J Neural Transm (2013) 120:211–224
DOI 10.1007/s00702-012-0822-y

DEMENTIAS - REVIEW ARTICLE

Folate and Alzheimer: when time matters

Margareta Hinterberger · Peter Fischer

Received: 9 March 2012 / Accepted: 6 May 2012 / Published online: 25 May 2012

Abstract Folate is necessary for DNA and mtDNA integrity and via folate/B12-dependent methionine cycle for methylation of multiple substrates (epigenetic DNA and enzymes) and methylation of homocysteine. During embryogenesis, folate deficiency is a risk factor for neural tube defects and late in life for cognitive decline and Alzheimer's dementia (AD). It induces several Alzheimer pathomechanisms like oxidative stress, Ca^{++} influx, accumulation of hyperphosphorylated tau and β-amyloid. But impact of folic acid supplementation on prevention or delay of dementia is a matter of debate. Six out of seven randomized controlled trials (RCT) with B vitamin intervention periods between 2 and 5.4 years reported about cognitive benefits in the supplemented groups mainly for those subjects with high homocysteine or low folate levels at baseline. This review tries to demonstrate the connection between folate deficiency and AD, analyses selected epidemiologic studies and RCT on folate/B12/homocysteine with long-observation periods ($\geq$2 years RCT; $\geq$4 years observational) and attempts to find explanations for the controversy in literature like short follow-up, heterogeneity of subjects concerning age, recruitment, baseline cognition, inclusion criteria and probably "misleading"(not representative for the past) folate/B12/homocysteine levels due to not reported short-term use of multivitamins or food-fortification. Population-based studies—epidemiologic and interventional—starting in the fourth decade would provide the best information about the impact of folate on later development of AD. Mandatory folate fortification areas will be important future field studies for—like neural tube defects—hopefully declining AD incidence and disproving safety concerns.

Keywords Folate · Alzheimer's disease · Dementia · Cognition · Vitamin B12 · Homocysteine

M. Hinterberger · P. Fischer
Ludwig Boltzmann Institute of Aging Research,
Langobardenstraße 122, 1220 Vienna, Austria
e-mail: margareta.hinterberger@utanet.at

P. Fischer (✉)
Psychiatric Department, Medical Research Society Vienna D.C.,
Danube Hospital, Langobardenstraße 122, 1220 Vienna, Austria
e-mail: p.fischer@wienkav.at

Introduction

Folate deficiency induces several pathophysiologic changes supposed to be pathogenetic in Alzheimer's dementia (AD) (Fig. 1) like mitochondrial dysfunction leading to oxidative stress, loss of calcium regulation, neuronal and synaptic impairment and accumulation of hyperphosphorylated tau and β-amyloid (Querfurth and LaFerla 2010). These specific features make low folate a probable candidate for long-term contribution to AD development.

Folate, the most important methyl group donator, is essential for maintaining integrity of DNA and mtDNA (Fig. 2). Multiple acceptors, like enzymes or DNA are methylated through the folate- and vitamin B12-dependent methionine–homocysteine cycle (Fig. 2). Folate is known to foster proper neural tube development during human embryogenesis (Mattson and Shea 2003) and lifelong neuronal cell growth and repair (Iskandar et al. 2004; Kruman et al. 2002, 2005). As a consequence, folic acid fortification of flour and grain has been introduced in several countries (USA and Canada 1998, Australia 2009, but not in Europe), reducing inadequate serum folate status from 16–26 % to 0.5–1.7 %, inadequate erythrocyte folate—a marker of folate intake over the past 90–120 days—from 39 to 3.7 %, high (>13 μmol/L)

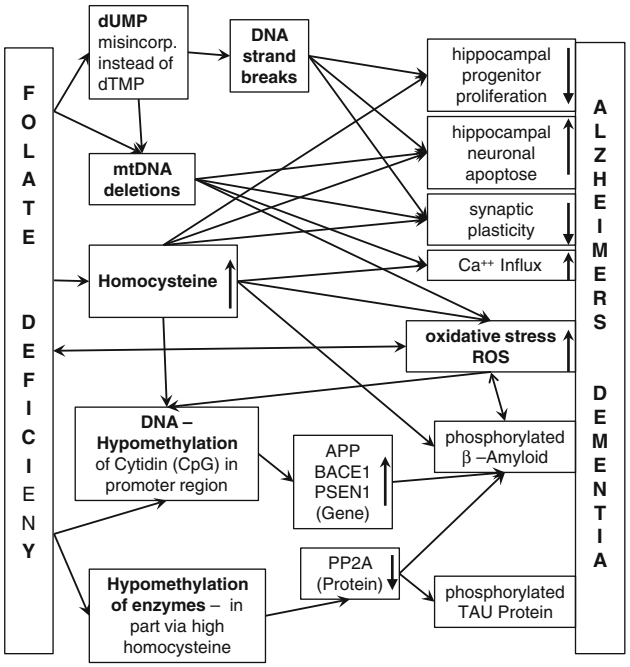


Fig. 1 Folate deficiency and Alzheimer's dementia. For depicting this figure, the following references were used: (Fenech 2010; Fleming et al. 2011; Fuso and Scarpa 2011; Ho et al. 2003; Kruman et al. 2002; Kruman et al. 2005; Mattson and Shea 2003; Pierrot et al. 2006; Sontag et al. 2007, 2008). Only direct folate and/or homocysteine-dependent actions are considered. *dUMP* deoxy-uridine monophosphate, *dTMP* deoxy-tymidine monophosphat, *mtDNA* mitochondriale DNA, *APP* amyloid precursor protein, *BACE1* beta site amyloid precursor protein cleavage enzyme 1, *PSEN1* presenilin1, *PP2A* protein phosphatase 2A

homocysteine from 18.7 to 9.8 % of the US population and reducing neural tube defects by 26 % (Dietrich et al. 2005; Jacques et al. 1999; Pfeiffer et al. 2005; Yetley and Johnson 2011). But fortification also has raised the problem of confusing ongoing trials (Bostom et al. 2001), appearance of unmetabolized folic acid of unknown significance in the blood and probably jeopardizing vitamin B12-deficient people (Morris et al. 2010).

