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1 SUMMARY

Recent studies, both epidemiological and experimental, have shown that hyperhomocysteinemia is a risk factor for atherosclerosis and thrombosis and therefore a factor in coronary heart disease. There are a number of factors that are considered to influence serum or plasma homocysteine levels in humans. Some are genetic and affect enzymes that influence the catabolism of homocysteine especially in methionine metabolism and in particular cystathionine beta-synthase (CBS) and Methylen-tetrahydrofolate reductase (MTHFR). It appears that vitamin B12 and folate deficiencies in the general population have a causative relationship to increased levels of homocysteine in the blood. There are other lifestyle factors that are involved in hyperhomocysteinemia such as smoking and coffee consumption. There is some evidence that also links hyperhomocysteinemia and vitamin B12 deficiency with vegetarianism.

This study looked at the serum levels of cystathionine (Cys), homocysteine (Hcy) and methylmalonic acid (MMA) and the serum levels of folate and vitamin B12 (B12) in the two distinct ethnic Groups. The first group is nationals of the Sultanate of Oman (Omanis) (total number 225) of median age 53 (33 – 75*)* years and roughly equal male and female (56 % male 44 % female). The second group is resident Indians from the Sultanate of Oman (total number 137**)** of average age 42 (39 – 57**)** years and mainly male (96 % male 4 % female). It should be noted that one group of Indians were omnivores and other group were vegetarians however, all the Omanis were omnivores.

The current study revealed that hyperhomocysteinemia, high levels of MMA and deficiency of Vitamin B12 are very common in Asian Indian residents in Oman who are

strict vegetarians. Contrastingly the Asian Indian residents who were omnivorous have much lower frequency of vitamin B12 deficiency than their vegetarian counterparts. It is also apparent from the study that Omanis have a much lower frequency of vitamin B12 deficiency and much lower levels of Hcy and MMA than the Asian Indian s in general but particularly the vegetarians. Omanis who are essentially omnivorous have a lower frequency of vitamin B12 deficiency than Asian Indian omnivores as demonstrated by the relatively increased levels of MMA and lower levels of vitamin B12 levels in Asian Indian omnivores as compared with Omanis. As vitamin B12 deficiency is known to be related to higher levels of Hcy, the Asian Indian vegetarians living in Oman should therefore consider vitamin B12 supplementation. There is no evidence of vitamin B12 deficiency in general Omani elderly population that is predominantly omnivorous. There is thus no need to screen the population for the deficiency given the low frequency of occurrence even in an ageing population.

Taken together the present work has shown that hyperhomocysteinemia (Hcy > 12 μmol/l) is not prevalent in Omani population and therefore no risk factor for Coronary vascular disease.

2 THE SCOPE OF THE STUDY

The Sultanate of Oman is a country of approximately 350,000 square kilometers that borders the southeastern fringe of the Arabian Peninsular. It has a long coastline along the Arabian Sea and the Gulf of Oman all part of the Indian Ocean. The country has mainly desert with a mountainous spine near the easterly coast which separates the narrow coastal plain from the mainly arid, desert interior.

The population of the country from the census of 2003 updated for the year 2004 is approximately 2.415 million of which 1.802 million are Omani nationals and 0.613 million are ex-patriate residents. The vast majority of the ex-patriate population is from the Indian sub-continent.

The general health of the population as measured by life expectancy and crude death rates has improved considerably over the last 30 years. Life expectancy has risen from 50 years in 1970 to 74 years in 2004 (Ministry of Health, Sultanate of Oman Annual Health Report, 2004). The death rate has reduced from 13.3 per 1,000 population in 1980 to 2.6 per 1000 population in 2004 (Ministry of Health, Sultanate of Oman annual Health Report, 2004). By far the greatest factor influencing the general health of the population and life expectancy has been the success achieved in the reduction of communicable diseases through highly effective child immunization programmes. The focus of health awareness has shifted to non-communicable diseases including cancer, diabetes and coronary artery disease. With increasing evidence that hyperhomocysteinemia is a risk factor in coronary artery disease any evidence of hyperhomocysteinemia in population groups will be of interest. Also of importance is the apparent relationship of other factors that have close metabolic relationships with homocysteine (Hcy) such as cystathionine (Cys), methymalonic acid (MMA), vitamin B12 (B12) and folate.

The study shows high levels of Hcy in the serum of a group of Indian ex-patriates living in the Sultanate of Oman and who are strict vegetarians. The study also shows vitamin B12 deficiency in the Indian vegetarians along with significantly higher levels of folate and MMA compared with Omanis and a group of omnivorous Indian ex-patriates. The relatively higher levels of folate in the group with B12 deficiency is in contrast with other studies that have shown that generally in hyperhomocysteinemia associated B12 deficiency lower levels of folate are seen. In the Omani group the levels of Cys, Hcy, MMA, vitamin B12 and folate were generally within the normal reference range.

3 INTRODUCTION

3.1 Historical review

Homocysteine was first described by Burtz and du Vigneaud in 1932 when they obtained homocysteine by treating methionine with concentrated acid. Homocysteine was identified in 1962 in the urine of some mentally retarded children (GERRITSEN et al., 1962; CARSON NAJ and NEILL DW, 1962). Some years later a genetic defect of cystathionine beta-synthase (CBS) was identified (MUDD SH et al., 1964). Patients with this genetic defect demonstrated homocysteinuria and very high levels of homocysteine in the plasma. These patients were found to have premature arteriosclerosis and frequently thromboembolisms. Over half had recurrent cardiovascular events and 25% died before reaching the age of 30 years (GIBSON JB et al., 1964; SCHIMKE RN et al., 1965).

Recent epidemiological studies have demonstrated that increased plasma or serum concentrations of homocysteine (hyperhomocysteinemia) is an independent risk factor for cardiovascular disease (DE BREE A et al.; 2002, BLUM A, 2001), neural tube defects, neuropsychiatric disorders (LASCALZO J, 2002) and cognitive disorders of the elderly such as dementia (MILLER JW, 2003). A moderate elevation of homocysteine is common in the general population and may be related to several inherited or acquired factors.

Hyperhomocysteinemia is mostly related to poor vitamin B status. Furthermore, elevated serum or plasma concentrations of Hcy has been linked to certain lifestyle factors such as smoking (BERGMARK C et al., 1997), malnutrition, lack of exercise (DE BREE A et al., 2001) and coffee consumption (NYGARD O et al., 1997). Hyperhomocysteinemia has been documented in individuals who have a poor diet (HERRMAN W et al., 2003). Renal insufficiency is the most important cause of hyperhomocysteinemia in the elderly.

Moreover, racial and ethnic differences in Hcy metabolism have been reported (UBBINK JB et al., 1995; CAPPUCCIO FP et al., 2002). Several mutations in genes encoding enzymes related to Hcy metabolism have been described. The most important mutation extensively investigated is a point mutation in the gene methylene tetrahydrofolate reductase (MTHFR C677T) (CHAMBERS JC et al., 2000). The complex manner of gene-environment interactions may determine serum concentrations of Hcy. Recent intervention studies documented better outcomes in patients receiving Hcy-lowering treatment (LEWERIN C et al., 2003). Therefore, with an increasing emphasis on disease prevention, it would be interesting to test for Hcy and B-vitamins in at-risk populations.

3.2 Biochemistry of homocysteine and its metabolism

Homocysteine is an endogenous sulphur-containing amino acid with a molecular weight of 135 Daltons. It is not a naturally occurring nor is it an "essential" amino acid in as much as it is not available from the diet but is formed entirely from the metabolism of methionine. Methionine is the only essential, sulphur-containing amino acid (aminothiol) found in the diets of mammals (FINKELSTEIN LD, 2000)

Figure 3.2.1. The chemical formulae of homocysteine, methionine and cystathionine

Homocysteine $HS\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)COOH$

Methionine $CH_3\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)COOH$

Cystathionine $HOOC\text{-}CH(NH_2)CH\text{-}S\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)COOH$

Homocysteine is produce as an intermediate step in the metabolism of methionine to cystathionine and cysteine as shown in the metabolic pathway illustrated (**Figure 3.2.2**.).

Methionine can also be generated by the methylation of homocysteine. Approximately 50% of homocysteine is re-methylated to methionine and 50% is transulphurated to cysteine (BOLANDER-GOUAILLE C et al., 2003). Cysteine is a major source of glutathione in humans.

Methionine is supplied mainly by food proteins and is activated in the presence of adenosine tri-phosphate (ATP) and converted to S-adenosylmethionine (SAM). SAM is a methyl donor in many trans-methylation reactions in humans. SAM is subsequently metabolized to S-adenosylhomocysteine (SAH) which is subsequently hydrolyzed to Hcy. This transmethylation pathway is the only metabolic pathway that is known to produce Hcy. After formation, Hcy is either re-methylated into methionine or catabolised into cystathionine (Cys).

Methionine synthetase (MS) and its methyl-B12 (i.e. methylcobalamin) catalyse the catabolism of Hcy via the re-methylation pathway. 5-methyltetrahydrofolate (5-MTHF) donates a methyl group to MS which then transfers it to Hcy forming methionine. Another route of Hcy re-methylation is mediated by beta-homocysteine methyltransferase that utilizes betaine as a methyl donor. This minor pathway takes place mainly in the liver **(Figure 3.2.2.)**

The trans-sulphuration pathway is in part mediated by cystathionine-β-synthetase (CBS) . The active form of vitamin B6, pyridoxal-5-phophate, is a cofactor for CBS. Cystathionine is produced as a result of this process and this in turn is converted into cysteine and α-ketobutyrate catalyzed by the enzyme cystathioninase which is another B6 dependant enzyme. Cysteine is further metabolised into glutathione which is very important in human antioxidant defense.

Several factors contribute to coordinative regulation of Hcy metabolism between the re-methylation and trans-sulphuration pathways. SAM is one of these regulators that enhances the activity of CBS and inhibits that of methylenetetrahydrofolate reductase (MTHFR). When there is methionine excess, such as occurs after a meal (methionine loading), the trans-sulphuration pathway is favored. However Hcy re-methylation to methionine is favored when there is a relative methionine deficiency in the cells such as might occur under fasting conditions. Hcy metabolism depends on the availability of B vitamins (JACOBSEN, 1998; TEMPLE et al., 2000) as well as a number of enzymes (MINER et al., 1997)

Hcy is very important in one-carbon metabolism. The role of Hcy in one-carbon metabolism is shared with 5-MTHF. Both Hcy and 5-MTHF are substrates for the production of tetrahydrofolate (THF). THF is the form that is used in the formation of purines in DNA synthesis.(STANGER, 2002).

When there is methionine excess, such as occurs after a meal (methionine loading), the trans-sulphuration pathway is favoured. However Hcy remethylation to methionine is favored when there is a relative methionine deficiency in the cells such as might occur under fasting conditions.

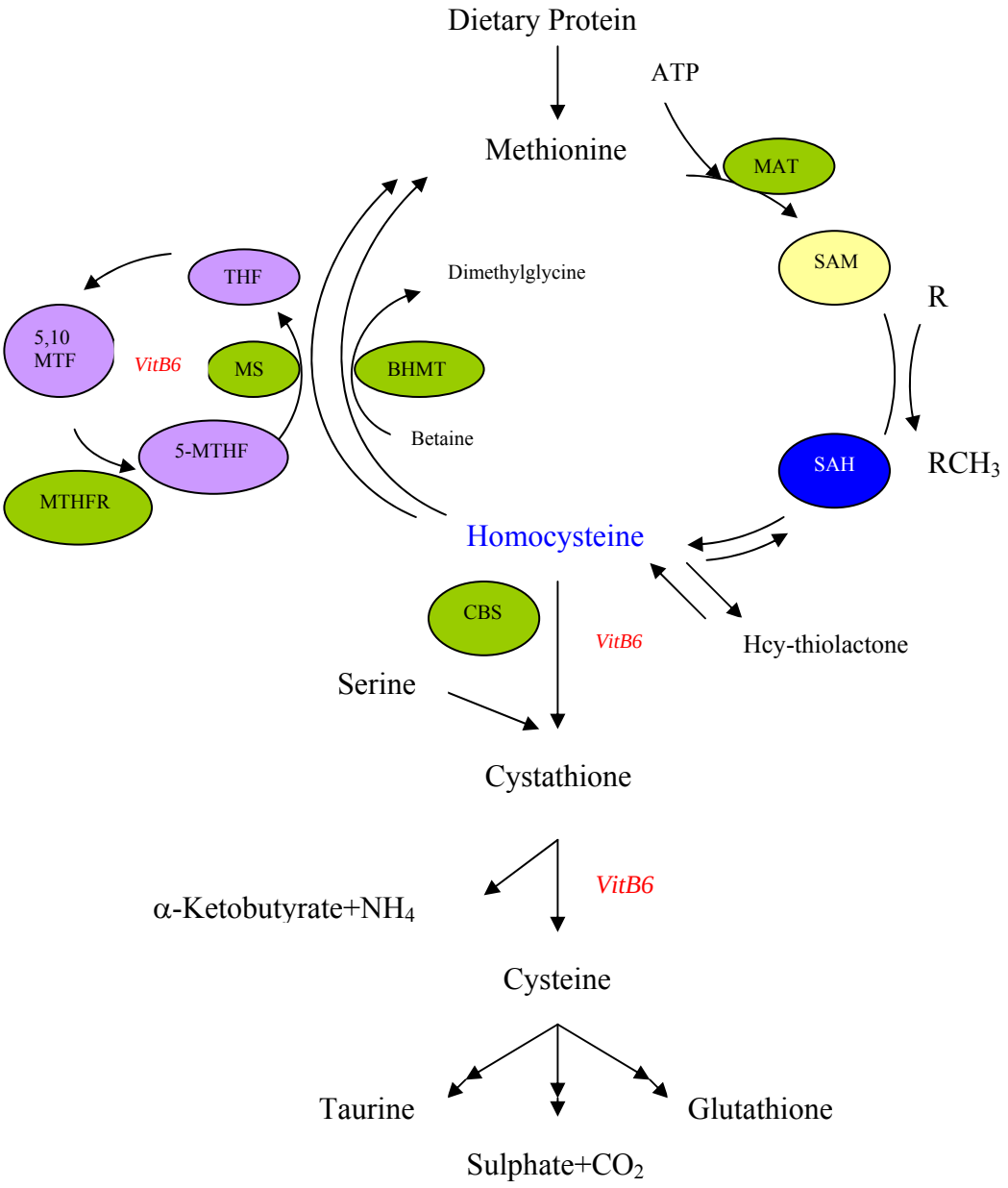
Homocysteine metabolism depends on the availability of the B vitamins (B12 & B6) and folate (JAKOBSEN, 1998; TEMPLE et al., 2000) as well as a number of enzymes (MINER et al., 1997). The trans-methylation pathway is extremely important in cellular metabolism. SAM is derived from an important ATP-dependant transfer of adenosine to methionine via methionine-adenosyl transferase **(Figure 3.2.2.)**. SAM is the universal methyl donor in numerous trans-methylation reactions in humans (MAT JM, 1997). Methyl acceptors include nucleic acids, proteins, phospholipids, neurotransmitters, myelin and

polysaccharides. Sam can be further de-methylated in SAH. SAH is converted to Hcy in a reversible reaction catalyzed by SAH-hydrolase. The conversion of Hcy to SAH is favored under conditions of hyperhomocysteinemia. Therefore, increases in Hcy due to B vitamin deficiencies may have a negative effect on cellular methylation reactions as a result of increased intracellular SAH (YI P et al., 2000). Conversely, hydrolysis of SAH into Hcy prevails under physiological conditions as long as the product, Hcy, is efficiently removed. Adenosine is converted to inosine by adenosine deaminase or it can be utilized in forming purine nucleotides (AMP and ATP) by adenosine kinase.

The metabolisms of Hcy SAM and SAH are functionally related and therefore disorder in one component will affect the other metabolites. In line with this SAM plays a role in coordinating Hcy catabolism by enhancing the activity of CBS and inhibiting MTHFR.

The ratio of SAM/SAH determines the methylation potential in the cell (FINKLESTEIN JD 1990, LEE ME et al., 1999, YI P et al., 2000) and signifies the flow methyl groups transferred from Sam to methyl receptors (KRAMER DL et al., 1990) SAH is a competitive inhibitor that binds to many transmethylases thus inhibiting the trans-methylation reactions (CHANG P et al., 1996). A recent review has address these issues and discusses present knowledge of DNA methylation in healthy cells and disease states (COSTELLO JF et al., 2001).

Figure 3.2.2. Homocysteine Metabolism

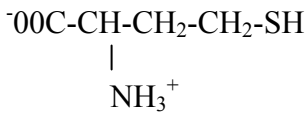
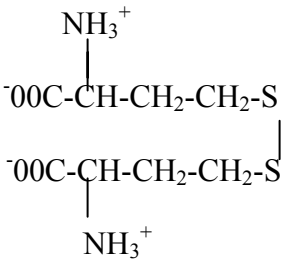
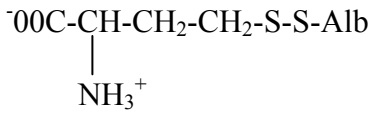
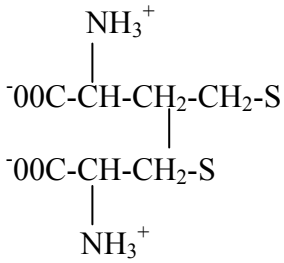


SAM	S-Adenosylmethionine	CBS	Cystathionine β-synthase
SAH	S-adenosylhomocysteine	MAT	Methionine adensosine transferase
THF	Tetrahydrofolate	MS	Methionine synthase
5-MTHF	5-Methyltetrahydrofolate	MTHFR	5,10 Methylenetetrahydrofolate reductase
5,10 MTHF	5,10 Metylenetetrahydrofolate	BHMT	Betaine homocysteine methyltransferase

3.3. In-vivo distribution of homocysteine

Homocysteine is found in plasma mainly as disulphide complexes (HCY-HCY). The sulphydryl groups are supplied by sulphydryl amino acids of proteins mainly albumin. (UELAND,1995). The free Hcy molecule (single molecule) is extremely unstable having a very low solubility at a neutral or physiological pH (GUTTORMSEN et al., 1993). The majority of plasma Hcy (80-90%) is protein bound, 70% to albumin in the mixed disulphide form. 5-10% is the unbound cysteine-homocysteine disulphides. 5-10% is found as the oxidized homo-disulphide (Hcy-Hcy). The oxidized homo-disulphide also called homocysteine constitutes 5 – 10 % of total Hcy and 1 – 2 % is found as free reduced homocysteine. **(Table 3.3.1) .**Intracellular homocysteine is mainly reduced or unbound homocysteine which has a highly reactive free thiol group. Extra cellular homocysteine is mainly present in the oxidized disulphide or bound to albumin. The transport of homocysteine between the cell and the extra cellular fluid appears to be controlled by a presumed *reduced homocysteine carrier* (BLOM HJ, 2000). In order for total Hcy (total Hcy) to be measured free Hcy has to be released from its disulphides. This step is essential before the assay of total Hcy can be made. Free Hcy is considered to be the main atherogenic proton. However it is highly oxidative and therefore unstable. It is therefore only practical in terms of concentration and stability to measure total Hcy.

Table 3.3.1. Total Plasma Homocysteine Constituents

Reduced Free Homocysteine	1-2%	
Oxidised Homocystine (disluphide)	5-10%	
Protein–bound Homocysteine	80-90%	
Cysteine-Homocysteine	5-10%	

3.4. Hyperhomocysteinemia in humans

Normal total fasting homocysteine is found in concentrations between 2 – 12 μmol/l in human plasma under physiological conditions. Hyperhomocysteinemia can be divided into 3 types; mild or moderate, intermediate and severe.

Table3.4.1. Hyperhomocysteinemia

Hcy	Plasma Hcy concentrations (μmol/l)	Most common causes
Mild or moderate	15 - 30	Old age, mild folate or vitamin B12 deficiency, MTHFR mutation, renal insufficiency
Intermediate	30 - 100	Renal failure, severe vitamin deficiency, MTHFR mutations
Severe	> 100	CBS mutation

3.5. Post methionine loading homocysteine

There are some instances when plasma total Hcy may be normal despite there being disturbances in Hcy trans-sulphuration. In such cases a post-methionine loading tests is needed to detect the abnormalities.

