

Review Article

Vitamin B₆: a challenging link between nutrition and inflammation in CVD

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Abstract

The objective of the present review is to highlight the relationship between low vitamin B₆ status and CVD through its link with inflammation. While overt vitamin B₆ deficiency is uncommon in clinical practice, increasing evidence suggests that marginal vitamin B₆ deficiency is rather frequent in a consistent proportion of the population and is related to an increased risk of inflammation-related diseases. Ample evidence substantiates the theory of atherosclerosis as an inflammatory disease, and low plasma vitamin B₆ concentrations have been related to increased CVD risk. Several studies have also shown that low vitamin B₆ status is associated with rheumatoid arthritis and chronic inflammatory bowel diseases, both of which hold an underlying chronic inflammatory condition. Furthermore, the inverse association observed between inflammation markers and vitamin B₆ supports the notion that inflammation may represent the common link between low vitamin B₆ status and CVD risk. In addition to the epidemiological evidence, there are a number of cell culture and animal studies that have suggested several possible mechanisms relating impaired vitamin B₆ status with chronic inflammation. A mild vitamin B₆ deficiency characterises, in most cases, a subclinical at-risk condition in inflammation-linked diseases which should be addressed by an appropriate individually tailored nutritional preventive or therapeutic strategy.

Key words: Vitamin B₆; Inflammation; Nutrition; CVD; Atherosclerosis; One-carbon metabolism

The two major derivatives of vitamin B₆ are the coenzyme species pyridoxamine 5'-phosphate and pyridoxal 5'-phosphate (PLP)⁽¹⁾. PLP is both the major coenzyme form of vitamin B₆ in plasma and the metabolically active coenzyme produced by the phosphorylation of the pyridoxal compound following the oxidation of other vitamin B₆ vitamers in the liver. Plasma PLP is considered as the most sensitive indicator for tissue vitamin B₆ status because it reflects liver PLP concentrations and stores^(2–6), while other measures of vitamin B₆ such as erythrocyte PLP, total plasma B₆ and urinary excretion of 4-pyridoxic acid are also useful markers of human vitamin B₆ status but are less commonly available in clinical practice^(7,8). Overt vitamin B₆ deficiency is a rare condition and is mainly defined by the appearance of specific clinical signs or symptoms, nonetheless a suboptimal vitamin B₆ status may influence the risk of development of several diseases. It has been previously suggested that vitamin B₆ deficiency

corresponds to plasma PLP concentrations below 20 nmol/l, while a borderline, marginal impairment of vitamin B₆ status may be observed for plasma PLP levels below 30 nmol/l^(7,9). However, there is no definite consensus for a value that unquestionably defines a deficient state of this nutrient. Vitamin B₆ deficiency is rather a notion in evolution since novel at-risk pathological conditions seem, due to a grade of impairment that fails, to meet the magnitude of deficit classically defined as a clear vitamin B₆-deficient status^(8,9).

Several reports have indicated a role of low plasma PLP concentrations in a number of pathological conditions. This suggests that not only does overt vitamin B₆ deficiency increase the risk of certain chronic diseases, but even mild vitamin B₆ deficiency could be associated with an increased risk of certain chronic diseases^(10–12).

Low plasma PLP concentrations have been reported to be inversely related to C-reactive protein (CRP), a major marker

Abbreviations: CAD, coronary artery disease; CRP, C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; MI, myocardial infarction; PLP, pyridoxal 5'-phosphate; RA, rheumatoid arthritis.

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of inflammation and a risk factor for atherosclerotic disease^(13,14). A number of studies have shown that low plasma vitamin B₆ levels are associated with typical inflammatory chronic diseases, such as rheumatoid arthritis (RA)⁽¹⁵⁾ and inflammatory bowel diseases⁽¹⁶⁾, and are inversely related to the markers of inflammation^(17,18). These studies suggest that impaired vitamin B₆ status in RA patients is not solely caused by either lower intake, malabsorption or excessive catabolism of the vitamin, but is rather the result of metabolic mechanisms caused by the inflammatory process^(18,19). Among other possible hypotheses, it has also been suggested that the underlying inflammatory condition itself may reduce circulating and hepatic concentrations of PLP by mobilising PLP from the liver and peripheral tissues to the sites of inflammation^(18,19). Chiang *et al.*⁽¹⁸⁾ observed that plasma PLP concentrations in rats with adjuvant arthritis were about 53% of the controls at acme of inflammation and related to the content of PLP in the liver, thus suggesting that the lower circulating PLP levels observed in RA could be a sign of a decrease in hepatic PLP pools, and that plasma PLP is a valuable indicator of liver vitamin B₆ status during inflammation. Further data showed that PLP in plasma seems a more relevant metabolic marker than PLP in the erythrocytes during inflammation⁽¹⁹⁾ as also confirmed by the observation that plasma, but not erythrocyte PLP concentrations, inversely relates to both clinical and biochemical indices of disease activity and severity in patients affected by RA⁽¹⁹⁾.

For decades, several studies have demonstrated that patients with RA or other inflammatory diseases have a higher risk of developing premature coronary artery disease (CAD)^(20,21) and traditional CVD risk factors did not, by themselves, clarify the increased prevalence of CAD in such patients⁽²²⁾. Through studying the relationship between RA, inflammation and CAD, some CVD prevention strategies in RA patients have been proposed, including anti-inflammatory therapy with cyclo-oxygenase-2-specific inhibitors and statins⁽²³⁾ as well as pyridoxine hydrochloride supplementation⁽²⁴⁾. Several studies, moreover, have confirmed the theory of atherosclerosis as an inflammatory disease, thus demonstrating that the link between atherosclerosis, RA and other inflammatory chronic diseases is through inflammatory processes. Inflammation may therefore be considered as a major pathogenic mediator underlying atherosclerosis and its complications^(25,26). The chronic inflammation in atherosclerotic-related disease, such as CAD and stroke, and its major clinical complications, namely myocardial infarction (MI), have also been associated with low plasma vitamin B₆ concentrations^(11,27–29).

The relationship between atherosclerosis and vitamin B₆-linked inflammatory mechanisms is indeed of great interest. Does low plasma PLP indicate the sole effect of inflammation? Or could it be a cofactor that promotes the development of inflammation, potentially contributing to a sustained chronic inflammatory response? The answer to these questions may open a way to novel and interesting approaches for dietary prevention and therapy.

