Homocysteine Day

Oxford, »That sweet City with her dreaming spires«

An update in relation to common diseases

Report from Homocysteine Day
Oxford 15–17 September 2006
by Dr David Pritchard

Homocysteine Day

PREFACE

Continuing from the successful meetings of previous years, Homocysteine Day 2006 was held on September 8th and 9th in the historic university city of Oxford. The meeting venue was St Anne's College of Oxford University, one of the newer colleges in Oxford being founded in 1952, developing out of "The Association for the Education of Women in Oxford" which was established in 1878 (Balliol and Merton are the oldest colleges and were established between 1249 and 1264). There is no clear date of foundation of Oxford University, but teaching existed in Oxford in 1096 and developed rapidly from 1167 when Henry II prevented English students attending University in Paris.

In addition to attending a series of excellent presentations and discussions, delegates had opportunity to explore "That sweet City with her dreaming spires",[1] in glorious September sunshine.

The aim of Homocysteine Day 2006 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 100 participants attended the meeting, most of the attendees were general practitioners, but other specialities represented included cardiologists, haematologists, geriatricians 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. Prof. Helga Refsum (Universities of Oxford and Oslo) then discussed homocysteine in children and the newborn. The first day's sessions concluded with a lively question and answer session where many issues pertaining to homocysteine were discussed.

On the first day of the conference, delegates had the opportunity of having blood taken for homocysteine analysis, and at the start of the second day Prof Refsum reported back the results, and provided information on how to interpret them. Prof Ebba Nexø of Aarhus University Hospital University, Denmark provided an update on the current status of vitamin B12 and holotranscobalamin testing. 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 the provocatively titled "Homocysteine. Time for a Funeral?"

[1] Description of Oxford in the poem "Thyrsis"; Mathew Arnold (1866)

Welcome and Introduction

Prof David Smith University of Oxford, Dept of Pharmacology
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 sulphydryl (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.

SH
|
CH2
|
CH–COOH
|
NH2

Figure 1. Chemical structure of homocysteine

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.

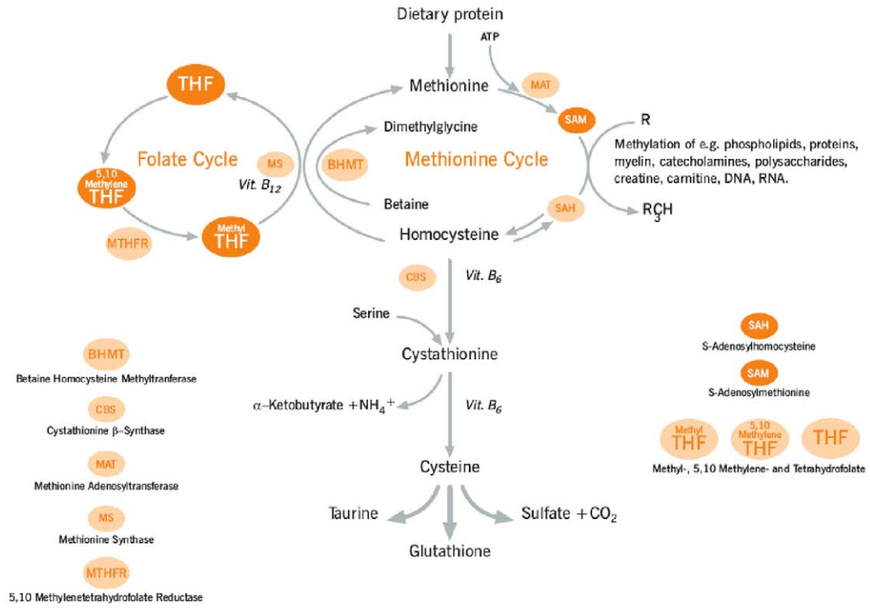


Figure 2. Metabolism of homocysteine.

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 µmol/l 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 this is currently unknown.

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

Population	Mean	Guideline upper limit
Children	6	10
Pregnancy	6	10
Adults	9	15
Elderly	12	20

Note that upper limits should not be used strictly.

Elevated homocysteine concentrations can result from non-modifiable and modifiable (lifestyle) factors.

Causes of elevated homocysteine. Non-modifiable 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 µmol/l 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?

