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Metabolic and Nutraceutical Approaches to preventing Cognitive Decline

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DEDICATION

I want to express my sincere gratitude to each of my team member for your dedication and hard work. Thank you for your help and support on this project. Your innovative ideas and creative solutions were key to this project's success. Your collaborative spirit and commitment did not go unnoticed.

To me, who dared to dream and the women I have become through this process and my parents, who planted the seed of knowledge in my mind, reminded me that words have the power to change the world. Your wisdom and patience have contributed to my growth. Your unconditional love and support inspired me to always do my best, be kind and take nothing for granted.

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Summary

The progressive ageing of the global population is associated with a marked increase in frailty and neurodegenerative disorders, including mild cognitive impairment and dementia. Cognitive decline is driven by complex interactions among metabolic dysfunction, chronic low-grade inflammation, oxidative stress, and lifestyle-related factors. Identifying early biomarkers and developing effective preventive strategies represent major challenges in contemporary biomedical and clinical research. Within this framework, metabolic regulation and nutraceutical interventions have emerged as promising, non-pharmacological approaches to preserve cognitive function and delay neurodegeneration.

This doctoral thesis, entitled "*Metabolic and Nutraceutical Approaches to Preventing Cognitive Decline*", investigates the contribution of metabolic alterations, peripheral biomarkers, and dietary factors to cognitive ageing and neurodegenerative disease prevention, integrating clinical, biochemical, and population-based perspectives.

In the first part of the thesis are introduced the biological basis of brain ageing, frailty, and neurodegenerative diseases, with a focus on the pathophysiological mechanisms linking systemic metabolic dysregulation to cognitive impairment. Metabolic approaches to preventing cognitive decline are addressed, examining the role of insulin resistance, lipid and energy metabolism, mitochondrial dysfunction, and metabolic comorbidities as modifiable risk factors for neurodegeneration. Nutraceutical approaches are also explored, examining evidence on bioactive compounds, dietary supplements, and functional foods with potential neuroprotective effects through the modulation of oxidative stress, neuroinflammation, and synaptic function.

The second part of the thesis concerns the experimental part and it is divided into three sections. **Section 1** outlines sex-specific metabolic signatures associated with Alzheimer's disease, with particular attention to circulating biomarkers involved in amino acid and lipid metabolism, in order to improve the understanding of biological differences in disease vulnerability and progression. **Section 2** critically examines the role of the cereal processing industry as a determinant of population nutrition and public health through the work conducted at Al-Faisal Flour and General Mill (Pakistan). Subsequently, **Section 3** presents a cross-sectional study conducted at Shifa Tameer-e-Millat University (Pakistan), evaluating whole-wheat flour intake as a dietary factor potentially associated with long-term dementia prevention, thus providing insights into the role of culturally specific dietary habits in cognitive health.

In conclusion, this thesis supports the relevance of integrated metabolic and nutraceutical strategies in the prevention of cognitive decline. The findings highlight the potential of peripheral biomarkers and dietary interventions for early risk stratification and personalized preventive

approaches, and outline future research directions aimed at translating these insights into clinical and public health practice.

INTRODUCTION

1. Ageing population

Populations across the globe are ageing rapidly due to decreased birth rates and longer life expectancies. This shift presents needs that require a holistic approach to tackle with the challenges face by ageing population. It is crucial to recognize various public health obstacles to meet the changing healthcare needs. Research reports that by 2050, people aged 65 and above will surpass children under 5 years old worldwide for the first time in history, The percentage of people globally over 60 years old in that same year will nearly double from 12% to 22% aaccording to the World Health Organization (WHO) (Aradhana 2025; Deliz et al. 2024).

The term “successful ageing” was coined to highlight the difference between typical and successful ageing. To age successfully, one has to be in good health free from any disease or disability and be physically and socially, cognitively active and contributing to society. This model of successful ageing presented by Rowe and Kahn (Rowe e Kahn 1997) was praised by Whitley et al. (Whitley et al. 2016). It was an objective measure of successful ageing. They further suggested successful ageing should be considered a spectrum, rather than a dichotomy which should allow age-related decline and compensation strategies to coexist (Waddell et al. 2024).

This profound demographic shift in the ageing population presents a number of complex public health challenges extracted from articles data. This information includes public health issues and needs impacting seniors such as chronic conditions, vulnerability to illnesses, disabilities, geriatric conditions, mental health challenges, other ailments that necessitate enhanced medical care and long-term services and support, increased dependency, caregiving demands, and social or environmental challenges (Z. Wu et al. 2025).

Ageing not only involves these factors but also confronts challenges, such as ageism, social isolation, financial insecurity, elder mistreatment, caregiving responsibilities, housing access, obstacles in transportation, and community engagement, effects on economies, social services, healthcare systems, communities and families (Aradhana 2025).

To tackle with the array of public health challenges proactively faced by rapidly growing ageing populations globally requires implementing collaborative, multisectoral policy solutions focused on improvements in issues concerning chronic disease management, healthcare infrastructure, improvising social support systems that demands immediate attention to meet the needs of elderly people and tap into their strengths (H. T. A. Khan et al. 2024).

Ageing and frailty

A major health concern linked to ageing populations is frailty due to the occurrence of

diseases. As individuals grow older, they are more prone to developing ailments (chronic disease) the frequency of these illnesses increases significantly with age. These includes heart disease, stroke, cancer, diabetes, arthritis, hypertension, dementia and lung disease. Chronic diseases are also referred as non-communicable diseases that are typically not curable but can be managed through continuous medical care and lifestyle adjustments. The illnesses cause limitations in functioning and restricted activities which result in a loss of independence, feelings of isolation and the necessity for ongoing care whether formal or informal (Lu et al. 2025).

The stigma around mental health and cognitive health issues poses significant public health challenges as populations age and prevents senior adults from seeking treatment. Common conditions such as depression, anxiety, dementia, delirium and substance abuse impact the quality of life. Depression affects up to 15% of older adults, if left untreated, can worsen outcomes of other conditions. Anxiety disorders also worsen chronic disease and disability while reducing social engagement. Dementia cases, mainly Alzheimer's disease, are projected to triple by 2050, requiring extensive caregiving. Delirium, an acute confused state, affects over 7 million hospitalized elderly people annually, increasing morbidity and mortality (H. T. A. Khan et al. 2024).

Falls which is a leading cause of injury for those aged 65 and above, is another significant marker of frailty which causes immediate physical injuries and immobility leading to a cascade of adverse outcomes both acute and chronic health impairment in elderly individuals. Millions of aged population requires emergency care annually for fall injuries such as fractures, head traumas, and dislocations (Harris et al. 2024).

An area that has been under research but been overlooked in the last few years is the impact of the menopause on women's health. It is considered a major life event that can have a detrimental impact on physical and mental health as well as on health issues such as osteoporosis leading to fractures, in later life. As populations age, more and more women are experiencing the menopause. Not all women will experience short- or long-term problems but research states that it is anticipated that by 2025, the number of postmenopausal women will be around 1.1 billion worldwide and it is claimed that many will present with complex medical issues beyond the scope of traditional gynaecologists and general practitioners (Choi et al. 2024).

Cancer is another increasing challenge faced by the ageing population is the growing burden of among individuals. It is natural process of cellular ageing and the accumulation of carcinogens. As people get older, they face a risk of developing prostate, breast, lung and colorectal cancers. Dealing with cancer presents challenges as ederly people often have other health issues chemotherapy tends to cause side effects in elderly patients leading to adjustments in dosage or early treatment cessation, that can complicate cancer treatments and make them harder to endure.

This collectively contribute to morbidity and mortality rates for cancers among older adults compared to younger individuals (Harris et al. 2024).

Caregiving gaps: meeting the needs of ageing populations

The growth of ageing populations worldwide presents significant public health challenges and places enormous strains on caregiving systems. Both paid and unpaid caregivers are both in short supply, leading to major care gaps. Mostly informal family caregivers provide most of the long-term care for elderly individuals. Due to fragmented families this workforce is overburdened and fewer family members are available to provide care (Cesari et al. 2024).

Staffing shortages and inadequate care and hospice/palliative services impacts quality of care home care workers leading to an extremely short supply of care workers which is a risk factor to deterioration of health in elderly people. It is clear that building and sustaining an adequate caregiving workforce is imperative to enable well-being, quality of life and dignity for ageing individuals. It presents daunting challenges but also provides opportunities to innovate systems and support (Choi et al. 2024).

According to Genworth, the cost of long-term in accessing healthcare include transportation limitations, shortages of geriatric specialists and care fragmentation has been rising steadily. This impedes the ability of elderly individuals to attend medical appointments, especially in rural or remote areas which leads to delayed care and poor health outcomes The nursing home care charges in the United States in 2021 averaging over $8910 per month (H. T. A. Khan et al. 2024).

Workforce shortages of geriatric specialists and primary care physicians trained in elder care also reduce access and quality of care. Although no single intervention alone can solve all the key issues highlighted but solutions to improve healthcare access for elderly individuals include expanding transportation services, funding more geriatric fellowships, integrating care teams, enhancing care transitions and investing in telehealth and outreach to bridge access gaps. With widespread population ageing, ensuring accessible, coordinated, high-quality healthcare for seniors is an urgent priority (H. T. A. Khan et al. 2024).

2. Neurodegenerative disorders

There is increasing evidence to suggest a role for the cerebellum in neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, Friedreich’s ataxia, spinal muscular atrophy, juvenile Batten’s disease, and multiple sclerosis, etc. (Iskusnykh et al. 2024).

Alzheimer's disease

Alzheimer's disease is a progressive brain disorder, the most common cause of dementia, constitutes about 60-70% of all cases of dementia worldwide and is an age-related neurodegenerative disease characterized by gradual memory loss thinking decline, and behavioural changes, leading to severe difficulty with daily tasks. The brain cell damage by the deposition of amyloid plaques and neurofibrillary tangles is the characteristic of Alzheimer's disease which appear in the cerebellum primarily during the later stages of the disease (Chamakuri e Janapana 2025). The cerebellum is more resistant to the neurotoxicity caused by the soluble amyloid-beta protein. It starts subtly during the early stages of Alzheimer's disease, the cerebellum compensates with enhanced activity that results in the improvement of some forms of memory but worsens to impact judgment, language, and recognition, with symptoms appearing years after brain changes begin. In addition, there is no cure but treatments to manage symptoms and slow progression. Over 6 million people in the United States have Alzheimer's dementia. By 2050, this number is projected to increase to about 13 million (Alzheimer's Association). It is estimated that more than 360,000 new cases occur in the United States each year (Cohen et al. 2024).

Parkinson's disease

Parkinson's disease is a progressive brain disorder in which the cerebellar dysfunction is associated with causing tremors, impaired balance, and unstable gait, stiffness, slow movement (bradykinesia), and balance issues due to the loss of dopamine-producing brain cells, affecting motor skills, mental health, sleep, and daily function. It is caused by aggregations of alpha-synuclein protein in the pre-cerebellar brainstem and the cerebellar nuclei (Deliz et al. 2024). Parkinson's disease patients, patients with postural instability and gait disorders exhibit cerebellar atrophy, specifically the reduced volume of the right crus II, right lobules IV and V, lobules VIII and IX, vermis VIII and IX, pyramis, and culmen. Some non-motor symptoms include depression, anxiety, apathy, sleep problems (insomnia, acting out dreams), constipation, fatigue, difficulty speaking (dysarthria) or swallowing (dysphagia), cognitive changes, and memory issues. It has no cure. The symptoms begin gradually, often on one side, and worsen over time, impacting walking, talking, and simple tasks but manageable through medications, therapy, lifestyle changes, and sometimes surgery with diagnosis based on medical history and neurological exams (Khani et al. 2024).

Huntington's disease

Huntington's disease is a rare, inherited neurological disorder caused appearing in mid-life (30s-40s) by genetic mutation (CAG repeat expansion in the HTT gene) which produces a toxic

huntingtin protein that kills brain cells, particularly in the striatum. Huntington's disease is characterized by uncontrollable movement (chorea), abnormal body posture, and functional deficits in behaviour, cognitive decline (memory, thinking), severe emotional/mood changes (depression, irritability), and personality changes (Moore 2025). While there's no cure, treatments focus on managing symptoms with therapies and medications to improve quality of life. In Huntington's disease, cerebellar degeneration was observed in the right crus I lobule, bilateral crus II lobules, and the left VIIb and VIIIa lobules, while another study noted significant volume loss in the cerebellar grey and white matter. Cerebellar volume loss is associated with a reduced number of Purkinje cells and granule cells. Notably, there was a detrimental change in gene expression, particularly of those factors that are responsible for exocytosis and vesicular fusion in granule cells (Martínez Lozada et al. 2024).

Amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis, or Lou Gehrig's disease, is a fatal, progressive neurological disorder. It is associated with progressive cerebellar degeneration (motor neurons) controlling voluntary muscles, leading to weakness, paralysis, and eventually respiratory failure, though cognition usually remains intact. Some scientists claims that genetics and environment play a role in causing Amyotrophic Lateral Sclerosis (Ludolph et al. 2024). It has no cure, but treatments, like riluzole and edaravone, can manage symptoms and potentially slow decline, with supportive care crucial for quality of life. The specific role of the cerebellum in this disorder may be related to the cerebellum-specific repeat expansion of several genes that determine (Wei et al. 2024).

Friedreich's ataxia

Friedreich's Ataxia is a rare, hereditary, progressive neurodegenerative disorder characterized by progressive motor dysfunction causing gradual loss of coordination (ataxia), muscle weakness, fatigue, and impaired speech due to damage to the spinal cord, nerves, and cerebellum, often starting in childhood/adolescence (Indelicato et al. 2025). It usually appears between ages 5 and 15, but can vary. Early onset often means faster progression. It is caused by a mutation in the *FXN* gene, commonly leads to severe heart problems (cardiomyopathy) and diabetes, significantly impacting life expectancy. Treatment focusses on symptom control and physical therapy, plus new treatments like omaveloxolone (Lima et al. 2025).

Spinal muscular atrophy

Spinal Muscular Atrophy is a rare genetic neuromuscular disorder which affects spinal cord motor neurons, causing their atrophy from inactivity. It leads to progressive muscle weakness and

wasting due to the loss of motor neurons in the spinal cord, leading to difficulties with movement, breathing, and swallowing, though it doesn't affect intellect. Loss of motor control in Spinal Muscular Atrophy patients involves the neurodegeneration of several brain regions, including the cerebellum. It's caused by mutations in the *SMN1* gene, resulting in low levels of the crucial Survival Motor Neuron protein, with severity and age of onset varying across types (Type 1 being most severe, Type 4 mildest). While, effective treatments and supportive care help manage symptoms, with recent advance as there's no cure of Spinal Muscular Atrophy (Schroth et al. 2024).

Juvenile Batten's disease

Juvenile Batten's Disease, also known as Juvenile Neuronal Ceroid Lipofuscinosis, is a rare, inherited, fatal neurodegenerative disorder. It onsets is in childhood (ages 5-10) characterised by vision loss, clumsiness, and seizures, leading to severe cognitive decline, motor impairment, dementia, and premature death, usually by the late teens or early twenties, due to a genetic mutation causing waste build-up in brain cells. It causes defects in lysosomal storage caused by mutations in the ceroid lipofuscinosis neuronal 3 (*Cln3*) gene, leading to the degeneration of cerebellar and retinal neurons. There's no cure, but supportive care and research for treatments helps manage the symptoms (Iskusnykh et al. 2024).

Multiple sclerosis

Multiple Sclerosis is a chronic autoimmune disease of the central nervous system (brain, spinal cord, optic nerves) in which cerebellar dysfunction starts in the early stages of the disease and the immune system attacks myelin, the protective coating of nerve fibers, disrupting nerve signals and causing varied symptoms such as vision problems, numbness, weakness, balance issues, and cognitive difficulties, with treatments focusing on managing relapses and slowing progression to maintain quality of life (Jellinger 2024).

Vascular dementia

Cerebrovascular diseases are increasingly prevalent in aging, tremendously impacting a patient's quality of life. It results from reduced blood flow to the brain, often as a result of a stroke or a series of strokes. It is the second most common form of dementia, and is believed to cause 10 to 20 per cent of cases (Al-Kuraishy et al. 2025). Cerebrovascular diseases such as stroke can impact the cerebellum due to cerebellar bi-directional interconnection to numerous brain regions, severely decreasing cerebellar function (Morgan e Mc Auley 2024).

3. Metabolic dysfunction as a driver of cognitive decline

Cognitive decline represents one of the most pressing challenges associated with population aging, with profound medical, social, and economic implications. Mild cognitive impairment and dementia, particularly Alzheimer's disease, affect hundreds of millions of individuals worldwide and currently lack disease-modifying therapies. As a result, increasing emphasis has been placed on prevention strategies targeting modifiable risk factors across the life course.

In recent years, a growing body of evidence has highlighted the central role of metabolic dysfunction in the pathogenesis of cognitive decline. Alterations in glucose metabolism, insulin signaling, lipid homeostasis, mitochondrial function, and systemic inflammation have emerged as shared mechanisms linking metabolic disorders, such as obesity, type 2 diabetes mellitus, and metabolic syndrome, to neurodegenerative diseases. These observations have led to the conceptualization of Alzheimer's disease as a "metabolic brain disease" sometimes referred to as "type 3 diabetes".

Within this framework, metabolic interventions aimed at restoring energy homeostasis and reducing systemic and cerebral metabolic stress represent promising strategies to delay or prevent cognitive deterioration. Therefore, this is possible through metabolomics analyses. Metabolomics, the comprehensive analysis of metabolites in biological samples, has emerged as a powerful tool to characterize metabolic alterations associated with cognitive aging and to identify biomarkers predictive of future cognitive impairment.

Metabolomics captures a snapshot of biochemical processes that reflect both genetic and environmental influences. In the context of aging and neurodegeneration, alterations in lipid metabolism, amino acid profiles, energy pathways, and diet-derived metabolites have been consistently associated with changes in cognition. Meta-analyses and systematic reviews indicate that circulating levels of lipids (e.g., phosphatidylcholines, sphingomyelins), amino acids (e.g., branched-chain amino acids), and steroid metabolites are significantly associated with cognitive performance and risk of dementia in longitudinal cohort studies (Jiang et al. 2019).

Metabolomics not only reveals potential biomarkers but also sheds light on perturbed biochemical pathways that might contribute to neurodegenerative processes, thereby providing targets for preventive strategies. Altered glucose metabolism, neuroinflammation-related metabolites, and gut microbiota-derived compounds have emerged as key metabolic nodes implicated in early brain changes preceding overt cognitive symptoms (H. Zhao et al. 2025).