Blood for plasma total Hcy is collected in the same way as conventionally but after 4 hours of an oral dose of methionine (100 mg/kg body weight). Under normal circumstances Hcy shows a peak value of 27 μmol/l 4 – 8 hours after the oral methionine dose. Concentrations greater than 38 μmol/l indicate a disturbance in the trans-sulphuration pathway as a result of vitamin B6 deficiency or heterozygote CBS mutation. Increased levels of Hcy after a methionine load may also indicate folate deficiency due to decreased SAM production.

3.6. Factors that determine homocysteine concentrations in humans

Factors that determine homocysteine levels in humans can be divided into 2 categories. They are genetic and acquired.

Table 1.6.2. Determinants of homocysteine levels in humans

Genetic factors	Effect	Reference (s)
Homo/hetero-zygosity for CBS mutation	↑↑	KOZICH et al., 1995
MTHFR defect (activity<20%)	↑↑	GOYETTE et al., 1995
Thermolabile MTHFR (activity<50%)	↕	FROST et al., 1995
Methyl cobalamin defective synthesis (Cbl C, D)	↑↑	BIANCHERI et al., 2001
Combined methyl- and adenosylCbl defects (Cbl E and G)	↑↑	WATKINS et al., 1988
Clinical conditions		
Renal insufficiency	↑↑	HENNING et al., 1999
Folate deficiency	↑	CHASAN-TABER et al., 1996
Cobalamin deficiency	↑→	UBBINK et al., 1993
Vitamin B6 deficiency	↑→	UBBINK et al., 1993
Thyroid function disturbances	↑	MORRIS et al., 2001
Physiologic factors		
Old age	↑→	HERRMANN et al., 1999
Male sex	↑	SILBERBERG et al., 1997
Post menopausal women	↑	HAK et al., 2000
Race	↔	UBBINK et al., 1995
Smoking	↑	BERGMARK et al., 1997
Coffee consumption	↑	NYGARD et al., 1997
Alcoholism	↑	SCHNEEDE et al., 2000
Physical activity	↓	DE BREE A et al., 2001
Vegetarian diet	↑	HERRMANN et al., 2001b
Medications Folate antagonists, Nitrrous oxide, Anti-epileptic drugs.	↑	DESOUZA et al., 2002; VILASECA et al., 2000

3.7. Significance of Hyperhomocysteinemia

Hyperhomocysteinemia was postulated as being involved in atherosclerosis (MCCULLY et al., 1975). Epidemiological studies have suggested the involvement of homocysteine in occlusive vascular disease, stroke and thrombosis (CARMEL et al., 2001; HERRMANN, 2001). Elevated serum levels of homocysteine have been shown to have some causative relationship with a number of neurological disorders including Alzheimer's disease (BUTTERFIELD DA et al., 2001) and other neuropsychiatric disorders (LOSCALZO J, 2002). A recent study has suggested an association between hyperhomocysteinemia and osteoporosis (RAISZ LG, 2004; McLEAN DR et al., 2004)

The trans-sulphuration pathway is absent in the human cardiovascular system. This important fact implies that the endothelial system is constantly under conditions of hyperhomocysteinemia (CHEN et al., 1999). Deficiencies of vitamin B12 (cobalamin) and folate will disturb the re-methylation of homocysteine and have critical consequences for the cardiac vascular endothelium. Homocysteine synthesis may rapidly exceed cell export resulting in cell injury or cell death.

In-vitro studies have suggested that hyperhomocysteinemia may enhance the recruitment of leukocytes at the site of damaged vascular endothelium (PODDAR et al., 2001). In-vitro studies also suggest that homocysteine may cause smooth muscle cells to proliferate and damage the vascular matrix (TSAI et al., 1994).

It is suggested that hyperhomocysteinemia and smoking carry the same degree of risk of coronary artery disease (BOUSHEY CJ et al., 1995). It has been assessed that the relative risk from Homocysteinemia is 1.5 for a 5.0 μmol/l increase (DURAND P et al., 2001). In a recent study, it has been suggested that patients with other risk factors such as renal disease, have a 3 % increase in risk of death for every 1 μmol/l increase in plasma

homocysteine (BUCCIANTI g et al., 2004). Studies have suggested lowering plasma homocysteine levels reduces the incidence of vascular disease in a dose-related manner (WILCKEN db et al., 1997), The causal association of hyperhomocysteinemia and vascular disease is essentially proven (WALD DS et al., 2002). However recent studies have suggested caution when inferring that homocysteine be treated in the same way as other independent risk factors even going further to suggest that homocysteine acts differently to other risk factors (RETERSTOL L et al., 2003). Another recent study concluded that hyperhomocysteinemia is a predictor of congestive heart failure (RAMACHANDRAN SV et al., 2003).

3.8. Influence of age and sex on homocysteine levels in humans

Homocysteine increases with age. This phenomenon is partly explained by the gradual decline in renal function with age. Plasma homocysteine increases linearly with age . This increase is very gradual up to 60 years. From childhood to old age homocysteine levels approximately double (REFSUM H et al., 2004). After 60 years of age the increase is much faster with a 1 μmol/l increase for every 10 years of life (de BREE A et al., 2002).

There is also a gender difference with men having higher levels than women approximately 2 μmol/l. However the gender difference becomes far less with increasing age such that in elderly men and women the proportion having levels of Hcy above a given upper reference range is similar (JACQUES PF et al, 1999)

4. BIOCHEMISTRY OF FOLATE AND ITS METABOLISM

4.1 Chemical Structure of Folic acid

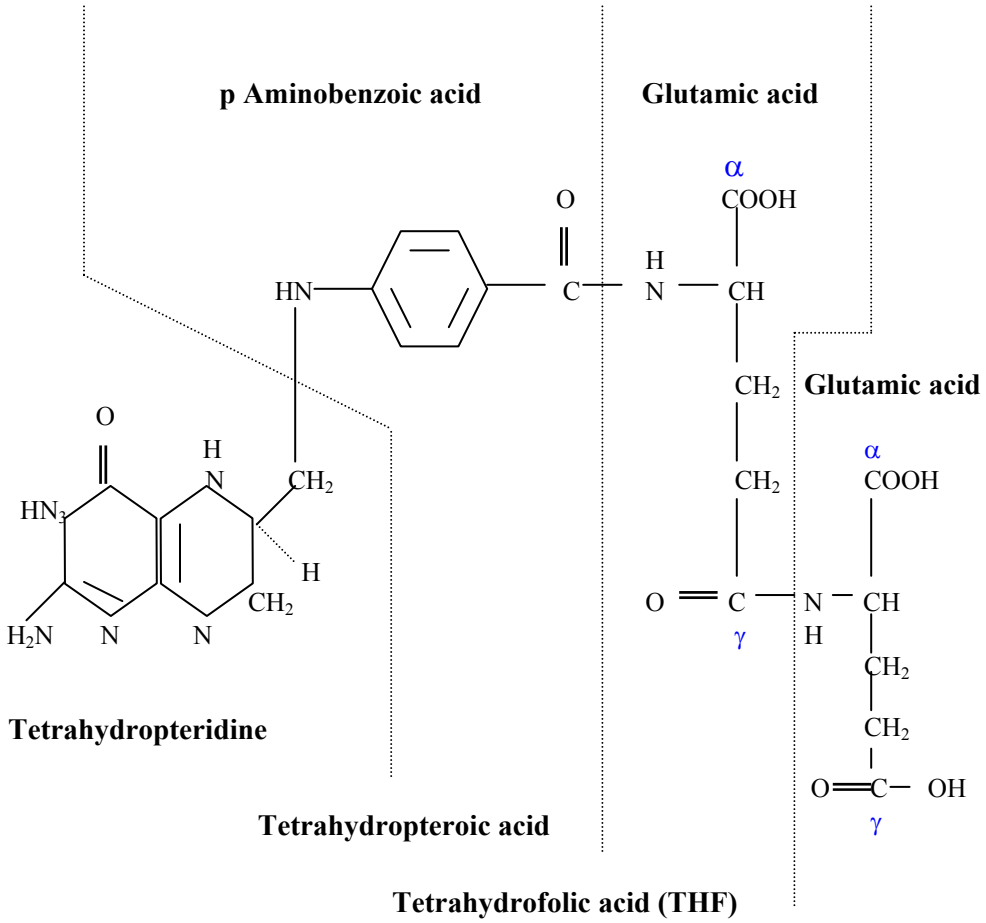


Figure4.1.1. Chemical Structure of folic acid

4.2. Reference range for plasma folate

The reference range for plasma folate is not clearly determined. Cut off points that indicate or separate sub-normal from normal have variously been attributed. The cut off point of 5nmol/l has been used as a classification for those at risk of folate deficiency. Also a borderline level of between 5 and 7 nmol/l has been used in a study on an elderly population (CLARKE RJ et al., 2003).

4.3. Biochemical structure of Folate (pteroylglutamic acid)

Tetrahydrofolate consists of a 2 amino-4hydroxy pteridine ring system linked from the C6 position to p-aminobenzoic acid **(Table 4.1.1).** In the polyglutamate forms from 2 to 11 glutamate residues may be attached.

4.4 Biochemistry of folate

As indicated in the metabolism of Hcy folate is intimately involved in conversion of Hcy to methionine. If there is a reduction in the recycling of Methyl-THF in the pool of active folates when there is inhibition of the enzyme MS. THF is required for the formation of 5,10-methylene-THF and 10-formyl-THF used in DNA base synthesis as two of the carbon atoms in purine bases are derived from folates (BOLANDER-GOUAILLE et al., 2003). Low MS activity leads to methyl-THF accumulation. Methionine synthase utilizes metyl-B12 (metylcobalamin) as a cofactor. Therefore the activity of this enzyme will be inhibited in vitamin B12 deficient subjects (HERBERT V et al., 1962) wherever the cells are unable to convert 5-methyl THF into its active form THF. The accumulation of folate in the extra-cellular milieu (plasma) is usually associated with sub-cellular folate deficiency. The folate trap hypothesis postulates that MS is inactive and that MTHFR is essentially irreversible (GREGORY et al., 2000). Therefore, vitamin B12 may cause a secondary folate deficiency. As with primary folate deficiency there is usually an associated megaloblastic anaemia. In subjects with vitamin B12 deficiency 60 % have low red blood cell folate and 20 % have low serum folate (CHANARIN I, 1979).

Reduced activity of methylene-tetrahyrofolate reductase (MTHFR) will result in the lower intra-cellular levels of methyl-THF (MTHF). As a consequence 5,10-methylene-THF

accumulates along with 10-formyl-THF. With the accumulation of these active folates DNA and RNA synthesis may be unaffected and megaloblastic anaemia may not result.

4.5. Natural sources of folate

Folate is present in a large number of foods of plant and animal origin. These include beans, nuts, meat, dairy products, fruits, grains and cereals. Yeast is the single richest source of dietaryfolate. It has been shown that folate in western diets varied from 69 – 601 µg per day. In food it occurs almost exclusively as polyglutamyl derivatives of tetrahydrofolic acid of which 5-methyl-tetrahydrofolate is the most important (LUCOCK, 2000). It should be noted that folic acid is the synthetic form of folate usually available as supplement.

Table 4.5.1. Recommended dietary allowance (RDA)[a] for folate (INSTITUTE OF MEDICINE, 1998)

Subgroup	DFE[b] (µg/day)
Infants	<150
1 – 3 years of age	150
4 – 8 years of age	200
9 – 13 years of age	300
14 – 18 years of age	400
Adults	400[c]
Pregnant women	600
Lactating women	500

[a] The recommended dietary allowance (RDA) is expressed in dietary folate equivalents (DFE). [b] 1 µg DFE = 1 µg of food folate = 0.6µg of synthetic folic acid fortifying food = 0.5 µg of synthetic folic acid supplement. [c] For women of reproductive age the RDA is expanded with recommended additional intake of 400 µg of folic acid supplement or folic acid fortified food

4.6. Folate Absorption

Absorption of folate from the gut is preceded by the hydrolysis of the polyglutamate to monoglutamate. This process is mediated by the enzyme pteroglutamate hydrolase located in the cells of the brush border. When the monoglutamate is absorbed it is rapidly transformed in the polyglutamate. As the most abundant and active form bioavailability of folate effectively decreases as the polyglutamate form increases (LOCOCK 2000). It is also the case that some foods contain conjugase inhibitors which prevent the formation of the polyglutamate. General poor nutritional status accompanied by vitamin deficiencies results in impaired utilisation of folate. Intestinal acidity is also an important factor in the bioavailability of folate (THIEN et al., 1977).

4.7. Folate Deficiency

Food folate is very unstable through the processes of cooking, storage and preservation. Therefore the intake of folate cannot be exactly estimated from the amount of food ingested. Recent studies have suggested an increase in recommended daily folate intake to between 400 and 600 μg daily folate equivalents (DEF) for adults **(Table 4.5.1.)**

There is clear evidence that folate deficiency leads to increased levels of homocysteine in humans given that studies have shown, particularly in the elderly, that folate supplementation can reduce elevated homocysteine levels (van OORT FVA et al., 2003).

In the USA and Canada in the 1990s the governments decided that all enriched grain products should be fortified with folic acid. This was mainly to reduce the risk of neural tube defects. This reduced the numbers of individuals with below average daily intake from 48.6 % to 7.0 %. It was also demonstrated that folate fortification lowered homocysteine as much as with an increase of consumption of folate-rich food.

5 BIOCHEMISTRY OF COBALAMIN

5.1. Chemical Structure of Cobalamin

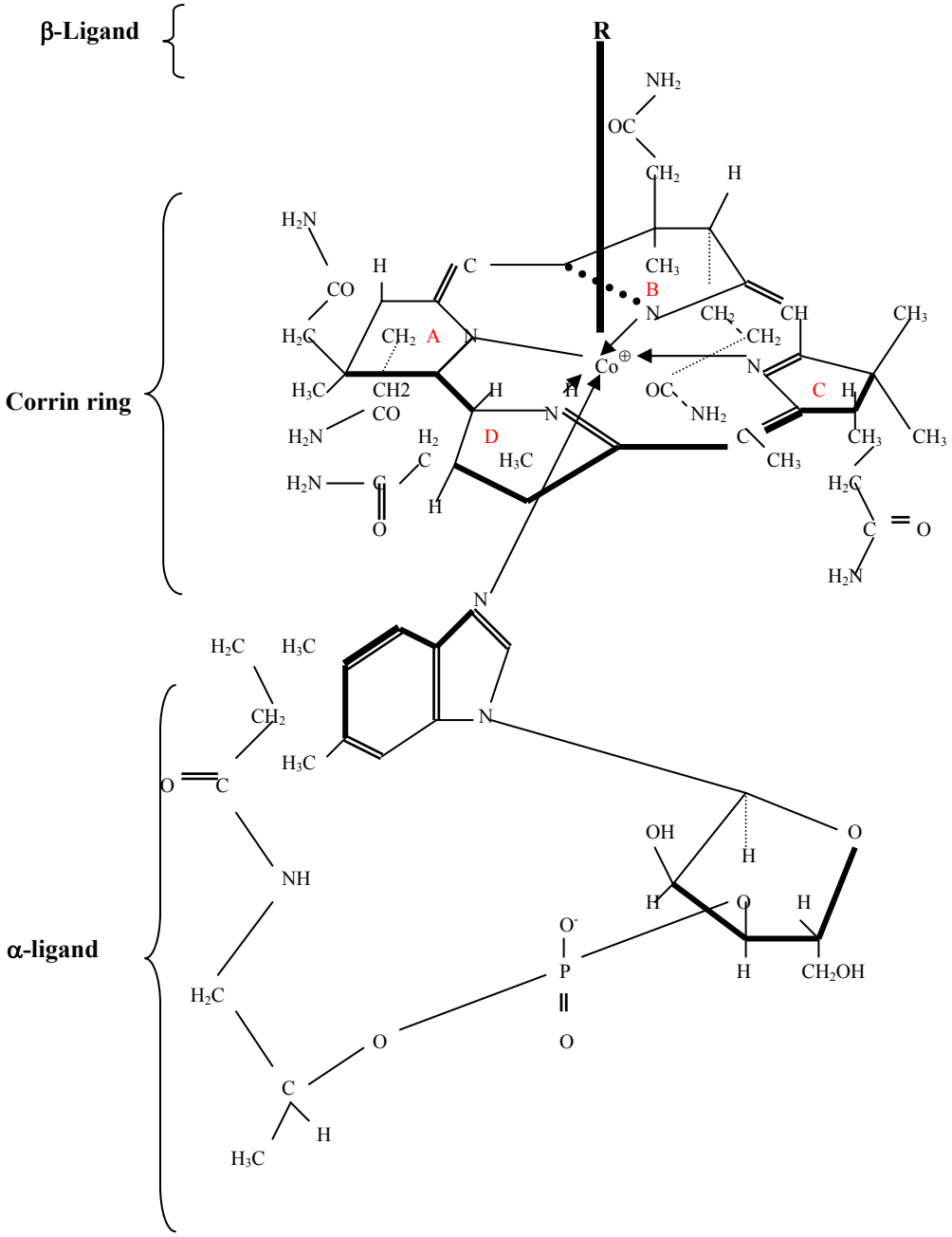


Figure 5.1.1. The Chemical structure of cobalamin. NB Changes in the β ligand determine the types of cobalamin.

Cobalamin is a tetrapyrole ring with a cobalt atom at its core. It is similar in configuration to the pyrol ring of haem which has iron at its core. There are a number of cobalamin derivatives defined by the different ligands that attach. Also cobalamin can exist with a mono, di and trivalent cobalt atom at the core (cob(I)alamin, cob(II)alamin and cob(III)alamin, respectively).

R-	Cobalamins
CH3	methylcobalamin
5′-deoxyadenosyl	Adenosylcobalamin
OH	Hydroxycobalamin
CN	Cyanocobalamin (vitamin B12)

Cobalamin is synthesized by bacteria in animals that are then eaten by humans. Plants, except for certain algae, appear not to synthesize cobalamin and it is therefore generally accepted that meat is the main source of cobalamin. A study concluded that there is a great deal of variation in dietary cobalamin but that it was almost exclusively sourced from meat (TUCKER et al 2000).

Cobalamin is relatively stable and relatively unaffected by food processing and preparation in particular cooking (CHANARIN 1979). As indicated previously this is not the case with dietary folate. It is estimated that, if the intrinsic factor is intact, 60 – 70% of dietary cobalamin is absorbed by humans.

5.2. Cobalamin Absorption and Transport

After ingestion cobalamin is released in the stomach by pepsin (del CORRAL et al 1990). Cobalamin is bound by haptocorrin for its transport to the duodenum where it is released by the action of pancreatic trypsin. Cobalamin is associated with intrinsic factor until it is released and absorbed from the ileum in association with transcobalamin II. Within 90 minutes of the absorption of cobalamin into the portal system it is distributed throughout the body (HALL CA, 1975). The liver and the kidneys are responsible for the largest accumulation of cobalamin.

Whilst intrinsic factor and transcobalamin II are mainly responsible for cobalamin transportation and absorption through the gut, transcobalamin I is almost exclusively responsible for transportation in the systemic system (CARMEL 1985).

Cobalamin acts as a cofactor for methionine synthase in the methylation of folate. It also becomes available as 5′-deoxyadenosylcobalamin in mitochondria for methylmalonyl CoA mutase. This mutase mediates the isomerisation reaction in proprionate metabolism whereby methylmalonyl CoA is converted to succinyl CoA. These are the only known metabolic reactions of cobalamin (CARMEL et al 2001).

5.3. Cobalamin Deficiency

There are 3 main causes of cobalamin deficiency. They are inadequate dietary intake, malabsorption and metabolic or transport disorders.

Deficiency due to dietary intake is unusual as cobalamin turnover in relation to its body store is small. Short periods when there is absence of dietary cobalamin will have little or no effect. It is only when the diet is almost devoid of cobalamin for a number of years that a clinically apparent deficiency will result. However sub-clinical deficiency may exist in some vegetarians and children display signs sooner and more often than adults

(ORTEGA RM, 1993). The average western diet of 5 – 15 μg/day is regarded as sufficient for the recommended dietary allowance of 2 μg/day (SNOW CF, 1999)

5.4. Biological Significance of Cobalamin Deficiency

Cobalamin deficiency and its pathology is most evident in the haematological disturbances which result. However the lack of availability of cobalamin at the cellular level is evident and has consequences for nerve tissue and myelination. This process is further complicated by the neurological disturbances that result from the inactivation of methionine synthase by nitric oxide (NO). Cobalamin deficiency has often been mistaken for folate deficiency (HERBERT, 1985). This complex inter-relationship between cobalamin and folate has been thoroughly researched (TISMAN et al., 1973).

