An update in relation to common diseases

Report from Homocysteine Day
Oxford 7 – 8 September 2007
by Dr. David Pritchard
Homocysteine Day
PREFACE

Continuing from the successful meetings of previous years, Homocysteine Day 2007 was held on September 7th and 8th at St Anne’s College in the historic university city of Oxford. In addition to attending a series of excellent presentations and discussions, delegates had opportunity to explore the many attractions of the bustling university city in glorious September sunshine. Some explored “That sweet City with her dreaming spires”, on foot whilst others took advantage of a bus tour. A number of people managed to gain access to the ancient college quadrangles, and some intrepid souls even tried their hand at punting on the River Cherwell.

The aim of Homocysteine Day 2007 was to provide an opportunity to update delegates with the latest advances in scientific research, academic and clinical knowledge, as well as facilitating networking with other members of the international medical and scientific community. More than 80 participants attended the meeting, with the majority of attendees being general practitioners, but other specialties represented included geriatricians, cardiologists, haematologists, and laboratory based physicians and scientists.

The purpose of this report is to briefly summarise the content of the seminar. Prof. David Smith of Oxford University opened proceedings by warmly welcoming delegates and providing an introduction to homocysteine. This was followed by Dr. John Chambers of Imperial College School of Medicine, London, talking on the evidence for the utility of homocysteine and other risk markers in cardiovascular disease. Prof. Smith then gave a presentation on the increasing evidence of association between homocysteine concentrations and dementia. Dr. Rosalie Dhonukshe-Rutten from the University of Wageningen, Netherlands discussed the association of homocysteine with osteoporosis and bone health. The morning session concluded with a lively question and answer session where many issues pertaining to homocysteine were discussed.

The afternoon session commenced with Prof. Helga Refsum (Universities of Oxford and Oslo) discussed homocysteine in children and the newborn, followed by Prof. Philip Cowen (University of Oxford) providing a psychiatrist’s view of the relationship between folic acid, homocysteine and depression. The first day’s sessions concluded with a presentation by Prof. Lindsay Allen (USDA, ARS Western Human Nutrition Research Center, University of California, Davis, USA) on the prevalence, causes and consequences of vitamin B12 and folate deficiency around the world.

Day 2 commenced with a presentation by Prof. Ralph Green of University of California, Davis, USA providing an update on the current status of vitamin B12 and holotranscobalamin testing. Prof. David Smith then gave a presentation considering whether folic acid fortification of food stuffs was desirable. The penultimate presentation was from Patrick Holford of the Brain Bio-centre, London providing a nutritionist’s view of homocysteine, and Prof. Refsum brought proceedings to a close with an overview of the association of homocysteine and disease with a review of the current status of homocysteine in clinical medicine.

1 Description of Oxford in the poem “Thyris”; Mathew Arnold (1866)
Homocysteine: an Introduction

Prof. David Smith, University of Oxford, Dept. of Pharmacology, UK
Oxford Project to Investigate Memory and Aging (OPTIMA)

Homocysteine is a risk factor common to all of the conditions listed below:

- Heart Disease
- Death
- Stroke
- Venous thrombosis
- Pregnancy complications
- Birth defects
- Osteoporosis
- Depression
- Schizophrenia
- Cognitive deficiency
- Alzheimer's Disease
- Vascular Dementia
- Brain atrophy

Homocysteine is an amino acid containing a highly reactive sulphhydryl (SH) group (Fig 1.) that acts as a metabolic intermediate, and is not incorporated into proteins. Homocysteine forms part of the methionine and folate cycles (Fig 2); methionine being an essential amino acid that is required for protein synthesis and as a methyl group donor for the synthesis of numerous methylated compounds.

In-vivo the majority of homocysteine is bound to protein (principally albumin) through a disulphide bonds, or linked to cysteine (again through a disulphide linkage). Only around 5% of total homocysteine in blood exists in a free form. Assays for homocysteine measure the total homocysteine (free and complexed).

Homocysteine is formed from methionine, an amino acid supplied by protein in food. Homocysteine is an intermediary in a number of critical processes, but elevated concentrations are associated with a number of adverse effects. The removal of homocysteine requires 4 vitamins: Folate, B2, B6 and B12, thus deficiencies of these vitamins result in elevated homocysteine levels.
Homocysteine levels in the general population are dependent upon a number of factors; the concentration tends to increase with age such that a typical value in the elderly is twice that found during childhood. There is also a gender effect, with males tending to have values 1 to 2 μM higher than females. Renal impairment, genetic polymorphisms and certain drugs and diseases resulting in impaired vitamin metabolism or renal function can also cause an elevation in homocysteine concentration. In pregnancy markedly lower homocysteine concentrations are observed, the reason for which currently unknown.