Whether folate supplementation can delay or prevent Alzheimer's disease is a matter of debate (Malouf and Grimley 2008). Epidemiologic studies found low folate or high homocysteine being a risk factor for later cognitive decline or AD (Fischer et al. 2008; Seshadri et al. 2002). However, reviews and meta-analyses of randomized controlled trials (RCT) on folate and cognition have provided inconclusive evidence (Dangour et al. 2010). Several weaknesses are running like a red thread through most of these studies: (1) heterogeneity of the subjects concerning age, recruitment and baseline cognition. (2) Different methods of assessment of cognition or dementia. (3) Different methods of determination of folate levels (food questionnaires, supplemental use and laboratory analyses). (4) Lack of a standardized threshold level and inconsistent definitions of "low" folate or "high" homocysteine levels with heterogeneous study entry criteria. (5) "Misleading" folate levels due to short-term use of multivitamins without reporting. (6) Mandatory folic acid fortification. (7) Short follow-up.

This review aims on the pathophysiologic connections between folate deficiency and different hypothesized AD pathomechanisms and on selected papers fulfilling the following criteria: longitudinal/observational studies or RCT on the impact of folate on cognition or AD incidence after a follow-up of at least 2 (RCT) or 4 and more (observational) years.

Folate metabolism (Fig. 2)

Folate is indispensable for purines syntheses and transformation of uracil into thymine (Herbig et al. 2002), thus maintaining DNA replication, repair and mtDNA integrity (Fenech 2010). Tetrahydrofolate (THF) transformation cycle and folate/vit B12-dependent methionine-methylation cycle necessary for methyl group donation to many acceptors are shown in Fig. 2. The impact of polymorphism of multiple folate metabolizing enzymes on AD is controversial (Friso et al. 2002; Kageyama et al. 2008; Hua et al. 2011; Naj et al. 2010).

Pathomechanism connecting folate deficiency and Alzheimer's dementia

Folate deficiency impairs DNA and mitochondrial DNA (mtDNA) causing oxidative stress and ROS generation—an accepted early hit in the AD pathogenesis (Querfurth and LaFerla 2010) followed by neuronal impairment and cell death in AD involved brain areas (Kruman et al. 2002, 2005). Folate deficiency causes hypomethylation of enzymes and promoter regions of genes reportedly involved in AD pathogenesis (Fuso and Scarpa 2011). Folate deficiency is accompanied by increase of cytosolic Ca^{++} and of homocysteine (Mattson and Shea 2003) which is a known risk factor for myocardial infarction, stroke and cognitive decline (Selhub 2008; Seshadri et al. 2002).

Folate deficiency and DNA

Lack of methyl group donation precludes conversion of dUMP (deoxy-uridine monophosphate) into dTMP (deoxy thymidine monophosphate) leading to uracil misincorporation into DNA causing double strand breaks prone to errors eventually leading to DNA damage and apoptosis (Fenech 2010; Quinlivan et al. 2005). Impaired DNA repair was found in folate-deficient hippocampal pyramidal

neurons (region CA1-3) and proliferation of adult hippocampal progenitors, synaptic plasticity and neuronal function is inhibited by folate deficiency in synergy with homocysteine (Ho et al. 2001, 2003; Kruman et al. 2002, 2005; Mattson and Shea 2003).

Folate deficiency has the same detrimental impact on mtDNA leading to reduced ATP generation and oxidative stress, with production of reactive oxygen species (ROS), dysregulation of calcium homeostasis, reduction of mitochondrial number and eventually apoptosis of the neuron (Fenech 2010; Ho et al. 2003; Ross 2005).

Folate deficiency and oxidative stress

In the brains of AD patients, ROS are responsible for damage of nuclear and mitochondrial DNA, protein oxidation, lipid peroxidation and β-amyloid aggregation (Christen 2000). APOE and folate have antioxidative capacities (Chan and Shea 2006a, b; Chan et al. 2009; Shea et al. 2002; Stanger and Wonisch 2012). Folate deprivation was shown to induce increase of ROS directly and via homocysteine (Ho et al. 2003; Mattson and Shea 2003). Tetrahydrofolate (THF) is very susceptible to oxidation leading to further intracellular and mitochondrial depletion of folate (Fuchs et al. 2001). Folate supplementation enhances antioxidant status and memory (Singh et al. 2011), prevents β-amyloid-induced oxidative stress and neurotoxicity (Ho et al. 2003) and enhances growth and repair mechanisms even in the adult central nervous system (Iskandar et al. 2004).

Folate deficiency and homocysteine

Folate or B12 deficiency increases homocysteine—a cytotoxic, atherogenic, non-proteinogenic amino acid—which induces Ca^{++} influx via NMDA channels and increases β-amyloid-induced Ca^{++} influx, potentiates glutamate excitotoxicity, induces oxidative stress and increases ROS-induced β-amyloid formation, increases hyperphosphorylated tau (Ho et al. 2003; Mattson and Shea 2003) and sensitizes hippocampal neurons to β-amyloid toxicity and apoptosis. During excessive oxidative stress, however, irreversible degradation with paradox reduction of plasma homocysteine by transsulfuration pathway in liver or gut (Fig. 2) occurs (Rogers et al. 2007). Lack of betaine-mediated conversion and irreversible transsulfuration of homocysteine in brain tissue aggravates local toxicity (Shea and Rogers 2002).

Folate deficiency and methyl group donation via methionine cycle

High homocysteine increases *S*-adenosyl homocysteine (SAH), which is a potent inhibitor of all methylation reactions (James et al. 2002). Genomic CpG dinucleotide methylation—regulating DNA stability, structure, and gene expression via promoter regions (Fenech 2010)—inversely correlates with homocysteine levels and directly with folate status (Friso et al. 2002). SAH/homocysteine-dependent DNA hypomethylation—which is an activating input on the promoter region—has already been demonstrated for APP (amyloid precursor protein) gene, PSEN1 (gene encoding presenilin 1) and BACE 1 (gene encoding beta site APP cleaving enzyme 1) leading to increased formation of β-amyloid (Fuso et al. 2005; Fuso and Scarpa 2011; Lin et al. 2009; West et al. 1995), but results are controversial (Brohede et al. 2010; Wang et al. 2008; Jung et al. 2011). Reduced methylation impedes protein phosphatase 2A (PP2A) leading to hyperphosphorylation of tau and APP (Sontag et al. 2007, 2008) rendering it more susceptible to β cleavage.

