of vitamin B6 is often observed. Such changes in IFN-γ activity or vitamin B6 levels have been associated with increased incidence of CVD. The aim of the study was to investigate systemic markers of IFN-γ-mediated immune activation, such as neopterin, the kynurenine-to-tryptophan ratio (KTR) and kynurenine pathway metabolites, as well as B6 vitamers in patients with PHPT.

Design/subjects. A total of 57 patients with PHPT and a control group of 20 healthy blood donors were included in this study. PHPT patients who responded positively to parathyroidectomy were followed for 6 months. Forty-three patients participated in the longitudinal study in which blood samples were taken at inclusion and 1, 3 and 6 months after surgery.

Results. Plasma concentrations of the B6 vitamers pyridoxal 5′-phosphate (PLP) ($P = 0.007$) and pyridoxal ($P = 0.013$) were significantly lower in the patient group compared to healthy control subjects. An increase in the KTR indicated that the kynurenine pathway of tryptophan metabolism was altered in PHPT patients ($P = 0.015$). During the initial 6 months after surgery, levels of PLP ($P < 0.001$) and anthranilic acid ($P < 0.001$) increased significantly, whereas neopterin decreased ($P = 0.018$).

Conclusions. The results of this study demonstrate altered levels of vitamin B6 and the KTR in PHPT patients, both of which may reflect cellular immune activation. These abnormalities should be considered in relation to the increased risk of CVD previously observed in patients with PHPT.

Keywords: inflammation, kynurenine-to-tryptophan ratio, neopterin, primary hyperparathyroidism, vitamin B6.

Introduction

Enhanced serum concentrations of parathyroid hormone (PTH) increase bone resorption. This results in elevated levels of serum calcium (Ca) and decreased bone density [1]. Furthermore, increased levels of inflammation may be observed in patients with primary hyperparathyroidism (PHPT). Elevated concentrations of inflammatory markers such as high-sensitivity C-reactive protein (CRP) and tumor necrosis factor-α were demonstrated in some [2] but not all studies [3]. In the majority of cases, PHPT is caused by either single adenomas or glandular hyperplasia producing excessive amounts of PTH. Parathyroidectomy has a cure rate as high as 90–95% [4]. Postoperatively there is a reversal of bone loss [5], but follow-up of patients 2 years after surgery has not shown beneficial effects on known inflammatory markers [2, 6].

It was shown in a long-term follow-up study that PHPT patients are at increased risk of death from

cardiovascular disease (CVD) [7]. Inflammatory pathways are centrally involved in atherogenesis and atherosclerotic plaques are infiltrated by activated macrophages and lymphocytes, which probably contribute actively to disease progression [8]. In patients with stable angina pectoris, the monocyte-activating cytokine interferon-γ (IFN-γ) has been linked to acute atherosclerotic complications including major coronary events and death [9, 10]. As IFN-γ has a short half-life, other circulating markers are often used as indicators of the activity of this cytokine. The conversion of tryptophan to kynurenine is mediated through the enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). Enhanced levels of IFN-γ lead to increased IDO activity and thus an increase in the kynurenine-to-tryptophan ratio (KTR) [11]. As IFN-γ enhances the production of neopterin by activated macrophages, neopterin is another marker of IFN-γ activity [12].

The kynurenine pathway of tryptophan catabolism is dependent on enzymes requiring either vitamin B6 or vitamin B2 as co-factors (Fig. 1). Pyridoxal 5′phosphate (PLP) is the most commonly used serum marker of vitamin B6 status. It is the active form of vitamin B6 and reflects the total body stores of this vitamin [13]. Low levels of PLP have been linked to conditions associated with inflammation, such as rheumatoid arthritis, CVD and the metabolic syndrome [14–16].

We have recently reported that genes involved in inflammation were up-regulated in adipose tissue from patients with PHPT. Many of the up-regulated genes were related to infiltration and activation of macrophages, possibly through enhanced IFN-γ activity [17]. To our knowledge, systemic markers of IFN-γ-induced inflammation have not been studied in patients with PHPT. The primary objective of this study was to investigate the IFN-γ markers KTR and neopterin as well as metabolites of the kynurenine pathway of tryptophan together with serum levels of B6 vitamers in patients with PHPT. A further aim was to determine the extent to which the levels of these biomarkers change following parathyroidectomy.