Table 1. Guideline homocysteine concentrations for different populations (µmol/l)

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean</th>
<th>Guideline upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Adults</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Elderly</td>
<td>12</td>
<td>20</td>
</tr>
</tbody>
</table>

*Note that upper limits should not be used strictly.*
Elevated homocysteine concentrations can result from non-modifiable and modifiable (lifestyle) factors.

- Increasing age.
- Male gender.
- Renal impairment.
  - Genetic Traits.
  - Homocystinuria.
- Genetic polymorphisms.*
- Certain drugs and diseases interfering with vitamin status or renal function.

* The best documented of the genetic polymorphism is the MTHFR 677C→T substitution. Around 10% of the population have this genotype and are at risk of hyperhomocysteinaemia and its associated diseases. Individuals with the TT polymorphism typically have homocysteines 2 to 3 μM higher than those with the CC genotype.

Causes of elevated homocysteine. Lifestyle (modifiable) factors.
- Dietary B vitamin deficiencies.
  - Poor diet (folate deficiency).
  - Vegetarianism (B12 deficiency).
- Smoking.
- Excessive coffee.
- Low physical activity.
- Low or excessive alcohol intake.

Conclusions.
- Homocysteine is critical for normal cell function, but in high concentrations it may cause harm.
- B vitamin intake, smoking and coffee are the most important modifiable factors determining homocysteine levels.
- A healthy lifestyle will not only lower homocysteine, it will reduce the risk of cardiovascular disease, cancer and other diseases.
- But, is homocysteine the cause of such diseases or is it just a marker?
- Should (every)one take B vitamin supplements?
Homocysteine and other risk factors for vascular disease: the evidence and recent trials

Dr. John Chambers, Imperial College School of Medicine, London, UK

Dr. Chambers asked the question whether further risk factors were required over and above the existing classical risk factors of smoking, cholesterol, hypertension, diabetes, obesity and physical inactivity? He pointed out that papers stating that most coronary heart disease (CHD) patients have at least one of the classical risk factors could be misleading, as whilst it is true that most CHD patients can be detected, the majority of adults who do not go on to develop CHD also have at least one of these risk factors.

The UK Medical Research Council states that a Biomarker is “an objective measurement that acts as an indicator of pathogenic processed or responses to therapeutic intervention.” For a biomarker to be valid it should fulfil the following criteria:

• Reproducible relationship to disease.
• Prospective validation.
• Additive information.
• Discriminates subjects well.
• Good assay.

Data was shown demonstrating that homocysteine fulfilled these criteria. Meta-analyses of clinical studies show a consistent relationship between elevated homocysteine and heart disease. This has shown to be a graded relationship for all concentrations of homocysteine, including those within the reference range. The association of homocysteine with CHD has been shown to be independent and additive to classical risk factors. This is illustrated in Figure 3, where addition of the homocysteine substantially enhances the risk prediction of the Framingham model, low homocysteine levels reducing, and high levels increasing, the risk. Plots of discrimination between cases and controls for homocysteine are similar to long established biomarkers such as HDL. A number of good assays for the determination of homocysteine are available.

Dr Chambers then addressed the issue as to whether reduction of homocysteine was beneficial. Whilst it is clear that treating patients who have homocystinuria is effective, at present we do not know if treatment to decrease homocysteine in those with moderately elevated concentrations results in an improved prognosis for cardiovascular disease. Evidence that lowering a moderately elevated homocysteine could be beneficial is provided by data on homocystinurics, animal studies and in-vitro/ex-vivo experiments.
Secondary prevention studies on vitamin supplementation (VISP and NORVIT and HOPE2) have to-date failed to show an improvement in prognosis even though a decrease in homocysteine was apparent. However, there are a number of problems with these studies; in particular they did not have adequate power to reliably detect the predicted risk reduction. Concerns have also been raised about whether the patient groups studied are the most appropriate. These studies have been in patients following an acute presentation, where the influence of risk factors is likely to be much reduced. For example, following MI the infarct size and extent of coronary artery disease are the primary determinants of survival.

Conclusions.
• There is more to life than conventional risk factors.
• Check homocysteine.
• Good evidence that homocysteine is a biomarker of increased vascular risk (~1.3 per 5 µmol/L).
  Be aware.
• No evidence that treating homocysteine in adults is beneficial, but also no evidence that it doesn’t work.
  - Treat conventional risk factors aggressively (it works).
  - Consider unproven B vitamin Rx.
Homocysteine and dementia: the evidence

Prof. David Smith, University of Oxford, UK
Oxford Project to Investigate Memory and Aging (OPTIMA)

Dementia is a major health issue worldwide, and with the ageing population the incidence of dementia is rapidly increasing. It is estimated that worldwide, there are currently 25 million sufferers and this is predicted to increase to 114 million by 2050. Alzheimer’s Disease is the most common form of dementia, and its cause is believed to be multifactorial. Only 1% of cases are believed to be directly attributable to inherited genes, with the remaining 99% resulting from a combination of genetically determined and non-genetic (modifiable) risk factors.