This chapter reviews current evidence on metabolic approaches to preventing cognitive decline, integrating mechanistic insights, translational research, and clinical data, with particular attention to dietary interventions, caloric modulation, insulin sensitization, and emerging metabolic therapies. Furthermore, the following chapter reviews state-of-the-art metabolomics

approaches relevant to preventing cognitive decline, integrating evidence from recent studies that link metabolic profiles with early detection and intervention.

Brain energy metabolism and aging

The human brain accounts for approximately 20-25% of total glucose consumption despite representing only 2% of body weight. Neuronal function critically depends on efficient glucose uptake and mitochondrial oxidative phosphorylation. Aging is associated with a progressive decline in cerebral glucose metabolism, detectable decades before the onset of clinical cognitive symptoms.

Positron emission tomography studies consistently demonstrate reduced glucose utilization in temporoparietal and frontal regions in individuals with mild cognitive impairment and Alzheimer's disease. These metabolic deficits precede amyloid deposition and neurodegeneration, suggesting that impaired energy metabolism is an early and potentially causal event in cognitive decline (Cunnane et al. 2011).

Insulin resistance and "Type 3 Diabetes"

Insulin plays a crucial role in the central nervous system, regulating synaptic plasticity, neurotransmitter release, and neuronal survival. Peripheral insulin resistance is frequently accompanied by impaired insulin signalling in the brain, leading to reduced glucose uptake, increased oxidative stress, and dysregulation of tau phosphorylation and amyloid-β clearance.

Epidemiological studies indicate that individuals with type 2 diabetes mellitus have a 50-100% increased risk of developing dementia. Moreover, insulin resistance has been independently associated with poorer cognitive performance even in non-diabetic populations. These findings support the hypothesis that targeting insulin sensitivity may be a viable strategy for cognitive preservation (Arnold et al. 2018).

Metabolic syndrome, inflammation, and vascular contributions

Metabolic syndrome, characterized by central obesity, dyslipidemia, hypertension, and insulin resistance, contributes to cognitive decline through multiple converging pathways. Chronic low-grade inflammation, endothelial dysfunction, and cerebrovascular damage impair cerebral perfusion and blood-brain barrier integrity, facilitating neurodegeneration.

Systemic inflammatory mediators such as tumor necrosis factor-alpha, interleukin-6, and C-reactive protein have been linked to accelerated cognitive aging, reinforcing the concept that metabolic and inflammatory processes are tightly intertwined in the pathophysiology of cognitive impairment (Livingston et al. 2020; Estruch et al. 2018).

Metabolomics for early prediction of cognitive decline

One of the most promising uses of metabolomics in dementia prevention is the identification of metabolic signatures predictive of future cognitive decline before clinical manifestation. Recent integrative metabolomics studies have used advanced analytical platforms (e.g., high-resolution mass spectrometry and nuclear magnetic resonance) to analyze blood metabolite profiles in amyloid-positive individuals who are cognitively normal at baseline. These studies successfully identified a signature of specific metabolites (including intermediates of energy metabolism and lipid species) that distinguished individuals who progressed to cognitive decline from those who remained stable more than three years before measurable cognitive deterioration (Tremblay-Franco et al. 2024).

Such predictive metabolite profiles, especially when integrated with neuroimaging and other biomarkers, offer the potential to stratify individuals by risk status, enabling earlier and more personalized preventive interventions.

Dietary interventions and cognitive health

The Mediterranean diet (MedDiet) is one of the most extensively studied dietary patterns in relation to cognitive aging. Characterized by high intake of fruits, vegetables, whole grains, legumes, olive oil, and fish, and low consumption of red meat and refined sugars, the MedDiet exerts beneficial effects on metabolic health, inflammation, and oxidative stress (Estruch et al. 2018).

Large observational cohorts and randomized controlled trials, including the PREDIMED study, have demonstrated that adherence to the MedDiet is associated with reduced risk of cognitive decline, mild cognitive impairment, and dementia. Notably, neuroprotective effects appear to be mediated by improvements in insulin sensitivity, lipid profiles, and vascular function.

Emerging evidence suggests that the MedDiet may also mitigate genetic risk, particularly in APOE $\varepsilon 4$ carriers, highlighting its relevance for personalized preventive strategies (Livingston et al. 2020).

The MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay) combines elements of the MedDiet and the DASH diet, emphasizing foods specifically associated with brain health, such as leafy green vegetables, berries, nuts, and fish.

Prospective studies indicate that even moderate adherence to the MIND diet is associated with significantly slower cognitive decline and reduced incidence of Alzheimer's disease. These benefits are thought to arise from synergistic metabolic and anti-inflammatory effects, reinforcing the importance of dietary quality over single nutrients.

Macronutrient composition and glycemic control

Beyond dietary patterns, macronutrient composition plays a critical role in modulating cognitive outcomes. Diets characterized by high glycemic load and excessive refined carbohydrate intake promote insulin resistance and glycemic variability, both of which have been linked to cognitive impairment.

Conversely, diets with balanced macronutrient distribution, adequate protein intake, and healthy fats, particularly omega-3 polyunsaturated fatty acids, support metabolic stability and neuronal membrane integrity (Jacka 2017).

Diet, nutritional metabolomics, and cognitive health

Diet profoundly influences circulating metabolite levels and, by extension, cognitive aging. Untargeted metabolomics approaches have identified diet-related metabolites (e.g., biomarkers of coffee, fish intake, and specific fatty acids) associated with slower cognitive decline over extended follow-ups in prospective cohort studies (Low et al. 2019).

Furthermore, metabolomics-based dietary indices have been developed to quantitatively capture adherence to protective dietary patterns, such as the Mediterranean diet. These metabolite scores correlate with reduced risk of cognitive decline, suggesting that metabolic phenotyping can provide objective measures of diet quality beyond self-reported food intake (Estruch et al. 2018).

Nutritional metabolomics thus offers a dual role: it uncovers potential mechanistic links between dietary patterns and brain health, and it may serve as a biomarker-based tool to monitor and optimize preventative nutritional interventions (Angeloni et al. 2020; Ghosh et al. 2026).

Nutraceuticals and metabolic modulators

Omega-3 fatty acids, particularly docosahexaenoic acid, are essential components of neuronal membranes and play a role in synaptic function and neuroinflammation regulation. Reduced docosahexaenoic acid levels have been observed in patients with Alzheimer’s desease.

While clinical trial results have been mixed, subgroup analyses suggest that omega-3 supplementation may be beneficial in early stages of cognitive decline and in individuals with low baseline intake, highlighting the importance of timing and metabolic context (Vauzour et al. 2017).

Polyphenols such as resveratrol, flavonoids, and curcumin exert antioxidant and anti-inflammatory effects and modulate metabolic signaling pathways including AMPK and sirtuins. These compounds have demonstrated neuroprotective effects in preclinical models, improving mitochondrial function and insulin sensitivity (Kullmann et al. 2020).

Although human evidence remains heterogeneous, ongoing trials are exploring optimized formulations and combination approaches to enhance bioavailability and clinical efficacy.

B-vitamins, particularly folate, vitamin B12, and vitamin B6, are critical for homocysteine metabolism. Elevated homocysteine levels have been associated with increased risk of cognitive decline and brain atrophy.

Intervention studies suggest that B-vitamin supplementation may slow cognitive deterioration in individuals with elevated homocysteine, underscoring the role of metabolic micronutrient balance in brain health (Jacka 2017; Vauzour et al. 2017).

Caloric restriction and fasting-based interventions

Caloric restriction and intermittent fasting improve metabolic efficiency by enhancing insulin sensitivity, promoting mitochondrial biogenesis, and activating cellular stress-response pathways such as autophagy. These mechanisms are highly relevant to neurodegeneration, where accumulation of damaged proteins and organelles contributes to neuronal dysfunction.

Animal models consistently demonstrate that caloric restriction delays cognitive aging and reduces amyloid and tau pathology. In humans, observational studies and short-term trials indicate that fasting-based interventions improve metabolic markers associated with cognitive risk, including insulin resistance and inflammation (Arnold et al. 2018).

While long-term cognitive outcomes remain under investigation, fasting-based approaches represent a promising avenue for metabolic prevention strategies.

Ketone metabolism in the aging brain

Ketone bodies provide an alternative energy substrate for the brain when glucose utilization is impaired. In aging and Alzheimer's disease, cerebral ketone uptake appears relatively preserved, suggesting that ketone-based strategies may compensate for glucose hypometabolism (Butterfield et al. 2022).

Ketogenic diets and exogenous ketone supplementation have demonstrated modest cognitive benefits in individuals with mild cognitive impairment and early Alzheimer's disease, particularly in APOE ε4-negative subjects. Mechanistically, ketones may enhance mitochondrial efficiency, reduce oxidative stress, and modulate neuroinflammatory pathways.

Despite concerns regarding long-term adherence and cardiovascular safety, refined ketogenic interventions and personalized protocols are under active investigation (Huang et al. 2025; Ardanaz et al. 2022).

Pharmacological metabolic interventions

Drugs commonly used in metabolic disorders, such as metformin and GLP-1 receptor agonists, have gained interest for their potential neuroprotective effects. Preclinical studies suggest

that these agents improve synaptic plasticity, reduce neuroinflammation, and enhance insulin signaling in the brain.

Several clinical trials are currently evaluating GLP-1 receptor agonists in patients with mild cognitive impairment and Alzheimer's disease, representing a promising example of translational repurposing (Barzilai et al. 2016).

Intranasal insulin delivery bypasses the blood-brain barrier and directly targets central insulin signaling. Early trials have shown improvements in memory and functional outcomes, although results vary depending on dosage, sex, and genetic background (Barzilai et al. 2016).

Multidomain lifestyle interventions

Evidence increasingly supports the effectiveness of multidomain interventions combining diet, physical activity, cognitive training, and metabolic risk management. The FINGER trial demonstrated that such integrated approaches can significantly slow cognitive decline in at-risk older adults, emphasizing the importance of addressing metabolic health within a broader lifestyle framework (Ngandu et al. 2015).

Gut microbiota-metabolite interactions

A growing body of evidence highlights the *gut microbiota-brain axis* as a key pathway linking peripheral metabolism to central nervous system function. Dysbiosis of the gut microbiome alters the production of microbial-derived metabolites that can enter systemic circulation, modulate inflammation, and influence brain function. Recent multi-omics studies integrating metabolomics and microbiome profiling have revealed metabolic signatures correlating with both structural brain changes and cognitive measures across the Alzheimer's disease continuum (H. Zhao et al. 2025).

These findings underscore the relevance of microbial metabolites, such as bile acids, short-chain fatty acids, and tryptophan derivatives, as potential early markers of cognitive deterioration and as targets for preventive modulation through diet, probiotics, and lifestyle.

4. Conceptual framework: biological pathways targeted by nutraceuticals

Cognitive decline represents a spectrum of age-associated impairments ranging from subtle decrements in memory and executive function to clinical syndromes such as mild cognitive impairment and dementia, including Alzheimer's disease. Given the limited success of pharmacological disease-modifying therapies, preventive strategies are increasingly prioritized in translational and clinical research. Among the modifiable factors influencing brain aging, dietary patterns and specific bioactive compounds, collectively termed *nutraceuticals*, have attracted substantial scientific interest due to their pleiotropic biological effects, relatively low risk profiles, and ease of integration into lifestyle interventions.

Nutraceuticals are food-derived bioactive compounds that exert health benefits beyond basic nutrition, including anti-oxidative, anti-inflammatory, neurotrophic, and metabolic modulatory actions. These effects align with key biological processes implicated in cognitive aging and neurodegeneration, such as oxidative stress, chronic inflammation, amyloid and tau pathology, synaptic dysfunction, and mitochondrial impairment (Sonnino et al. 2025; Kheirouri e Alizadeh 2025). This chapter critically examines current evidence on nutraceutical approaches to preventing cognitive decline, focusing on mechanistic insights, preclinical and clinical findings, and translational potential.

Oxidative stress and neuroinflammation

Age-related increases in reactive oxygen species and pro-inflammatory signaling contribute to neuronal damage and synaptic loss. Nutraceuticals with antioxidant and anti-inflammatory properties, such as polyphenols, carotenoids, and omega-3 polyunsaturated fatty acids, modulate redox balance and attenuate microglial activation (Figure 1). Elevated peripheral and central inflammatory markers, including interleukin-6 and C-reactive protein, have been consistently associated with cognitive impairment, strengthening the rationale for targeting neuroinflammation in preventive strategies (Reza-Zaldívar e Jacobo-Velázquez 2023; Mekhora et al. 2024).

Neurotransmission and synaptic plasticity

Bioactive compounds may enhance neuronal signaling and plasticity. For example, polyphenols and omega-3 polyunsaturated fatty acids influence brain-derived neurotrophic factor, which supports synaptic health. Modulation of neurotransmitter systems, including cholinergic and glutamatergic pathways, may also underpin cognitive effects attributed to specific nutraceuticals.

Metabolic dysregulation

Metabolic dysfunction, including insulin resistance and impaired glucose utilization, is implicated in cognitive aging. Nutraceuticals that improve metabolic parameters or provide alternative energy substrates (e.g., ketone precursors) may indirectly support cognitive health by stabilizing systemic and cerebral energy metabolism.

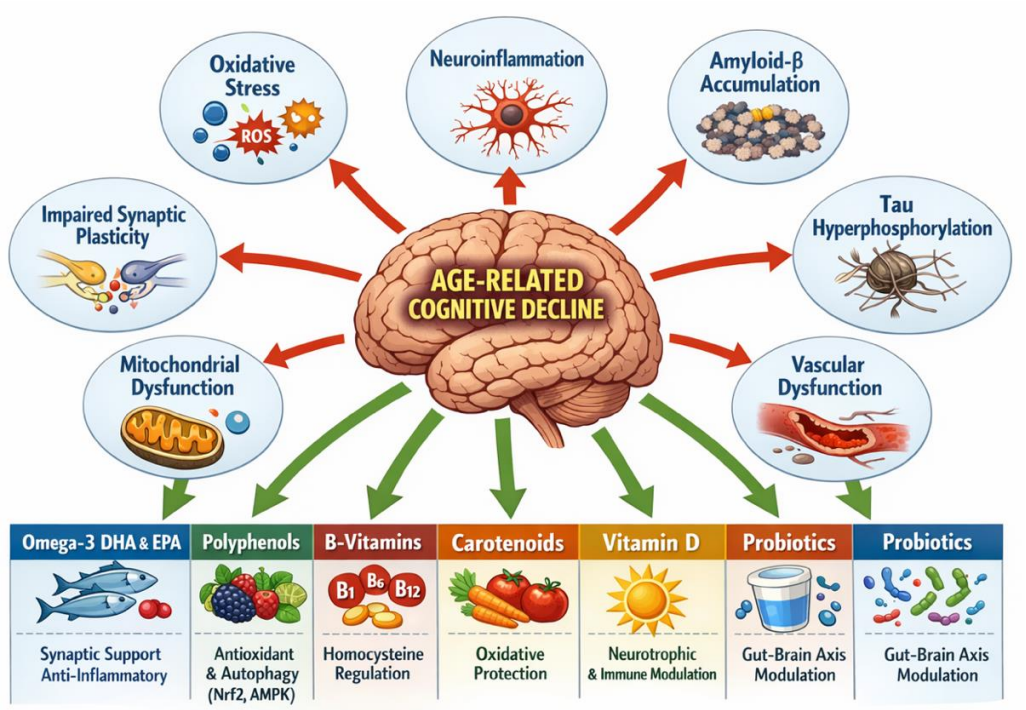


Figure 1. Schematic overview of age-related cognitive decline mechanisms and the multi-target neuroprotective actions of nutraceuticals.

Omega-3 polyunsaturated fatty acids

Omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, present mainly in fish, are structural components of neuronal membranes and influence inflammation, synaptic plasticity, and vascular function. (Castellanos-Perilla et al. 2024), Epidemiological evidence suggests that higher dietary intake of long-chain omega-3 polyunsaturated fatty acids is associated with lower risk of cognitive decline and dementia (Mulargia et al. 2025; Welty 2023).

Although results are mixed, systematic reviews of randomized controlled trials indicate modest cognitive benefits, especially in cognitively healthy older adults and individuals with mild cognitive impairment. A recent overview across multiple randomized controlled trials (n ≈ 26,881 participants) found statistically significant but modest improvements in global cognition with omega-3 polyunsaturated fatty acids supplementation, supporting its role as a complementary strategy within multi-domain interventions (Barros et al. 2025).

Omega-3 polyunsaturated fatty acids modulate membrane fluidity, reduce pro-inflammatory eicosanoid production, and may enhance cerebral blood flow. They also interact with lipid receptors and signaling pathways linked to amyloid processing and tau phosphorylation.

B-Vitamins and homocysteine modulation

Elevated homocysteine is a well-established risk factor for cognitive decline. B-vitamins such as folate, vitamin B12, and B6 are essential cofactors in homocysteine metabolism. Intervention studies demonstrate that B-vitamin supplementation lowering homocysteine levels can favourably impact cognitive performance, particularly in individuals with elevated baseline levels (Kang et al. 2021).

Vitamins D, E and other micronutrients

Vitamin D has neuroprotective attributes, including modulation of neurotrophins like brain-derived neurotrophic factor and anti-inflammatory effects. Systematic analyses suggest associations between adequate vitamin D status and improved cognitive function, although long-term randomized controlled trials evidence remains limited (Fu et al. 2024).

Vitamin E, a lipid-soluble antioxidant, also emerges in some studies as potentially beneficial in slowing cognitive decline, though evidence is variant and context-dependent.

Polyphenols and flavonoids

Polyphenolic compounds, including flavonoids (e.g., catechins in green tea, resveratrol in grapes), exhibit potent antioxidant and anti-inflammatory properties. They influence multiple pathways relevant to brain health, such as enhancing cerebral blood flow, reducing oxidative damage, and modulating signaling proteins associated with synaptic plasticity and neurogenesis (Godos et al. 2024).

Systematic reviews of specific polyphenols, including epigallocatechin gallate, fisetin, and spermidine, highlight their potential to modulate autophagy, reduce neuroinflammation, and improve markers of neuronal health in preclinical models (Gruendler et al. 2020).

Carotenoids and phytochemicals

Carotenoids such as lutein and zeaxanthin exhibit antioxidant capacity and have been correlated with cognitive performance in observational studies. Combined supplementation with omega-3s and carotenoids has shown promising enhancements in working memory and related cognitive domains in older adults.