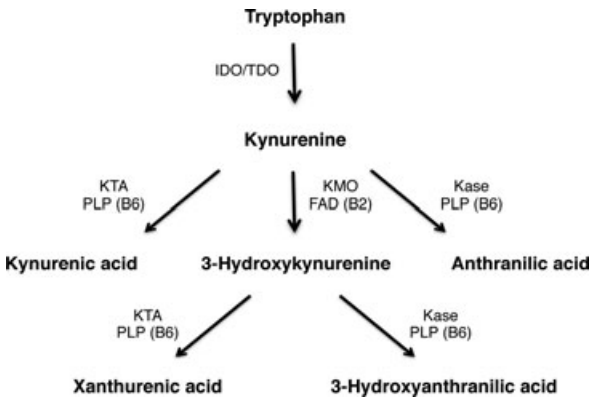

Fig. 1 *The kynurenine pathway of tryptophan metabolism. IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan 2,3-dioxygenase; KTA, kynurenine transaminase; KMO, kynurenine 3-monooxygenase; Kase, kynureninase; PLP, pyridoxal 5′-phosphate; FAD, flavine adenine dinucleotide; B2, vitamin B2; B6, vitamin B6.*

Materials and methods

Participants

A total of 57 (48 women and nine men) patients undergoing surgery for PHPT were included in the study; 20 (15 women and five men) healthy blood donors participated as control subjects. All participants were recruited from September 2007 to December 2010 and patients underwent surgery at the Department of Endocrine Surgery, Haukeland University Hospital, Bergen, Norway. Study participants were consecutive consenting patients and controls who met the inclusion criteria. The diagnosis of PHPT was based on serum levels above the reference ranges of both PTH (reference range: 1.3–6.8 pmol L^{-1}) and albumin-corrected Ca (reference range: 2.20–2.55 mmol L^{-1}). Exclusion criteria were known systemic inflammatory disease, such as inflammatory bowel disease, rheumatoid diseases and chronic obstructive lung disease, as well as genetically confirmed multiple endocrine neoplasia type I (MEN-1 syndrom), based on verified mutations in the *MEN1* gene. Blood donors with serum levels of PTH or corrected Ca outside the reference range were also excluded from the control group. Anthrophometric data and medical history were recorded prior to surgery, and blood samples were collected on the day before surgery. Participants were not required to fast before blood sampling.

In 43 (five men and 38 women) patients, blood samples were additionally drawn 1, 3 and 6 months after surgery. Patients received a written request for blood samples to be collected at these prescheduled timepoints. Exclusion criteria for participating in the longitudinal study were persistently elevated corrected serum Ca levels during the 6 months of follow-up.

The study was approved by the Western Norway Regional Committee for Medical Research Ethics. All enrolled subjects provided informed written consent,

and the study was performed according to the principles of the Declaration of Helsinki.

Biochemical analysis and anthrophometric data

All blood samples were centrifuged within 2 h after collection and EDTA-anticoagulated plasma was frozen at −80 °C. Levels of Ca, albumin, phosphate, total alkaline phosphatase (ALP), alanine transaminase (ALAT) and CRP in serum were analysed immediately at the Laboratory of Clinical Biochemisty, Haukeland University Hospital, using the Modular P-system from Roche Diagnostics (Basel, Switzerland). PTH was measured at the Hormone Laboratory, Haukeland University Hospital, using a two-site chemiluminescent immunometric assay for intact PTH (Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL, USA). The inter-assay variation was 6.3% and 8.8% at concentrations of 5.6 and 40 pmol L^{-1}, respectively. Plasma concentrations of tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, neopterin, PLP, pyridoxal (PL), riboflavin and flavin mononucleotide were analysed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) at Bevital A/S, Bergen, Norway [18]. Neopterin was analysed using the method of protein precipitation by trichloroacetic acid, which oxidizes 7,8-dihydroneopterin to neopterin. This method thus yields total neopterin concentration, and the values are therefore higher than those obtained using assays measuring only neopterin [19]. Because IFN-γ induces a step that precedes formation of 7,8-dihydroneopterin in the neopterin pathway, neopterin alone and total neopterin (neopterin plus 7,8-dihydroneopterin) both reflect inflammation. The use of total neopterin is further supported by the strong correlation between total neopterin levels in serum and in urine [20]. Serum cobalamin was determined using a *Lactobacillus leichmannii* microbiological assay [21] and serum folate using a *Lactobacillus casei* microbiological assay [22]. Creatinine concentration was determined by including creatinine and its deuterated internal standard (d3-creatinine) in an established LC-MS/MS assay using the ion pairs 114/44.2 and 117/47.2, respectively [23].