Cobalamin deficiency is said to develop through 4 stages. Stage I and II develop when cobalamin intake is in negative balance (daily intake is lower than the daily loss). There is no clinical evidence at these stages. If a negative balance continues cellular deficiency may develop. At this stage serum and tissue deficiency is manifest and metabolic markers such as homocysteine and methylmalonic acid become elevated in serum (HERBERT, 1985). Neurological symptoms also develop as a result of vitamin B12 deficiency. Continued vitamin B12 deficiency leads to a clearer picture of disease as seen in Stage IV.

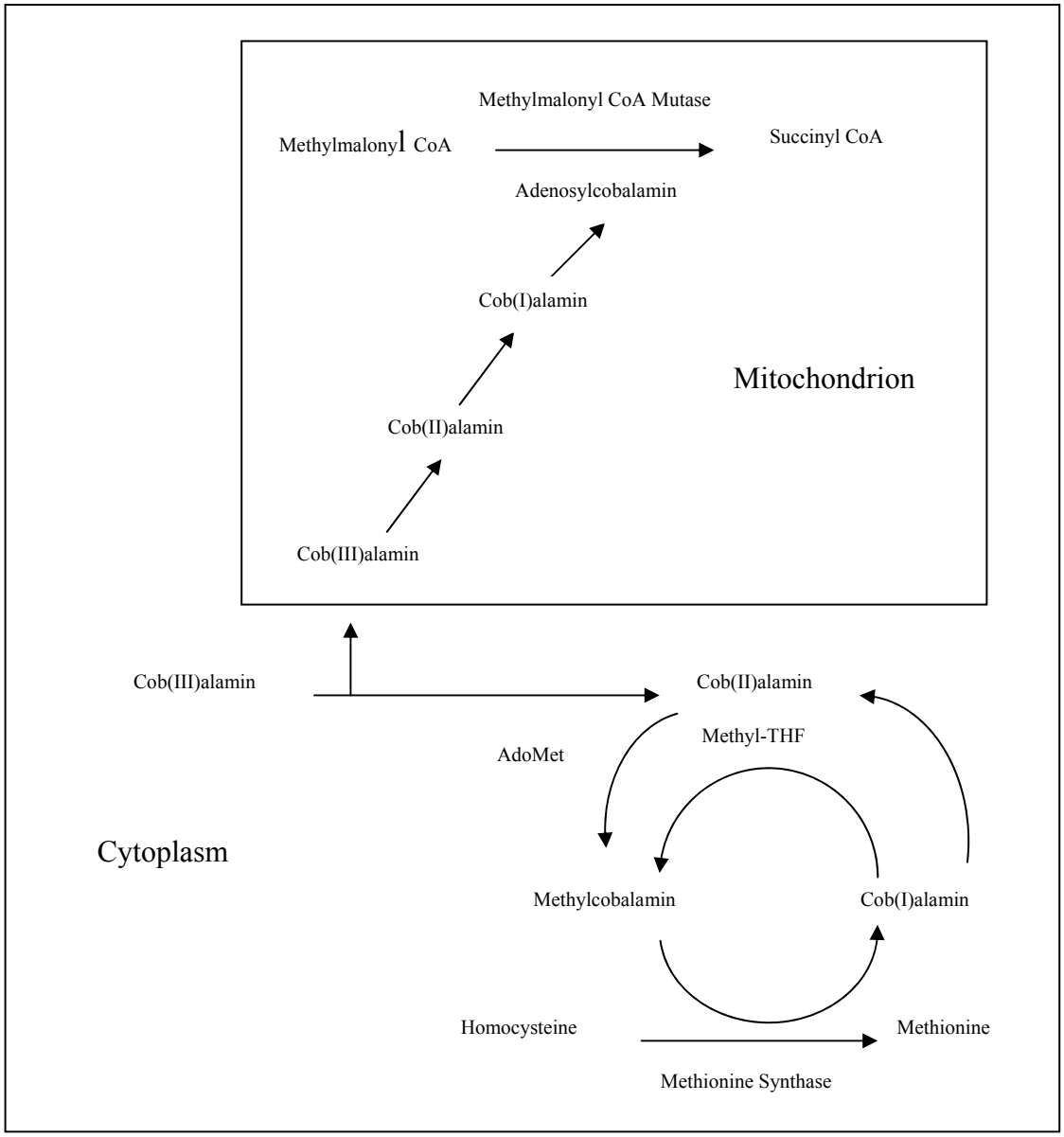
Cobalamin deficiency is far more prevalent in the elderly due to the increased incidence of atrophic gastritis and the concomitant malabsorption (CARMEL 1997). It is reported that there is a 5-15% prevalence of cobalamin deficiency in the elderly (PENNYPACKER LC et al., 1992; JOOSTEN E et al., 1993). Vegetarians who have eliminated all animal food from their diet also develop cobalamin deficiency. This process takes many years (HERBERT 1987 & 1994). Children breast fed by strict vegetarian (vegan and lacto-vegan)

mothers may also develop cobalamin deficiency or may be born with low levels of the vitamin. Finally those who have had a partial or total gastrectomy may also develop a deficiency (SUMNER AE et al., 1996). Deficiency results from mal-absorption due to the decrease or absence of the secretion of gastric acid vital in the absorption of vitamin B12.

Treatment of patients with suspected cobalamin deficiency is controversial (CARMEL, 1996). It has been seen that subtle deficiency may not worsen. Studies are underway that are testing the effectiveness of vitamin supplements in food for the general population or those groups at particular risk such as vegetarians, the elderly, pregnant women and patients with cerebra-vascular disease. Reductions in Hcy and MMA levels after oral vitamin B12 supplementation would seem to confirm that the absorption process is intact. However, researchers have found that vitamin B12 dietary supplementation has limited health-related effects (HVAS A-M et al., 2003). The research found only a limited improvement in general health after four weeks of injected supplementation.

5.5. Metabolism of cobalamin

Cob(I)alamin, Cob(II)alamin and Cob(III)alamin are cobalamin with Cobalt in its mono, di and trivalent forms.



6 HOMOCYSTEINE AND METHYL MALONIC ACID

6.1. Methylmalonic acidemia

Cobalamin is a cofactor along the enzyme methylmalonyl-CoA mutase in the conversion of methylmalonyl-CoA to succinyl-CoA. When cobalamin is deficient methylmalonyl-CoA is converted to methylmalonic acid rather than succinyl-CoA (STABLER SP, 1995). Cobalamin deficiency therefore leads to increased methylmalonic acid (MMA) and methylmalonic acidemia and is the main cause in MMA increases in the plasma in humans (JOOSTEN E et al., 1993).

There are few known causes of increased plasma MMA other than cobalamin deficiency. These include renal failure (MOELBY L, et al., 1992), congenital methylmalonic aciduria (FENTON WA et al., 1995) and small bowel overgrowth with bacteria producing large amounts of propionic acid, the precursor of MMA (HOVERSTAD et al., 1985). However there is still some concern as to whether elevated MMA can be taken as proof of vitamin B12 deficiency (HVAS AM et al., 2001).

In humans only 2 cobalamin dependant metabolic reactions have so far been identified. One is the synthesis of methionine from homocysteine and the other is the conversion of methylmalonyl-CoA to succinyl CoA using adenosyl-cobalamin as a cofactor.

6.2. Homocysteine and methylmalonic acid as surrogate markers for cobalamin and folate deficiency

The first demonstration of the association between folate deficiency and hyperhomocysteinemia came in 1987 (KANG SS et al., 1987). Subjects with low folate were found to have very high levels of homocysteine in the blood. Many studies have since demonstrated the association between hyperhomocysteinemia and cobalamin deficiency. A

recent study has shown that high homocysteine levels in blood can be lowered by treatment with high oral doses of vitamin B12 (JOHNSON MA et al., 2003).

Controversy still surrounds the clinic value of serum cobalamin and it has been suggested that other tests need to be done in conjunction with cobalamin (LINDENBAUM J et al., 1988). It has been demonstrated that low levels of cobalamin alone may overestimate the number of patients with true deficiency and that a surrogate marker such as methylmalonic acid (MMA) is useful in differentiating those patients with true deficiency (MOELBY L et al., 1990). It has been suggested that patients with low to low-normal levels of cobalamin benefit diagnostically from the measurement of MMA (HØLLELAND G et al., 1999). Indeed it has been suggested that raised levels of plasma homocysteine and MMA could be indicators of very early cobalamin deficiency in the absence of conventional symptoms (BJORKEGREN K et al., 2003).

As stated previously homocysteine may increase when re-methylation to methionine is inhibited by lack of cofactors in the conversion of cobalamin and folate. Therefore increased homocysteine may be an early marker for the detection of cobalamin and folate deficiency (FLYN MA et al., 1997). This finding has been investigated by folate fortification trials. It is contended that increasing dietary folate will reduce homocysteine levels in the elderly (JACQUES PF et al., 1999). Further to these findings a randomized controlled trial has suggested that homocysteine can be lowered with cobalamin and dietary folate supplementation and can improve the outcome after percutaneous coronary intervention (SCHNYDER G et al., 2002).

6.3. Homocysteinemia and Vegetarianism

Folate, vitamin B12 and B6 play an important role in the metabolism of homocysteine. Cobalamin is vital in the re-methylation of homocysteine to methionine.

Folate is found in many foods of plant and animal origin. However, it is very unstable and can be lost to a large extent in the cooking process. Cobalamin is found almost exclusively in meat in the diet of humans. Plants do not appear to synthesize cobalamin.

Long term vegetarian diet causes or is associated with deficiency of vitamin B12 and B6 and could result in reduced intake of folate.

Vegetarians can be classified into 4 main groups:

1	Lactovegetarians:	Those who have no meat in their diet but consume dairy products
2	Ovovegetarians:	Those who have no meat but include eggs in their diet
3	Lactoovovegetarians:	Those vegetarians who eat eggs and dairy products
4	Vegans:	Those who do not eat meat, dairy products or eggs

As a result of poor diet or diet poor in cobalamin and folate, there may be significant interference in the metabolism of methionine. In these circumstances it could be assumed that increased levels of homocysteine in the blood may result. There is evidence that homocysteine is generally increased in blood in people who are strict vegetarians (HERRMANN 2001, HERRMAN 2003, BISSOLI et al., 2002).

Research has shown that hyperhomocysteinemia is an independent risk factor for coronary heart disease (REFSUM H et al., 1998). There is evidence to suggest that reducing the level of homocysteine in the blood promotes increased endothelial function in humans and slows the progress of atherosclerosis (STANGER O et al., 2002). Studies have shown that

cobalamin deficiency in vegetarians is associated with raised plasma methylmalonic acid (MILLER DR et al., 1991), in infants breast-fed by vegetarian mothers (HIGGINBOTTOM MC et al., 1978) and in children fed on a macrobiotic diet (SCHNEEDE J et al., 1994). These and other studies demonstrate that cobalamin is generally lower and in many cases clinically deficient in strict vegetarians. Also Homocysteinemia and raised levels of plasma methylmalonic acid are associated with low cobalamin. It is also the case that in the UK for example Asian Indians who had been resident for many years showed increased incidence of coronary heart disease compared with their British white counterparts (CHAMBERS JC, 2000). It is also known that a significant number of the Asian populations in the UK are vegetarians.

There has been some doubt raised about low total serum vitamin B12 as a marker for cobalamin deficiency. However, studies have shown that overt megaloblastic anaemia is evident in a community of strict vegetarians (MATTHEWS JH et al., 1984, CHANARIN I et al., 1985). The link between megaloblastic anaemia and cobalamin deficiency has been clearly understood for a long time. It is evident that vegetarian may develop cobalamin deficiency and that the stricter the vegetarian diet the more likely the deficiency is to result. It is clear that many of these subjects will develop overt deficiency as demonstrated by low vitamin B12 levels, hyperhomocysteinemia and methylmalonic acidaemia.. It is evident that hyperhomocysteinemia results from a long-term strict vegetarian diet. Vegetarian diets are rich in anti-oxidants that may be protective against coronary heart disease. Anti-oxidants may prevent the oxidation of homocysteine and thus lower the risk (LOSCALZO J, 1996). It is also surmised that vegetarians who have selected to adopt their dietary regime or follow it for ethical or religious reasons are less likely to smoke and less likely to pursue other high coronary risk habits.

Other sequel resulting from cobalamin deficiency, particularly psychomotor dysfunction and learning disabilities can extend through infancy and into adolescence in children breast-fed by strict, long-term vegetarian mothers. 20 % of these children continue to have low levels of cobalamin well into their teenage years despite reverting to an omnivorous diet after their 6th birthday (LOUWMAN MW et al., 2000). Low cobalamin status persists into adolescence in infants fed a macrobiotic diet (DAGNELIE PC et al., 1994).

6.4 Racial and ethnic differences in homocysteine levels

Homocysteine levels in the blood of white American males are significantly higher than in black American males. This has been attributed to MTHFR polymorphism (ESTRADA DA et al., 2001). Another study has supported the contention that racial differences in homocysteine levels is due to genetic differences that effect methionine metabolism (SAW S-M et al., 2001).

Care must be taken in simple transcription of ethnic differences on cobalamin and folate deficiencies particularly in the heterogeneous population of India (ANTHONY CA, 2001). It has been postulated that young black South Africans may metabolize homocysteine more efficiently than their white South African counterparts. This may explain the lower incidence of coronary heart disease in the black population despite the increase in other risk factors such as smoking, hypertension and diabetes (URBINK JB et al., 1995).

A study conducted in Canada of South Asian (Indian) immigrants and East Asian (Chinese and Japanese) immigrants demonstrated that there were differences in the plasma homocysteine levels between the two groups in subjects where there was evidence of coronary heart disease. The study also suggested that the contribution made by

hyperhomocysteine to the development of coronary heart disease appears less in the East Asian group than in the South Asian group in Canada (SENARATNE MPJ et al., 2001).

When considering race or ethnicity, great care must be taken not to attribute differences in cobalamin status and hyperhomocysteinemia strictly to race or ethnicity (CARMEL R, 1999). A study conducted in the UK demonstrated distinct difference in homocysteine levels between white, black and South Asians resident in the UK. However the study suggests that within the South Asian population the Hindus had higher homocysteine levels than the Muslims. This was attributed to the larger numbers of vegetarians amongst the Hindus (CAPPUCCIO FP et al., 2002). Another study compared white UK residents and Indian Asian UK residents in relation to coronary heart disease risk and hyperhomocysteinemia. The study concluded that novel genetic factors and/or environmental factors influence homocysteine metabolism in Indian Asians residing in the UK (CHAMBERS JC et al., 2000).

7. SUBJECTS, SAMPLING AND ANALYTICAL METHODS

7.1. Subjects

The subjects for the study were elderly Omanis attending clinics in a number of extended health centers in a number of locations for a variety of conditions including diabetes, hypertension and other non-specified complaints. The 3 extended health centers where sampling took place were in Muscat, Sohar and Nizwa (See Map). The age range of the Omanis was from 40 years to late 70s. 225 Omani patients were recruited for the study. All the Omani subjects were omnivores having a diet of vegetables, grains and meat. 137 expatriate Indians were also recruited for the study. They were mainly male subjects and in the age range of 35 – 55 years. A substantial minority of the expatriate subjects were vegetarians (59). Blood samples were collected from each subject according to the protocols later described.

7.2 Sampling

Informed consent was obtained from all subjects and ethical approval was obtained from the Ministry of Health. A 12 hour fasting blood sample was obtained from all subjects and collected into a 5 ml plain vacuum blood collection tube (Becton Dickinson) and a 4 ml EDTA vacuum blood collection tube (Becton Dickinson). The blood samples were immediately placed on ice and centrifuged within 1 hour. The samples stored at $4^{o}C$ until the samples were centrifuged at 3,000 rpm at room temperature within 1 hour of the samples being obtained. The plasma and serum samples were placed in pilot tubes and stored at $-70^{o}C$. The samples were then shipped to Germany packed in dry ice and stored at

– 7^{o}C until analysis could be performed. All samples were analyzed within 6 months of collection.

7.3. Analytical Methods

7.3.1. Homocysteine, Cystathionine and Methylmalonic Acid Assays

The assays of homocysteine, cystathione and methylmalonic acid were performed by Gas Chromatography-Mass Spectrometry (GCMS) using a method described by Allen and Stabler (ALLEN et al. 1993; STABLER et al., 1993) and slightly modified in our laboratory. Homocysteine and cystathionine were assayed simultaneously in the same test vial while methylmalonic acid was assayed using the same apparatus but in a separately treated sample. Deuterium labeled homocysteine, cystathionine and methylmalonic acid were simultaneously used with the samples as internal standards.

The assay depends on obtaining volatile derivatives of the compounds to be assayed. These compounds are then separated by gas chromatography. The chromatographic process uses the phenomenon of selective absorbance and absorbance onto a stationary phase between the different derivatives. This delay the relative movement of the component parts of the assay mixture such that the various derivatives become separated. They will then arrive at the detection device (mass spectrometer) at different times.

The derivatives are then quantified by mass spectrometry. The mass spectrometer works by ionizing the separated derivatives in a beam of electrons. The derivative may then split into a number of different species or parts. The spectrum of parts will be “unique” to the separated derivative and can therefore be identified in relation to standard assays. Each component part or species is separated in a mass filter and counted by a detector. The signal generated is proportional to the total number of ions for any given mass.

The labeled internal standards behave in a similar way to the native compounds differing only in their mass/charge as a result of the hydrogen isotope. As the labeled compounds are of known concentration the concentration of compounds in the sample can be calculated.

7.3.2. Sample Preparation

Sample volume (serum, plasma or urine)	400 µl
Preparation time (washing, elution, derivitization)	approximately 5 hours
Measurement time by GCMS (for a batch of 20 samples)	7 hours

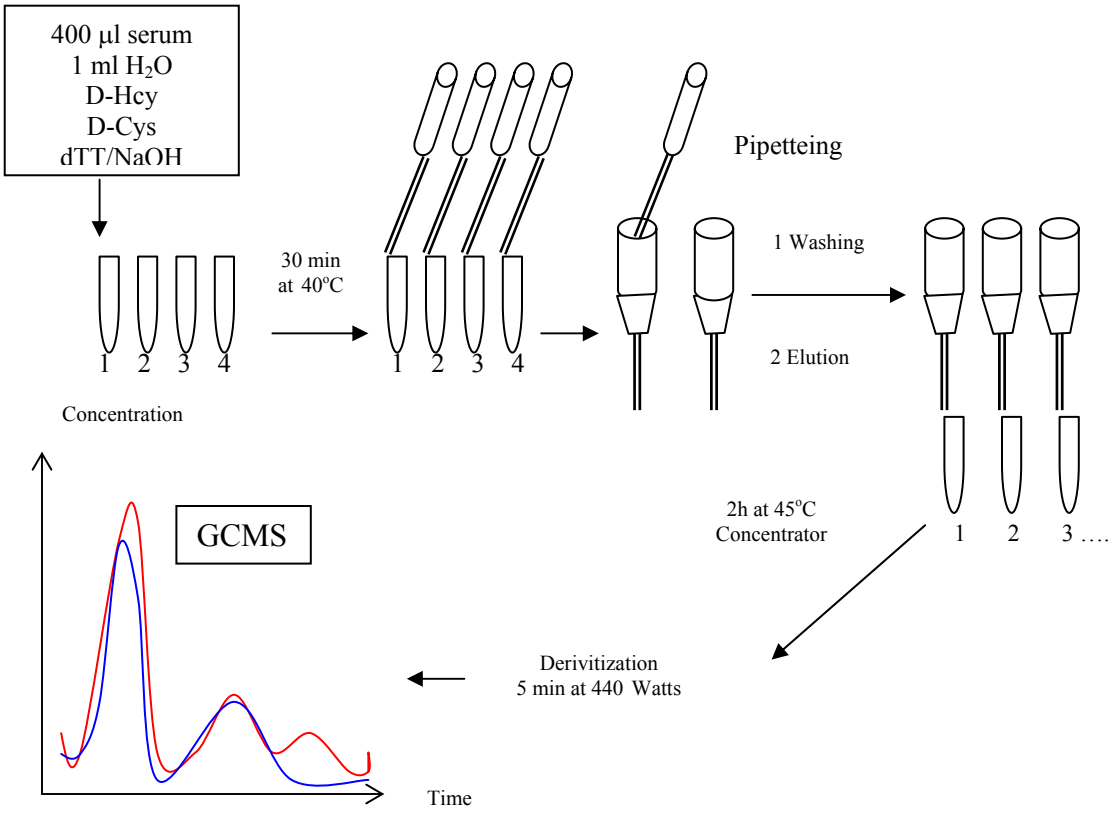


Figure7.3.3. Schematic of sample preparation for gas chromatographic assays

7.3.3. Materials

1 Gas chromatography column HP 5MS (Cross-linked 5% PH ME Siloxane). Column length 30 m, film thickness 0.25 um, phase ratio 250, column internal diameter 0.25 mm. (Cat. No. 19091S-433, Agilant Technologies®).

