

# Role of homocysteine, cystathionine and methylmalonic acid measurement for diagnosis of vitamin deficiency in high-aged subjects

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## Abstract

**Background** Intracellular B-vitamin and folate deficiency indicated by hyperhomocysteinemia is very frequent in the elderly population. Hyperhomocysteinemia increases the risk of atherothrombotic diseases and neuropsychiatric complications. Our aim was to evaluate the prevalence of increased serum metabolite concentrations in subjects of a higher age, and whether the measurement of metabolite concentrations is more effective in diagnosing B-vitamin deficiency than mere homocysteine.

**Materials and methods** Homocysteine (HCY), cystathionine (CYS) and methylmalonic acid (MMA) were investigated in serum together with vitamin B-12, B-6 and folate in 90 high-aged subjects (85–102 years), 92 seniors (65–75 years), and in 50 younger subjects (19–50 years).

**Results** Elderly subjects (high-aged and senior) had elevated serum concentrations of metabolites. High-aged subjects had a higher frequency of pathological increases than seniors: HCY 62% vs. 24%; MMA 62% vs. 23%; CYS 81% vs. 36%. Folate and vitamin B-6 concentrations were significantly decreased in both elderly groups; vitamin B-12 was only decreased in high-aged subjects. Utilising vitamin B-6, B-12 and folate for diagnosis of intracellular vitamin deficiency, the rate was 30% in seniors and 55% in high aged subjects. However, utilising the metabolites (HCY, MMA and CYS) for the diagnosis of intracellular vitamin deficiency, there was a distinctly increased rate of 55% in seniors respective to 90% in high-aged subjects. Backward multiple regression analysis revealed that only folate, MMA, creatinine and age were independent variables influencing the HCY concentration. Furthermore, the MMA concentration was significantly and independently influenced by folate, vitamin B-12, HCY and creatinine, and the serum concentration of CYS by vitamin B-12, creatinine and age.

**Conclusion** The metabolites HCY, MMA and CYS are sensitive indicators diagnosing impaired remethylation of homocysteine to methionine with parallel activation of catabolic pathway. Compared to mere HCY or B-vitamins in serum, the efficiency of diagnosing a disturbed HCY metabolism increases very much in utilising the metabolites HCY, MMA and CYS. For differential diagnosis, parallel measurement of folate and creatinine is recommended. The early and correct diagnosis of B-vitamin deficiency in elderly subjects is of high clinical relevance.

**Keywords** Cystathionine, folate, high age, homocysteine, methylmalonic acid, vitamin B-12. *Eur J Clin Invest* 2000; 30(12): 1083–1089

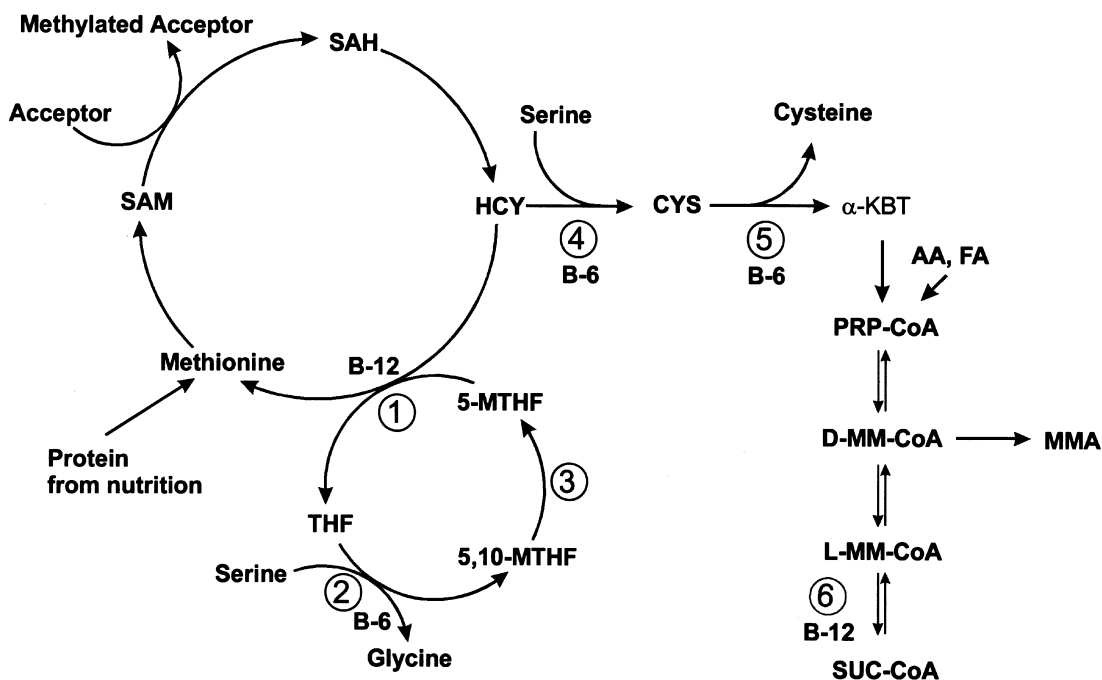
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## Introduction

