CHAPTER 2

REVIEW OF LITERATURE

Neurodegenerative diseases are engulfing the world swiftly being the cause of enormous mortality and morbidity. For alleviating these escalating episodes, rigorous efforts towards discovering of valuable healthy foods may seem to be the only answer at present. Till date, the therapeutic and functional foods are feasibly acknowledged for their beneficial health effects in numerous disease conditions. Amongst these, vitamin B12 therapy and omega-3 rich foods such as flaxseeds are apparent to be the key therapeutic approaches having huge potential towards enhancing memory functions and might have a concrete function in reducing the burden of cognitive decline.

The categories of literature search available under this chapter are amassed as follows:

2.1 Global and national prevalence of neurodegenerative disorders

2.2 Dementia-prevalence, types, signs and symptoms, complications, treatment

2.3 Mild Cognitive Impairment- definition, criteria, types, prevalence, risk factors, progression cascade

2.4 Neuroimaging and biomarkers for MCI detection

2.5 Overview of brain –gut axis: the perplexing connection

2.6 Miscellaneous preventative strategies for Mild Cognitive Impairment

2.7 Vitamin B12, their types, clinical manifestations and aetiology of B12 deficiency, its types and normal absorption mechanism

2.8 Omega-3 fatty acids, their classification and associated health benefits

2.9 Emergence of flaxseeds as pro-active nutraceutical or functional food and augmenting effect in Mild Cognitive Impairment

The present chapter shall encompass, the literature pertaining to the emergence of Mild Cognitive Impairment and the effective strategies for preventing MCI. In perspective to this, the present study was designed with the title, "**Vitamin B12 and Omega-3 fatty acid Interventions for Cognition in Elderly- a V.O.I.C.E. trial**".

2.1 Global and National prevalence of neurodegenerative disorders

> ***"People with mental disorders experience disproportionately higher rates of disability and mortality. Prompt recognition and treatment of mental and neurological disorders in older adults is essential."***
> ***- WHO 2016***

Neurodegenerative disease is an umbrella term provided for the series of neuron affecting primary conditions in the human brain. *Neurodegenerative diseases are defined as debilitating and incurable conditions characterized by loss of neuronal cell function and often associated with atrophy of the affected central nervous system structures causing progressive nervous system dysfunction* (Griffin 2006). The nervous system includes the brain and spinal cord wherein the neurons serve as the building blocks. Neurons are irreproducible and irreplaceable by the human body, if damaged or dead. Examples of neurodegenerative diseases include Alzheimer's, Parkinson's and Huntington's disease. Neurodegenerative diseases are incurable and debilitating conditions result in progressive degeneration and / or death of nerve cells causing problems with movement (called ataxias), or mental functioning (called dementias) (European Union Joint Programme- Neurodegenerative Disease Research 2014).

According to the WHO Fact Sheet 2016, Dementia is a syndrome in which there is deterioration in memory, thinking, behaviour and the ability to perform everyday activities. Although dementia mainly affects older people, it is not a normal part of ageing. Worldwide, 47.5 million people have dementia and there are 7.7 million new cases every year. Alzheimer's disease is the most common cause of dementia and may contribute to 60–70% of cases. Dementia is one of the major causes of disability and dependency among older people worldwide. The total number of people with

dementia is projected to increase to 75.6 million in 2030 and 135.5 million in 2050, with majority of sufferers living in low- and middle-income countries. Dementia has significant social and economic implications in terms of direct medical costs, direct social costs and the costs of informal care. In 2010, the total global societal costs of dementia were estimated to be US$ 604 billion. This corresponds to 1.0% of the worldwide gross domestic product (GDP) or 0.6% if only direct costs are considered. The total cost as a proportion of GDP varied from 0.24% in low-income countries to 1.24% in high-income countries.

The world is noticing a rapid population ageing. The proportion of the world's older adults between 2015 and 2050 is estimated to almost double from about 12% to 22%. In absolute terms, this is an expected increase from 900 million to 2 billion people over the age of 60. There is a need for recognition of the special physical and mental health challenges which the older people face. Over 20% of adults aged 60 and over suffer from a mental or neurological disorder (excluding headache disorders) and 6.6% of all disability (disability adjusted life years-DALYs) among over 60s is attributed to neurological and mental disorders. These disorders in the elderly population account for 17.4% of Years Lived with Disability (YLDs). The most common neuropsychiatric disorders in this age group are dementia and depression (WHO 2016). The Table 2.1.1 illustrates the total number of DALYs (in thousands) associated with neurological disorders. Alzheimer and other dementias globally were 11.78 million in 2005 increased to 13.54 million in 2015 and expected to reach to 18.4 million by 2030, showing a 66% increase.

WHO (2006) reported that amidst neurological disorders, cerebrovascular disease accounted for more than half of the burden in DALYs, 12% was contributed by Alzheimer and other dementias and 8% each by epilepsy and migraine. Neurological disorders contributed to 10.9%, 6.7%, 8.7% and 4.5% of the global burden of disease in high, upper-middle, lower-middle and low-income countries, respectively. The higher burden in the lower-middle category reflects the double burden of communicable diseases and non-communicable diseases. For neurological disorders DALYs per 100 000 population were highest for lower middle and low income countries (1514 and 1448, respectively) (Table 2.1.2).

Table 2.1.1: Number of DALYs for neurological disorders and as percentage of global DALYs projection

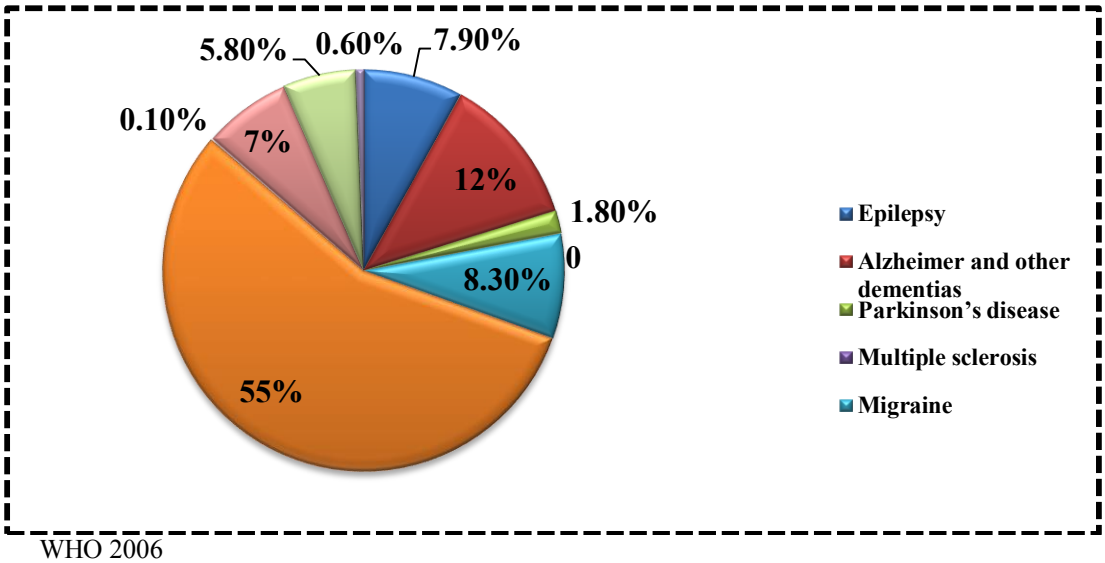
Cause category	2005		2015		2030	
	No. of DALYs (000^s)	Percentage of total DALYs	No. of DALYs (000^s)	Percentage of total DALYs	No. of DALYs (000^s)	Percentage of total DALYs
Epilepsy	7308	0.50	7419	0.50	7442	0.49
Alzheimer and other dementias	11078	0.75	13540	0.91	18394	1.20
Parkinson's disease	1617	0.11	1762	0.12	2015	0.13
Multiple sclerosis	1510	0.10	1586	0.11	1648	0.11
Migraine	7660	0.52	7736	0.52	7596	0.50
Cerebrovascular disease	50785	3.46	53815	3.63	60864	3.99
Poliomyelitis	115	0.01	47	0.00	13	0.00
Tetanus	6423	0.44	4871	0.33	3174	0.21
Meningitis	5337	0.36	3528	0.24	2039	0.13
Japanese encephalitis	561	0.04	304	0.02	150	0.01
Total	92392	6.29	94608	6.39	103335	6.77

WHO 2006

Table 2.1.2: DALYs per 100,000 population for neurological disorders globally and by World Bank income category

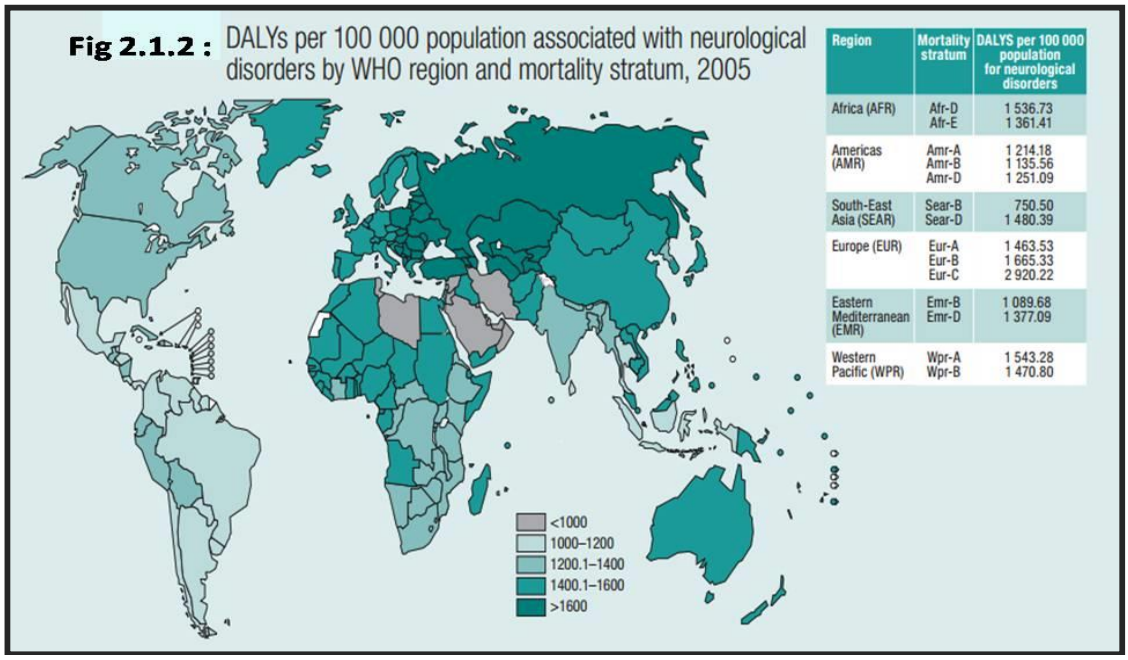
Cause category	World (100,000 population)	Income Category			
		Low	Lower Middle	Upper Middle	High
Epilepsy	113.4	158.3	80	139.2	51.3
Alzheimer and other dementias	172	90.7	150.7	166.9	457.3
Parkinson's disease	25.1	15.1	19.7	17.5	70.8
Multiple sclerosis	23.4	20.1	23.3	24.9	32.5
Migraine	118.9	114	106.8	147.1	146.3
Cerebrovascular disease	788.4	662.5	1061.2	612.2	592
Poliomyelitis	1.8	2.6	1.6	0.9	0.6
Tetanus	99.7	228.6	10.8	1.3	0.1
Meningitis	82.9	143.2	51.2	39.7	10.7
Japanese encephalitis	8.7	13	9	0.4	0.6
Total	1434.3	1448.1	1514.3	1150.1	1362.2

WHO 2006



WHO 2006

Figure 2.1.1: DALYs for individual neurological disorders as percentage of total neurological disorders



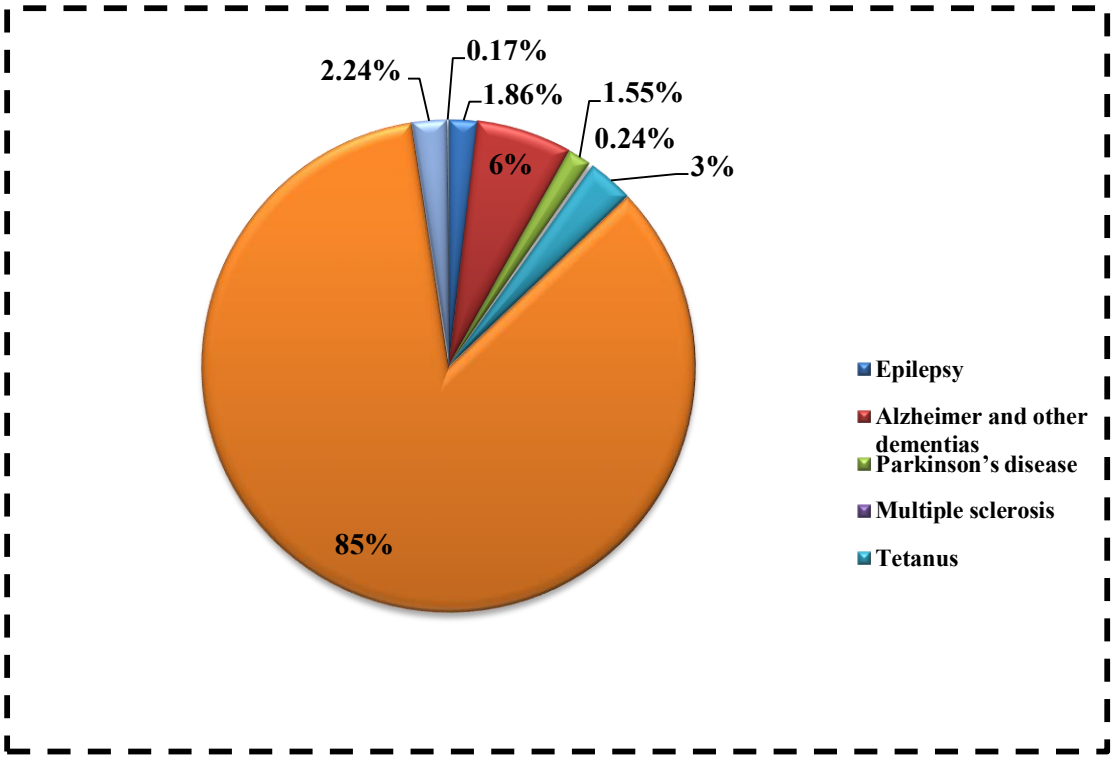
Region	Mortality stratum	DALYS per 100 000 population for neurological disorders
Africa (AFR)	Afr-D Afr-E	1 536.73 1 361.41
Americas (AMR)	Amr-A Amr-B Amr-D	1 214.18 1 135.56 1 251.09
South-East Asia (SEAR)	Sear-B Sear-D	750.50 1 480.39
Europe (EUR)	Eur-A Eur-B Eur-C	1 463.53 1 665.33 2 920.22
Eastern Mediterranean (EMR)	Emr-B Emr-D	1 089.68 1 377.09
Western Pacific (WPR)	Wpr-A Wpr-B	1 543.28 1 470.80

WHO 2006

Estimation of deaths by neurological disorders

Globally, neurological disorders constituted 12% of total deaths and were an important cause of mortality. It was observed that in lower- middle income countries, neurological disorders constituted 16.8% of the total deaths as compared to 13.2% of

the total deaths in high-income countries. In the year 2005 among the neurological disorders, Alzheimer's and other dementias constituted 2.84% of the total deaths in high-income countries. Cerebrovascular disease constituted 15.8%, 9.6%, 9.5% and 6.4% of the total deaths in lower-middle, upper-middle, high and low-income countries respectively (WHO 2006).



WHO 2006

Figure 2.1.3: Deaths from selected neurological disorders as percentage of total neurological disorders

Alzheimer and other dementias were estimated by WHO (2006) to cause 0.73% of the total deaths in the world with 0.34% in lower-middle income countries like India and the highest 2.84% in the higher income countries (Table 2.1.3).

Table 2.1.3: Deaths attributable to neurological disorders as percentage of total deaths by World Bank income category

Cause category	World (%)	Income Category			
		Low (%)	Lower Middle (%)	Upper Middle (%)	High (%)
Epilepsy	0.22	0.28	0.17	0.20	0.11
Alzheimer and other dementias	0.73	0.41	0.34	0.46	2.84
Parkinson's disease	0.18	0.06	0.18	0.15	0.60
Multiple sclerosis	0.03	0.01	0.02	0.05	0.10
Migraine	0	0	0	0	0
Cerebrovascular disease	9.90	6.41	15.81	9.64	9.48
Poliomyelitis	0	0	0	0	0
Tetanus	0.33	0.64	0.04	0.01	0.00
Meningitis	0.26	0.39	0.18	0.16	0.04
Japanese encephalitis	0.02	0.03	0.01	0	0
Total	11.67	8.23	16.77	10.67	13.18

WHO 2006

Epidemiology of neurodegenerative diseases in India

The prevalence rates from different Indian regions provided the rough estimates of over 30 million people with neurological disorders (excluding neuroinfections and traumatic injuries). This spectrum of neurological disorders ranged from 967–4,070 having a mean of 2394 per 100000 population (Gourie-Devi 2014). Few institutionalized and hospital-based studies stated psychiatric morbidity prevalence in older adults to be 49.28 per cent (Sood et al 2006) and 8.6 per cent in the geriatric population (Avasthi et al 1998). Similar studies determined 43.32 % psychiatric morbidity in rural elderly (Tiwari 2000), 49.2 % in urban area of New Delhi (Chowdhury and Rasania 2008) and 19.3% prevalence in urban elderly from Lucknow (Tiwari and Tripathi 2009). Gururaj et al 2005 also observed a wide range of estimates of neurodegenerative diseases ranging from 22 to 333 (per 1000 age-specific population). Previous study conducted on the elderly population by Shaji et al

(1995) in their Kerala based study on priority mental disorders reported the prevalence of 95/1000 population. Meanwhile, Nandi et al. (2000) found an astonishing 61% of their study subjects to have been 'mentally ill'. The higher rate from these surveys could possibly due to the studying of priority disorders in a rapidly greying population. The community-based study estimates are further hampered by the non-recognition of mental illness of the elderly while reporting of such diseases. Another meta-analysis study by Chandrashekhar and Isaac (1998) estimated the prevalence of mental disorders to be 31/1000 among the 60+ years age group.

2.2 Dementia –prevalence, types, signs and symptoms, complications, treatment

> ***"Rising life expectancy is contributing to rapid increases in numbers, and is associated with increased prevalence of chronic diseases like dementia. Proportionate increases in the number of people living with dementia will be much steeper in low and middle income countries than in high income countries."***
>
> **-ADI 2015**

Dementia is a collective name for progressive degenerative brain syndromes affecting memory, thinking, behaviour and emotions. Symptoms may include loss of memory, difficulty in finding the right words or understanding what people are saying, difficulty in performing previously routine tasks, personality and mood changes (Alzheimer's Disease International 2015). WHO (2016) defines dementia as a syndrome, usually of a chronic or progressive nature, caused by a variety of brain illnesses that affect memory, thinking, behavior and ability to perform everyday activities. Dementia is caused by a variety of diseases and injuries that primarily or secondarily affect the brain, such as Alzheimer's disease or stroke. The dementia has been the major topic of research interest since several decades. In one of the few early description by Folstein et al (1975) referred dementia as a clinical syndrome which is characterized by global deterioration of intellect, occurring in clear consciousness.

Worldwide Prevalence

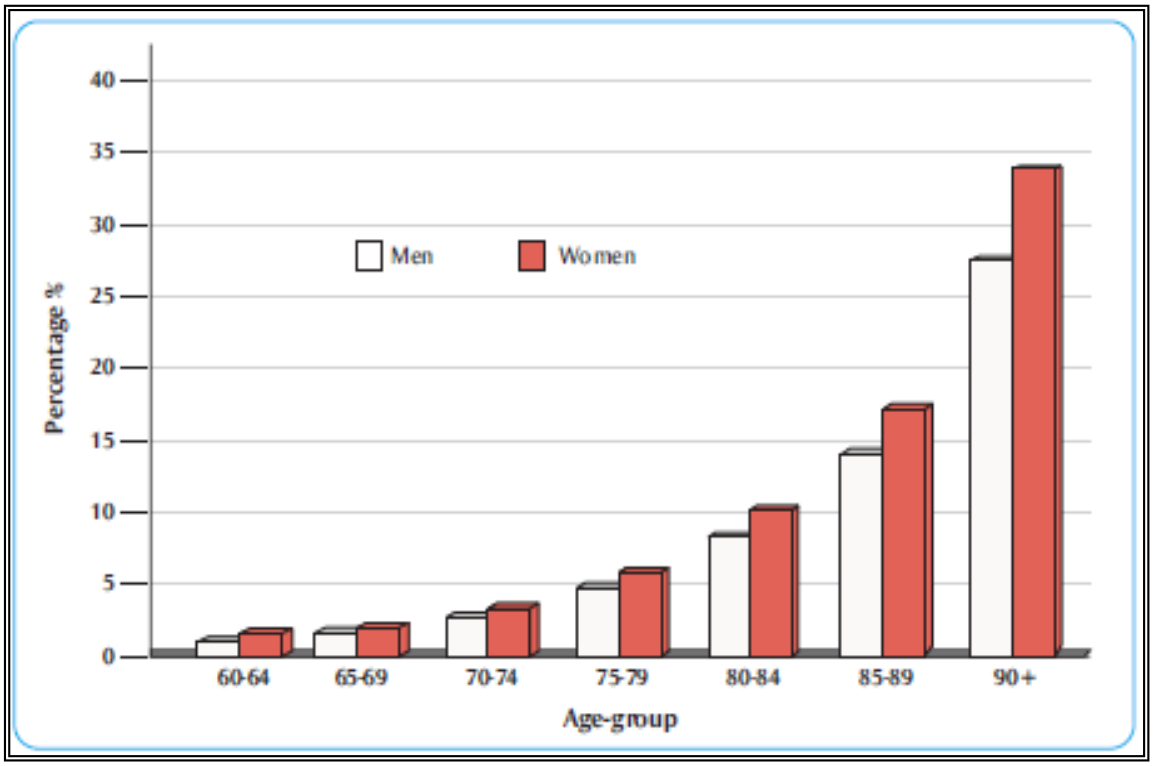
Today, over 46 million people live with dementia worldwide. This number is estimated to increase to 131.5 million by 2050 (ADI 2015). It is expected that this number will double in every 20 years. A new case is reported every three seconds. Much of the increase will take place in low and middle income countries (LMICs): in 2015, 58% of all people with dementia live in LMICs, rising to 63% in 2030 and 68% in 2050. According to the WHO (2016), currently, over 47.5 million people worldwide are affected with dementia. Nearly 7.7 million new cases of dementia are added worldwide each year, implying that 1 new case every 4 seconds. The general population aged 60 and over with dementia at a given time is estimated to have a proportion between 5 to 8 per 100 people. The increase in people with dementia has been expected to increase to 75.6 million in 2030 and 135.5 million in 2050. High dementia costs predicting future increase in cases will challenge the dealing health systems. ADI (2015) describes the number of peoplewith dementia based on the World Bank income wise listing of the countries where LMIC like ours has been projected to tremendously have 31.54 million people with dementia by 2050 (Table 2.2.1).