Evidence from systematic reviews and trials

Comprehensive reviews indicate that omega-3 fatty acids and B vitamins have the strongest evidence base among nutraceuticals for cognitive preservation, while other compounds (e.g.,

vitamins D/E, polyphenols) show potential but require further rigorous trials (D'Cunha et al. 2018).

The heterogeneity of study designs, dosages, intervention durations, participant baseline cognitive status, and outcome measures complicates comparisons across studies. Genetic factors, such as apolipoprotein E genotype, may modify responses to nutraceuticals, highlighting the need for personalized strategies and larger, well-powered randomized controlled trials.

Gut-brain axis and microbiome modulation

Emerging research suggests that nutraceuticals may exert effects through modulation of the gut microbiome, which influences metabolite production, immune signaling, and blood-brain barrier integrity. Polyphenols and prebiotic fibers alter microbiome composition favourably, with downstream effects on neurofunctional outcomes.

Oxidative stress pathways and neuroprotection

By enhancing endogenous antioxidant defences and reducing oxidative damage to lipids, proteins, and DNA, nutraceuticals may stabilize neuronal function. Many polyphenols also activate transcription factors like Nuclear factor erythroid 2-related factor 2, which regulate antioxidant gene expression.

5. Whole Grain Consumption as a Modifiable Dietary Factor in Dementia Prevention

Dementia represents a growing global public health challenge, with increasing prevalence in ageing populations and substantial social and economic burden. Preventive strategies are urgently needed, particularly those based on modifiable lifestyle factors such as diet (Johnstone et al. 2025). Among these, dietary patterns that emphasize whole grains have attracted considerable interest, as they may confer neuroprotective benefits via multiple mechanisms, including reduced inflammation, improved vascular health, and metabolic regulation (Solfrizzi et al. 2011; K. Wang e Zhou 2023).

Several epidemiological studies suggest that higher whole grain consumption is associated with a lower risk of cognitive decline and dementia. For example, in the Framingham Offspring Cohort, individuals with the highest intake of whole grains had a significantly lower risk of developing dementia and Alzheimer's disease compared to those with the lowest intake (K. Wang et al. 2023). In addition, in a biracial cohort drawn from the Chicago Health and Aging Project, greater whole-grain consumption was linked to a slower rate of decline in global cognition, perceptual speed, and episodic memory, especially among African American participants (X. Liu et al. 2023).

Systematic reviews of observational and interventional studies further explore these associations. Though the overall evidence is still considered limited and of low-to-moderate strength, several studies point to potential cognitive benefits of whole-grain intake (Ross et al. 2023). Moreover, population-based reviews of dietary patterns (such as the Mediterranean and MIND diets), which often include whole grains as a core component, consistently report neuroprotective effects and reduced incidence of cognitive disorders (Townsend et al. 2023).

At a biological level, whole grains are rich in dietary fiber, B-vitamins, polyphenols, and other bioactive compounds which may contribute to neuroprotection. These nutrients have been implicated in mechanisms such as antioxidant activity, reduced oxidative stress, and improved endothelial function, all relevant to delaying or preventing neurodegenerative processes (Foscolou et al. 2019).

Despite promising data from Western cohorts, there is a paucity of research exploring whole grain intake in low- and middle-income countries, especially in regions where wheat-based products dominate dietary patterns. Pakistan, in particular, is characterized by high consumption of wheat flour (often refined), but little is known about the prevalence of whole wheat flour intake and its potential long-term implications for cognitive health in the local population.

6. Translational and clinical implications

From a translational perspective, metabolic approaches to cognitive prevention align with precision medicine principles, allowing interventions to be tailored based on metabolic profiles, genetic risk, and disease stage. Early identification of metabolic dysfunction may enable timely preventive strategies before irreversible neurodegeneration occurs. Furthermore, the translation of metabolomics research into clinical preventive strategies depends on several factors. Standardization of analytical platforms, larger longitudinal cohorts with replication across diverse populations, and integration with other omics layers (e.g., genomics, proteomics) are necessary to improve robustness and interpretability (Jiang et al. 2019).

Nonetheless, the identification of early metabolic alterations offers several actionable opportunities:

- risk stratification: metabolite signatures can complement genetic and imaging biomarkers to identify individuals at high risk for future cognitive decline;
- monitoring interventions: changes in metabolomic profiles may serve as sensitive readouts of preventive interventions (e.g., diet, exercise);
- mechanistic insights: metabolite pathways reveal biochemical processes that could be directly targeted by therapeutics or lifestyle modifications.

Precision nutrition approaches that integrate genetic profiling, metabolic biomarkers, and lifestyle contexts may optimize nutraceutical effectiveness. For example, individuals with elevated homocysteine or specific metabolic risk profiles may particularly benefit from targeted B-vitamin regimens.

Nutraceutical strategies are often most effective when integrated with broader lifestyle interventions, including physical activity, cognitive engagement, and cardiovascular risk management. Synergistic effects among these domains may enhance cognitive resilience (Figure 2).

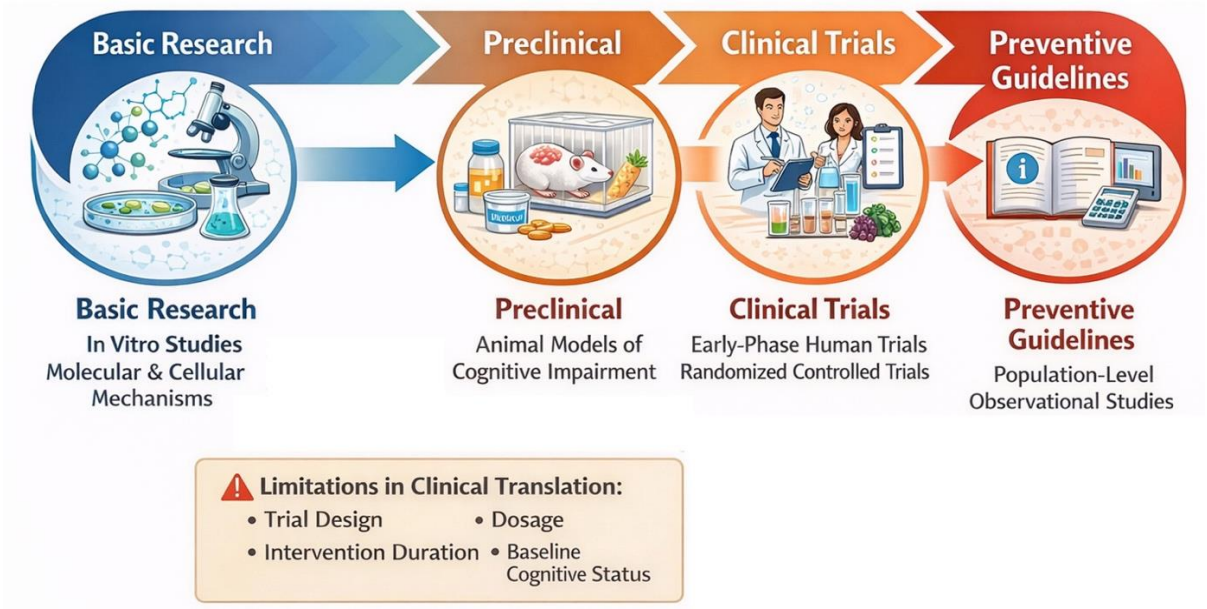


Figure 2. Translational pathway of nutraceutical research from mechanistic studies to clinical and population evidence.

7. Future directions and challenges

While promising, several challenges persist. The *causal interpretation* of metabolomic associations remains complex, as metabolite levels reflect convergent influences of genetics, environment, and aging. Moreover, analytical heterogeneity across studies limits comparability. Future research should emphasize harmonized protocols, large-scale multi-center cohorts, and integrative computational frameworks to address missing data and pathway complexity (Y. Zhang et al. 2025).

Integrating metabolomics with real-world digital health data (e.g., continuous monitoring) and machine learning tools also holds promise for dynamic risk prediction and personalized prevention.

Metabolic dysfunction plays a central role in the development and progression of cognitive decline. Interventions targeting glucose metabolism, insulin resistance, lipid homeostasis, and systemic inflammation offer promising avenues for prevention and early intervention. Integrating metabolic strategies into clinical practice may significantly alter the trajectory of cognitive aging and reduce the global burden of dementia.

Metabolomics has emerged as a key approach in understanding and preventing cognitive decline. By capturing systemic metabolic alterations linked to diet, gut microbiota, energy metabolism, and neurodegeneration, metabolomics provides both mechanistic insights and practical biomarkers for early detection and targeted prevention. Continued advancement in analytical methods, longitudinal studies, and translational frameworks will be essential to fully harness the potential of metabolomics in maintaining cognitive health across the lifespan.

Despite promising data, several areas require advancement:

- longitudinal randomized controlled trials with standardized outcomes and biomarker analyses to clarify efficacy and dose-response relationships;
- mechanistic studies in humans to validate pathways observed in preclinical models;
- research on combinatorial nutraceutical regimens and interactions with medications or dietary patterns;
- inclusion of diverse populations to assess generalizability and potential subgroup effects.

Nutraceutical approaches offer a compelling framework for mitigating cognitive decline through multi-target biological mechanisms. While evidence for specific compounds such as omega-3 polyunsaturated fatty acids and B vitamins is relatively robust, ongoing research is needed to establish definitive guidelines, optimal dosages, and synergistic strategies. Integrating nutraceuticals into personalized, multi-domain preventive paradigms holds promise for addressing the growing global burden of cognitive aging and dementia (Figure 3).

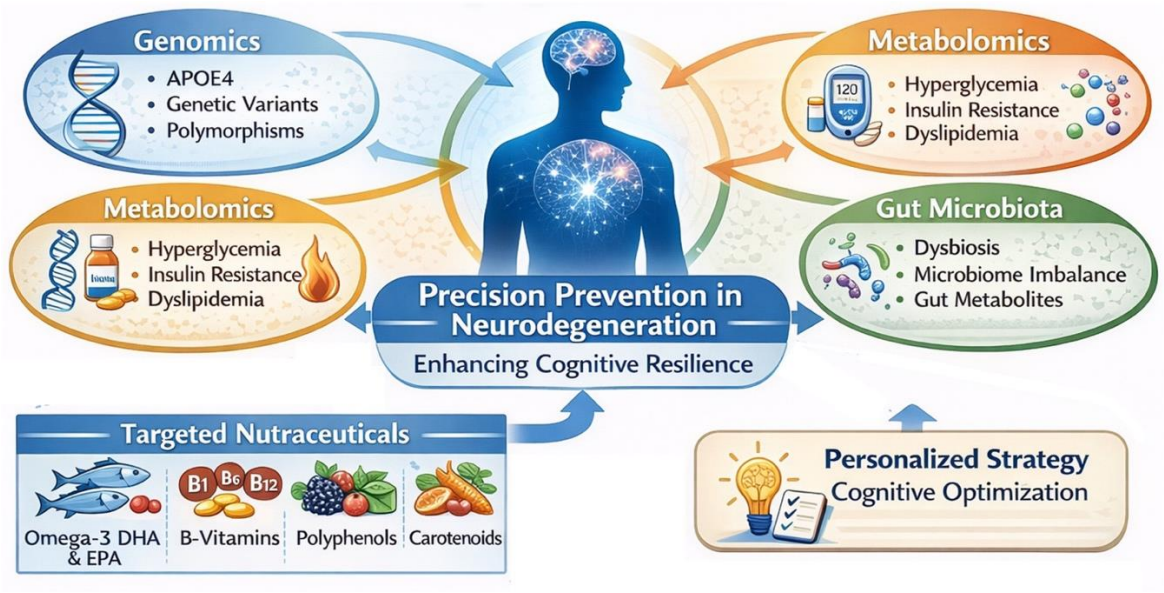


Figure 3. Conceptual model of personalized nutraceutical strategies for precision prevention of cognitive decline.

PURPOSE OF THE RESEARCH

The aim of this PhD thesis was to investigate how metabolic alterations and nutrition-related factors may contribute to the prevention or modulation of cognitive decline, with a particular focus on Alzheimer's disease and population-based preventive strategies.

Specifically, this work aimed to:

(**i**) characterize sex-specific metabolic signatures associated with Alzheimer's disease, with particular attention to circulating biomarkers involved in amino acid and lipid metabolism, in order to improve the understanding of biological differences in disease vulnerability and progression (***Section 1***);

(**ii**) critically examine the role of the cereal processing industry as a determinant of population nutrition and public health, through industrial training and professional experience at a large-scale flour mill in Pakistan, highlighting opportunities for nutritional improvement of staple foods (***Section 2***); and

(**iii**) assess dietary whole wheat flour intake in the Pakistani population and explore its potential implications for long-term dementia prevention, within the framework of modifiable lifestyle and dietary risk factors (***Section 3***).

By integrating clinical metabolic profiling, applied industrial experience, and population-based dietary analysis, this thesis sought to provide a multidisciplinary perspective on how metabolic regulation and nutraceutical strategies may be leveraged to reduce the risk of cognitive decline and support healthier brain ageing at both individual and population levels.

SECTION 1

SEX-SPECIFIC METABOLIC SIGNATURES IN ALZHEIMER'S DISEASE

Materials and methods

Study population and clinical assessment

One hundred and twenty-one participants were consecutively recruited from the Center for Cognitive Disorders and Dementia and the Center for Research and Training in Medicine of Aging, University of Molise, Italy. All subjects were diagnosed with probable Alzheimer's disease or mild cognitive impairment due to Alzheimer's disease according to the National Institute on Aging-Alzheimer's Association (McKhann et al. 2011) diagnostic criteria. The cohort included individuals across a wide spectrum of disease severity, ranging from mild cognitive impairment to severe dementia.

To exclude alternative causes of cognitive impairment, participants underwent comprehensive clinical, neuropsychological, and laboratory evaluations, as well as brain imaging. Depressive symptoms were assessed using the Geriatric Depression Scale-Short Form, and individuals with scores indicative of major depression were excluded.

Global cognitive function was assessed using the Mini-Mental State Examination, while functional autonomy was evaluated using the Basic Activities of Daily Living and Instrumental Activities of Daily Living scales. Medical comorbidities and their severity were quantified using the Cumulative Illness Rating Scale. Demographic variables, lifestyle factors, vascular risk factors, and pharmacological treatments were systematically recorded.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Institutional Review Board (IRB Prot. N. 16/2020). Written informed consent was obtained from all participants or their legal caregivers.

Neuropsychological assessment and mass spectrometry in the diagnosis of neurodegenerative diseases

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, frontotemporal dementia, and dementia with Lewy bodies, represent a major and growing public health challenge worldwide. These disorders are characterized by progressive neuronal dysfunction and loss, leading to cognitive, behavioural, and motor impairments. Given the irreversible nature of neuronal damage, early and accurate diagnosis is crucial for patient management, prognostic evaluation, and the development and monitoring of disease-modifying therapies.

Currently, the diagnostic process for neurodegenerative diseases relies on a combination of clinical evaluation, neuropsychological testing, neuroimaging, and biochemical biomarkers. Among these, neuropsychological tests play a central role in the assessment of cognitive and functional impairment, while advanced analytical techniques such as mass spectrometry are increasingly employed to identify molecular biomarkers associated with neurodegenerative processes. This chapter provides an overview of the most widely used neuropsychological tests and discusses the emerging role of mass spectrometry in the diagnosis and characterization of neurodegenerative diseases.

Neuropsychological assessment in neurodegenerative diseases

Neuropsychological testing is a cornerstone of the clinical evaluation of patients with suspected neurodegenerative disorders. These tests aim to quantify cognitive deficits across multiple domains, including memory, attention, executive function, language, visuospatial abilities, and praxis. The pattern and severity of cognitive impairment can provide valuable information for differential diagnosis and disease staging (Petersen et al. 1999).

Global cognitive screening tests

Global cognitive screening instruments are commonly used in both clinical and research settings due to their ease of administration, relatively short duration, and ability to provide an overall estimate of cognitive functioning.

Mini-Mental State Examination

The Mini-Mental State Examination (MMSE) is one of the most widely used cognitive screening tools in clinical practice. It assesses several cognitive domains, including orientation to time and place, immediate and delayed recall, attention and calculation, language, and visuoconstructive abilities. The total score ranges from 0 to 30, with lower scores indicating greater cognitive impairment (Figure 4).

Despite its widespread use, the MMSE has several limitations. It shows reduced sensitivity in detecting mild cognitive impairment and early stages of dementia, and its performance can be influenced by age, education, and cultural background. Furthermore, the MMSE provides limited information on executive functions, which are often impaired in non-Alzheimer neurodegenerative disorders (Valles-Salgado et al. 2024).

MINI MENTAL STATE EXAMINATION

Name: _____

Patient ID: _____ Date of evaluation: _____

Orientation *score* *max*

1. What is the year ___ 1
season ___ 1
date ___ 1
day ___ 1
month ___ 1

2. Where are we state ___ 1
county ___ 1
town ___ 1
hospital ___ 1
floor ___ 1

Registration

3. Name 3 objects (key, table, coin): 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct answer. Then repeat them until he learns all 3. *score* *max*
___ 3

Attention and calculation

4. Serial 7's. Ask the patient: 'What is 100-7'. Upon reply 'x'. Ask: 'What is x-7'. 1 point for each correct. Stop after 5 answers. Alternatively spell 'world' backwards.
score *max*
___ 5

Recall

5. Ask for the 3 objects repeated above. 1 point for each correct.
score *max*
___ 3

Total score: _____ **of max 30.**

Language

6. Show the patient a watch. Ask what it is. Repeat for pencil ___ 2

7. Repeat the following: "'No ifs, ands or buts" ___ 1

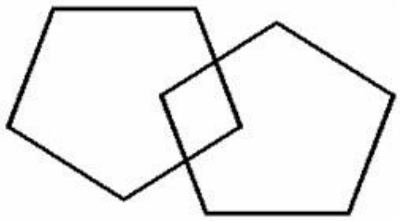
8. Follow a 3-stage command: "Take a paper in your right hand, fold it in half, and put it on the floor" ___ 3

9. Read and obey the following:
CLOSE YOUR EYES ___ 1

10. Write a sentence:

___ 1

11. Copy this drawing



___ 1

Figure 4. Examples of questions on the MMSE

Montreal Cognitive Assessment

The Montreal Cognitive Assessment (MoCA) was developed to overcome some of the limitations of the MMSE, particularly in the detection of mild cognitive impairment. It includes tasks assessing executive function, attention, memory, language, visuospatial skills, and abstract reasoning (Figure 5). The MoCA has demonstrated higher sensitivity than the MMSE in identifying early cognitive decline, making it particularly useful in prodromal stages of neurodegenerative diseases (Valles-Salgado et al. 2024).