Vitamin B₆ and coronary artery disease, myocardial infarction and ischaemic stroke

Vitamin B₆ and coronary artery disease

Table 1 summarises the human studies which addressed the relationship between vitamin B₆ status and CAD. Low plasma vitamin B₆ concentrations are not only associated with an increased risk for atherosclerotic diseases⁽³⁰⁾ and more specifically with higher CAD incidence^(27,31,32) but the higher risk also appears to be independent of other recognised risk factors for CAD, including homocysteine⁽²⁷⁾. Moreover, adequate vitamin B₆ levels emerged as a protective factor for CAD⁽³¹⁾. In a prospective design study, Folsom *et al.*⁽³³⁾ demonstrated that the risk of developing CHD within 5 years significantly decreased in parallel with increasing concentrations of plasma PLP⁽³³⁾. The association between low PLP and CAD was, however, not confirmed in all studies^(34,35) (Table 1).

In addition to several epidemiological studies demonstrating a role for low PLP as an independent risk factor for CAD, some studies have shown that low PLP may be linked to CAD through inflammation. In an observational study conducted in an Italian population, a cohort of patients with angiography-defined, severe, multivessel CAD were compared with CAD-free subjects to evaluate the relationship between CAD risk and both plasma PLP concentrations and major markers of the acute-phase reaction⁽¹¹⁾. An inverse relationship between plasma PLP and both high-sensitivity CRP (hs-CRP) and fibrinogen has been found, confirming previous findings of an inverse association between inflammatory markers and vitamin B₆⁽¹³⁾. The prevalence of low PLP (defined as PLP concentrations below 36.3 nmol/l, which was the median value in the control group) was significantly higher among CAD patients compared with controls⁽¹¹⁾. The association between low plasma PLP concentrations and increased CAD risk was also independent of the major classical risk factors for atherosclerosis, including total plasma homocysteine. This association continued to be significant even when hs-CRP and fibrinogen were included in the multiple logistic regression models. The strength of this independent relationship was confirmed even after adjustments for a number of other conditions known to be associated with low concentrations of plasma PLP, including ageing, smoking status and impaired renal function. Results from the present study also showed that the combined presence of low PLP along with other major risk factors for CAD, such as higher hs-CRP and elevated LDL:HDL ratio, further increased the risk for CAD in a graded manner⁽¹¹⁾.

These plasma PLP levels could be described as a mild PLP impairment compared with previous studies that had defined PLP impairment by concentrations as low as 20 nmol/l⁽²⁷⁾, suggesting that even a moderate impairment of this vitamin is sufficient to confer a higher risk for CAD. In a case–control study performed by Lin *et al.*⁽³⁶⁾, low PLP concentrations (defined as PLP below 30 nmol/l) were associated with a significantly increased risk of CAD for angiography-documented patients compared with healthy controls, even after adjustments for hs-CRP⁽³⁶⁾. In a case–control study evaluating

Table 1. Vitamin B₆ and risk of CAD

Authors and references	Study design	Main findings	Low vitamin B ₆ association with CVD risk
Cheng <i>et al.</i> ⁽³⁷⁾	Case-control	Risk of CAD was higher in subjects with plasma PLP <20 nmol/l (OR 2.39, 95% CI 1.24, 4.60) and those with hs-CRP 0.6 mg/dl (>6 mg/l) (OR 3.37, 95% CI 1.52, 7.46), also after adjustment for potential confounders. The combined presence of low PLP and high hs-CRP levels increased CAD risk (OR 4.62, 95% CI 1.28, 16.71). Plasma PLP concentrations were significantly and negatively associated with hs-CRP after adjusting for potential risk factors ($\beta = -0.001$, $P=0.03$) in healthy controls	Yes
Folsom <i>et al.</i> ⁽³³⁾	Prospective	Risk of developing CHD within 5 years significantly decreased with an increasing concentration of vitamin B ₆ . HR = 0.73 ($P<0.01$) for an increase in vitamin B ₆ by a 1 standard deviation increment (3.51 nmol/l)	Yes
Lin <i>et al.</i> ⁽³⁶⁾	Case-control	PLP <30 nmol/l was associated with a significantly higher risk of CAD, as documented angiographically (OR 1.85, 95% CI 1.16, 2.95), after adjusting for homocysteine and hs-CRP. The association between PLP and risk of CAD remained significant even after adjustment for lipid profile	Yes
Friso <i>et al.</i> ⁽¹¹⁾	Case-control	There was a significant, inverse, graded relationship between PLP and both hs-CRP and fibrinogen ($P<0.001$). PLP concentrations <36.3 nmol/l were significantly higher for CAD patients (n 475) than for CAD-free subjects (n 267) ($P<0.001$). After adjustment for major classic CAD risk factors, including hs-CRP and fibrinogen, low PLP was still significantly associated with higher risk (OR 1.89, 95% CI 1.18, 3.03; $P=0.008$)	Yes
Folsom <i>et al.</i> ⁽³¹⁾	Prospective case-cohort	In a population of middle-aged men and women, the incidence of CHD (definite or probable MI, silent MI, fatal CHD and revascularisation procedures) was recorded over an average of 3.3 years of follow-up. Risk of CHD was significantly lower in the highest quintile of PLP compared with the lowest quintile (RR 0.28, 95% CI 0.1, 0.7; $P=0.001$)	Yes
Rimm <i>et al.</i> ⁽⁵⁶⁾	Prospective case-cohort	Highest quintile of vitamin B ₆ intake was associated with a reduced risk for CAD compared with lowest quintile, adjusted for CVD risk factors (RR 0.67, 95% CI 0.53, 0.85; $P=0.002$). Women who regularly used multiple vitamins also had a reduced CAD risk (RR 0.76, 95% CI 0.65, 0.90)	Yes
Robinson <i>et al.</i> ⁽³⁰⁾	Case-control	Lowest quintile of PLP levels (<23.3 nmol/l) was associated with an increased risk of atherosclerosis, independent of tHcy. CAD, peripheral vascular disease and cerebrovascular disease were documented clinically and/or by angiography	Yes
Siri <i>et al.</i> ⁽³⁵⁾	Case-control	Among patients with severe CAD, as documented by angiography, those in the lowest quartile (≤ 25 th percentile) of vitamin B ₆ did not have a significantly higher risk of coronary atherosclerosis (OR 0.86, 95% CI 0.33, 2.22)	No
Verhoef <i>et al.</i> ⁽³⁴⁾	Case-control	Plasma PLP was lower in CAD patients (as documented by angiography) than that in controls, but the difference was not statistically significant	No
Dalery <i>et al.</i> ⁽³²⁾	Case-control	PLP levels were significantly lower in CAD patients (as documented by angiography) than those in controls ($P<0.005$)	Yes
Robinson <i>et al.</i> ⁽²⁷⁾	Case-control	Patients with low PLP (<20 nmol/l) had a significantly higher risk of CAD, adjusting for multiple risk factors, including tHcy (OR 4.3, 95% CI 1.1, 16.9; $P<0.05$)	Yes

CAD, coronary artery disease; PLP, pyridoxal 5'-phosphate; hs-CRP, high-sensitivity C-reactive protein; HR, hazard ratio; MI, myocardial infarction; RR, relative risk; tHcy, total homocysteine.

whether plasma PLP exerts an independent or a synergic effect with inflammation in elevating the risk of CAD, Cheng *et al.*⁽³⁷⁾ confirmed that low PLP levels (below 20 nmol/l) are independently associated with higher CAD risk. The inverse relationship between PLP and hs-CRP was observed only within the control group.