Data from a number of studies was presented, and these provided convincing evidence that elevated homocysteine concentrations were associated with dementia. 77 cross sectional studies have been reported since 1998 and 90% of these have found an association between homocysteine concentration and cognitive impairment or dementia. Prospective studies (Framingham and Conselice) have shown that elevated homocysteine significantly increases the risk of developing dementia (for example in the Conselice study, individuals with homocysteine greater than 15 μM were at around 5 times the risk of developing dementia as those with homocysteine concentration below 10 μM). Of 17 prospective studies since 1998, 14 have found an association between elevated homocysteine and future dementia or cognitive impairment.

Some data was indicative of a causal relationship between homocysteine and dementia. For example one study demonstrated that scores in an episodic memory test declined over a 6 year period for individuals whose homocysteine increased over that time, whilst individuals whose homocysteine decreased demonstrated improved scores.

Data was presented from the OPTIMA study demonstrating that homocysteine concentration is associated with the speed of disease progression. Elevated homocysteine is associated with a greater rate of brain atrophy and cognitive decline in patients with Alzheimer’s Disease. In elderly patients who did not show impairment at baseline, elevated homocysteine was associated with an increased rate of brain shrinkage and greater cognitive impairment over a 6 year period. Data from intervention trials to assess whether lowering homocysteine slows age-related cognitive decline are now starting to be available. The FACIT trial (Durga, Lancet 2007) concluded that “Folic acid significantly improved domains of cognitive function that tend to decline with age”.

Conclusions.
- Homocysteine is a risk factor for:
  - Conversion from normal ageing to cognitive impairment.
  - Conversion from cognitive impairment to dementia.
  - Incident dementia.
  - Faster rate of cognitive decline in Alzheimer’s Disease.
  - Cerebral atrophy and faster rate of atrophy.
Homocysteine, osteoporosis and bone health

Dr. Rosalie Dhonukshe-Rutten, Wageningen University, Netherlands

Osteoporosis is a multifactorial disease and is defined as “a systemic skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures”. Osteoporosis is associated with increased morbidity and mortality, decreased quality of life and high economic cost.

Data from a number of sources indicates that vitamin B12 is involved in bone metabolism. In-vitro experiments have demonstrated that vitamin B12 stimulates osteoblast proliferation and alkaline phosphatase activity (marker of bone formation). In patients with pernicious anaemia, supplementation with vitamin B12 improves alkaline phosphatase and osteocalcin activity. Early onset of osteoporosis is observed in patients with homocystinuria. Children who are on a strict vegetarian (macrobiotic) diet have low bone mineral densities.

Dr. Dhonukshe-Rutten presented data on a number of clinical studies that she had conducted. In adolescents low bone mineral density is associated with lower vitamin B12 and increased MMA (methylmalonic acid, a marker of vitamin B12 deficiency) concentration. In a study of frail elderly women the adjusted prevalence odds ratio for osteoporosis was around 4 for patients with marginally deficient vitamin B12 status and more than 6 for patients who were definitely vitamin B12 deficient. In a prospective study examining the cumulative incidence of fractures in the general older population it was found that having a homocysteine in the highest quartile doubled the risk of fracture. These data are consistent with a number of other studies, including the Framingham study where men with homocysteine in the highest quartile were at 4 times the risk of fracture, whilst women with homocysteine in the highest quartile had a 2-fold increased risk. Data from an intervention study suggests that folate and vitamin B12 supplementation is effective in reducing the risk of fracture. 628 elderly hemiplegic patients were randomised to vitamin supplementation or placebo, and it was found that the placebo group had 3 times the incidence of hip fracture over a 2 year follow up.

Hypotheses were put forward that vitamin B12 has a direct influence on osteoblast proliferation, whilst homocysteine interferes with collagen crosslinking resulting in altered bone matrix and bone fragility.

Conclusions
• There is growing evidence – from different populations – for an association between markers of vitamin B12 status and bone health.
• Whether this association is causal is still unclear. Intervention studies in the general population are needed.
• Association between markers of vitamin B12 status and bone health may have important implications for the prevention of fractures.
Homocysteine as a risk factor in CVD, dementia and osteoporosis – Discussion:

**Question.** In the studies presented on osteoporosis and fractures was the level of vitamin D taken into account?

**Response.** Yes, vitamin D was measured and accounted for in the analyses. In studies on children subjected to a macrobiotic diet, low concentrations of both vitamin D and vitamin B12 were observed. Vitamin D could be a confounding factor, but the results of the statistical analyses did not support this.

**Question.** Is there a correlation between 25-hydroxy vitamin D and homocysteine as they can both be viewed as factors affected by the lifestyle of individuals?