An increasing prevalence of low serum vitamin B-12 (cobalamin), vitamin B-6 (pyridoxal phosphate) and folate concentration in advancing age has been reported in many investigations [1–3]. The homocysteine (HCY) elevation with age may result from an age-related decline in cystathionine b-synthase [4], substantial reduction of the amount of vitamin B-12 on transcobalamin II responsible for the transcellular traffic of vitamin B-12 [5] and possibly



**Figure 1** Metabolism of homocysteine. SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; HCY, homocysteine; CYS, cystathionine;  $\alpha$ -KBT,  $\alpha$ -ketobutyrate; PRP-CoA, propionyl-CoA; MMA, methylmalonic acid; D-MM-CoA, D-methylmalonyl-CoA; L-MM-CoA, L-methylmalonyl-CoA; SUC-CoA, succinyl-CoA; THF, tetrahydrofolate; 5-MTHF,

5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; AA, amino acids; FA, fatty acids. 1, methionine synthase; 2, serine-hydroxymethyltransferase; 3,  $N^5,N^{10}$ -methylene-tetrahydrofolate reductase; 4, cystathionine- $\beta$ -synthase; 5, cystathionase; 6, L-methylmalonyl-CoA-mutase.

other involved enzymes [6]. In the elderly, metabolic evidence of intracellular vitamin B-12, vitamin B-6 and folate deficiency is more frequent than low serum vitamin concentrations [1,7].

Figure 1 illustrates the interaction of folate and vitamin B-12 in metabolism. The essential amino acid methionine provides methyl group to other synthetic steps within the cell and HCY is formed. The cycle is completed by remethylation of HCY to methionine. The remethylation of HCY to methionine, catalysed by methionine synthase, requires 5-methyltetrahydrofolate – which is formed from 5,10-methylenetetrahydrofolate by the methylenetetrahydrofolate reductase (MTHFR) – and vitamin B-12 in the form of methylcobalamin. In the catabolic pathway, HCY is condensed with serine to form cystathionine (CYS). This reaction is catalysed by cystathionine  $\beta$ -synthase, which is vitamin B-6 dependent. Cystathionine is hydrolysed in another vitamin B-6 dependent reaction to cysteine and  $\alpha$ -ketobutyrate [2]. Blockage of either pathway results in elevated HCY concentration. This may occur in the case of inborn errors of metabolism or deficiency of folate, vitamin B-12 or vitamin B-6 [2,3,5,8,9]. HCY may also be elevated in patients with renal insufficiency [10–12]. As shown in Fig. 1, another metabolic pathway also requires vitamin B-12: the rearrangement of L-methylmalonyl-CoA to succinyl-CoA is catalysed by L-methylmalonyl-CoA mutase, a vitamin B-12 dependent enzyme. In vitamin B-12 deficiency, L-methylmalonyl-CoA mutase is impaired due

to a lack of adenosylcobalamin. Increased concentrations of methylmalonyl-CoA are hydrolysed and lead to elevated amounts of methylmalonic acid (MMA). Increased serum concentration of HCY is an indicator of functional intracellular deficiency of vitamin B-12 and folate, whereas enhanced MMA concentration is a more specific indicator for vitamin B-12 deficiency, only [2,7,13,14]. Increased serum concentration of CYS occurs for both vitamin B-12 and vitamin B-6 deficiency [7,15]. HCY and CYS are expected to be increased in intracellular vitamin B-6 shortage [16].