Vitamin B₆ and myocardial infarction

A specific association between low plasma PLP and incidence of MI, the main thrombotic complication of CAD, is supported by early reports^(38–41) and by subsequent case–control and prospective studies^(42–45), as listed in Table 2. Moreover, an association between lower dietary vitamin B₆ intake and higher risk of MI has also been suggested⁽⁴²⁾.

A number of studies have observed an inverse association between plasma concentrations of vitamin B₆ and risk of MI, independent of known CAD risk factors^(44,46), though not all studies confirmed this independent association⁽⁴⁷⁾. In a study evaluating heart transplant recipients, it has been observed that about 21% of the patients showed lower PLP concentrations and that only 9% of those with lower plasma PLP levels did not have CVD complications⁽⁴⁸⁾. Subjects in the highest quintile of PLP had a significantly reduced risk of MI but adjustment for either low-grade inflammation or smoking diminished this association⁽⁴⁷⁾. With regard to the relationship between MI, vitamin B₆ and major markers of inflammation, results from early studies have shown that low plasma PLP levels are related to acute-phase reactants in the initial phase of MI development^(41,49).

Vitamin B₆ and stroke

In 1995, Selhub *et al.*⁽²⁸⁾ described a relationship between lower vitamin B₆ concentrations and extracranial carotid artery stenosis through its role in homocysteine metabolism. Moreover, recent case–control studies have described a possible relationship between low plasma vitamin B₆ concentrations and the onset of cerebrovascular disease, specifically ischaemic stroke and transient ischaemic attack (TIA)^(29,50). A strong association between stroke/TIA and low PLP (defined as levels less than 20 nmol/l) was independent of other well-established vascular risk factors, including total plasma homocysteine concentrations. Furthermore, results from that study identified a possible protective effect for higher PLP concentrations⁽²⁹⁾.

In the context of the Health Professional Follow-up Study, He *et al.* evaluated dietary intakes of vitamin B₆ together with the intake of other B vitamins by a semiquantitative FFQ⁽⁵¹⁾ in relation to the risk of ischaemic and haemorrhagic stroke⁽⁵²⁾. Unlike data related to other B vitamins, the intake of vitamin B₆ was not associated with the risk of ischaemic stroke after adjustments for lifestyle and dietary factors⁽⁵²⁾.

Data from the Spanish National Nutrition Survey, which was designed to assess the association between dietary intake of B vitamins including vitamin B₆ and coronary heart and cerebrovascular mortality, failed to prove a definite association between vitamin B₆ impairment and cardiovascular

mortality⁽⁵³⁾ even though data on vitamin B₆ supported a limited protective effect, only with respect to cerebrovascular mortality in men⁽⁵³⁾.

Vitamin B₆ and peripheral artery disease

Reports on the relationship between vitamin B₆ and peripheral artery disease are few⁽⁵⁴⁾. Wilmink *et al.*⁽⁵⁴⁾ reported that daily vitamin B₆ intake is lower in patients with peripheral artery disease, as defined by an ankle-brachial pressure index below 0.9, and appears as an independent predictor of peripheral artery occlusive disease. An increase in daily vitamin B₆ intake by 1 standard deviation significantly decreased the risk of peripheral artery disease by 29%.

Vitamin B₆ supplementation, inflammation and CVD prevention

An early observation of patients given vitamin B₆ for inflammatory diseases or degenerative diseases found that subjects supplemented with vitamin B₆ had a lower risk of developing MI compared with patients who had not taken vitamin B₆⁽⁵⁵⁾. Table 3 summarises the main studies in which B vitamin supplementation, including supplementation with vitamin B₆, has been performed. In the Nurses' Health Study, among women with no prior history of CAD, users of multivitamins containing folate and vitamin B₆ had a reduced risk of CAD⁽⁵⁶⁾.

Very few human studies have been performed to evaluate the modifications of major markers of inflammation during supplementation with B vitamins, including vitamin B₆, despite the association with either folate and/or vitamin B₁₂^(57,58). Furthermore, results from these studies have shown that CRP and pro-inflammatory IL levels are unchanged after vitamin supplementation^(57,58). Antioxidant activity of vitamin B₆ supplementation has been observed in a study performed on rats, but the exact mechanism is unclear⁽⁵⁹⁾.

Several large, prospective trials have been conducted in recent years with the principal aim of studying the effects of lowering serum homocysteine concentrations with the use of B vitamins, including vitamin B₆, on cardiovascular events^(60,61).

Overall, the most compelling data from vitamin supplementation studies have demonstrated that vitamin B₆ is not effective for preventing the recurrence of cardiovascular events, including CAD, peripheral vascular disease and stroke^(54,62,63).

Only a few small trials, performed with renal transplant patients that have hyperhomocysteinaemia and subjects at risk for cerebral ischaemia, have demonstrated the effectiveness of vitamin B₆ supplementation with folate and vitamin B₁₂ on carotid artery intima-media thickness progression^(64,65). This marker for subclinical atherosclerosis was also evaluated in another recent double-blind, placebo-controlled, randomised clinical trial⁽⁶⁶⁾. Vitamin supplementation significantly reduced subclinical atherosclerosis progression only in subjects at low risk for CVD, with total plasma homocysteine concentrations equal to or above 9.1 μmol/l⁽⁶⁶⁾. Other trials evaluated CAD patients for the effects of B vitamin supplementation, though the interpretation of the results

Table 2. Vitamin B₆ and risk of myocardial infarction (MI)