Observational and interventional studies

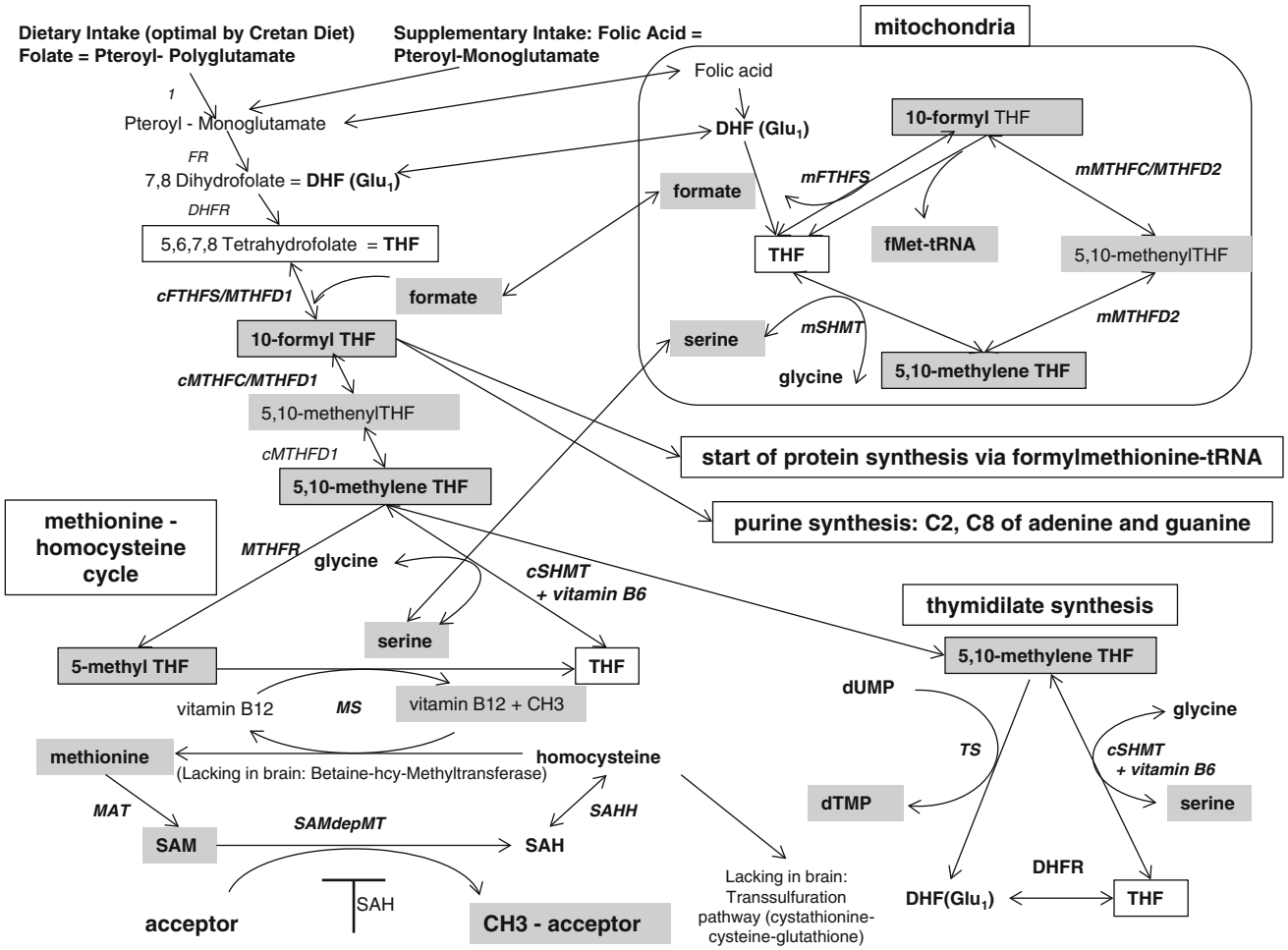
Folic acid supplementation in atherosclerotic diseases has generally fallen short of expectations (Maron and Loscalzo 2009). Interventional studies on folic acid with and without B12 for preventing dementia or cognitive decline in healthy or demented persons seemingly are not doing better (Malouf and Grimley 2008; Shea et al. 2012). Heterogeneity of methods in these studies almost precludes final comparisons and conclusions. In addition, it is not surprising that interventional studies with follow-up periods of weeks are failing when the disease triggered by a suspected deficiency takes decades or probably the whole life to evolve (Lahiri and Maloney 2010).

Recent mandatory folic acid fortification or short-term use of multivitamins without reporting produce "misleading" folate, B12 and—to a much lesser extent—homocysteine levels (Jacques et al. 1999) not representative for the vitamin exposure during the past. In the VITA study (longitudinal, community-based birth cohort study) about 5–10 % of those without vitamin history at baseline supposedly did not report about it (Hinterberger, personal communication). Therefore, levels of folate and B12 might not depict the real, long-lasting, possibly deficient levels but are probably only a short flashlight of 1 day or 1 week. Sporadic supplementation might be explained by increasing concerns of older people about their decreasing health and memory conditions.

Population-based studies with observation periods of ≥4 years (Table 1)

Studies using food frequency questionnaires (FFQ)

Five studies analysed elder subjects free of dementia at baseline using food frequency questionnaires (FFQ) to



quantify impact of folate and B12 intake on incident AD and/or dementia or cognitive decline after 4–9 years (Table 1). Corrada et al. (2005) and Luchsinger et al. (2007) found a reduced risk of AD with higher total (dietary and supplementary) folate intake, but not with B12. Both studies started long before the start of mandatory folic acid fortification and ended 1 (Corrada et al. 2005) or 2 years afterward (Luchsinger et al. 2007).