Megaloblastic anaemia is often an early symptom of vitamin B-12 shortage. Because a shortage of vitamin B-12, but not folate, can cause neurological damage, it is clinically important to diagnose the actual deficiency, which is helpful in order to prescribe the correct therapy [17–19]. The prevalence of elevated metabolite concentrations and potential risk factors for related diseases, such as neuropsychiatric [18,20] and atherothrombotic disorders [8], are very frequent in the elderly.

In recent years, multiple studies have associated moderate hyperhomocysteinemia with premature cerebrovascular, peripheral, and coronary artery disease [8,21–26]. In a study of 1041 elderly subjects from the Framingham Study cohort, it was found that higher plasma HCY concentrations were related to poor status for the vitamins B-6, B-12 and folate as well as to increased prevalence of carotid-artery stenosis, a risk factor for stroke [25,27].

Therefore, a disturbed methionine metabolism with moderate hyperhomocysteinemia, regardless of cause, is accepted as an independent atherogenic risk factor.

The aim of this study was to evaluate the prevalence of increased serum concentration of HCY and other related metabolites in two age groups: seniors and high-aged subjects. Furthermore, we wanted to clarify whether the determination of HCY in combination with the other metabolites tells us more about intracellular impairment of HCY metabolism in advanced age than B-vitamin serum concentrations.

## Subjects

We investigated 90 high-aged individuals (also called 'longeval'; 65 female, 25 male; median age 89 [85–102] years), 92 seniors (51 female, 41 male; median age 71 [65–75] years) and 50 younger subjects (37 female, 13 male; median age 28 [19–50] years). All elderly subjects were living at home independently and were able to carry out all usual daily activities on their own or with minimal help without any acute illness. None of them had a clinical history of acute cerebrovascular event or myocardial infarction. No active malignant process was present. There was no concomitant treatment with iron, vitamins or cytotoxic drugs. The most frequent chronic diseases in the high-aged subjects were arterial hypertension (24%), cardiac insufficiency (56%), coronary heart disease (31%) and NIDDM (24%). The most frequently-used prescribed drugs in the group of high aged subjects were as follows: digitalis 38%, diuretics (28%), ACE inhibitors (33%), nitrate (27%), acetyl salicylic acid (21%), antidiabetes drugs (14%) and antihypertension drugs (19%).

## Laboratory tests

All tests were carried out in serum that was separated from whole blood (4 °C) by centrifugation within 45 min after vein puncture and stored at –70 °C.

Serum metabolites, total HCY, CYS and MMA, were assayed by a modified capillary gas chromatography and mass spectrometry method according to Allen *et al.* and Stabler *et al.* [15,28] (capillary gas chromatograph 6890 with a mass selective detector 5973, Hewlett-Packard, Germany). Vitamin B-6 was investigated by HPLC. Peaks were separated under isocratic conditions and detected with a fluorescence detector. Folate and vitamin B-12 were measured with an Abbott IMX Analyser (II, USA) utilising the ion capture method and MEIA method respectively. Serum creatinine was measured using the Jaffé method without deproteinization (Roche Diagnostics, Germany) on a Hitachi Analyser.

## Statistical analysis

Median values, 5% and 95% percentiles, Mediantest, Mann–Whitney test, correlation analysis by Spearman-Rho and

backward regression analysis were calculated with the software package SPSS (version 8.0 for Windows; SPSS Inc., Chicago, IL, USA).