Authors and references	Study design	Main findings	Low vitamin B ₆ independent association with MI risk
Page <i>et al.</i> ⁽⁴⁴⁾	Nested case-control	Fasting concentrations of PLP were significantly inversely associated with subsequent risk of MI: highest v. lowest quartile (RR 0.22, 95% CI 0.09, 0.55; <i>P</i> =0.047). In a conditional logistic regression model, the effect of adding PLP with conventional MI risk factors was statistically significant (χ^2 15.8; <i>P</i> =0.001). The relationship between PLP and risk of MI was stronger among women under 60 years old than among older women: highest v. lowest quartile (RR 0.05, 95% CI 0.004, 0.61)	Yes
Dierkes <i>et al.</i> ⁽⁴⁷⁾	Nested case-cohort	Subjects were recruited at random from the general population and excluded if they had a history of MI and stroke at baseline. The highest PLP quintile had a significantly reduced risk of MI (HR 0.50, 95% CI 0.29, 0.83). Adjustment for either low-grade inflammation or smoking diminished this association. When adjusted for both low-grade inflammation and smoking, the association between PLP and risk of MI was abolished. Adjustment for established risk factors also abolished the association	No
Tavani <i>et al.</i> ⁽⁴³⁾	Case-control	Risk of acute MI was significantly lower for the highest tertile of vitamin B ₆ intake compared with the lowest tertile of intake (OR 0.34, 95% CI 0.19, 0.60)	Yes
Nahlawi <i>et al.</i> ⁽⁴⁸⁾	Follow-up	21% of heart transplant recipients had PLP deficiency. Only 9% of the patients with low PLP did not have CVD complications/death (<i>P</i> =0.05). Risk for CVD events, including death, was 2.7 times higher for patients with low vitamin B ₆ (PLP ≤20 nmol/l) (95% CI 1.2, 5.9; <i>P</i> =0.02)	Yes
Folsom <i>et al.</i> ⁽³¹⁾	Prospective case-cohort	In a population of middle-aged men and women, incidence of CHD (definite or probable MI, silent MI, fatal CHD and revascularisation procedures) was recorded over an average of 3.3 years of follow-up. Risk of CHD was lower in the highest quintile of PLP v. the lowest quintile (RR 0.28, 95% CI 0.1, 0.7; <i>P</i> =0.001)	Yes
Chasan-Taber <i>et al.</i> ⁽⁴²⁾	Case-control	The lowest quintile of PLP (<28.9 nmol/l) had a higher risk of MI when adjusting for multiple variables (RR 1.5, 95% CI 1.0, 2.2). If adjusted for folate, RR was 1.3 (95% CI 0.9, 2.1)	Yes
Verhoef <i>et al.</i> ⁽⁴⁶⁾	Case-control	Patients with first MI had lower dietary and plasma vitamin B ₆ than controls. OR was 0.97 for the lowest quintile of plasma PLP (≤29.7 nmol/l) when adjusted for age and sex and 0.97 (95% CI 0.40, 2.33) when adjusted for multiple variables. OR was 0.32 for the highest quintile of plasma PLP (>88.9 nmol/l) when adjusting for age and sex and 0.51 (95% CI 0.19, 1.36) when adjusting for multiple variables	Yes
Kok <i>et al.</i> ⁽³⁸⁾	Case-control	Patients with MI (<i>n</i> 84) were compared with control subjects (<i>n</i> 84). MI was more than five times more likely among subjects in the lowest quartile of plasma PLP (<20 nmol/l) (OR 5.2, 95% CI 1.4, 18.9)	Yes

PLP, pyridoxal 5'-phosphate; RR, relative risk; HR, hazard ratio.

Table 3. Vitamin B supplementation, including vitamin B₆, and CVD

Authors and references	Study design	Supplementation	Main findings	Benefit of vitamin supplementation
Albert <i>et al.</i> ⁽⁶²⁾ WAFACS	Double-blind, placebo-controlled, randomised clinical trial	2.5 mg folic acid + 1 mg vitamin B ₁₂ + 50 mg vitamin B ₆ Time: 7.3 years	Population of 5442 women with a history of CVD, or three or more coronary risk factors. Patients receiving vitamin supplementation had a similar risk to patients receiving a placebo for the composite CVD primary endpoint (MI, stroke, coronary revascularisation, CVD mortality) (RR 1.03, 95% CI, 0.90, 1.19; <i>P</i> =0.65)	No benefit on CVD risk
Ebbing <i>et al.</i> ⁽⁶⁹⁾ WENBIT	Double-blind, controlled, randomised clinical trial Two-by-two factorial design	Four groups: (1) 0.8 mg folic acid + 0.4 mg vitamin B ₁₂ + 40 mg vitamin B ₆ ; (2) 0.8 mg folic acid + 0.4 mg vitamin B ₁₂ ; (3) 40 mg vitamin B ₆ ; (4) Placebo Time: 7 years (but terminated early, after 38 months)	Population of 3090 patients undergoing coronary angiography with double or triple vessel disease, stable angina pectoris or acute coronary syndromes. Patients receiving vitamin supplementation had a similar risk to subjects receiving a placebo for the composite endpoint (all-cause death, non-fatal AMI, acute hospitalisation for unstable angina pectoris, non-fatal thromboembolic stroke) Comparing group 2 v. 4: HR 1.09, 95% CI 0.90, 1.32; <i>P</i> =0.36. Comparing group 3 v. 4: HR 0.90, 95% CI 0.74, 1.09; <i>P</i> =0.28	No benefit on CVD risk
Bønaa <i>et al.</i> ⁽⁶¹⁾ NORVIT	Double-blind, controlled, randomised clinical trial Two-by-two factorial design	Four groups: (1) 0.8 mg folic acid + 0.4 mg vitamin B ₁₂ + 40 mg vitamin B ₆ ; (2) 0.8 mg folic acid + 0.4 mg vitamin B ₁₂ ; (3) 40 mg vitamin B ₆ ; (4) Placebo	Population of 3749 subjects with an acute MI within 7 d before randomisation. Supplementation with vitamin B ₆ was not associated with any significant benefit on recurrent MI, stroke or death attributed to CAD (RR 1.14, 95% CI 0.98, 1.32; <i>P</i> =0.09). In the group given folic acid, vitamin B ₁₂ and vitamin B ₆ , there was a trend towards an increased risk (RR, 1.22, 95% CI, 1.00, 1.50; <i>P</i> =0.05)	No benefit on CVD risk
Lonn <i>et al.</i> ⁽⁷⁰⁾ HOPE-2	Double-blind, placebo-controlled, randomised clinical trial	2.5 mg folic acid + 1 mg vitamin B ₁₂ + 50 mg vitamin B ₆ or placebo Time: 5 years	Population of 5522 patients with vascular disease or diabetes. Compared with placebo, active treatment did not significantly decrease the risk of death from cardiovascular causes (RR 0.96, 95% CI 0.81, 1.13) or MI (RR 0.98, 95% CI 0.85, 1.14)	No benefit on CVD risk
Toole <i>et al.</i> ⁽⁶⁰⁾ VISP	Multicentre, double-blind, controlled, randomised clinical trial	Two groups: (1) High-dose multivitamin formulation (<i>n</i> 1827): 2.5 mg folic acid + 0.4 mg vitamin B ₁₂ + 25 mg vitamin B ₆ ; (2) Low-dose multivitamin formulation (<i>n</i> 1853): 20 µg folic acid + 6 µg vitamin B ₁₂ + 200 µg vitamin B ₆ Time: September 1996–May 2003	Population of 3680 adults with non-disabling cerebral infarction. There was a difference between two groups in the mean reduction of tHcy, but there was no treatment effect on any endpoint. Mean reduction of tHcy was 2 µmol/l greater in the high-dose group than in the low-dose group. The unadjusted RR for stroke, CAD or death was 1 (95% CI 0.8, 1.1)	No benefit on recurrent cerebral infarction or CVD risk
Hodis <i>et al.</i> ⁽⁶⁶⁾	Double-blind, placebo-controlled, randomised clinical trial	5 mg folic acid + 0.4 mg vitamin B ₁₂ + 50 mg vitamin B ₆ or placebo Time: 3.1 years	Population of 506 subjects with tHcy > 8.5 µmol/l without diabetes and CVD. Among subjects with tHcy ≥ 9.1 µmol/l, those randomised to supplementation had a statistically significant lower average rate of cIMT progression, as assessed using high-resolution B-mode US, compared with placebo (<i>P</i> =0.02). Among subjects with tHcy < 9.1 µmol/l, there was no significant effect of vitamin supplementation on subclinical progression of atherosclerosis	Limited benefit on subclinical atherosclerosis progression
Till <i>et al.</i> ⁽⁶⁵⁾	Double-blind, placebo-controlled, randomised clinical trial	2.5 mg folic acid + 0.5 mg vitamin B ₁₂ + 25 mg vitamin B ₆ Time: 1 year	Population of 50 patients with cIMT ≥ 1 mm. In the treatment group, cIMT significantly decreased after supplementation (<i>P</i> =0.034). The mean changes in cIMT differed significantly (<i>P</i> =0.019) between vitamin supplementation and placebo groups. Multiple regression analysis revealed that the observed effect on cIMT depended only on medication. This effect was independent of tHcy concentration	Benefit on cIMT in patients at risk
Marcucci ⁽⁶⁴⁾	Double-blind, placebo-controlled, randomised clinical trial	5 mg folic acid + 0.4 mg vitamin B ₁₂ + 50 mg vitamin B ₆ or placebo Time: 6 months	Population of fifty-six stable hyperhomocysteinaemic RTR. cIMT of common carotid arteries, an early sign of atherosclerosis, was measured with high-resolution B-mode US. In the treatment group, cIMT significantly decreased after supplementation (<i>P</i> <0.0001). In hyperhomocysteinaemic patients without vitamin supplementation, there was a significant increase in cIMT after 6 months (<i>P</i> <0.05)	Benefit on cIMT in the group of RTR