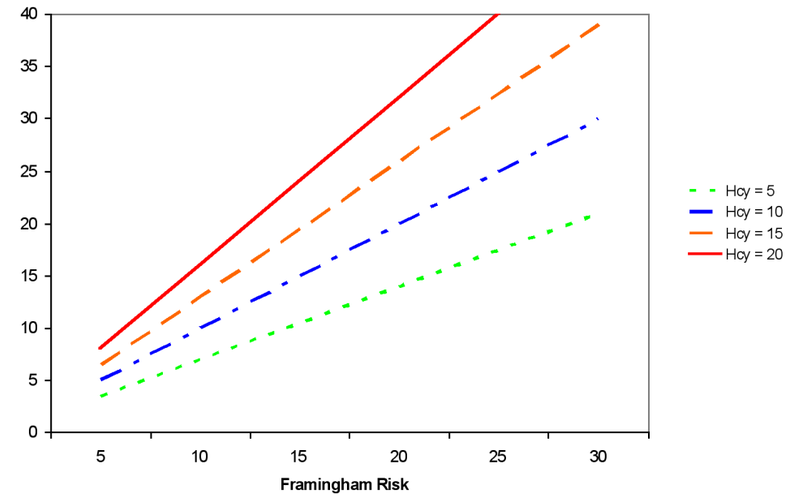
Homocysteine and other risk factors for vascular disease:

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.

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.

Figure 3. Biomarker enhancement of risk prediction. (µmol/l)



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 homocysturia, animal studies and in-vitro 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.

The issue that was next addressed was that the importance of homocysteine as a cardiovascular risk factor is not equal in all patients. This was exemplified by comparing Rural Indian Asians with Indian Asians and Caucasians resident in the UK. A much higher percentage of rural Indian Asians have elevated homocysteine concentrations, and the cardiovascular risk attributable to this is around 3 times that for people resident within the UK (Figures 4 and 5).

Figure 4. Percentage of different ethnic groups with homocysteine > 15 µmol/l.

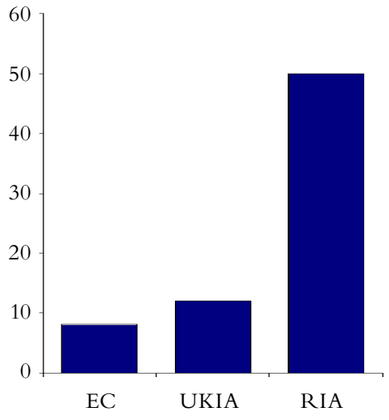
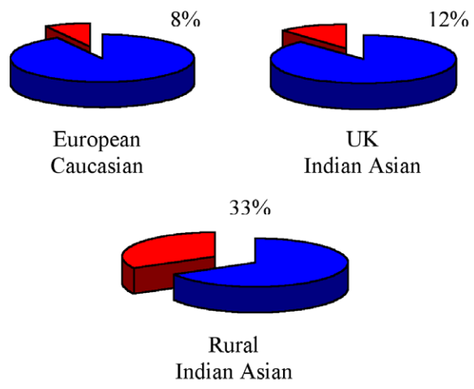


Figure 5. Percentage population risk for cardiovascular disease attributable to homocysteine



Conclusions

- There is more to life than conventional risk factors
- Check homocysteine
- Good evidence that homocysteine is a biomarker of increased vascular risk. 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
    - Consider unproven B vitamin Rx.

Homocysteine and dementia: the evidence

Prof David Smith, University of Oxford,
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 µmol/l were at around 5 times the risk of developing dementia as those with homocysteine concentration below 10 µmol/l). 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.

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 is not a diagnostic marker but it is a strong prospective marker of subsequent brain atrophy, cognitive decline and dementia.
- Clinical trials of homocysteine-lowering treatments are needed to see if raised homocysteine is one of the causes of dementia.

Homocysteine, osteoporosis and bone health

Dr Rosalie Dhonukshe-Rutten. Wageningen, Netherlands

Osteoporosis is a multifactorial disease and is defined as "a systemic skeletal disease characterised by low bone mass and microarchitectual 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 general population are needed.
- Association between markers of vitamin B12 status and bone health my have important implications for the prevention of fractures.

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.