**Response.** This raises an important point as in nutrition it is often difficult to separate out effects. It is quite likely that intake of vitamin D could be related to intake of vitamin B12 and/or folate, but there is no reason to believe that vitamin D is linked to homocysteine through biochemical mechanisms. A recent study in the USA showed a strong correlation of vitamin D deficiency to cardiovascular associated factors such as hypertension and hypertriglyceridemia.

**Question.** Dr. Chambers showed an example of a patient with the homocysteine concentration increasing from 15 to 26 over a 4 month period despite receiving vitamin supplementation, how can this be explained?

**Response.** It was suspected that this apparent increase was an artefact of improper sample collection, and that the whole blood sample had been left at room temperature for a significant period of time before the assay was performed. Homocysteine is generated in the erythrocytes, and therefore plasma should be separated from the cells as rapidly as possible (and at the most within 2 hours of collection). The physician had been advised to repeat the test ensuring that optimal sample collection conditions were used.

**Question.** Is coffee consumption itself a risk factor for cardiovascular disease, and how is it correlated to homocysteine?

**Response.** Coffee may act as a confounding factor, the panel were unsure if coffee itself was a risk factor in its own right, but it is unequivocally a cause of elevated homocysteine. The causal effect of coffee consumption on elevating homocysteine levels has been demonstrated.

**Question.** Are there studies other than the Hordaland study looking at the impact of coffee consumption?

**Response.** Yes, for example the Framingham population.

**Question.** How strong is the evidence for the relationship between low folate and B12, elevated homocysteine and bone disorders?
Response. The consensus was that it is still early days, but there are some very promising data. For example the data from the Japanese intervention study provides an “amazing result”, but very high levels of vitamin supplementation were used and it was a small and specific patient group (stroke patients with hemiplegia). In a number of studies an associated effect of vitamin D was not observed, but this could still be underlying some of the results. Folate, vitamin B12 and homocysteine concentrations may be reflecting the overall nutritional status and thus it is possible that other factors may be contributing to the data.

Question. At what level is homocysteine neurotoxic?

Response. Neurotoxicity has been demonstrated in cell culture and animal studies with very high homocysteine concentrations. Homocysteine concentration is generally low in CSF, but we do not know the intracellular concentration of homocysteine within the brain. Children treated with methotrexate have very high CSF homocysteine concentrations. Neurotoxicity of homocysteine is probably not associated with a threshold concentration. Other consequences of elevated homocysteine are continuous concentration related effects and there is no reason to suspect that neurotoxicity would be different.

Question. Low dose methotrexate is often used in rheumatoid arthritis. What is the best dose of folic acid to use in conjunction with this?

Response. Folic acid is used to protect against some of the adverse side effects of methotrexate therapy. The panel did not feel qualified to advise on the best dosage, but typical dose regimes were 1-5 mg folic acid daily or 3 mg folinic acid weekly.

Question. Are there differences in plasma and CSF levels of homocysteine?

Response. Yes, CSF homocysteine is only around 5% the concentration of plasma homocysteine. There is no clear data on dementia and the concentration of homocysteine in CSF.

Question. If a patient with Alzheimer’s disease was showing a rapid decline in cognitive function, would you treat with B12 and folate if the individual had normal levels of these?

Response. A range of opinions were expressed on this. There was a consensus that if levels were low, then vitamin supplementation should be used. Where plasma concentrations were normal, one response was that they would not treat the patient with these vitamins, but would inform them and/or their family of some of the research in this area but stress that before a definitive position on utility of vitamin supplementation could be reached, results from well designed clinical trials were required. The patient (or their family) could then buy over the counter vitamin supplements if the wished to do so. Another view expressed was that a physician’s first responsibility was not to harm the patient, and that by treating with these vitamins it was very unlikely that any harm would result (but see section on folic acid supplementation), and it might have a beneficial effect.
Homocysteine in newborns and children: what does it tell us?
Prof. Helga Refsum, University of Oxford & University of Oslo

Prof. Refsum started her presentation by highlighting changes in homocysteine and related factors over the first few years of life. Throughout infancy and childhood homocysteine typically is at a concentration of around 60% that of adults. In newborns, cobalamin (vitamin B12) tends to be lower than in adults, and it drops further so that over the first year of life concentrations are about half those found in adults. There is then a marked increase such that children aged between 2 and 8 years typically have cobalamin concentrations around 50% higher than those in adults. There is a gradual decrease in cobalamin concentration from around 8 years onwards, until adult levels are reached at around the age of 16. Folate levels in the newborn are around 3 times higher than those found in adults; levels continue to increase until around 6 months, thereafter concentrations decrease until they reach adult values at around 2 years. Methylmalonic acid (MMA) levels are very high during the first two years of life, thus making it difficult to use as an indicator of vitamin B12 status. From around 2 years onwards, MMA levels are similar to those for adults.

Table 2. Normal lower and upper limits for folate, B12, homocysteine and MMA levels.