WAFACS, Women's Antioxidant and Folic Acid Cardiovascular study; MI, myocardial infarction; RR, relative risk; WENBIT, Western Norway B Vitamin Intervention Trial; AMI, acute myocardial infarction; HR, hazard ratio; NORVIT, Norwegian Vitamin Trial; CAD, coronary artery disease; HOPE-2, Heart Outcomes Prevention Evaluation 2; VISP, Vitamin Intervention for Stroke Prevention randomised control trial; tHcy, total homocysteine; cIMT, carotid intima-media thickness; US, ultrasound; RTR, renal transplant recipients.

was ambiguous. Supplementation with vitamin B₆, folate and vitamin B₁₂ after coronary angioplasty decreased the rate of restenosis and the need for revascularisation⁽⁶⁷⁾, while supplementation after coronary stenting increased the risk of in-stent restenosis and the need for target-vessel revascularisation⁽⁶⁸⁾.

Despite the advantage of large randomised intervention studies, wisdom from the studies of cancer chemoprevention with folate clearly suggests that two critical factors should be taken into account in the aforementioned trials: time and dose of B vitamin supplementation. Nutritional intervention is indeed considered to be a two-edged sword, where a beneficial effect may be observed with nutritional support in the prevention phase and a disease-aggravating effect may be observed after the onset of illness, with nutritional support actually fuelling the disease process. This observation may certainly apply to folate supplementation, which can effectively prevent the onset or progression of disease in the early phase, while it may accelerate the progression of disease in the late phase. It can therefore be speculated that continuous supplementation with a single non-physiological form of vitamin supplements might contribute to unexpected or even harmful outcomes, even in the at-risk condition of having an impaired vitamin B₆ status. Furthermore, the major endpoints of these studies were those of evaluating the effect of lowering plasma homocysteinaemia on the recurrence of established disease, mainly by the simultaneous use of various B vitamins that have many additional functions other than that of lowering total plasma homocysteine. Little information, however, is available regarding both the effects of vitamin B₆ supplementation on inflammatory markers and the correct dose and timing to be followed to avoid the possible harmful effect of excessive or inappropriate vitamin B₆ supplementation. This information that would be needed for a possible primary or secondary preventive approach using vitamin B₆ is to be developed. Further specific studies are required to deepen our knowledge in this regard.

Even if there is consistently a lack of benefit in secondary prevention of CVD with B vitamin supplementation, with or without vitamin B₆ (Western Norway B Vitamin Intervention Trial⁽⁶⁹⁾, Women's Antioxidant and Folic Acid Cardiovascular study⁽⁶²⁾, Vitamin Intervention for Stroke Prevention randomised controlled trial⁽⁶³⁾, Norwegian Vitamin Trial⁽⁶¹⁾, Heart Outcomes Prevention Evaluation 2⁽⁷⁰⁾), it should also be considered that all the aforementioned studies are quite diverse from one another in terms of the time period of supplementation. In fact, one may raise the point that the time period of supplementation is especially critical to reach positive outcomes or avoid possible harmful effects in terms of the rate of CVD events⁽⁷¹⁾. However, despite the apparently clear outcomes⁽⁷¹⁾, it could be argued that a potential benefit that modifies secondary prevention outcomes may not be observed over a period of moderate duration (between 2 and 5 years for the Norwegian Vitamin Trial, Heart Outcomes Prevention Evaluation 2 and Vitamin Intervention for Stroke Prevention studies or of about 7 years for the Women's Antioxidant and Folic Acid Cardiovascular study).