## Results

### Serum concentration of metabolites and B-vitamins

Multiple comparison (Mediantest) of the three groups has shown significant differences (1% level) between the groups for the following parameters: HCY, MMA, CYS, folate, vitamin B-6 and creatinine. For vitamin B-12 we could only find a significant difference between the younger subjects and the longeval (high-aged) subjects. The serum concentrations of HCY, MMA, CYS and creatinine increased from younger subjects over seniors to longeval subjects, while the vitamin concentrations decreased in this order, Table 1. Statistical evaluation of our data revealed for elderly subjects (> 65 years) no gender dependent significant difference in metabolite concentrations. Subdividing the age groups according to their creatinine concentration, we found that in both elderly groups, subjects with normal serum creatinine had lower concentrations of the metabolites HCY, CYS and MMA than those with increased creatinine concentrations. However, vitamin B-12, vitamin B-6 and folate did not significantly differ within the same age group subdivided into normal and increased serum creatinine. The frequency of pathologically increased metabolites rose strongly from seniors to high-aged subjects, Table 2. However, the frequency of lowered vitamin B/folate concentrations in senior and longeval subjects also raised significantly but less markedly. Utilising vitamin B-6, B-12 and folate to diagnose intracellular vitamin deficiency, the rate was 30% in seniors and 55% in longeval subjects. However, when using metabolites HCY, MMA and CYS, the rate was found much higher: 50% in seniors and 90% in longeval subjects.

### Relations of B-vitamins and metabolites

There was a significant inverse correlation between serum concentration of vitamin B-12 with MMA, HCY and CYS as well as a positive correlation with folate and vitamin B-6. Additionally, MMA correlated negatively with vitamin B-6, vitamin B-12 and folate but positively with HCY, CYS and creatinine. The serum concentration of vitamin B-6 significantly correlated inversely with HCY, CYS, MMA and creatinine but positively with folate and vitamin B-12. The CYS concentration correlated significantly with HCY and MMA but inversely with folate, vitamin B-6 and vitamin B-12.

### Multiple regression analysis

Backward multiple regression analysis revealed that only folate, MMA, creatinine and age were variables that

**Table 1** Medians\* of serum metabolite and vitamin concentrations for different age groups

	Younger subjects (19–50 years; <i>n</i> = 50)	Seniors (65–75 years; <i>n</i> = 92)	Longeval subjects (85–102 years; <i>n</i> = 90)	Significance
HCY (mmol L <sup>-1</sup> ) normal: 5–15 mmol L <sup>-1</sup>	7.5 (5.0/10.0)	12.4 (8.1/17.9)	16.5 (10.1/37.8)	s
CYS (nmol L <sup>-1</sup> ) normal: 65–301 nmol L <sup>-1</sup>	144.0 (79.8/247.6)	247.1 (123.6/504.9)	529.2 (212.0/2689.2)	s
MMA (nmol L <sup>-1</sup> ) normal: 73–271 nmol L <sup>-1</sup>	135.1 (92.7/294.0)	186.1 (89.4/378.7)	342.7 (138.2/1691.4)	s
Vitamin B-6 (mg L <sup>-1</sup> ) normal: 5.0–36.9 mg L <sup>-1</sup>	15.4 (6.2/34.5)	7.3 (2.6/15.7)	6.0 (1.7/22.9)	s
Vitamin B-12 (ng L <sup>-1</sup> ) normal: 179–1132 ng L <sup>-1</sup>	334.5 (223.3/641.4)	345.0 (159.5/745.6)	262.6 (141.0/1005.2)	ns
Folate (mg L <sup>-1</sup> ) normal: 2.8–16.9 mg L <sup>-1</sup>	8.7 (5.5/12.4)	6.1 (3.4/9.5)	5.5 (3.3/11.3)	s
Creatinine (mg dL <sup>-1</sup> ) normal: < 1.2 mg L <sup>-1</sup>	0.9 (0.8/1.1)	1.0 (0.8/1.4)	1.3 (0.9/2.9)	s
Age (years)	27.5 (19/43)	71.0 (65/75)	88.5 (85/96)	s

\*5% and 95% percentile in parenthesis. HCY, homocysteine; CYS, cystathionine; MMA, methylmalonic acid; s, significance within a 1% level between the groups (Mediantest); ns, not significant.

influenced the HCY concentration significantly and independently while the serum concentration of B-vitamins did not have an independent significant influence on the HCY concentration in serum, Table 3. The same analysis has also shown that the MMA concentration was significantly and independently influenced by folate, vitamin B-12, HCY and creatinine. The serum concentration of CYS was independently modulated by vitamin B-12, creatinine and age.

groups of elderly subjects and in a group of younger persons together with their serum concentrations of B-vitamins, folate and creatinine. This investigation confirms a high prevalence of hyperhomocysteinemia in elderly subjects and adds a further strong increase of the prevalence as well as of the degree of hyperhomocysteinemia in higher age, which is accompanied by a further increase of vitamin shortage that concerns its serum concentration as well as its frequency.