Table 2.2.1: Number of people with dementia (millions) according to 2015 World Bank Classification

World Bank Income Group	Number of people with dementia in millions							
	2015	**2020**	**2025**	**2030**	**2035**	**2040**	**2045**	**2050**
Low Income	1.19	1.42	1.68	2.00	2.41	2.90	3.55	4.35
Lower Middle Income	9.77	11.52	13.72	16.35	19.48	23.12	27.18	31.54
Upper Middle Income	16.32	19.36	23.33	28.39	34.28	40.43	46.90	53.39
High Income	19.50	21.97	24.73	27.95	31.72	35.71	39.14	42.18
World	46.78	54.27	63.45	74.69	87.88	102.15	116.78	131.45

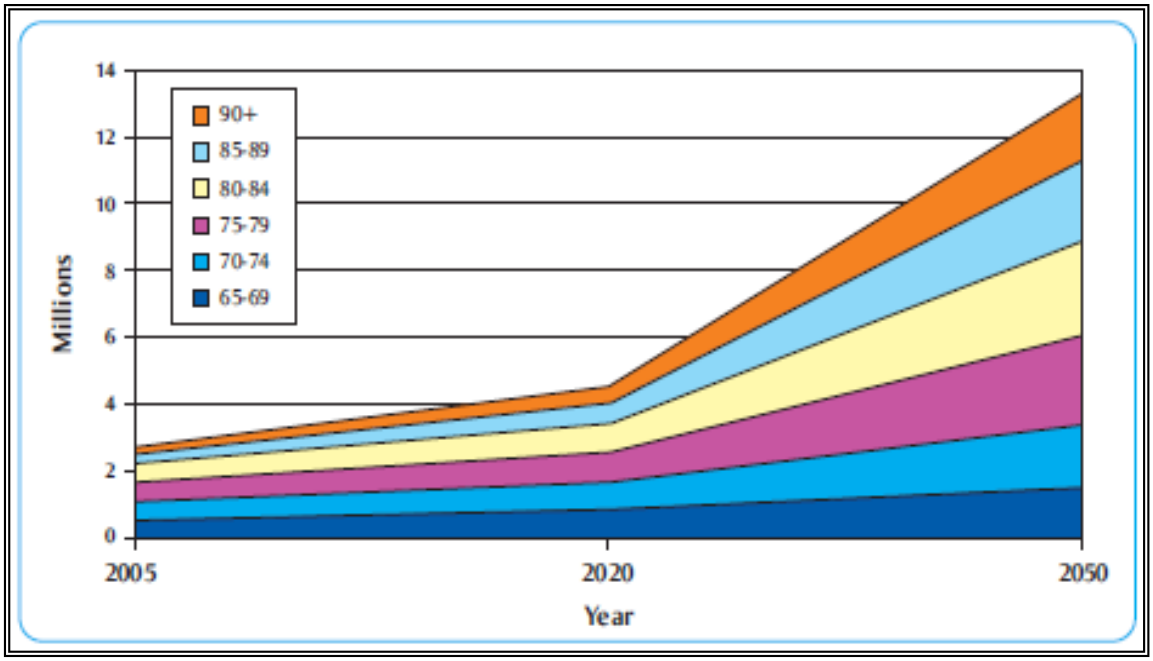
ADI 2015

An estimated 3.7 million Indian people aged over 60 have dementia (2.1 million women and 1.5 million men). Steady increases in the prevalence rate of dementia with increase in age were observed more among older women than men. But, age-specific studies have revealed no significant difference in the incidences of dementia among older women and men. Therefore, ruling out that gender does not appear to be a risk factor for Alzheimer's disease (AD) or dementia for the older age group. People in younger age groups, 60-75 years, are expected to observe a steady rise in dementia over time and a sharp increment amongst age groups over 75 years can be predicted after 2030. The number of persons with dementia is projected to increase every year because of the stable growth in the older population and constant increment in life expectancy. Thus, an estimated twofold increase by 2030 and threefold by 2050 can be anticipated. Dementia UK Report (2007) projected UK to have 1 million people with dementia by the year 2025 (Knapp et al 2007). According to current estimates, India has more than 3 million people with dementia and is projected to overtake USA in number of people by 2015 [Figure 2.2.1 (a) - (d)].



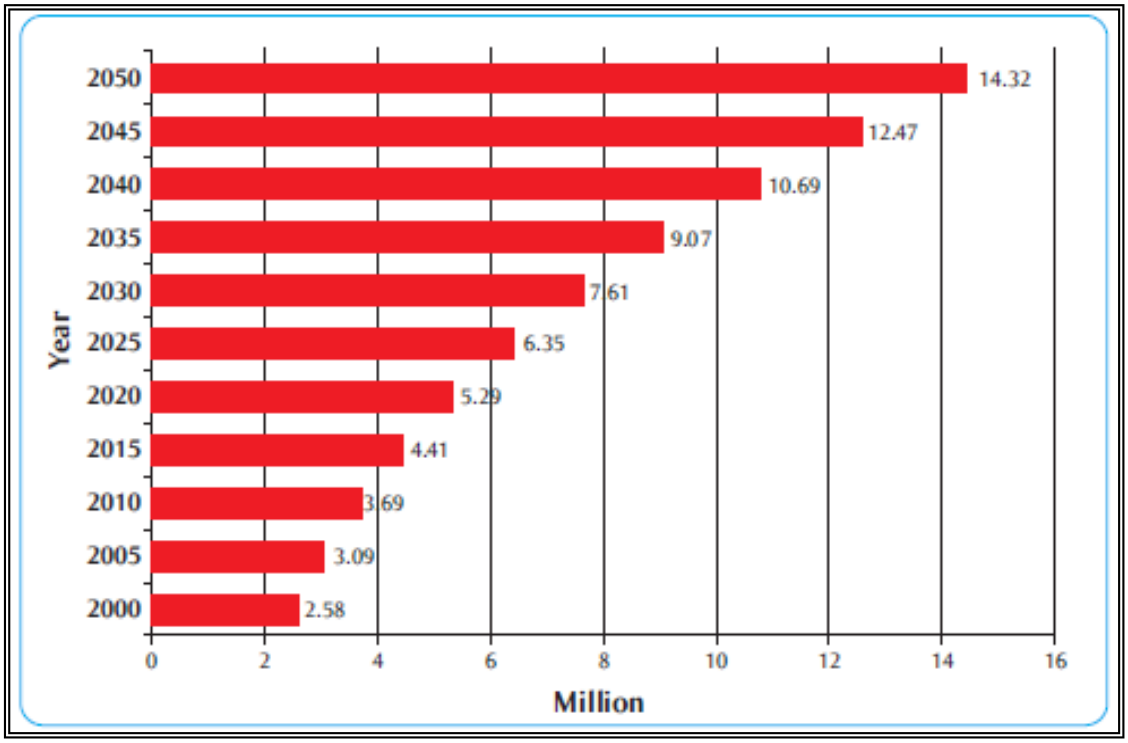
ARDSI 2010

Figure 2.2.1(a): Prevalence of Dementia in India, 2010



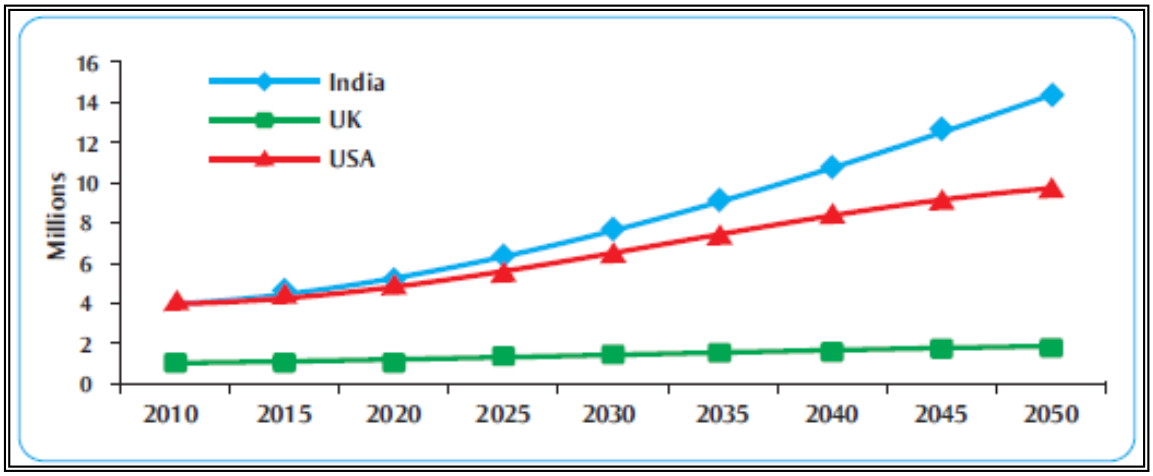
ARDSI 2010

Figure 2.2.1(b): Trend in dementia prevalence in age over time



ARDSI 2010

Figure 2.2.1(c): Estimation of number of people with dementia over 60 years in India between 2000 and 2050



ARDSI 2010

Figure 2.2.1(d): Estimation of people with dementia > India, US, UK

India stood at the third position with 4.1 million amongst the top ten countries being home to over a million people with dementia after China (9.5 million) and USA (4.2 million) according to the revised 2015 estimates (Alzheimer's Disease International, 2015). India will experience relatively rapid growth trends owing to its massive population base and higher demographic ageing. According to the WHO estimates, almost 60% of the demented persons live in low- and-middle income countries. Majority of this increase can be attributed to the rising numbers of persons in low- and middle-income countries living with dementia (WHO 2016). Dementia affects older people chiefly, although about two per cent of cases begin before the 65 years of age. The prevalence doubles every five years with over a third of all people aged 90+ years being affected. There is an exponential increase in the Indian elderly (60+ years) population and is expected to rise to 198 million in 2030 and 326 million in 2050, dementia poses an alarming public health challenge, whose severity is underscored (ARDSI 2010).

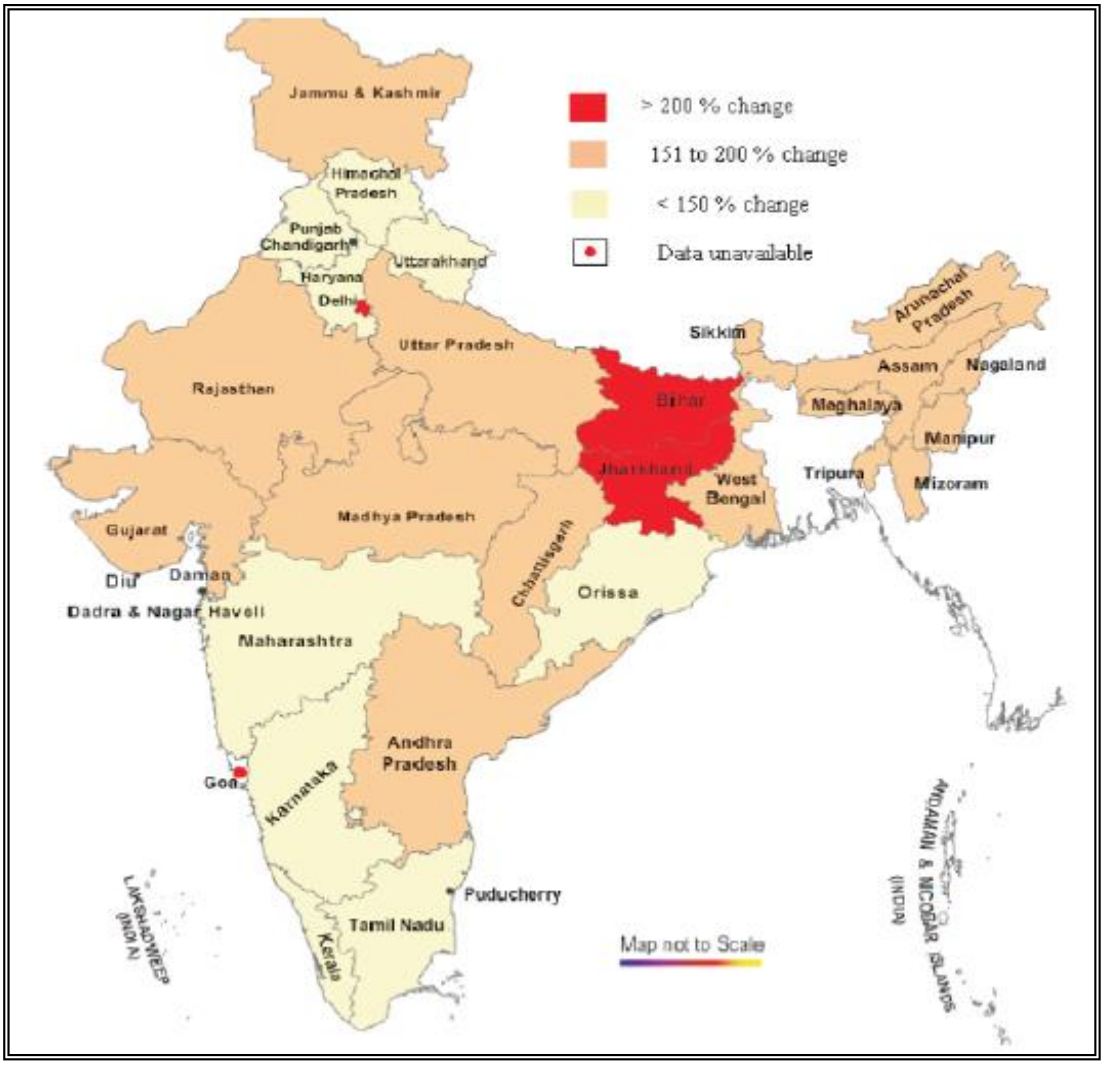
The first attempt in India for multi-centric dementia diagnosis was in 1999, by the 10/66 Dementia Research Group who launched a large-scale pilot study in 25 centers in LMIC (lower-middle income countries) wherein developing and validating the 10/66 dementia diagnosis as a tool was performed especially suited for studies based in low education populations in the developing world or designed to make valid

comparisons across countries and cultures (Prince et al 2003). The subsequent population-based study in Chennai, diagnosed 75 people with 10/66 dementia and 193 with cognitive impairment but no dementia (CIND) reassessing for the criteria of diagnostic status, clinical severity, cognitive function, disability and needs for care (Jotheeswaran et al 2010). A large amount of Indian population-based studies reported a low prevalence of dementia compared to the American or European studies (Ferri et al 2005). Few of the geriatric psychiatric regional community-based studies studies focusing on dementia provide substance into this disorder to be at varying levels from 0.8 to 4.1 per cent in those aged above 60years. The prevalence of dementia was found to be 3.5% in population aged above 60 years from rural Chennai (Rajkumar et al. 1997) while another study in urban South India showed the overall prevalence of 3.4% (Shaji et al. 2005). A study from rural North India reported a prevalence of 1.36% among those 65+ years of age (Chandra et al. 1998) and further added with the prevalence rate of 1.8% for males and 1.25% for females. The study in Kashmir suggested 1.83% prevalence for the elderly over 60 years (Raina et al 2010). Vas et al. (2001) in Mumbai found the prevalence of dementia to be 2.44% for those over 65 years of age while another from urban Pune demonstrated a higher prevalence of 4.1% in the same age group (Saldanha et al 2010). In similar studies from urban Kolkata, the prevalence came to be 0.8% (Das et al 2008) for those aged 60 years and 1.28% (Banerjee et al 2008) in those above 60 years of age. The different Indian regions of India differ widely in terms of the dementia prevalence rates (PRs) as being evidenced from the several regional studies. The multiethnic, multicultural, and environmental differences may be true for consideration of a common protocol and conduction of multi-centric study on dementia prevalence and incidence may neglect distinctive region specific differences (Das et al 2012).

Very few long-term studies on dementias and cognitive dysfunction have been carried out in India. One of the North Indian study on AD depicted an incidence of 4.7 per 1000 person-years as compared to 17.5 per 1000 person-years in Monongahela valley, USA being the twin studies with similar methodology but contrary in life expectancy and sample literacy (Chandra et al 2001). An incidence study in Dogra population of

North India documented an incidence rate of 5.34 per 1000 person-years (Raina et al 2009).

ARDSI (2010) in their state-wise estimates made using meta-analyzed prevalence estimation for India projected the number of people aged 65 and older with dementia for years 2011, 2016 and 2026 to fluctuate by state and region and corresponding variability in number of people with dementia was also observed. The percentage change in dementia between base year 2006 and each of the subsequent time periods were analysed. By 2026, more than 500,000 older people with dementia are expected to be living in Uttar Pradesh and Maharashtra. In other states like Rajasthan, Gujarat, Bihar, West Bengal, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu round about 200,000 to 400,000 people with dementia are expected within the next 26 years. As compared to 2006, Delhi, Bihar and Jharkhand are expected to experience 200% (or greater) increment in total number of dementia cases over the 26 year period (Figure 2.2.2). Other states (Jammu and Kashmir, Uttar Pradesh, Rajasthan, Madhya Pradesh, West Bengal, Assam, Chhattisgarh, Gujarat, Andhra Pradesh, Haryana, Uttaranchal, Maharashtra, Karnataka and Tamil Nadu) are predicted to experience 100% (or more) change in number of older persons with dementia. The rapidly increasing number of people with dementia will pose a striking impact on infrastructural and healthcare facilities of these states, which in several regions presently remain ill prepared. The projected increases for the Southern region are not as marked as in other Indian regions, however, large percentage of population aged 65 would result in more demented people (Figure 2.2.2).



ARDSI 2010

Figure 2.2.2: Projected changes between 2006 and 2026 in number of people living with dementia by state

Dementia – The Gujarat Scenario

There is an acute scarcity of data on dementia prevalence in relation to the Gujarat region. The estimates reveal that an approximate number of 20,000 to 40,000 people are projected to be having dementia within the time of next 26 years. Meanwhile, the Gujarat state is also projected to notice 100% (or more) alterations in the number of elderly to be suffering with dementia (ARDSI 2010).

As far as MCI is concerned, there is deeper lack of the studies carried out in Gujarat state per se for giving the insight on the prevalence data of this expansive disorder

mainly affecting disorder amongst the elderly population. But, few studies have been carried out in Gujarat region and summarised in the Table 2.2.2 below:

Table 2.2.2: Studies carried out in the Gujarat region (Vadodara) for prevalence estimation of lower cogntion status

Researcher	Year	Population Setting	Percent prevalence of lower cognition levels
Chauhan et al	2013	Urban	40
Chauhan et al	2012	Urban	30
Chauhan et al	2012	Institutionalised	47.7
Vasavda et al	2010	Urban	50 moderately depressed
Mehta et al	2009	Institutionalised	34.2 moderately depressed
Chauhan et al	2007	Urban	21
Chauhan et al	2007	Institutionalised	28 moderately depressed
Mehta et al	2006	Urban	24.8 moderately depressed

Types of dementia

Neurodegenerative disorders such as Alzheimer's disease (AD), frontotemporal disorders, and Lewy body dementia result in a progressive and irreversible loss of neurons and brain functions. Currently, there are no cures for these progressive neurodegenerative disorders. The National Institute on Aging (2013) defines the following types of dementia:

i. Alzheimer's disease: In some dementias, a protein called tau clumps together inside nerve cells in the brain, causing the cells to stop functioning properly and die. In AD, the tau protein becomes twisted and aggregates to form bundles, called neurofibrillary tangles, inside the neurons. Abnormal clumps (plaques) of another protein, called amyloid, are prominent in spaces between brain cells and are a hallmark of the disease.

ii. Vascular dementia: Vascular dementia and vascular cognitive impairment (VCI) are caused by injuries to the vessels supplying blood to the brain. These disorders can be caused by brain damage from multiple strokes or any injury to the small vessels carrying blood to the brain. Vascular dementia and VCI arise as a result of risk factors that similarly increase the risk for cerebrovascular disease (stroke), including atrial fibrillation, hypertension, diabetes, and high cholesterol. Symptoms of vascular dementia and VCI can begin suddenly and progress or subside during one's lifetime.

iii. Lewy body dementia: This involves protein aggregates called Lewy bodies, balloon-like structures that form inside of nerve cells. The initial symptoms may vary, but over time, people with these disorders develop very similar cognitive, behavioral, physical, and sleep-related symptoms. Lewy body dementia is one of the most common causes of dementia, after Alzheimer's disease and vascular disease.

iv. Frontotemporal disorders (FTD) are caused by a family of brain diseases that primarily affect the frontal and temporal lobes of the brain; they account for up to 10 percent of all dementia cases. Some, but not all, forms of FTD are considered tauopathies. In some cases, FTD is associated with mutations in the gene for tau (MAPT), and tau aggregates are present.

Table 2.2.3: Common subtypes of irreversible dementia

Dementia Subtypes	Early, characteristic dementia	Neuropathology	Proportion of symptoms case
Alzheimer's disease	Impaired memory, apathy and depression. Gradual onset	Cortical amyloid plaques and neurofibrillary tangles	50-75%
Vascular dementia	Similar to AD but memory less affected and mood fluctuations more prominent. Physical frailty Stepwise progression.	Cerebro-vascular disease Single infracts in critical regions or more diffuse multi-infarct disease	20-30%
Dementia with Lewy bodies (DLB)	Marked fluctuations in cognitive ability, visual hallucinations, Parkinsonism (tremor and rigidity)	Cortical Lewy bodies (alpha-synuclein)	<5%
Frontotemporal dementia (FTD)	Personality and mood changes, Disinhibition, Language difficulty	No single pathology damage limited to frontal and temporal lobes	5-10%

Signs and symptoms

Acccording to WHO fact sheet (2016), dementia affects each person in a different way, depending upon the impact of the disease and the person's personality before becoming ill. The signs and symptoms linked to dementia can be understood in three stages.

i. *Early stage:* the early stage of dementia is often overlooked, because the onset is gradual. Common symptoms include:
   - Forgetfulness
   - Losing track of the time and becoming lost in familiar places.

ii. *Middle stage***:** as dementia progresses to the middle stage, the signs and symptoms become clearer and more restricting. These include:
   - Becoming forgetful of recent events and people's names
   - Becoming lost at home
   - Having increasing difficulty with communication
   - Needing help with personal care and experiencing behaviour changes, including wandering and repeated questioning.

iii. *Late stage***:** the late stage of dementia is one of near total dependence and inactivity. Memory disturbances are serious and the physical signs and symptoms become more obvious. Symptoms include:
   - Becoming unaware of the time and place
   - Having difficulty recognizing relatives and friends
   - Having an increasing need for assisted self-care
   - Having difficulty walking and experiencing behaviour changes that may escalate and include aggression.

Complications

Dementia being the major cause of disability and dependency among older people worldwide is devastating not only for the patients who are suffering but also for their caregivers and families. There is often an acute lack of awareness and understanding regarding dementia, resulting in stigmatization and obstacle to diagnostic care. The

impact of dementia on caregivers, family and societies can be physical, psychological, social and economic (WHO 2016).