Nelson et al. (2009), however, did not find any association with incident AD, but CCMS started 1995 2 to 3 years before mandatory folic acid fortification possibly beneficially changed folate and homocysteine levels (Yetley and Johnson 2011) during the following 6 years thus confounding former low folate and high homocysteine exposure. The problem of "misleading" folate might apply to CCMS, too. The rate of multivitamin users dramatically increases with increasing folate quintiles from lowest (1.7 %) to highest (95 %) without differentiation between long-term exposure and only short-term use. The AD incidence in CCMS seems to be rather small compared to epidemiologic data (Ferri et al. 2005). Only 5.8 % (212 out of 3,634) of a population with a median age of 75 years and a follow-up of 9 years. The MMSE screening policy and drop outs might have underestimated the AD incidence. There remains, however, the possibility of reduced AD incidence because of advantageous impact of fortification. In a comparable study, Morris et al. (2006) reported about 161/1,041 incident AD (15.5 %) after 3.9 years. Dietary intake of folate or B12 again was not associated with AD incidence, but like CCMS this study crossed the fortification start, too, and had a high rate of supplementation users.

However, in contrast to the studies above Morris et al. (2005) from Chicago reported fastest cognitive decline in the highest total folate intake quintile compared to the lowest but reduced cognitive decline in the oldest subjects with high total B12 intake. There might be several explanations to these results: (1) the highest quintile of total folate intake also presented with exceedingly good cognitive performance at baseline. A greater regression to the mean from maximum is not surprising and possibly with less clinical relevance than a smaller decline from a worse starting point. (2) Again in the highest folate quintile there are 96.9 % multivitamin users, compared to 3.1 % in the lowest quintile with the uncertainty of duration of the

◀ **Fig. 2** Folate-mediated one-carbon metabolism. For depicting this figure, the following references were used: (Appling 1991; Herbig et al. 2002; MacFarlane et al. 2009; Mattson and Shea 2003; Morris and Tangney 2007; Quinlivan et al. 2005; Ulrey et al. 2005). The chemical reactions are simplified and incomplete. Dietary "folate" intake, highest with "Cretan diet", provides the body with pteroyl-polyglutamate.Cleavage by several intestinal y-Glutamyl-Carbopeptidases (*1*) from gut, liver and pancreas into pteroyl-monoglutamate is necessary before resorption (only about 50 %). Supplemented "folic acid" (pteroyl-monoglutamate) is readily absorbed (100 %), converted to dihydrofolate (*DHF* Glu_1 dihydro-pteroyl-monoglutamat) (both able to enter mitochondria) and tetrahydrofolate (*THF* tetrahydro-pteroyl-monoglutamat)—the bioactive starting point of methylation—by FR and DHFR. Glu_1 (monoglutamat) is only explicitly written for *DHF* (dihydrofolate), and omitted for all other active THF-metabolites. THF-polyglutamate and 5-methyl-THF-polyglutamate are inactive intracellular storage forms not shown in this figure. For activation, they have to be transformed into monoglutamates by folyl-polyglutamyl-hydrolase first. *Cytoplasm*: In cytoplasm the trifunctional enzyme MTHFD1 bidirectionally catalyzes transformation into 10-formyl THF (cFTHFS/MTHFD1), the starting point for purine synthesis supplying C2 and C8 of adenine and guanine and for protein synthesis producing fMet-tRNA. cMTHFC/MTHFD1 and MTHFD1 bidirectionally transform 10-formylTHF into 5,10-methyleneTHF, starting point for thymidilate synthesis. *Mitochondria*: The analog enzyme found in mitochondria is only bifunctional: MTHFD2 with mMTHFC activity. mFTHFS bidirectionally tranforms 10-formylTHF and THF and generates formate and fMet-tRNA. THF is bidirectionally transformed into 5,10-methyleneTHF by vitamin B6 highly dependent cSHMT or B6 less dependent mSHMT using serine as a methyl group donor. Formate, serine, folic acid and DHF can pass mitochondrian membrane. *Thymidilate synthesis*: 5,10methylenTHF is methylating dUMP by TS forming dTMP and DHF Glu_1, which is again reduced to THF by DHFR. *Methionine-homocysteine cycle*: 5-methyl-THF is the methyl group donor to transform homocysteine into methionine by MS and vitamin B12. Methionine is transformed by MAT into SAM, which is the methylgroup donor for many acceptors like phospholipide, myelin, DNA, RNA, protein, enzymes (PP2A), choline, acetylcholine, catecholamine, melatonin. SAM is transformed to SAH when the acceptor is methylated by *SAMdepMT*. Accumulation of SAH which has an equilibrium to homocysteine strongly inhibits further methylation. *SAH* is hydrolyzed to homocysteine by *SAHH*. Lack of two major routes of homocysteine degradation in brain tissue (betaine-mediated conversion and transsulfuration into cystathionine and cysteine). *FR* Folatreductase, *DHFR* dihydrofolate reductase, *MTHFD1* cytoplasmatic trifunctional enzyme: methylene-THF dehydrogenase (NADP + dependent)1 with FTHFS and MTHFC function: *cFTHFS/MTHFD1* cytoplasmic formyl-THF synthase function of MTHFD1, *cMTHFC/MTHFD1* cytoplasmic methenyl-THF cyclohydrolase function of MTHFD1; *MTHFD2* mitochondrial bifunctional enzyme: methylene-THF dehydrogenase (NADP + dependent) 2 with MTHFC function: *mMTHFC/MTHFD2* mitochondrial methenyl-THF cyclohydrolase function of MTHFD2, *mFTHFS* mitochondrial formyl-THF synthase, *fMet-tRNA* formyl-methionine-tRNA, *mSHMT* mitochondrial serine hydroxymethyltransferase, *cSHMT* cytoplasmic serine hydroxy methyltransferase, *MTHFR* methylene THF reductase, *MS* methionine synthase, *hcy* homocysteine, *MAT* methionine adenosyltransferase, *SAM* *S*-adenosy-methionine, *SAMdepMT* SAM-dependent methyltransferase; *SAH* *S*-adenosyl-homocysteine, *SAHH* SAH hydrolase, *dUMP* deoxy-uridine monophophate, *dTMP* deoxy thymidine monophosphate, *TS* thymidilate synthase, *acceptor*: phospholipide, myelin, DNA, RNA, protein, *enzymes*: (e.g.: PP2A), choline, acetylcholine, catecholamine, melatonin. *Gray background* indicates CH3 carrier, *rectangle box* bioactive THF derivative

supplementation. (3) No increased AD incidence was reported as mentioned above (Morris et al. 2006).