## Discussion

In the present study, we have measured serum concentrations of the metabolites HCY, MMA and CYS in two

## Vitamin B-12 and methylmalonic acid

Ageing is accompanied by a higher prevalence of vitamin B-12 deficiency [2,3,6,7,29,30]. In a trial on older patients

**Table 2** Frequency of pathological serum concentrations of metabolites and vitamins in subjects of different age groups

	Younger subjects ( <i>n</i> = 50)	Seniors ( <i>n</i> = 92)	Longevals ( <i>n</i> = 90)
Frequency of increased concentrations (%)			
HCY	0	24	62
MMA	4	23	62
CYS	4	36	81
Frequency of decreased concentrations (%)			
Folate	0	0	1
B-12	2	9	20
B-6	4	23	40
Frequency of B-vitamin shortage diagnosed by:			
metabolite determination* (%)	8	55	90
determination of serum vitamin concentration† (%)	6	30	50

HCY, homocysteine; MMA, methylmalonic acid; CYS, cystathionine. \*At least one of the metabolites (HCY, MMA, CYS) was increased. †At least one of the vitamins (B-6, B12, folate) was decreased.

**Table 3** Backward multiple regression analysis

Independent variable	Variables in the order of their removal	Variables with significant influence (10%)	
HCY	Vitamin B-12, Vitamin B-6, MTHFR, CYS	Age	0.000
		MMA	0.000
		Folate	0.002
		Creatinine	0.000
MMA	CYS, Vitamin B-6, MTHFR, Age	HCY	0.000
		Folate	0.070
		Vitamin B-12	0.056
		Creatinine	0.005
CYS	Vitamin B-6, MMA, Folate, MTHFR, HCY	Age	0.001
		Vitamin B-12	0.075
		Creatinine	0.000

(mean age 80 years), about 90% of those with serum vitamin B-12 < 150 pmol L<sup>-1</sup> showed evidence of tissue vitamin B-12 deficiency (increased HCY concentration, neutrophil hypersegmentation, or elevated MCV) [5]. Vitamin B-12 deficiency passes different stages from low serum vitamin B-12, low cell-stored vitamin B-12, biochemical vitamin B-12 deficiency, to clinical vitamin B-12 deficiency. Vitamin B-12 deficiency became manifest in older patients at relatively higher serum concentrations of vitamin B-12 than in younger subjects, possibly because of lower concentrations of holotranscobalamin II in higher age. Allen *et al.* [3] described subclinical vitamin B-12 deficiency as the most important deficiency in the elderly population although vitamin B-6 and folate deficiencies appear to play significant roles [27]. Elevated concentrations of the metabolites HCY and MMA are described as sensitive markers of vitamin B-12 status and suitable to identify clinical as well as subclinical or cellular deficiency [17,31]. Joosten *et al.* [7] have shown in an European study that elderly healthy subjects have elevated concentrations of HCY and MMA while serum vitamin B-12 and folate concentrations were in the low-normal range. Berg *et al.* [32] reported increased serum MMA concentration in 26% of healthy elderly subjects, but in 39% of geriatric patients, while vitamin B-12 was only lowered in 6% res. 5%. In our study, healthy seniors had a frequency of increased MMA concentration of 23% and in high-aged subjects a further increase to 62% was observed. Conversely, serum vitamin B-12 was decreased in 9% res. 20%. Thus, the MMA concentration is more sensitive in diagnosing an intracellular vitamin B-12 deficiency than the measurement of vitamin B-12 concentration in serum. MMA is a marker which mirrors the biochemical stage of vitamin B-12 deficiency but does not represent vitamin B-12 depletion, which precedes deficiency. Additionally, backward regression analysis revealed that MMA is a significant and independent, but not specific parameter of intracellular vitamin B-12 deficiency. Folate also influences the MMA concentration. When using MMA as a marker for vitamin B-12 deficiency, creatinine should be normal and folate above 5 mg L<sup>-1</sup> (in our opinion the lower normal value for folate of 2.8 mg L<sup>-1</sup> suggested by

the test kit manufacturer is too low and should be elevated to 5.0 mg L<sup>-1</sup>).