<table>
<thead>
<tr>
<th></th>
<th>Newborns</th>
<th>Infants</th>
<th>2 Years old</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Limit</td>
<td>Serum folate, nM</td>
<td>25</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Serum B12, pM</td>
<td>125</td>
<td>125</td>
<td>240</td>
</tr>
<tr>
<td>Upper Limit</td>
<td>Homocysteine, μM</td>
<td>10</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>MMA, μM</td>
<td>0.50</td>
<td>2.00</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Serum cobalamin (vitamin B12) is transported via 2 binding proteins, transcobalamin and haptocorrin. Holotranscobalamin (holoTC) is the physiologically active form and typically represents 20 to 25% of the total cobalamin. The majority of the serum cobalamin exists as holohaptocorrin (holoHC) whose function is currently unknown. Changes in cobalamins during early childhood are shown in Figure 4. Since holoHC, the form that is not utilised, remains high, the low holoTC at 6 -12 months probably reflects efficient use of holoTC rather than deficient supply of cobalamin.

Figure 4. Changes in cobalamins during early childhood.
To a large extent, homocysteine and vitamin B12 concentrations in the newborn are determined by maternal vitamin B12 status. Low neonatal B12 is associated with elevated homocysteine and MMA. Breastfed babies often have particularly low B12 levels, and the effect of breastfeeding is dependent upon the vitamin B12 status of the mother.

Acute symptoms of vitamin B12 deficiency in infancy include vomiting, apnoea, cyanosis, hypotonia, movement disorder and seizures. Chronic symptoms include failure to thrive, irritability and delayed psychomotor development. However, prognosis is excellent if treatment is started early. Failure to diagnose and treat the condition results in a longstanding deficiency which may result in intellectual impairment.

A number of autosomal recessive inborn errors of metabolism can result in homocystinuria, a condition characterised by severely elevated homocysteine in blood and urine. The most common of these errors is cystathionine β-synthase (CBS) deficiency. Prof. Refsum suggested that CBS deficiency might be much more common than previously thought and that homocysteine testing should be done in children presenting with any of the symptoms associated with this disorder.

Figure 5. What to do with elevated homocysteine levels in newborns and infants.

Conclusions.
• Folate and B12 status change markedly during childhood, particularly the 1st year.
• Folate and MMA are higher and vitamin B12 is lower in breastfed compared to non-breastfed infants.
• Exclusively breastfed babies of vegetarian mothers are at risk of developing neonatal vitamin B12 deficiency.
• Keep in mind homocystinuria in children. A single test can make all the difference.
Folic acid and depression

Prof. P Cowen, University of Oxford, UK

Plasma and red cell folate have been measured in a number of studies on psychiatric patients. Generally it has been found that folate levels are lower in patients with depression, and a meta-analysis of around 15,000 subjects provided an overall odds ratio of 1.55 (95% confidence limits 1.26 to 1.91) for an association between low folate and depression. There are also a number of publications demonstrating that elevated homocysteine concentrations are associated with depression.

As is often the case with homocysteine, the question arises as to whether these lower folate and elevated homocysteine levels are a contributory causative factor to the depressive state or a consequence of it (for example due to poor diet during the period of depression). One way of exploring this is to look at the disease incidence in relation to the MTHFR polymorphism, if homocysteine is actively involved as a causative factor of the disorder one would expect to observe higher frequency of disease in those with the TT polymorphism (where the MTHFR has reduced activity thus resulting in higher homocysteine concentrations). Table 3 shows odds ratios for depression related to MTHFR genotype, and indeed a statistically significant relationship is apparent, thus providing evidence that the altered biochemistry is a contributory causative factor for the depression.

Table 3. Rates of MTHFR polymorphisms in individuals ever diagnosed with depression.

<table>
<thead>
<tr>
<th>Genotype (n)</th>
<th>Odds ratio (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (1565)</td>
<td>1.0</td>
</tr>
<tr>
<td>CT (1520)</td>
<td>1.2 (0.99 – 1.46)</td>
</tr>
<tr>
<td>TT (402)</td>
<td>1.4 (1.01 – 1.80)</td>
</tr>
</tbody>
</table>

There are a number of possibilities as to what biochemical mechanism might be involved with the association between homocysteine and depression.

- Homocysteine may act as a direct neurotoxin.
- S-adenosylmethionine (SAM) is itself used as an antidepressant therapy. Decreased endogenous levels of SAM (along with elevated homocysteine) are associated with a less efficient methionine cycle (see Figure 6).
- Tetrahydrobiopterin (BH₄, a pterin) is a cofactor for the production of the neurotransmitters 5HT (5-hydroxytryptamine) and noradrenaline, thus the reduced levels of BH₄ may contribute to the depressive state.
Figure 6. Biochemical mechanism of folate associated depression.