Albumin-corrected Ca was calculated using Payne's formula: corrected Ca = measured serum Ca+ 0.025* (42 − measured albumin) [24]. The isotope dilution mass spectrometry traceable formula developed by the Modification of Diet in Renal Disease Study Group was used to calculate estimated glomerular filtration rate (eGFR): $eGFR = 175 \times (\text{s-creatinine}/88.4)^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ if female [25]. The KTR was calculated by dividing the plasma concentration of kynurenine (nmol L^{-1}) by that of tryptophan (μmol L^{-1}). Body mass index (BMI) was calculated as weight divided by the square of height (kg m^{-2}). CRP levels below the lowest detection limit for this analysis (CRP < 0.5 mg L^{-1}) were included in the calculations as 0.4 mg L^{-1}.

Statistical analysis

Continuous variables are reported as median (25th–75th percentile) or mean (standard deviation) values and categorical variables as numbers (percentages). To assess differences in continuous variables at baseline between patients and controls, we used the independent samples *t*-test. Where variables were not normally distributed, the Mann–Whitney *U*-test was used. Age- and gender-adjusted analysis of covariance (ANCOVA) was used to assess differences in the kynurenine pathway of tryptophan, neopterin and the B vitamins. Where distributional assumptions were violated we used distribution-free median regression estimates [26]. Correlations among continuous variables were assessed by Spearman rank correlation corrected for age, gender and eGFR. Linear trends over time between repeated end-point measures were estimated with a random intercept mixed model. Paired samples *t*-test was used to assess differences between longitudinal samples at inclusion and after 1 or 6 months. For variables with skewed distribution (CRP, PTH and ALP), Wilcoxon signed-rank test was used for comparisons. All tests were two-sided and $P < 0.05$ was considered to be statistically significant.

Statistical analyses were performed using SPSS Statistics 19 for Mac (IBM Corporation, New York, NY, USA) and R version 2.14.1 (R Foundation for Statistical Computing, Vienna, Austria). The random intercept mixed model was computed using the NLME-package version 3.1-103 in R (version 2.14.1).

Results

Subject characteristics

Baseline characteristics of the study population are shown in Table 1. Serum levels of PTH, corrected Ca and ALP were increased in patients compared to controls, whereas phosphate and creatinine levels were lower in patients. Of note, eGFR did not differ significantly between the two groups, indicating that kidney function was not impaired in the PHPT patients. Two of the patients had dietary regulated type II diabetes mellitus. One patient previously

Table 1 *Baseline characteristics of PHPT patients and control subjects at inclusion*

	PHPT (n = 57)	Controls (n = 20)	P-value
Females[a]	48 (84.2)	15 (75.0)	
Age (years)	59.7 (12.2)	56.8 (4.34)	0.12
BMI (kg m^{-2})	26.0 (4.35)	25.3 (2.97)	0.48
PTH (1.3–6.8 pmol L^{-1})	12.4 (9.65–17.9)	3.8 (2.60–4.08)	<0.001
Corrected Ca (0.2–2.55 mmol L^{-1})	2.68 (0.17)	2.29 (0.12)	<0.001
Phosphate (0.85–1.50 mmol L^{-1})	0.83 (0.17)	1.19 (0.20)	<0.001
ALP (35–105 U L^{-1})	87.0 (71.0–116.5)	64.5 (53.5–74.0)	<0.001
Albumin (34–48 g L^{-1})	45.7 (2.44)	47.2 (4.33)	0.17
Creatinine (45–90 μmol L^{-1})	65.4 (59.1–76.3)	75.8 (65.5–90.7)	0.042
eGFR (>60 mL min^{-1} per 1.73 m^2)	83.7 (21.6)	74.5 (16.2)	0.085
ALAT (10–70 U L^{-1})	26.0 (18.0–31.8)	21.5 (16.3–31.0)	0.32

PHPT, primary hyperparathyroidism; BMI, body mass index; PTH, parathyroid hormone; corrected Ca, albumin-corrected calcium; ALP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; ALAT, alanine transaminase.
Values are given as mean (SD) or median (25th–75th percentile) unless otherwise indicated. Reference values are shown in parentheses in the left-hand column. P-values are based on Student's t-test or Mann–Whitney U-test.
[a]Values are numbers (percentages).

underwent coronary artery bypass surgery and another patient previously had a cerebrovascular accident. Atherosclerotic disease was not reported in the other patients or in any control subjects.

Inflammatory markers and correlations at baseline

Levels of the vitamin B6 forms PLP and PL were significantly lower in the patient group than in the control group at inclusion. Furthermore, levels of kynurenine and the KTR were significantly higher and levels of tryptophan, anthranilic acid and xanthurenic acid were lower in patients compared to controls. Neopterin levels did not differ between the two groups (Table 2).