### Vitamin B-6 and cystathionine

Our results emphasise that vitamin B-6 and B-12 deficiencies strongly increase with advancing age. In subjects with intracellular vitamin B-6 deficiency, HCY and CYS concentrations in serum should be increased but the results are not consistent [16,33]. In patients with homozygous state for cystathionine-b-synthase deficiency, high concentrations of serum HCY were found and low or low-normal concentrations of serum CYS [28]. To study the effect of a selective vitamin B-6 deficiency on transsulfuration, Ubbink *et al.* [34] performed oral methionine load tests on vitamin B-6 deficient asthma patients treated with theophylline. Compared with younger subjects, methionine loading resulted in significantly higher increases in circulating HCY and CYS concentrations in vitamin B-6 deficient patients. Thus, vitamin B-6 deficiency may contribute to impaired transsulfuration. In our study, vitamin B-6 had a significant inverse correlation with HCY, CYS and MMA but correlated positively with folate. The latter correlation suggests that the increase of HCY and CYS by vitamin B-6 deficiency is possibly also induced by inhibition of the serin-hydroxymethyl-transferase, a vitamin B-6 dependent enzyme in the folate cycle (Fig. 1). This was very recently confirmed by tracer experiments on rats [35]. These data have shown that in vitamin B-6 deficient rats the formation of methionine from serine via cytosolic serine-hydroxymethyl-transferase and remethylation pathway was reduced by 81%. The deficiency did not significantly reduce cystathionine-b-synthase activity and *in vivo* hepatic transsulfuration flux increased by over two-fold. From this it follows that in vitamin B-6 deficiency, less 5-methyltetrahydrofolate is formed, leading to a disturbed remethylation of HCY and increased metabolite concentrations in serum, while the transsulfuration pathway was relatively insensitive to vitamin B-6 deficiency. Additionally, backward regression analysis of our data revealed that the CYS concentration was significantly and independently

influenced by vitamin B-12, creatinine and age. This confirms that in elderly subjects, the CYS concentration is not directly modulated by vitamin B-6 level. Therefore CYS is not a specific indicator for vitamin B-6 deficiency, but for in B-vitamin/folate deficiency in general.

### Renal function and homocysteine

The concentration of HCY does not only depend on age, gender, menopausal or vitamin status [36] but also seems to be influenced by renal clearance [37]. The renal clearance capacity substantially declines with age. About 2/3 of our high aged subjects and 1/3 of our senior subjects had increased serum creatinine, indicating a reduced glomerular filtration rate. In our study, serum creatinine correlated highly significantly with metabolite concentrations, confirming the role of the kidney as an important organ in HCY metabolism. The aetiology of hyperhomocysteinemia in patients with chronic renal disease is not well understood. Under normal conditions, 20–30% of plasma HCY is freely filtered, but < 1% of the produced total HCY is excreted into the urine, excluding decreased renal excretion as a factor for hyperhomocysteinemia in renal disease [10,11,38]. The remethylation of HCY in the renal metabolism plays an important role for the HCY clearance [10,11,37,39]. The renal uptake and metabolism could account for approximately 70% of daily HCY elimination from plasma [11]. The loss of normal renal metabolism plays a crucial role in the B-vitamin refractory hyperhomocysteinemia frequently seen in end-stage renal disease [11,40]. Further experiments will be necessary to characterise the potential circulating inhibitors of HCY remethylation in renal failure, which are largely unknown [41]. Backward regression analysis of our data revealed that the serum concentrations of HCY, MMA and CYS were significantly and independently influenced by creatinine. This suggests, that in elderly subjects with renal insufficiency, the remethylation of HCY to methionine is strongly impaired, causing hyperhomocysteinemia with simultaneous activation of the catabolic pathway. In our study we found an inverse correlation of HCY with folate and B-vitamins. However, from backward regression analysis, it follows that only folate influences the HCY concentration significantly and independently.