In part, similar results were reported by Morris et al. (2007, 2010) from Boston in a cross-sectional study using serum values instead of FFQ. They found worse cognitive performance in subjects with fortification-induced high levels of folate and unmetabolized free folic acid but only in subjects with concomitant low B12. Those with high folate but normal B12 levels, however, showed better cognitive performance. In the elderly, B12 deficiency (<148 pmol/L) was reported to occur in about 6 %, marginal depletion (148–221 pmol/L) in more than 20 % (Allen 2009) probably due to gastritis-induced worse B12 uptake (Selhub et al. 2000). Therefore, some experts have already suggested mandatory B12 fortification (Refsum and Smith 2008; Selhub and Paul 2011) to protect the B12-deficient elderly from the harm of elevated folate intake.

Studies using serum/plasma/erythrocyte levels of folate

Nine studies analysed influence of baseline folate, B12 and homocysteine levels on incident AD/dementia or cognitive decline after 4–8 years (Table 1). Three studies reported low folate (Fischer et al. 2008, personal communication; Kado et al. 2005; Ravaglia et al. 2005), three reported B12 (Elias et al. 2005; Haan et al. 2007; Hooshmand et al. 2010) and six reported homocysteine as independent risk factor for cognitive decline, dementia or AD (Elias et al. 2005; Haan et al. 2007; Kivipelto et al. 2009; Nurk et al. 2005, 2010; Ravaglia et al. 2005; Seshadri et al. 2002). Only three studies crossed the fortification start before closure and five took place in unfortified areas (Table 1).

Kado et al. (2005) reported worse cognitive outcome in subjects with excellent physical and cognitive performance when in the lowest folate quartile at baseline.

Elias et al. (2005) showed an association of higher homocysteine and low B12 with worse cognitive performance after 7.6 years only in the oldest of three age groups (60–82 years) demonstrating the long time necessary for high homocysteine to produce clinically measurable cerebral damage. Folate showed no association. However, folate plasma levels significantly increased from the youngest to the oldest age group with increasing SD and again might be a hint at confounding short-term supplementation.

VITA study (Fischer et al. 2008, personal communication) found folate being an independent risk factor for AD in multiple adjusted models, also including homocysteine and B12.

Haan et al. (2007) demonstrated that the risk of homocysteine-induced dementia or cognitive decline may be modified by plasma vitamin B12. No impact of red cell

Table 1 Longitudinal, observational studies on folate/vitamin B12/homocysteine and cognition with follow-up periods of ≥4 years

Author	*N*	Age [mean (SD) or range] (baseline)	Follow-up (years)	Cognitive start	End Point	Food Frequency Questionnaire (FFQ) or plasma/serum (at baseline)
Corrada et al. (2005), Baltimore Longitudinal Study of Aging (BLSA)	579	69.6 (range 49–93)	9.3 (0.4–14.6)	Free of dementia	Incident AD NINCDS-ADRDA (nonAD-dementia censored)	7 days FFQ + supplements
Luchsinger et al. (2007), Washington Heights-Inwood Columbia Aging Project (WHICAP)	965	75.8 (±5.8)	6.1 (± 3.3)	Free of dementia	Incident AD NINCDS-ADRDA (nonAD-dementia censored)	FFQ + supplements serum
Nelson et al. (2009), Cache County Memory, Health Aging Study (CCMS)	3,634	75 (±6.5)	9	Free of dementia	Incident dementia/AD screening (MMSE) Dementia Questionnaire	FFQ
Morris et al. (2005), Chikago Health and Aging Project (CHAP)	3,718	74.3	5.5	Free of dementia	Cognitive decline (MMSE, memory, perceptual speed, attention)	FFQ + supplements (14 % after folic acid fortification has started
Morris et al. (2006), Chikago Health and Aging Project (CHAP)	1,041	72.5	3.9	Free of dementia	Incident AD by CERAD + NINCDS-ADRDA without excluding co-existing condition causing dementia	FFQ + supplements median 1.2 years after baseline
Elias et al. (2005), Framingham Offspring Study	2,096	40–49 50–59 60–82	7.6 (±1.02)	Free of dementia and stroke	Cognitive decline neuropsychol test battery (12 tests, only once after 7,6 years)	Plasma (fasting)
Kado et al. (2005), MacArthur Studies of Successful Aging	370	70–79	7	Free of dementia top third (cognitive and physical)	Cognitive decline (=being in bottom quartile) neuropsychol test battery (5 tests)	Plasma (non fasting)
Fischer et al. (2008), personal communication VITA 2012	492	75 (±0.4)	7.5	Free of dementia	Incident AD by CERAD + NINCDS-ADRDA	Serum (fasting)
Haan et al. (2007), Sacramenta Area Latino (SALSA)	1,779	60–69: $n = 790$ 70–79: $n = 597$ 80–101: $n = 147$	4.5	Free of dementia	Dementia and CIND; screening (MMSE)DSM 3, NINCDS-ADRDA	Plasma (B12, homocysteine) red blood cell folate
Hooshmand et al. (2010), Cardiovasc.,Aging, Dementia (CAIDE)	271	70.7 (±3.6)	7.4	Free of dementia	Incident AD screening (MMSE) NINCDS-ADRDA	Serum (+holotranscobalamin = holoTC)
Kivipelto et al. (2009), Kungsholmen Project	213	81 (±4.6)	6.7	Free of dementia	Dementia/AD DSM 3	Plasma, (+holoTC)
Nurk et al. (2005), Hordaland Homocysteine study	2,189	66–67	6	Free of dementia	Assessment of episodic memory (KOLT) once	Plasma (non fasting)
Ravaglia et al. (2005), Conselice study of brain aging (CSBA)	816	73.6 (±6.3)	4	Free of dementia	Incident dementia/AD DSM IV, NINCDS-ADRDA	Plasma(hcy) serum (vit)
Seshadri et al. (2002), Framingham Study	1,092	76 (±6)	8	Free of dementia	Incident AD screening (MMSE) DSM IV, NINCDS-ADRDA	Plasma (non fasting)