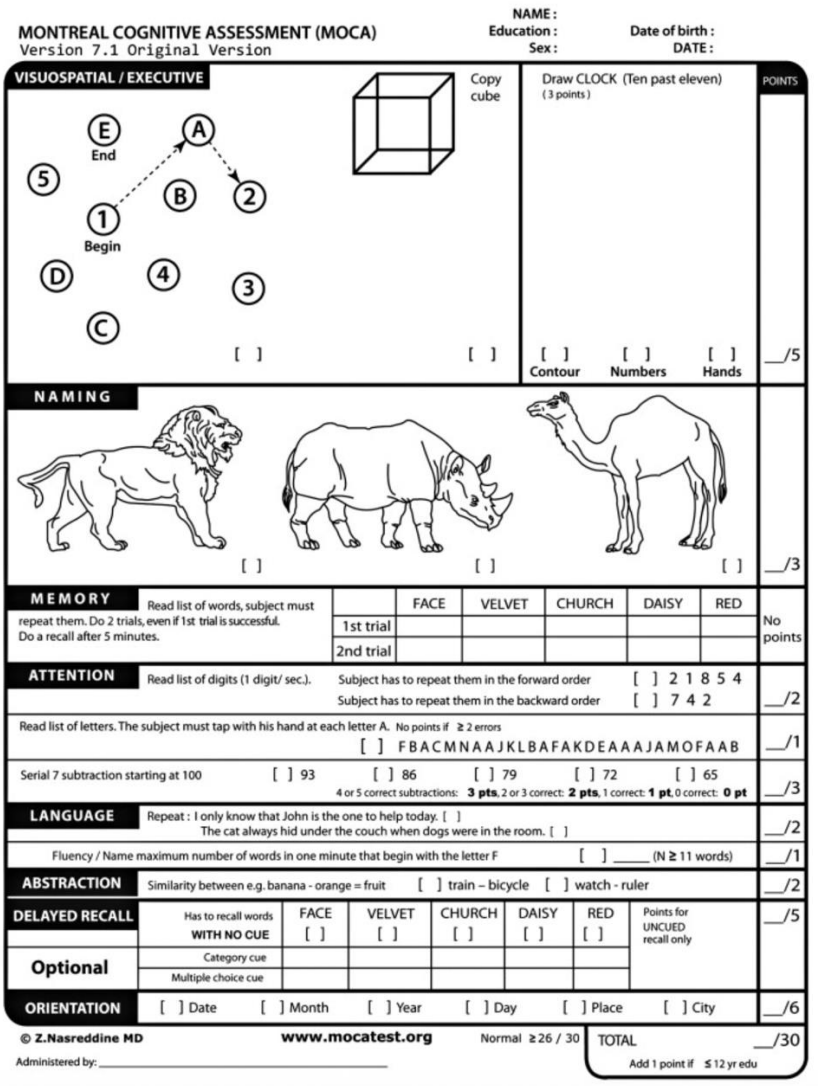


Figure 5. Example of tests present in the MoCA.

Domain-specific neuropsychological tests

In addition to global screening instruments, comprehensive neuropsychological batteries include domain-specific tests that allow a more detailed characterization of cognitive deficits.

Memory Assessment

Episodic memory impairment is a hallmark of Alzheimer's disease. Tests such as the Rey Auditory Verbal Learning Test and the Free and Cued Selective Reminding Test are commonly used to assess verbal learning, recall, and recognition. Distinct patterns of memory impairment can help differentiate Alzheimer's disease from other dementias, such as frontotemporal dementia, where memory may be relatively preserved in early stages.

Executive Functions and Attention

Executive dysfunction is particularly prominent in frontotemporal dementia and Parkinson's disease dementia. Tests such as the Trail Making Test, Stroop Color-Word Test, and verbal fluency tasks (phonemic and semantic) are used to assess cognitive flexibility, inhibitory control, and processing speed.

Visuospatial and Constructional Abilities

Visuospatial deficits are commonly observed in Alzheimer's disease and dementia with Lewy bodies. The Clock Drawing Test is a simple and effective tool for assessing visuospatial abilities, planning, and executive function (Figure 6). Patients are typically asked to draw a clock face with a specific time indicated. Errors in number placement, hand positioning, or overall organization can provide insight into underlying cognitive dysfunction.

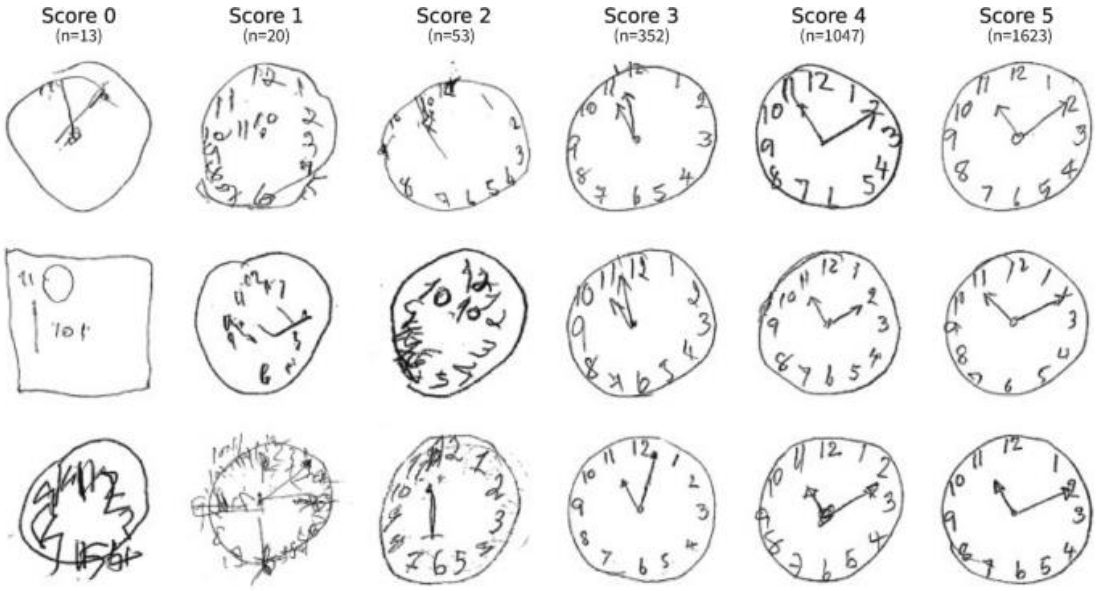


Figure 6. Examples of drawings made by patients during the Clock Drawing Test.

Limitations of neuropsychological testing

While neuropsychological tests are essential for clinical diagnosis, they are inherently indirect measures of brain pathology. Cognitive performance can be influenced by non-neurological factors such as depression, anxiety, fatigue, and sensory impairments. Moreover, neuropsychological assessment alone cannot provide information about the underlying molecular mechanisms of neurodegeneration. These limitations highlight the need for complementary biomarker-based approaches.

Blood collection and biochemical analyses

Venous blood samples were collected in the morning (between 7:30 and 8:00 a.m.) after an overnight fast of approximately 12 hours. Routine hematological and biochemical parameters were analyzed as part of the standard diagnostic work-up for cognitive disorders. These included markers of liver and kidney function, electrolyte balance, inflammatory status, vitamin levels, and immune-related parameters.

Non-routine analyses focused on circulating amino acid and fatty acid profiles, given their emerging relevance in neurodegenerative diseases. Plasma amino acids were quantified using standardized laboratory procedures, while fatty acid composition was determined in serum/plasma lipid fractions, including saturated, monounsaturated, and polyunsaturated fatty acids.

Amyloid beta 42 (Aβ42) levels were measured using a commercially available ELISA kit. Aβ40 was not included in the analysis due to insufficient detectability in a substantial proportion of samples.

Two analytical strategies were adopted:

1. Comparison between females and males with Alzheimer's disease to identify sex-specific differences;
2. Evaluation of deviations from established physiological reference ranges to detect disease-related metabolic alterations.

Biomarkers in neurodegenerative diseases

Biomarkers are objectively measurable indicators of biological processes, pathogenic mechanisms, or responses to therapeutic interventions. In the context of neurodegenerative diseases, biomarkers can reflect key pathological features such as amyloid-beta deposition, tau pathology, synaptic dysfunction, neuroinflammation, and neuronal loss.

Traditional biomarkers include cerebrospinal fluid measurements of amyloid-beta, total tau, and phosphorylated tau, as well as neuroimaging markers obtained through magnetic resonance imaging and positron emission tomography. In recent years, advances in analytical chemistry have enabled the identification of novel biomarkers using mass spectrometry-based approaches (Mangoni e Zinellu 2025; Azevedo et al. 2022).

Mass spectrometry in neurodegenerative disease research and diagnosis

Mass spectrometry is a powerful analytical technique that allows the identification and quantification of molecules based on their mass-to-charge ratio. A typical mass spectrometer consists of an ion source, a mass analyzer, and a detector. When coupled with separation

techniques such as liquid chromatography (LC-MS), mass spectrometry enables the analysis of complex biological samples with high sensitivity and specificity (Jang et al. 2023).

Proteomics and neurodegeneration

Proteomic approaches using mass spectrometry have significantly advanced the understanding of neurodegenerative diseases. By analyzing protein expression, post-translational modifications, and protein–protein interactions, MS-based proteomics can reveal disease-specific molecular signatures (Schumacher-Schuh et al. 2022).

In Alzheimer's disease, mass spectrometry has been used to quantify amyloid-beta peptides and different tau isoforms in cerebrospinal fluid and plasma. Similarly, in Parkinson's disease, MS-based studies have focused on alpha-synuclein and related pathways (Azevedo et al. 2022). These approaches not only improve diagnostic accuracy but also provide insights into disease mechanisms and progression.

Metabolomics and lipidomics

Beyond proteomics, mass spectrometry is widely used in metabolomics and lipidomics to investigate alterations in small molecules and lipid species associated with neurodegeneration. Changes in energy metabolism, oxidative stress markers, and lipid homeostasis have been reported in several neurodegenerative conditions. Such metabolic signatures may serve as early biomarkers, potentially detectable before the onset of overt cognitive symptoms (Shao et al. 2021; Schumacher-Schuh et al. 2022).

GC-MS analysis

Metabolite extraction, purification, and derivatization were performed using the *MetaboPrep kit* (Theoreo, Montecorvino Pugliano, SA) according to the manufacturer's instructions (Troisi et al. 2020). Briefly, 200 μL of extraction mixture supplemented with an internal standard were added to the cell pellet, followed by shaking at 1250 rpm for 30 min and sonication for an additional 30 min. Extracts were centrifuged at 16,000 rpm for 5 min at temperatures below 4 °C, and 200 μL of the supernatant were transferred to a purification tube, mixed at 1250 rpm for 30 s, and rapidly centrifuged at 16,000 rpm below 4 °C to prevent solid-phase solubilization. Subsequently, 175 μL of the liquid supernatant were transferred to glass vials and lyophilized overnight. For Gas Chromatography-Mass Spectrometry (GC-MS) analysis, dried extracts were derivatized by adding 50 μL of the first derivatization reagent (methoximation step) and shaking at 1200 rpm at room temperature for 90 min, followed by the addition of 25 μL of the second derivatization reagent (silylation step) and further shaking under the same conditions for

90 min. The derivatized samples were transferred to 100 μL glass inserts, centrifuged again at 16,000 rpm for 5 min below 4 °C, and 2 μL were injected into a GC-MS system (GC-2010 Plus gas chromatograph coupled to a 2010 Plus single-quadrupole mass spectrometer; Shimadzu, Kyoto, Japan). Chromatographic separation was achieved on a CP-Sil 8 CB fused silica capillary column (30 m × 0.25 mm i.d., 1.0 µm film thickness; Agilent J&W) using helium as the carrier gas, with an initial oven temperature of 100 °C held for 1 min, ramped to 320 °C at 6 °C/min, and held for 2.33 min. The carrier gas flow was set to a constant linear velocity of 39 cm/s with a split ratio of 1:5. Mass spectra were acquired under electron impact ionization (70 eV) in full-scan mode (m/z 35–600) with a scan speed of 3333 amu/s and a solvent delay of 6.0 min; the total GC run time was 40 min. Samples were analyzed in batches of up to 10, each including three quality controls: a blank run (2 μL hexane), an injection of a standard mixture containing metabolites (organic acids, sugars, amino acids, sterols, fatty acids, and vitamins), and a randomly selected sample analyzed in duplicate. Linear retention indices were determined at the beginning and end of each analytical sequence and every samples using an even-carbon-number n-alkane mixture according to Kovats. All samples, including replicates, were analyzed in triplicate. Batches were considered valid if no peaks were detected in blanks, standard peak area ratios (normalized to the internal standard) were within 90-110% of expected values, and the variance of normalized area ratios for the 100 most intense peaks in repeated injections was below 15%. Due to the lack of baseline resolution, chromatograms were subjected to spectral deconvolution using major mass fragments for each metabolite, applying the following criteria: area > 10,000, slope > 100/min, and width > 1 s. Metabolite areas were calculated using SIM chromatograms, and 2-isopropylmalic acid (SIM m/z 147) was used as the internal standard.

Integrating neuropsychological testing and mass spectrometry

An integrated diagnostic approach combining neuropsychological assessment and mass spectrometry-based biomarkers holds great promise for improving the diagnosis and management of neurodegenerative diseases. Neuropsychological tests provide functional information about cognitive impairment, while mass spectrometry offers molecular-level insights into underlying pathology.

Such a multimodal strategy may enhance early detection, improve differential diagnosis among neurodegenerative disorders, and enable personalized monitoring of disease progression and therapeutic response. In research settings, the correlation between cognitive performance and molecular biomarkers can also contribute to a deeper understanding of disease heterogeneity (Palmqvist et al. 2012).

Neuropsychological testing and mass spectrometry represent complementary and increasingly interconnected approaches in the field of neurodegenerative disease diagnosis. While traditional cognitive assessments remain indispensable for clinical evaluation, mass spectrometry-based techniques are expanding the biomarker landscape and paving the way toward more precise and earlier diagnoses. Future research efforts should focus on integrating these methodologies within standardized diagnostic frameworks, ultimately contributing to improved patient outcomes and the development of targeted therapeutic strategies.

Statistical analysis

Descriptive statistics were expressed as mean ± standard deviation. Group comparisons between females and males were performed using unpaired t-tests or chi-square tests, as appropriate. Multiple comparisons were corrected using Sidak's or Tukey's methods when applicable. Spearman's correlation analysis was used to explore associations between metabolic variables, cognitive and functional scores, and other biochemical parameters. Bonferroni correction was applied. Statistical analyses were conducted using GraphPad Prism software, and significance was set at $p < 0.05$ after correction.

Results

Clinical and demographic characteristics

The study included 121 individuals with Alzheimer's disease, comprising 60 females and 61 males. The two groups were comparable in terms of age, body mass index, educational level, disease duration, cognitive performance, functional status, comorbidities, and pharmacological treatments. Smoking prevalence was higher in males, while no other lifestyle or vascular risk factors differed significantly between sexes (Table 1).

Importantly, Mini-Mental State Examination, Basic Activities of Daily Living, and Instrumental Activities of Daily Living scores did not show sex-related differences, indicating a comparable clinical severity and functional impairment across groups (Table 1).

Table 1. Clinical features of patients with AD.

	Females (n=60)	**Males (n=61)**	***p*-value**
Aβ42 (ng/ml)	0.07±0.04	0.11±0.06	0.0001
Age (years)	78.15±7.48	78.13±6.90	NS
BMI (kg/m^2)	25.22±5.63	25.71±4.94	NS
Age of onset (years)	72.60±13.46	73.23±7.52	NS
Duration of disease (years)	3.17±2.12	3.67±2.80	NS
MMSE			
>24 normal			

24-20 mild dementia 20-13 moderate dementia <13 severe dementia	16.56±7.86	19.54±7.00	NS
BADL (/6)	4.76±1.57	4.52±1.80	NS
IADL (/8)	4.21±2.59	3.77±2.69	NS
CIRS (IS)	1.39±0.36	1.41±0.39	NS
CIRS (IC)	1.43±1.81	1.69±2.19	NS
Comorbidities			
Smoke	16/60	32/61	0.003
Dyslipidaemia	23/60	22/61	NS
Diabetes	14/60	11/61	NS
Hypertension	32/60	32/61	NS
TIA	3/60	7/61	NS
Ischemia	2/60	5/61	NS
MCI	13/60	23/61	NS
MD	12/60	13/61	NS
MdD	16/60	11/61	NS
SD	20/60	13/61	NS
Medications (n. of patients)			
Antidepressant	18/60	13/61	NS
Antihypertensive	27/60	28/61	NS
Hypolipidemic	19/60	21/61	NS
Hypoglycaemics	12/60	6/61	NS
Anti inflammatory	0/60	2/61	NS
Antiacid	11/60	14/61	NS
Antiplatelet	18/60	23/61	NS
Supplements	27/60	28/61	NS

Abbreviations: Aβ42: amyloid beta-42; BMI: body mass index; MMSE: Mini-Mental State Examination. BADL: basic activities of daily living; IADL: instrumental activities of daily living; CIRS-IC: Cumulative Illness Rating Scale for Comorbidities and CIRS-IS: Cumulative Illness Rating Scale for Severity; TIA: transient ischemic attack; MCI: mild cognitive impairment; MD: mild dementia; MdD: moderate dementia; SD: severe dementia; NS: non-significant.

Hematological and metabolic profiles

The majority of routine hematological and biochemical parameters did not differ significantly between females and males after correction for multiple comparisons. Similarly, most amino acid concentrations were comparable between sexes and fell within or slightly above physiological reference ranges (Table 2).

Among amino acids, citrulline and alanine emerged as the most frequently altered metabolites, exceeding reference values in a substantial proportion of both female and male patients. These alterations were not sex-specific but were consistently observed across disease stages.

Table 2. Blood metabolites expressed in males and females with Alzheimer's disease.