2 Mass Spectrometry with electron ionizer system

3 Poly-Prep Chromatographic Columns Cat No. 731-1550 (Bio-RAD®)

4 Anionic Resin AG MP-1M Resin (BioRAD®)

5 Derivitizing agent: N-methyl-butyldimethylsilyl-Tri-fluoroacetamide (MBDSTFA) (Machery and Nagel®)

6 DL-homocysteine (3,3,3#,3#,4,4,4#,4#,D8) as an internal standard (working concentration of 110.544 mg/l equivalent to 400 μmol/l (MW 276.36)

7 DL-(2-amino-2c-carboxyethyl) homocysteine (DL-cystathionine) as an internal standard (working concentration of 1.78 mg/l equivalent to 7.86 μmol/l (MW 226.3)) DL-methylmalonic acid as an internal standard (working concentration 4 mg/l equivalent to 33.03 nmol/l (MW 121.11))

8 1,4-Dithiotriol ($C_4H_{10}O_2S_2$, MW 154.2; DTT) from ROTH®: Cat No. 6908.1, used as a reducing agent with working concentration of 10 mg/ml (in 1N NaOH)

9 Acetonitril, methanol, acetic acid and chromatography water were from MERCK®

7.3.4. Procedure for homocysteine (Hcy) and cystathionine (Cys)

An anionic ion exchange resin (Bio-RAD®) was prewashed with 1N HCl and methanol and left to dry at 60°C for 4 hours.

5 ml glass test tubes were labeled from 1 to 20. To each tube was added 1ml HPLC grade water, 400 µl of test serum, 20 µl of DL-Homocysteine, 50 µl of DL-Cystathionine and 30 µl dTT (10 mg/ml in 1N NaOH).

All tubes were mixed and incubated for 35 minutes at 45°C for the reduction of Hcy.

The mixtures were then transferred to the anionic resin columns, pre-equilibrated with 1 ml of methanol, then 3 ml of water then washed 3 times with 3 ml of water and once with 3 ml of methanol.

The columns were then eluted with 1.1 ml of 0.4 N acetic acid/methanol solution. The elutions were then dried in an Eppindorf ® concentrator 5301 for 2 – 3 hours.

The samples were derivatized by adding 30 µl of N-methyl-butyldimethylsilyl-Tri-fluoroacetamide and acetonitril (1:2 V/V).

These samples were then mixed and incubated in a microwave oven at 440 watts for 5 minutes.

The samples were then loaded onto the GCMS for the final assay.

Reference Ranges (serum/plasma)

Homocysteine: 2 – 12 µmol/l

Cystathionine: 65 – 301 nmol/l

7.3.5 Procedure for methymalonic acid assay (MMA)

This test was done in a similar way to homocysteine and cystathionine but with the following modifications:

1. An anionic ion exchange resin (Bio-RAD®) was pre-washed with 1N HCl and methanol and left to dry at 60°C for 4 hours.
2. 5 ml glass test tubes were labeled from 1 to 20. To each tube was added 1ml HPLC grade water, 400 µl of sample and 50 µl of DL-MMA as an internal standard.
3. After transferring samples to the chromatography/ion exchange columns the columns were washed once with water, three times with 0.01 N acetic acid/methanol solutions.
4. The columns were then eluted with 1.1 ml of a solution of 90 ml 4 N acetic acid/methanol and 10 ml 1N HCl.
5. Subsequently the same steps were followed as for the assays of homocysteine and cystathionine (drying, derivitization and GCMS).

Reference Range (serum/plasma)

Methylmalonic acid: 73 – 271 nmol/l

7.4. Procedures for the Quality Control of the Assays

Pooled normal serum was aliquoted and one aliquot was used with each batch of assays. 24 independent measurements for each analyte were performed during the course of the study on 24 separate days. The following results were derived from all aliquots assayed during the analysis process:

Table 7.7.1. Intra-day Coefficients of Variation for each assay

Assay	Intra-day Coefficient of Variation (CV%)
Homocysteine	5.3
Cystathionine	4.8
Methylmalonic acid	3.2

Table 7.7.2. GCMS characteristics for Hcy, cystathionine and MMA assays

Features	Homocysteine	Cystathionine	Methylmalonic acid
Column head pressure	53.3 Psi	53.3 Psi	53.3 Psi
Initial/max. temperature	80/310^{o}C	80/310^{o}C	80/310^{o}C
Temperature rise rate	15^{o}C/minute	15^{o}C/minute	15^{o}C/minute
Retention time (minutes)	13.4	17.2	9.0
Major ion fragments (MZ)			
For the labeled molecule	424/396	366/625	292/334
For the main molecule	420/392/318	362/621/302	289/331/189/147
Standard concentrations	0.01568 µmol/µl	400 nmol/50µl	1.635 nmol/50µl
Quantification*	A1/A2 x 39.2 (µmol/l)	A1/A2 x 1000 (nmol/l)	A1/A2 x 4087.5 (nmol/l)

* A1 = area under the curve for the sample, A2 = area under the curve for the standard

7.5. Folate and Vitamin B12 Assays

7.5.1. Folate Assay (ADVIA Centaur®)

The assay method used was a competitive chemi-luminescence immunoassay. It works on the principle that Avidin-bound folic acid will compete with the folate in the test serum for a limited number of antigen binding sites on a folate binding protein that is labeled with Biotin. The folate in the sample will be inversely related to the chemi-luminescence detected.

Reference Range (plasma/serum): 5– 25 nmol/l

7.5.2. Vitamin B12 Assay (ADVIA Centaur®)

The assay is a chemi-luminescence immunoassay in which the Vitamin B12 in serum, after being released from its binding proteins, competes with acridinimester labeled Vitamin B12 on a limited amount of solid-phase bound Vitamin B12. The amount of bound labeled Vitamin B12 is proportional to the B12 in the sample serum being tested.

Reference Range (serum/plasma): > 156 pmol/l

7.6 Haemoglobin, Creatinine, Total Cholesterol and Triglyceride Assays

7.6.1 Haemoglobin Assay

Haemoglobin was measured using a modified Cyanmeth method on a Celldyn CD 3500 automated 5 population WBC differential analyzer (Abbott Laboratories ®, USA).

Reference Range: Men 14.0 – 17.0 g/dl, Women 12.0 – 15.0 g/dl

7.6.2 Creatinine, Total Cholesterol and Triglyceride Assays

Analysis of creatinine, total cholesterol and triglyceride were performed using Hitachi 911E and 912E automated biochemistry analyzers (Roche Diagnostics ®, Switzerland).

The creatinine method is a modified Jaffe reaction.

Creatinine + alkaline picrate solution $\longrightarrow$ creatinine-picrate complex (yellow)

Reference Range (serum/plasma): Men 60 120 µmol/l Women 40 – 90 µmol/l

Total Cholesterol Assay

The total cholesterol method is an enzymatic reaction utilizing cholesterol oxidase

$$\text{total cholesterol} \xrightarrow[\text{(Cholesterol esterase)}]{} \text{free cholesterol} \xrightarrow[\text{(Cholesterol oxidase)}]{} (H_2O_2 + \text{phenol/aminophenazone}) \text{ (red)}$$

The phenol/aminophenazone peroxide complex gives a red colour the intensity of which is directly proportional to the total cholesterol.

Reference Range: < 5.7 mmol/l

Triglycerides Assay

The triglyceride method was an enzymatic procedure

Reference Range: < 1.5 mmol/l

7.7 Statistical Analysis

Statistical analysis was performed using SPSS (version 9.0) for windows and Microsoft® Excel version 2000. All parameters were skewed and hence they were expressed as median (5^{th} /95^{th}) percentiles. Correlations between different parameters were established using Spearman-Rho test. All tests were two-tailed and were considered statistically significant when $P < 0.05$ and very significant when $P < 0.01$. When it was required to compare different parameters between different groups then the two-tailed Mann-Whitney test was used. Log- transformation was used to correct for skew in some of the data chart presentations

8 RESULTS

Study population and main characteristics

In this study 373 subjects were tested for metabolic markers of methionine metabolism as well as haemoglobin, creatinine, total cholesterol and triglycerides. All subjects were fasting for 12 hours before blood was extracted.

226 of the subjects were indigenous Omanis of which 100 were female and 126 were male. The median age was 53 years. All of the Omani subjects were omnivorous. 147 subjects were Asian Indian expatriates working in Oman of whom 130 were male and 17 female. The median age in this group was 43 years. 63 of the Asian Indians were strict vegetarians of which 57 were male and 6 female. The median age was 42 years. 84 of the Asian Indian were non-vegetarians (omnivores). Of these 73 were male and 11 female. The median age of this group was 43 years.

Table 8.1. Main characteristics of the study population

	Omanis	Asian Indians
Number	226	147
Females, n (%)	100 (44%)	17(12%)
Age*, years	53	43
Omnivores, n (%)	226(100%)	84(57%)
Vegetarians, n (%)	0(0%)	63(43%)

Data are median (5^{th}-95^{th}) percentiles

Metabolic markers and vitamin status in the Omanis

The median age of the Omani group was 53 years. The median levels of homocysteine (Hcy), methylmalonic acid (MMA) and cystathionine (Cys) were observed to be 8.2 μmol/l, 220 nmol/l and 171 nmol/l respectively, which were within normal limits. The median levels of folate and B12 were observed to be 20.4 nmol/l and 334 pmol/l respectively, which were again within normal limits. The median value of haemoglobin in the Omani group was found to be 13.5 g/dl which was marginally lower than the normal limits, which could be due to a significant portion of females (44%) in this sample population. The median levels of creatinine, cholesterol and triglycerides were observed to be 69 μmol/l, 5.7 mmol/l and 1.5 mmol/l respectively, which were again within normal limits.

Table 8.2. Concentrations of biomarkers of the vitamins B

	Omanis
Number	225
Age (years)	53 (40, 70)
Hcy (μmol/l)	8.2 (5.0, 14.8)
Cys (nmol/l)	220 (113, 457)
MMA (nmol/l)	171 (101, 373)
Folate (nmol/l)	20.4 (10.7, 43)
Vitamin B12 (pmol/l)	334 (168, 625)
Haemoglobin (g/dl)	13.5 (10.9, 16.1)
Creatinine (μmol/l)	69 (42, 107)
Cholesterol (mmol/l)	5.7 (4.2, 7.7)
Triglyceride (mmol/l)	1.5 (0.72, 4.6)

Median (5^{th}-95^{th}) percentiles.

Metabolic markers and vitamin status in the Omanis according to sex

Within the Omani group the age range of males and females was almost identical (males median age 53 years, females median age 52 years). The median level of Hcy was significantly higher in the male group compared to the female group (9.2 vs. 7.7 μmol/l). The median level of haemoglobin was higher in the male group as compared with the female group (14.0 vs. 13.0 g/dl). Also the median creatinine level was higher in the male group as compared to the female group (77.5 vs. 54.0 μmol/l). The rest of the metabolic markers did not show any significant differences between the two sexes.

Table 8.3 - Concentrations of biomarkers in Omani subjects according to Sex

	Females	Males
Number	105	120
Age (Years)	52 (40, 67)	53 (40, 68)
Homocysteine (μmol/l)	7.7 (4.7, 12.2)	9.2 (5.7, 17.3)
Cystathionine (nmol/l)	198 (75, 356)	242 (116, 449)
Methylmalonic acid (nmol/l)	170 (96, 292)	171 (110, 621)
Folate (nmol/l)	21.9 (12.2, 40.9)	19.6 (11, 35)
Vitamin B12 (pmol/l)	359 (195, 889)	324 (163, 643)
Haemoglobin (g/dl)	13.0 (12, 16)	14.0 (11.7, 16.4)
Creatinine (μmol/l)	54 (33, 79)	77.5 (43, 104)
Cholesterol (mmol/l)	5.8 (4.1, 7.6)	5.7 (4.4, 7.5)
Triglyceride (mmol/l)	1.5 (0.8, 2.9)	1.5 (0.9, 5.5)

medians and 5^{th} and 95^{th} percentiles

Metabolic markers and vitamin status in the Omanis according to age

Of the 225 subjects in the Omani group there were 125 males and 100 were females. The median ages of both groups were 53 and 52 respectively. The subjects were divided into 4 age groups as shown in **Table 8.4 and Figure 8.1** and the metabolic markers compared. It can be seen that Hcy tends to increase as age increases and this is particularly pronounced between the ages of 60 and 80 years. Cys and MMA appear to increase in a similar way although the increase appears to occur linearly. There does not appear to be any evidence that folate levels change with age the median level of folate remaining relatively unchanged from the age of 40 through to 80 years of age. It appears that vitamin B12 tends to decrease with age and the decrease appears to be linear over the age range of 40 to 80 years, however, it is statistically insignificant. (Fig.8.2)

Table 8.4 - Median values for each age group in Omanis for metabolic markers

Age groups (years)	Hcy µmol/l	Cys nmol/l	MMA nmol/l	Folate nmol/l	B12 pmol/l
40 – 49 (n = 78)	7.8	182	150	20.1	364
50 – 59 (n = 62)	8.0	212	171	21.0	346
60 – 69 (n = 46)	8.6	256	190	20.8	331
70 – 79 (n = 32)	11.2	320	220	22.9	321

7 subjects < 40 years old excluded from the data

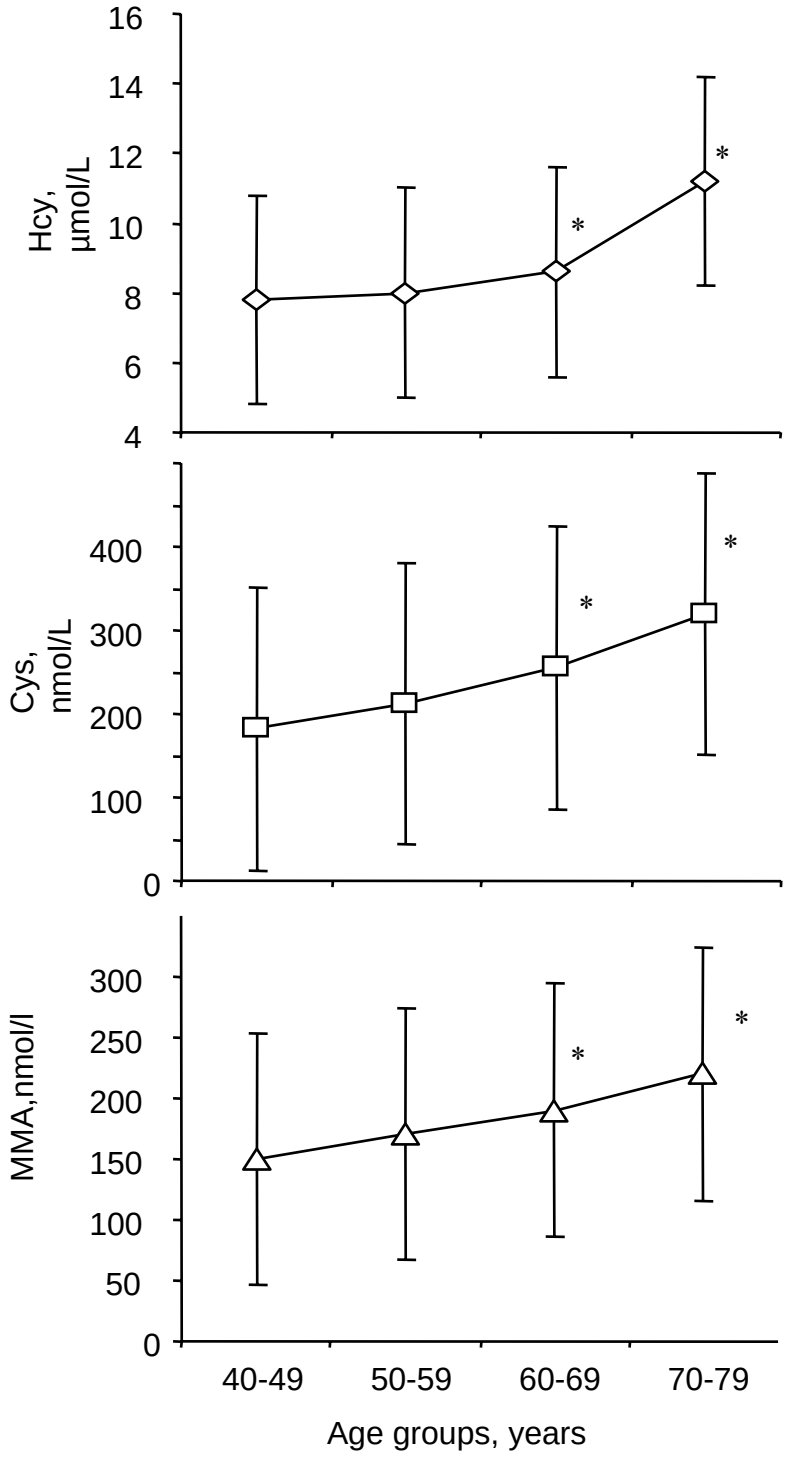


Figure 8.1 - Mean (SD) of Hcy, Cys, MMA concentrations according to the age of the Omani subjects. (* $p < 0.05$ when compared to the youngest group)

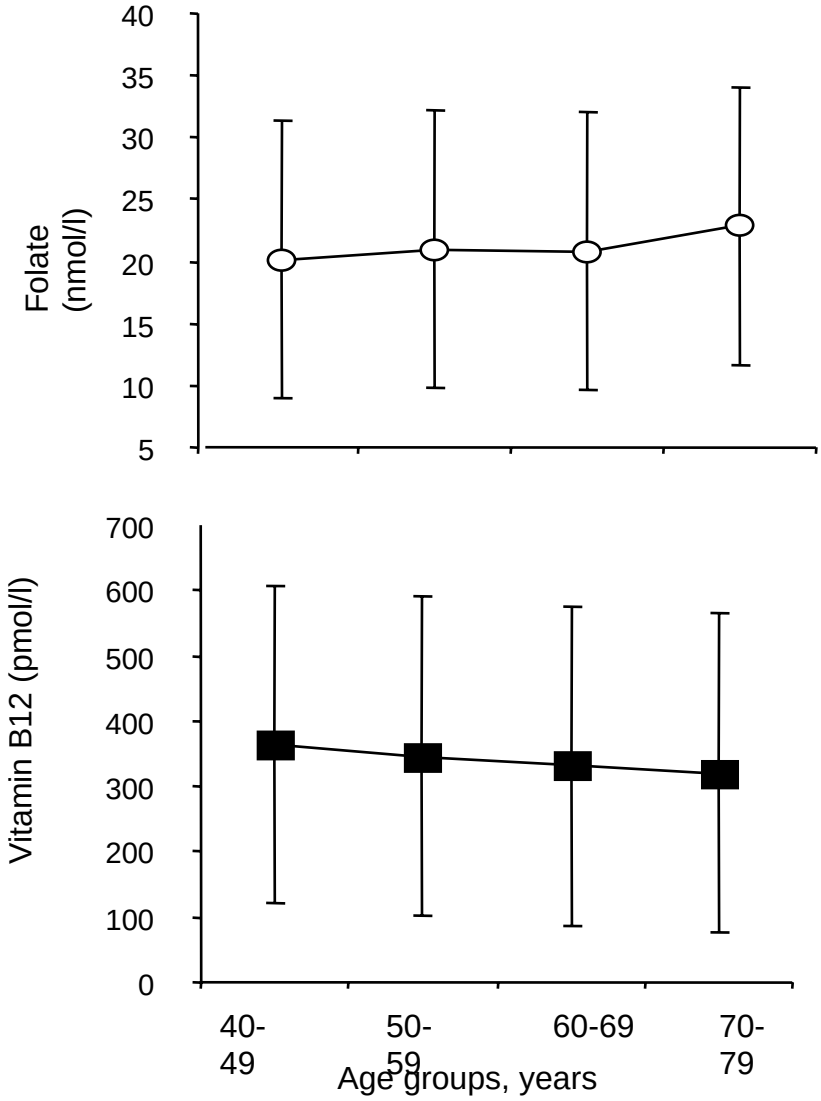


Figure 8.2 - Mean (SD) of folate and vitamin B12 concentrations according to the age of the Omani subjects.