Table 1 continued

Author	Significant risk factor for AD or cognitive decline			FA fortification since 1997/98 in USA and Canada Date of study	AD incidence or comment	Supplement use folic acid (FA), Vitamin B12(B12)
	Folate	B12	Homocysteine			
Corrada et al. (2005), Baltimore Longitudinal Study of Aging (BLSA)	60 % reduced risk of AD for subjects with folate uptake at or above RDA (multiple adjusted, B12 included)	No	Not done	Start: 1984, end: 1999	57 AD (9.8 %) (non-AD dementia not stated)	FA: 31 %, B12: 34 %
Luchsinger et al. (2007), Washington Heights-Inwood Columbia Aging Project (WHICAP)	Highest quartile of total folate intake related to lower risk of AD (multiple adjusted, B12 and hcy included)	No	No	Start: 1992/1994, end: ≈2001/02	192 AD (19.9 %) (non-AD dementia not stated)	FA: NoAD: 32.7 %, AD 29.2 %, B12: No AD:31.6 % AD:27.1 %
Nelson et al. (2009), Cache County Memory, Health Aging Study (CCMS)	No	No	Not done	Start: 1995, end: 2004	212 AD (5.8 %) (353 all cause dementia)	Multivitamin use female: 38.6 %, male: 45.6 %
Morris et al. (2005), Chikago Health and Aging Project (CHAP)	Highest quintile has fastest cognitive decline but also by far the best cognitive performance at baseline	High supplementation reduces cognitive decline in the oldest	Not done	Start : 1993–1997, end: 2002	Highest folate quintile has 96.9 % multivitamin users versus overall 33 %	FA: 31.6 %
Morris et al. (2006), Chikago Health and Aging Project (CHAP)	No	No	Not done	Start:1993-97, end:2002	161 AD (15.5 %) (non-AD dementia not excluded)	No data (Morris et al. 2005: FA: 31.6 %)
Elias et al. (2005), Framingham Offspring Study	No	Positively related to cognition (6/12 tests better) in oldest group (60–82)	Cognitive performance (8/12 tests) worse in oldest group with higher homocysteine (multiple adjusted, folate, B12 included)	Start:1991/94, end:1999/ 2002	Highest folate plasma levels in oldest group	No data
Kado et al. (2005), MacArthur Studies of Successful Aging	Lowest quartile: increased risk of being in the bottom quartile of cognition,RR 1.6 (multiple adjusted, B12 & hcy included)	No	No (only univariate significant, not with folate in the model)	Start 1988/89, end:1995	For logistic regression: lowest or highest quartiles vs. rest	No data
Fischer et al. (2008), personal communication VITA 2012	Independent risk factor multiple adjusted, B12 and hcy included: OR: 0.9, (95 %CI:0.85–0.96) $p = 0.002$	No	No	No fortification area (Austria)	115 AD (23.4 %) (124 all cause dementia)	FA: 6.3 %, B12: 12.3 %

Table 1 continued

Author	Significant risk factor for AD or cognitive decline			FA fortification since 1997/98 in USA and Canada Date of study	AD incidence or comment	Supplement use folic acid (FA), Vitamin B12(B12)
	Folate	B12	Homocysteine			
Haan et al. (2007), Sacramenta Area Latino (SALSA)	No	U-shaped (middle tertile best); but also interaction: highest B12 tertile reduces hcy associated risk	Independent risk factor (multiple adjusted, B12 included)	Start:1997/99, end; 2002/04	62 dementia and 55 CIND analysed together	Multivitamin use: 24.5 %
Hooshmand et al. (2010), Cardiovasc.,Aging, Dementia (CAIDE)	No	Independent risk factor (multiple adjusted, folate and hcy included)	Significant only in below median holoTC group	No fortification area (Finland/ Suomi)	17 AD (6.2 %) (non-AD dementia not stated)	No vitamins used (not stated as exclusion criteria)
Kivipelto et al. (2009), Kungsholmen Project	No	U-shaped: 3rd tertile associated with reduced risk of AD and dementia (multiple adjusted, folate & hcy included)	Highest quartile has doubled risk of AD (multiple adjusted, folate, B12 included)	No fortification area (Sweden)	61 AD (28.6 %) (83 all cause dementia)	B12 supplemented ($n = 15$; 6.6 %) excluded
Nurk et al. (2005), Hordaland Homocysteine study	Lowest quintile at baseline associated with memory deficits when multiple adjusted, significance lost when hcy enters the model	No	Highest quintile at baseline associated with memory deficits, (multiple adjusted, folate, B12 included)	No fortification area (Norway)	Cognition not tested at baseline	Multivitamin or any type of B vitamin 11.1 %
Ravaglia et al. (2005), Conselice study of brain aging (CSBA)	Independent risk factor for both, AD or dementia (multiple adjusted, hcy included)	No	Independent risk factor for both, AD or dementia (multiple adjusted, folate, B12 included)	No fortification area (Italy)	70 AD (8.5 %) (112 all cause dementia)	No data
Seshadri et al. (2002), Framingham Study	No	No	Independent risk factor for both, AD or dementia (multiple adjusted, folate, B12 included)	Pre-start: 1979/82, start:1986/ 90, end:2000	83 AD (7.6 %) (111 all cause dementia)	No data

AD Alzheimeŕs dementia, *FA* folic acid, *MMSE* Mini Mental State Examination and CERAD: Morris et al. 1989; *NINCDS-ADRDA* McKhann et al. 1984, *RDA* recommended dietary allowance, *KOLT* Kendrick Object Learning Test, *CIND* Cognitive Impaired No Dementia; *hcy* homocysteine, *DSM* Diagnostic and statistical manual of mental disorders 3rd or 4th edition of the American Psychiatric Association

folate could be demonstrated probably because the study started after the beginning of the folate fortification.

Hooshmand et al. (2010) reported only holotranscobalamin (active form of B12) but not folate being an independent risk factor for incident AD. Homocysteine lost its significance when holotranscobalamin entered the model but remained significant in the below median group of holotranscobalamin.