	Females (means±SD)	**Males (means±SD)**	***p*-value**
Basal blood glucose *60-105 mg/dl*	104.10±23.30	102.40±9.55	NS
GPT *0-37 UI/L*	19.25±8.07	18.90±5.12	NS
GOT *0-45 UI/L*	20.02±10.0	19.28±5.00	NS
Reuma test *0-20 UI/ml*	16.51±4.25	16.43±3.00	NS
Urea *20-50 mg/dl*	46.59±13.61	47.28±10.22	NS
Creatinine *0.7-1.3 mg/dl*	0.87±0.18	1.01±0.21	NS
Uric Acid *3.5-8.5 mg/dl*	4.95±1.35	5.66±1.32	NS
Alkaline Phosphatase *Male 53-128 U/L* *Female 42-141 U/L*	84.6±19.9	85.5±21.2	NS
Creatine phosphokinase (CPK) *38-171 UI/L*	95.61±51.46	102.00±34.59	NS
Amylase *30-110 U/L*	78.5±26.8	78.0±17.5	NS
Lipase *8-57 U/L*	27.7±8.2	28.3±5.5	NS
Blood cell count			
Total Proteins *6.3-8.2 g/dL*	6.34±0.36	6.50±0.35	NS
WBC *3.60-11 10^3/ml*	6.26±2.08	7.01±2.15	NS
NE% *37-70*	60.70±7.83	62.92±9.58	NS
LY% *20-45*	29.98±6.99	26.93±8.32	NS
MO% *1.1-12*	6.62±1.79	7.31±1.59	NS
EO% *0.1-8.0*	2.45±2.09	2.63±1.62	NS
BA% *0-3*	0.25±0.10	0.27±0.17	NS
NE *1.4-7 10^3/ml*	3.94±1.47	4.51±1.94	NS

LY *1.2-3.4 10^3/ul*	1.87±0.53	1.79±0.55	NS
MO *0.1-1.2 10^3/ml*	0.42±0.16	0.51± 0.22	NS
EO *0.00-0.80 10^3/ml*	0.15±0.19	0.17±0.11	NS
BA *0.00-0.30 10^3/ml*	0.02±0.01	0.02±0.009	NS
RBC *4.20-5.40 10^6/ml*	4.65±0.44	4.96±0.59	NS
HGB *14-18 g/dL*	13.58±1.23	14.62±1.81	NS
HCT *42-52*	41.40±3.72	44.35±5.25	NS
MCV *80-99 fL*	89.07±5.93	89.45±5.25	NS
MHC *27-33 pg*	29.20±2.12	29.51±2.29	NS
MCHC *31.0-37.0 g/dL*	32.79±0.74	32.96±0.90	NS
RDW *11.5-15.5%*	14.25±1.34	14.24±1.17	NS
PLT *120-450 10^3/ml*	247.20±54.01	218.10±42.26	NS
MPV *7.4-10.4 fL*	9.53±1.04	9.45±0.89	NS
Albumin *4.02-4.76 g/dL*	3.86±0.26	3.95±0.35	NS
α1 globulin *0.21-0.35 g/dL*	0.26±0.03	0.27±0.07	NS
α2 globulin *0.51-0.85 g/dL*	0.66±0.10	0.64±0.12	NS
β1 globulin *0.34-0.52 g/dL*	0.38±0.05	0.38±0.06	NS
β2 globulin *0.23-0.47 g/dL*	0.28±0.06	0.30±0.07	NS
γ globulin *0.80-1.35 g/dL*	0.88±0.22	0.95±0.24	NS
α/ γ globulin *1.10-2.40 g/dL*	1.59±0.23	1.58±0.26	NS
C reactive protein *0.0-5.0 mg/L*	4.44±1.75	5.08±2.97	NS

Transferrin *200-400 mg/L*	294.80±38.62	303.10±70.16	NS
Ions			
Magnesium *1.6-2.6 mg/dL*	1.84±0.19	1.86±0.18	NS
Calcium *8.4-10.2 mg/dL*	8.91±0.26	8.87±0.22	NS
Sodium *136-154 mEq/L*	141.40±1.84	140.40±1.82	NS
Potassium *3.5-5.3 mEq/L*	4.29-0.42	4.36-0.39	NS
Chloride *98-110 mEq/L*	101.50 1.21	101.40 1.27	NS
Phosphorus *2.7-4.5 mg/dL*	3.61±0.62	3.29±0.43	NS
Insulin *<20 mcUI/ml*	13.09±4.06	13.42±3.65	NS
C3 *80-170 mg/dL*	120.50±24.69	119.80±23.63	NS
C4 *12-36 mg/dL*	28.4±4.17	29.15±4.34	NS
Anti-cytoplasmic antibodies (c-ANCA) *absent: <0.8; present: >1.2*	0.33±0.21	0.35±0.22	NS
p-ANCA *absent: <0.8; present: >1.2*	0.43±0.18	0.45±0.17	NS
Anti-cardiolipin (ACLA) IgG *<10*	3.53±1.99	3.71±1.93	NS
Anti-cardiolipin (ACLA) IgM *<7*	3.33±1.22	3.58±1.24	NS
Extractable Nuclear Antigen (ENA) Screening *Negative < 0.7* *Positive >1.0*	0.30±0.16	0.29±0.19	NS
ENA screening antibodies ratio: *Negative < 0.7* *Positive >1.0*	<0.7	<0.7	NS
Ceruloplasmin *20-60 mg/dL*	25.21±6.97	23.83±3.90	NS
Vitamin E *5.5-17 mg/L*	9.82±3.03	9.85±2.87	NS
Lupus-like anticoagulant *Negative <1.2*	0.98±0.13	1.01±0.09	NS

Mild 1.2-1.5			
Moderate 1.5-2			
Severe > 2			
Procalcitonin	0.07±0.1	0.09±0.2	NS
Normal value < 0.10			
Low risk of sepsi <0.50			
Increased risk of sepsi 0.50-2.0			
High risk of sepsi > 2.0			
LDH	312.20±45.09	288.80±40.25	NS
<480 U/L			
Lactic Acid	19.12±3.37	18.12±3.54	NS
5.7-22 mg/dL			
Pyruvic Acid	82.23±10.88	87.73±12.54	NS
60-100 mmol/L			
Vitamin A	0.52±0.14	0.53±0.10	NS
0.20-0.80 mg/L			
Vitamin B1	46.26±12.45	44.95±12.68	NS
32-95 mcg/L			
Cobalamin (Vit B12)	505.30±237.60	524.00±189.10	NS
179-1162 pg/ml			
Vitamin D	24.45±11.25	24.29±7.13	NS
15-130 ng/ml			
Folic Acid	7.51±3.82	7.83±3.91	NS
4.6-34.8 ng/ml			
Vitamin B6	9.52±3.65	11.70±11.37	NS
3.6-18 mg/L			
Amino acids			
Homocysteine	13.78±6.49	13.30±2.99	NS
6-15 mmol/L			
Aspartic Acid	9.42±6.85	8.23±5.73	NS
<15 mM			
Glutamine	568.50±176.90	586.00±162.80	NS
200-800 mM/L			
Glutamic Acid	116.30±117.60	119.20±144.80	NS
30-120 mM/L			
Ornithine	98.63±41.14	99.38±45.36	NS
50-200 mM/L			
Citrulline	38.88±14.94	38.85±10.45	NS
10-35 mM/L			
Asparagine	41.67±1.0	44.41±10.33	NS
25-60 mM/L			
Methionine	25.06±9.65	28.02±8.60	NS

10-50 mM/L			
Serine	113.60±46.64	108.50±26.27	NS
35-190 mM/L			
Glycine	268.60±69.59	236.0±65.73	NS
120-340 mM/L			
Threonine	139.20±38.27	136.90±36.97	NS
40-220 mM/L			
Histidine	79.13±20.93	81.69±25.36	NS
40-140 mM/L			
Alanine	413.80±116.80	417.80±119.40	NS
150-400 mM/L			
Taurine	63.02±19.85	58.50±16.75	NS
20-100 mM/L			
Arginine	77.05±24.18	69.16±20.66	NS
30-90 mM/L			
α-aminobutyric acid	20.91±7.51	21.40±7.27	NS
10-40 mM/L			
Tyrosine	71.35±37.14	73.66±21.11	NS
30-200 mM/L			
Valine	225.40±43.25	241.40±49.46	NS
150-320 mM/L			
Tryptophan	25.22±25.49	25.11±25.18	NS
5-40 mM/L			
Phenylalanine	61.63±16.46	63.57±15.51	NS
30-80 mM/L			
Isoleucine	79.00±28.94	89.31±29.36	NS
30-100 mM/L			
Leucine	125.80±35.68	136.20±34.59	NS
50-190 mM/L			
Lysine	148.60±61.32	138.80±61.86	NS
80-300 mM/L			

Abbreviations: GOT, glutamic-oxaloacetic transaminase; GPT, glutamate pyruvate transaminase; WBC, white blood cells; NE, neutrophils; LY, lymphocytes; EO, eosinophils; MO, monocytes; BA, basophils; RBC, red blood cell; HGB, haemoglobin; HT, haematocrit test; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; MCH, mean Corpuscular Haemoglobin; RDW, red cell distribution width; PLT, platelets; MPV, mean platelet volume; LDH, lactate dehydrogenase; NS, non-significant.

In contrast, lipidomic analysis revealed a more complex pattern. While absolute fatty acid concentrations did not differ significantly between sexes for most analytes, several lipid species

exceeded physiological ranges more frequently in females. Notably, docosahexaenoic acid, gamma-linolenic acid, and dihomo-gamma-linolenic acid were elevated exclusively in female patients. Among these, gamma-linoleic acid levels were significantly higher in females than in males (Table 3).

Table 3. Lipids expressed in males and females with Alzheimer's disease

	Females (means±SD)	**Males (means±SD)**	***p*-value**
Saturated fatty acids of serum/plasma lipids			
14:0 Myristic Acid *24-64 mg/L*	35.03±17.12	27.83±15.27	NS
16:0 Palmitic Acid *609-893 mg/L*	870.10±270.40	784.70±189.40	NS
18:0 Stearic Acid *186-279 mg/L*	280.20±69.45	247.10±59.50	NS
20:0 Arachidic Acid *5.0-9.0 mg/L*	7.34±3.08	5.89±1.86	NS
22:0 Behenic Acid *14-26 mg/L*	23.15±11.97	20.14±7.88	NS
Omega-7 and Omega-9 fatty acids of serum/plasma lipids			
16:1w7 Palmitoleic Acid *48-124 mg/L*	82.66±38.58	61.71±34.75	NS
18:1w9 Oleic Acid *593-916 mg/L*	1042.00±394.90	920.20±284.80	NS
24:1w9 Nervonic Acid *24-41 mg/L*	38.59±17.58	31.19±10.50	NS
Omega-3 fatty acids of serum/plasma lipids			
18:3w3 Alpha-linolenic Acid *13-33 mg/L*	14.11±7.72	12.60±5.53	NS
20:5w3 Eicosapentaenoic (EPA) *16-61 mg/L*	24.99±12.03	23.22±12.58	NS
22:6w3 Docosahexaenoic Acid (DHA) *44-111 mg/L*	115.80±51.09	110.80±41.99	NS
Omega-6 fatty acids of serum/plasma lipids			
18:2w6 Linoleic Acid *781-1167 mg/L*	1117.00±423.10	938.80±229.10	NS
18:3w6 Gamma-linolenic Acid (GLA) *8-25 mg/L*	26.09±11.59	17.56±8.18	0.0001
20:3w6 Dihomo-gamma-linolenic Acid (DGLA) *37-75 mg/L*	87.07±31.80	68.64±20.81	NS

20:4w6 Arachidonic Acid (AA) *188-343 mg/L*	459.50±149.80	386.20±134.30	NS
Quotients			
Saturated/unsaturated fatty acids ratio *0.4-0.6*	0.41±0.06	0.43±0.06	NS
Omega-6/Omega-3 fatty acids ratio *5.0-14.0*	11.54±3.47	10.53±3.36	NS
Arachidonic Acid/Eicosapentaenoic Acid ratio (AA/EPA) *<4*	22.54±14.63	19.94±8.45	NS
Omega-3 Index *6.0-8.0*	3.54±1.21	3.84±1.33	NS

Amyloid beta and disease stage

Circulating Aβ42 levels were significantly lower in females compared with males (Table 1). This difference was already evident at the mild cognitive impairment stage, despite similar cognitive performance, and became more pronounced with disease progression. No sex-specific correlations were observed between individual metabolites and Mini-Mental State Examination scores.

Correlation analyses revealed strong associations between cognitive scores and functional measures (Basic Activities of Daily Living and Instrumental Activities of Daily Living), confirming the clinical validity of these scales. Additionally, citrulline levels correlated positively with threonine and asparagine in females, while folic acid levels showed an inverse relationship with homocysteine in both sexes.

Discussion

This study provides a comprehensive evaluation of sex-specific metabolic alterations in Alzheimer's disease, integrating routine clinical biomarkers with targeted amino acid and lipidomic profiling. While most circulating metabolites did not differ significantly between females and males, distinct patterns emerged for Aβ42 and selected lipid species, suggesting subtle but potentially meaningful sex-related differences in Alzheimer's disease pathophysiology.

The observation of lower circulating Aβ42 levels in females, already apparent at the prodromal stage, is particularly noteworthy. Reduced peripheral Aβ42 is generally interpreted as a surrogate marker of increased cerebral amyloid deposition, suggesting that females may experience a more pronounced amyloid burden earlier in the disease course. This finding aligns with epidemiological and experimental evidence indicating faster amyloid accumulation and disease progression in women (Doecke et al. 2020; Chuang et al. 2024).

Importantly, the observed sex difference in Aβ42 does not appear to be driven by systemic

organ dysfunction or pharmacological factors, as liver and kidney markers, inflammatory indices, and treatment regimens were comparable between sexes. Instead, biological mechanisms such as sex hormone regulation, blood-brain barrier integrity, and differential amyloid clearance pathways are likely contributors (Y.-R. Wang et al. 2017; Swaminathan et al. 2018; Parodi-Rullán et al. 2020; Bellaver et al. 2023).

Lipidomic alterations were more prominent in females, particularly within omega-6 fatty acid metabolism. Elevated gamma-linolenic acid and dihomo-gamma-linolenic acid levels may reflect sex-specific vulnerabilities in lipid regulation, potentially influenced by hormonal status. Estrogens are known modulators of lipid metabolism and amyloid processing, and their decline during menopause may contribute to the observed metabolic shifts. Although hormone levels were not assessed in this study, the findings strongly support the need to integrate endocrine parameters in future investigations (Vesga-Jiménez et al. 2022).

Alterations in amino acid metabolism, especially elevated citrulline and alanine, were observed irrespective of sex, suggesting a disease-related metabolic signature rather than a sex-specific phenomenon. Elevated citrulline may indicate dysregulation of the urea cycle, nitric oxide metabolism, or intestinal barrier function, all of which have been implicated in neurodegeneration. The sex-specific correlation between citrulline and threonine further suggests a potential gut–brain axis involvement in female patients (Mukherjee et al. 2021; D. Zhao et al. 2024).

Similarly, increased alanine levels may reflect impaired glucose metabolism and mitochondrial dysfunction, processes that are central to Alzheimer's disease pathology. Whether these circulating changes mirror cerebral metabolic alterations or represent compensatory systemic responses remains an open question (Hata et al. 2019).

Despite its strengths, this study is limited by sample size, which may have reduced the power to detect subtle sex-specific effects, particularly across different disease stages. Nonetheless, the consistency of certain findings, such as reduced Aβ42 and elevated gamma-linolenic acid in females, provides a strong rationale for larger, longitudinal studies.

In conclusion, the present findings highlight that while most circulating metabolic markers are shared between females and males with Alzheimer's disease, specific differences in amyloid burden and lipid metabolism may contribute to sex-related vulnerability and disease progression. These results underscore the importance of incorporating sex as a biological variable in metabolic and biomarker research and may inform the development of more personalized diagnostic and therapeutic strategies in Alzheimer's disease.

SECTION 2

INDUSTRIAL TRAINING AND PROFESSIONAL EXPERIENCE AT AL-FAISAL FLOUR AND GENERAL MILL (PAKISTAN)

This chapter describes the industrial experience carried out at Al-Faisal Flour and General Mill, Pakistan, as part of the doctoral training program, to observe the milling and fortification and distribution process of wheat flour.

Al-Faisal Flour and General Mill (Figure 7) is a well-established industrial facility operating in the Pakistani agri-food sector, primarily focused on wheat milling and the production of flour for both domestic and commercial markets. The mill plays a significant role in the regional supply chain, contributing to food security and supporting local agriculture through the procurement of raw wheat from domestic producers.

The facility is equipped with modern milling machinery, storage silos, and packaging lines, enabling continuous production under varying demand conditions. In addition to flour milling, the company engages in general grain handling and distribution activities, ensuring product availability across different market segments.

Whole wheat grain that comes from different fields contains bran, germ, and endosperm fractions. Germ and bran fractions generally contain higher concentration of bioactive phytochemicals. Refined grain flour contains only the endosperm. The process of refining flour removes the germ and the bran. Refining grain flour provides for a longer shelf life and a finer texture that is discussed in the study further.

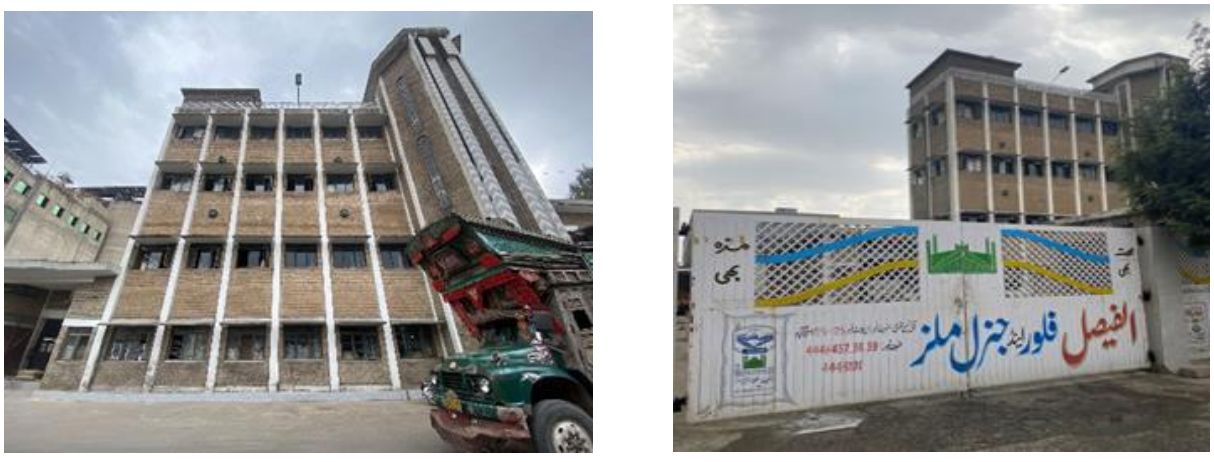


Figure 7. Al-Faisal Flour and General Mill.