		Newborns	Infants	2 Years old	Adults
Lower Limit	Serum folate, nmol/l	25	25	10	5
	Serum B12, pmol/l	125	125	240	150
Upper Limit	Homocysteine, µmol/l	10	12	7.5	15
	MMA, µmol/l	0.50	2.00	0.30	0.27

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.

Table 3. Folate, B12, homocysteine and MMA levels at birth and 6 months.

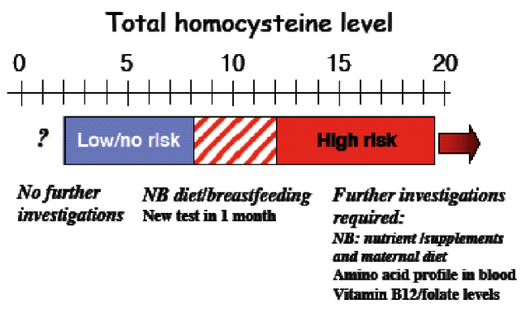
		6 Months		
	Birth	Not Breastfed	Breastfed + other food	Exclusively Breastfed
Folate nmol/l	45	50	55	69
Homocysteine µmol/l	6.2	5.8	7.0	7.5
MMA µmol/l	0.26	0.28	0.48	0.45
Vitamin B12 pmol/l	302	355	243	236

Prof. Refsum highlighted the importance of vitamin B12 deficiency in infancy with a case history where an exclusively breast-fed 9 month old baby of a strict vegetarian mother presented with dystrophy, muscular weakness, loss of tendon reflexes, psychomotor regression and haematological abnormalities. Biochemical investigation demonstrated that the baby had severe methylmalonic aciduria and homocystinuria, and a low vitamin B12. The mother had moderate methylmalonic aciduria and low vitamin B12. Studies of infants on a strict vegan diet have demonstrated impaired growth and development of these children.

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 ß-synthase (CBS) deficiency. Prof. Refsum suggested that CBS deficiency might be much more common than previously thought (only the most severe variants are identified by newborn methionine screening), and that homocysteine testing should be done in children presenting with thromboembolic disease, mental retardation, psychiatric abnormalities, Marfan like skeletal abnormalities, osteoporosis or lens dislocation.

Figure 6. What to do with elevated homocysteine levels in newborns and infants. (µmol/l).



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.

Discussion:

Homocysteine as a risk factor in CVD, dementia and osteoporosis

Question: Why are cut-off levels for homocysteine in the USA lower than those quoted in Europe?

There are two factors contributing to this. In the USA an estimate has been made of an ”optimal” homocysteine level and this as often quoted as the cut-off, whereas in Europe quoted cut-off levels are generally statistically derived from actual homocysteine measurements in the population. The second factor is that in the USA, all cereals are fortified with folate and that this has the effect of reducing homocysteine levels in the general population.

Question: What is the effect of homocysteine on nitric oxide?

Homocysteine directly reacts with nitric oxide, thus blocking its action. There is considerable debate on whether homocysteine and folate individually have an independent effect upon nitric oxide, but a recent publication by Helga Refsum and Keith Channon suggests that this is the case.

Question: Does cooking food with microwaves affect the vitamin B12?

It was thought that as vitamin B12 is a fairly stable molecule, it would be unaffected by exposure to microwaves.

Question: Should women take vitamin B6 and B12 supplements during pregnancy?

Yes, the use of a supplement containing B6 and B12 as well as folate will be advantageous for the child.

Question: How would you explain normal vitamin B12 and folate results but an elevated homocysteine?

There are a number of factors that need to be taken into consideration in this scenario. It might be due to renal impairment, drugs such as anti-epileptics, possession of the MTHFR TT genotype or a combination of these factors. It is also possible that the patient could have a homocystinuric enzyme defect.

However, possibly a more likely explanation is that vitamin levels within the reported ”normal” range can be clinically deficient, thus if vitamin levels are at the lower end of the ”normal” range deficiency should not be ruled out. This is particularly the case for vitamin B12 where quoted cut-off levels vary between 150 and 280 pM. This situation can be clarified by measuring Holo transcobalamin (HoloTC). HoloTC is the physiologically active form of vitamin B12 and measurement of this avoids the clutter introduced by the inactive forms that are also measured by the vitamin B12 assays. A commercial assay is now available enabling routine measurement of HoloTC. This assay performs well and can be of great assistance in determining patients who are vitamin B12 deficient.