Kivipelto et al. (2009) reported a doubled risk of AD in the highest homocysteine quartile, and a reduced risk of dementia and AD in the third holotranscobalamin quartile. The U-shaped association probably might be due to infrequent and unreported supplement use or parenteral administration. Only 15/228 subjects reported supplementation with B12 and folic acid and were obviously excluded from analysis. But only 6.6 % supplemented subjects in a 80-year-old study group seems to be less than reported from FFQ studies (Table 1) or our own experience (VITA study: 80-year old: folate: 24.9 %, B12: 25.7 %).

Nurk et al. (2005) reported significantly increased risk of memory deficits for the highest homocysteine quintile at baseline. Folate association was lost when controlled for homocysteine.

RCT with at least 2 years of intervention

Clarke et al. (2010) on behalf of the B vitamin treatment trialists' collaboration reported in the meta-analysis no benefit of B vitamins (folate, B6, B12) in vascular diseases but also no adverse effects especially on cancer or survival which is important information about the general safety of B vitamin trials.

Only 9 RCTs with folic acid supplementation with or without B12 and B6 lasting 2–5.4 years with different inclusion criteria have been reported up to now (Table 2). Only one was confounded by folic acid fortification (Kang et al. 2008).

Two population-based studies without exclusion of cognitively impaired subjects found cognitive benefits in the vitamin group of moderately hyperhomocysteinemic subjects (Durga et al. 2007) or in the subgroup with low dietary intake of B vitamins (Kang et al. 2008).

Durga chose well-considered exclusion criteria: normal homocysteine $\leq$13 or excessive homocysteine $\geq$26 μmol/L with concomitant low B12 levels ($\leq$200 pmol/L). Mild cognitive impaired (MCI) or demented subjects were neither diagnosed nor excluded from analysis, but only 7 subjects had a dementia compatible MMSE (0–30; Morris et al. 1989) below 24, 6 of them accidentally randomised into the placebo group. Supplementation of 818 subjects with folic acid for 3 years significantly improved 3 year change of cognitive function.

Kang et al. (2008) reported about possible cognitive benefits of supplementation among women with a low dietary intake of B vitamins but no effects in the total cohort. This might be explained by increased background dietary intakes due to mandatory folic acid fortification starting just 3 months earlier.

Two other population-based studies in high-functioning subjects found no effect after 2 years of supplementation (MacMahon et al. 2006; Ford et al. 2010).

MacMahon's study group was small and the test results were excellent at baseline and at follow-ups, making it impossible to find any difference within only 2 years (Clarke 2006).

Ford et al. (2010) did not find any cognitive benefit of 2 years of supplementation in older hypertensive men after 8 years of follow-up. However, there was a small immediate benefit after 1 and 2 years of active supplementation in two subtests.

Walker et al. (2012) reported significantly improved cognitive performance after 2 years of supplementation in elderly, psychological distressed, but not severely depressed subjects. These results are all the more remarkable since subjects with folate or B12 deficiency at baseline, probably benefitting most, were excluded.

Kwok et al. (2011) found a cognitive benefit after 2 years of supplementation in a small sample of mild to moderate AD/VD in one subtest only in the subgroup with elevated homocysteine.

de Jager et al. (2011) reported 2 years of supplementation slowing down cognitive and clinical decline in MCI, in particular in those with elevated homocysteine. Smith et al. (2010) found in the same study group that 2 years of supplementation slowed down the rate of brain atrophy. This has been already suggested by Snowdon et al. (2000) and has been confirmed by Blasko et al. (2012) who found fewer converters from MCI to AD in those supplemented with folic acid and B12.

Conclusion concerning the studies

Eleven out of 14 epidemiologic studies with observation periods between 4 and 9.3 years reported significant, independent and beneficial impact of at least 1 of the 3 players—folate/B12/homocysteine—on cognition. Six out of seven RCTs with B vitamin intervention periods between 2 and 5.4 years reported about cognitive benefits of the supplemented groups (cognitively healthy or MCI or AD) mainly for those subjects with high homocysteine levels or low folate intake at baseline. Only one study found no difference. These are encouraging results. It is of utmost importance to design further studies avoiding the general pitfalls of short follow-up, heterogeneous study

Table 2 Double blind placebo controlled randomized trials on cognitive decline with folic acid with or without B12/B6 with follow-up of ≥2 years

Author	*N*	Age	FU years	Selection criterion	Cognitive start	End point	Intervention	Outcome	FA fortification/ date of study start and end	Comment
de Jager et al. (2011), Homocysteine and B vitamins in cognitive impairment (VITACOG)	266	76.7 (±5)	2	MCI according to Petersen et al. (2009) excluded: dementia, antidementia drugs, stroke, cancer,methotrexate, vitamin supplementation: FA >300 µg/d, B6 >3 mg/d, B12 >1.5 µg/d	Mild cognitive impaired (MMSE: ≥24/30) 17 ≤ TICS-M ≥ 29	Cognitive decline, neuropsychol. Test battery TICS-M, MMSE, CDR, 4 additional tests (episodic memory, fluency, executive function, constructional praxis)	0.8 mg FA and 0.5 mg B12 and 20 mg B6 versus placebo for 2 years	Significant cognitive decline in placebo group versus vit B treatment group in subjects with homocysteine above median (≥11.3 µmol/L) or in upper homocysteine quartile (≥13.1 µmol/L):	No fortification area (Great Britain)	43 (16 %) without follow-up; baseline: placebo group significantly higher depression score [GDS: (0–30) 7.5 vs. 5.7)
Durga et al. (2007), Folic acid carotis intima media Trial (FACIT)	818	60 (±5.5)	3	Participants from municipal and blood bank registries with moderately elevated homocysteine:≥13; ≥26 µmol/L only if B12 levels normal (≥200 pmol/L)	No cognitive exclusion MMSE(0-30): 29 ± 1 range:15 – 30 (7 subjects below 24: 6/7 in placebo group)	Cognitive decline; neuropsychol. test battery (12 tests)	0.8 mg FA versus placebo for 3 years	Supplementation significantly improves 3-year change in memory, information processing speed, sensorimotor speed and global cognitive function	No fortification area (Netherlands)	24 (3 %) without follow-up
Ford et al. (2010), Health in Men study	299 and 73	79 (± 2.8)	2 and 8	Community representative, hypertensive men, Exclusion: depression (BDI ≥ 18) MMSE ≤ 24, B vitamin supplemented, severely ill	Free of dementia	Cognitive decline, dementia; 2 years: ADAS-cog, MMSE, verbal memory,digit cancelation, clock drawing,8 years: telephone interview TICS	2 mg FA and 0.4 mg B12 and 25 mg B6 versus placebo for 2 years	No cognitive benefit after 2 years (ADAS-cog, 241 men); no cognitive benefit after 8 years (TICS, 73 men) intermediate benefit after the 1st (digit cancellation) and 2nd year (immediate recall) of active supplementation in two additional subtests	Start: 2000, end: 2008 Mandatory fortification in Australia started 2009	58 (19 %) without follow-up at 2 years; Placebo group significantly younger, 78.7 versus 79.3 ($p = 0.05$)
Kang et al. (2008), Women's Antioxidant and Folic Acid Cardiovascular study(WAFACS)	2009	71.3 (±4.2)	6.6	Female health professionals with cardiovascular disease (CVD) or 3 risk factors for CVD	Free of dementia	Cognitive decline, dementia telephone interview: TICS, verbal memory, fluency, MMSE,	2.5 mg FA and 1 mg B12and 50 mg B6 versus placebo	No overall cognitive benefit; in the subgroup with low baseline dietary intake of folate and/or B12 and/or B6 cognitive benefit in the vitamin group	Start: 1998–2000, end: 2005 Mandatory fortification in USA started 1998	121 (6 %) without follow-up; first cognitive assessment 1.2 years after randomization