In conclusion, the early and correct diagnosis, as well as treatment of impaired HCY metabolism due to intracellular B-vitamin deficiency, is of clinical importance because it can prevent irreversible neurological damages in subjects with vitamin B-12 deficiency, which is very frequent in elderly persons. The measurement of B-vitamins in serum fails to sensitively diagnose intracellular disturbances in B-vitamin dependent HCY metabolism. The measurement of the metabolite concentrations HCY, CYS and MMA is much more effective in diagnosing an impaired HCY metabolism caused by intracellular B-vitamin deficiency as well as renal insufficiency. Compared to mere HCY or B-vitamin measurement in serum, the diagnostic efficiency of identifying subjects with

impaired HCY metabolism increased by 50–100% when utilizing all three metabolites. Instead of the methionine load test (difficult to practise in higher age), which identifies subjects who already have a functionally disturbed HCY metabolism but normal fasting HCY, the determination of the metabolites helps to diagnose a functionally impaired HCY metabolism with latent vitamin deficiency. The measurement of folate and creatinine in combination with metabolites helps to differentiate B-vitamin deficiency from folate shortage and/or renal insufficiency.

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### References

- Herrmann W, Quast S, Ullrich M, Schultze H, Bodis M, Geisel J. Hyperhomocysteinemia in high-aged subjects: relation of B-vitamins, folic acid, renal function and the methylenetetrahydrofolate reductase mutation. *Atherosclerosis* 1999;144:91–101.
- Stabler SP, Lindenbaum J, Allen RH. Vitamin B-12 deficiency in the elderly: current dilemmas. *Am J Clin Nutr* 1997;66:741–9.
- Allen RH, Lindenbaum J, Stabler SP. High prevalence of cobalamin deficiency in the elderly. *Trans Am Clin Climatol Assoc* 1995;107:37–45.
- Nordstrom M, Kjellstrom T. Age dependency of cystathionine beta-synthase activity in human fibroblasts in homocyst(e) inemia and atherosclerotic vascular disease. *Atherosclerosis* 1992;94:213–21.
- Metz J, Bell AH, Flicker L, Bottiglieri T, Ibrahim J, Seal E *et al.* The significance of subnormal serum vitamin B12 concentration in older people: a case control study. *J Am Geriatr Soc* 1996;44:1355–61.
- Araki A, Sako Y, Fukushima Y, Matsumoto M, Asada T, Kita T. Plasma sulfhydryl-containing amino acids in patients with cerebral infarction and in hypertensive subjects. *Atherosclerosis* 1989;79:139–46.
- Joosten E, van-den Berg A, Riezler R, Naurath HJ, Lindenbaum J, Stabler SP *et al.* Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. *Am J Clin Nutr* 1993;58:468–76.
- Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P *et al.* Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. *Circulation* 1998;97:437–43.
- Kang SS, Wong PW, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Annu Rev Nutr* 1992;12:279–98.
- Yeun JY. The role of homocysteine in end stage renal disease. *Semin Dialysis* 1998;11:95–101.
- Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997;52:10–20.
- Manns BJ, Burgess ED, Parsons HG, Schaefer JP, Hyndman ME, Scott-Douglas NW. Hyperhomocysteinemia, antidiabetic lipids