The main objectives of the experience at Al-Faisal Flour and General Mill were:

1. to evaluate the impact of industrial cleaning and fortification processes on the nutritional quality of wheat flour;
2. to determine the detailed nutrient composition of whole and refined wheat flours through laboratory analysis.

Materials and methods

During the training period, several activities were undertaken across different operational units of the mill. These included:

Wheat reception and storage

The initial phase of production involves the reception, inspection, and storage of raw wheat, included *cleaning* and *grading* and removing of the sticks, stones, any foreign matter and other such impurities from the wheat which can be seen in the Figure 8. Activities included monitoring grain quality parameters such as moisture content, impurity levels, and kernel integrity. Particular attention was given to storage conditions, as improper temperature and humidity control can significantly affect both processing efficiency and final product quality.

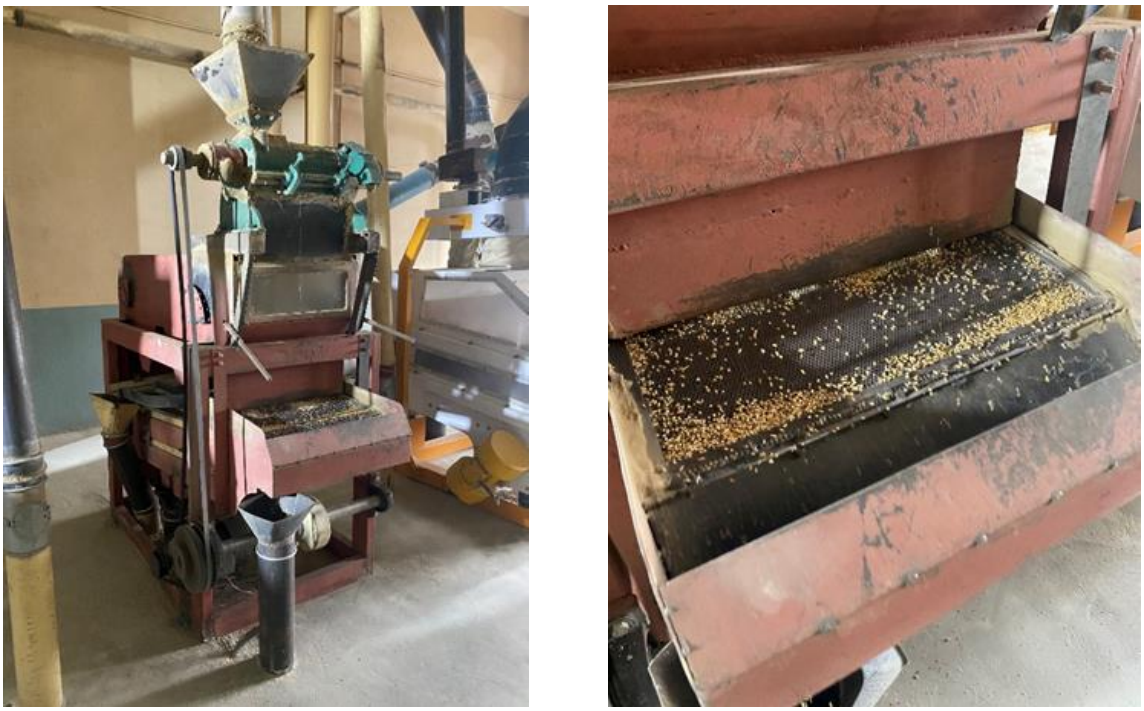


Figure 8. Cleaning and grading of wheat flour.

Milling process

The core of the experience focused on the milling process, including cleaning, conditioning, grinding, sifting, and blending. The functioning of roller mills, plansifters, and purifiers was

analyzed in detail. Emphasis was placed on *conditioning* and *tempering* where the wheat was soaked in water to easily remove the bran as seen in the Figure 9. The wheat grains is conditioned by adjusting their moisture content, which helps to facilitate the separation of the bran and germ from the endosperm during milling. The conditioned wheat is often stored for a period to allow the moisture to distribute evenly.

Figure 9. Conditioning and tempering of wheat flour.

Quality control and laboratory analysis

The quality control laboratory plays a crucial role in ensuring compliance with national and international standards. Participation in routine laboratory analyses allowed the evaluation of flour characteristics such as ash content, protein level, gluten quality, and particle size distribution. This phase highlighted the strong interdependence between process parameters and product performance.

Breaking and *sifting* was done to separate the endosperm (flour) from the bran and germ. The wheat kernels were broken down into smaller particles using roller mills, separating the bran and germ from the endosperm. The broken wheat was then sifted to separate the different fractions, including the flour, bran, and germ (Figure 10).

Figure 10. Breaking and sifting.

The last stage involved *grinding* of wheat kernels to produce flour of the desired fineness as seen in the Figure 11. The flour may undergo further refinement processes to remove any remaining bran or germ particles.

Figure 11. Grinding of wheat flour.

Packaging and distribution

Flour can be *enriched* and *packed* for final distribution. Fortification is done at the milling stage, where micronutrients are added to the flour before it is packed and distributed. The specific types and amounts of micronutrients added are determined based on the nutritional needs of the target population and public health priorities.

The final stage of production involves packaging and distribution (Figure 12). Observations were made regarding packaging materials, labelling requirements, and logistics management. This stage emphasized the importance of traceability and supply chain coordination in maintaining product integrity and customer satisfaction.

Figure 12. Enriching and packaging of wheat flour.

Enrichment

Wheat flour is enriched with vitamins and minerals known as fortification. It involves adding essential micronutrients like iron, folic acid, and other B-complex vitamins to improve its nutritional value. Wheat is a staple food for many populations worldwide, making it a good vehicle for delivering essential nutrients and to address public health concerns, particularly in areas with high rates of micronutrient deficiencies as it's an effective, simple, and inexpensive strategy for improving the nutritional quality of the food supply. Fortification can eliminate deficiencies in iron, folic acid, and other micronutrients that are common in many populations.

Food Fortification Program (FFP) Pakistan

Widely consumed wheat flour is fortified based on the public demand to reduce deficiencies among women and children. Commonly fortified micronutrients include iron, folic acid, and B-complex vitamins (thiamine, riboflavin, niacin). Other micronutrients, such as vitamin A, D and zinc, may also be added depending on the specific needs of the population. Table 4 displays worldwide addition of fortificants by different industries.

Table 4. Common Fortificants.

Micronutrient	Fortificant	Level of Quantity (mg/kg)
Vitamin A	Palmitate CWS 250	2.0
Folic Acid	Folic Acid	2.08
Vitamin B12	Vitamin B12 0.1% WS	0.01
Thaimine	Thaimine Mononitrate	8.4.
Riboflavin	Riboflavin	4.5
Niacin	Nicotinamide	59.0
Iron	Ferrous fumarate	58.5
Zinc	Zinc Ocide	28.3
NaFeEdta	Sodium Iron Edta	0.2

A food fortification program has been started in Pakistan, consisting of two components:

(i) oil ghee fortification with vitamin A and Vitamin D;

(ii) wheat flour fortification with iron, zinc, folic acid and B12.

Addition of common fortificants added to the whole wheat flour in Pakistan observed in Al-Faisal Flour and General Mills, as recommended by World Health Organization, is mentioned as below. It provides 15 ppm iron to the flour, 30 ppm zinc, 1 ppm folic acid and 0.008 ppm B12 to the final product of the flour.

Over 80 countries have legislation to fortify wheat flour, and estimates indicate that a significant portion of the world's industrially milled wheat flour is fortified with at least iron or folic acid. In Pakistan, there is a focus on institutionalizing wheat flour and edible oil fortification to ensure more people have access to critical micronutrients. It Improves overall nutritional status, particularly among vulnerable populations like women and children by reducing the risk of iron-deficiency anemia. Moreover, it is a cost-effective public health strategy to address micronutrient deficiencies on a large scale. Decisions about which nutrients to add and the appropriate amounts to add is based on a number of factors which includes, the nutritional needs, resources and the costs involved as discussed in the Section 3 of this thesis (Table 8 and Figures 14, 15 and 16).

To assess wheat flour fortification, a questionnaire was developed that covered the demographics, food consumption patterns, and knowledge/attitudes towards fortification of wheat flour, to get information on demand of enrichment and its effectiveness. Response was taken from the Chief Executive Officer of Al-Faisal Flour and General Mill. Given below is a breakdown of potential questions for a questionnaire on wheat flour fortification.

Results

Nutritive profiling of wheat grain

Different blends of wheat flour were collected from Al-Faisal Flour and General Mills. For comparison, samples were also purchased from a local Chaki and a General Store. One was milled

in the factory and brought to the market. It was compared with another sample milled at Chaki (small scale, less refining). A sample which was a total refined product after removing husk and another sample which was only the husk that was removed from the wheat grains was collected from Chaki. A number of total 6 samples were screened for their nutritive and antioxidants (nutraceuticals) properties. Whole Grained and Refined flour was compared for bioactive compounds present in them. Chokar was taken as reference sample.

Proximate analysis

The *ash*, fats and the total proteins was accessed by AOAC (1990) methods. Moisture was taken by gravimetric heating (105°C for one hour) using 2 g of sample. Aluminium cans was pre-dried in a hot air oven and cooled in a desiccator for 30 minutes. The hot cans were then transferred into the desiccator to cool with the help of tongs. Total *carbohydrate* is calculated using an equation from AOAC (1975), i.e. (Sharoba 2021),

$$100 - (\text{Fat} + \text{Ash} + \text{Protein} + \text{Moisture})$$

The main components of wheat flour are *starch***,** *protein* and *water*. Starch is the primary source of energy in wheat flour. Lipids are present in relatively small amounts but play a role in dough texture and stability (Khalid et al. 2023). Refined samples and those that went through extreme milling including *Plain Flour* (PF01), *Refined from Chaki* (RC02), *Milled from Industry* (MI03) contain 98 grams of carbohydrate on every 2 g of wheat sample. Whereas samples that went through less refining and milling including *Stoned Milled* (SM04), *Stoned and Chokar* (SC05), *Chokar* (CK06) and *Mixed Flour* (MF07) have 99 grams of carbohydrate in them.

5 g of each sample was taken into the thimble/flask to determine *fat*. The sample was dried in an oven at 102ºC for 5 hours. The thimble was inserted in a Soxhlet liquid/solid extractor. 150 ml round cleaned and dried bottom flash was taken and about 90 ml of petroleum ether was filled in the flask. The extraction unit was assembled over in a water bath. The solvent was heated in the flask until it boiled (heat was adjusted so that solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second). The extraction process was continued for 6 hours. The extraction unit was removed from the heat source and the extractor was detached (the flask on the heat source was replaced and the solvent was evaporated). The flask was placed in an oven at 60 to 80ºC; the contents was dried until a constant weight was obtained (for 1 to 2 hours). The flask was cooled in a desiccator. The weight of the flask with contents was taken (Macmurray e Morrison 1970).

As shown in the Table 5, PF01 has the highest amount of fat with 0.04 g per 5 grams of wheat flour. However, sample RC02 has 0.02 g of fat. SC05 also showed 0.031 g of fats. CK06 contained only 0.01 g of fats. Whereas, the sample MF07 that is a mixture of grains has only 0.001

g of fat. Samples MI03 and (SM04) shows a fat content of 0.012 g and 0.02 g per 5 grams of wheat sample.

To determine the protein content in wheat samples, the Kjeldahl method was performed according to the method 981.10 of the AOAC International. 1 g of each blend was taken and hydrolysed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block at 420°C for 2 h. After cooling, H_2O was added to the hydrolysates before neutralization and titration. The amount of total nitrogen in the raw materials were multiplied with both the traditional conversion factor of 6.25 and species-specific conversion factors in order to determine total protein content (Javid Iqbal et al. 2022).

The sample MI03 showed the highest content of protein with 0.20 g per gram of wheat. Whereas, sample SM04 lost considerable proteins and showed a total of 0.1 g of protein / g. MF07 that was a mixture of grains also had the highest content with 0.20 g/g of wheat. Blend of flour RC02 had 0.2 g / g same as the whole wheat grain blend SC05 with some chokar added in it with 0.2 g / g of wheat protein. PF01 had the least amount of protein with 0.1 g (Saini et al. 2023).

High moisture content in stored wheat can lead to grain deterioration, mold growth, and insect infestations, which can damage the crop and the storage facility. The sample which was a mixture of grains showed a moisture content of 0.9 g per 2 grams of sample which indicates high chances of deterioration. All samples except RC02 showed 0.1 grams. Rest of the samples showed significant increase of 1 gram of moisture content in them (Moya et al. 2024).

Ash content refers to the mineral content of the flour, including elements like phosphorus and calcium. PF01 shows the highest amount of 1.6 g of ash in it. RC02 has 1.3, MI03 with 1.4 and SM04 with 1.2 g of ash in it. Sample SC05, CK06 and MF07 has the lowest amount of ash with a ratio of 0.7, 0.6 and 0.7 grams.

The pH was determined using pH paper. Sample was prepared. It was made sure that the sample was liquid was at room temperature and mixed well. A strip of pH paper was dipped into the solution. The pH paper was removed and excess solution was allowed to drain. Color change of the paper was compared with the provided pH color chart. The color of the paper was matched with the closet value on the chart. Approximate pH was recorded. All samples showed a pH of 7 which is considered as basic, neutral or alkaline. However, MF07, CK06 and SC05 represents a slightly acidic pH of 6 (Alfaris et al. 2022).

Water activity (aw) in food science is a measure of how readily the water in a food product is available for microbial growth and other reactions. It determines the shelf life of product. The aw of the samples was determined using a Tunable Diode Laser (TDL, AquaLab, Series 4TE). 2 grams of starch sample was taken in the sealed chamber. The TDL shines light through the headspace above the sample and the detector of the light provides a direct measure of the vapor

pressure. The TDL is a spectroscopic method in which it sweeps across a single water vapor absorption line at 1854 nm. The absorption at that wavelength is specific to water vapor, which makes it insensitive to the presence of other volatiles. Temperature during testing remained 24°C. The activity was followed by 3 replications for each sample. PF01 which is the refined flour has a water activity of 14% which mean it has the lowest shelf-life span. Sample MI03 and CK06 has the water activity of 13%. Rest of the samples had the aw of 10% (Marynin et al. 2021).

Table 5. Composition of wheat flour blends.

	Fats (g)	Protein (g)	Carbohydrate (g)	Moisture	Ash	pH	aw (%)
PF01*	0.04	0.1	98	0.2	1.6	7	14
RC02*	0.02	0.2	98	0.1	1.3	7	10
MI03*	0.01	0.2	98	0.2	1.4	7	13
SM04*	0.02	0.1	99	0.2	1.2	7	10
SC05*	0.03	0.2	99	0.2	0.7	6	10
CK06*	0.01	0.2	99	0.2	0.6	6	13
MF07*	0.001	0.2	99	0.9	0.7	6	10

*Wheat Flour (WF), Plain flour (PF), Refined from Chaki (RC), Milled from Industry (MF), Stoned milled (SM), Stoned with chokar (SC), Chokar (CK), Mixed Flour (MF)

Pasting properties of wheat

The viscosity was analyzed using a Rapid Visco-Analyzer (RVA, Charpa, Series - 4TE). RVA is widely used as it requires a small sample size and takes approximately 3 minutes to draw out the results. The sample was diluted with 25 ml distilled water in a cup. A disposable plastic stirring handle was used to mix the sample solution by rotating for 30 seconds approximately. The cup was placed in the RVA. The copper block automatically clamps once the test cycle activates. The stirring paddle spins to disperse the sample for seven seconds at 900 rpm and slowed down to 160 rpm after seven seconds. Sample was equilibrated for 3 minutes at 30°C before the test. The suspension was heated and recorded at 95°C at the speed of 1°C per 40 seconds. The temperature was kept on 95°C for 30 minutes and lowered to 50°C at 1°C per 40 seconds (Suh e Jane 2003). Wheat flour has been used in the formation of chapati, tortillas and bakery products due to its excellent baking properties. Moreover, its nutraceutical potential makes it vulnerable for people with neurodegenerative disorders (Raigond et al. 2015). All samples showed the highest peak at 12 rpm which represents good baking properties. However, peaks of SC05 and CK06 were high as compared to other samples. In starch pastes, the swelling of starch granules during heating and

the formation of hydrogen bonds between molecules contribute to the increasing viscosity. As the swelling reaches its maximum and the structure stabilizes, the viscosity reaches its peak (Table 6).

Table 6. Pasting properties of blends of wheat flour.

Viscosity	**(cP*)**			
	6 rpm*	**12 rpm***	**30 rpm***	**60 rpm***
PF01**	0	18 (3.2 %)	0	7 (6 %)
RC02**	0	54 (10%)	8 (4%)	9 (8 %)
MI03**	0	51 (10 %)	8 (4 %)	7 (6%)
SM04**	0	27 (5%)	15 (6 %)	9 (8%)
SC05**	0	42 (9 %)	10 (4 %)	12 (10.3 %)
CK06**	165 (15 %)	116 (22 %)	54 (23 %)	36 (32 %)
MF07**	0	0	16 (6 %)	4 (3%)

*cP-control point, rpm-rotation per minute

**Plain flour (PF), Refined from Chaki (RC), Milled from Industry (MI), Stoned milled (SM), Stoned with chokar (SC), Chokar (CK) and Mixed Flour (MF).

Antioxidants Activity

Antioxidant activity (AOA) was measured using a modified version of the method described by Brand-Williams, Cuvelier, and Berset (1995). 100 mg of wheat flour sample was extracted with 1 mL methanol for 2 h and centrifuged at 3000 g for 10 min. 100 ml supernatant was reacted with 3.9 mL of a 6 x 10^{-5} mol/L of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution. Absorbance (A) at 515 nm was read once at 0 minutes and then after 30 minutes using a methanol blank. Antioxidant activity was calculated as % discoloration. Percentage was calculated using the difference between readings taken at 0 and 30 minutes (Sharma e Gujral 2014).