Correlations between different plasma indices were assessed in patients with PHPT at inclusion. PTH was positively correlated with corrected Ca (r = 0.60, P < 0.001), but negatively correlated with the vitamin B6 form PLP (r = −0.27, P = 0.048) and with CRP (r = −0.313, P = 0.024). PLP was not correlated with either CRP (P = 0.12) or the KTR (P = 0.75), but was negatively correlated with neopterin (r = −0.27, P = ;0.048). KTR and neopterin values were strongly correlated (r = 0.50, P < 0.001), as were kynurenine and neopterin (r = 0.27, P = 0.047).

Changes in parathyroid status after surgery

All patients underwent surgery for PHPT caused by an adenoma or multiglandular hyperplasia. Patients were considered cured if serum-corrected Ca was within the normal range 6 months after surgery. Of the 57 patients included, 43 completed the follow-up study. The remaining patients were excluded from the longitudinal study because of either persistently elevated serum levels of corrected Ca (n = 4) or unavailability of blood samples at the prescheduled time-points after surgery (n = 10). Median age in this subgroup of 43 patients was 61.0 years and median BMI was 26.3 kg m^{-2}.

Six months after surgery, patients in the longitudinal study had significantly lower levels of PTH (median 3.90 vs. 12.6 pmol L^{-1}, P < 0.001), corrected Ca (mean 2.32 vs. 2.69 mmol L^{-1}, P < 0.001), ALP (median 71.0 vs. 87.0 U L^{-1}, P < 0.001) and albumin (mean 44.5 vs. 45.5 g L^{-1}, P = 0.004) compared to the levels at inclusion.

Changes in inflammatory markers and vitamin B6 status during follow-up

Changes in inflammatory markers and B6 status after parathyroidectomy and recovery from PHPT were evaluated during 6 months of follow-up (Table 3). A significant increase in the levels of PLP, anthranilic acid and CRP occurred after 6 months of follow-up, whereas neopterin concentration decreased significantly. PLP (P = 0.006) and anthranilic acid (P = 0.003) were already increased at 1 month after surgery, compared to the respective baseline concentrations. At 6 months after surgery,

Table 2 *Levels of kynurenines, inflammatory markers and B vitamins in PHPT patients and control subjects at inclusion*

	PHPT (n = 57)	Controls (n = 20)	P-value
Tryptophan and kynurenines			
Tryptophan (μmol L^{-1})	61.0 (13.0)	69.6 (9.93)	0.007
Kynurenine (μmol L)	1.58 (1.38–1.98)	1.50 (1.42–1.74)	0.029
Kynurenic acid (nmol L^{-1})	50.0 (40.3–62.8)	52.8 (43.3–56.2)	0.55
Anthranilic acid (nmol L^{-1})	12.7 (3.81)	15.4 (5.98)	0.013
3-Hydroxykynurenine (nmol L^{-1})	39.1 (31.2–53.0)	35.3 (31.7–45.3)	0.86
Xanthurenic acid (nmol L^{-1})	14.9 (10.4–19.6)	20.3 (15.0–24.0)	0.013
3-Hydroxyanthanilic acid (nmol L^{-1})	33.2 (26.9–44.0)	38.4 (30.3–53.4)	0.57
Markers of inflammation			
KTR (nmol μmol^{-1})	28.4 (8.28)	23.2 (4.47)	0.015
Neopterin (nmol L^{-1})	11.2 (6.89–14.5)	10.0 (8.54–13.1)	0.36
CRP (mg L^{-1})	1.00 (0.40–3.00)	1.00 (0.40–3.00)	1.00
B vitamins			
Pyridoxal 5′-phosphate (B6) (nmol L^{-1})	39.3 (21.7)	54.2 (28.7)	0.015
Pyridoxal (B6) (nmol L^{-1})	8.82 (6.75–11.7)	13.1 (9.47–16.1)	0.011
Riboflavin (B2) (nmol L^{-1})	10.3 (6.82–15.3)	10.9 (7.72–21.6)	0.82
Flavin mononucleotide (B2) (nmol L^{-1})	10.6 (7.90–12.1)	12.1 (7.93–15.7)	0.51
Cobalamin (B12) (pmol L^{-1})	422 (361–545)	405 (343–491)	0.57
Folate (nmol L^{-1})	5.64 (3.72–9.17)	7.87 (5.5711.0)	0.08

PHPT, primary hyperparathyroidism; KTR, kynurenine-to-tryptophan ratio, CRP, C-reactive protein.
Values are given as mean (standard deviation) or median (25th–75th percentile). P-values are based on ANOVA or quantile regression where appropriate, adjusted for age and gender.

there were no significant correlations between PLP and PTH, CRP or the KTR (all $P > 0.2$).