- antibody status, and risk for vascular access thrombosis in hemodialysis patients. *Kidney Int* 1999;**55**:315–20.
- 13 Koehler KM, Pareo-Tubbeh SL, Romero LJ, Baumgartner RN, Garry PJ. Folate nutrition and older adults: challenges and opportunities. *J Am Diet Assoc* 1997;**97**:167–73.
  - 14 Markle HV. Cobalamin. *Crit Rev Clin Lab Sci* 1996;**33**:247–356.
  - 15 Allen RH, Stabler SP, Savage DG, Lindenbaum J. Elevation of 2-methylcitric acid I and II levels in serum, urine, and cerebrospinal fluid of patients with cobalamin deficiency. *Metabolism* 1993;**42**:978–88.
  - 16 Miller JW, Ribaya-Mercado JD, Russell RM, Shepard DC, Morrow FD, Cochary EF et al. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. *Am J Clin Nutr* 1992;**55**:1154–60.
  - 17 Stabler SP. Screening the older population for cobalamin (vitamin B12) deficiency. *J Am Geriatr Soc* 1995;**43**:1290–7.
  - 18 Carmel R, Gott PS, Waters CH, Cairo K, Green R, Bondareff W et al. The frequently low cobalamin levels in dementia usually signify treatable metabolic, neurologic and electrophysiologic abnormalities. *Eur J Haematol* 1995;**54**:245–53.
  - 19 Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. 1988 (classical article). *Nutrition* 1995;**11**:181.
  - 20 Allen RH, Stabler SP, Lindenbaum J. Relevance of vitamins, homocysteine and other metabolites in neuropsychiatric disorders. *Eur J Pediatr* 1998;**157** (Suppl. 2):S122–S126.
  - 21 Coull BM, Malinow MR, Beamer N, Sexton G, Nord F, deGarmo P. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. *Stroke* 1990;**21**:572–6.
  - 22 Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T et al. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation* 1989;**79**:1180–8.
  - 23 Herzlich BC, Lichstein E, Schulhoff N, Weinstock M, Pagala M, Ravindran K et al. Relationship among homocyst(e)ine, vitamin B-12 and cardiac disease in the elderly: association between vitamin B-12 deficiency and decreased left ventricular ejection fraction. *J Nutr* 1996;**126**:1249S–1253S.
  - 24 Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;**338**:1042–50.
  - 25 Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995;**332**:286–91.
  - 26 Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;**337**:230–6.
  - 27 Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;**270**:2693–8.
  - 28 Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood* 1993;**81**:3404–13.
  - 29 Nilsson-Ehle H, Landahl S, Lindstedt G, Netterblad L, Stockbruegger R, Westin J et al. Low serum cobalamin levels in a population study of 70- and 75-year-old subjects. Gastrointestinal causes and hematological effects. *Dig Dis Sci* 1989;**34**:716–23.
  - 30 Hanger HC, Sainsbury R, Gilchrist NL, Beard ME, Duncan JM. A community study of vitamin B12 and folate levels in the elderly. *J Am Geriatr Soc* 1991;**39**:1155–9.
  - 31 Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994;**96**:239–46.
  - 32 Berg A, Rietzler R, Naurath HJ. Vitamin-B-Mangel. *Munch Med Wschr* 1993;**135**:506–9.
  - 33 Smolin LA, Benevenga NJ. Accumulation of homocyst(e)ine in vitamin B-6 deficiency: a model for the study of cystathionine beta-synthase deficiency. *J Nutr* 1982;**112**:1264–72.
  - 34 Ubbink JB, van der Merwe MA, Delport R, Allen RH, Stabler SP, Rietzler R et al. The effect of a subnormal vitamin B-6 status on homocysteine metabolism. *J Clin Invest* 1996;**98**:177–84.
  - 35 Martinez M, Cuskelly GJ, Williamson J, Toth JP, Gregory JF III. Vitamin B-6 deficiency in rats reduces hepatic serine hydroxymethyltransferase and cystathionine beta-synthase activities and rates of *in vivo* protein turnover, homocysteine remethylation and transsulfuration. *J Nutr* 2000;**130**:1115–23.
  - 36 Andersson A, Brattstrom L, Israelsson B, Isaksson A, Hamfelt A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. *Eur J Clin Invest* 1992;**22**:79–87.
  - 37 Arnadottir M, Hultberg B, Nilsson EP, Thysel H. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest* 1996;**56**:41–6.
  - 38 Janssen MJB, Stehouwer CD, Boers GH. Hyperhomocysteinaemia: a role in the accelerated atherogenesis of chronic renal failure? *Neth J Med* 1995;**46**:244–51.
  - 39 Guttormsen AB, Schneede J, Ueland PM, Refsum H. Kinetics of total plasma homocysteine in subjects with hyperhomocysteinemia due to folate or cobalamin deficiency. *Am J Clin Nutr* 1996;**63**:194–202.
  - 40 Bostom AG, Shemin D, Lapane KL, Sutherland P, Nadeau MR, Wilson PW et al. Hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein (a) excess in maintenance dialysis patients: a matched case-control study. *Atherosclerosis* 1996;**125**:91–101.
  - 41 Henning BF, Rietzler R, Tepel M, Langer K, Raidt H, Graefe U et al. Evidence of altered homocysteine metabolism in chronic renal failure. *Nephron* 1999;**83**:314–22.