Furthermore, the negative results of vitamin supplementation trials, including vitamin B₆ use, do not preclude the possibility of a protective effect in primary prevention. It could be difficult, however, to demonstrate that vitamin B supplementation is ineffective in patients who have had a clinical vascular event, while effective in those without a clinical event or with subclinical atherosclerosis. Clinical trials for primary prevention may require a longer duration and larger populations in order to answer the key question on whether vitamin B₆ supplementation is effective in preventing CAD before the first vascular event or in younger life.

Studies are needed to find the specific time and optimal dose of vitamin B₆ in order to maximise efficacy, minimise adverse effects and identify targets for vitamin B₆ interventions on the basis of genetic susceptibility and environmental factors. A better understanding of the mechanisms underlying the relationship between CVD and vitamin B₆ may indeed be extremely helpful in designing the most accurate preventive strategies.

Inflammation and vitamin B₆-related atherogenesis

PLP functions as a coenzyme in more than 100 reactions that are involved in the metabolic pathways of neurotransmitters as well as in the metabolism of amino acids, lipids and carbohydrates⁽⁷²⁾. PLP also takes part in other significant pathways related to immune function⁽⁷³⁾, thrombosis^(74,75) and inflammation⁽¹⁷⁾, all of which are crucial mechanisms in every stage of the atherosclerotic process.

Furthermore, PLP is implicated in the synthesis and repair of both nucleic acids and proteins. Low vitamin B₆ concentrations could thus reflect an increased consumption of PLP in the accelerated synthesis of cytokines⁽⁷⁶⁾ and in the activation and proliferation of lymphocytes, both of which are key events in the inflammatory process^(77,78).

Considering the epidemiological evidence of a role of vitamin B₆ in inflammatory-related diseases and the observed relationship with inflammatory markers^(11,16,18,79), it is plausible that vitamin B₆ plays a role in CVD pathogenesis through mechanisms linked to inflammation (Table 4).

Therefore, with the knowledge that systemic acute-phase markers are solid and independent risk factors for CAD^(80,81), that inflammation exerts an essential role in all stages of the atherosclerotic process⁽⁸²⁾ and the possibility that vitamin B₆ has a role in inflammatory processes, several mechanisms were proposed linking low vitamin B₆ and CVD using cell culture studies, animal studies⁽⁷²⁾ and clinical trials.

Animal studies have reported that inflammation reduces circulating and hepatic concentrations of vitamin B₆. Plasma PLP is considered as a sensitive indicator of tissue vitamin B₆ status^(3-7,15,18). As shown in Fig. 1, it is thus possible that, in patients in an inflammatory state, PLP is mobilised from the liver and peripheral tissues to the sites of inflammation⁽¹⁹⁾. Plasma PLP levels are known to be inversely related to TNF- α production and other inflammatory cytokines in RA⁽¹⁷⁾. It has also been reported that low plasma PLP concentrations are inversely related to both plasma fibrinogen⁽⁸³⁾ and CRP^(11,13), with a robust and independent association of

Table 4. Vitamin B₆ and inflammation

Authors and references	Study design and participants	Main findings	Conclusions
Morris <i>et al.</i> ⁽⁷⁹⁾	Observational study on a proportion of the participants in 2003–4 NHANES (general US population); 2686 adults were eligible	Higher vitamin B ₆ intakes were protective against inflammation (as measured by CRP concentrations). After multivariate-adjusted analysis, the prevalence of vitamin B ₆ inadequacy was <10% when serum CRP concentrations were ≤3 mg/l and about 50% if serum CRP concentrations were >10 mg/l (<i>P</i> <0.001)	Low vitamin B ₆ status in inflammation-related illnesses appears to be caused, not by lower intake or excessive catabolism of PLP, but by the inflammatory process underlying the disease itself
Shen <i>et al.</i> ⁽⁹²⁾	Cross-sectional study on 1205 Puerto Rican adults living in Massachusetts	Plasma PLP was related to plasma CRP in a clear dose–response relationship. Plasma CRP significantly decreased with increasing quartiles of plasma PLP (<i>P</i> <0.001)	Low PLP concentrations are associated with markers of inflammation and may influence CAD risk through different mechanisms than homocysteine
Cheng <i>et al.</i> ⁽³⁷⁾	Case–control study with 184 CAD patients and 516 CAD-free control subjects	In the controls but not the cases, PLP was negatively associated with hs-CRP (<i>P</i> =0.03). Risk of CAD was 2.39 times higher for low PLP and 3.37 times higher for high hs-CRP. The co-occurrence of low PLP and high hs-CRP was associated with 4.35 times higher risk of CAD	PLP and hs-CRP are independently associated with CAD risk. Low PLP and high hs-CRP occurring together increased the risk of CAD
Vasilaki <i>et al.</i> ⁽⁹¹⁾	Case–control study on 96 critically ill patients and 126 control subjects	PLP and PL were significantly lower in critically ill patients than in control subjects (<i>P</i> <0.001 and <i>P</i> <0.01, respectively). Among patients, the PLP:PL ratio was significantly lower in plasma than in erythrocytes (<i>P</i> =0.001)	The inflammatory condition may induce an increased utilisation of vitamin B ₆
Chiang <i>et al.</i> ⁽²⁴⁾	Double-blind study on subjects with RA and low vitamin B ₆ levels randomised to receive either active vitamin B ₆ (50 mg of pyridoxine, <i>n</i> 14) or placebo (<i>n</i> 14) tablets for 30 d	Subjects taking methotrexate or prednisone treatment were stratified in two subgroups, and the subjects in each group were randomised to receive either active vitamin B ₆ or placebo treatment. There were significant improvements in vitamin B ₆ parameters only in the active treatment group. In patients with RA, PLP and net increase in plasma homocysteine (post-methionine load test) were related to CRP	Vitamin B ₆ supplementation should be considered in RA patients to reduce the potential for adverse consequences of vitamin B ₆ deficiency. CRP could be a potential target for vitamin B ₆ supplementation
Friso <i>et al.</i> ⁽¹¹⁾	Case–control study on 742 participants that either had severe multivessel CAD (<i>n</i> 475) or were CAD-free (<i>n</i> 267)	A significant, inverse relationship was observed between PLP and both hs-CRP and fibrinogen (<i>P</i> <0.001). The prevalence of PLP <36.3 nmol/l was significantly higher in CAD patients than in CAD-free subjects (<i>P</i> <0.001). The OR for CAD risk related to low PLP concentrations after adjusting for the major classic CAD risk factors, including hs-CRP and fibrinogen, was 1.89 (95% CI 1.18, 3.03; <i>P</i> =0.008)	Low plasma PLP is inversely related to markers of inflammation and independently associated with an increased risk of CAD
Chiang <i>et al.</i> ⁽¹⁹⁾	Cross-sectional study on thirty-seven patients with RA	PLP levels were inversely correlated with ESR (<i>r</i> –0.37, <i>P</i> =0.02), CRP (<i>r</i> –0.52, <i>P</i> =0.002) and with other markers of disease activity and severity (disability score, morning stiffness and degree of pain)	In patients with RA, there is a consistent association between PLP and several indicators of inflammation. Impaired vitamin B ₆ status may be a result of inflammation
Folsom <i>et al.</i> ⁽⁸⁸⁾	Cross-sectional study on 519 healthy middle-aged adults in the ARIC Study	Plasma PLP was not associated with CRP concentrations, but it was significantly inversely associated with both factor VIIIc and leucocyte count	B vitamin status does not correlate strongly with circulating levels of inflammatory markers
Saibeni <i>et al.</i> ⁽¹⁶⁾	Case–control study on sixty-one patients with IBD	Median vitamin B ₆ levels were significantly lower in IBD patients than in controls (<i>P</i> <0.01). Low vitamin B ₆ levels were significantly more frequent in patients with active disease than in patients with quiescent disease (<i>P</i> <0.001). Low PLP levels were significantly correlated with CRP (<i>P</i> <0.01)	Low PLP is frequent in patients with IBD, especially those with active disease
Friso <i>et al.</i> ⁽¹³⁾	Observational study on 891 participants from the population-based Framingham Heart Study cohort	Mean plasma PLP levels were lower in subjects with CRP ≥6 than in subjects with CRP <6 mg/l (mean values were 36.5 and 55.8 nmol/l, respectively; <i>P</i> <0.001). After multiple logistic regression, including adjustment for tHcy, the association between PLP and CRP remained highly significant (<i>P</i> =0.003)	Low plasma PLP is associated with higher CRP levels independent of tHcy. Vitamin B ₆ may be decreased due to its increased utilisation in the site of inflammation