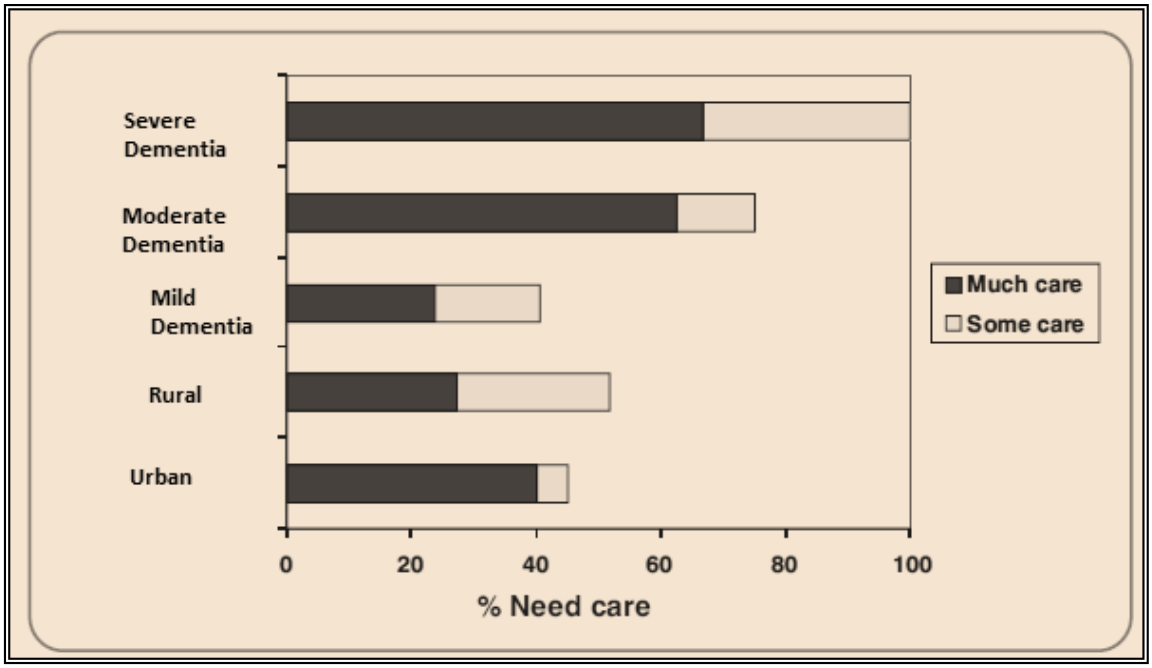
Treatment and care

WHO fact sheet (2016) describes that there is no treatment currently available to cure dementia or to alter its progressive course. Numerous new treatments are being investigated in various stages of clinical trials. Much can be, however, offered to support and improve the lives of people with dementia and their caregivers and families. The principal goals for dementia care are:

- Early diagnosis in order to promote early and optimal management
- Optimizing physical health, cognition, activity and well-being
- Identifying and treating accompanying physical illness
- Detecting and treating challenging behavioural and psychological symptoms
- Providing information and long-term support to caregivers.

Dementia care- who are the targets?

Nearly all diagnostic definitions state that all persons with dementia experience functional disability to a certain degree. But, this does not imply here that they all should be requiring care as assessed in the 10/66 Dementia Research Group's population-based studies in Latin America, India and China. The needs for care among those with dementia (Clinical Dementia Rating 1 or above indicating mild or > mild dementia) from the Indian centers are summarized in Figure 2.2.3. Among 50 and 70% of persons with dementia in most places needed 'much care' being changing by level of dementia, having 30% with mild dementia, 69% with moderate dementia, and 88% with severe dementia who required much care. In the urban Indian centre of this study, 78.5% of those with dementia needed much care. In the case of the rural Vellore, proportion requiring great care was 33.3 % (ARDSI 2010).



10/66 Dementia Research Group population-based studies, data release 2.2

Figure 2.2.3: Need for care among people with dementia

2.3 Mild Cognitive Impairment- Definition, Criteria, Types, Prevalence, Risk Factors , Progression Cascade

"Mild cognitive impairment (MCI) and dementia are reaching epidemic proportions in Asia. Lack of awareness and late presentation are major obstacles to early diagnosis and timely intervention."

-Ho et al 2015

Mild cognitive impairment referred to the transitional state between the cognitive changes of normal aging and the fully developed clinical features of dementia (Petersen 2007). Mild cognitive impairment (MCI) is a syndrome of cognitive decline greater than expected for an individual's age and education level but that does not interfere notably with activities of daily life (Gauthier et al 2006). The concept of MCI has been evolving in an ever changing fashion. The first attempt towards the characterization of cognitive changes with the normal tail dates back to 1962, when Kral used the term "benign senescent forgetfulness" to describe early memory concerns with aging (Kral 1962). Successive to this was the National Institute of

Mental Health workgroup in 1986 who proposed the term "age-associated memory impairment" (AAMI) referring to the memory changes felt as a variant of normal aging. AAMI included impairment restricted only with the memory domain and comparing the memory function in older adults to the performance of young adults, which served as its shortcomings. AAMI was powerless for demarcating individuals at risk of developing pathological conditions from those undergoing the normal aging processes. The term "age-associated cognitive decline" was coined by the International Psychogeriatric Association in an effort to bypass many of the AAMI's recognized shortcomings (Levy 1994). Operational criteria for age-associated cognitive decline indicated a variety of cognitive domains presumed to decline in normal aging and included age- and education-adjusted normative values. Then again, the Canadian Study of Health and Aging coined the term "cognitive impairment-no dementia" (CIND) describing persons with impaired cognitive function but possessing insufficient severity to constitute dementia. This CIND label in fact includes a wider population subset and in various respects these "in-between" persons resemble MCI subjects but (Graham et al 1997). CIND assemblage encompasses individuals with lifelong cognitive impairment, static encephalopathy, and learning disability. Few investigators have defined subsets of CIND individuals very closely resembling the MCI subjects (Fisk et al 2003).

In the late 1980s, the term 'MCI' was initially used by Reisberg and colleagues for describing individuals with a Global Deterioration Scale (GDS) rating of 3 (Reisberg et al 1982; Flicker et al 1991; Reisberg et al 1988). Amongst the various definitions been proposed to ascertain the clinical signs and symptoms attributed to the earliest stages of dementia, the Mayo Clinic description of MCI, launched by the seminal works of Petersen and collaborators (1999) is perhaps the most widely used term in the recent literature (Forlenza et al 2010). Originally, this definition stressed on the presence of memory complaints, with objective demonstration of lower than expected memory tests performance; global preservation of intellectual function and no evidence of functional impairment. A higher risk to progress to AD upon follow-up (approximately 10% per year) was attributed to subjects diagnosed as with MCI. Subsequently, the definition of MCI was broadened encompassing deficits in other cognitive domains, such as language, attention and executive functions, and also to

differentiate cases with association of deficits on one, two or more cognitive domains (that is, amnestic and non-amnestic, single-domain or multiple-domain MCI) (Petersen et al 2001; Winblad et al 2004). Therefore, MCI has emerged as the representative stage of impairment beyond what is considered normal for age, but of insufficient magnitude so as to affirm the diagnosis of dementia or AD (Petersen 2003).

MCI is a transitional stage between normal aging and dementia. Persons with MCI are at higher risk to develop dementia. Identifying MCI from normal aging has become a priority area of research. Neuropsychological assessment could help to identify these high risk individuals (Tripathi et al 2015). Among persons with incident MCI, patterns of neuropsychiatric symptoms may increase the likelihood of progression to dementia (Forrester et al 2015).

The rise in incidence and prevalence with age is known, but interesting to see is that the incidence and prevalence do not rise in a parallel manner with age as simple logic would assume. Between the ages of 60 and 90, the incidence in men increases two times and in women 41 times, prevalence increase in men is 55.25-fold and in women 77-fold. Regarding the women/men ratio, the incidence is 20.5-fold increased, and prevalence is merely 1.3936-fold increased. These numbers raise concerns about the evolution of the disease. Regarding mild cognitive impairment (MCI)/AD ratio, only about 1 in 2 people get AD (raising) issues about the pathogenic disease relatedness (Cornutiu 2015). Individuals with MCI those who have memory impairment as a prominent feature in their cognitive profile (i.e. amnestic MCI) have the highest probability of developing Alzheimer's disease in the future (Fields et al 2011). The rationale for the study of MCI is consequentially based on the assumption that the sooner a degenerative process one intervenes, the more likely the damage done to the central nervous system can be prevented. As such, early diagnosis becomes paramount in trying to prevent subsequent disability (Petersen et al 2008). Identification of MCI is thought to be crucial to early intervention. MCI is associated with an increased risk of dementia (Petersen et al 2001), as well as with future disability (Purser et al 2005) and mortality (Hunderfund et al 2006).

MCI syndrome possesses an expression of an incipient disorder that may lead to dementia, is extremely heterogeneous and may coexist with systemic, neurologic, or psychiatric disorders that can cause cognitive deficits. The different clinical forms of MCI presentation of the syndrome were accounted by the clinical criteria designed (Lopez 2013). The critical contemporary challenge in the management of AD is to establish its early diagnosis, or in the ideal sense to identify the cases of AD prior to the actual onset of dementia. This necessitates towards development of new diagnostic tools predicting the dementia outcome is required among older people with very mild symptoms of cognitive dysfunction, or even asymptomatic individuals. Although a few promising and experimentally validated methods have been available, the translation of the current knowledge into clinical practice still requires methodological pruning and guidance by operational criteria (Forlenza et al 2010).

The classification of MCI has been given by Petersen et al (2004; 2009) to identify the disease spectrum including impairment in both memory and non-memory cognitive domains: Cognitive complaints, decline or impairment; objective evidence of impairment in cognitive domains; essentially normal functional activities; not demented.

Table 2.3.1: Criteria for MCI and CIND

MCI ▪ Cognitive complaint, cognitive decline or impairment ▪ Objective evidence of impairment in cognitive domains: memory, executive function/attention, language, or visuospatial skills ▪ Essentially normal functional activities ▪ Absence of dementia
CIND ▪ Participant or informant-reported significant decline in cognition or function; ▪ Physician-detected significant impairment in cognition ▪ Cognitive test score(s) at least 1.5 SD below the mean of published norms ▪ No clinically important impairment in activities of daily living assessed by physician/informant ▪ Absence of dementia

Petersen et al 2004; 2009

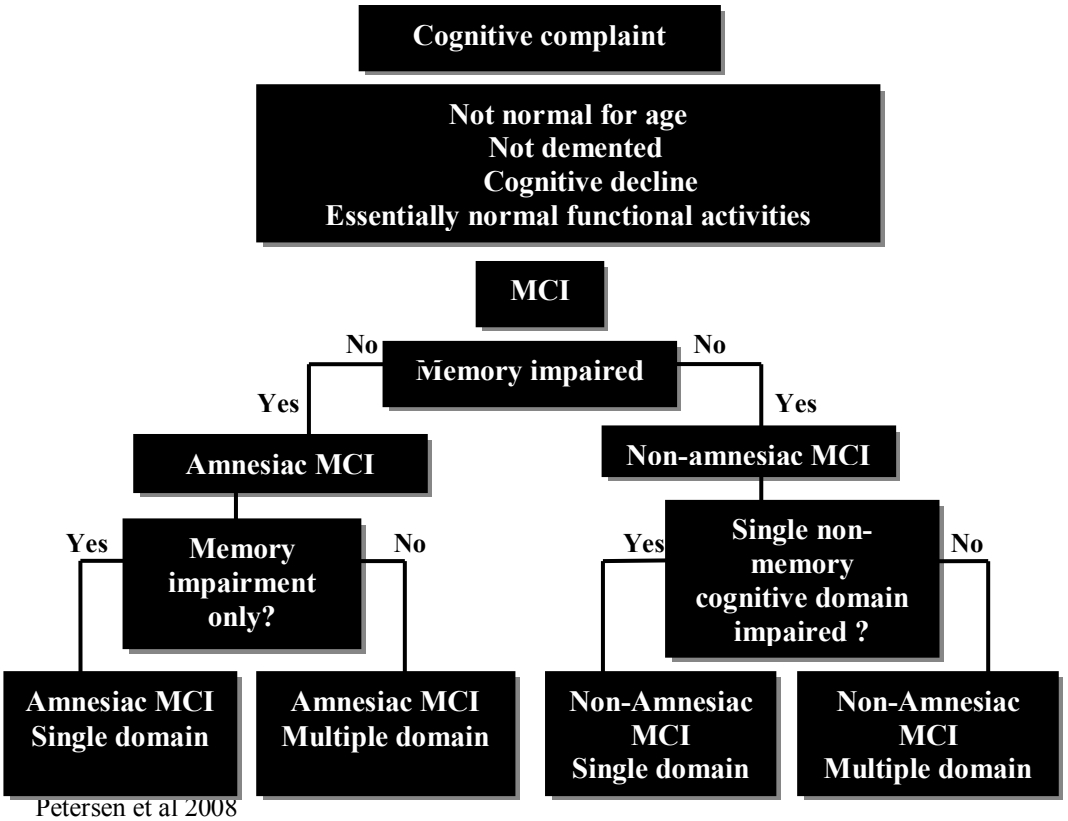
The International Psychogeriatric Association and the World Health Organization proposed the term “age-associated cognitive decline” (AACD) to describe the subjects with a wider range of cognitive deficits (Levy 1994).

Table 2.3.2: Criteria developed to characterize cognitive impairments in non-demented elderly subjects

Criteria	Year
Benign senescent forgetfulness	1962
Age-associated memory impairment	1986
Late-life forgetfulness	1989
Mild cognitive impairment	1991
Mild cognitive decline[a]	1993
Age-associated cognitive decline	1994
Age-related cognitive decline	1994
Mild neurocognitive decline[a]	1994
Cognitive impairment no dementia	1995
Mild cognitive impairment	1996
Modified mild cognitive impairment (four subtypes[b])	2004
Modified mild cognitive impairment (three subtypes[c])	2004
Diagnostic guidelines for mild cognitive impairment due to Alzheimer disease from the National Institute on Aging and Alzheimer's Association[d]	2011

[a] Criteria developed to identify mild cognitive deficits in subjects with neurologic or medical disorders.
[b] (1) Deficits in memory functions, (2) deficits in memory functions plus another cognitive domain, (3) deficits in a single nonmemory domain, and (4) deficits in more than one nonmemory domain.
[c] (1) MCI amnestic, (2) MCI multiple domain, and (3) MCI single nonmemory domain.
[d] Evidence of lower performance in one or more cognitive domains (ie memory, executive functions, language, visuospatial functions) for the patient's age and education level.

Lopez 2013



Petersen et al 2008

Figure 2.3.1: Diagnostic algorithm for diagnosing and subtyping MCI

Types of MCI

Basically, two subtypes of MCI have emerged: a clinical presentation with memory impairment is characterized as amnestic MCI (aMCI), whereas the absence of memory impairment with presence of impairment in one or more non-memory cognitive domains including executive function/attention, language, and visuospatial skills domains, is characterized as non-amnestic MCI (naMCI). This classification by subtype relates to the underlying etiology and pathology, the clinical presentation, and outcomes (Table 2.2.5). In addition, MCI may consist of impairment in a single cognitive domain or multiple cognitive domains. The number of affected domains has important implications for understanding the extent of the underlying brain disease or pathology, disease severity, and likelihood of progression to dementia. Multiple-domain MCI denotes a greater extent of disease than single domain MCI, which in turn has implications for a higher rate of progression from MCI to dementia. Information from both the MCI phenotype (aMCI vs. naMCI) and the number of cognitive domains affected (single vs. multiple) is hypothesized to determine future outcomes. Single or multiple domain aMCI is hypothesized to progress to AD if there is an underlying degenerative etiology. naMCI may progress to non-AD dementias such as frontotemporal dementia if a single domain is affected with a degenerative etiology or dementia with Lewy bodies if multiple domains are affected with a degenerative etiology (Petersen 2009). The impairment that encompasses subjects meeting criteria for MCI as well as others who are cognitively impaired but do not meet all the criteria for MCI is defined as cognitively impaired, not demented (CIND) (Graham et al 1997; Unverzagt et al 2001; Plassman et al 2008).

Table 2.3.3: MCI subtypes by etiology, pathology, presentation and outcomes

Variables	Amnestic	Non-amnestic
Aetiology	Neurodegenerative disease	Vascular damage
	APOEε4	Cerebrovascular disease
	Neurodegenerative	Cerebrovascular
Pathology	Amyloid β plaques	Cortical infarctions
	Neurofibrillary tangles	Subcortical infarctions
	Hippocampal atrophy	White matter hyperintensities
	Reduced brain volume	
Presentation	Memory impairment present	Impairment in non-memory domains
Long term outcomes	Alzheimer's dementia (AD)	Non-Alzheimer dementia: Vascular dementia
		Lewy body, Frontotemporal

Roberts et al 2013

Some people with MCI seem to remain stable or return to normal over time, but more than half progress to dementia within 5 years. MCI can thus be regarded as a risk state for dementia, and its identification could lead to secondary prevention by controlling risk factors such as systolic hypertension. The amnestic subtype of MCI has a high risk of progression to Alzheimer's disease, and it possibly could constitute a prodromal stage of this disorder (Gauthier et al 2006).

MCI Subtypes

	Etiology			
	Degener-ative	**Vascular**	**Psychiatric**	**Medical conditions**
Single domain Amnestic MCI	**AD**		**Depr**	
Multiple domain	**AD**	**VaD**	**Depr**	
Single domain Non-amnestic MCI	FTD			
Multiple domain	DLB	VaD		

Petersen et al 2008

MCI=mild cognitive impairment; AD=Alzheimer's disease; Depr=depression; VaD=vascularvdementia; FTD=frontotemporal dementia; DLB=dementia with Lewy bodies.

Figure 2.3.2: Predicted outcome of MCI subtypes according to presumed aetiology

MCI – The Global Scenario

Hanninen et al (2002)in their population-based sample study in (Kuopio) Finland on 806 elderly aged 60-76 years reported the overall MCI prevalence of 6.5% with those in the age of 60–64 years to be 2.4%, 65–69 for 4.8% and 8.4% for those in the 70–76 years of age. Out of which the males constituted as 7.1% and women were 4.1%. In the population-based, prospective community dwelling cohort named as the Leipzig Longitudinal Study of the Aged, Germany by Busse et al (2003) on a number of 929 subjects more than 75 years found the total MCI prevalence of 5.1%.The subjects aged 75–79years were found to be 5.6% and more than 85 years were 5.2%. A similar prospective, population-based, multi-ethnic Cardiovascular Health cohort (US) by Lopez (2003) on 2470 urban subjects aged >75 years revealed the overall 18.8% to be having MCI with 75-79 year olds to be 14.7% , 80-84 to be 22.6% and ≥85 were 25.9%. Males were 19% and females were 18.7%. Similar studies have also estimated the MCI prevalence falling in the range between to be 16% -20% (Petersen

et al 2010; Ganguli et al 2010). But, there is also the evidence from few studies to have described very high estimates that could be due to issues with non-participation or elements peculiar to the study (Sachdev et al 2012). Moreover, the estimates derived from the studies conducted in urban sites, multiethnic cohorts, and in clinic-based studies were also at the higher end of the spectrum of up to 42% (Manly et al 2005; Luck et al 2007; Sachdev et al 2012 and Artero et al 2008).

MCI – The Indian Scenario

The first ever incidence of MCI in India was determined by Das et al (2007) in their urban Kolkata based study cross-sectional study on amnestic MCI meeting the multiple domain criteria [*]on 745 subjects suggested the total prevalence of aMCI to be 14.9% with 65-69 year olds to be in the 11.7% bracket, 70-74 years in 12.3%, 75-79 in 17.9% and ≥80 years of age to be 10.6%. The males were 7.6% and females represented to be 4.5% whereas 6.3% males and 11.4% females were affected with the multiple domain MCI. Hence, MCI is emerging as a major health problem as the people with MCI are three to four times more likely to develop Alzheimer's disease (6% to 25% annually). Various studies have shown that the prevalence of MCI lies between 0.5% and 36% depending upon the diagnostic criteria used and nature of the study population (Singh 2014). Estimating the population prevalence of MCI in LAMICs is a public health priority as rapid demographic ageing is predicted to result in a large majority of people residing in these regions being at risk of dementia and cognitive decline. If so, this will have significant implications with regard to social support and future health care costs, especially as systems are not in place to cope with increased neurodegenerative disease and health resources at present are already extremely limited (Sosa et. al 2012).

Risk factors for MCI

The quest for the viable risk factors for causation of MCI has been ongoing since several decades as it becomes mandatory to identify risk factors prior interventions. The modifiable risk factors such as hypertension, diabetes and depression represent potential areas for therapeutic interventions to minimize the progression of MCI

(Campbell et al 2013). The further categorization of risk factors has been enlisted below (Table 2.3.4).

Table 2.3.4: Contributing factors for MCI

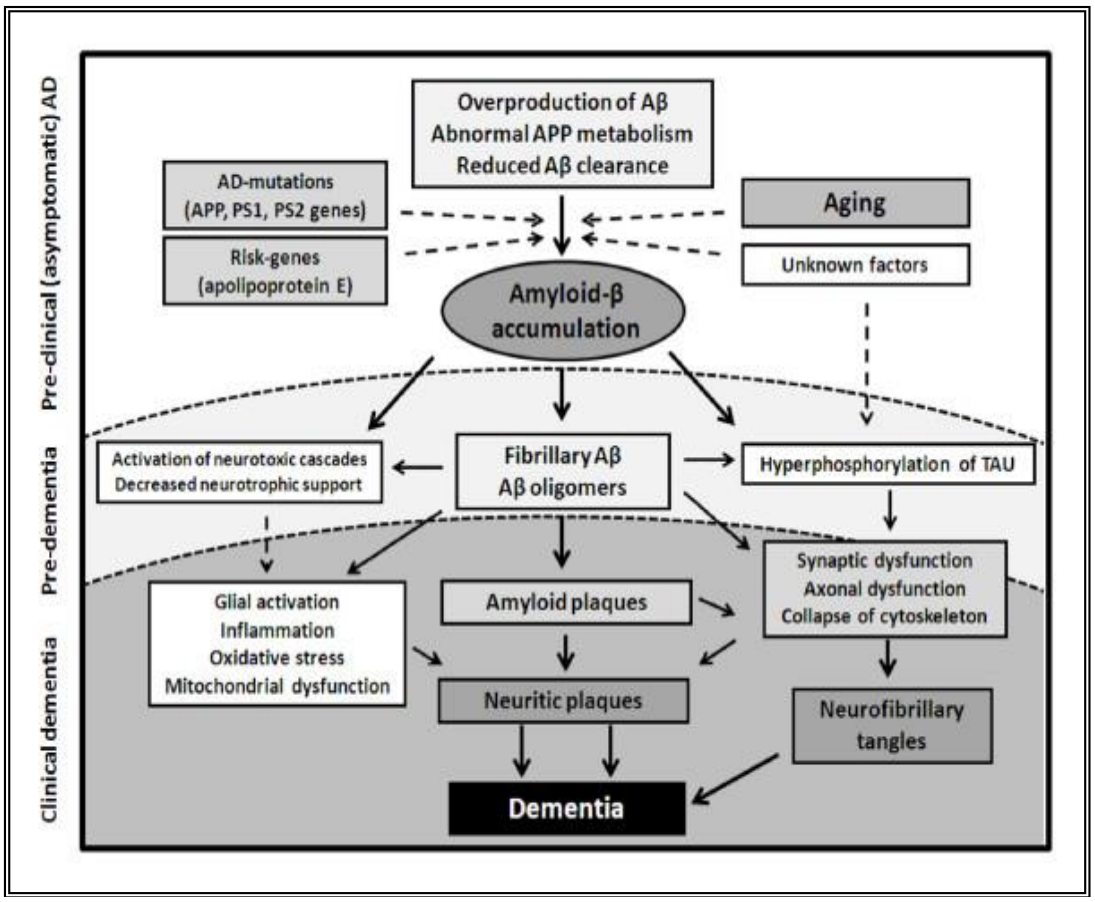
Risk factors for MCI	Protective factors
Older age Apoplipoprotein ε4 allelle	Higher education
Sex: Higher in men, higher in women, and no sex difference	Cognitively stimulating activities
Low number of years of education	Physical exercise/ activities
Vascular risk factors: Type 2 diabetes, hypertension, obesity, dyslipidemia, smoking	Dietary factors: mono and polyunsaturated fatty acids
Cardiovascular disease outcomes: coronary artery disease, atrial fibrillation, congestive heart failure, cerebrovascular disease	Mediterranean diet
Systemic inflammation: C-reactive protein	

Roberts et al 2013

Progression cascade from MCI to dementia

Several studies have demonstrated that subjects with MCI progress to dementia at a higher rate than cognitively normal subjects ranging from low estimates of 3% to very high estimates of 40% to 50% in samples defined according to the Mayo Clinic diagnostic criteria for MCI (Roberts et al 2013; Bruscoli and Lovestone 2004; Mitchell and Shiri-Feshki 2009). AD is defined as a chronic neurodegenerative disease having well-defined pathological markers, which mostly affect medial temporal lobe and associative neocortical structures. Amidst the pathological hallmarks of AD, the neuritic plaques and neurofibrillary tangles are related primarilty to the overproduction and aggregation of the amyloid β peptide (Aβ) within the brain and to the hyperphosphorylation of Tau protein in affected neurons. These abnormalities lead to the activation of neurotoxic cascades and to cytoskeletal changes which eventually cause neuronal dysfunction and dealth. Neurofibrillary tangles seem to appear first in allocortical structures, whereas amyloid plaques may first be found in the neocortex (Nelson et al 2009). Synaptic dysfunction leading to neuronal dystrophy in addition to amyloid accumulation and neurofibrillary pathology, are the phenomena proxy to the structural changes of the brain, which

ultimately triggers the clinical syndrome that characterizes incipient AD (Jack et al 2010). The cognitive manifestations associated with this process are compatible with subtle damage to hippocampal and related limbic and prefrontal structures, amd may last for many years until the functional burden becomes severe enough to surmount the dementia threshold (Blass 2002).