Prof. Cowen discussed using therapeutic agents to correct for folate deficiency in depression: A study using SAM administered either orally or IV demonstrated similar effects upon depression scores as treating with Imipramine (a tricyclic antidepressant). It should be noted that there was no placebo group in this study so the data is not entirely convincing. However, SAM is better tolerated than many other drugs, so therapy with this is an attractive option. Meta-analysis of trials where folate supplementation has been used to augment other therapies shows a benefit of this therapeutic approach. One hypothesis is that impairment of methylation due to folate deficiency reduces the efficacy of tricyclic antidepressants, SSRIs (selective serotonin reuptake inhibitors) etc, which can be counteracted by folate supplementation.

Conclusions:
- Low folate and high homocysteine levels are associated with depression in the community.
- Allelic variation in the MTHFR gene makes a small contribution to the heritability of depression.
- Folate treatment probably has a modest ability to potentiate the effects of antidepressant medication.
- A more general role for folate in the prevention of depression at a community level is worth exploring.
WHO report on folate and B12 deficiencies around the world

Prof. Lindsay Allan, University of California, Davis, USA

Professor Allan gave a presentation on the prevalence, causes and consequences of vitamin B12 and folate deficiency around the world.

The purpose of a WHO consultation on folate and vitamin B12 was:
• To review prevalence and causes of folate and vitamin B12 deficiency.
• To review the functional consequences of these deficiencies and of elevated homocysteine, through life span.
• To derive public health recommendations.

Average concentrations and the prevalence of low values for both vitamin B12 and folate are strongly associated with geographical location as shown in Figure 7. In India approximately 50% of adults are deficient in vitamin B12, and in many countries the average vitamin B12 concentration is below 300 pM.

Figure 7. Measure of central tendency of serum vitamin B12 concentrations and prevalence of low values in adults.

Vitamin B12 deficiency can result from inadequate dietary intake (e.g. vegans, lacto-ovo vegetarians, low animal source food intake), malabsorption (e.g. gastric atrophy, pernicious anaemia), infections/medical problems (e.g. H. pylori, Giardia, fish tapeworm, bacterial overgrowth, malaria, HIV/AIDS), medication (e.g. cimetidine, omeprazole, lansoprazole) or polymorphisms (e.g. HoloTC 776C>G).
Data was shown for vitamin B12 to indicate that:

- Low animal source food diets can cause depletion more readily and rapidly than usually assumed and is probably the main cause of deficiency in the poor.
- Food bound malabsorption is probably the main cause of deficiency in industrialised countries (particularly in the elderly).
- Maternal depletion in pregnancy and lactation is a strong risk factor for deficiency in infancy and childhood.
- Ethnic differences and polymorphisms affect vitamin B12 status.
- Repletion of stores is slow with low doses.
- Effects of marginal vitamin B12 status are unknown.

**Figure 8. Measure of central tendency of serum folate concentrations and prevalence of low values in adults.**

Folate deficiency can result from low dietary intake, alcoholism, infections (e.g. sprue, Giardia, bacterial overgrowth, malaria, HIV/AIDS), smoking, lactation, medication (e.g. methotrexate, anticonvulsants, sulfasalazine, NSAIDs) or polymorphisms (e.g. MTHFR 677C>T). A theory to explain the variability in folate status is that deficiency is more likely with rice or wheat based diets where legume, fruit and green vegetable intake is low.

Other results from WHO consultation.

- Deficiency cut-offs: serum folate 4.5 nmol/L, vitamin B12 150pmol/L.
- Megaloblastic anaemia is rare unless severe deficiency.
- Vitamin B12 deficiency may contribute to NTD and poor infant development.
- High homocysteine consistently related to abnormal pregnancy outcomes, and folate deficiency to low birthweight.
- Folic acid and Vitamin B12 fortification of flour should be based on usual intake and/or evidence of poor status.
Is folic acid fortification of food the best solution?

Prof. David Smith, University of Oxford, Dept. of Pharmacology
Oxford Project to Investigate Memory and Aging (OPTIMA)

Professor Smith commenced his presentation by warning that some of the issues he would be discussing could be considered controversial. Folic acid fortification of food is widespread in the developed world, in some countries such as the USA and Canada this is mandatory, whilst in others such as the UK and Australia it is voluntary. A number of countries are currently considering introducing mandatory folic acid fortification.

Folic acid fortification is primarily aimed at reducing neural tube defects (NTD), and there is considerable evidence that it had been successful in this (although the extent by which risk is reduced appears to be dependent upon geographical location). Mandatory fortification has been shown to markedly increase serum folate levels and to reduce homocysteine concentration. Following mandatory fortification in the USA and Canada an increase in the rate of decline in stroke mortality was observed, i.e. there were fewer deaths than predicted.

Figure 9. Mortality from stroke in Canadian females 1990 to 2002 (Yang, Circulation 2006).