Table 2 continued

Author	*N*	Age	FU years	Selection criterion	Cognitive start	End point	Intervention	Outcome	FA fortification/ date of study start and end	Comment
Kwok et al. (2011)	140	78 (±7)	2	Hospital recruited mild to moderate dementias, according NINCDS-ADRDA and NINDS-AIREN, MMSE, no individual additional B vitamins during RCT, Lewy body dementias excluded	Mild/ moderate AD (64 %), mixed dementia (19 %), VD (17 %) with (40 %) and without dementia related therapies	Cognitive decline; Mattis Dementia Rating Scale, MMSE	5 mg FA and 1 mg B12 versus placebo	No overall cognitive benefit; in subjects with high homocysteine (>13 µmol/L) the vitamin group had significantly less cognitive decline in one subtest (construction)	No fortification area (Hong Kong)	28 (20 %) without follow-up; low number of patients. equally distributed in both groups, started cholinesterase inhibitors during RCT
MacMahon et al. (2006)	276	73.5 (± 5.8)	2	Only participants with elevated homocysteine ≥ 13 µmol no supplemental vitamins, no therapy for depression or diabetes	Free of dementia	Cognitive decline; neuropsychol. test battery (8 tests)	1 mg FA and 0.5 mg B12 and 10 mg B6 versus placebo for 2 years	No difference in 7 out of 8 different tests. Only time of completion of Reitan Trail making Test B was significantly shorter (!) in the placebo group, also after adjustment for sex and education	No fortification area (New Zealand)	23 (8.3 %) without follow-up
Walker et al. (2012), Beyond Ageing Project	900	65.9 (± 4.2)	2	Electoral register recruited, elevated psychological stress K10 between 16 and 29; no severe depression, no physical activity, no vitamin deficits: erythrocyte folate > 250 nmol/L, B12 > 130 nmol/L, no vitamin supplementation	Free of dementia	Cognitive decline; telephone interview (TICS-M) and processing speed, (BTACT)	0.4 mg FA and 0.1 mg B12 versus placebo	Overall cognitive benefit and benefit in immediate and delayed recall tests in vitamin group after 2 years, but not after 1 year; higher baseline homocysteine independently associated with worse outcome	Start:2005/06, end: 2008 Mandatory fortification in Australia started 2009	123 (13.5 %) without follow-up; vitamin group had higher B12 levels at baseline; higher baseline depression scores independently associated with worse outcome

FA folic acid, *B12* vitamin B12, *MCI* mild cognitive impaired, *AD* Alzheimeŕs dementia, *VD* vascular dementia, *MMSE* Mini Mental State Examination (Morris et al. 1989), *CDR* cognitive dementia rating (Morris 1993), *K10* Kessler 10 measures non-specific psychological distress, *BDI* Becks Depression Inventory, *ADAS-cog* cognitive subscale of Alzheimeŕs Disease Assessment Scale, *TICS* Telephone Interview for Cognitive Status, *TICS-M* modified TICS (maximum 39), *BTACT* telephone interview of processing speed (Backward counting from 100 by ones), *FU* follow-up, *NINCDS-ADRDA* McKhann et al. (1984), *NINDS-AIREN* Roman et al. (1993)

entry criteria, and of folate/B12/homocysteine levels not representative for midlife exposure due to short-term supplementation or fortification. Deduced from these results, we suggest that serum levels of folate and B12 should exceed low normal values (folate >14 nmol/L; B12 >220 pmol/L) at any age and homocysteine should not exceed 13 μmol/L by means of (1) Mediterranean ("Cretan") diet rich in vegetables and fruits for adequate folate intake (Morris and Tangney 2007) in countries without mandatory folate—fortification of cereal—grain products, (2) dairy products for B12 intake (Allen 2009), (3) voluntarily fortified breakfast cereals for both or (4) low dose supplementation (folic acid ≤0.4 mg/day, B12 ≥0.003 mg/day). An artificial folic acid load exceeding 1 mg/d in concomitant B12 deficiency (vegans, age beyond 60 years) must be avoided until proved to be safe. Mandatory folate fortification has already started 1998 in several countries and rapidly reduced neural tube defects. It has also offered the unique opportunity of a huge field study hopefully finding an AD incidence lower than historical controls when the mid-fourth generation of 1998 reaches the age of sporadic AD.

Acknowledgments The Vienna Transdanube Aging (VITA) study is supported by the Ludwig Boltzmann Society, Vienna-Austria, and is carried out at the Ludwig Boltzmann Institute of Aging Research which is located at the Donauspital, Vienna-Austria.

Conflict of interest Hinterberger and Fischer declare that they have no conflict of interests.

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