Incidence of abnormal metabolic markers and vitamin B status in the Omanis according to gender

In the Omnai group 33 of the 221 subjects (15.6 %) had elevated levels of homocysteine, 53 of the 211 subjects (25.1 %) had elevated levels of cystathionine and 31 of the 205 subjects tested (15.5 %) had elevated levels of methylmalonic acid.

In the Omani group, the only group to have significant numbers of females (47 %) tested for Hcy and Cys and 45.4 % of subjects tested for MMA, there were differences noted in the number of subjects with elevated levels. For example, whereas 20.7 %, 31.5 % and 17.9 % of males had elevated levels of Hcy, Cys and MMA respectively, in female Omanis the incidence of elevated results were 10 %, 18 % and 11.8 %, respectively.

Table. 8.5 – Incidence of abnormal vitamin B status in the Omanis according to gender

	Hcy >12 µmol/l	Cys > 301 nmol/l	MMA > 271 nmol/l	Folate < 7 nmol/l	Vitamin B12 < 156 pmol/l
Omanis (total)	33/211 (15.6 %)	53/211 (25.1 %)	31/205 (15.2 %)	2/224 (0.9 %)	9/225 (4 %)
Omani males	23 (111) (20.7 %)	35/111 (31.5 %)	20/112 (17.9 %)	2/119 (1.7 %)	5/120 (4.2 %)
Omani females	10/100 (10 %)	18/100 (18 %)	11/93 (11.8 %)	0/105 (0 %)	4/105 (3.8 %)

Correlations between concentrations of different markers in Omanis

Table 8.6 shows that there was a strong positive correlation between age and Hcy, Cys and MMA in the Omani group. There was also strong positive correlation between Hcy and Cys and MMA in the Omani group. There was strong inverse correlation between Hcy and folate. Cys correlated strongly and positively with Hcy and MMA and there was inverse correlation between Cys with folate and B12. There was strong inverse correlation between MMA and vitamin B12.

Table 8.6 Spearman-Rho correlations between different markers in Omanis (n = 226)				
	Age	Hcy	Cys	MMA
Hcy	0.386		0.583	0.440
Cys	0.399	0.583		0.360
MMA	0.319	0.440	0.360	
Folate	ns	-0.307	-0.177	Ns
Vit. B12	ns	Ns	-0.132	-0.220
Data are correlation coefficients according to Spearman test. All p values were < 0.05; ns= not significant.				

Metabolic markers and B-vitamins status in Asian-Indians immigrants in Oman

The median age of the Asian Indian non-vegetarian and vegetarian group was 44 and 42 years respectively. The median levels of homocysteine (Hcy), methylmalonic acid (MMA) and cystathionine (Cys) in Asian Indian non-vegetarians were observed to be 8.9 μmol/l, 221 nmol/l and 239 nmol/l respectively, which were within normal limits. The same parameters in the Asian Indian vegetarian group were observed to be 14.2 μmol/l, 467 nmol/l and 234 nmol/l respectively, which were all higher than normal limits. The median levels of folate and B12 in Asian Indian non-vegetarians were observed to be 21.5 nmol/l and 227 pmol/l respectively, which were again within normal limits. However, in the Asian Indian vegetarian group folate levels were observed to be 32.6 nmol/l which were higher than normal limits, whereas the same for B12 were observed to be 110pmol/l, which were lower than the normal limits. The median values of haemoglobin, creatinine, cholesterol and triglycerides in both the groups were found to be with in normal limits as shown in Table 8.7.

Table 8.7 Concentrations of biomarkers of the vitamins B in Asian-Indians immigrants in Oman according to the type of the diet

	Asian Indian (non-vegetarians)	Asian Indian (vegetarians)
Number	63	84
Age (years)	44 (40, 55)	42 (37, 55)
Hcy (μmol/l)	8.9 (5.6, 18.5)	14.2 (7.4, 36.5) *
Cys (nmol/l)	239 (137, 419)	234.5 (151, 391)
MMA (nmol/l)	221 (117.4, 843)	466.5 (167, 3619) *
Folate (nmol/l)	21.5 (6.9, 49)	32.6 (13, 59) *
Vitamin B12 (pmol/l)	226.5 (104, 386)	110 (60, 246) *
Haemoglobin (g/dl)	15.6 (13.3, 17.1)	14.5 (11.3, 18.4)
Creatinine (μmol/l)	77.3 (52, 104)	76.4 (51, 103)
Cholesterol (mmol/l)	5.2 (3.7, 6.9)	5.1 (3.6, 6.8)
Triglyceride (mmol/l)	1.9 (0.9, 4.1)	1.4 (0.7, 5.1)

Median (5^{th}-95^{th}) percentiles. * indicates significantly different from Indian non-vegetarians

Frequency of abnormal concentrations of metabolic markers according to the type of diet and gender among Asian Indian group -

In the Asian Indian group (both vegetarian and non-vegetarians) 46 of the 136 subjects (33.8 %) had elevated levels of homcysteine (Hcy > 12 μmol/l), 28 of 136 (20.5 %) had elevated levels of cystathionine (Cys > 301 nmol/l) and 62 of the 130 tested (47.7 %) had elevated levels of methylmalonic acid (MMA > 271 nmol/l) (Table 8.8)

It was clear from simple observation of the results from the Asian group that there was a marked difference between the vegetarians and non-vegetarians in this group. For example 34 of the 57 Asian Indian vegetarians tested (59.6 %) had elevated levels of Hcy compared with 12 of the 79 (15.2 %) non-vegetarian Asian Indians in the group, 9 of the 57

vegetarians (15.8 %) had elevated Cys compared with 19 of the 79 (24 %) non-vegetarians and 38 of the 52 vegetarian Asian Indians in the group (73 %) had elevated MMA compared with 24 of the 78 (30.8 %) non-vegetarian Asian Indians.

None of the 59 vegetarian Asian Indians in the group (0 %) had low folates compared with 4 of the 89 non-vegetarian Asian Indians (4.5 %). Also 39 of the 59 vegetarian Asian Indians (66.1 %) had deficient levels of vitamin B12 compared with 14 of the 89 non-vegetarian Asian Indians (15.7 %).

Correlations between concentrations of different markers in Asian-Indians

Table 8.8 -The incidence of elevated or decreased concentrations of metabolic markers and B-vitamins in Asian-Indians immigrant in Oman according to the type of the diet.

	Hcy >12 µmol/l	Cys > 301 nmol/l	MMA > 271 nmol/l	Folate < 7 nmol/l	Vitamin B12 < 156 pmol/l
Asian-Indian omnivores (total)	12/79 (15.2 %)	19/79 (24 %)	24/78 (30.8 %)	4/89 (4.5 %)	14/89 (15.7 %)
Asian-Indian omnivore ♂	12/68 (17.6 %)	18/68 (25.6 %)	23/68 (33.8 %)	7/78 (9 %)	14/78 (18%)
Asian Indian omnivore ♀	0/11 (0 %)	1/11 (9.1 %)	1/10 (10 %)	0/11 (0 %)	0/11 (0 %)
Asian-Indian vegetarians (total)	34/57 (59.6 %)	9/57 (15.8 %)	38/52 (73 %)	0/59 (0 %)	39/59 (66.1 %)
Asian-Indian vegetarian ♂	33/52 (63.5 %)	9/52 (17.3 %)	35/46 (76 %)	0/53 (0 %)	35/53 (66 %)
Asian-Indian vegetarian ♀	1/5 (20 %)	0/5 (0 %)	3/6 (50 %)	0/6 (0 %)	4/6 (66.7 %)

Table 8.9 shows that there was strong positive correlation between Hcy and MMA in the Asian Indian non-vegetarian group. There was strong inverse correlation between Hcy and vitamin B12. There was positive correlation between Cys and MMA in the non-vegetarian Indians. There was strong positive correlation between MMA and Hcy, positive correlation of MMA with Cys and folate. However, there is a strong inverse correlation between MMA and B12.

Table 8.9 - Spearman-Rho correlations between different markers in Indian non-vegetarians (n = 84)

	Hcy	Cys	MMA	Folate
Hcy		ns	0.363	Ns
Cys	ns		0.299	Ns
MMA	0.363	0.299		0.235
Folate	ns	ns	0.235	
Vit. B12	-0.396	ns	-0.564	-0.227

Data are correlation coefficients according to Spearman test. All p values were < 0.05; ns= not significant.

Table 8.10 shows that there is strong positive correlation between Hcy and Cys whilst there is strong inverse correlation between Hcy and vitamin B12 in the Asian Indian vegetarian group. There is inverse correlation between MMA and vitamin B12. There was no correlation between Age and the metabolic markers in this group.

Table 8.10 - Spearman-Rho correlations between different markers in Indian vegetarians (n = 63)				
	Age	Hcy	Cys	MMA
Hcy	ns		ns	0.621
Cys	ns	ns		Ns
MMA	ns	0.621	0.337	
Folate	ns	ns	ns	Ns
Vit. B12	ns	-0.659	ns	-0.689
Data are correlation coefficients according to Spearman test. All p values were < 0.05; ns= not significant.				

Comparing B-vitamin status between Omanis and Asian-Indians living in Oman

Table 8.11 – Median values for all parameters in various groups(5^{th} to 95^{th} percentile)			
	Omanis	Asian Indian (non-veg.)	Asian Indian (veg.)
Number	225	63	84
Age (years)	53 (40, 70)	44 (40, 55) **§**	42 (37, 55) §
Hcy (µmol/l)	8.2 (5.0, 14.8)	8.9 (5.6, 18.5)	14.2 (7.4, 36.5) §*
Cys (nmol/l)	220 (113, 456.9)	239 (136.8, 419)	234.5 (150.6, 391.4)
MMA (nmol/l)	171 (101, 372.7)	221 (117.4, 842.8)	466.5 (167.4, 3619) §*
Folate (nmol/l)	20.4 (10.7, 42.6)	21.5 (6.9, 49)	32.6 (12.9, 59.3) §*
Vitamin B12 (pmol/l)	334 (168.2, 625)	226.5 (104, 386.3) §	110 (60.2, 246) §*
Haemoglobin (g/dl)	13.5 (10.9, 16.1)	15.6 (13.3, 17.1)	14.5 (11.3, 18.4)
Creatinine (µmol/l)	69 (41.9, 106.9)	77.3 (52.4, 103.9)	76.4 (50.9, 102.9)
Cholesterol (mmol/l)	5.7 (4.2, 7.7)	5.2 (3.7, 6.9)	5.1 (3.6, 6.8)
Triglyceride (mmol/l)	1.5 (0.72, 4.6)	1.9 (0.9, 4.1)	1.4 (0.7, 5.1)
§ indicates significantly different from Omanis. * indicates significantly different from Indian non-vegetarians			

As indicated in Table 8.11 and figure 8.3, The median age of the Omani group was significantly higher (53 years) than that in the Asian Indian groups both vegetarian and non-vegetarian (42 and 44 years, respectively). The median levels of homocysteine (Hcy) and methylmalonic acid (MMA) were significantly higher in the Asian Indian vegetarian

group than in both the Asian Indian non-vegetarian group and the Omani group (Hcy 14.2 vs, 8.9 and 8.2 μmol/l) and (MMA 466.5 vs. 221 and 171 nmol/l) respectively. The median levels for folate were significantly higher in the Asian Indian vegetarian group than in both the Asian Indian non-vegetarian group and the Omani group (32.6 vs. 21.5 and 20.4 nmol/l) respectively. The median level of vitamin B12 in the Asian Indian vegetarian group was significantly lower that in both the Asian Indian non-vegetarian group and the Omani group (110 vs. 226.5 vs. 334 pmol/l). The median level of the vitamin B12 in the Asian Indian non-vegetarian group was significantly lower than that in the Omani group (226.5 vs. 334 pmol/l). The median value for haemoglobin in the Omani group was lower that that in the Asian Indian vegetarian group (13.5 vs. 14.5 g/dl) and significantly lower than that in the Asian Indian non-vegetarian group (13.5 vs. 15.6g/dl). The median creatinine level of the Omani group was lower that that in both the Asian Indian vegetarian and non-vegetarian groups (69 vs. 76.4 and 77.3 μmol/l respectively).The median level of cholesterol in the Omani group was higher in than those in the Asian Indian groups both vegetarian and non-vegetarian (5.7 vs. 5.1 and 5.2 mmol/l respectively). The median level of triglyceride in the Asian Indian non-vegetarian group was higher than those in both The Omani group and the Asian Indian vegetarian group (1.9 vs. 1.5 and 1.4 mmol/l respectively)

Concentration of metabolic markers in Omani, Asian Indian vegetarian

and Asian Indian non-vegetarian groups.

Fig. 8.4 shows that the median value of Hcy was significantly higher in Asian Indian vegetarian group than that of Asian non-vegetarian and Omani, while there was no significant difference between Indian non-vegetarians and Omanis. There was no

significant difference between the median values of Cys in all the three groups. MMA levels were significantly high in Asian Indian vegetarian group than in other two groups while there was no significant difference in values between Asian Indian non-vegetarian and Omani groups. Folate values in Indian Asian vegetarian group are significantly higher than in other two groups. It is interesting to note that if the Asian Indian vegetarian group does exhibit a cobalamin deficiency, than folate may become trapped. This appears to result in high level of plasma folate. Vitamin B12 level is significantly lower in Indian vegetarian group compared with the Asian Indian non-vegetarians and Omanis. It is also of interest to note that median level of vitamin B12 in Asian Indian non-vegetarian group is significantly lower than Omanis. There is no significant difference between Haemoglobin, Creatinine, Total cholesterol and Triglyceride levels of the three groups.

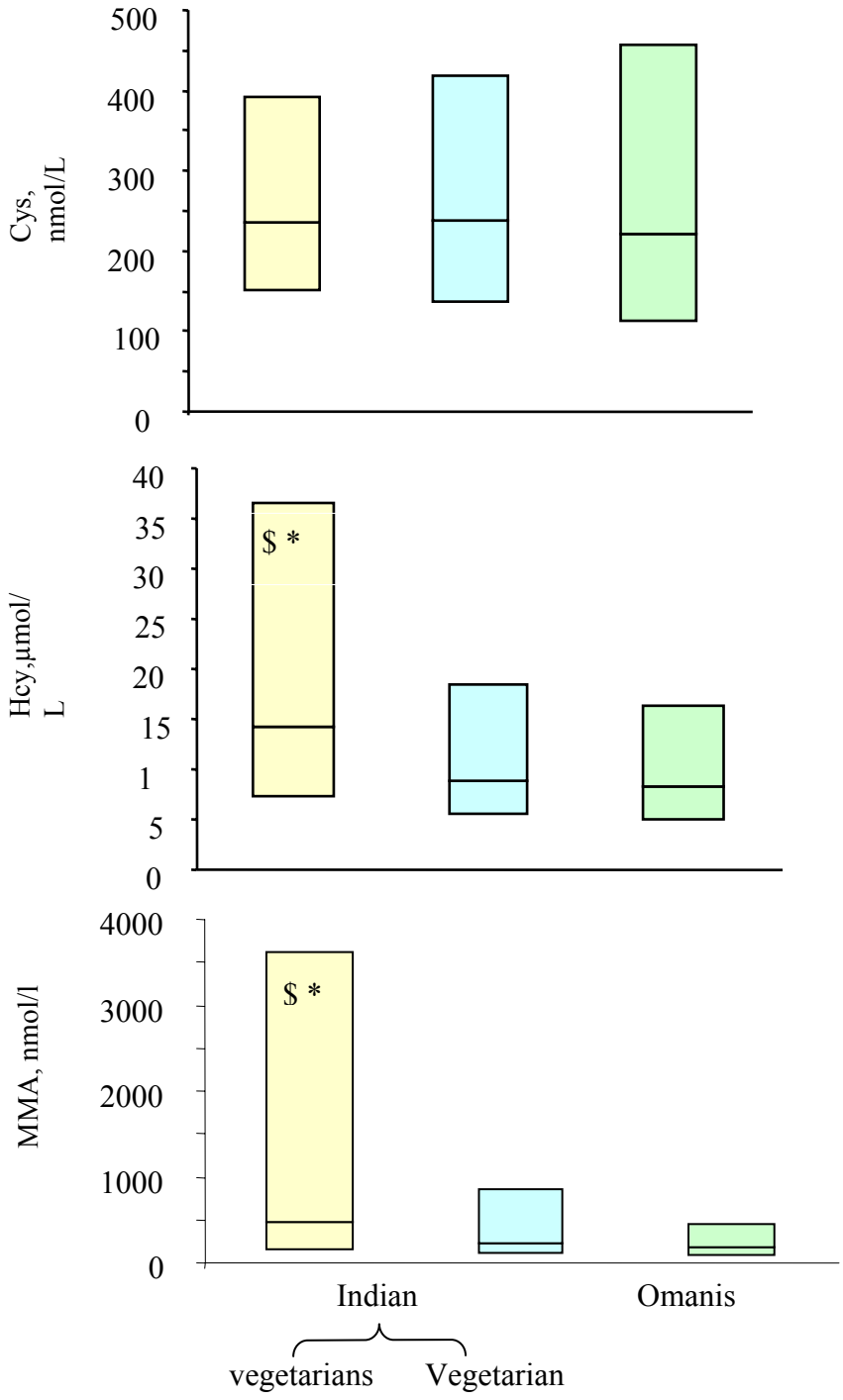


Chart 8.3. Concentration of Hcy ,Cys,MMA [presented as median and 5th to 95th percentile] in Omani, Asian Indian vegetarian and Asian Indian non-vegetarian groups.* indicates significant difference compared with Omanis.$ indicates significant difference compared with Asian Indian non- vegetarian (Mann-Whitney Test)

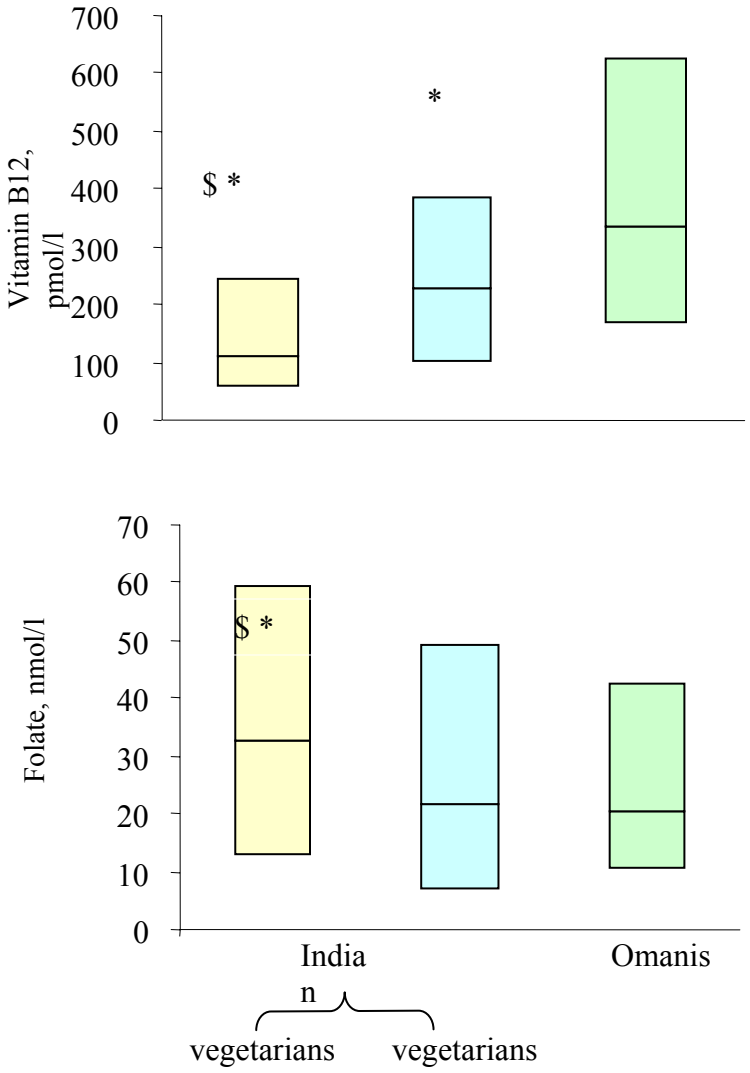


Chart 8.4. Concentrations of folate and vitamin B12 [presented as median and 5th to 95th percentile] in Omani, Asian Indian vegetarian and Asian Indian non-vegetarian groups. * indicates significant difference compared with Omanis. $ indicates significant difference compared with Asian Indian non-vegetarians (Mann-Whitney Test).