Question: How would you explain low vitamin B12 and folate results but a normal homocysteine?
Again it was suggested that measurement of HoloTC would be useful in this scenario. In the same way that a normal vitamin B12 but a low HoloTC level is indicative of a deficiency, a low vitamin B12 is not indicative of deficiency if the HoloTC is normal. It was also suggested that repeating the measurements 6 months later may be helpful in determining whether there is any deficiency.

Question: What is the significance of a high vitamin B12?
There are a number of conditions such as liver disease and leukaemia where high vitamin B12 levels are encountered, or the elevation might be unrelated to an underlying pathology. Again it was suggested that measurement of the physiologically important HoloTC could be useful.

Question: What is the optimal level of alcohol intake for having a low homocysteine
The Hordaland study conducted by Prof. Refsum was on a population that had very low alcohol consumption so she felt unable to provide an answer.

Question: Smoking has an influence on homocysteine levels, does use of snuff have a similar effect?
As far as the panel were aware, the effect of snuff upon homocysteine levels has not been evaluated.

Question: What is the mechanism underlying the influence of alcohol intake, coffee intake and smoking upon homocysteine?
Smoking leads to impairment of activity of a number of the enzymes involved in homocysteine metabolism. Whilst it has been shown that drinking coffee increases homocysteine, and stopping drinking coffee results in lower homocysteine values the underlying mechanism is currently unknown.

Results from homocysteine assays: how to interpret them

Prof Helga Refsum University of Oxford & University of Oslo

28 people attending Homocysteine Day voluntarily had blood samples taken for homocysteine testing on the first day of the conference, and at the start of the second day's proceeding Prof Refsum reported back the results,[2] along with some interpretation of these.

11 individuals (9 females and 2 males) had homocysteines of below 10 μmol/l. These are well within the normal range (unless any of the donors were pregnant in which case a value approaching 10 could be in the high normal range).

9 individuals (2 females and 7 males) had homocysteine concentrations between 10 and 12 μmol/l. If individuals were taking folate supplements these values may be at the high end of the normal range, but this is related to age. For example a 35 year old had a homocysteine of 11.79 which would be a little high if taking folate, whilst for a 68 year old individual a homocysteine of 12.00 is quite normal. If homocysteine values of between 10 and 12 μmol/l were obtained from a pregnant individual this would be considered borderline elevated. Homocysteines of between 10 and 12 μmol/l for non-pregnant adults not receiving folate supplementation are considered normal.

8 individuals (5 females and 3 males) had homocysteine concentrations greater than 12 μmol/l, of whom 2 (both males) had values greater than 15 μmol/l (15.52 & 16.25). If taking folate supplements a homocysteine greater than 12 μmol/l is considered borderline or elevated. A homocysteine of this level would definitely be considered elevated during pregnancy. Homocysteines of between 12 and 15 μmol/l for non-pregnant adults not receiving folate supplementation are considered to be at the high end of the normal range. The 2 individuals with homocysteines of 15.52 & 16.25 μmol/l would be considered to be borderline elevated.

<u>How to treat patients with borderline or elevated homocysteines.</u>

<u>Subjects without symptoms.</u>
- Doctor to give advice on lifestyle.
- Consider follow-up test in 6 - 12 months if the subject has a high CVD risk, or is relatively young.
- In pregnancy: use supplements

<u>Subjects with symptoms or with definitely elevated homocysteine.</u>
- Measure additional biomarkers: Folate + B12 marker (HoloTC, MMA or B12) + creatinine.
- If folate or B12 marker indicates deficiency, treat with appropriate supplement, retest in 1 - 3 months

[2]Data was anonymous and reported using a sample ID number known only to the person whose sample it was.