Discussion

Kynurenine levels and the KTR were higher and levels of tryptophan, anthranilic acid, xanthurenic acid and B6 vitamers were lower in the 57 patients with PHPT, compared to the levels in 20 healthy control subjects. During 6 months of follow-up in 43 PHPT patients after parathyroidectomy, concentrations of PLP and anthranilic acid increased. During the same time period, the concentration of neopterin decreased whereas the KTR was essentially unchanged. These observations suggest that IFN-γ-mediated cellular immune activation was enhanced and vitamin B6 status was altered in patients with PHPT. Within months after surgery, the vitamin B6 status appeared to be normalized and neopterin concentration reduced, but some changes in tryptophan metabolism persisted.

The observed changes in tryptophan and its metabolites, in particular the KTR, in patients with PHPT, may be explained by increased conversion of tryptophan to kynurenine catalysed by IDO or TDO [27]. The increased KTR probably mainly reflects IFN-γ stimulation of IDO linked to increased cellular Th1 immune activation. This is supported by the strong correlation that we observed between the KTR and neopterin, which is in agreement with previous results [28] and explained by IFN-γ being the main inducer of both neopterin and kynurenine formation [11]. A decrease in neopterin concentration during the 6 months after surgery may be explained by a decrease in IFN-γ activity after parathyroidectomy. Of note, levels of CRP actually increased 6 months after surgery among those patients with available samples, although levels were identical in patients and controls at inclusion. This observation is in line with earlier reports describing elevated levels of CRP and interleukin-6 (IL-6) 1 year after parathyroidectomy [3, 29]. It is possible that incomplete inflammatory recovery at 6 months after surgery may also explain the modest, nonsignificant reduction in the KTR seen in our study after parathyroidectomy.

PTH stimulates the release of inflammatory cytokines such as IL-6 from bone cells and haematopoietic cells

Table 3 *Longitudinal changes in levels of kynurenines, inflammatory markers and B vitamins in PHPT patients after surgery*

		Time after surgery (months)			
	Inclusion	1	3	6	
	(n = 43)	(n = 37)	(n = 37)	(n = 41)	P-value
Tryptophan and kynurenines					
Tryptophan (μmol L^{-1})	61.1 (13.0)	63.3 (11.4)	65.4 (12.3)	63.5 (11.0)	0.29
Kynurenine (μmol L^{-1})	1.67 (0.44)	1.72 (0.39)	1.70 (0.38)	1.69 (0.44)	0.88
Kynurenic acid (nmol L^{-1})	51.1 (20.1)	56.7 (28.6)	50.3 (22.7)	56.0 (27.9)	0.11
Anthranilic acid (nmol L^{-1})	12.3 (3.82)	14.4 (5.47)	13.4 (4.29)	15.0 (4.94)	<0.001
3-Hydroxykynurenine (nmol L^{-1})	41.7 (14.5)	39.5 (13.4)	41.8 (13.1)	39.3 (11.1)	0.24
Xanthurenic acid (nmol L^{-1})	15.7 (8.45)	17.5 (8.18)	17.8 (10.1)	17.2 (8.78)	0.20
3-Hydroxyanthranilic acid (nmol L^{-1})	36.2 (15.1)	36.5 (14.3)	38.2 (16.2)	35.8 (15.3)	0.87
Markers of inflammation					
KTR (nmol μmol^{-1})	28.1 (8.25)	27.9 (7.70)	26.8 (7.50)	26.8 (6.16)	0.054
Neopterin (nmol L^{-1})	12.4 (5.63)	12.7 (6.03)	11.6 (8.00)	10.9 (4.26)	0.018
CRP (mg L^{-1})[a,b]	1.0 (0.4–0.3)			2.0 (1.0–5.0)	0.014
B vitamins					
Pyridoxal 5′-phosphate (B6) (nmol L^{-1})	37.0 (23.0)	48.0 (29.6)	46.2 (24.3)	52.7 (33.1)	<0.001
Pyridoxal (B6) (nmol L^{-1})	12.1 (14.3)	12.3 (11.6)	12.7 (13.9)	14.8 (17.6)	0.21
Riboflavin (B2) (nmol L^{-1})	15.2 (17.0)	12.4 (9.25)	13.3 (10.8)	16.7 (26.5)	0.86
Flavin mononucleotide (B2) (nmol L^{-1})	10.5 (3.36)	11.5 (5.04)	11.8 (3.99)	10.6 (3.26)	0.68
Cobalamin (B12) (μmol L^{-1})	479 (203)	535 (163)	495 (157)	481 (154)	0.52
Folate (μmol L^{-1})	12.5 (25.4)	10.1 (12.2)	10.6 (22.7)	10.6 (17.0)	0.42

Values are given as mean (standard deviation) unless otherwise indicated. P-values for trend over time estimated with a random intercept mixed model, except for CRP where P-value was based on Wilcoxon signed-rank test.
PHPT, primary hyperparathyroidism; KTR, kynurenine-to-tryptophan ratio.
[a]C-reactive protein measurements at 1 and 3 months after surgery were not available.
[b]Median (25th–75th percentile).