The incidence of abnormal metabolites, folate and vitamin B12 in Omanis and Asian Indians both vegetarians and non-vegetarians

Fig.8.5 to 8.7 show that the incidence of abnormal level of Hcy, MMA and B12 in Indian vegetarian group was very high and there was a much higher percentage of abnormal values than seen in Indian non-vegetarian group. The incidence of abnormal level of Cys was similar for all groups and was between 16% to 25 %. There was a low incidence of abnormal folate in general with no abnormal levels seen Asian Indian vegetarian group. Although there was a lower incidence of abnormal levels of MMA and B12 in Asian Indian non-vegetarian group compared to Asian Indian vegetarian group. The non-vegetarian Indian group showed significantly higher incidence of abnormal levels of MMA and B12 compared with Omani group(31%).

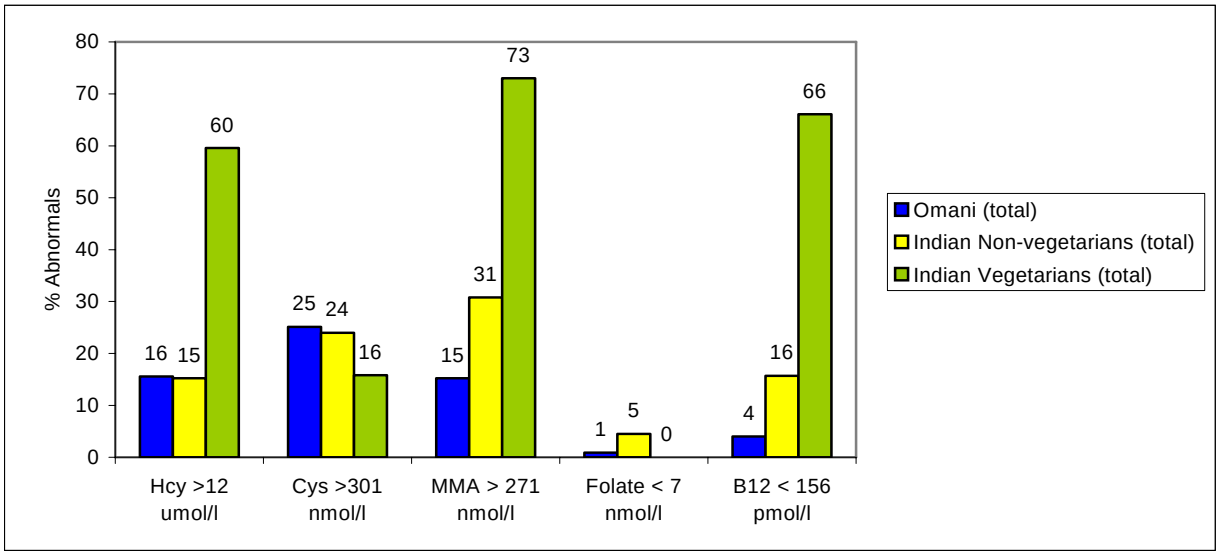


Fig. 8.5 - Incidence (%) of abnormal parameters in Omanis, Asian Indian omnivores and Asian Indian vegetarians

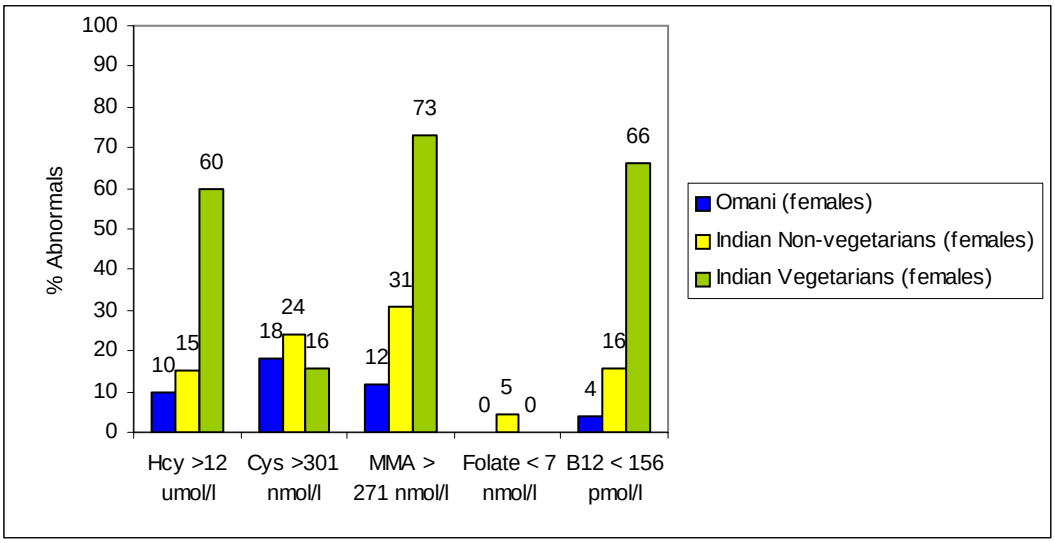


Chart 8.6 - Incidence (%) of abnormal parameters in female subjects according to ethnic origin and type of diet

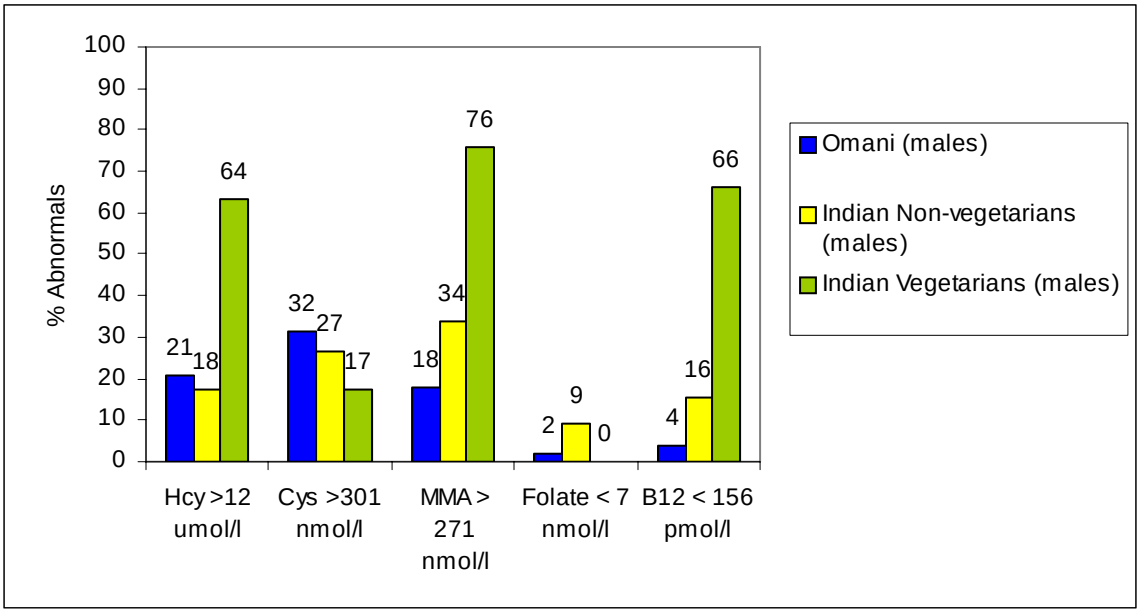


Chart 8.7 - Incidence (%) of abnormal parameters in male subjects according to ethnic origin and type of diet

Correlation between Hcy and vitamin B12 in Indian vegetarian and Omani groups

Fig. 8.8 and 8.9 show the correlations between Hcy and vitamin B12 and MMA and vitamin B12 respectively in Asian Indian vegetarian and Omani groups. It can be seen that the two groups form two separate populations. Whereas the Asian Indian vegetarians in general have high levels of Hcy and MMA and low or deficient levels of vitamin B12. The Omani group has generally normal levels of Hcy and MMA and normal levels of vitamin B12. It should also be noted that there is a significant inverse, negative correlation between HCY and MMA and vitamin B12.

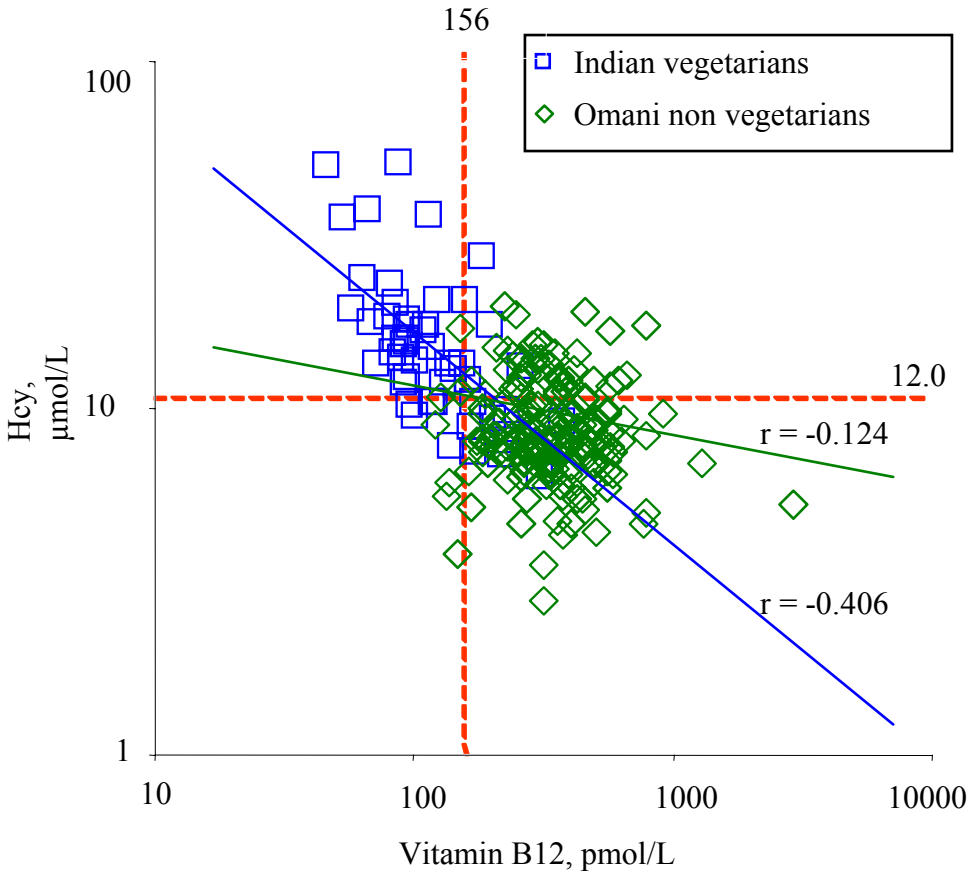


Figure 8.8 - Correlation between Hcy and vitamin B12 in Indian vegetarian and Omani groups

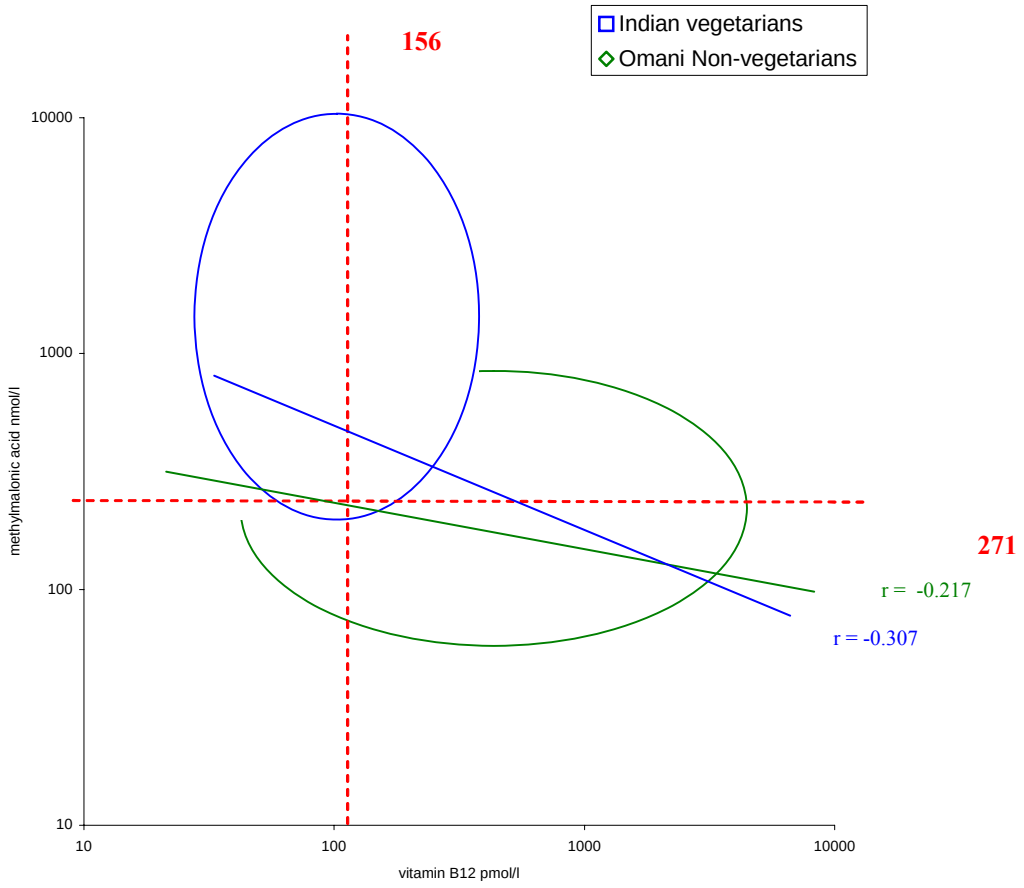


Figure 8.9 - Correlation between MMA and vitamin B12 in Asian Indian vegetarian and Omani groups

Fig.8.10 -Correlations between Hcy and vitamin B12 in Omani group (A), Asian Indian non-vegetarian group (B) and Asian Indian vegetarian group (C)

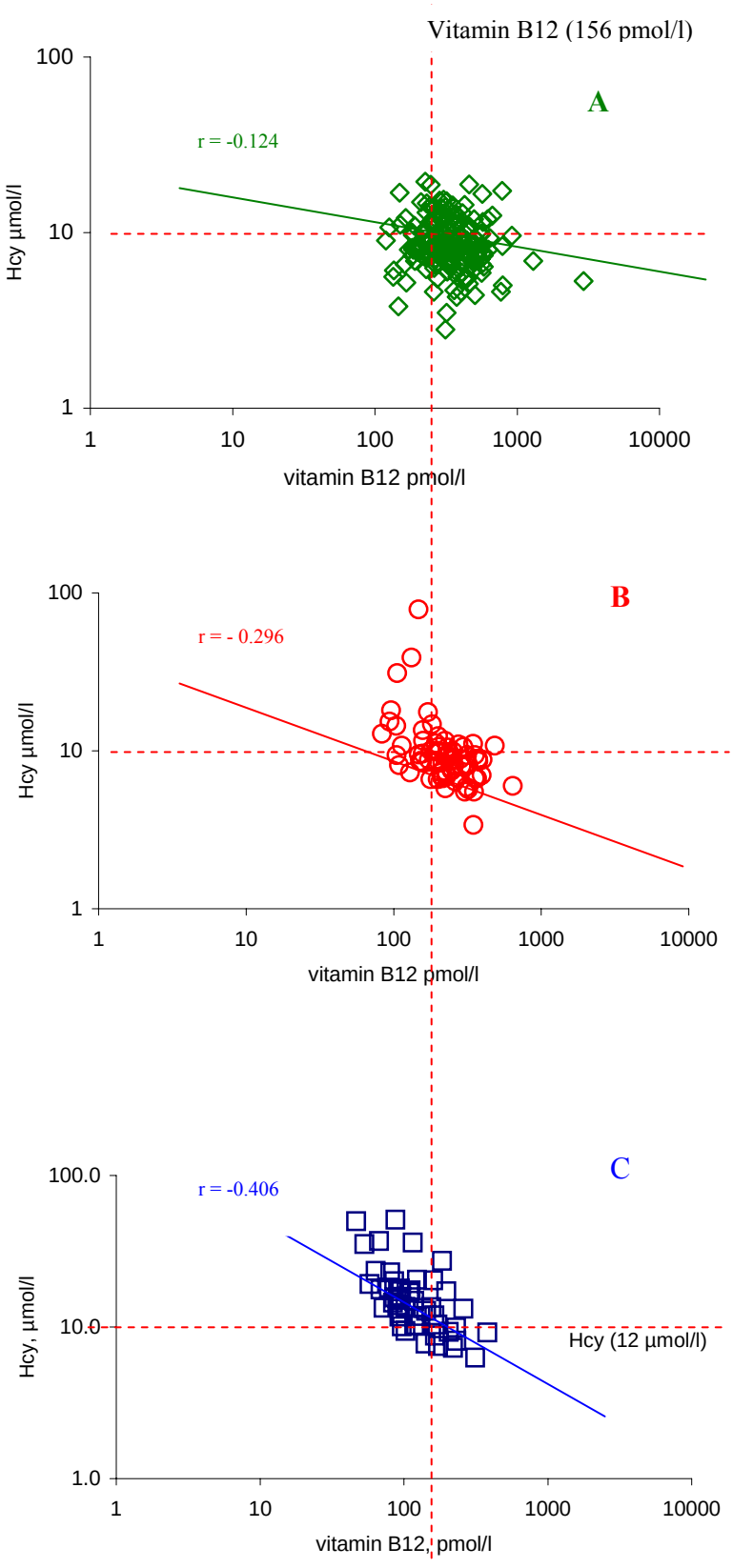


Figure 8.11 - Correlation between MMA and vitamin B12 in Omani group (A), Asian Indian non-vegetarian group (B) and Asian Indian vegetarian group (C)