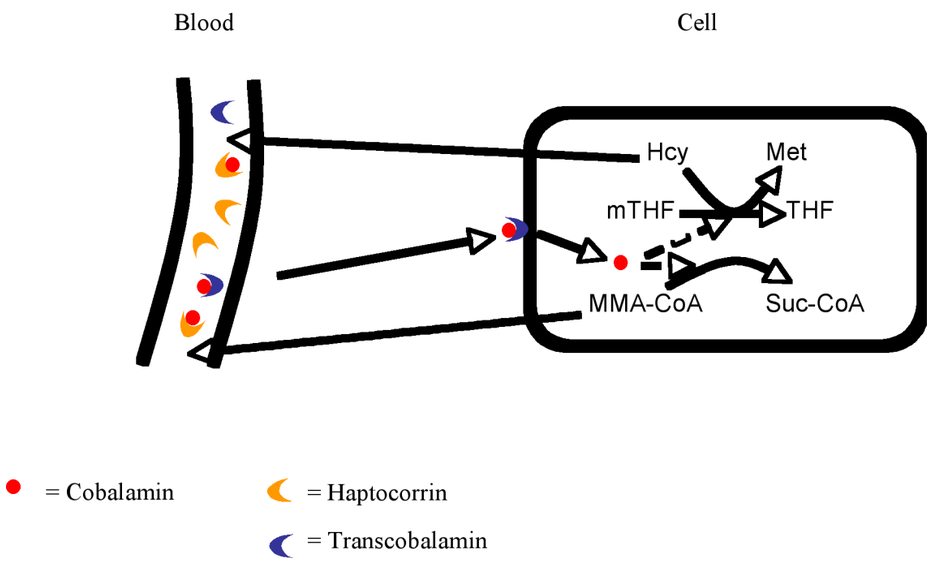
Vitamin B12 and holotranscobalamin: Current status and clinical significance

Prof Ebba Nexø (Aarhus University Hospital, Denmark)

Vitamin B12 deficiency is an important and common cause of pathology, particularly given the aging population and the increased number of people with vegetarian and vegan diets. Early detection is important as once patients have developed neurological symptoms, these may be irreversible.
As shown in Figure 7 there are 2 binding proteins for vitamin B12 (cobalamin) in the blood;

- Transcobalamin in the blood is responsible for the transport of cobalamin to the cells.
- Although the majority of the cobalamin in the blood is bound to haptocorrin, the function of this is unknown.

Figure 7. Uptake and transport of vitamin B12.



Inadequate uptake and transport of vitamin B12 in to the cells will lead to reduced conversion of homocysteine to methionine and of MMA to succinate, therefore elevated levels of MMA and homocysteine may be evident in the blood.

Existing measures of vitamin B12 deficiency are problematic. Measurement of total B12 is predominantly a measure of the haptocorrin bound cobalamin which is thought to be biologically inactive. Methylmalonic acid (MMA) and homocysteine are surrogate markers of B12 deficiency, but have their own limitations. Measurement of MMA is not widely available, is expensive and is affected by kidney function, whereas elevated homocysteine may result from causes other than B12 deficiency. If one were to address which moiety is likely to be the best marker for early diagnosis of B12 deficiency from a theoretical point of view, then the obvious analyte would be holotranscobalamin as this represents the physiologically utilisable vitamin. However, until recently it has not been possible to measure HoloTC, but an easy to use HoloTC assay suitable for clinical laboratories has recently been launched.

Data was presented from a number of clinical studies and indicated that measurement of HoloTC is a very promising marker for the diagnosis of vitamin B12 deficiency, and that it is an effective early marker of deficiency.

Prof Nexø discussed the Cobasorb test, a new HoloTC based non-radioactive alternative to the Schillings test for investigation of defects of B12 absorption. Measurement of HoloTC before and after oral intake of vitamin B12 appears to be a promising method in evaluating absorption of vitamin B12.
There was discussion as to which marker should be considered the gold standard test for vitamin B12 deficiency. Prof Nexø said that a number of people were passionate proponents of MMA, whilst others disagreed with this. It has been demonstrated that low levels of HoloTC are observed before elevated MMA concentrations are apparent, thus HoloTC may prove to be the "Gold Standard Test" for vitamin B12 deficiency, particularly for early diagnosis.

<u>Conclusions.</u>

- HoloTC seems to be the test of the future. It may be able to replace combined testing with cobalamins, MMA and homocysteine.
- HoloTC is a better marker than cobalamins for detecting metabolic Vitamin B12 deficiency.
- HoloTC is an early marker of vitamin B12 deficiency.
- In the studies presented HoloTC performed better than total cobalamins
- The HoloTC test is now available but has yet to be introduced in most laboratories.
- Measurement of HoloTC after intake of vitamin B12 can be used to judge absorption of the vitamin.