However, there are a number of theoretical concerns about potential negative effects of fortification:

- Masking of vitamin B12 deficiency.
- Epigenetic effects.
- Antagonism of anti-folate drugs.
- Cancer.
Data was presented that in simplistic terms indicated that if an individual's B12 status was good then folate supplementation was beneficial, whereas if the B12 status was poor, extra folate had detrimental effects. Reference was made to studies indicating that high maternal folate resulted in epigenetic effects, one example being increased insulin resistance in children. Many drugs are anti-folates (particularly for treatment of cancer as folate is required for nucleic acid synthesis and cell division and growth), and the effect of folate fortification on these is worthy of consideration.

Many studies suggest that higher folate concentrations protect against developing cancer, but there are some striking exceptions where higher folate levels are associated with a marked increase in risk. One hypothesis that may explain this is outlined in Figure 10, where folate has a dual role dependent upon the stage of the cancer. In this hypothesis high folate protects against the development of normal cells to preneoplastic cells but increases the risk of preneoplastic cells becoming cancerous.

Figure 10. Kim's hypothesis: folate has a dual role in cancer, depending on stage and concentration (Kim, Nutr. Rev. 2006).

The inescapable fact is that for each NTD pregnancy prevented by fortification, around 500,000 people will be exposed without choice to extra folic acid. Within the 500,000 how many:

- Have precancerous cells in their colon?
- Have precancerous cells in their prostate?
- Are being treated with anti-folate drugs for cancer, rheumatoid arthritis, psoriasis?
- Have low B12 status?

Prof. Smith concluded that in his view, it was time for more research not for fortification.
Vitamin B12 and holotranscobalamin: Current status and clinical significance

Prof. Ralph Green, University of California, Davis, USA

Prof. Green gave a wide ranging presentation on vitamin B12 and holotranscobalamin (holoTC) encompassing the past, present and future of diagnosing vitamin B12 deficiency. Vitamin B12 deficiency is a significant health problem particularly in the elderly, and is likely to become ever more common as the world population ages. In the USA 30 – 40% of those aged over 65 have food B12 malabsorption, and 2-3% have pernicious anaemia.

There are several different forms of vitamin B12 (cobalamin), but only two of these are metabolically active: methyl- and adenosylcobalamin. Adenosylcobalamin mediates the mitochondrial conversion of methylmalonyl-CoA to succinyl-CoA, which enters the tricarboxylic acid cycle. Adenosylcobalamin is therefore of importance for lipid and carbohydrate metabolism. Deficiency of adenosylcobalamin leads to accumulation of methylmalonic acid (MMA). Methylcobalamin is a co-factor for methionine synthase in the conversion of homocysteine to methionine in the methylation cycle. This reaction requires methylenetetrahydrofolate as a substrate, and constitutes a unique point of interaction between two vitamins, vitamin B12 and folate. A deficiency of methylcobalamin and/or MTHFR causes increased total levels of homocysteine.

Figure 11. Vitamin B12 dependent reactions.

\[
\text{Homocysteine}^* + \text{MethylTHF} \rightarrow \text{Methionine} + \text{THF}
\]

\[
\text{Methylmalonyl-CoA} \rightarrow \text{Succinyl-CoA}
\]

\[
\text{Methylmalonic Acid}^*
\]

*Levels rise in B12 deficiency

Figure 12 summarises the absorption and transport of vitamin B12. Dietary vitamin B12 enters the stomach bound to animal proteins and is released from them by the action of pepsin and hydrochloric acid. The free vitamin B12 is then captured by the binding protein haptocorrin (HC). In the small intestine haptocorrin is degraded by pancreatic enzymes and the released vitamin B12 is complexed with intrinsic factor (IF) a protein synthesised by gastric parietal cells. The IF-vitamin B12 complex is then internalised in the small intestine by a receptor mediated mechanism and thereafter IF is subject to proteolysis, subsequently only vitamin B12 and not IF enters the circulation. In the circulation vitamin B12 is bound to two proteins, transcobalamin (TC) and haptocorrin (HC). Vitamin B12 bound to transcobalamin is known as holotranscobalamin (holoTC). HoloTC is the biologically active fraction that is delivered to all tissues of the body whereas the function of haptocorrin is unknown.
There are a number of theoretical advantages of measuring holoTC in the diagnosis of B12 deficiency:

- TC delivers B12 to all tissues, haptocorrin does not.
- Genetic TC deficiency leads to life-threatening functional B12 deficiency, genetic haptocorrin deficiency is relatively benign.
- HoloTC has a short half-life (~6 min) and is therefore expected to fall early during states of B12 malabsorption.

The sequences of changes in developing B12 deficiency are believed to be:

1. **Early**: low holoTC.
2. **Cellular**: low serum B12, depletion of body stores.
3. **Metabolic**: increased homocysteine and methylmalonic acid.
4. **Clinical**: macrocytic anaemia, neurological impairment.