[30, 31]. IL-6 is the primary inducer of the acute phase response of the innate immune system [32] and may contribute to the mild inflammation observed in PHPT patients.

The most important observation in this study is a marked reduction in circulating PLP and PL in PHPT patients; PLP was normalized 1 month after parathyroidectomy. Other B vitamins were not affected. PHPT is thus another example of the many chronic inflammatory conditions characterized by a low concentration of vitamin B6 [16].

The mechanisms by which PLP is decreased during inflammation are not fully understood. Chronic inflammatory diseases are complex and the causes of reduced levels of PLP may be different from those of elevated levels of CRP. In this study, we did not observe an inverse correlation between CRP and PLP, as described by others [15]. However, our results are in line with those of a study of patients with coronary artery disease, in which lower levels of PLP were found to be associated with an increased risk of coronary artery disease, independent of levels of CPR [14]. Supplementation with pyridoxine does not normalize levels of inflammatory markers [33] and does not attenuate the inverse relation between such markers and PLP [34]. These observations from intervention studies indicate that inflammation and immune activation do not cause vitamin B6 deficiency but rather change the distribution of B6 vitamers between and within intracellular and extracellular compartments. Low vitamin B6 levels during inflammation and immune activation may reflect increased demand and cellular uptake of PLP due to quantitative changes in protein turnover and involvement of PLP

in cytokine production and lymphocyte proliferation [33, 35]. Altered distribution of vitamin B6 is in line with low levels of the kynurenine metabolites anthranilic acid and xanthurenic acid in PHPT patient, as both metabolites are formed by enzymes requiring PLP as a cofactor [36]. After surgery, we observed an increase in the level of anthranilic acid, a metabolite that has previously been shown to increase in response to pyridoxine supplementation [37].

In line with earlier studies [38], we observed higher levels of ALP in patients with PHPT. Higher serum concentrations of ALP probably reflect increased bone turnover and may be related to disease severity [39]. Also, inflammatory processes and an increased incidence of aortic calcification have been correlated with enhanced serum levels of ALP [40–42], but whether ALP *per se* has a role in the inflammatory response or is a product of the inflammatory processes is not clear [43]. It is well documented that in conditions that are accompanied by increased plasma ALP, such as bone and liver diseases, PLP levels are reduced [44]. This is explained by hydrolysis of PLP to PL catalysed by ALP. PL is the transport form of vitamin B6 for most tissues [45], and enhanced ALP may therefore promote cellular uptake and affect the distribution of this vitamin. Thus, increased ALP may reduce plasma PLP, but does not explain the low level of PL that we observed in PHPT patients.

A strength of this study is the measurement of two vitamin B6 species to assess its status; this is a particular advantage under conditions of elevated ALP which may affect PLP level [44]. Another strength is the simultaneous measurement of several kynurenines, some of which are metabolized by vitamin B6-dependent enzymes. Furthermore, the metabolic abnormalities observed in PHPT patients were monitored at three time-points during a follow-up period of 6 months after parathyroidectomy. However, only 43 of 57 (75%) patients completed the longitudinal study; the remaining patients were excluded because of either lack of postoperative blood samples or consistently elevated serum levels of corrected Ca after surgery.

Because food intake may influence plasma levels of vitamin B6 [46] and tryptophan [47], a possible limitation of this study is that patients were nonfasting and we did not have information on time since the last meal. However, measurement of the ratio between kynurenines and tryptophan (KTR) may correct for possible postprandial changes [11]. Gender distribution was uneven between the patient and control groups. This was due to strict inclusion criteria in the control group and a limited number of available blood donors. Changes in kynurenines, inflammatory markers and B vitamins were adjusted for gender to correct for this. Additionally, the results were unchanged in a subgroup analysis of women only (data not shown).

In summary, patients with PHPT had decreased vitamin B6 levels and an elevated KTR. The latter points to an increased IFN-γ-mediated cellular immune activation in these patients. Some but not all abnormalities in levels of kynurenines, inflammatory markers and B vitamins were corrected 6 months after parathyroidectomy. Low plasma vitamin B6 concentration and an elevated KTR are associated with increased risk of CVD and should be evaluated as CVD risk factors in PHPT.