Folic acid and depression

Prof P Cowen University of Oxford

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, but not all studies agree. 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 4 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.

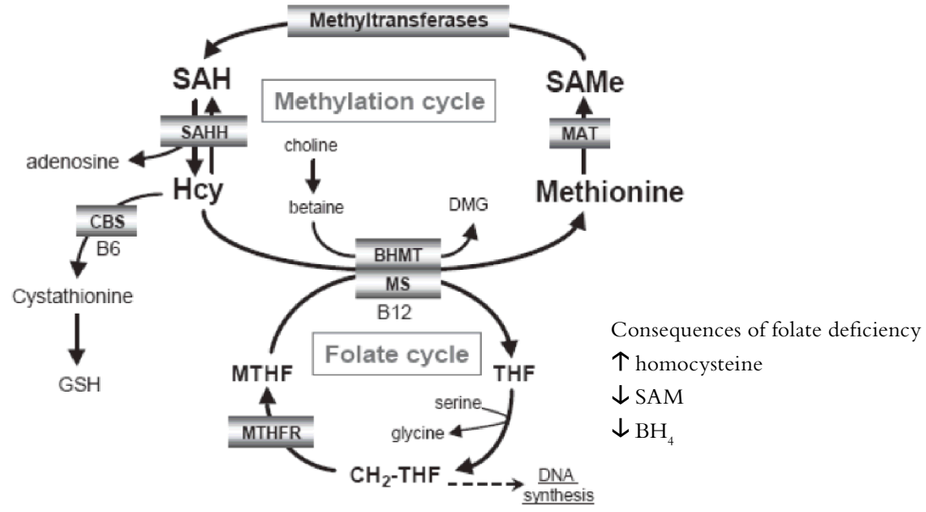
Table 4. Rates of MTHFR polymorphisms in individuals ever diagnosed with depression.

Genotype (n)	Odds ratio (95% confidence limits)
CC (1565)	1.0
CT (1520)	1.2 (0.99 - 1.46)
TT (402)	1.4 (1.01 - 1.80)

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 8).
* Tetrahydrobiopterin (BH_4, a pterin) is a cofactor for the production of the neurotransmitters 5HT (5-hydroxytryptamine) and noradrenaline, thus the reduced levels of BH_4 may contribute to the depressive state.

Figure 8. 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.

Conclusions

- Low folate and perhaps to a greater extent 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.

The H-factor solution: a nutritionist's perspective

Patrick Holford, (Brain Bio Centre, London)
Patrick Holford provided a thought provoking and stimulating 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 7.5µmol/l.

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 5.

Table 5. Percentage reduction in homocysteine with differing vitamin supplementation regimes.

Supplement	Reduction in homocysteine
Folate alone	17.3%
B12 alone	18.7%
B12 + Folate	57.4%
B12 + Folate + B6	59.9%

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. Time for a Funeral?

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

In this final session 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.

Reanalysis of data from intervention studies indicates that the initial negative conclusions may have been arrived at somewhat prematurely. In the VISP study, the initial conclusion was that there was "no effect of high dose B vitamins versus low dose B vitamins on stroke events", However, when one looks at mortality the data suggest that those on high dose vitamins have lower mortality rates It was suggested that the trial may have been stopped too early to properly study this outcome. In the HOPE2 trial a conclusion was "B vitamins do not reduce the risk of major CVD events", but the authors demonstrated a reduction of stroke events by 25% ($p=0.03$). The incidence of stroke has decreased in the USA since the introduction of folic acid fortification, leading some researchers to conclude that "the uncontrolled experiment of folic acid fortification in USA suggests that up to 13,000 stroke deaths are prevented each year".

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 levels reflect an individual's health.
- Raised homocysteine (particularly when pronounced) is never good, suggesting B vitamin deficiency, serious diseases or an unhealthy lifestyle
- People with raised homocysteine require medical attention on some way or another.
- For scientists, the question remains. Is homocysteine causative of disease, a consequence of it or a bit of both?

Prof Refsum's overall conclusion was that far from being time for a funeral, homocysteine was still in its infancy.

Notes

Homocysteine Day

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Axis-Shield Diagnostics
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Postboks 205
1372 Asker
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