Blass 2002

Figure 2.3.3: Hypothetical model of the pathological processes in Alzheimer's disease (AD), focusing on the amyloid β peptide (Aβ) cascade. Dotted arrows indicate possible or secondary mechanisms affecting core pathological processes within amyloid cascade. Background shades of gray separated by dotted lines are schematic representation to integrate progression of pathological events along with development of AD. Three clinical phases of disease are defined: presymptomatic (or preclinical) AD may last for several years or decades until overproduction and accumulation of Aβ in brain reaches critical level that triggers amyloid cascade; in predementia phase, compatible with definition of MCI secondary to AD, early stage pathology is present in varying degrees, from mild neuronal dystrophy to early stage Braak pathology. Finally, in clinically defined dementia phase, there is progressive accumulation of classical pathological hallmarks of AD (that is, neuritic plaques and neurofibrillary tangles), bearing relationship with progression of cognitive deficits d magnitude of functional impairment. APP = amyloid precursor protein; PS1/2 = presenilin 1/2; TAU = microtubule-associated protein Tau.

Many studies have confirmed of a long preclinical phase in AD, in which the abnormalities gradually accumulate in affected brain areas prior to the presentation of significant cognitive decline and dementia. The models based on neuropathological,

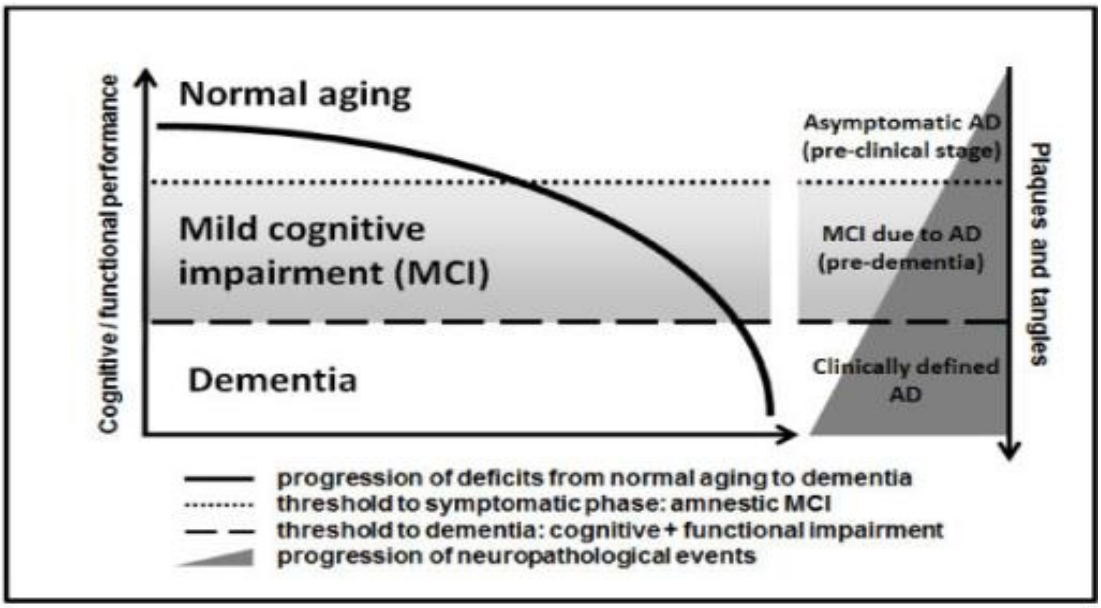
biochemical and neuroimaging methods have encouragingly proposed that intracerebral amyloidosis precedes the onset of cognitive symptoms by several years, if not decades. From the autopsy studies it has been demonstrated that intracerebral amyloidosis may be observed in some subjects as early as in the third or fourth decades of life, with increasing magnitude in late middle age, and highest estimates in old age (Thal et al 2000; Thal et al 2002; Knopman et al 2003).

There is uncertainty regarding the exact proportion of amyloid-positive normal adults who will ultimately develop AD and critically dependent on the age and genetics of the cohort; yet, cortical amyloid load in cognitively normal older adults seems to be associated with a higher progression rate to symptomatic AD in the long term (Morris et al 2009).

Patients with AD in the early stages may present with mild but persistent (and often progressive) cognitive deficits, albeit not severe enough to warrant the diagnosis of dementia. In the literature available, individuals in this pre-dementia stage of AD have been most commonly categorized according to the definition of MCI (Petersen et al 1999). Most of the neurodegenerative illnesses are specified with insidiously progressive nature, among which AD represents the most prevalent condition. It is reasonably assumed that most patients prone to become demented will present at early stages with symptoms compatible with the definition of MCI.

Nevertheless, given the fact that many persons who fulfill diagnostic criteria for MCI at one particular assessment will not evolve to dementia at all, the reciprocal assumption may not be true (Forlenza et al 2010).

The neuropathological features of subjects with amnestic MCI are intermediate between those found in cognitively normal and demented individuals (Figure 2.3.3). In a clinico-pathological study, most of the patients with amnestic MCI did not meet the neuropathologic criteria for AD, but their pathological findings suggested a transitional state of evolving AD, given the involvement of medial temporal lobe structures likely to be accounting for the memory impairment (Petersen et al 2006a).



Forlenza et al 2010

Figure 2.3.4: Relationship between the progression of cognitive and functional symptoms and the neuropathological events in the transition from asymptomatic Alzheimer's disease (AD) to mild cognitive impairment due to AD and clinically manifest dementia of the AD type.

2.4 Neuroimaging and Biomarkers for MCI detection

"Neuroimaging-biomarkers of Mild Cognitive Impairment (MCI) allow an early diagnosis in preclinical stages of Alzheimer's disease (AD). The dynamic measures of these imaging biomarkers are used to predict the disease progression in early stages and improve assessment of therapeutic efficacy in these diseases in futute clinical trials."

-Ruan et al 2016

Neuroimaging

In asymptomatic or mildly symptomatic patients, the subtle changes related to the pathological process may be quantified by the assessment of humoral fluids, mostly cerebrospinal fluid (CSF), or by using advanced neuroimaging methods. Therefore, the rationale for the search for biological markers in AD is to increase diagnostic

accuracy at early stages of the disease process. (Forlenza et al 2010). Neuroimaging potentially is a powerful tool for the differential diagnosis of cognitive impairment and for monitoring change. Cross-sectional and longitudinal studies have used structural (computed tomography [CT] and magnetic resonance imaging [MRI]) and functional (single photon emission computed tomography [SPECT], positron emission tomography [PET], and magnetic resonance spectroscopy [MRS]) modalities in the evaluation of MCI (Jack 2010). The link of the MCI syndrome to a specific etiology by the use of biomarkers for AD in combination with additional information from structural magnetic resonance imaging, PIB-PET, FDG-PET and cerebrospinal fluid biomarkers determines the certainty with which a person with MCI has the underlying AD pathology. This amount of certainty is determined from; 1) evidence of amyloid β accumulation in the brain assessed by PET, decreased cerebrospinal fluid (CSF) levels of amyloid β (Aβ42), and 2) evidence of neuronal injury assessed as increased CSF tau (total and phosphorylated), brain hypometabolism assessed from fluorodeoxyglucose PET, and hippocampal atrophy from structural magnetic resonance imaging (Sachdev et al 2012). The utility of this classification is the potential prognostic value for future dementia outcomes. Subjects with a high likelihood of MCI due to AD have a greater certainty of progression to AD (Roberts et al 2013).

The prognosis of the MCI subtypes is a principal issue on debate. The early definition of amnestic MCI carried the notion that the patients would present with signs of episodic memory impairment at the early stages of AD and progress linearly to a full-blown dementia syndrome. A similar assumption was attributed to other MCI subtypes and respective (theoretical) outcomes (Petersen 2004) (Figure 2.3.5). Nevertheless, epidemiological and clinical studies have questioned the association between MCI subtypes and specific dementia outcomes (Busse et al 2006; Fischer et al 2007). Studies confer that individuals initially diagnosed as with MCI may show a long-term stability of cognitive deficits or even return to normal standards over time (Palmer et al 2002; Loewenstein et al 2007 and Diniz et al 2009). In a future evaluation, a substantial proportion of such patients may in fact be reclassified as cognitively normal. These cases are usually reported as 'unstable MCI'. Whether the

first diagnosis was a false-positive artifact of cognitive testing, or these individuals do recover normal cognitive function after having transient, subtle impairment still needs to be defined. As the case is, the diagnostic instability is found in 5% to 20% of longitudinal samples of MCI (Diniz et al 2009).

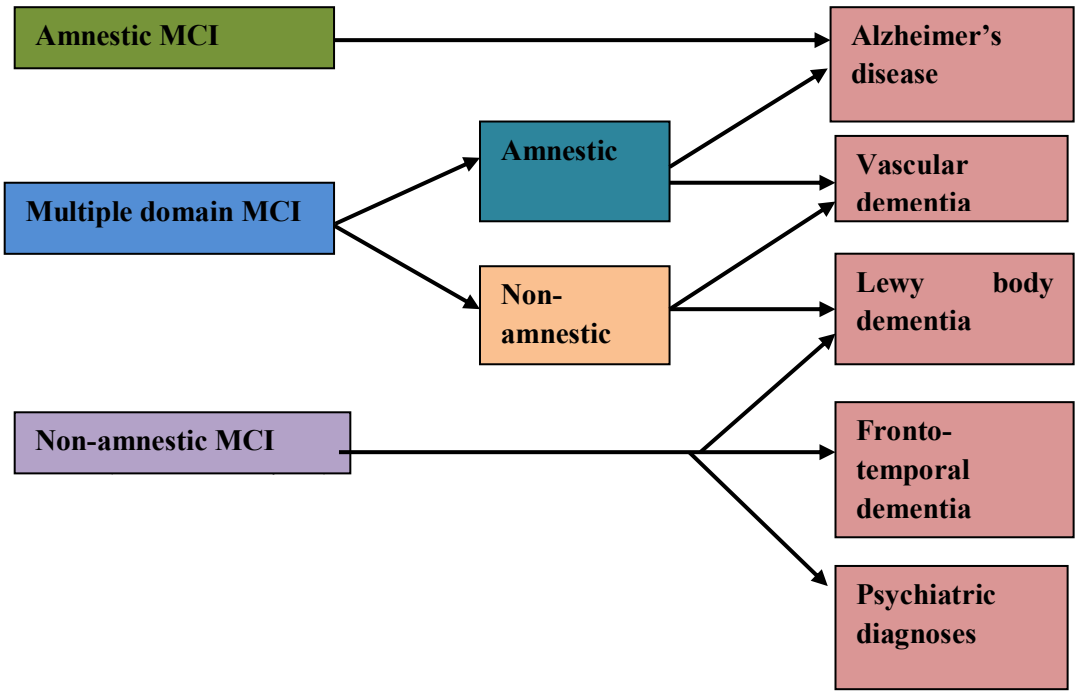


Figure 2.4.1: Hypothetical outcomes according to distinct mild cognitive impairment (MCI) subtypes

Search for biological biomarkers for Alzheimer's disease

The development of biomarker research in AD is a good example to successfully translate the knowledge of key pathophysiological mechanisms of the disease into clinical applications. A biomarker is a measurable characteristic and evaluated as an indicator of the pathogenetic processes, or to ascertain the effect of pharmacological interventions on predefined biological cascades (Wagner 2009). The ideal diagnostic marker for AD should at least meet the three basic requirements: (i) reflect core neurobiological changes that characterize the disease process; (ii) be validated by post mortem studies, assuming that the neuropathological findings are gold standards of abnormalities affecting the same cascade; and (iii) be measurable as early as possible

in the disease continuum, ideally at presymptomatic stages. Additional requirements include being non-invasive and simple to perform, precise and reliable, and adequate for large-scale screenings. Among many candidate markers, none has so far achieved universal acceptance, nor fully met the above mentioned criteria. Nonetheless, there has been significant progress toward this goal in the areas of CSF and neuroimaging biomarker identification, with attention focusing on the prediction of AD in the prodromal stages of disease and in high-risk groups (Forlenza et al 2010).

i. CSF biomarkers

The CSF may be considered an ideal source for viable biomarkers in AD. It is in intimate contact with the cerebral tissue, and pathological changes in the brain are often reflected in the CSF (Reiber 2001). Among several potential diagnostic biomarkers, the most consistent findings have been obtained with the measurement of CSF concentrations of Aβ peptide ($A\beta_{42}$), total Tau (T-Tau) and phosphorylated Tau (P-Tau) (Wiltfang et al 2005).$A\beta_{42}$ is a byproduct of the abnormal processing of the amyloid precursor protein (APP) leading to amyloidogenesis and formation of neuritic plaques. In addition, decreased concentrations of $A\beta_{42}$ likely reflect its deposition in plaques, preventing its clearance through the CSF. P-Tau illustrates the cytoskeletal changes that arise from the deregulation of microtubule homeostasis and ultimately cause axonal dysfunction and neuronal death. This marker is more specifically associated with AD, given the central role of Tau hyperphosphorylation in the formation of paired helical filaments (PHFs) and neurofibrillary tangles (Blennow and Hampel 2003). More than 100 published studies have supported the notion that the AD-positive CSF pattern has a good diagnostic accuracy to distinguish between normal ageing and AD (> 85%) and a positive predictive value (> 90%) to determine the dementia outcome in patients with MCI. Large-scale longitudinal studies of MCI cohorts too have established consistently that the presence of the 'AD signature' in the CSF has a good diagnostic accuracy (that is, >80%) discriminating patients with MCI who progress to AD ('MCI converters') from those who remain cognitively stable ('MCI-stable' patients) and healthy controls (Hansson et al 2006), as well as those MCI patients who progress to non-AD dementias (Riemenschneider et al 2002).

Diverse research groups worldwide have widely replicated these data sets for final conclusions (Arai et al 2000; Hampel et al 2004 and Shaw et al 2009).

ii. Structural and functional neuroimaging

Non-invasive methods to ascertain the pathological changes that evolve in the AD brain have been decisively searched from the development in the neuroimaging technologies. These advances result from new protocols for the analysis of structural imaging (such as volumetric assessments of regions of interest and voxel-based morphometry based on statistical maps) and functional imaging with PET, addressing the metabolic changes to temporomedial structures (Table) that presumably antedate structural damage and the investigation of AD-specific biomarkers has been made possible with PET tracers that allow the *in vivo* intracerebral imaging of amyloid and Tau (Busatto et al 2008).

MCI patients show a relative preservation of cerebral structures in comparison to those with AD; however, these MCI patients may have mild, but significant, volumetric changes and decreased cortical thickness in specific brain regions (Apostolova et al 2006; Seo et al 2007 and Singh et al 2006).

The new technological development to visualize and quantitate Aβ and Tau deposits *in vivo* within the brain is undoubtedly a major achievement in the field AD biomarker research. The first compound to be developed for human experimentation was the 'Pittsburgh Compound B' (PiB) (Mathis et al 2003), which is an ^{11}C-labelled compound that binds intracerebral Aβ in mature amyloid plaques (Klunk et al 2004). Important studies have shown negative correlations between intracerebral amyloid content (as shown by PiB scans) and CSF concentrations of $A\beta_{42}$ in patients with mild AD as compared to controls (Fagan et al 2006; Fagan et al 2009).In one of the prospective study, PiB-positive MCI patients had a higher conversion rate than PiB-negative patients; in addition, the amyloid load was negatively associated with time to conversion (Okello et al 2009).

Table 2.4.1: Putative clinical and biological markers of the distinct stages in the AD continuum (from normal cognition to dementia), and respective therapeutic interventions (clinically supported therapies and potential interventions with candidate drugs/strategies that still require experimental validation)

Clinical stage	*Underlying pathological mechanisms*	*Putative clinical and biological markers*	*Potential therapeutic interventions*
Asympto-matic (pre-clinical AD)	Intracerebral accumulation of amyloid-β	-CSF concentrations of $A\beta_{42}$ -Molecular imaging (PiB-PET) -Autosomal dominant mutation(APP, PS1, PS2 genes)	-Cognitive reserve (education and level of intellectual functioning) -Lifestyle changes (nutrition, physical fitness, reduction of stress) -Management of underlying factors (cardiovascular risk factors, toxic and comorbid conditions)
Prodromal (pre-dementia AD)	Aβ-related pathology (amyloid cascade)	-Episodic memory impairment (amnestic MCI) - CSF concentrations of $A\beta_{42}$ -Molecular imaging(PiB-PET) - Autossomal dominant mutation(APP, PS1, PS2 genes)	-Anti-amyloid therapy: * immunotherapy anti-Aβ * modulation of β- and γ-secretase * anti-fibrillization agents and chelators
	Tau-related pathology (neuro-degeneration)	-Multiple-domain amnestic MCI - CSF concentrations of Tau(total and phosphorylated Tau) - Brain metabolism (FDG-PET) - Medial temporal lobe atrophy(volumetric MRI, VBM	- All above -Neuroprotective approaches(antioxidants, anti-inflammatory drugs) -Tau-related therapies(GSK inhibitors, lithium -Neurorestorative approaches(NGF, BDNF, stem cells)
Clinical dementia	Neuritic plaques Neurofibrillar y tangles	- Neuropsychological tests - Functional assessment - Structural imaging (CT/MRI) - Neuropathology	- Antidementia drugs (cholinesterase inhibitors, memantine) - Cognitive training - Functional rehabilitation (ADLs) - Psychoeducation (caregivers)

AD, Alzheimer's disease; MCI, mild cognitive impairment; Aβ, amyloid-beta peptide; CSF, cerebrospinal fluid; APP, amyloid precursor protein; PS, pre-senilins 1 and 2; PET, positron emission tomography; PiB, Pittsburgh compound B; FDG, fluorodeoxyglucose; CT, computerized tomography scan; MRI, magnetic resonance imaging; VBM, voxel-based morphometry.

Forlenza et al 2010

2.5 Overview of brain–gut axis: the perplexing connection

> ***"Commensal bacteria of the digestive tract are separated from the brain by multiple barriers. Despite that bacteria residing in the intestine and the neurons of the brain interact by neural and humoral pathways".***
> ***-Tomova et al 2015***

An increasing amount of evidence has shown that gut microbiota also play a role in the function of the central nervous system (CNS) through metabolic, neuroendocrine and immune pathways (Mayer 2014). These pathways are under the influence of the gut microbiota and together, they comprise the brain-gut-microbiota axis (Grenham et al 2011). A cardinal function of the gut microbiota is the development and maintenance of the intestinal barrier across the lifespan (Ohland and Macnaughton 2010; Swanson et al 2011; Shifrin et al 2012). There is an association between gut flora composition and cognitive processes such as learning and memory. Intestinal microbiota additionally contributes to the early development of normal social and cognitive behaviors (Gareau 2014). In particular, more than 100 trillion bacteria reside in the human gastrointestinal (GI) tract, which is remarkably 10–100 times more than the quantity of eukaryotic cells in our bodies (Qin 2010). Numerous years of co-evolution have led to a mutualistic symbiosis between host and microorganism. As such, gut microbiota contribute to various important developmental and homeostatic processes in adult life. For example, they influence metabolism by breaking down complex polysaccharides in the diet (Gill 2006).