Prof. Green mentioned 2 potential replacements for the Schilling test (which is unavailable in many countries) for investigation of defects of B12 absorption. The Cobasorb assay measures HoloTC before and after oral intake of vitamin B12, whilst the other technique utilises accelerator mass spectrometry (AMS) to measure orally ingested 14C labelled material thus allowing determination of the absorption, transport, metabolism and turnover of vitamin B12.
The H-factor solution: a nutritionist’s perspective

Professor Patrick Holford, CEO, Brain Bio Centre, London, UK
Visiting Professor, University of Teesside

Professor Holford provided an interesting perspective on nutritional aspects of homocysteine. He stressed that due to the rapid turnover rates of body tissues, we become what we eat, and that the most direct influence on an individual’s environment is what they put in their mouth.

The association of homocysteine with a large number of physical and mental health problems was highlighted, and it was stressed that homocysteine could easily be lowered with optimal nutrition. The presentation contained several anecdotal examples where changes in nutrition significantly lowered homocysteine, and were associated with significant improvement in health and quality of life. There was discussion of work which had been performed indicating that the optimal homocysteine concentration was below 7.5μM.

Folate provided as a supplement is more effective at decreasing homocysteine, than the same quantity of folate in food. Supplementation of multiple vitamins, providing a synergistic effect is more effective at decreasing homocysteine levels as shown in Table 4.

Table 4. Percentage reduction in homocysteine with differing vitamin supplementation regimes.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Reduction in homocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate alone</td>
<td>17.3%</td>
</tr>
<tr>
<td>B12 alone</td>
<td>18.7%</td>
</tr>
<tr>
<td>B12 + Folate</td>
<td>57.4%</td>
</tr>
<tr>
<td>B12 + Folate + B6</td>
<td>59.9%</td>
</tr>
</tbody>
</table>

8 ways to lower you homocysteine.

1. Eat less fatty meat, more fish and vegetable protein.
2. Eat your greens.
3. Have a clove of garlic a day.
4. Cut back on tea and coffee.
5. Limit alcohol intake.
6. Reduce your stress.
7. Stop smoking.
8. Supplement homocysteine lowering nutrients daily.
Homocysteine in clinical medicine: current status

Prof. Helga Refsum, University of Oxford, University of Bergen & University of Oslo

Prof. Refsum commenced this final session by discussing results from intervention trials designed to lower homocysteine concentrations. Neither NORVIT nor WENBIT demonstrated a reduction in primary end points upon vitamin supplementation, and a meta-analysis of randomised controlled trials concluded “Folic acid supplementation has not been shown to reduce risk of cardiovascular diseases or all-cause mortality among participants with prior history of vascular disease”.

The conclusion from the HOPE2 study had been that “B vitamins do not reduce the risk of CVD events” but the study had demonstrated a statistically significant reduction in the risk of stroke by 25% upon B vitamin supplementation. Data from the uncontrolled experiment of mandatory folic acid fortification in the USA and Canada suggested that between 5 and 10% of all stroke deaths may be prevented by fortification. A meta-analysis of the efficacy of folic acid supplementation in stroke prevention concluded that folic acid significantly reduces the risk of stroke by 18%.

Prof. Refsum summarised homocysteine’s association with a number of disease states. She highlighted that elevated homocysteine was associated with increased mortality both in low and high cardiovascular disease risk populations. The association of elevated homocysteine levels with pregnancy complications, depression, reduced bone mineral density, memory loss and a number of other conditions were discussed.

Whilst there are mixed and on occasion partially untrue signals coming from various “lobby groups”, it can be stated that a good folate (and vitamin B12) balance is critical, that there are indications and contra-indications (e.g. data suggesting increased risk of colo-rectal cancer) for the use of folic acid and that folic acid should be used with care, in the right way for the right groups.

Prof. Refsum asked the audience whether they would be happy with a high homocysteine level, and there was a universal response of “No”. Prof. Refsum suggested that if they would not be happy with an elevated homocysteine, then equally patients or their clinicians should be concerned about a high homocysteine.

Conclusions
• Homocysteine is a biomarker of overall health.
• Raised homocysteine (particularly when pronounced) is never good, suggesting B vitamin deficiency, serious diseases or an unhealthy lifestyle.
• Indications and contra-indications for use of folic acid/B vitamin supplements are now apparent.
• Hence, such supplements should optimally be used only after medical evaluation.

Homocysteine determination is an excellent way to identify those that have B vitamin deficiency, and those that may benefit from supplements.
Homocysteine Day

Recip AB
Lagervägen 7
S-136 50 Haninge
Sweden
www.recip.se

Axis-Shield Diagnostics
Luna Place
The Technology Park
Dundee
DD2 1XA
Scotland
www.axis-shield.com