NHANES, National Health and Nutrition Examination Survey; CRP, C-reactive protein; PLP, pyridoxal 5'-phosphate; hs-CRP, high-sensitivity CRP; CAD, coronary artery disease; PL, pyridoxal; RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; ARIC, Atherosclerosis Risk in Communities; IBD, inflammatory bowel disease; tHcy, total homocysteine.

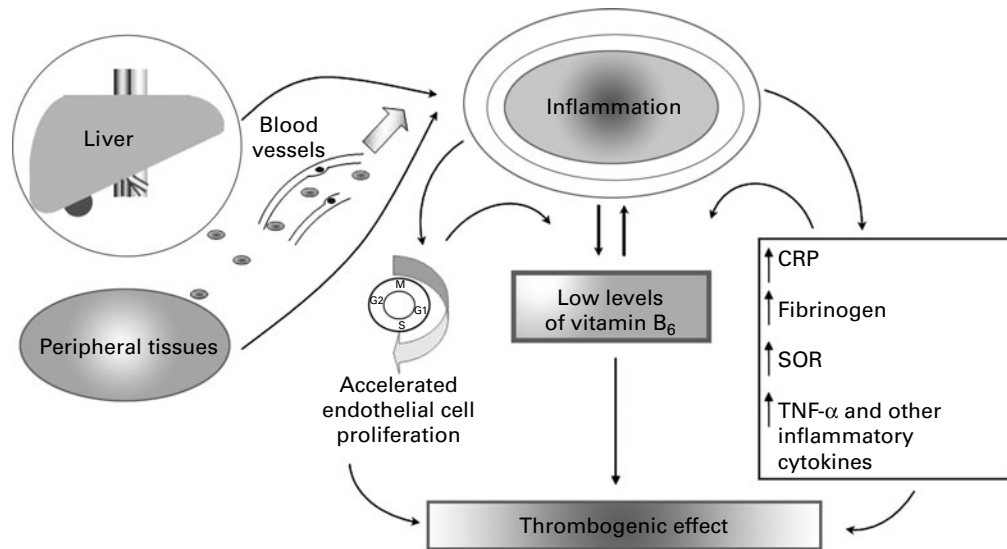


Fig. 1. A simplified scheme of the proposed possible mechanisms for the relationship between low vitamin B₆ and CVD. This figure is a simplified representation of some of the possible mechanisms through which an impaired vitamin B₆ status has been hypothesised to exert its effect in atherosclerotic disease process. As shown, it is possible that vitamin B₆ is mobilised from the liver and peripheral tissues to the sites of inflammation which is characterised by an increase in C-reactive protein (CRP), fibrinogen, superoxide radicals (SOR), TNF- α as well as other inflammatory cytokines. Plasma vitamin B₆ (pyridoxal-5'-phosphate) concentrations are reported to be inversely related to TNF- α and other inflammatory cytokine production as well as major markers of inflammation such as CRP and fibrinogen, in chronic inflammatory processes. The inflammatory status, furthermore, increases oxidant stress, which results from an imbalance between oxidant production (SOR) and antioxidant defences and may induce the consumption of vitamin B₆ with a consequent reduction of the active form of vitamin B₆ (pyridoxal-5'-phosphate) plasma levels and its antioxidant effect and, at the same time, it may facilitate a thrombotic effect which then triggers the impairment of endothelial function, a key event in the pathogenesis of atherosclerosis. Vitamin B₆ has also been described to have an inhibitory effect on endothelial cell proliferation; therefore, the reduction of vitamin B₆ (pyridoxal-5'-phosphate) plasma levels induced by inflammatory process may further favour the mechanisms leading to atherogenesis. \odot Cellular cycle; \bullet , vitamin B₆; M, mitosis cell phase; S, synthesis cell phase; G1, gap 1 cell phase; G2, gap 2 cell phase.

other major biomarkers related to vitamin B₆ metabolism⁽¹¹⁾. Moreover, inflammatory status increases oxidant stress, which results from an imbalance between oxidant production and antioxidant defences (Fig. 1). All these conditions characterised by the increase in inflammatory cytokines, fibrinogen, CRP and superoxide radicals may induce the consumption of vitamin B₆, with a consequent reduction of PLP plasma levels and its antioxidant effect, and, at the same time, they may favour a thrombotic effect, thus triggering an impairment of endothelial function which is a key event in the pathogenesis of atherosclerotic processes (Fig. 1)⁽⁸⁴⁾. PLP has also been described to have an inhibitory effect on endothelial cell proliferation^(85,86). The persistence of a chronic inflammatory condition may result in the depletion of vitamin B₆, which might then contribute to a sustained chronic inflammatory response (Fig. 1).

Most of the evidence demonstrates a potential role for vitamin B₆ in inflammatory processes where the low vitamin B₆ status in inflammation-related illnesses appears to result not from lower intake or excessive catabolism of PLP but from the inflammatory process underlying the disease itself⁽⁷⁹⁾.