Using metagenomic sequencing on faecal samples, the number of microbial species present in the adult gut has been estimated to be around 1000, with each individual harbouring roughly 160 of these species (Qin 2010). Although the variety of individual microorganisms varies greatly between individuals, it has been proposed they fall into three separate enterotypes, each characterised by a single microbial genus; namely *Bacteroides*, *Prevotella* or *Ruminococcus* (Arumugam 2011). The reciprocal communication between gut and brain involves neurological, metabolic, hormonal, and immunological signalling pathways and disturbance in these systems can result in altered behaviour. For example, gut inflammation is associated with

changes in gut-brain interactions and there is a high co-morbidity between inflammatory bowel disorders with anxiety. The complex relationship between gut flora and the host has given rise to the notion of the microbiota-gut-brain axis (Rhee 2009). Mental processes, such as the stress response, may affect the composition and function of intestinal bacteria via the brain-gut axis. On the other hand, intestinal bacteria can influence the processes in the brain through the gut-brain axis. Disruption of these interactions may be involved in various alterations both in the function of the gastrointestinal tract and the brain function (Tomova et al 2015).

a) Blood Brain Barrier (BBB)

Originally, the BBB was thought of as a 'barrier' between the blood and the brain, preventing environmental insults and metabolic changes in the body from disturbing the brain's chemistry and function. New work revealed that the BBB is not really a barrier at all, but instead an extensive set of transport carriers, located on the membranes of the cells that make up the capillaries of the brain, that promotes or restricts the entry of an endless number of molecules into brain, as well as their removal (Fernstrom and Fernstrom 2004). Structural similarities exist between the intestinal, placental and blood brain barriers (BBB) (Doran et al 2013). The BBB is a complex neurovascular unit (Bauer et al 2014) consisting of central nervous system (CNS) endothelial cells which separate the lumen of blood vessels from the CNS parenchyma. Tight junctions, astrocytes and pericytes seal the capillary endothelial cells of the BBB (Daneman and Rescigno 2009). The tight junctions transmembrane proteins claudins, tricellulin, and occludin restrict paracellular diffusion of watersoluble substances from blood to the brain (Hawkins and Davis 2005). The preclinical evidence from (Germ Free) GF mice suggests that the microbiota can modulate the BBB. Exposure of GF adult mice to the fecal microbiota from pathogen-free donors decreased BBB permeability and increased the expression of tight junction proteins. Moreover, monocolonization of the intestine of GF adult mice with short chain fatty acid (SCFA)- producing bacterial strains normalized BBB permeability whilst sodium butyrate was associated with increased expression of occludin in the frontal cortex and hippocampus (Braniste et al 2014).

b) Microbiota in Aging

The interaction between the immune system, the gut microbiota and the intestinal barrier may be of particular importance to health at the other extreme of life, aging. Aging is characterized by chronic low-grade inflammation (termed "inflammaging") as evidenced by increased circulating levels of Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 and C-reactive protein (CRP); inflammatory molecules known to affect mood and cognition (Frasca and Blomberg 2015). The fact that the gut microbiota are key regulators of immune function and inflammatory responses, it is likely that a change in the composition of the gut microbiota during aging plays a role in the gradual activation of the immune system and consequently inflammaging (Prenderville et al 2015), possibly via an impact on intestinal permeability. The gut microbiota composition modifies as its host matures: it is relatively stable from 20 to 75 years old whereas microbial organisation in centenarians is significantly different from the adult-like pattern (Biagi 2010). Though both *Bacteroidetes* and *Firmicutes* are dominant in the adult and elderly gut (95% and 93% respectively), distinct changes occur in the *Firmicutes* subgroups, with multiple members in this phylum decreasing in number (Mariat 2009). There is also evidence of an increase in proportions of facultative anaerobes, such as *Staphylococcus* and *Bacillus*, and *Proteobacteria* such as *E. coli* in older age. A reason for the transformation in gut microbiota composition is the decline in GI function witnessed in old age. In the elderly, a decrease in intestinal motility affects defecation which in turn leads to constipation (Brocklehurst 1972). As a result, there is a reduction in bacterial excretion, leading to an increase in the breakdown of pancreatic enzymes which adversely alters gut function. Other age-related factors such a decline in salivary function, digestion, and dentition may also affect the GI microbiome through time. Additionally, age-related neurone degeneration within the enteric nervous system can alter GI motor function (Lovat 1996). However, findings into the variation of gut bacteria composition through age have not been consistent. For example, differing results have been found in the numbers of *Bacteroides* between young and elderly subjects. The ELDERMET consortium demonstrated that there is greater inter-individual variation in the composition of the GI microbiome in adults over 65 years

old compared to younger adults (Claesson 2011). Of note, differences in microbiota composition were more pronounced between frail elderly subjects and healthy elderly subjects. Moreover, certain gut microbiota signatures were linked to measures of frailty, co-morbidity, nutritional status, and markers of inflammation (Claesson et al 2012).

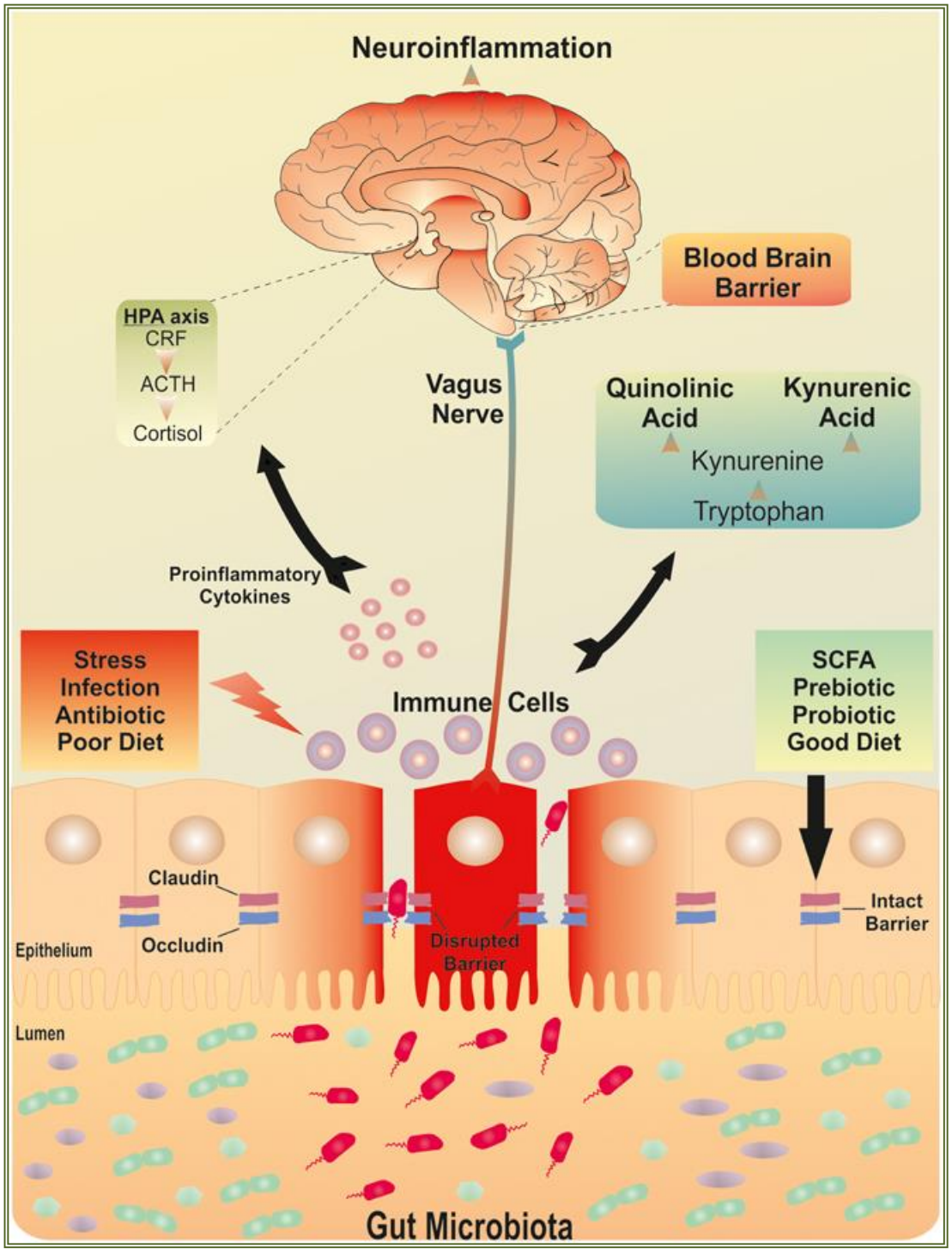
Due to the huge number of confounding factors affecting the composition of gut microbiota, it is difficult to control for these variants and elicit the influencing power of age. For these reasons, determining whether age-related changes in gut flora contribute to the parallel decline in cognitive function is challenging. A combination of events, such as antibiotic treatment or infection, may have a more significant impact on mental health in the elderly. In fact, more than three quarters of those aged 65 or older use at least a single prescription medication which can lead to unfavorable side effects on the GI tract (Chrischilles 1992).

c) Microbiota and CNS function

The microbiota produces several bioactive metabolic products, including polysaccharides, lycosylceramides, nucleic acids, structural proteins, and short chain fatty acids (SCFAs) (Olle 2013; Russell et al 2013). SCFAs (butyrate, acetate, and propionate) are neurohormonal signaling molecules produced by certain classes of bacteria such as *Bacteroides*, *Bifidobacterium*, *Propionibacterium*, *Eubacterium*, *Lactobacillus*, *Clostridium*, *Roseburia*, and *Prevotella* (Macfarlane and Macfarlane 2012). SCFAs are transported by monocarboxylate transporters, which notably are expressed at the BBB (Steele 1986; Vijay and Morris 2014). Indeed a recent preclinical imaging study demonstrated that microbiota-derived acetate can cross the BBB where it can subsequently alter hypothalamic gene expression (Frost et al 2014). SCFAs which is the main product of dietary fibre fermentation by gut microbes in the large intestine, have neuro-modulatory and epigenetic effects through histone acetylation and SCFAs has been shown to improve cognitive function in animal models for neurodevelopmental and neurodegenerative diseases (Stilling 2014). It has, however, proven difficult thus far to demarcate the CNS consequences of SCFA-mediated effects on intestinal barrier function from a direct action in the brain. It is

also notable that there is still considerable debate surrounding the ability of physiological levels of SCFAs to impact substantially on relevant behaviors via central mechanisms, albeit those higher doses do have clear behavioral consequences (Macfabe 2012; MacFabe et al 2007). Microbiota can also affect the CNS through alterations in adult hippocampal neurogenesis (AHN). It has been discovered that the adult brain has the capacity to generate new neurons in discrete areas within the hippocampus and lateral ventricles. AHN is involved in learning and memory and affected in a variety of neurological disorders such as epilepsy, depression, Alzheimer's disease, and Parkinson's disease (Zhao 2008). A dysfunctional intestinal barrier could permit a microbiota driven proinflammatory state with implications for the brain (Figure 2.5.1). The sequence of this process is not yet clear. An increase in gut permeability could precede mucosal inflammation to induce the inflammatory response and thus culminate in a feed-forward cycle between inflammatory responses and barrier dysfunction. This could subsequently maintain and exacerbate the low grade inflammatory response. Alternatively, systemic inflammation could increase intestinal barrier permeability and thus allow translocation of commensal bacteria with further implications for systemic inflammation. Indeed, the source of the low grade inflammation which has been reported in depression has not been isolated to a particular source. Irrespective of the sequence, both processes could engage the gut microbiota (Kelly et al 2015).

Preclinical research points to a role of the gut microbiome in brain function and behavior with a number of potential pathways being investigated. The possibility that these effects might be mediated by alterations in intestinal permeability is supported by converging lines of evidence. This includes evidence linking stress to both compromised barrier function and microbiota disruption with the ensuing systemic inflammation mediating the impact on the expression of neuropsychiatric symptoms (Figure 2.5.2). Indeed, there is growing evidence that certain probiotic strains as well as prebiotics can benefit barrier function, albeit largely in healthy controls or non-psychiatric populations including stress-related GI disorders (Kelly et al 2015). Alternative approaches such as psychobiotics may target other brain-gut axis pathways independent of any effect on the intestinal barrier.



Kelly et al 2015

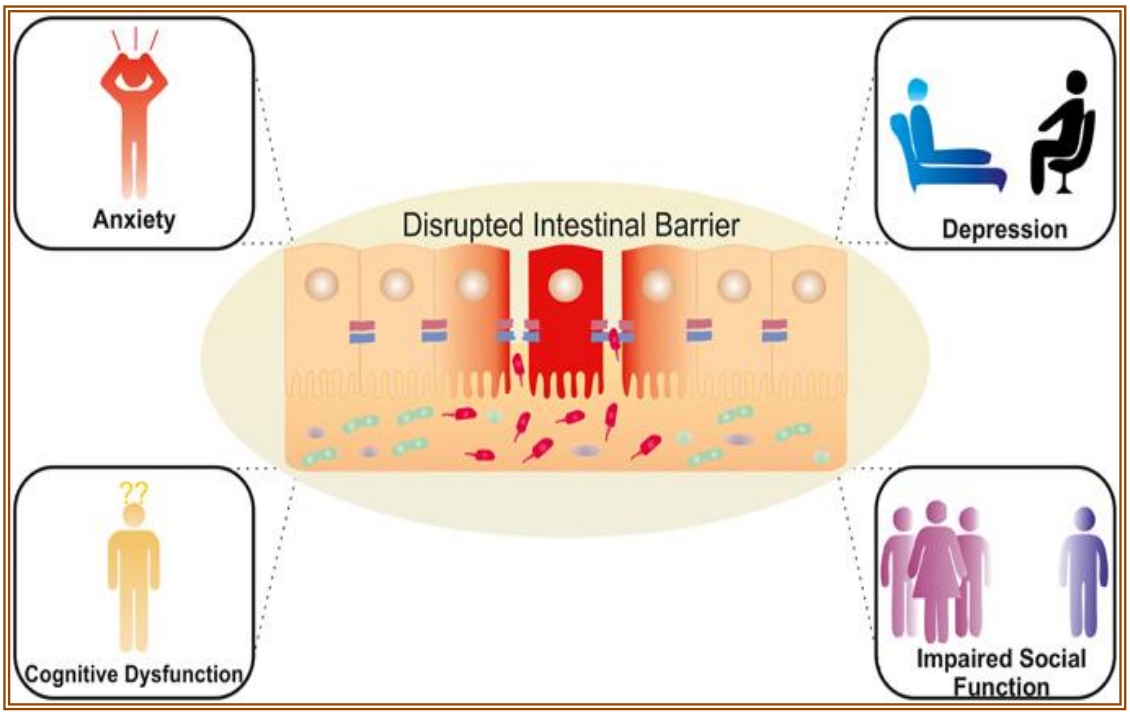
Figure 2.5.1: The brain-gut-microbiota axis. Postulated signaling pathways between gut microbiota, intestinal barrier and the brain. A dysfunctional intestinal barrier or "leaky gut" could permit a microbiota-driven proinflammatory state with implications for neuroinflammation.

Intervention studies utilizing therapeutic modulation of the gut microbiota or its metabolites to restore "normal" intestinal permeability may be of benefit in stress-related disorders. One clear example of a distinct alternative signaling mechanism between the gut microbiota and the CNS is via the vagus nerve .Vagus nerve, also known as the tenth cranial nerve, is responsible for the function of several organs, including the control of the heart rate and gut motility (Bravo 2011).

Vagus-dependent pathways have been shown to be involved in microbiota-brain communication, with vagotomy preventing microbiota-modulated changes in behaviour (Bravo 2011). Since gut microbiota can exert direct effects on the immune system, immune activation is a likely pathway in conveying the influence of bacteria on the CNS (Forsythe 2010).

The immune system plays a crucial role in maintaining the homeostasis of the GI tract and thus the maintenance of health. There is a decline in immune function in ageing, and so microbiota-brain communication may be altered in the elderly, leading to changes in behavior. Supporting this is the fact, that behavioral changes are observed in those with systemic infections as, similar to the gut, there is reciprocal communication between the CNS and the immune system (Bravo 2011).

Metabolites of gut microbiota can modulate the maturation and function of microglia, thereby affecting CNS function. Gut microbes can also regulate the permeability of the BBB, a highly selective barrier essential in protecting the brain from potential toxins. Given that the function of both the BBB and microglia may deteriorate through age, these mechanisms are particularly relevant with regards to the role of microglia in elderly cognitive decline (Harry 2013). However, the clinical implications and the role of the intestinal barrier have yet to be fully established (Julio-Pieper et al., 2014; Mayer et al., 2015).



Kelly et al 2015

Figure 2.5.2: Potential neuropsychiatric consequences of a dysregulated intestinal barrier. Activation of brain-gut-microbiota Axis signaling pathways via a compromised intestinal barrier with potential effects on mood, anxiety, cognition and social interaction.

2.6 Miscellaneous preventative strategies for Mild Cognitive Impairment

"Considering that a high proportion of risk factors are preventable, it is essential that physicians and health care personnel, educate their patients on MCI risk reduction through dietary measures, exercise, engagement in cognitively stimulating activities, stroke prevention and initiate non-therpeutic and therapeutic interventions as these are potential measures to reduce the MCI risk, with direct beneficial implications for progression from MCI to dementia, and reduced mortality from MCI."

-Roberts and Knopman 2013

Mild Cognitive Impairment has started gaining impetus due to its insidious characteristic of cognitive dysfunction turning irreversible in severe stages. Hence, the primary prevention management demands initial screening and diagnosis of MCI.

Recently, strategies to curb MCI comprise of diet, nutraceutical approaches and exercise.

Studies have well established that mortality rates in older people are reduced by physical activity, i.e. 75 minutes or more of moderate exercise weekly. The growing evidence suggests that physical activity can help improve cognition. A meta-analysis of 16 prospective studies supported an association between physical activity and reduced incidence of dementia in follow-up for at least three years (Hamer and Chida 2009). Additionally, a randomized controlled trial (RCT) in Perth studied 170 people (mean age 68 years) with subjective memory impairment for a six month programme of physical activity which provided a 'modest but significant improvement in cognition over a period of 18 months' (Lautenschlager and Cox 2008). Since then the same research group has published RCTs of exercise in older people with mild cognitive impairment, all giving cautious optimism to a positive effect on cognition, especially executive function requiring high levels of intellectual skill, e.g. problem solving now turning to strategizing to enthuse sedentary elders to exercise beyond the duration of research trials (Lautenschlager and Cox 2013). These outcomes point towards the effect of exercise in the prevention against MCI.

Many studies have strongly linked the actions of antioxidants against neurodegeneration by limiting the production of toxic substances and by reducing damage by free radicals. There are relatively fewer antioxidant enzymes specifically focused on neuronal protection, suggesting that antioxidant nutrients may have a more prominent role in older and ageing brains than in other organ systems (Mao 2013; Olanow 1990). Most of the researches investigating the relationship between antioxidants and cognitive function have focused on vitamin E. Findings of a review inclusive of 51 eligible studies on association between intake and serum levels of tocopherols and tocotrienols with age-related pathologies as osteoporosis, sarcopenia and cognitive impairment revealed that low intake and serum levels of tocopherols and tocotrienols correlated with the detrimental age-specific morbidities. The lifestyle changes comprising of optimum diet therapy with focussed body weight maintenance, increased vitamin E rich food sources such as wheat germ oil, olive oil, hazelnuts, walnuts, almonds and vitamin E rich cereals or supplements proved to be a

cornerstone in the prevention of vitamin E related deficient pathologies in elderly subjects and promoted healthy ageing without any adverse effects (Rondanelli et al 2015). Moreover, clinical trials have reported an inverse association between vitamin E levels and the incidence of cognitive decline or dementia. The evidence is not as conclusive and consistent in the studies that have assessed vitamin E status using biochemical levels (concentrations of individual tocopherols). It has been proposed that this may be due to the fact that total tocopherols levels, rather than the individual components, may be more important for neuroprotection. This seems to be supported by studies that have reported a significant relationship between low levels of total tocopherols, cognitive decline and incident dementia (Morris et al 2005).

Cohort studies have scrutinised the role of flavonoids and vitamin C but once again they provide a very mixed and inconclusive evidence, with highly heterogeneous studies (e.g. deficiency definition, populations, follow-up time, investigated outcomes, etc.) showing inconsistent results for both types of antioxidants. (Williams and Spencer 2012). The Nurses' Health study, which followed up 16,010 older participants for an average 6.4 years, published the association between antioxidants and cognition. Vitamin C and E were assessed using a semi-quantitative food frequency questionnaire, but no consistent associations between vitamin C and E intake and cognitive decline were identified (Devore et al 2013). In the same study, with regard to flavonoids (berries in particular), a higher intake of strawberries and blueberries was associated with slower cognitive decline at follow up.

As surfaced from several animal model based studies, range of polyphenols including curcumin, resveratrol and its pinostilbene and piceatannol metabolites, proanthrocyanidin and ferulic acid have emerged to be the therapeutic targets for combating age-related cognitive decline through their modulatory effects on serotonergic transmission, hypothalamic-pituitary-adrenal (HPA) axis activity, protects both brain-derived neurotrophic factor (BDNF) levels and hippocampal neurogenesis implications (Xu et al 2007; Deng et al 2010; Chao et al 2010; Moriya et al 2011; Speisman et al 2013; Ebrahimi et al 2012).

In view of the another phytochemical gaining concern is the flavonol fisetin (3, 3',4'7'-tetrahydroxylflavone) abundantly present in many plants as acacia, strawberries and mangoes which protects neuronal cells from oxidative stress induced cell death and promotes long term potentiation and memory reducing post ischemia infarct size in rats (Maher 2006).

Since decades, the worth of Mediterranean diet has been proven to be a cornerstone in the prevention of cognitive impairment. The Mediterranean diet could reduce the risk of dementia by affecting the vascular system, reducing cardiovascular disease, which in itself is a risk factor for dementia. However, this hypothesis does not seem to be supported by Scarmeas and colleagues who have shown that the association between Alzheimer's disease and the Mediterranean diet is not mediated by vascular co-morbidity (Scarmeas et al 2006). Oxidative stress has also been suggested as a potential mechanism to explain an association between the Mediterranean diet and dementia. Cognitively impaired brains show evidence of injury mediated by highly unstable reactive oxygen and nitrogen species. Some of the components of the Mediterranean diet (e.g. vegetables, olive oil, wine and fruits) are rich in antioxidants. Neutrophins, which are basic proteins, usually protect neurons against oxidative stress, and some have proposed that the Mediterranean diet could increase the concentration of plasma neutrophins (Sanchez-Villegas 2011).

Findings of the study conducted on Mediterranean dietary pattern adherence indicated its association with slower cognitive decline, reduced risk of progression from mild cognitive impairment to AD, reduced risk of AD, and decreased mortality in AD patients (Frisardi et al 2010). On the contrary, some studies have described that (RCTs) show inconsistent data whether certain nutrients such as omega-3 fatty acids, antioxidants or B vitamins, and dietary patterns (Mediterranean diet) have any protective or general effect on brain vascular health or directly interfere with the aetiopathogenesis of AD against dementia (Otaegui-Arrazola et al 2014). These outcomes designate that diet rich in omega-3 fatty acids, B vitamins or Mediterranean dietary pattern constitute a crucial component for controlling MCI. In a randomized PREDIMEDNAVARRA trial of 285 participants (95 randomly allocated to each of 3 groups) with high vascular risk were nutritionally intervened comparing two Med

Diets (supplemented with extra virgin olive oil [EVOO] or mixed nuts) versus a low fat control diet. A long term intervention with an EVOO rich Med Diet resulted in a significantly better cognitive function in these group participants across all domains and had less MCI (OR=0.34 95% CI: 0.120.97) than controls (Martínez-Lapiscina et al 2013).

Epidemiological studies have advocated that diets rich in olive oil slow the onset and progression of age-related cognitive decline and Alzheimer's disease because of its putative active ingredient hydroxytyrosol which has exhibited neuroprotective properties in both *in vivo* and *in vitro* analysis (Schaffer et al 2012).

In another study it has been shown that probiotics have also been promising as a potential preventative strategy for depression in human trials (Steenbergen et al 2015) there is evidence that the concentration of fatty acids, namely arachidonic acid and docosahexaenoic acid, are increased in mice that received a strain of *Bifidobacteriumbreve*. It is known that both fatty acids contribute to several neurodevelopmental processes, including neurogenesis and neurotransmission, and can impact cognitive functions such as learning and memory (Yurko-Mauro et al 2010).

Mechanistic evidence till date suggests that several nutritional components can effectively counteract the processes of membrane/synaptic degeneration, abnormal protein processing (amyloid beta, tau), vascular risk factors (hypertension, hypercholesterolemia), inflammation, and oxidative stress, e.g., by promoting membrane formation and synaptogenesis, enhancing memory/behaviour, improving endothelial function, and cerebrovascular health. Further, the need for early intervention in AD was reinforced and suggested that multinutritional intervention during the earliest possible phase in the development of the disease, targeting multiple aspects of the neurodegenerative process, is likely to have the greatest therapeutic potential (Kamphuis and Scheltens 2010).

A review article evidenced in support of a proactive epigenetics diet and nutraceuticals program holds promise as potential buffer against the negative impact of aging and stress responses on cognition, and can optimize the vascular, metabolic,

and brain health in the community. The stress was laid on the classification of AD into preclinical, mild cognitive impairment, to be offering a window for intervention to prevent, delay, or modify the course of AD (Chiu et al 2014).