Least percentage of 70% AOA was found in the sample that was PF01. Antioxidant potential was also tested in CK06, which refers to wheat bran, the outer hardest part of the grain had the highest (97%) AOA (Ye et al. 2021). It was taken as the reference sample. Chokar was added in another sample (SC05) that was stoned milled showed 98% of AOA. 97% of free radical scavenging activity was found in the sample which contained multiple grains (MF07). It is mostly used for making wheat products for diabetic patients. Factory milled wheat that goes through refining (MI03) showed significant increase of 90% as compared to stoned milled wheat sample (SM04) which had 84% as displayed in the Table 7. Refined flour from Chaki (RC02) had 91% which was which was slightly more than wheat that is refined in mills (RF03). Results signifies

that refining of flour removes wheat bran which is rich in antioxidants that decrease the AOA of wheat flour.

DPPH radical scavenging activity (%) = (1- (A of sample t = 30/A of control t = 0)) x 100

Table 7. Determination of antioxidants in blends of wheat flour.

Absorbance	T-0*	T-30*	%
PF01**	0.446	0.343	70
RC02**	0.269	0.291	91
MI03**	0.263	0.289	90
SM04**	0.236	0.275	84
SC05**	0.313	0.315	98
CK06**	0.281	0.284	97
MF07**	0.371	0.375	97

*T-0 (time at 0 minutes), T-30 (Time at 30 minutes)

**Plain flour (PF), Refined from Chaki (RC), Milled from Industry (MI), Stoned milled (SM), Stoned with chokar (SC), Chokar (CK) and Mixed Flour (MF).

Polyphenols

Figure 13 displays both (**a**) a ferulic acid standard and (**b**) ferulic compounds in white flour. The total content of polyphenols was evaluated in free and bound phenolic extracts supported by individual phenolics determination using HPLC analysis. The highest content of total phenolic content was detected in free phenolic fractions found in plain flour ranged 311044.00 uV. Phenolic compounds are generally present in wheat flour, particularly whole wheat flour, are a group of phytochemicals with potential antioxidant and health-promoting properties. These compounds, including phenolic acids and flavonoids, exist in various forms: free, soluble conjugates, and insoluble bound forms. The most prevalent phenolic acid in wheat is ferulic acid, accounting for a significant portion of the total.

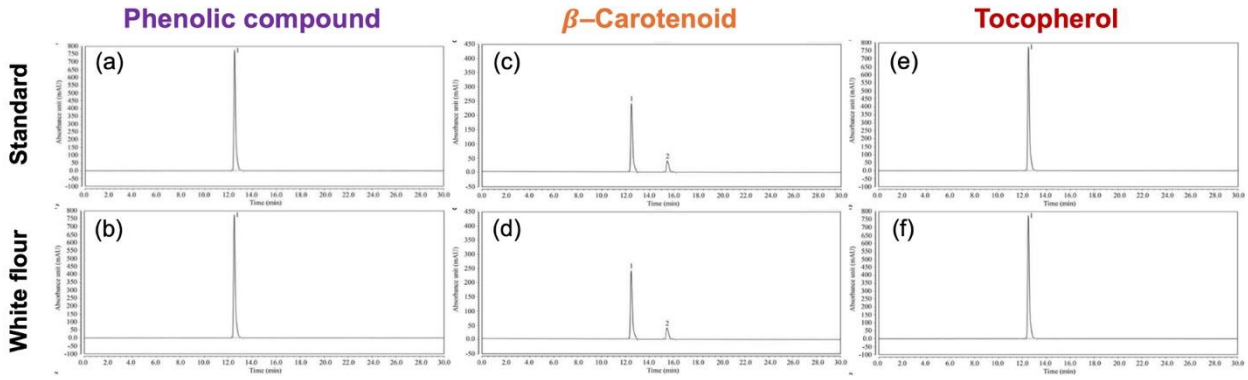


Figure 13. (**a-b**) Phenolic compound, (**c-d**) β–carotenoid, (**e-f**) Tocopherol in standard and white flour.

Carotenoids

Figure 13 displays both (**c**) carotenoid standard and (**d**) carotenoids in plain flour. Carotenoid profile was obtained for the white wheat species which exhibited the highest level of carotenoid, averaging 359309.00 uV. Carotenoids in wheat flour are primarily lutein and zeaxanthin, with smaller amounts of beta-carotene and other carotenoids. The specific carotenoid profile and concentration can vary depending on the wheat variety and environmental conditions. Lutein is often the most abundant carotenoid, especially in bread wheat, while durum wheat may have a higher proportion of beta-carotene.

Tocopherol

A rapid normal-phase high-performance liquid chromatography was used for determination of the total content of tocopherols in wheat. Figure 13 show both the results of standard (**d**) and white flour (**e**) ranging 311041 Uv. Tocopherols, a type of vitamin E, are present in wheat flour, primarily in the germ and bran fractions. The main forms found are alpha-tocopherol and beta-tocotrienol, with alpha-tocopherol being the most active form of vitamin E. While wheat flour is a good source of tocopherols, the amount and composition can vary based on factors like milling process, wheat variety, and growing conditions.

Discussion

The industrial training and professional experience conducted at Al-Faisal Flour and General Mill provided a valuable opportunity to bridge academic knowledge with real-world industrial practice within the agri-food sector of Pakistan. This experience allowed for an in-depth understanding of the operational, technological, and managerial aspects of flour milling, highlighting both the strengths and the challenges inherent in large-scale cereal processing in a developing-country context.

One of the most significant outcomes of this training was the direct exposure to the wheat-to-flour production chain, from raw material procurement and quality assessment to milling, packaging, and distribution. Observing these processes in an industrial environment reinforced the importance of process standardization, equipment maintenance, and quality control in ensuring product consistency and food safety. The experience emphasized how theoretical principles of cereal chemistry, grain technology, and food engineering are applied in practice, often under constraints related to cost, infrastructure, and supply chain variability.

A key aspect that emerged during the training was the predominance of refined wheat flour production, reflecting prevailing consumer preferences and market demand in Pakistan. While this aligns with affordability and sensory expectations, it raises important nutritional considerations, particularly in light of growing evidence linking whole grain consumption to improved metabolic and cognitive health. The industrial setting highlighted the technological and economic barriers to large-scale production of whole wheat flour, including shorter shelf life, oxidative stability issues, and consumer acceptance. These observations underscore the need for innovation in milling technology and storage solutions to support the production of nutritionally superior products without compromising safety or profitability.

From a quality assurance perspective, the training reinforced the central role of routine physicochemical analyses in maintaining compliance with national and international food safety standards. However, it also revealed potential gaps in the systematic integration of advanced quality monitoring tools and data-driven decision-making processes. Addressing these gaps could enhance traceability, reduce waste, and improve overall production efficiency. The experience thus highlighted how investments in analytical capacity and staff training could translate into measurable improvements in product quality and consumer trust.

From a broader perspective, this training experience highlights the strategic role of the milling industry in national food security and public health. Given the centrality of wheat-based products in the Pakistani diet, flour mills represent a critical point of intervention for nutritional improvement at the population level. The integration of research-driven approaches, such as nutrient fortification, promotion of whole grain products, and process optimization, could significantly enhance the health impact of staple foods.

In conclusion, the industrial training at Al-Faisal Flour and General Mill was instrumental in contextualizing academic research within an applied industrial framework. The experience not only strengthened technical competence in cereal processing but also provided critical insights into the challenges and opportunities facing the food industry in Pakistan. These insights reinforce the importance of academia-industry collaboration and support the translation of scientific research

into sustainable, health-oriented industrial practices. Such integration is essential for addressing both current and emerging challenges in food systems, nutrition, and public health.

SECTION 3

DIETARY WHOLE WHEAT FLOUR INTAKE IN PAKISTAN: A CROSS-SECTIONAL INSIGHT INTO LONG-TERM DEMENTIA PREVENTION

Materials and methods

Study design

This study employed a two-phase, cross-sectional design to explore dietary whole wheat flour consumption patterns in Pakistan and their potential association with long-term dementia prevention. Data were collected using structured, interviewer-administered questionnaires focused on dietary habits and whole wheat flour intake.

Phase I: population-level dietary survey

In the initial phase, 144 adult participants were recruited from various urban and semi-urban regions of Pakistan using convenience sampling. Individuals aged ≥18 years and willing to provide informed consent were eligible for inclusion. The survey assessed general dietary patterns, frequency of whole wheat flour consumption, socioeconomic characteristics, and lifestyle factors (appendix A). The demographic and clinical characteristics of the study group are presented in Table 8. Participants were interviewed in person, and all responses were recorded using a standardized questionnaire developed for this study.

Table 8. Demographic profile and health conditions of the Pakistani population examined.

Clinical features:	
Gender	
Males	34/144
Females	110/144
Age (years)	38.7 ± 14.7
21-30	34%
31-40	37%
41-50	4%
>50	25%
Marital status	
Unmarried	41/144
Married	103/144
Income group	
<50k	36%
>50k	64%

Education	
None	1%
Faculty of Arts	5%
Bachelor's degree	30%
Master's degree	40%
PhD	24%
Allergies	
Yes	14/144
No	130/144
BMI at enrolment (kg/m^2)	24.6 ± 4.7
Underweight	7%
Normal	50%
Overweight	31%
Obese	12%
Daily physical activity	
Gym	22/144
Brisk walk	35/144
Cleaning or gardening	9/144
Cooking and whashing up	50/144
None	28/144
Pathologies:	
Hypertension	20/144
Cardiovascular disease	5/144
Diabetes mellitus	14/144
Irritable bowel disease	9/144
Obesity	20/144
Celiac disease	1/144
Gluten intolerance	3/144
Dementia	3/144
None	90/144
Other	18/144
Symptoms experienced in daily routine	
Language problems	0/144
Increasing confusion	6/144
Reduced concentration	9/144
Personality or behavior issues	2/144
Loss of ability to do everyday tasks	6/144
Apathy and withdrawal or depression	2/144
Not recognizing friends and family	0/144
Problems with planning and decision-making	4/144
Not remembering where they live or where they are	1/144
Memory problems, particularly remembering recent events	16/144
None	98/144
Other	0/144

Brain health	
Bad	2/144
Poor	5/144
Average	41/144
Good	67/144
Excellent	29/144
Use of multivitamins	
Yes	69/144
No	75/144

Phase II: case–control dietary assessment

In the second phase, 60 participants were enrolled, comprising 30 clinically diagnosed dementia patients and 30 HLT controls matched for age and sex. Dementia diagnoses were established by qualified neurologists according to standard clinical criteria. Control participants had no history of cognitive impairment and scored within the normal range on routine cognitive screening. All participants in this phase completed the same dietary questionnaire used in Phase I, with additional questions focusing on long-term whole wheat flour intake, concomitant dietary habits, and medical history (appendix B). Caregivers assisted dementia patients when needed to ensure accurate reporting. The demographic and clinical characteristics of patients with dementia and HLT controls are presented in Table 9.

Table 9. Demographic profile and health conditions of patients with dementia *vs.* HLT.

Clinical features:	**HLT** n=30	**Patients with dementia** n=30
Gender		
Males	11/30	12/30
Females	19/30	18/30
Age		
<50	50%	40%
>50	50%	60%
Marital status		
Unmarried	0/30	0/30
Married	30/30	30/30
Income group		
<50k	10%	20%
>50k	90%	80%
Education		
Yes	97%	60%
No	3%	40%

Daily physical activity		
Yes	29/30	11/30
No	1/30	19/30

Other pathologies:

Hypertension	5/30	16/30
Cardiovascular disease	0/30	2/30
Diabetes mellitus	7/30	9/30
Irritable bowel disease	0/30	0/30
Obesity	3/30	0/30
Celiac disease	0/30	0/30
Gluten intolerance	0/30	0/30
None	18/30	5/30
Other	2/30	8/30
Symptoms experienced in daily routine		
Language problems	0/30	19/30
Increasing confusion	0/30	16/30
Reduced concentration	0/30	1/30
Personality or behavior issues	0/30	8/30
Loss of ability to do everyday tasks	0/30	8/30
Apathy and withdrawal or depression	0/30	1/30
Not recognizing friends and family	0/30	5/30
Problems with planning and decision-making	0/30	12/30
Not remembering where they live or where they are	0/30	5/30
Memory problems, particularly remembering recent events	0/30	2/30
None	30/30	0/30
Other	0/30	0/30
Stage of dementia		
No cognitive decline	30/30	0/30
Very mild cognitive decline	0/30	3/30
Mild cognitive decline	0/30	8/30
Moderate cognitive decline	0/30	7/30
Moderately severe cognitive decline	0/30	7/30
Severe cognitive decline	0/30	5/30
Very severe cognitive decline	0/30	0/30
Average age of clinical diagnosis	/	58.3±15.4
<30	/	7%
31-40	/	7%
41-50	/	17%
51-60	/	36%
61-70	/	13%
>70	/	20%

Use of multivitamins

Yes	16/30	12/30
No	14/30	18/30

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and approved by the appropriate institutional ethics committee (IRB # 367-24). Written informed consent was obtained from all participants or, when applicable, from legally authorized representatives.

Data Management

All questionnaire responses were anonymized and entered into a secure database. Data analysis was performed using GraphPad Prism (v. 4.00 GraphPad Software, Inc., San Diego, CA, USA). Data are expressed as percentage of respondents in each group.

Results

Demographics and health overview of Pakistani population

As summarized in Table 8, a total of 144 Pakistani participants were included in the study. The sample consisted of 23.6% males (34/144) and 76.4% females (110/144). The mean age was 38.7±14.7 years, with most individuals between 31-40 years (37%), followed by 21-30 years (34%), while 25% were older than 50 years and 4% were between 41-50 years.

Regarding marital status, 71.5% of participants were married (103/144), whereas 28.5% were unmarried (41/144). Most respondents belonged to the higher income group (>50k; 64%), while 36% reported a monthly income below 50k. Educational attainment varied: 40% held a master's degree, 30% a bachelor's degree, 24% had a PhD, 5% a Faculty of Arts qualification, while only 1% reported no formal education.

Allergies were reported by 9.7% (14/144) of the population. The mean BMI at enrolment was 24.6±4.7 kg/m², with 50% classified as normal weight, 31% overweight, 12% obese, and 7% underweight.

Daily physical activity patterns showed that 50/144 participants engaged in cooking or household tasks, 35/144 performed brisk walking, and 22/144 attended a gym, while 28/144 reported no regular physical activity. Cleaning or gardening was practiced by 9/144 participants.

With respect to medical conditions, 90/144 individuals reported no diagnosed pathology. Among the remaining participants, the most prevalent conditions included hypertension (20/144), obesity (20/144), diabetes mellitus (14/144), and irritable bowel disease (9/144). Less frequent conditions were cardiovascular disease (5/144), gluten intolerance (3/144), dementia (3/144), celiac disease (1/144), and other unspecified disorders (18/144). Regarding disease duration, 23% of the population had been diagnosed for less than 10 years, whereas 9% had been living with their condition for more than 10 years. When asked about potential causes of their illnesses, 36%

attributed their condition to poor diet, 9% to aging, and 6% specifically to the consumption of refined flour.

Symptoms affecting daily routine were uncommon, with 68% reporting no symptoms (98/144). The most frequently reported issues were memory problems related to recent events (16/144), reduced concentration (9/144), and increasing confusion (6/144). Other symptoms, such as personality changes, apathy, loss of everyday functioning, or planning difficulties, were each reported by fewer than 6 participants.

Self-perceived brain health was rated as good or excellent by 96/144 participants (66.7%), while 41/144 rated it as average. Only 7 individuals assessed their brain health as poor or bad. Use of multivitamins was reported by 47.9% (69/144) of participants.

Wheat product intake, flour preferences, and chapati consumption habits in Pakistan

The majority of participants reported consuming three meals per day (78%), while 21% indicated eating twice daily and only 1% reported eating once a day (Figure 14a). This pattern is in line with what might be expected in a "standard" three-meals-a-day dietary scheme. However, in the context of dietary studies in Pakistan, evidence suggests considerable variability in meal frequency and diet composition depending on socioeconomic status, urban *vs*. rural residence, and seasonal availability of food (Hussain et al. 2014; Ali et al. 2019). The predominance of a three-meals-per-day pattern may reflect relative food security and stable daily routines in the surveyed population. Nonetheless, meal frequency alone does not provide information about portion sizes, energy density, or nutritional balance. Given the high reliance on wheat-based foods, it is plausible that energy intake is sufficient, but nutrient density (especially in terms of fiber, micronutrients, and protein quality) remains suboptimal.

Among Pakistani respondents, the data show a high reliance on wheat-based foods in their daily diet. Chapati was the most frequently consumed item (87%), followed by bread (62%). Other commonly consumed wheat products included pasta or pizza (40%), cookies (35%), and cakes or pastries (23%) (Figure 14b). These findings highlight the central role of wheat products in the dietary patterns of the Pakistani population surveyed. The predominance of wheat-based staples aligns with findings from recent community-based research among Pakistani adults, which identified distinct dietary patterns, including "discretionary" or "processed food" patterns, alongside more traditional ones (Rafique et al. 2022). National-level data confirm that wheat remains the main staple crop and food source in Pakistan, contributing substantially to the average household caloric intake (Hameed et al. 2021; Bengali et al. 2010). Moreover, the non-negligible proportion of participants consuming processed wheat-based foods (pasta/pizza, cookies, pastries) suggests a "hybrid diet" combining traditional staples (chapati, bread) with modern or convenience

foods. This pattern aligns with recent observations of nutrition transition in urban and peri-urban Pakistani populations, characterized by increased consumption of processed and discretionary foods (Rafique et al. 2022; Bhagtani et al. 2025).

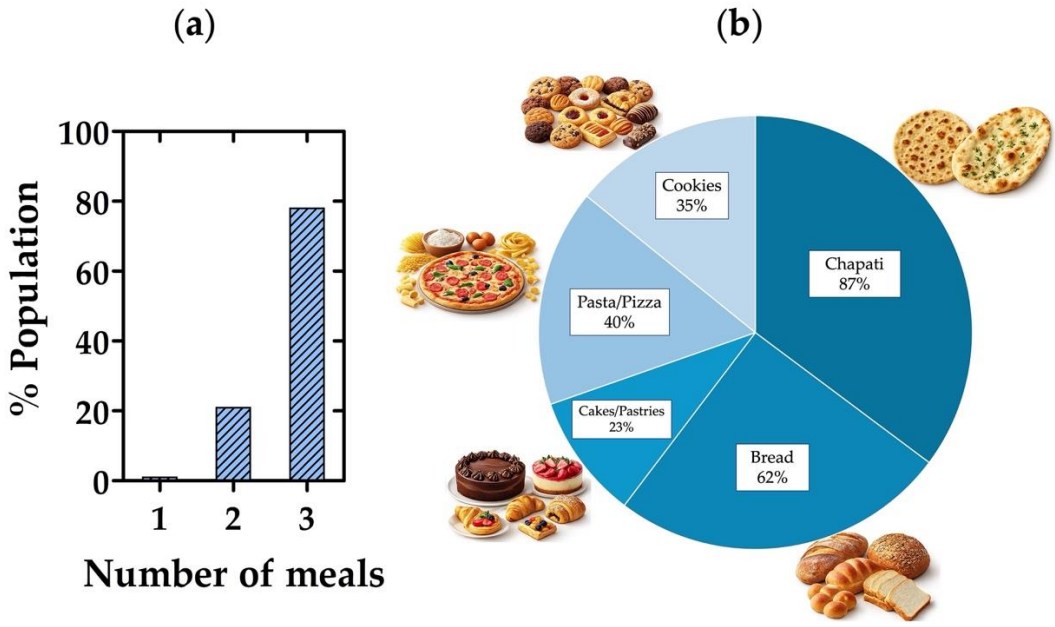


Figure 14. Dietary survey results from a Pakistani population. (**a**) Distribution of respondents by the number of daily meals. (**b**) Frequency of consumption of common wheat-based food products, including chapati, bread, pasta/pizza, cakes/pastries, and cookies.