Conflict of interest statement

No conflict of interest to declare.

Acknowledgements

We are very grateful for excellent statistical help from Reinhard Seifert at the Department of Heart Disease, Haukeland University Hospital. We also thank the staff at the Hormone Laboratory, Haukeland University Hospital, in particular, Hege Skavøy Hoff, for technical assistance. Funding for this project was provided by Samarbeidsorganet Helse Vest RHF and by Familien Blix Fond til Fremme av Medisinsk Forskning, Oslo, Norway.

References

1 Moosgaard B, Vestergaard P, Heickendorff L, Melsen F, Christiansen P, Mosekilde L. Vitamin D status, seasonal variations, parathyroid adenoma weight and bone mineral density in primary hyperparathyroidism. *Clin Endocrinol (Oxf)* 2005; **63:** 506–13.
2 Ogard CG, Engelmann MD, Kistorp C, Nielsen SL, Vestergaard H. Increased plasma N-terminal pro-B-type natriuretic peptide and markers of inflammation related to atherosclerosis in patients with primary hyperparathyroidism. *Clin Endocrinol (Oxf)* 2005; **63:** 493–8.
3 Farahnak P, Larfars G, Sten-Linder M, Nilsson IL. Mild primary hyperparathyroidism: vitamin D deficiency and cardiovascular risk markers. *J Clin Endocrinol Metab* 2011; **96:** 2112–8.
4 Rodgers SE, Lew JI, Solorzano CC. Primary hyperparathyroidism. *Curr Opin Oncol* 2008; **20:** 52–8.
5 Bollerslev J, Jansson S, Mollerup CL *et al.* Medical observation, compared with parathyroidectomy, for asymptomatic primary hyperparathyroidism: a prospective, randomized trial. *J Clin Endocrinol Metab* 2007; **92:** 1687–92.