Indeed, the growing body of studies have suggested the intriguing possibility of regular consumption of nutrients as the ω3 fatty acids, antioxidants (vitamin C and zinc), members of the vitamin B family (Vitamin B12 and folic acid) and magnesium in preventing the onset of oxidative damage to mitochondria and lipids in the neuronal circuits associated with cognitive behaviours. These have important implications for enhancement of neurocognitive function in military and civilian populations, or significantly augment the therapeutic effect of available antidepressants for vulnerable individuals (Du et al 2014).

2.7 Vitamin B12, their types, clinical manifestations and aetiology of B12 deficiency, its types and normal absorption mechanism

> ***"The detection and correction of vitamin B12 deficiency prevents megaloblastic anemia and potentially irreversible neuropathy and neuropsychiatric changes".***
> ***-Harrington 2016***

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin involved in the optimal functioning of the hemopoetic, neuro-cognitive and vascular systems. It is involved in DNA synthesis, fatty acid metabolism and energy production (Yamada 2013). Vitamin B_{12} exerts its physiological effects by facilitating the methylation of homocysteine to methionine which is later activated into S-adenosyl methionine that donates its methyl group to methyl acceptors (Bottiglieri et al 2000). Similarly, vitamin B_{12} mediates the conversion of methyl malonyl coenzyme A (coA) to succinyl coA, a process when hindered, results in accumulation of serum methylmalonic acid (MMA) thereby causing defective fatty acid synthesis of the neuronal membranes (Malouf and Areosa 2003).

Vitamin B12 (cobalamin) plays an important role in DNA synthesis and neurological function. Deficiency can lead to a wide spectrum of hematologic and neuropsychiatric

disorders that can often be reversed by early diagnosis and prompt treatment (Robert and Brown 2003). Adequate serum levels are necessary for nervous system maintenance and the development of normal red blood cells (Butler et al 2006). Vitamin B12 cannot be synthesised in the body and must therefore be obtained from the diet. B12 deficiency can cause several forms of anemia, most notably pernicious anemia. The earlier treatments of pernicious anemia have a rather fascinating story. Up until the late 1920's pernicious anemia was untreatable and fatal. Three American physiologists (William Murphy, George Minot and George Whipple) devised the concept that food could be used to treat pernicious anemia. The diet they constructed containing liver "in such quantities [that] seemed very outrageous" had dramatic beneficial effects on the once untreatable pernicious anemia (Minot and Murphy 1926). In 1934, the three colleagues were awarded the Nobel Prize in physiology and medicine. Despite the proven efficacy of the liver therapy, a more satisfactory method of treatment than the daily consumption of a half pound of liver was needed. An American doctor named William Bosworth Castle devised an idea that enabled him to investigate the pathophysiology of this disease. Liver seemed to be necessary for the patients' bone marrow to function properly and Castle questioned why normal people did not have to eat such large amounts of liver to stave off pernicious anemia. Castle proposed a theory that an "intrinsic factor" is secreted by the stomach of normal healthy individuals which is required for the formation of an "anti-pernicious anemia complex [from an] extrinsic factor", present in beef and liver (Castle 1929). The B12 molecule was first isolated in its cyano-form in 1948 and was then identified as the active component of the "extrinsic factor" proposed by Castle (Smith 1948). The chemical structure of the B12 molecule was later confirmed, using X-ray crystallography by Dorothy Hodgkin in 1956. She was subsequently awarded the Nobel Prize in chemistry for her significant findings (Smith 2008).

a) Coenzyme forms/Types of Vitamin B12

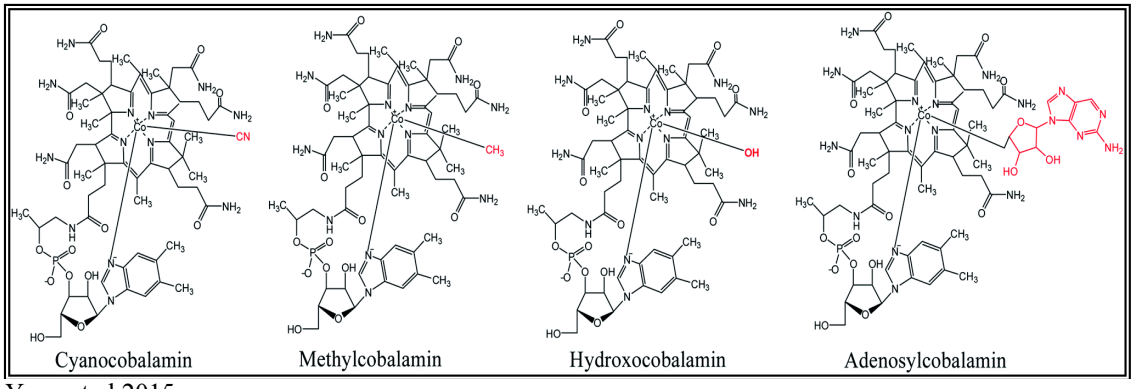
Vitamin B12 is a rather large molecule. One part of its structure is a "corrin" nucleus, which resembles the "haem" of haemoglobin. While the haem moiety in haemoglobin holds an atom of iron, in B12 the corrin group holds an atom of cobalt. In order to be true B12, cobalamin must have one of a number of attachments to the corrin group:

depending on the attachment, cobalamin can be cyanocobalamin, hydroxycobalamin, aquacobalamin, nitritocobalamin, methylcobalamin, or adenosylcobalamin. Only two of these cobalamins are active as co-enzymes in the human body: methylcobalamin and adenosylcobalamin. Most supplemental B12 is supplied as cyanocobalamin, in which form it is stable; cyanocobalamin must be converted to methyl- or adenosylcobalamin before it is biologically active (Harrington 2012).

Vitamin B12 is synthesised by micro-organisms and enters the diet with food of animal origin. Plants do not require B12 for any function, and therefore have no mechanisms to produce or store B12. The biosynthesis of this nutrient never seems to have made the transition to the higher, eukaryotic forms of life. In humans, the vitamin is required in trace amounts (approximately 1 µg/day) to act as a co-enzyme to two enzymes, methionine synthase and (R)-methylmalonyl-CoA mutase (Ehrenpreis 2002; Schjonsby 1989). B12 is the only co-enzyme for methylmalonyl-CoA mutase, which catalyses the conversion of methylmalonyl-CoA to succinyl-CoA. When adequate B12 is not available, methylmalonyl-CoA production increases. Because it is toxic, methylmalonyl CoA is then rapidly converted to methylmalonic acid (MMA), which accumulates in the blood and urine. Since this reaction only requires B12 as a co-enzyme, MMA levels are a good indicator of B12 status (Harrington 2012).

Vitamin B12 is a complex compound that is converted into several coenzymes. It is used for shifting of hydrogen between carbon atoms, usually in conjunction with a shift of some other group (e.g., NH2, or CH3); Vitamin B12 can also act as a methyl group carrier, accepting the carbon from tetrahydrofolate derivatives. In humans, vitamin B12 has only two known functions: 1) synthesis of methionine from homocysteine and 2) the rearrangement of methylmalonyl-CoA (from odd chain fatty acid metabolism and some amino acids) to succinyl-CoA. The structures below include the structure of the actual vitamin and of the major coenzyme forms found in humans. (The cyanide group in cyanocobalamin is not necessarily present, and is typically an artifact of purification.) 5′-Deoxyadenosyl cobalamin is the coenzyme required by methylmalonyl-CoA mutase, while methylcobalamin acts as the methyl-group acceptor and donor during the methionine synthase reaction (Brandt 2011).

Vitamin B12 is a collective term for a group of cobalt-containing compounds known as corrinoids which when assembled with 5th and 6th position ligands are known as cobalamins. Vitamin B12 (cyanocobalamin, CNCbl) was discovered in the first half of the 20th century and identified as the anti-pernicious anemia factor. The two coenzyme forms, 5 - deoxy-5 -adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), were discovered later on (Hodgkin et al 1956; Lenhert and Hodgkin 1961). Cobalamin (Cbl) is necessary for synthesis of DNA bases, transfer of methyl group [i.e. regeneration of S-adenosylmethionine (SAM)], as well as metabolism of branched chain amino acids and fatty acids with an odd number of carbon atoms. There are a variety of Cbl forms, which share the core structure of Cbl but contain different upper ligands. Also the physiological forms of cobalamin (hydoxycobalamin, adenosylcobalamin and methylcobalamin) are available as supplements with different routes of administrations (Obeid et al 2015) as:



Yang et al 2015

*The corrin ring of vitamin B12 is similar but not identical to the porphyrin ring found in heme-containing proteins. Vitamin B12 is not used as a source of heme.

Figure 2.7.1: Chemical structures of vitamin B12 forms

1. Cyanocobalamin (CNCbl)

This synthetic version of vitamin B12 is created in a lab, which makes it the cheapest supplement option. It offers the most stable form of B12, although it does so through the presence of a cyanide molecule. While the amount of cyanide is not dangerous, it does require the body to expend energy to convert and remove it. CNCbl is a stable and inexpensive synthetic form commonly used for food fortification and oral or parenteral supplements.

2. Methylcobalamin (MeCbl)

This is the most active form in the human body. It converts homocysteine into methionine, which helps protect the cardiovascular system. Methylcobalamin also offers overall protection to the nervous system. This B12 form can also cross the blood-brain barrier–without assistance–to protect brain cells. It contributes essential methyl groups needed for detoxification and to start the body's biochemical reactions. In mammalian cells, MeCbl is a cofactor for the cytosolic methionine synthase.

3. Hydroxocobalamin (HOCbl)

Hydroxycobalamin is an abundant and physiologically relevant intermediate form. Exposure of aerated aqueous solutions of MeCbl and AdoCbl to ambient light causes formation of HOCbl in vitro. Bacteria naturally create this form of vitamin B12, making it the main type found in most foods. It easily converts into methylcobalamin in the body. Hydroxocobalamin is commonly used via injection as a treatment for B12 deficiency as well as a treatment for cyanide poisoning.

4. Adenosylcobalamin (AdoCbl)

AdoCbl is a cofactor for the mitochondrial methylmalonyl-CoA (MM-CoA) mutase. The energy formation that occurs during the Citric Acid cycle requires this form of B12. Although naturally occurring, it is the least stable of the four types of B12 outside the human body and does not translate well into a tablet-based supplement. It can be difficult to find this one in supplement form.

When supplemented, CNCbl needs to be converted into MeCbl and AdoCbl in order to exert its anticipated biological effect on the cell. The concept of replacing CNCbl/HOCbl with the coenzyme forms as ready-to-use sources of the cofactors has recently emerged. Supplementation of MeCbl and AdoCbl is postulated to be more effective than that of CNCbl/HOCbl (Thakkar and Billa 2015; Zhang and Ning 2008).

Clinical Manifestations:

Vitamin B12 deficiency is associated with hematologic, neurologic, and psychiatric manifestations (*Table 2.7.1*). It is a common cause of macrocytic (megaloblastic) anemia and, in advanced cases, pancytopenia. Neurologic sequelae from vitamin B12

deficiency include paresthesias, peripheral neuropathy, and demyelination of the corticospinal tract and dorsal columns (subacute combined systems disease). Vitamin B12 deficiency also has been linked to psychiatric disorders, including impaired memory, irritability, depression, dementia and, rarely, psychosis (Lee 1999; Lindenbaum et al 1988). In addition to hematologic and neuropsychiatric manifestations, vitamin B12 deficiency may exert indirect cardiovascular effects. Similar to folic acid deficiency, vitamin B12 deficiency produces hyperhomocysteinemia, which is an independent risk factor for atherosclerotic disease (Nygard et al 1997). Although the role of folic acid supplementation in reducing homocysteine levels as a method for preventing coronary artery disease and stroke continues to be a subject of great interest, there has been little emphasis on the potential role of vitamin B12 deficiency as a contributing factor in the development of cardiovascular disease. This possibility becomes especially important when considering vitamin replacement therapy. Folic acid supplementation may mask an occult vitamin B12 deficiency and further exacerbate or initiate neurologic disease. Therefore, clinicians should consider ruling out vitamin B12 deficiency before initiating folic acid therapy (Tucker et al 1996).

Table 2.7.1: Clinical Manifestations of Vitamin B12 Deficiency

Hematologic
Megaloblastic anemia
Pancytopenia (leukopenia, thrombocytopenia)
Neurologic
Paresthesias
Peripheral neuropathy
Combined systems disease (demyelination of dorsal columns and corticospinal tract)
Psychiatric
Irritability, personality change
Mild memory impairment, dementia
Depression
Psychosis
Cardiovascular
Possible increased risk of myocardial infarction and stroke

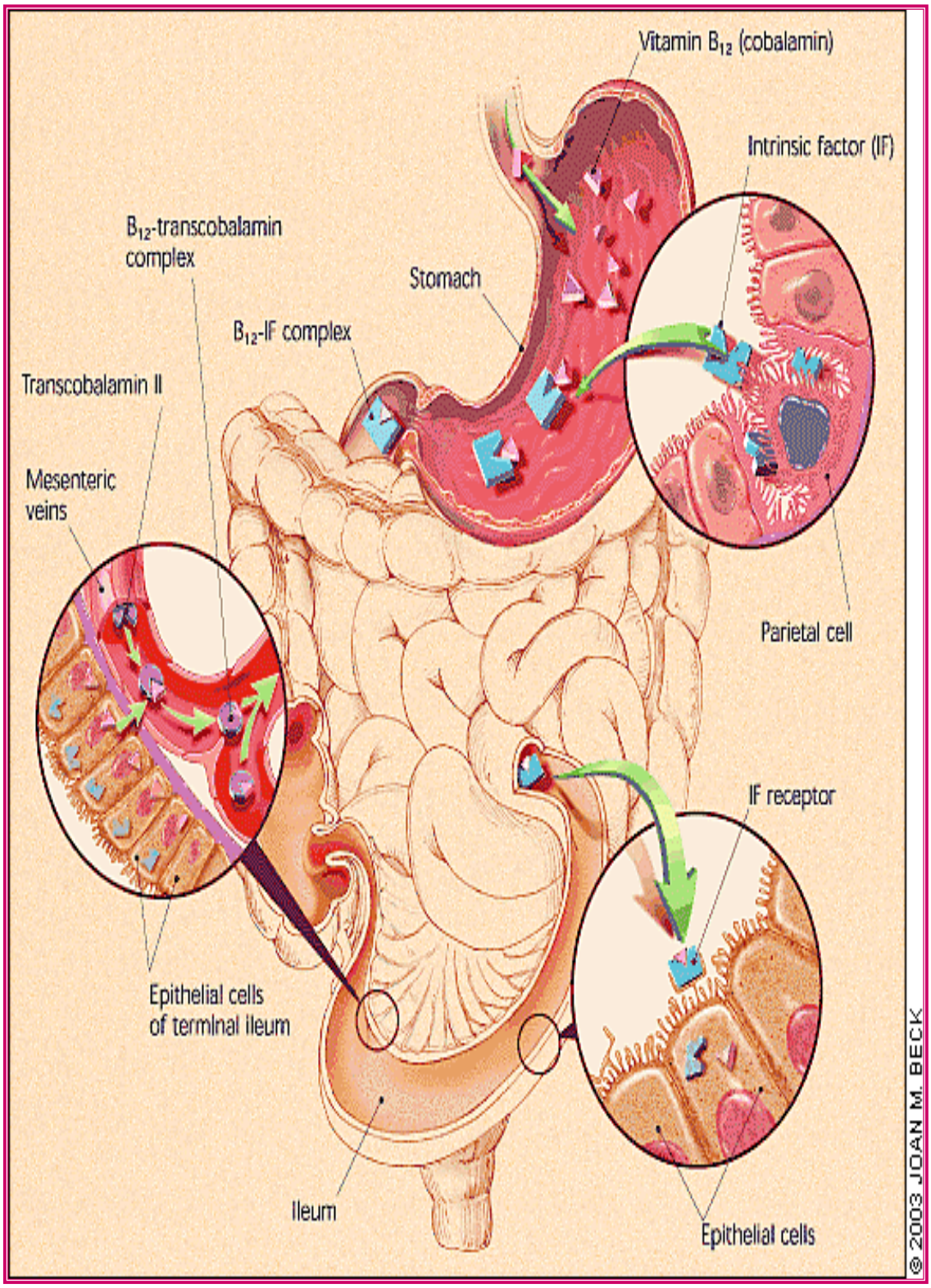
Robert and Brown 2003

Normal absorption mechanism of vitamin B12 in action

The main dietary sources of B12 are dairy products, meat (especially liver) and eggs. When humans eat animal foods, the B12 they consume is protein bound. The acidic environment of the stomach enables the release of B12 that is bound to food (Robert and Brown 2003).

The free B12 is then rapidly bound by intrinsic factor (IF), a muco-polysaccharide secreted by the gastric parietal cells that line the stomach. The binding of B12 to IF occurs in the duodenum causing the formation of the IF-B12 complex (Butler et al 2006). This complex is resistant to digestion by gastric juices. Upon reaching the terminal ileum, it binds to and is absorbed by the intestinal microvilli. Approximately 1 percent of a large oral dose of vitamin B12 is absorbed by this second mechanism (Elia 1998).

This pathway is important in relation to oral replacement. In the plasma, about 20% of the absorbed B12 binds to the serum protein holotranscobalamin (Holo-TC) for transport (Hin et al 2006). Holo-TC is the protein that delivers bound B12 to all cells in the body. The majority of B12 (80%) circulating in the blood binds to the serum protein haptocorrin and is biologically unavailable for most cells (Hvas and Nexo 2006). The function of haptocorrin remains unknown. The interruption of one or any combination of these steps places a person at risk of developing deficiency (Figure 2.7.2).

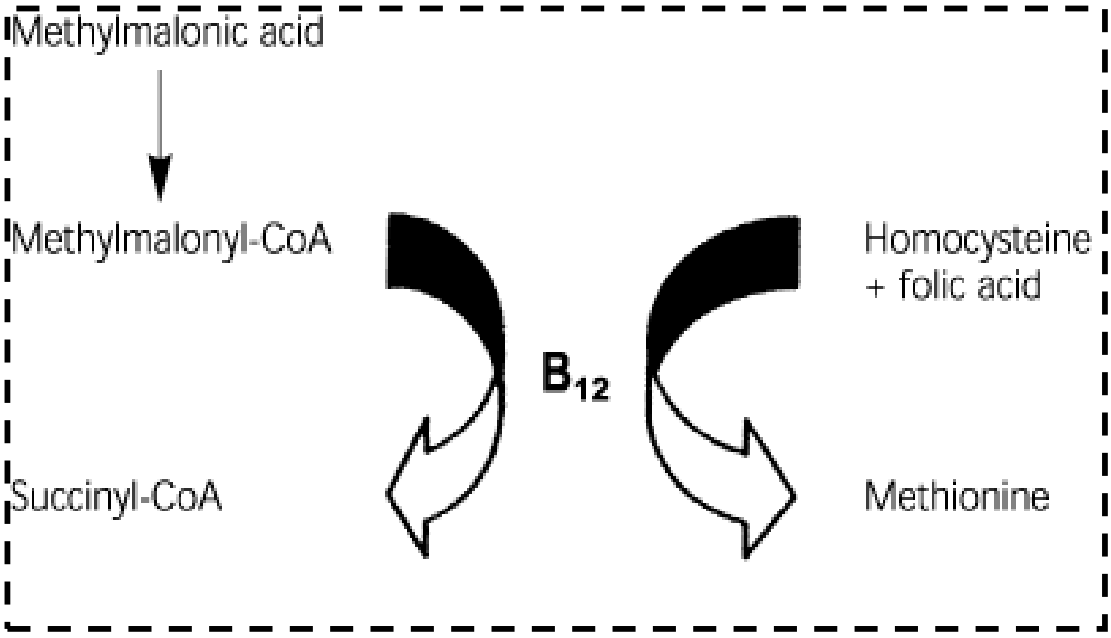


Robert and Brown 2003

Figure 2.7.2: Vitamin B12 absorption and transport.

Methylmalonic Acid and Homocysteine

In humans, only two enzymatic reactions are known to be dependent on vitamin B12. In the first reaction, methylmalonic acid is converted to succinyl-CoA (a necessary component of the citric acid cycle) using vitamin B12 as a cofactor (Figure 2.7.3). Vitamin B12 deficiency, therefore, can lead to increased levels of serum methylmalonic acid. In the second reaction, homocysteine is converted to methionine by using vitamin B12 and folic acid as cofactors. The latter reaction is accompanied by the conversion of methyltetrahydrofolate to tetrahydrofolate, which is necessary for efficient DNA synthesis (Hvas and Nexo 2006). Therefore, a deficiency in B12 can impair DNA synthesis and may lead to increased homocysteine levels (Robert and Brown 2003; Martin 1998).



Stabler 1995

Figure 2.7.3: Vitamin B_{12} deficiency leads to a serum build-up of methylmalonic acid. Deficiency of vitamin B_{12} or folic acid can lead to increased homocysteine levels.

It has been hypothesized that a pathway of oxidation of homocysteine to homocysteic acid is the potential explanation of the dangerous effect of homocysteine. Elevated levels of homocysteine in the blood predispose to arteriosclerosis and stroke (Lipton

et al 1997). Indeed it has been recently estimated that as many as 47% of patients with arterial occlusions manifest modest elevations in plasma homocysteine. Included among the many causes are genetic alterations in enzymes such as cystathionine beta-synthase, a defect found in 1-2% of the general population, and deficiencies in vitamins B6, B12, and folate whose intake is suboptimal in perhaps 40% of the population (Perry et al 1995).

Aetiology of Vitamin B12 deficiency- The causes of vitamin B12 deficiency can be divided into three classes: i) nutritional deficiency, ii) malabsorption syndromes and iii) other gastrointestinal (GI) causes (Snow 1999).

i). Nutritional deficiency: Dietary sources of vitamin B12 are primarily meats and dairy products. Normally, humans maintain a large vitamin B12 reserve, which can last two to five years even in the presence of severe malabsorption. Nevertheless, nutritional deficiency of B12 can occur in specific populations. Elderly patients with "tea and toast" diets and chronic alcoholics are at especially high risk due to the dietary deficits of B12 frequently found within these groups. The dietary limitations of strict vegans make them another, less common at-risk population.