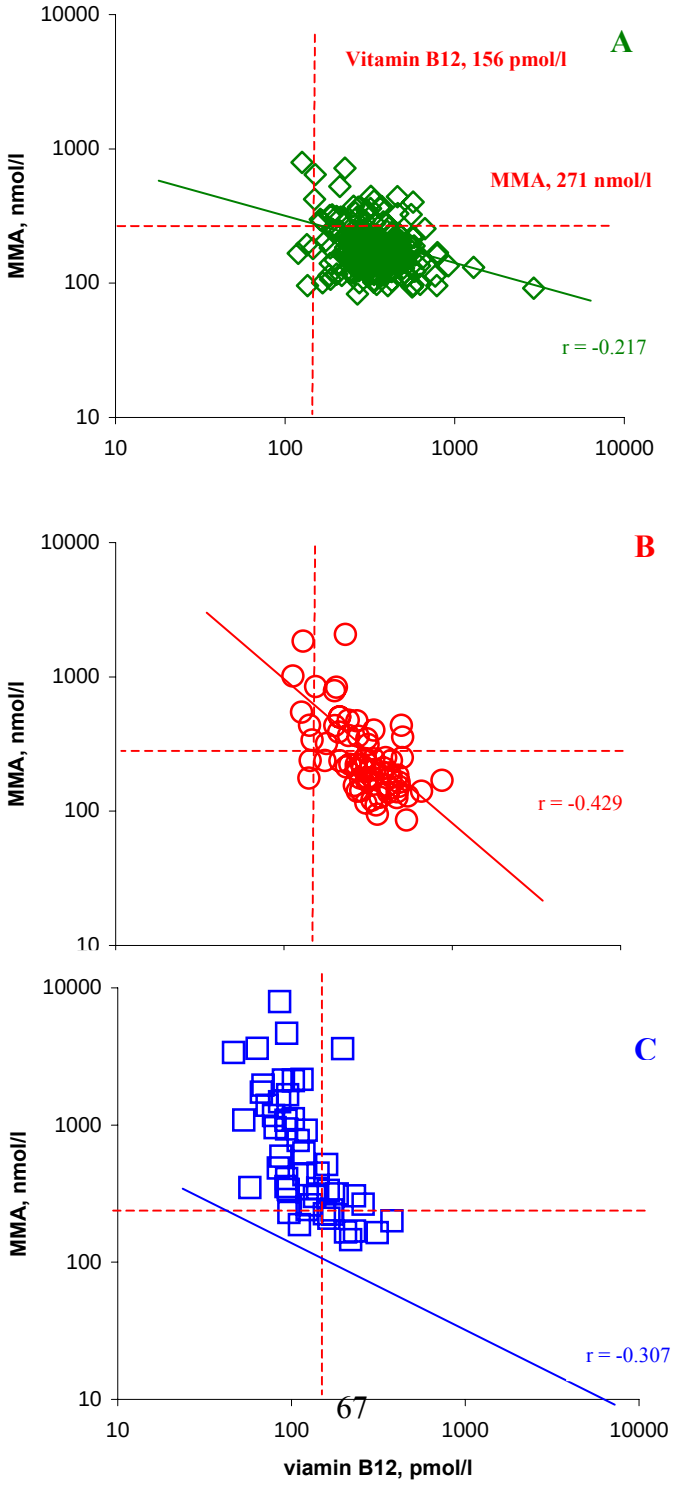
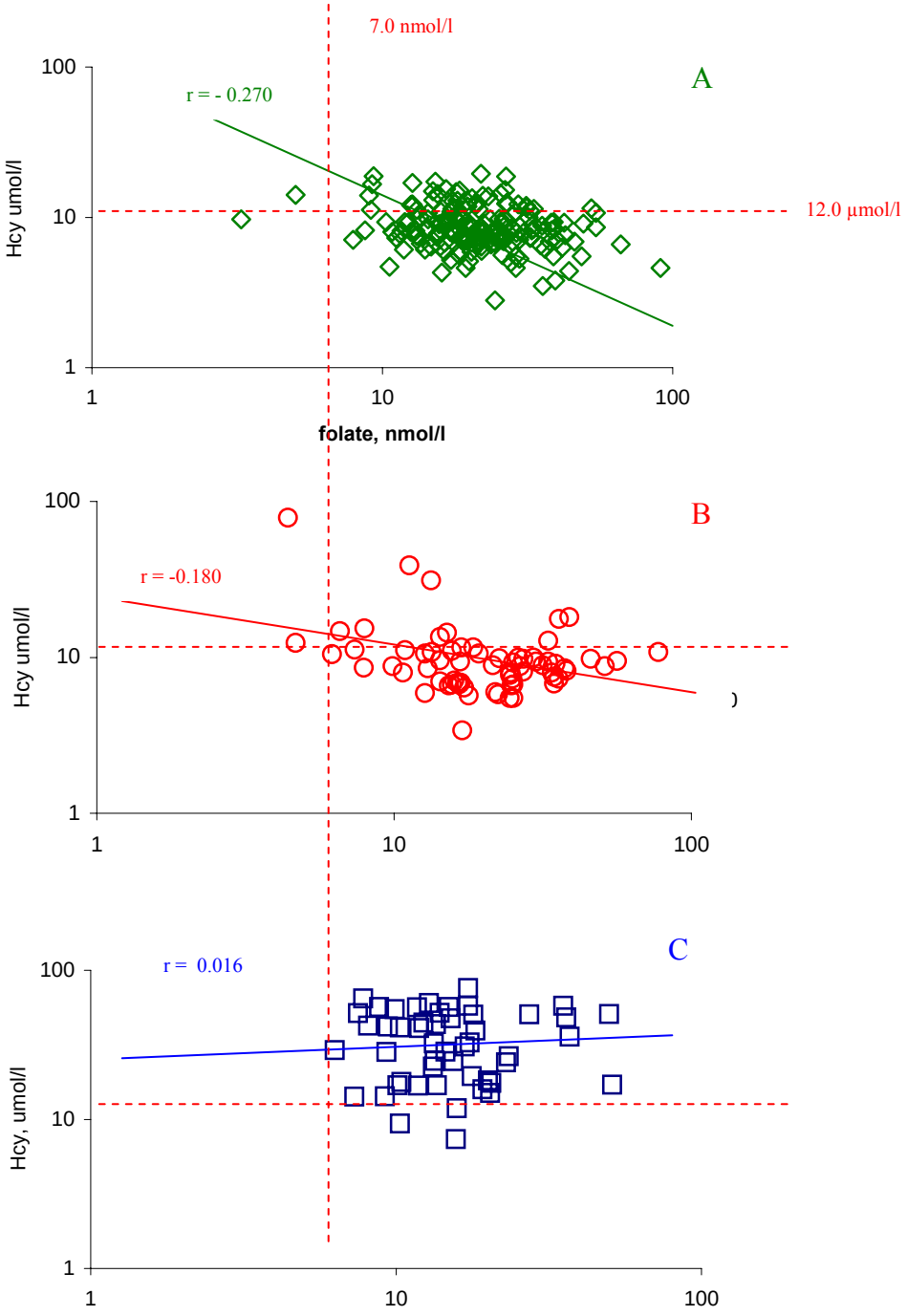


Fig. 8.10 demonstrates that the Omani and Asian Indian non-vegetarian groups have generally normal Hcy and vitamin B12 levels the Asian Indian vegetarian group have generally low or deficient levels of vitamin B12 and generally high levels of Hcy. There is a reverse correlation between Hcy and vitamin B12 in Asian Indian vegetarian group and the Asian Indian non-vegetarian group indicating the as vitamin B12 decreases so Hcy tends to increase. There was no correlation between the levels of Hcy and vitamin B12 in the Omani group.

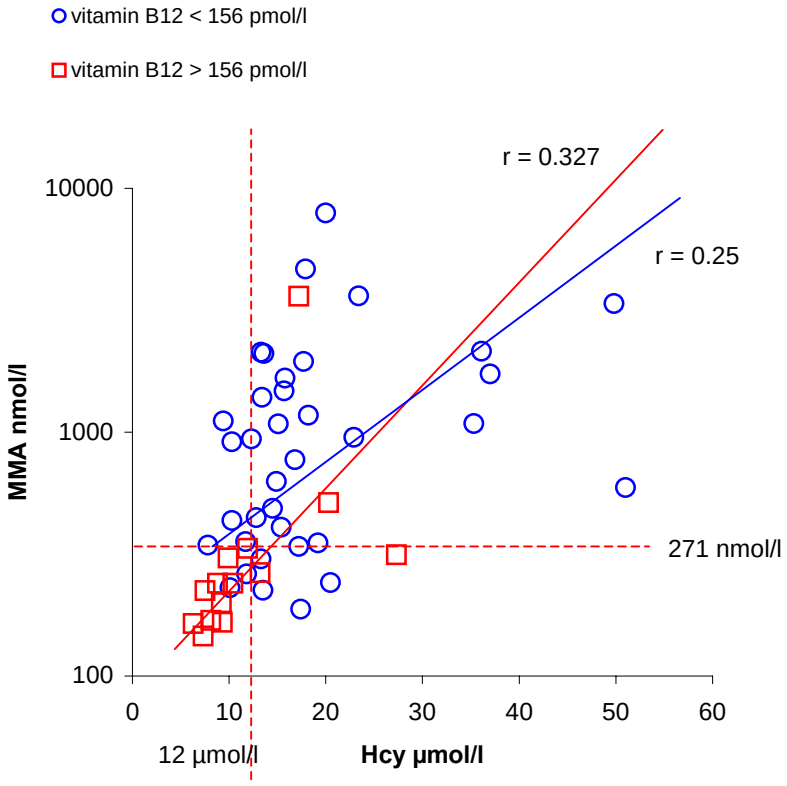
Fig.8.11. demonstrates that a similar pattern exists regarding the relationship between MMA and vitamin B12 as is seen between Hcy and vitamin B12 in the three groups (A, B and C). It can be seen that the Omani and Asian Indian non-vegetarian groups have generally normal levels of vitamin B12 and MMA. In the Asian Indian vegetarian group the levels of MMA are generally high and the vitamin B12 levels are generally low. In all three groups there is a reverse correlation between MMA and vitamin B12 indicating that as vitamin B12 decreases MMA tends to increase.

Fig 8.12 -Correlation between Hcy and folate in Omani group (A), Asian Indian non-vegetarian group (B) and Asian Indian vegetarian group (C)



Correlations between Hcy and folate are shown in **fig 8.12**. There was a strong inverse correlation between Hcy and folate in the Omani group. It can be seen that in the Asian Indian vegetarian group, who were generally vitamin B12 deficient (66 %), there were generally high levels of serum homocysteine and all had normal levels of folate.

Fig.8.13 -Correlation between Hcy and MMA in Asian Indian vegetarian group



There were 36 subjects within the Asian Indian vegetarian group who had low levels of vitamin B12 (vitamin B12 < 156 µmol/l) (median value 95) and 14 subjects with normal levels (median value 194). The median and mean values are shown in **Table 8.12**

Fig.8.13. demonstrates clearly that subjects with low vitamin B12 generally have high (abnormal or elevated) levels of Hcy and MMA. Those subjects with normal levels of vitamin B12 have generally normal levels of Hcy and MMA.

Table 8.12 Median values (5th and 95th Percentiles) for Hcy and MMA in Asian Indian vegetarian group with (A) low vitamin B12 levels and (B) normal vitamin B12 levels

	(A) Low vitamin B12 levels (<156 pmol/l) (n = 36)		**(B) Normal vitamin B12 levels (> 156 pmol/l) (n = 14)**	
	vitamin B12 median value 95 µ mol/l (57.3, 142.3)		**vitamin B12 median value 197 µ mol/l (162, 280)**	
	Hcy (µmol/l)	MMA (nmol/l)	Hcy (µmol/l)	MMA (nmol/l)
Median	15.4	926	9.6	240
Mean	18.8	1335	11.9	492
5th Percentile	9.9	229	7.0	158
95th Percentile	40.2	3888	22.8	1598

Fig.8.14 -Correlation between and folate and vitamin B12 in Omani group (A), Asian Indian non-vegetarian group (B) and Asian Indian vegetarian group (C) (folate axis log adjusted).

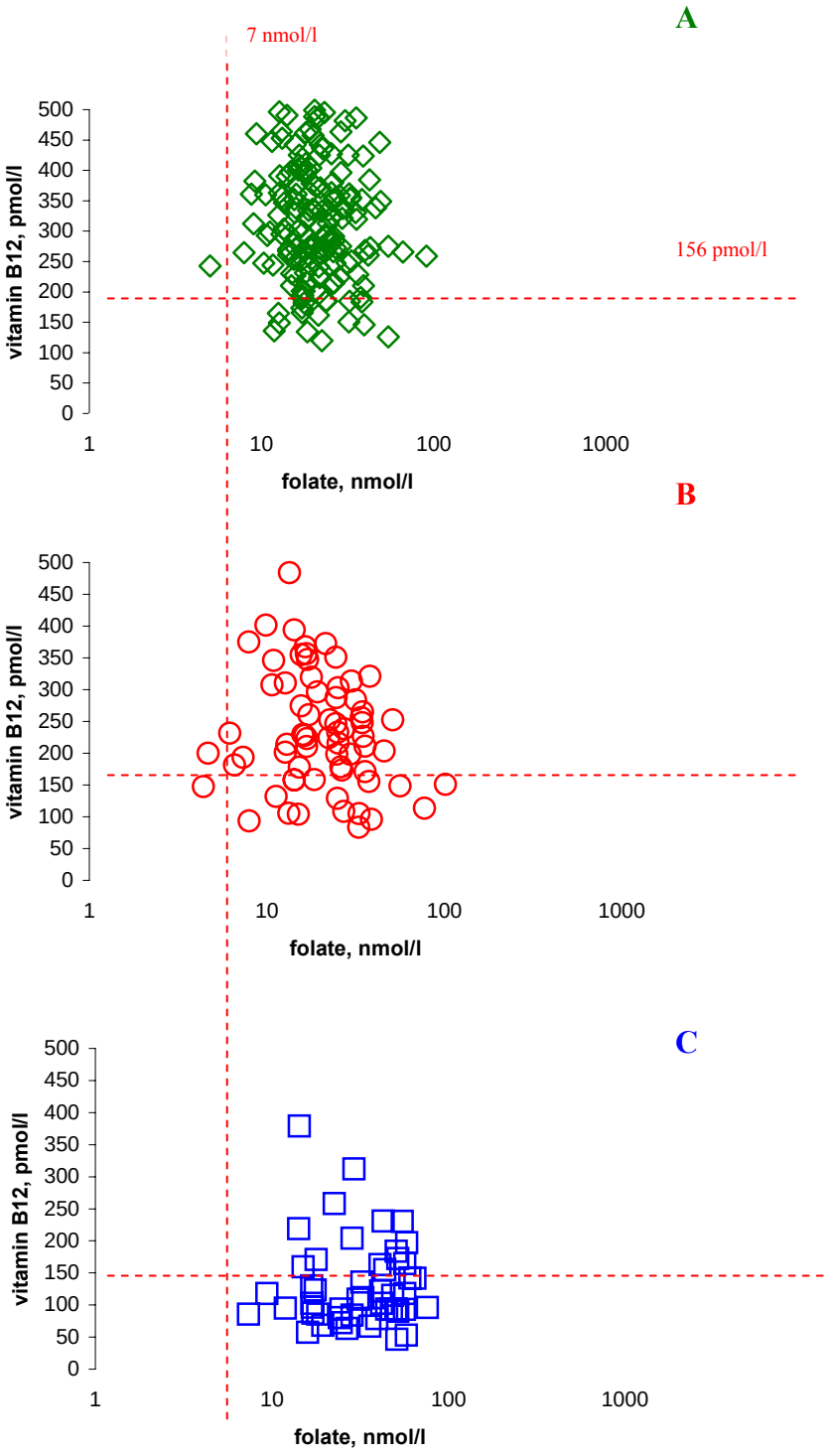
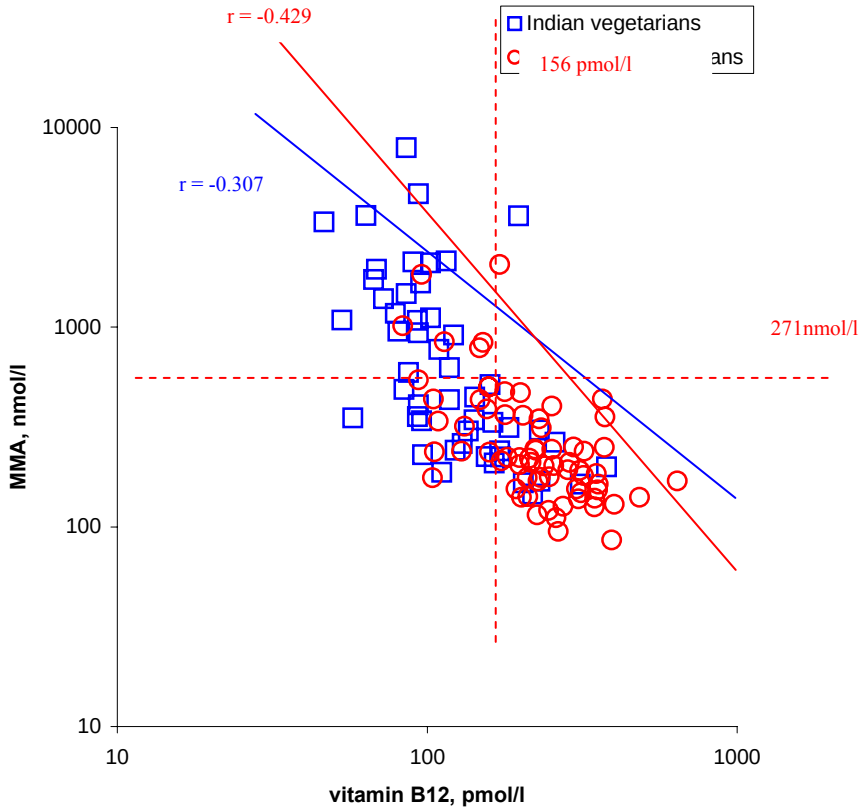


Fig.8.14 shows that there were no Asian Indian vegetarian subjects with deficient levels of folate despite the fact that a significant number (59.6 %) had hyperhomocystememia and 73 % of this group had elevated MMA. In contrast in the Omani and Asian Indian non-vegetarian groups where there were far fewer subjects with elevated Hcy (15.6 % and 15.2 % respectively), there were very few subjects with folate deficiency as would be expected. The chart also shows that there were a significant number of Asian Indian vegetarians with low levels of vitamin B12 (66.1 %)

(All charts show median values in each age group with + or – 1 standard deviation)

Fig.8.15 -Correlation between vitamin B12 and MMA in Asian Indian vegetarian group and Asian Indian non-vegetarian group (axes log-adjusted)

Fig.8.15 demonstrates that MMA increases as vitamin B12 decreases in the Asian Indian groups both vegetarian and non-vegetarian. It can be seen that the Asian Indian vegetarian



group generally have increased MMA (73 %) and are predominantly vitamin B12 deficient (66 %). The Asian Indian non-vegetarian group generally has normal levels of MMA (69 %) and vitamin B12 (84 %). There is a strong inverse correlation between MMA and vitamin B12 in both Asian Indian groups.

Sensitivity and Specificity of Hcy and MMA as markers for vitamin B12 deficiency

Table 8.13 Number of subjects with low and normal levels of vitamin B12

All subjects with low vitamin B12 (n = 58)	True positive (Hcy > 12.0 µmol/l)	True positive (MMA > 271 nmol/l)
	39	47
	False Positive (Hcy < 12.0 µmol/l)	False negative (MMA < 271 nmol/l)
	19	11
All subjects with normal vitamin B12 (n = 248)	True negative (Hcy < 12.0 µmol/l)	True negative (MMA < 271 nmol/l)
	213	207
	False negative (Hcy > 12.0 µmol/l)	False negative (MMA < 271 nmol/l)
	35	41

All subjects who have vitamin B12 levels below 156 µmol/l are defined as being deficient. In order to measure the sensitivity and specificity of Hcy and MMA as markers for vitamin B12 deficiency the following formulae can be used:

$$\text{Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positive + number of false negatives}} \times 100\ (\%)$$

$$\text{Specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives + number of false positives}} \times 100\ (\%)$$

True positives are defined as all subjects with low levels vitamin B12 (<156 pmol/l) with elevated levels of Hcy (> 12.0 μmol/l) or elevated levels of MMA (> 271 nmol/l).

True negatives are defined as all subjects with normal levels of vitamin B12 (> 156 pmol/l) and normal levels of Hcy (< 12.0 μmol/l) and normal levels of MMA (< 217 nmol/l).

False positives are defined as all subjects with low levels of vitamin B12 (< 156 pmol/l) with normal levels of Hcy (< 12.0 μmol/l) and normal levels of MMA (< 271 nmol/l).

False negatives are defined as all subjects with normal levels of vitamin B12 (> 156 pmol/l) with elevated levels of Hcy (> 12.0 μmol/l) and elevated levels of MMA (> 271 nmol/l).

Using the formulae it was established that the sensitivity of Hcy as a marker for vitamin B12 deficiency was **67.2 %** and the specificity **81 %.** The sensitivity of MMA as a marker was **85.9 %** and the specificity **83.5 %.** These findings seem to indicate that MMA is a more sensitive marker than Hcy for vitamin B12 deficiency.

7. Discussion

The Sultanate of Oman is a country with an approximate area of 350,000 square kilometers. It has a population of approximately 2.415 million (Ministry of National Economy, Sultanate of Oman) of which 1.802 million are indigenous Omanis and 0.613 million (25.4 %) are expatriate workers. The expatriate workforce is predominantly from the Indian sub-continent and most of these expatriates are from India.

Several reports from the Middle East have documented a high prevalence of coronary vascular diseases. Non-traditional risk factors including the nutritional status are one important risk factor for human diseases. Reports from Syria, Jordan, and Israel outlined a very high incidence of vitamin B12 deficiency in these populations (GIELCHINSKY Y et al., 2001; JOURBAN R et al., 1998). Low vitamin B12 can cause hyperhomocysteinemia (HHCY), the risk factor for degenerative vascular diseases. Vitamin B12 deficiency and HHCY in the Middle East populations have been linked to the risk of thrombosis, coronary heart disease, pregnancy complications and poor pregnancy outcome. No reports are available from Omanis about vitamin B12 and folate status as tow major determinants of homocysteine concentrations.

HHCY has been recognized as an independent risk factor in coronary heart disease (de BREE A et al., 2002) and has been implicated in neural tube defects, neuropsychiatric disorders (LOSCALZO J, 2002), cognitive dysfunction and dementia (MILLER JW, 2003). Several life style factors may influence concentrations of homocysteine in blood such as; smoking and coffee consumption (CRAVO ML et al., 1996), lack of exercise, poor diet

(HERRMANN W et al., 2003), and certain medications. Nevertheless, the most important determinants of Hcy concentrations in human are folate and vitamin B12 status or intakes.