6 Bollerslev J, Rosen T, Mollerup CL *et al.* Effect of surgery on cardiovascular risk factors in mild primary hyperparathyroidism. *J Clin Endocrinol Metab* 2009; **94:** 2255–61.
7 Hagstrom E, Hellman P, Larsson TE *et al.* Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation* 2009; **119:** 2765–71.
8 Bu DX, Erl W, de Martin R, Hansson GK, Yan ZQ. IKKbeta-dependent NF-kappaB pathway controls vascular inflammation and intimal hyperplasia. *FASEB J* 2005; **19:** 1293–5.
9 Delneste Y, Charbonnier P, Herbault N *et al.* Interferon-gamma switches monocyte differentiation from dendritic cells to macrophages. *Blood* 2003; **101:** 143–50.
10 Pedersen ER, Midttun O, Ueland PM *et al.* Systemic markers of interferon-gamma-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol* 2011; **31:** 698–704.
11 Schrocksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta* 2006; **364:** 82–90.
12 Huber C, Batchelor JR, Fuchs D *et al.* Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. *J Exp Med* 1984; **160:** 310–6.
13 Lui A, Lumeng L, Aronoff GR, Li TK. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *J Lab Clin Med* 1985; **106:** 491–7.
14 Cheng CH, Lin PT, Liaw YP *et al.* Plasma pyridoxal 5′-phosphate and high-sensitivity C-reactive protein are independently associated with an increased risk of coronary artery disease. *Nutrition* 2008; **24:** 239–44.
15 Chiang EP, Selhub J, Bagley PJ, Dallal G, Roubenoff R. Pyridoxine supplementation corrects vitamin B6 deficiency but does not improve inflammation in patients with rheumatoid arthritis. *Arthritis Res Ther* 2005; **7:** R1404–11.
16 Shen J, Lai CQ, Mattei J, Ordovas JM, Tucker KL. Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. *Am J Clin Nutr* 2010; **91:** 337–42.
17 Christensen MH, Dankel SN, Nordbo Y *et al.* Primary hyperparathyroidism influences the expression of inflammatory and metabolic genes in adipose tissue. *PLoS One* 2011; **6:** e20481.
18 Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2009; **23:** 1371–9.
19 Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Simultaneous determination of neopterin and creatinine in serum with solid-phase extraction and on-line elution liquid chromatography. *Clin Chem* 1987; **33:** 2028–33.
20 Fuchs D, Milstien S, Kramer A *et al.* Urinary neopterin concentrations vs total neopterins for clinical utility. *Clin Chem* 1989; **35:** 2305–7.
21 Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *J Clin Pathol* 1991; **44:** 592–5.
22 Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol* 1997; **281:** 43–53.
23 Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. *Clin Chem* 2003; **49:** 286–94.
24 Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 1973; **4:** 643–6.
25 Levey AS, Coresh J, Greene T *et al.* Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 2007; **53:** 766–72.
26 Koenker RW. *Quantile Regression.* Cambridge, UK: Cambridge University Press, 2005.
27 Ball HJ, Sanchez-Perez A, Weiser S *et al.* Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene* 2007; **396:** 203–13.
28 Fuchs D, Moller AA, Reibnegger G *et al.* Increased endogenous interferon-gamma and neopterin correlate with increased degradation of tryptophan in human immunodeficiency virus type 1 infection. *Immunol Lett* 1991; **28:** 207–11.
29 Almqvist EG, Bondeson AG, Bondeson L, Svensson J. Increased markers of inflammation and endothelial dysfunction in patients with mild primary hyperparathyroidism. *Scand J Clin Lab Invest* 2011; **71:** 139–44.
30 Greenfield EM, Gornik SA, Horowitz MC, Donahue HJ, Shaw SM. Regulation of cytokine expression in osteoblasts by parathyroid hormone: rapid stimulation of interleukin-6 and leukemia inhibitory factor mRNA. *J Bone Miner Res* 1993; **8:** 1163–71.
31 Pirih FQ, Michalski MN, Cho SW *et al.* Parathyroid hormone mediates hematopoietic cell expansion through interleukin-6. *PLoS One* 2010; **5:** e13657.
32 Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci (Lond)* 2012; **122:** 143–59.
33 Lotto V, Choi SW, Friso S. Vitamin B6: a challenging link between nutrition and inflammation in CVD. *Br J Nutr* 2011; **13:** 1–13.
34 Ulvik A, Midttun O, Ringdal Pedersen E, Nygard O, Ueland PM. Association of plasma B-6 vitamers with systemic markers of inflammation before and after pyridoxine treatment in patients with stable angina pectoris. *Am J Clin Nutr* 2012; **95:** 1072–8.
35 Kwak HK, Hansen CM, Leklem JE, Hardin K, Shultz TD. Improved vitamin B-6 status is positively related to lymphocyte proliferation in young women consuming a controlled diet. *J Nutr* 2002; **132:** 3308–13.
36 Price JM, Brown RR, Yess N. Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites. *Adv Metab Disord* 1965; **2:** 159–225.
37 Midttun O, Ulvik A, Ringdal Pedersen E *et al.* Low plasma vitamin B-6 status affects metabolism through the kynurenine pathway in cardiovascular patients with systemic inflammation. *J Nutr* 2011; **141:** 611–7.
38 Christiansen P, Steiniche T, Brixen K *et al.* Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. *Bone* 1997; **21:** 93–9.
39 Nakaoka D, Sugimoto T, Kobayashi T, Yamaguchi T, Kobayashi A, Chihara K. Prediction of bone mass change after parathyroidectomy in patients with primary hyperparathyroidism. *J Clin Endocrinol Metab* 2000; **85:** 1901–7.
40 Iba K, Takada J, Yamashita T. The serum level of bone-specific alkaline phosphatase activity is associated with aortic calcification in osteoporosis patients. *J Bone Miner Metab* 2004; **22:** 594–6.
41 Nayeem F, Anderson KE, Nagamani M, Grady JJ, Lu LJ. Alkaline phosphatase and percentage body fat predict circulating C-reactive protein in premenopausal women. *Biomarkers* 2010; **15:** 663–70.

42 Shantouf RS, Budoff MJ, Ahmadi N *et al.* Total and individual coronary artery calcium scores as independent predictors of mortality in hemodialysis patients. *Am J Nephrol* 2010; **31:** 419–25.
43 Martins MJ, Azevedo I. Let's think in alkaline phosphatase at heart function. *Int J Cardiol* 2010; **144:** 333–4.
44 Bitsch R. Vitamin B6. *Int J Vitam Nutr Res* 1993; **63:** 278–82.
45 Ink SL, Henderson LM. Vitamin B6 metabolism. *Annu Rev Nutr* 1984; **4:** 455–70.
46 Brussaard JH, Lowik MR, van den Berg H, Brants HA, Bemelmans W. Dietary and other determinants of vitamin B6 parameters. *Eur J Clin Nutr* 1997; **51(Suppl 3):** S39–45.
47 Ashley DV, Barclay DV, Chauffard FA, Moennoz D, Leathwood PD. Plasma amino acid responses in humans to evening meals of differing nutritional composition. *Am J Clin Nutr* 1982; **36:** 143–53.

Correspondence: Monika H. E. Christensen, Hormone Laboratory, Haukeland University Hospital, N-5021 Bergen, Norway.
(fax: +4755975814; e-mail: monika.christensen@med.uib.no).