Table 2.7.2: Etiologies of Vitamin B12 Deficiency

Nutritional deficiency
Inadequate intake (e.g., alcoholics, elderly, vegans)
Malabsorption syndromes
Food-bound B12 malabsorption
Prolonged use of proton pump inhibitors
Prolonged use of histamine H2 receptor blockers
Lack of intrinsic factor or parietal cells
Pernicious anemia
Atrophic gastritis
Postgastrectomy
Other gastrointestinal causes
Ileal malabsorption
Enteritis (Crohn's disease)
Ileal resection
Biologic competition
Bacterial overgrowth
Tapeworm infestation
Defective transport
Transcobalamin II deficiency

Snow 1999

ii.) Malabsorption syndromes: The primary example of a malabsorption syndrome is pernicious anaemia. This condition is the result of an autoimmune disease in which antibodies attack the parietal cells of the stomach, almost completely blocking the release of IF as a result. This hindered IF release prevents the formation of the IF-B12 complex, subsequently impairing B12 absorption. Researchers now believe there is an age-associated decline in the intestinal absorption of B12 (Hin et al 2006). Therefore, it comes as no surprise that B12 deficiency has been reported in about 15% of adults older than 65 years. Laboratory evidence of parietal cell antibodies is approximately 85 to 90 percent sensitive for the diagnosis of pernicious anemia. However, the presence of parietal cell antibodies is nonspecific and occurs in other autoimmune states. Intrinsic factor antibody is only 50 percent sensitive, but it is far more specific for the diagnosis of pernicious anemia (Robert and Brown 2003). The phenomenon of food-bound malabsorption occurs when vitamin B12 bound to protein in foods cannot be cleaved and released. Any process that interferes with gastric acid production can lead to this impairment. Atrophic gastritis, with resulting hypochlorhydria, is a major cause, especially in the elderly (Stabler 1995). Subtotal gastrectomy, once common before the availability of effective medical therapy for peptic ulcer disease, also can lead to vitamin B12 deficiency by this mechanism. The widespread and prolonged use of histamine H2-receptor blockers and proton pump inhibitors for ulcer disease also may cause impaired breakdown of vitamin B12 from food, causing malabsorption and eventual depletion of B12 stores. Studies in the past have confirmed that long-term use of omeprazole can lead to lower serum vitamin B12 levels (Marcuard et al 1994; Termanini et al 1998). While more studies are needed to identify the incidence and prevalence of vitamin B12 deficiency in this subset of patients, screening for subclinical B12 deficiency should be a consideration in patients who have received long-term acid-suppression therapy (Bradford and Taylor 1999).

iii.) Other GI causes: Although quite rare, certain GI conditions can also cause B12 deficiency. If a patient has an intestinal parasite infestation such as *Diphyllobothrium latum* (fish tapeworm) this may compete with the intestinal microvilli for the absorption of B12 (Snow 1999). Similarly, other etiologies of vitamin B12 deficiency, although less common, deserve mention. Patients with evidence of vitamin B12

deficiency and chronic gastrointestinal symptoms such as dyspepsia, recurrent peptic ulcer disease, or diarrhea may warrant evaluation for such entities as Whipple's disease (a rare bacterial infection that impairs absorption), Zollinger-Ellison syndrome (gastrinoma causing peptic ulcer and diarrhea), or Crohn's disease. Patients with a history of intestinal surgery, strictures, or blind loops may have bacterial overgrowth that can compete for dietary vitamin B12 in the small bowel, as can infestation with tapeworms or other intestinal parasites. Congenital transport-protein deficiencies, including transcobalamin II deficiency, are another rare cause of vitamin B12 deficiency (Robert and Brown 2003).

Vitamin B12 deficiency is common in the elderly, affecting as much as 10% to 15% people over the age of 60 as a consequence of inadequate intake or malabsorption (Baik and Russell 1999). The incidence, however, appears to increase with age (Robert and Brown 2003).Vitamin B12 deficiency causes a classical neurological and hematological syndrome (Langan and Zawistoski 2011; Savage and Lindenbaum 1995) and has long been associated with cognitive and psychiatric disturbances (Strachan and Henderson 1965). Vitamin B12 deficiency has been widely implicated in the milder forms of cognitive decline as observed in a consecutive series of patients attending a memory clinic, 3.3% of the MCI patients had low serum vitamin B12 values (Pereira et al 2006). In another studies, non-demented cognitively impaired elderly patients with vitamin B12 deficiency had lower verbal fluency scores as compared to those with normal values of vitamin B12 and poorer performances in spatial copying test (Eastley et al 2000; Riggs et al 1996). In a community-based sample of non-demented elderly subjects, both normal and cognitively impaired, the values of methylmalonic acid (MMA), which inversely reflects the level of vitamin B12, were associated with worse performances in language and praxis tests (McCracken et al 2006). Similarly, in a sample of non-demented subjects older than 75 years, low levels of vitamin B12 were associated with decreased performance in a modified block design test, which evaluates abstraction and visuospatial abilities (Robins et al 2001). Thus, vitamin B12 profoundly affects the various cognitive domains in the non-demented elderly.

Mode of Vitamin B12 therapy

In a cohort study, all (n=15) vitamin B12 deficient MCI patients were started on vitamin B12 therapy (1 mg orally or 1 mg by intramuscular administration every month) and the normalization of serum levels was achieved (Silva et al 2013). B12 supplementation is now widely used for the treatment of B12 deficiency. Mostly the B12 deficient individuals are treated with an intramuscular B12 injection (Butler et al 2006). Treatment schedules for intramuscular administration vary widely but usually consist of initial loading doses followed by monthly maintenance injections. One regimen consists of daily injections of 1,000 mcg for one to two weeks, then a maintenance dose of 1,000 mcg every one to three months (Robert and Brown 2003).

Table 2.7.3: Schedule for Vitamin B12 Therapy

Route of administration	***Initial dosage***	***Maintenance dosage***
Oral	1,000 mcg per day for one to two weeks	1,000 to 2,000 mcg per day for life
Intramuscular	100 to 1,000 mcg every day or every other day for one to two weeks	100 to 1,000 mcg every one to three months

Robert and Brown 2003

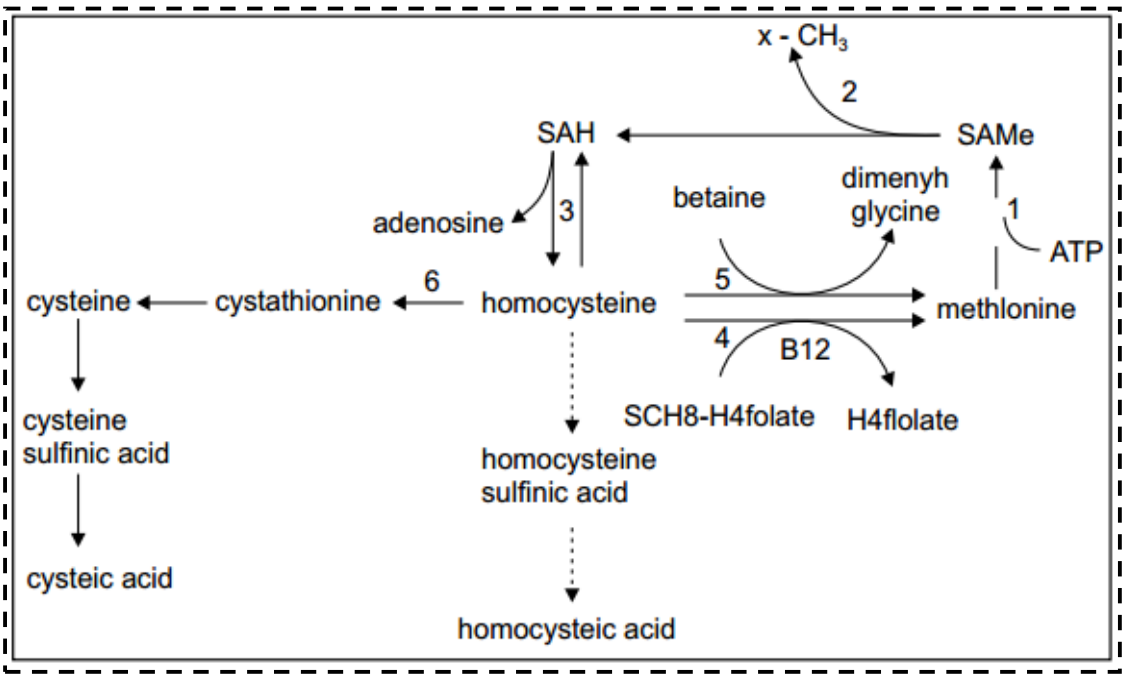
Beneficial impact of Vitamin B12

Experiments carried out largely have highlighted that B vitamins are important for the methylation and assembly of phospholipids (da Silva et al 2014; Akesson et al 1982). Vogiatzoglou et al (2008) in their study confirmed that the atrophy rate of the brain is faster at low plasma vitamin B-12 concentrations. In a brain aging study involving 49 cognitively normal individuals (age 25–72 years, 69% women) with dietary information, 11C-PiB (11C-Pittsburgh Compound-B- PiB: a measure of amyloid-β (Aβ) load) and 18F-FDG PET (18F-fluorodeoxyglucose –FDG: a proxy of neuronal activity) scan examinations illustrated that the higher intake of vitamin B12 from food sources was associated with lower Aβ load in cognitively normal individuals (Mosconi et al 2014).

Role of Vitamin B and homocysteine

Some studies have shown that higher intakes of vitamin B12 and folate are related to better cognitive functioning or lower AD risk in the elderly (Bryan et al 2004; Corrada et al 2005; La et al 1997; Luchsinger et al 2007; Morris et al 2005; Durga et al 2007; Morris et al 2006), possibly due to their ability to reduce homocysteine levels, although results are not conclusive (Morris et al 2006).

The central nervous system requires a constant supply of glucose, and adequate brain function and maintenance depend on almost all the essential nutrients. For those B vitamins that participate in one-carbon metabolism (i.e. folate, vitamin B12, and vitamin B6) deficiency of the enzymes involved in these pathways is associated with severe impairment of brain function. As shown in figure 2.7.4 vitamin B12 participate in methylation process. The de novo synthesis of methionine requires vitamin B12, which is involved directly in the transfer of the methyl group to homocysteine (Wang 2001).



Wang 2001

Figure 2.7.4: Metabolic relationship between folate, Vitamin B12 and homocysteine

In case of folate or vitamin B12 deficiency, the methionine synthetase reaction is severely impaired. In particular, vitamin B12 is the necessary coenzyme, adequate for

the correct functioning of the methyl donation from 5 methyltethrahydrofolate in tetrhahydrofolate, necessary for methionine synthetase. The folic acid "obliges" the entire vitamin B12 to subserve as coenzyme, and therefore enforces the otherwise limited damage caused by the vitamin B12 defect (Moretti 2001).

Diagnosis of vitamin B12 deficiency

Serum vitamin B12 (cobalamin) testing has long been – and continues to be – recommended as part of the routine screening of patients with dementia (American Psychiatric Association 2007). The diagnosis of vitamin B12 deficiency has traditionally been based on low serum vitamin B12 levels, usually less than 200 pg per mL (150 pmol per L), along with clinical evidence of disease. However, studies indicate that older patients tend to present with neuropsychiatric disease in the absence of hematologic findings (Lee 1999; Lindenbaum et al 1988). The APA recommendation reflects the widespread assumption that B12 deficiency is a reversible cause of dementia, or at least is commonly associated with cognitive impairment that may be partially correctable. A full diagnostic work-up is frequently not undertaken until a dementing illness is well established; and when B12 deficiency is diagnosed in a demented patient, it is usually not the only (or even primary) etiological process involved. Furthermore, B12 deficiency is often incidentally detected during the course of a dementia of other etiology (e.g. Alzheimer's disease). In such circumstances there is little prognostic data available to guide clinicians regarding the likely response to B12 replacement therapy (Dyck et al 2009). Further diagnostic challenges are posed by patients who present with subtle B12 deficiency (Carmel et al 1987) or B12-related neuropsychiatric syndromes without anemia or macrocytosis (Lindenbaum et al 1988). These diagnostic uncertainties prompted the quest for biochemical markers of tissue B12 deficiency and the development of assays for two metabolites that accumulate if B12 is lacking: methylmalonic acid (MMA) and homocysteine (Hcys) (Stabler et al 1986; 1988). The measurement of serum MMA and Hcys in combination with serum B12 levels has enlarged the understanding of B12 deficiency considerably beyond the classically described megaloblastic anemia and subacute combined systems disease (Dyck et al 2009). The MMA and Hcys metabolite measurements have been shown to be more sensitive in

the diagnosis of vitamin B12 deficiency than measurement of serum B12 levels alone (Stabler 1995; Savage et al 1994; Sumner et al 1996; Frenkel and Yardley 2000; Lindenbaum et al 1990 ; Snow 1999). This finding suggests that MMA and Hcys levels can be early markers for tissue vitamin B12 deficiency, even before hematologic manifestations occur. Vitamin B12 or folic acid deficiency can cause the Hcys level to rise. Also, MMA levels can be elevated in patients with renal disease (the result of decreased urinary excretion); thus, elevated levels must be interpreted with caution (Savage et al 1994).

In the cross-sectional Banbury B12 study carried by Dr. Harold Hin and his colleagues (2006) on 1,000 community-dwelling individuals more than 75 years, the associations of cognitive impairment, depression and neuropathy with blood measurements of B12 in elderly were examined. 13% (125 free-living) participants having the serum B12 concentration of less that 133 pmol L^{-1} were deemed to be B12 deficient. Low B12 concentrations correlated with cognitive impairment. A further finding was that participants with B12 levels in the bottom quartile had a two-fold risk of cognitive impairment, when compared to those in the top quartile. Low B12 levels were also associated with peripheral neuropathy, based on the findings that the B12 deficient participants were observed to have missing knee and ankle jerk reflexes.

Vitamin B12 and neuropsychiatric symptoms

Data obtained from the literature state that vitamin B12 is somehow bound to cognition and to the implementation of active strategies to coordinate and do well in active problem solving (Savage et al 1994). Psychiatric symptoms attributable to vitamin B12 deficiency have been described for decades. These symptoms seem to fall into several clinically separate categories: slow cerebration, confusion, memory changes, delirium with or without hallucinations and or delusions, depression, acute psychotic states, and more rarely, reversible manic and schizophreniform states (Nillson 1998.

A higher prevalence of lower serum vitamin B12 levels have been found in subjects with AD, other dementias and in people with different cognitive impairments, as compared with controls (Bell et al 1998). In the prevalence of low vitamin B12 serum

levels is consistent with that found in community-dwelling elderly persons in general but is associated with greater overall cognitive impairment. (Whyte et al 2002). Furthermore, some intervention studies have shown the effectiveness of vitamin B12 supplementation in improving cognition in demented or cognitively impaired subjects.

Chronic dementia responds poorly but should nevertheless be treated if there is a metabolic deficiency (Nillson etal 1998) However, a treatment effect was demonstrated among the patients presenting with cognitive impairment, improving when compared to matched patients on the verbal fluency test. A prospective investigation by Cunha et al (1995) on a total of 181 consecutive, demented (DSMIII or DSMIIIR criteria and score below 24 on the MMSE) elderly outpatients demonstrated beneficial effects on cognitive function of demented and cobalamin deficient patients after cobalamin replacement. The frequency of vitamin B12 deficiency (less than 200 pg/mliter) was 25% (46 patients) and the treatment outcome obtained in 19 patients (19 of 46) showed improvement (MMSE returned to normal values) with all having the mild dementia history of less than 2 years. The implications from this study suggest that screening for B12 deficiency should be considered in patients with recent onset of mild mental status changes.

The extensive investigations conducted in the Homocysteine and B Vitamins in Cognitive Impairment (VITACOG), randomized clinical trial with homocysteine-lowering B vitamins in older people with MCI, showed that treatment with high doses of B vitamins markedly reduced the global brain atrophy rate, as well as atrophy rates in those gray matter regions most commonly associated with AD (Smith et al 2010, Douaud et al 2013). In 2012, Blasko and co-workers in a prospective cohort with a retrospective vitamin intake evaluation of older adults (N=81) with MCI from the Vienna Transdanube aging study reported that users of vitamin B12 or folate, independent of time and pattern of use, had lower grades of peri-ventricular hyper-intensities and deep white matter lesions associated with a lower conversion rate to dementia as compared to nonusers.

Reports of patients with cognitive deficits showed notable improvement, namely in language and frontal lobe functions, after the vitamin B12 supplementation (Eastley et al 2000).

Several cross-sectional and longitudinal studies performed in both healthy and cognitively impaired older subjects reported inverse associations between vitamin B12 levels and the degree of cognitive impairment (Engelborghs et al 2004; Elias et al 2006; Hin et al 2006; Tangney et al 2009). On the contrary, some studies found no association between the two (Ariogul et al 2005; Paulionis et al 2005; Kang et al 2006; Faux et al 2011).

Moreover, a review also found that vitamin B12 deficiency is associated with cognitive impairment, but supplementation did not improve cognitive function in patients with previous deficits (Moore et al 2012).

Similarly the findings from a retrospective monocentric study conducted on 125 cobalamin deficient elderly patients over 14 months having received oral (88.8%) and intramuscular (11.2%) cobalamin supplementation, depicted a significant increase of Vitamin B12 levels ($p<0.001$) but it proved to be less effective in patients with dementia ($p=0.04$) (Couderc et al 2015).

Contrary to this, a review based on evidence from the limited studies stated that daily oral dose of 2000 mcg vitamin B12, an initial daily 1000 mcg doses, thereafter weekly and then monthly may be as effective as intramuscular administration for obtaining short term haematological and neurological responses in vitamin B12 deficient patients (Vidal-Alaball et al 2005). Wellmer et al (2006) suggested from their case study of successful oral vitamin B12 that a monitored oral substitution therapy should be used as the first line therapy for neurological disorders related to vitamin B12 deficiency. It could be concluded that that vitamin B12 treatment may improve frontal lobe and language function in patients with cognitive impairment, but rarely reverses dementia.

2.8 Omega-3 fatty acids, their classification and associated health benefits

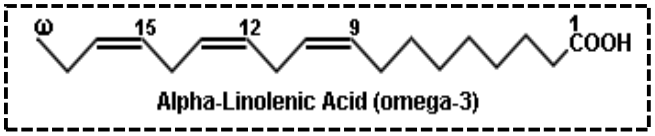
> ***"Omega-3 fatty acids improve neuron functions during aging and in patients affected by mild cognitive impairment."***
>
> ***- Yin et al 2016***

In polyunsaturated fatty acids (PUFAs) the first double bond may be found between the third and the fourth carbon atom from the o carbon; these are called omega-3 fatty acids. Omega-3 fatty acids cannot be interconverted, and re essential nutrients (Rustan and Drevon 2005).

Growing researches have demonstrated the effects of omega-3 polyunsaturated fatty acids (PUFA) on key metabolic functions, including inflammatory and immune response, coagulation and cell signaling (Klek 2016). The beneficial effects of omega-3-fatty acids on cardiac and extra cardiac organs have been extensively studied in the last two decades, and continue to show great promise in the primary and secondary prevention of cardiovascular diseases (CVDs). Omega-3-fatty acid supplementation has been proven to have beneficial action on lipid profile, cytokine cascade, oxidant-anti-oxidant balance, parasympathetic and sympathetic tone and nitric oxide synthesis (Chopra et al 2013). There are benefits of long-chain ω-3 polyunsaturated fatty acid (LC-PUFA) intake on brain functioning.There is only limited evidence to support that ω-3 LC-PUFA supplementation is beneficial in brain disorders, such as Alzheimer′s Disease, Attention Deficit/Hyperactivity Disorder, Major Depressive Disorder and schizophrenia (Bos et al 2016).

Classification of omega-3 fatty acids

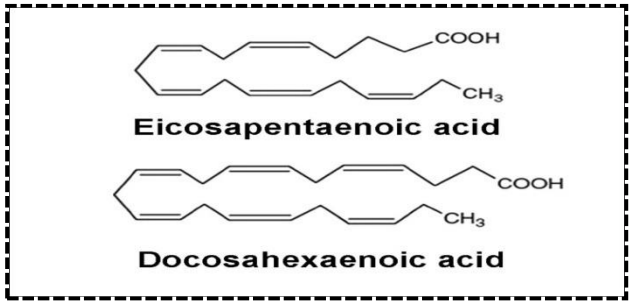
α-Linolenic acid (ALA): The chemical structure of ALA is C18:3n-3. ALA has 18 carbon atoms (C) and three double bonds, the first of which is located 3 carbon atoms from the terminal methyl group (omega [ω] end) which defines it as an omega-3 fatty acid (Food and Nutrition Board 2002). This plant derived omega-3 fatty acid can be converted to EPA and DHA (Chopra et al 2013).



Zamora 2016

Figure 2.8.1: Structure of α-Linolenic acid (ALA).

Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA): Eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6 ω-3) are major fatty acids of marine algae, fatty fish and fish oils (Rustan and Drevon 2005). The fish/fish oil-based ω-3 polyunsaturated fatty acids consist of EPA, (20 carbon atoms, 5 double bonds) and DHA, (22 carbon atoms, 6 double bonds) (Holub 2002). The chemical structure of EPA is C20:5n-3 and DHA is C22:6n-3 (USDA 2015). It is noted that EPA and DHA can be designated in abbreviated form as 20:5ω-3 or 20:5n-3 (i.e., 20 carbon atoms with 5 double bonds of the omega-3 configuration) and 22:6n-3, respectively. The number before the colon indicates the number of carbon atoms in the fatty acid chain and the number after the colon indicates the number of double bonds between adjacent carbon atoms (unsaturated sites) in the structure. Hence, the X:Y designation for DHA is 22:6 and the n-3 indicates that the first of six double bonds begins at the third carbon atom when beginning the carbon counting at the methyl end of the fatty acid structure (Holub 2002).



Chopra et al 2013

Figure 2.8.2: Structures of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)

The DHA and EPA are synthesized from the n-3 precursor a-linolenic acid (ALA; 18:3) (Ruxton et al 2004). These are further metabolized in the body by the addition of carbon atoms and by desaturation (extraction of hydrogen). Mammals have desaturases that are capable of removing hydrogens only from carbon atoms between

an existing double bond and the carboxyl group (Figure 2.8.3). β-oxidation of fatty acids may take place in either mitochondria or peroxisomes (Rustan and Drevon 2005). This enzyme, which is vital for the conversion of ALA to DHA and EPA, has a preference for ALA but the presence of high levels of plasma LA (caused by high n-6 PUFA intakes) can shift its actions towards the n-6 pathway (Budowski, 1988).

Although data on the required intake of essential fatty acids are relatively few, the adequate intakes of linoleic acid (18:2 o-6) and a-linolenic acid (18:3 o-3) should be 2% and 1% of total energy, respectively.

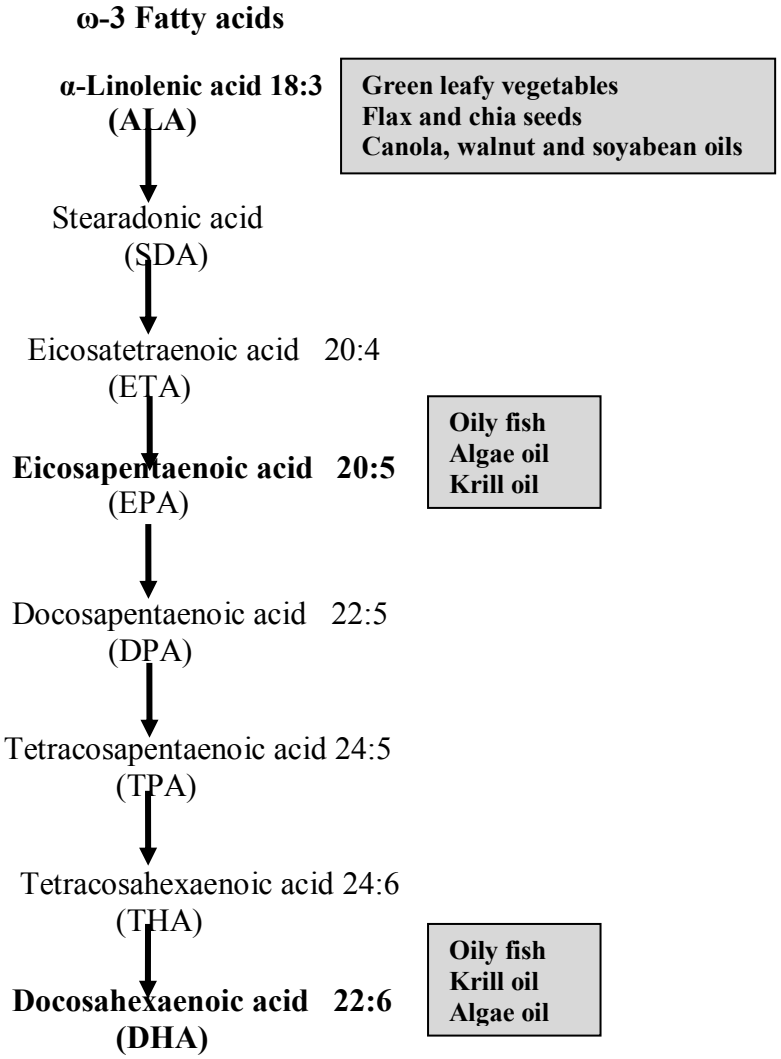


Figure 2.8.3: Synthesis of ω-3 polyunsaturated fatty acids (PUFAs). There is a family of essential fatty acids that are metabolized in the body as shown in this figure. Retroconversion, e.g. DHA → EPA also takes place. Dietary sources of different fatty acids are listed.