Although vitamin B₆ represents an important coenzyme in the metabolism of homocysteine, a recognised risk factor for thrombosis, the role of PLP in atherosclerosis is only partly related to its function in the one-carbon pathway. Several studies have thus supported a role for vitamin B₆ in the risk of CVD^(27,31,42,46) and stroke⁽²⁹⁾, independent of homocysteine and other risk factors. A hypothesis is that vitamin B₆ could

be directly implicated as a cofactor in an anti-inflammatory mechanism where the utilisation of the vitamin results in its consumption, and the consequent low vitamin B₆ concentrations could support and amplify the inflammatory process, thereby leading to chronic progression of inflammatory disease. Indeed, a mild deficiency of vitamin B₆ may be associated with an increased risk, not only of atherosclerosis, but also of other chronic inflammatory diseases such as RA^(18,87) and inflammatory bowel diseases⁽¹⁶⁾. A number of studies have highlighted the relationship between PLP and inflammation, showing an inverse association between vitamin B₆ and major markers of inflammation, including plasma fibrinogen concentration⁽⁸³⁾, erythrocyte sedimentation rate and CRP⁽¹³⁾. In the population-based Framingham Heart Study cohort, the association between PLP and CRP was strong and independent of other major biomarkers related to vitamin B₆ metabolism⁽¹³⁾, supporting a possible role for plasma PLP in inflammatory processes.

Some reports did not support this observation⁽⁸⁸⁾, such as the study by Folsom *et al.*⁽⁸⁸⁾, conducted among healthy middle-aged adults in the Atherosclerosis Risk in Communities Study, which did not find an inverse association between CRP and PLP. However, the authors did observe that lower plasma PLP and dietary vitamin B₆ were associated with a higher leucocyte count, though this association was not found with the use of vitamin supplements⁽⁸⁸⁾. It should also be taken into account that some diversity in the observations reported by the studies may be due to the different methods utilised to measure plasma PLP, although the methods used by most

studies are considered highly reliable for the assessment of vitamin B₆ status^(11,15,88–90). The majority of studies, however, confirmed the inverse correlation between PLP and major markers of inflammation in various inflammation-related diseases. In subjects affected by RA^(19,24), PLP was associated with erythrocyte sedimentation rate, CRP levels and other markers of disease activity and severity, suggesting that impaired vitamin B₆ status is a result of inflammation. It also appeared that the relationship between lower vitamin B₆ and increased inflammation in these RA patients could be tissue-specific⁽²⁴⁾.

Plasma PLP concentrations are also altered in subjects who have acute diseases with an evident underlying inflammatory condition. Vasilaki *et al.*⁽⁹¹⁾ observed that in patients admitted to an intensive therapy unit, high concentrations of CRP, PLP and intracellular pyridoxal levels were significantly lower in those critically ill patients than in the group of subjects taken as controls.

Further support for the hypothesis of a link between vitamin B₆ and inflammation can be found in an analysis of data from a large population-based survey from participants in the 2003–4 National Health and Nutrition Examination Survey. Results showed that higher vitamin B₆ intakes are protective against inflammation, as indicated by hs-CRP concentrations. Moreover, the level of vitamin B₆ intake that was associated with maximum protection against vitamin B₆ inadequacy was elevated in the presence of inflammation compared with its absence⁽⁷⁹⁾.

A strong inverse association between vitamin B₆ status, as measured by plasma PLP concentration, and the inflammatory marker CRP was also observed recently in a cohort of elderly Puerto Ricans living in Massachusetts⁽⁹²⁾. In the present study, chronic inflammatory conditions, such as the metabolic syndrome, diabetes and obesity, were significantly associated with lower plasma PLP. The patients affected by such diseases were also significantly more likely to demonstrate vitamin B₆ inadequacy. Furthermore, lower PLP plasma concentrations were associated with oxidative stress, as indicated by higher urinary concentrations of 8-hydroxydeoxyguanosine, a marker of DNA damage and oxidative stress⁽⁹²⁾. Authors concluded that vitamin B₆ status may influence CAD risk through mechanisms that link vitamin B₆ to inflammatory processes rather than mechanisms related to the role of vitamin B₆ in homocysteine metabolism⁽⁹²⁾.

Conclusions

Several studies have demonstrated an association between mild vitamin B₆ deficiency with inflammation-related diseases, including CVD^(11,16,18,79), by highlighting an inverse relationship between vitamin B₆ and inflammatory markers^(11,19,79). In a consistent number of studies, this association between impaired vitamin B₆ status and higher risk of CVD is independent of other major traditional atherosclerosis risk factors^(13,27,37,46,44) and is inversely related to the major markers of inflammation⁽¹¹⁾. This evidence suggests a link between impaired vitamin B₆ and CVD through inflammation. Because vitamin B₆ is involved in a large number of

physiological reactions, it could be essential to design appropriate studies to define the exact mechanisms underlying the inter-relationships among suboptimal vitamin B₆ status, as defined by both plasma and tissue PLP concentrations, and biochemical–molecular alterations leading to the development of inflammation-related diseases. A research priority may be that of investigating the kinetics and regulation of B₆ vitamers and enzymes in different body compartments during inflammatory processes.

Mild vitamin B₆ deficiency is not a rare occurrence in population-based studies⁽¹²⁾. This issue, therefore, deserves further investigation, especially in terms of prevention strategies, for the purpose of promoting specific public health policies. Current clinical trials have indicated that vitamin B₆ supplementation seems not to be effective for the prevention of recurrence of CVD, although the appropriate dosage and timing for possible beneficial effects through the use of vitamin supplements still remains to be discussed. Other crucial issues pertain also to the evaluation of the actual effect of supplementation with vitamin B₆ in synthetic form, as well as to whether such approach may be as effective as an adequate dietary vitamin B₆ intake. The question of whether supplementation with vitamin B₆ may be useful for primary prevention of CVD is yet another key issue to be defined. The importance of considering vitamin B₆ status in relation to the risk for CVD may nevertheless open new insights for the potential identification of innovative preventive and therapeutic strategies. In order to help tailor an adequate nutritional approach on an individual basis, both dose and timing as well as the possible harmful effect of vitamin B₆ supplementation should be carefully considered in the design of future *ad hoc* clinical trials that are aimed at identifying appropriate vitamin B₆ supplementation.

The notion of a definite vitamin B₆ deficiency is a concept to be considered *in fieri* since the risk of certain diseases seems to be associated with a degree of vitamin B₆ impairment that falls short of the classical definition of a clear vitamin B₆-deficient state.

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