Our study is the first report about B-vitamin status from Oman. Our results demonstrated that serum concentrations of homocysteine are much lower than values reported from other population in the Middle East. Median (5^{th}-95^{th} percentiles) concentrations of Hcy were 8.2 (5.0-14.8) in Omani and 11.2 (6.9-24.7) in Syrians (HERRMANN et al. 2003;). Moreover, we found that concentrations of folate and vitamin B12 (medians, 20.4 nmol/L and 334 pmol/L, respectively) were within the values usually found in white population from Europe or America (ESTRADA D; et al., 2001). As has been seen in other population groups, we found significant differences in Hcy, cys, and vitamin B12 between males and females from this population (ESTRADA D; et al., 2001).

In addition to the effect of gender, we found that subject's age is a main determinant of serum concentrations of Hcy, cys and MMA (Table 8.4 and Figure 8.1). In contrast, concentrations of folate and vitamin B12 were not related to the age of the subjects (Figure 8.2). Therefore, variations of the three metabolites (Hcy, cys, and MMA) with age could be related to the physiological decline in renal function with age. This suggestion is supported by the notice that concentrations of creatinine correlated to that of Hcy ($r=0.55$, $p<0.001$), MMA ($r=0.28$, $p<0.001$), and cystathionine ($r=0.53$, $p<0.001$).

A recent study in Oman investigated the prevalence of genetic polymorphisms in enzymes that participate in homocysteine metabolism (PATHARE A et al,., 2006). The study documented a high incidence of methylene tetrahydrofolate reductase gene polymorphism MTHFR C 677 T and the cystathionine beta synthase (CBS 844 ins 68) in patients with thrombosis (PATHARE A et al,., 2006). These mutations are generally associated with higher concentrations of Hcy in subjects with a low or a marginal vitamin

status (HERRMANN et al. 2001). Therefore, it would be interesting to test whether elevated concentrations of homocysteine are a risk factor for thrombosis in this population.

Higher rates of mortality and morbidities from cardiovascular diseases have been reported in Asian-Indians. This was not explained by traditional risk factors for coronary vessel disease such as, smoking and hypercholesterolemia. A causal link has been reported between poor nutritional status and vascular disease, dementia, and several other disorders. Moreover, about 50% of the Asian-Indians adhere to a life-long strict vegetarian diet for cultural and religious reasons. Even the omnivores in India consume less animal products when compared to the western populations (Refsum et al 2004.). A vegetarian diet may be deficient in certain micronutrients such as iron and vitamin B12. Furthermore, people who adhere to a strict vegetarian diet will develop vitamin B12 deficiency unless they supplement their diet with synthetic vitamin B12. That why investigating non-traditional risk factors such as nutritional factors of great interest in this population.

A number of studies have shown differences in cobalamin, folate, homocysteine and methylmalonic acid according to the ethnic origin (CARMEL R et al., 1999; ESTRADA DA et al., 2001; ANTONY A, 2001). Several studies have been carried out on Middle Eastern and Arabic populations (GIELCHINSKY Y et al., 2001; JOURBAN R et al., 1998; OBEID R, 2002; FORA MA et al., 2005). However there is no data available for various ethnic groups living in Oman. Our current study investigated the relationship between homocysteine, cystathionine and methylmalonic acid with folate and cobalamin in two population groups of different ethnic backgrounds.

The two ethnic groups, Omanis and Asian Indians, were compared for their metabolites (Hcy, Cys and MMA) and for their B vitamin status (folate and vitamin B12). It was noted that 43 % of the Asian-Indians were strict vegetarians. Because it is well

established that strict vegetarian diets can lead to HHCY and B vitamin deficiency, the vegetarian and the non vegetarian Asian Indians were considered separately.

The study shows a significantly higher prevalence of HHCY in the Asian Indian vegetarian group than in the Asian Indian non-vegetarian group and the Omani group (59.6 % vs 15.2 % and 15.6 %, respectively). Vegetarian Asian-Indians showed a high prevalence of increased MMA (73 %) and a low vitamin B12 (66%) when compared to the Asian Indian non-vegetarian (31% and 16 % for MMA and B12, respectively) or to the Omani group (15% and 4 %, respectively). The prevalence of elevated cystathionine was 15.8 % in the Asian Indian vegetarian group, 24 % in the Asian Indian non-vegetarian group and 25.1 % in the Omani group.

In contrast to a low vitamin B12 status, low levels of folate was detected in 5% of the Asian Indian non-vegetarians, less than 1% of the Omanis and in non of the Asian Indian vegetarians. Moreover, the median level of folate in the vegetarians was significantly higher than that in the non-vegetarians and the Omanis (32.6, 21.5 nmol/l, and 20.4 nmol/l, respectively). This finding of normal folate levels in vegetarians with low levels of vitamin B12, hyperhomocysteimia and elevated levels of MMA is in accordance with a previous study on vegetarians from Germany and the Netherlands (HERRMANN W et al., 2003). The results may suggest that folate elevation is secondary to vitamin b12 deficiency. In this case, folate deficiency can not be excluded. In the case of cobalamin deficiency, folate may become “trapped” (HERBERT V, 1965) and this will result in normal to high normal plasma levels of 5-methyl THF whereas intracellular folate may be low.

There was a significantly higher prevalence of elevated MMA in the Asian Indian vegetarians compared with the Asian Indian non-vegetarians and Omanis (73 % vs 30.8 % and 15.2 %, respectively). Elevated levels of MMA confirm that cobalamin deficiency is

very common in vegetarian Asian Indians. Plasma vitamin B12 lacks the sensitivity and the specificity as a marker for cobalamin deficiency (HERRMANN W et al., 2001). However, it was noted that the higher incidence of elevated MMA was a more sensitive and specific indicator of cobalamin deficiency (**Table 8.6**). These findings of relative sensitivity and specificity were similar to those in a study done in 2000 (BOLAN BJ et al., 2000). The fact that MMA is influenced by renal function may be considered when evaluating vitamin B12 renal patients or in elderly people (OBEID et al., 2004). However, our Asian Indian subjects were relatively young (in non vegetarians; r=0.30, p=0.011) and in vegetarians (r=0.12, p=0.38).

Hcy levels and the incidence of HHCY were similar in the Asian Indian non-vegetarian and Omani groups (median levels 8.9 vs 8.2 μmol/l and 15.2 % vs 15.6 % respectively). However there was a significantly higher incidence of low levels of vitamin B12 in the Asian Indian group compared with the Omani group (15.7 % vs 4 %). Also the median level of vitamin B12 is significantly lower in the Asian Indian non-vegetarian group than the Omani group (226.5 vs 334.0 pmol/l). This is reflected by the fact that the incidence of elevated MMA is significantly higher in the Asian Indian non-vegetarian group compared with the Omani group (30.8 % vs 15.2 %). Both groups are omnivores and therefore diet cannot be suggested as the cause of the differences seen. It may be that some genetic factors have contributed to this apparent increased incidence of cobalamin deficiency. It may be that some differences in lifestyle are contributing factors or it may be a combination of genetic and induced factors. The subjects in the Asian Indian non-vegetarian group were predominantly male (85 %) whereas there were 56 % males in Omani group. Comparison of the results of the markers and other parameters between males and females in the Omani group showed that there was no significant difference

between the genders for MMA (**Table 8.3**). The results in this table show that the median level of vitamin B12 is lower in males than in females (324 vs 359 pmol/l). However, this difference does not account for the difference seen between the 2 groups and it can be concluded that the increased incidence of cobalamin deficiency in the Asian Indian non-vegetarian group compared with the Omani group is not attributable to gender.

The effect of aging on metabolic markers and B vitamins (**Figure 8.1. to 8.2** and **Table 8.4**) demonstrate that Hcy Cys and MMA increase with age. There was no significant change in the levels of folate as age increased. Vitamin B12 levels appear to decrease with age although the decrease is very gradual. There were positive strong correlations between age and Hcy, Cys and MMA ($r = 0.382$, p 0.000, $r = 0.192$, p 0.005 and $r = 0.208$, p 0.003 respectively). There was no significant difference between the median ages of the subjects in the Indian Asian groups and therefore any differences in Hcy , MMA, folate and vitamin B12 cannot be directly attributable to age. It should noted that there was no correlation between age and the metabolic markers in the Asian Indians. However the median age of the Omani group was significantly higher than the Asian Indian vegetarian and non-vegetarian groups (53 vs 42 and 44 years respectively). Data in this study suggests that Hcy increases by approximately 0.7 µmol/l for every increment of 10 years life after the age of 40 years. Also MMA increases by an average of19 nmol/l for every increment of 10 years of life after the age of 40 years. The increase seen in Hcy with age is in line with findings given in 2004 (REFSUM H et al., 2004). Given that the median age of the Indian Asian non-vegetarian group is approximately 10 years less than that in the Omani group it might be assumed that the median value for Hcy would be lower in the Indian group. However, the predominance of males in the group is likely to have increased the median value. There is no significant difference in median values of MMA between

males and female Omanis (171 vs 170 nmol/l respectively). Also the median age is lower in the Asian Indian non-vegetarian group which would tend to lower the median value of MMA in this group. The fact that the median value is significantly higher in the Asian Indian non-vegetarian group compared with the Omani group (221 vs 171 nmol/l) would suggest that there is a greater prevalence of cobalamin deficiency in the Indian group despite the fact there is no significant difference in the levels of Hcy. The incidence of vitamin B12 deficiency evidenced by hyperhomocysteinemia, elevated MMA and low levels of vitamin B12 is much lower in the Omani group than in the Asian Indian groups both vegetarian and non-vegetarian. 4 % of the Omani subjects had low vitamin B12 levels whilst 15.6 % and 15,2 % had elevated Hcy and MMA respectively. In other studies done on Arab populations very high prevalence of vitamin B12 deficiency has been demonstrated. A study on Jordanians showed that 48.1 % of 216 randomly selected Jordanians had sub-optimal levels of vitamin B12 (< 164 pmol/l) (FORA MA et al, 2005). Another study showed low vitamin B12 levels in 33 % of healthy Syrian volunteers (GIELCHINSKY Y et al., 2001). The frequency of only 4 % Omanis with low vitamin B12 is more akin with that found in Caucasian populations in the west.

CONCLUSIONS

1. The results suggest that hyperhomocysteinemia; methylmalonic academia and vitamin B12 deficiency is very common in Asian Indians resident in Oman who follow a strict vegetarian diet.
2. Asian Indian residents in Oman who are essentially omnivorous have much lower frequency of vitamin B12 deficiency than the Asian Indian vegetarians.
3. The Omanis have a much lower frequency of vitamin B12 deficiency and much lower levels of Hcy and MMA than the Asian Indians both omnivores and vegetarians but particularly the vegetarians.
4. Omanis have a much lower frequency of vitamin B12 deficiency than that seen in apparently health Jordanians and Syrians the frequency being more akin to that found in western populations.
5. Whilst it is apparent that there is no significant difference in Hcy between the Omanis and the Asian Indian omnivores there is a significant difference in levels of MMA. This suggests that the incidence of vitamin B12 deficiency is significantly higher in the Asian Indian omnivores compared with the omnivorous Omanis. This contention is also supported by the significantly lower

levels of vitamin B12. This cobalamin deficiency is attributable to genetic or induced (lifestyle) factors or a combination of both.

6 The difference in frequency of vitamin B12 deficiency between the Asian Indian vegetarians and the Asian Indian non-vegetarians must be as a consequence of the difference in the diet of each group.

7 The results demonstrate that vitamin B12 deficiency is very common in vegetarians and may present a major health problem for those who pursue a strict vegetarian diet.

8 Many Asian Indian vegetarians living in Oman have a cobalamin deficiency that is evidenced by the high levels of homocysteine and methylmalonic acid and low levels of vitamin B12. These results would suggest that the differences mainly as a result of diet. Further, the findings suggests that vegetarians should consider vitamin B supplements to reduce the risk of CAD and other diseases implicated in the disturbance of methionine metabolism and hyperhomocysteinemia.

9 With a frequency of cobalamin deficiency of 15 % in the Asian Indian non-vegetarian residents living in Oman this group should also consider B vitamin supplements.

10 There is no evidence that cobalamin deficiency is a major problem in the general Omani population that is predominantly omnivorous. It appears that there is no need to screen for cobalamin deficiency given the low frequency of occurrence even in an aging population

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13 RESUME

NAME	:	Dr. Nayyar Ali Akhtar Niyaz
DATE OF BIRTH	:	1st December 1956
PLACE OF BIRTH		Karachi, Pakistan
FATHER'S NAME		**Akhtar**
MOTHER'S NAME		**Shamima**
NATIONALITY	:	Omani
MARITAL STATUS	:	Married
PERMANENT ADDRESS	:	P.O. Box1078, AL Hamria131 Sultanate Of Oman Tele. (Residence) 564407 Tele. (Office) 694176
PRESENT POSITION	:	Head of Diagnostic Laboratory Services, Ministry of Health, Sultanate of Oman & Senior Specialist Haematologist & Head of Pathology Department, Khoula Hospital

ACADEMIC QUALIFICATIONS

1982		:M.B.B.S., University of Karachi - Medicine, Surgery, Obstetrics & Gynaecology, Ophthalmology, Oto-rhino-laryngology, Pathology, Pharmacology, Anatomy, Community & Forensic Medicine, Physiology and Biochemistry. (Five Years course)
1988	:	DCP (MSc.), London University, Haematology
1989	:	DCP London University, Chemical Pathology, Microbiology
1990	:	DCP London University, Clinical Immunology/Virology

GENERAL EDUCATION

1975	:	Higher School Certificate Examination (F.Sc.)
Board	:	Karachi Board
Subjects	:	Urdu, English, Physics, Chemistry, Biology
Distinction	:	Physics & Chemistry
1972	:	General Education
Board	:	Karachi Board
Subjects	:	Urdu, English, Islamiat, Pak. Studies, Physics, Chemistry, Mathematics & Biology
Distinction	:	Islamiat, Physics, Chemistry & Mathematics

PROFESSIONAL EXPERIENCE

Jun - Dec 82	Resident House Physician, Civil Hospital, Karachi
Dec 82 - Jun 83	Resident House Surgeon, Civil Hospital, Karachi
Jun 83 - Mar 84	Medical Officer, Rab Private Clinic, Pakistan
Apr 84 - Mar 86	Medical Officer, Al Nahda Hospital, Muscat, Sultanate of Oman.

Duties included 2 months in each of the following disciplines : Pathology, General Medicine, Dermatology, Ophthalmology, E.N.T., Radiology, Neonatology & Paediatrics and Clinical Haematology & Transfusion

Mar 86 - Sep 88	Registrar in Haematology, Western Infirmary, Glasgow, Scotland
Sep 88 - Jul 89	Post Graduate Student (DCP), University of London
Sep 89 - Jul 90	Post Graduate Medical School, Hammersmith Hospital, London
Oct - Dec 90	Honorary Registrar, Wessex Regional Transfusion Centre, Southampton General Hospital
Feb - Nov 91	Senior Registrar, St. George's Hospital Medical

	School, London
Dec 91 - Dec 95	Specialist in Haematology, Royal Hospital, Muscat Sultanate of Oman
Jan 96 - Present	Head of Diagnostic Laboratory Services, Ministry of Health, Sultanate of Oman

PROFESSIONAL EXPERIENCE IN DETAIL

I spent 1 year working in Karachi immediately after obtaining my MBBS. I then came to work in the Sultanate of Oman as a Medical Officer in Al Nadha Hospital.

In 1986 I was awarded a scholarship to work in the UK to further my studies in pathology.

I worked in a number of hospital medical schools from 1986 to 1991 before returning to Oman to take up a post as Specialist in Haematology at the Royal Hospital in Muscat. My work in the Royal Hospital involved both clinical and laboratory responsibilities. I helped set up and run a haematology oncology clinic with the Consultant Haematologist. During this time I was asked to take on extra responsibilities in helping to organize and administer a regional pathology service. This led to the official organization of a regional laboratory service of which I am currently the head.

As Head of the Diagnostic Laboratory Service in the Ministry of Health I have overall responsibility for 105 laboratories in health institutions around the country with a staff of approximately 450 medical laboratory technicians and 20 pathologists. There are 10 administrative regions in the country and each has one large regional referral hospital and a number of smaller hospitals and health centers. My duties are to administer the pathology laboratories under my supervision and to advise the Ministry of Health on the maintenance and up-grading of the services that are offered. This includes the planning, staffing and equipping of new hospital laboratory projects of which there have been 5 in the last 5 years. The planning of these projects starts with laboratory design, specification and selection of equipment, estimation of staffing levels, recruitment of staff and final commissioning of the project.

The administration of such a large organization is, by definition, difficult. I visit the laboratories of the regions at least once per year and prepare an annual report to H.E. the Minister. I have also set up a system of regional administration to ensure adequate day to day running of the major regional referral hospitals and their satellites. We hold quarterly meetings over 2 days when we discuss issues based on a formal agenda and we spend one of the days having free discussion and contributory seminars.

I have been the country's representative to the Eastern Mediterranean Region (EMRO) WHO committee on Laboratory Services since 1992. I initiated and published the first

Newsletter for EMRO on Laboratory Instrument and Consumables Specification and Standardization which was circulated to all member counties in 1997. I recently took part in representing Oman in the hosting of a WHO Quality Assurance workshop and meeting in Oman.

TEACHING EXPERIENCE

While in Royal Hospital, teaching the postgraduate students in haematology. From June 1999 teaching Institute of Health Sciences students in Haematology till now.

CONFERENCES/ SYMPOSIUMS/COURSES

ATTENDED RECENTLY

2001 May	Executive war College on Lab. & Pathology Management course, Cincinatti, USA
2001 July	AACC, Chicago, USA
2001 November	MEDICA, Dusseldorf, Germany
2000 July	American Association of Clinical Pathology, San Francisco, USA
2000 November	AABB/53rd Annual Meeting, Washington D.C, USA
2000 November	MEDICA- Latest development in Lab. Equipment, Dusseldorf, Germany
2000 December	ASH, San Francisco, California, USA
1999 August	AABB Annual meeting in San Francisco, USA
1999 July	AACC in New Orleans, USA
1999 (01 Mar to 05 Mar)	DIAMED. Blood Transfusion Technique, Training. Motern, Switzerland.
1998 (Nov)	MEDICA Laboratory Instruments Exhibition, Dusseldorf, Germany
1998 (Oct/Nov)	American Association of Blood Banks (AABB) annual meeting , Philadelphia, USA
1998 (July)	American Association of Clinical Pathology, Chicago, USA
1997 (Oct)	AABB Management Course in Blood Bank, Denver, Colorado, U.S.A.

1997 (Jun)	American Association of Clinical Pathology, Atlanta, Georgia, U.S.A.
1997 (Mar)	Specialist Short Course on Diagnostic Haemopathology, Hammersmith Hospital, University of London
1996 (Dec)	ASH Meeting, - Haematology Laboratory Management, Orlando, Florida, U.S.A.
1996 (Nov)	MEDICA - latest developments in laboratory equipment, Dusseldorf, Germany
1996 (Oct)	AABB Management Course in Blood Banking, Orlando, Florida, U.S.A.
1996 (Aug)	American Association of Clinical Pathology Management, Chicago, U.S.A.
1996 (June)	Represented Oman in WHO (EMRO) Director of Laboratories Meeting, Damascus, Syria
1995 (Dec)	ASH Course in Haematology, Seattle, U.S.A.
1995 (Nov)	AABB Blood Transfusion Management, San Diego, U.S.A.
1994 (Dec)	American Society of Haematology, Annual Meeting, Nashville, U.S.A.
1994 (Nov)	Annual Meeting of the American Association of Blood Banks, San Diego, U.S.A.
1994 (July)	American Association of Clinical Pathology, New Orleans, U.S.A.
1994 (July)	Represented Oman in WHO (EMRO) Director of Laboratory Services Meeting, Cyprus
1994 (June)	Advanced Course in Haematology, Hammersmith Hospital, London
1994 (April)	WHO Workshop on Haemoglobinopathies, Riyadh, Saudi Arabia
1993 (June)	Advanced Course in Haematology, Hammersmith Hospital,

	London
1993 (March)	Blood Transfusion Management, Phoenix, Arizona, U.S.A.
1992 (May)	Represented Oman at WHO Quality Assurance in Laboratories Workshop and Meeting, Cyprus

14 MAP OF THE SULTANATE OF OMAN