Rustan and Drevon 2005; Flock et al 2013

Present evidence suggests that 0.2–0.3% of the energy should be derived from preformed very long-chain o-3 PUFAs (EPA and DHA) to avoid signs or symptoms of deficiency. This corresponds to approximately 0.5 g of these o-3 fatty acids per day. It should be stressed that this is the minimum intake to avoid clinical symptoms of deficiency (Table 2.8.1).

It has been suggested that the ratio between o-3 and o-6 fatty acids should be 1:4 as compared to 1:10 in modern dietary habits, but the experimental basis for this suggestion is rather weak (Rustan and Drevon 2005).

Table 2.8.1 Recommended intake of essential PUFA[a]

	Intake as % of energy		Intake (mg day^{-1})	
	ω-3	**ω—6**	**ω--3**	**ω-6**
Minimum	0.2–0.3	1–3	400–600	2400–7200
Optimum	1–2	3–5	2400–4800	7200–12 000

[a] The numbers are based on data from patients with essential fatty acid deficiency and on estimation of required and optimal intake in healthy, normal individuals with energy intake of 9.2 MJ day^{-1}

Rustan and Drevon 2005

Selected food sources of a-linolenic acid (ALA) are given in Table 2.8.2**.** Some of the common plant oils have significant levels of ALA - e.g., 7% by weight in soybean oil, 10% in canola oil, and approximately 20% in hemp oil. Much higher amounts are found in the oils from flax, perilla (Japan and elsewhere), and chia (Argentina and elsewhere) with approximately 50-60% of the fatty acids being in the form of ALA. Furthermore, strains of flaxseed oils have become available which contain approximately 70% by weight of the oil as ALA which is significantly higher than the 50-55% found in conventional flax oil varieties. (Kris-Etherton et al 2000).

Table 2.8.3 gives the levels of EPA plus DHA in a few selected fish and seafood. Apart from herring, mackerel the white fish also contain omega-3 and can be included too. Examples include cod, haddock, plaice, pollack, coley, dover sole, dab, flounder, red mullet and gurnard (Williams and Burdge 2006).

Table 2.8.2. Apha-linolenic acid content of various foods

Source (100 g raw edible portion)	ALA (g)	Source (100 g raw edible portion)	ALA (g)
Flaxseed	22.8	Oats, germ	1.4
Walnuts, English/Persian	6.8	Seaweed, Spirulina (dried)	0.8
Chia seeds	3.9	Wheat , germ	0.7
Soyabeans, green (raw)	3.2	Spinach (raw)	0.1
Soyabeans (dry)	1.6	Mustard	0.1

Kris-Etherton et al 2000

Table 2.8.3. Fish and sea food sources of DHA plus EPA

Source (100 g portion)	DHA+ EPA (g)	Source (100 g portion)	DHA+ EPA (g)
Trout	0.9	Swordfish	0.8
Herring	2.0	Salmon	2.14
Catfish	0.1	Mussel	0.7
Clams	0.3	Oyster	0.4
Shrimp	0.3	Crab	0.4
Tuna, cooked/fresh	0.3	Octopus	0.3
Sardine	0.9	Mackerel	1.8

Williams and Burdge 2006

Associated health benefits of omega-3 fatty acids

Several of the messages have included the positive role of n-3 PUFA. The lower rate of cardiovascular diseases (CVD) seen in fish-eating communities, e.g. the Japanese, prompted investigations into how fish and its nutritional components might lower the risk of CVD (Ruxton et al 2004). Omega-3 PUFAs decrease platelet and leucocyte reactivity, inhibit lymphocyte proliferation, and slightly decrease blood pressure. They may also beneficially influence vessel wall characteristics and blood rheology, prevent ventricular arrhythmias and improve insulin sensitivity (Rustan and Drevon 2005).

Consumption of omega-3 fatty acids from fish has been associated with a variety of beneficial effects, including a reduction in overall mortality. This is largely due to

protection against coronary heart disease, in part through lowered heart rate and blood pressure (Torpy 2006). Other beneficial effects associated with fish consumption include reduced risk of stroke, depression, and mental decline with aging. (McNamara 2006).

Omega-3 fatty acids provide many beneficial effects to the body and brain (Ruxton et al 2004; Dyall and Michael-Titus 2008). Individuals with self-reported diets high in omega-3 fatty acids decline at a slower rate on the MMSE as compared to omega-3 deficient individuals (van Gelder et al 2007). Several human and animal studies have speculated about the additive or multiplicative benefits that might arise from combining omega-3 administration or supplementation with greater amounts of physical activity (Gómez-Pinilla and Feng 2012).

Omega- 3 effects for the cardiac functions

A study by Bulliyya (2002) compared fish-eaters (n = 500) with non fisheaters (n = 500) based in South Indian villages having found that the mean high-density lipoprotein (HDL) concentrations were consistently but modestly higher, whereas low-density lipoprotein (LDL) concentrations were lower in the fish-eaters compared with the non fish-eaters. There was also an improvement in the HDL: LDL ratio. Additionally, comparable effects on HDL were noted by Dewailly et al (2003) in their comparison of three ethnic groups in Quebec. Interestingly, the Inuit – regular fisheaters – demonstrated the lowest CVD risk despite a high prevalence of obesity and smoking.

Various studies provide compelling evidence regarding the beneficial effects of omega-3 fatty acids in reducing risk of cardiac death (Mozaffarian et al 2011). Omega-3 fatty acids may improve cardiac function by their anti-triglyceridemic, antihypertensive, hemostatic, antiarrhythmic and anti-atherogenic effects (deLeiris et al 2009; Kromhout et al 2012, Di Minno et al 2010). They may also confer cardiovascular benefits through enrichment of membrane phospholipids, vasodilation, antithrombotic potential and anti-inflammatory endothelial effects (Harris 2007; Din et al 2008; Pauwels and Koskiewicz 2008). Omega-3 fatty acids reduce the synthesis and secretion of very-low-density lipoprotein (VLDL) particles, and increase TG

removal from VLDL and chylomicron particles through the up-regualtion of enzymes, such as lipoprotein lipase (Bays et al 2008).

Role of omega-3 fatty acids in the brain

Interestingly, there is more to n-3 PUFAs than their well-recognized role in ameliorating the risk of heart disease (Ruxton et al 2004). The mouse models tudy signiifciantly indicated that providing either iron or ALA alone to double-deficient rats affected serotonin pathways and cognitive performance differently from combined provision (Baumgartner et al 2014).

Case-control studies have revealed associations between DHA or EPA and brain volume and lower degrees of white matter hyperintensities (Virtanen et al 2013; Tan et al 2012). In prospective studies, red blood cell DHA and EPA concentrations were positively correlated with higher total brain and hippocampal volumes 8 years later (Pottala et al 2014), and higher relative concentrations of plasma EPA were associated with a reduced brain atrophy rate in the medial temporal lobe (Samieri et al 2012). However, results from randomized clinical trials including n-3 supplementation are not equally convincing (Huang 2010, Sydenham et al 2012). One reason for this discrepancy may be a failure to identify the relevant subgroups that are likely to benefit from supplementation (Frautschy and Cole 2011).

The role of n-3 fatty acids in cognitive decline and dementia have been a debatable for quite some time now. Epidemiologic evidence is consistent with a protective role of dietary intake of fish, oils rich in n-3 fatty acids such as eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) (Beydoun et al 2014; Huang 2010).

Witte and coworkers (2014) described that daily fish-oil supplementation (880 mg DHA and 1320 mg EPA) in healthy elderly for 26 weeks prevented the loss of total gray matter volume. Mosconi and colleagues (2014) in their brain aging study comprising 49 cognitively normal individuals (age 25–72 years, 69% women) with dietary information, 11C-PiB and 18F-FDG PET scan examinations illustrated that the higher intake of ω-3 PUFA, EPA from food sources was associated with lower Aβ load in cognitively normal individuals. These data indicate that a healthy diet rich in natural folate, ω-3 PUFA and vitamin B12 might be particularly useful to support

healthy brain ageing. Similarly, plethora of studies have depicted that the intake of polyunsaturated fatty acids, especially ω-3 PUFA, is known to decrease risk of cognitive decline (Gu et al 2010; Gu et al 2012; Bowman et al 2011; Morris et al 2003).

About 50 to 60 percent of the dry weight portion of the human brain consists of lipids. PUFAs constitute approximately 35 percent of that lipid content. (Lauritzen et al 2001). Omega -3 fatty acids, particularly EPA and DHA, play important roles in the development and maintenance of normal central nervous system (CNS) structure and function. DHA is a major constituent of neuronal membranes, making up about 20 percent of the brain's dry weight (Yehuda et al 1999).

Release of n-3 fatty acids is involved in the phospholipase A2 cycle following activation of various neurotransmitter receptors. DHA is also important for normal cognitive development. PUFAs in general play important roles in structural and functional maintenance of neuronal membranes, neurotransmission, and eicosanoid biosynthesis, (Lauritzen et al 2001) as well as in the maintenance of membrane fluidity and flexibility and in the modulation of ion channels, receptors, and ATPases. The importance of PUFAs in maintenance of adequate membrane rigidity is evidenced by the loss of fluidity that follows decreased in PUFAs, leading to changes in the orientation and function of receptors and ion channels, such as calcium and sodium channels (Bourre et al 1991)

Numerous animal studies on mouse models have demonstrated superior learning and memory in animals fed omega-3 fatty acids compared with control animals (Hashimoto et al 2002). In transgenic mouse models, dietary DHA improved memory, increased synapse density and decreased amyloid beta toxicity, thus providing evidence of protection against AD and cognitive decline (Calon 2004).

Omega- 3 fatty acids in neurological disorders

Deficiencies in omega-3 fatty acids and/or an imbalance in the ratio of omega-6 fatty acids to omega-3 fatty acids have been implicated in a variety of disorders affecting the CNS, including AD (Cooper 2013) and several psychiatric disorders, Parkinson's

disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, ischemic brain injury, and multiple sclerosis (MS). Indeed, dietary intake of omega-3 fatty acids has been associated with a reduced incidence of MS since the early studies carried by Swank in the 1950s (Hag 2003).

Amongst nutritionally important polyunsaturated n-3 fatty acids, α-linolenic acid (ALA) are highly concentrated in the brain and have anti-oxidative stress, anti-inflammatory and antiapoptotic effects. They are involved in many bodily processes and may reportedly lead to neuron protection in neurological diseases. aged or damaged neurons and in Alzheimer's disease. Their effect in cognitive and behavioral functions and in several neurological and psychiatric disorders has been also proven.This process is associated with beneficial effects on cognition, mood and chronic pharmacological treatment. The exposure to n-3 fatty acids enhances adult hippocampal neurogenesis associated with cognitive and behavioral processes, promotes synaptic plasticity by increasing long-term potentiation and modulates synaptic protein expression to stimulate the dendritic arborization and new spines formation. Moreover they have pointed out for their possible use as a new therapeutic approach for neurodegenerative diseases (Crupi et al 2013).

Various animal and human studies have suggested several possible biological mechanisms for the role of fatty acids in disease processes. Evidence for a positive association between intake of omega-3 fatty acids and reduction of cardiovascular risk and adverse outcomes, along with the finding that certain forms of dementia have been related to cardiovascular risk factors, suggest that one mechanism linking fatty acids and cognitive function or dementia may be atherosclerosis and thrombotic events (Bretler 2003). Inflammation is another mechanism that may explain the role that omega-3 fatty acids play in dementia (Blok et al 1996). A study by Wurtman et al (2010) directed towards the improvement of memory in mild and very mild patients of AD stimulating the cellular and synaptic plasticity by the consumption of combination based on choline, uridine and DHA, along with B vitamins possessing beneficial effects in age–related cognitive decline.

Epidemiological findings have demonstrated of high PUFA intake to have a protective effect against the development of MCI. Moreover, findings from n-3 PUFA

supplementation based clinical trials showed efficacy on cognitive symptoms only in very mild AD subgroups, MCI patients, and cognitively unimpaired subjects non-APOE epsilon4 carriers. Numerous hypotheses well explain the association between dietary unsaturated fatty acids and cognitive functioning, comprising mechanisms through the co-presence of antioxidant compounds in fatty acid rich food groups, via atherosclerosis and thrombosis, inflammation, accumulation of beta-amyloid, or via a maintenance effect on the structural integrity of neuronal membranes, determining the fluidity of synaptosomal membranes that thereby regulate neuronal transmission. Collation of this data together with epidemiological evidence supported for a possible role of fatty acid intake in maintaining adequate cognitive functioning and prevention and management of cognitive decline and dementia, but not when the AD process has already taken over (Solfrizzi et al 2010).

In a study by Fiala et al (2015) investigated a significant immune and biochemical effects of 4-17 month supplementation with ω-3 fatty acids and antioxidants (Smartfish drink; Smartfish AS, Oslo, Norway) on the 12 patients with MCI (MMSE ≥19), 2 patients with preMCI (normal MMSE) and 7 patients with AD (MMSE <19). In patients with MCI and preMCI, phagocytosis of Aβ reported increase of monocytes from 530 to 1306 mean fluorescence intensity units (P=0.016) and similarly the lipidic mediator RvD1, which stimulates Aβ phagocytosis in vitro, too increased in macrophages in 80% of these patients. The mean MMSE score of MCI and preMCI patients was 25.9 at baseline and 25.7 after 4-17 months.

Freemantle et al (2006) contemplated that ALA and EPA may well have useful supporting roles in maintaining brain function during aging but not by their conversion to DHA. ALA is an efficient ketogenic fatty acid, while EPA promotes fatty acid oxidation. By helping to produce ketone bodies, the effects of ALA and EPA could well be useful in strategies intended to use ketones to bypass problems of impaired glucose access to the brain during aging.

Furthermore, a mouse model study illustrated that threshold changes for brain DHA metabolism and concentration were maintained at or below 09% dietary ALA

suggesting presence of homeostatic mechanisms to maintain brain DHA metabolism when dietary ALA intake is low (Taha et al 2016).

2.9 Emergence of flaxseeds as pro-active nutraceutical or functional food and augmenting effect in Mild Cognitive Impairment

> ***"The rising interest of the food industry in flaxseeds as nutraceutical is primarily because of functional nutrients, such as alpha-linolenic acid and lignans, which have health benefits due to their lipid-lowering properties."***
>
> ***-Ribas et al 2016***

Flaxseed (Linum usitatissimum) belonging to family Lineaceae. Flax seed is a blue flowering crop that produces small, flat seeds ranging in colour from golden yellow to reddish brown. The texture of flaxseed is crisp and chewy possessing a pleasant nutty taste (Carter 1996). Flaxseed is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications. Seed contains oil which after refining is used for edible purpose. The stem yields fiber of good quality possessing high strength and durability (Singh 2012). In India flaxseed is mainly cultivated in Madhya Pradesh, Maharashtra, Chattisgarh and Bihar. Flaxseed is considered a good nutritional food because of its exceptionally high content of alpha-linolenic acid (ALA), dietary fiber, high quality protein and phytoestrogens. Flaxseeds contain about 55% ALA, 28– 30% protein and 35% fiber (Rabetafika et al 2011). Flaxseed is establishing importance in the world's food chain as a functional food. Functional food can be defined as the food or food ingredients that may provide physiological benefits and helps in preventing and/or curing of diseases. ALA is one of the essential polyunsaturated fatty acid and reported to exhibit antiinflammatory, anti-thrombotic and anti-arrhythmic properties. Flaxseed serves as the best omega-3 fatty acid source to the non-fish eaters. Edible flaxseed products include the whole flaxseed, ground meal and extracted oil or mucilage. Most of the benefits reported from flaxseed consumption are believed to be due to the following three important components found in flaxseeds, fiber, lignans, and α-linolenic acid (ALA) (Al-Okbi 2005).

Dietary Fibre in Flaxseeds

Flaxseed is a rich source of dietary fiber both soluble as well as insoluble fibers. Insoluble fiber consists of cellulose, hemicellulose and lignin (Oomah 1993). Total dietary fibre content of flaxseed is 40 grams per 100 grams out of which 10 grams are soluble and 30 grams are insoluble. High amount of dietary fiber adds bulk to waste products in the gut and increases bile movement in the gastrointestinal movement. Low glycemic index foods containing soluble fiber not only prevent certain metabolic ramifications of insulin resistance but also reduce insulin resistance (Reaven 1999). Soluble fiber and other components of flaxseed fraction could potentially affect insulin secretion and its mechanism of action in maintaining plasma glucose homeostasis. Flaxseed was shown to reduce the post prandial blood glucose response in humans. Bread containing 25% flaxseed gave a glycemic response that was 28% lower than the control (no flaxseed) bread (Jenkins 1999). Only 10 g of flaxseed in the daily diet increases the daily fiber intake by 1g of soluble fiber and by 3 g of insoluble fiber. Insoluble fiber helps improve laxation and prevent constipation, mainly by increasing fecal bulk and reducing bowel transit time (Greenwald 2001). Studies have shown that the high intake of dietary fibers is beneficial for the prevention of obesity in both men and women (Du 2010).

Lignans

Flaxseed is the richest source of plant lignans. Secoisolariciresinol diglycoside (SDG) is the predominant lignan in flaxseed with minor amount of pinoresinol and matairesinol (MAT). SDG was found 2653 mg/100 g of non-defatted flaxseed extract lignans of flaxseed are phytoestrogens and serve as precursors in the production of mammalian lignans. The lignan content in flaxseed differs between varieties, but has also been shown to depend on growing location and year (Westcott et al 1996). Flaxseed lignans convert to mammalian lignans enterolactone and enterodiol by intenstinal flora (Wang and Rosell 2002). The lignan rich flaxseed may influence oestrogen metabolism and reduce the incidence of breast and colon cancer and possibly other disease (Horwitz and Walker 1984). Lignan may act to prevent oxygen radical production, thus effectively reducing atherosclerosis. Lignans have antioxidant activity and thus may contribute to the anticancer activity of flaxseed (Kaangas et al

2002). The mammalian lignans stimulate the synthesis of sex hormone binding globulin, which binds sex hormones and reduce their circulation in blood stream, and decrease their biological activity and thus reducing the risk of developing cancer (Thompson 1996).

α-Linolenic acid (ALA)

Flaxseed oil differs from whole and ground flaxseed by being devoid of both fiber and lignans. It is a unique oil in that it is composed of 73% polyunsaturated fatty acids (PUFA), 18% monounsaturated fatty acids (MUFA) and 9% saturated fatty acids (SFA), making it a low-saturated fat food. It is also the richest known source of the omega 3 (n-3) fatty acid, ALA, which comprises 55% of the total fatty acids. In fact, the percent of fat as ALA in flaxseed oil is 5.5 times higher than the next highest sources, walnuts and canola oil. Alpha-linolenic acid is the main functional component of flaxseed. It serves as an exclusive source of omega-3 fatty acid in the vegetarian diets. Fatty acids are termed as essential because both they are required by the body but body cannot synthesize them, therefore need to be supplied in the diet. Human body lacks the enzymes which are required for the synthesis of these essential fatty acids (Riediger 2009).

Flaxseeds contain a mixture of fatty acids. It is rich in polyunsaturated fatty acids, particularly ALA, the essential omega-3 fatty acid, and linoleic acid (LA), the essential omega-6 fatty acid. ALA and linoleic acid constitutes 57% and 16.0% of total fatty acids respectively in flax making the richest source of ALA. These two polyunsaturated fatty acids are essential for humans – that is, the body needs them. 12 g of ALA was taken three times a day by group of healthy young women in the flaxseed oil capsules and compared with group given in flaxseed flour supplemented products. Impressive reductions in blood lipids were observed in both cases (Cunnane and Ganguli 1993). Ground flaxseed is high in omega-3 fatty acids which have been shown to reduce hypertension, cholesterol and triglyceride level (Oomah 1998). The clinical studies revealed that n-3 polyunsaturated fatty acids are helpful in prevention of coronary heart diseases, atherosclerosis, rheumatoid arthritis and asthma. Daily intake of 3 g EPA and DHA for more than 12 weeks was found to be effective in reducing the inflammation of rheumatoid arthritis (Kremer 2000). Flaxseed and its oil

reduces the growth of tumors at the later stage of carcinogenesis; whereas, mammalian lignan precursor hold inhibitory potential for new tumor growths. Diverse studies advocated cholesterol lowering, antioxidant and hepato-protective benefits of flaxseed meal. EPA and DHA are clinically proven vital for reducing depressive symptoms (Thompson 1996). Laboratory research advocates ALA as antioxidant, free radical scavenging, preventing inflammatory and brain cell damaging conditions of stroke, multiple sclerosis, Alzheimer's Disease (AD) and dementia. Longer clinical trials reveal beneficial supplementation of 600 mg ALA combination with omega-3 fatty acids in AD patients, showing less cognitive and functional decline over one year compared to placebo (Rondenalli 2012).

Copious amount of animal studies instead of human trials have been carried out demonstrating ALA efficacy for benefitting memory functions. In a mouse model study, 2 weeks ALA treatment improved learning and memory and reduced oxidative stress in very old mice (Kim 2010). Similarly, 12 months dose-dependent ALA supplementation on aging rats improved spatial performance in working memory tasks. These results indicated that long-term dietary intake of ALA enhances cAMP response element-binding protein (CREB)/ brain-derived neurotrophic factor (BDNF)/ tyrosine kinase B (TrkB) pathway contributing to protective effects on cognitive deficits in natural aging (Gao et al 2015).

Examination of recent synergistic models using vitamin B12 and flaxseeds, undeniably associate them to cognitive, neurological, glycemic and lipemic responses. With reference, neutraceutical approaches using the vitamin B12 and flaxseeds are extensive in protective management of intermingled complex disorders as MCI, obesity, cardio-vascular disorders, diabetes and metabolic syndrome. This translates to inter-disciplinary animal based researches proving the efficacious supplementation of vitamin B12 and flaxseeds in ameliorating these disease states. Besides, acute deficiency of data on humans also restricts our existing understanding of exact linkage of vitamin B12 and flaxseeds inclusive diet, cognition, memory and associated metabolic diseases. In conjunction to these lacunae, longer duration human interventional clinical trials are necessitated to further establish the impact of vitamin B12 and flaxseeds on cognition and its related metabolic aberrations.