Most respondents reported using whole-grain wheat flour (77%), making it the predominant choice, whereas white flour accounted for 21% of use, and diet atta and multigrain flour were each selected by 1% of participants (Figure 15a). The preference for whole-wheat flour within the Pakistani population was largely motivated by health considerations, with 72% indicating that perceived health benefits were the primary reason for their choice, despite most respondents reporting limited awareness of the nutraceutical components of whole-grain flour, the survey indicated a general preference for whole-wheat flour; notably, many participants stated they were already using whole-wheat flour, and a substantial proportion expressed willingness to switch to it if advised by healthcare professionals. Other factors played a comparatively minor role, including taste (13%), commercial availability (9%), economical price (3%), and appearance or fine texture (3%) (Figure 15b). Regarding purchasing patterns, 46% of respondents typically obtained wheat flour from supermarkets, while 34% relied on local markets and 20% sourced it from village-level vendors. Together, these findings illustrate not only a clear inclination toward whole-grain flour but also the strong influence of health-related motivations on consumer decisions (Figure 15c).

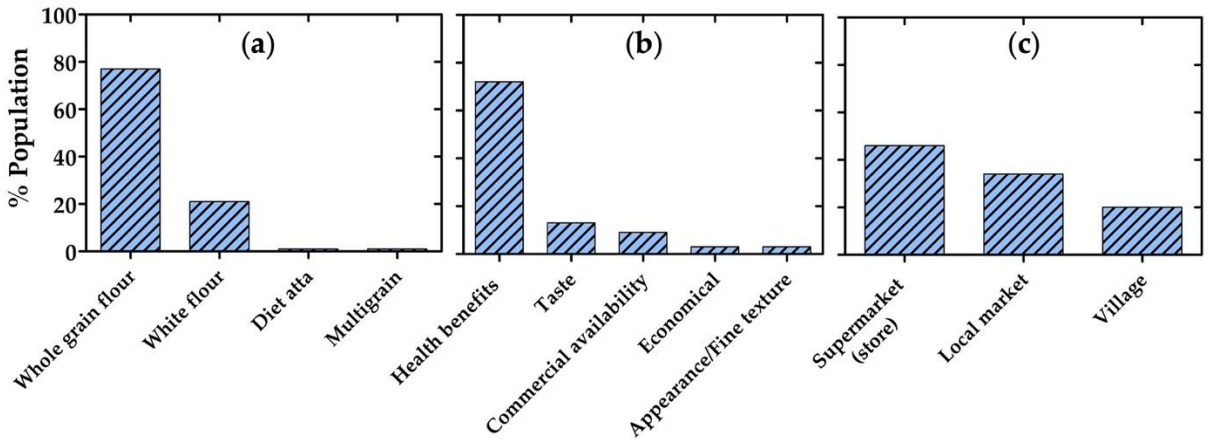


Figure 15. Survey results from a Pakistani population regarding flour preferences and purchasing habits. (**a**) Types of flour commonly consumed. (**b**) Primary factors influencing flour selection. (**c**) Main locations where flour is purchased.

Given the high consumption of chapati within the Pakistani population (see Figure 14b), additional analyses were conducted to explore its role in daily dietary habits. The majority of respondents reported eating chapati once (41%) or twice (45%) per day, while a smaller proportion consumed it three times daily (14%) (Figure 16a). In terms of portion size, most individuals typically ate the equivalent of one chapati per meal (69%), whereas 27% consumed two and only 4% consumed three (Figure 16b). Preferences regarding chapati dimensions were almost evenly split: 49% favoured a 15 cm chapati, while 51% preferred the larger 30 cm size (Figure 16c). These findings highlight not only the widespread integration of chapati into daily eating patterns but also the consistency in portion size and dimensional preferences among consumers.

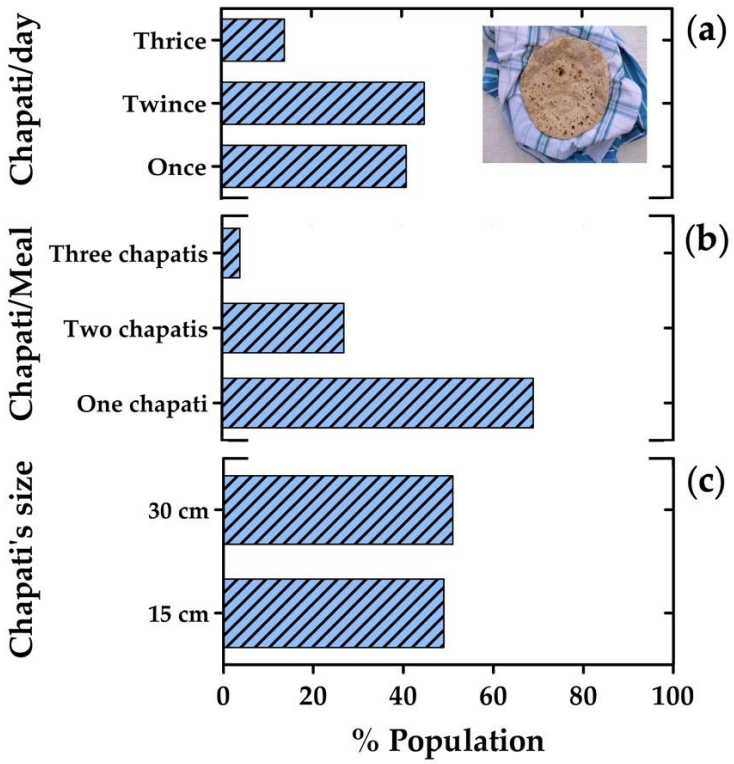


Figure 16. Chapati consumption patterns in a Pakistani population. (**a**) Frequency of daily chapati intake (in the insert, chapati prepared from whole wheat flour). (**b**) Number of chapatis typically consumed per meal. (**c**) Preferred chapati size.

Demographic and clinical profile of HLT vs. subjects with dementia enrolled in the study

The demographic and clinical characteristics of healthy controls (HLT) and patients with dementia are presented in Table 9. The gender distribution was comparable between groups, with a slightly higher proportion of females in both cohorts (HLT: 63.3%; dementia: 60%). Patients with dementia were generally older, with 60% being older than 50 years compared with 50% in the HLT group.

All participants were married. A higher percentage of patients with dementia belonged to the lower-income group (<50k), representing 20% of the cohort compared with 10% among HLT. Educational attainment differed markedly between groups: 97% of HLT reported having formal education, whereas only 60% of patients with dementia did so.

Daily physical activity was substantially reduced in the dementia group. While 96.7% of HLT engaged in regular physical activity, only 36.7% of patients with dementia reported doing so. Consistent with this, 63.3% of patients with dementia reported being physically inactive compared with only 3.3% of HLT.

Comorbidities were more prevalent among individuals with dementia. Hypertension (53.3% *vs.* 16.7%), cardiovascular disease (6.7% *vs.* 0%), and diabetes (30% *vs.* 23.3%) were all more

common in the dementia group. Only 16.7% of HLT reported no comorbid conditions, compared with 60% of HLT.

Characteristic dementia-related symptoms were reported exclusively by the dementia group. These included language difficulties (63.3%), increasing confusion (53.3%), problems with planning and decision-making (40%), personality and behavioral changes (26.7%), and loss of ability to perform daily tasks (26.7%). All HLT participants reported the absence of these symptoms.

Cognitive staging revealed that all HLT individuals presented with no cognitive decline, whereas dementia patients exhibited a spectrum of impairment: very mild (10%), mild (26.7%), moderate (23.3%), moderately severe (23.3%), and severe cognitive decline (16.7%). The average age at clinical diagnosis in the dementia group was 58.3±15.4 years, with the highest proportion diagnosed between 51 and 60 years (36%).

Use of multivitamin supplements was similar between groups, reported by 53.3% of HLT and 40% of dementia patients.

Eating habits in life (lifelong diet)

During the survey, both the HLT and individuals with dementia were asked to describe the type of diet they had followed throughout their lives, prior to the onset of dementia. Participants with dementia reported having historically consumed a more monotonous diet, largely based on refined flours, whereas healthy controls tended to report a higher intake of whole-grain products and more varied meals.

Figure 17a shows the frequency of outdoor dining. As can be seen, clear differences emerge between HLT and patients with dementia. HLT show a more varied distribution, with a substantial proportion reporting monthly (27%) or no outdoor meals (23%), and over one third indicating weekly outdoor meals (1-3 times/week: 44%). In contrast, individuals with dementia predominantly report no outdoor meal consumption (63%), with only a minority indicating occasional participation, either once per week or once per month (17% each). Notably, none of the patients with dementia report consuming outdoor meals more than once per week, whereas this pattern is relatively common among HLT (27%). These findings align with existing literature suggesting that engagement in social and outdoor activities tends to decline in dementia, potentially reflecting both reduced functional autonomy and diminished participation in lifestyle habits established earlier in life (Heikkilä et al. 2024; Chaudhury et al. 2021).

Both HLT and individuals with dementia were asked which type of flour they preferred for preparing chapati at home and for how long they had been consuming it. Participants reported using the same type of flour since the beginning of their habitual diet. As shown in Figure 17b, all

HLT reported the use of whole wheat flour, whereas patients with dementia predominantly reported long-term consumption of refined white flour (73%) or diet atta (27%). These findings are consistent with scientific literature indicating that diets richer in whole grains are more common among healthier populations, while more refined and less varied dietary patterns are frequently observed among individuals with cognitive decline (K. Wang et al. 2023; X. Liu et al. 2023; Ross et al. 2023; Kim et al. 2025).

Furthermore, both the HLT group and individuals with dementia were asked why they preferred a specific type of flour for preparing chapati. As shown in Figure 17c, HLT predominantly chose whole wheat flour due to its perceived health benefits (90%), including higher fiber content and the presence of nutraceuticals and vitamins. In contrast, patients with dementia tended to prefer refined white flour, for greater commercial availability (47%), more appealing color and appearance (33%), and lower cost (11%). These patterns align with scientific literature indicating that healthier populations are more likely to prioritize nutritional value, whereas individuals with cognitive decline may rely more on convenience, sensory appeal, and affordability when making dietary choices (Hyun et al. 2023; Gomes Gonçalves et al. 2023; Yeh et al. 2025; Crichton et al. 2015). However, the 9% of dementia patients who reported choosing their flour for its beneficial effects are those who use “diet atta”, a blended flour formulated for low-calorie diets. Such mixes are typically lower in carbohydrates and richer in fiber, protein, and other nutrients, which may explain why this small subset of patients associated their flour choice with health benefits.

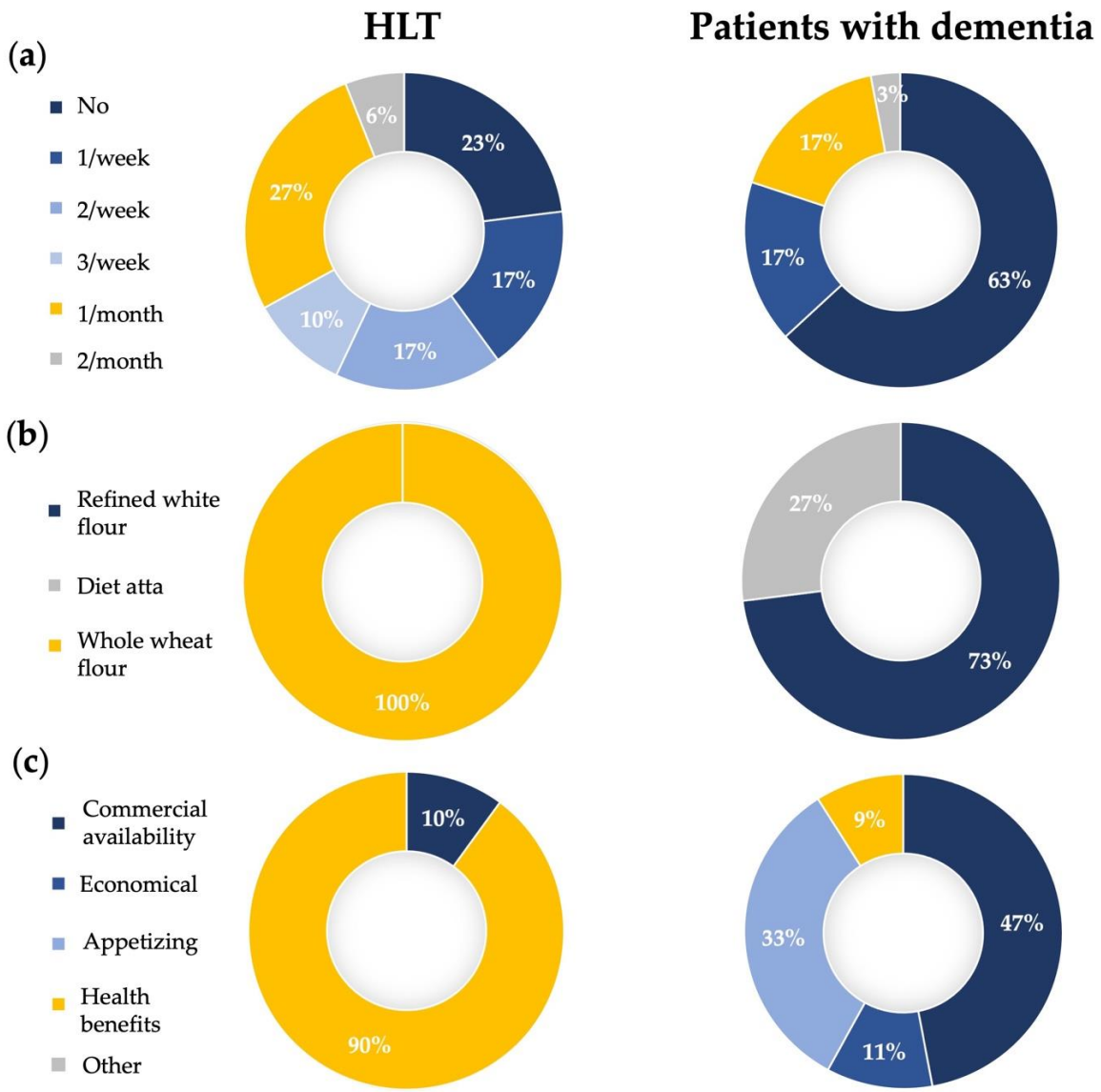


Figure 17. Comparison of food consumption habits and flour preferences between HLT and patients with dementia. (**a**) Frequency of outdoor dining. (**b**) Type of flour typically used. (**c**) Main factors influencing flour choice.

Current eating habits linked to brain health

Figure 18a shows clear differences between HLT and patients with dementia in their daily consumption of foods typically associated with better cognitive health. Overall, HLT report higher intake frequencies for nearly all brain-supportive foods, such as nuts, green leafy vegetables, whole-grain flour, green tea, and blueberries, while patients with dementia show markedly lower consumption rates. These findings align with the scientific literature, which highlights the neuroprotective role of diets rich in antioxidants, omega-3 fatty acids, polyphenols, and fiber, such as those emphasized in the Mediterranean and MIND diets (Loughrey et al. 2017; Picone et al. 2024; Siervo et al. 2021; Fekete et al. 2025; L. Wu e Sun 2017; Zuliani et al. 2026; Masana et al. 2017; Valls-Pedret et al. 2012). The lower intake of these foods among dementia patients may

reflect long-term dietary patterns that contribute to cognitive decline, or alternatively, reduced ability to maintain healthy eating habits after the onset of symptoms. In contrast, the consistently higher consumption reported by HLT reinforces the growing evidence that sustained adherence to nutrient-dense dietary patterns is associated with better brain aging and reduced dementia risk.

A substantial difference between HLT and patients with dementia in their use of additional foods, specifically chosen to support healthy brain function, is showed in Figure 18b. While 63% of HLT report consuming other brain-supportive foods, only 30% of patients with dementia do so, suggesting that HLT participants are more attentive to incorporating targeted dietary elements into their daily routines. Notably, among the HLT respondents, who answered positively, the most commonly mentioned items were olive oil and honey, both frequently highlighted in the scientific literature for their antioxidant, anti-inflammatory, and neuroprotective properties (Navarro-Hortal et al. 2025; Zamri et al. 2023; Fadzil et al. 2023; Gonçalves et al. 2024). In contrast, the higher percentage of negative responses among dementia patients may reflect reduced dietary variety, diminished nutritional awareness, or changes in eating habits that often accompany cognitive decline.

The Figure 18c shows that awareness of the benefits of nutraceuticals in whole grains is high in both groups, though slightly higher among HLT (97%) compared to patients with dementia (93%). This small gap may indicate that HLT are marginally more exposed to or engaged with nutritional information, while patients with dementia may experience reduced access to such knowledge or memory-related difficulties in recalling it. According to recent scientific literature, whole grains contain a variety of nutraceutical compounds, including dietary fiber, vitamins, minerals, phenolic acids, lignans, and antioxidants, that contribute to reduced inflammation, improved metabolic health, and potentially enhanced cognitive function (J. Khan et al. 2024; Călinoiu e Vodnar 2018; Allai et al. 2022; J. Khan et al. 2022; Chung et al. 2022; Slavin 2004). These components support vascular health and help counteract oxidative stress, both of which play essential roles in maintaining brain integrity (Chung et al. 2022; Anderson 2003; Basile et al. 2023). The high level of awareness observed in both groups suggests that the perceived health value of whole grains is broadly recognized, even though actual dietary habits may differ.

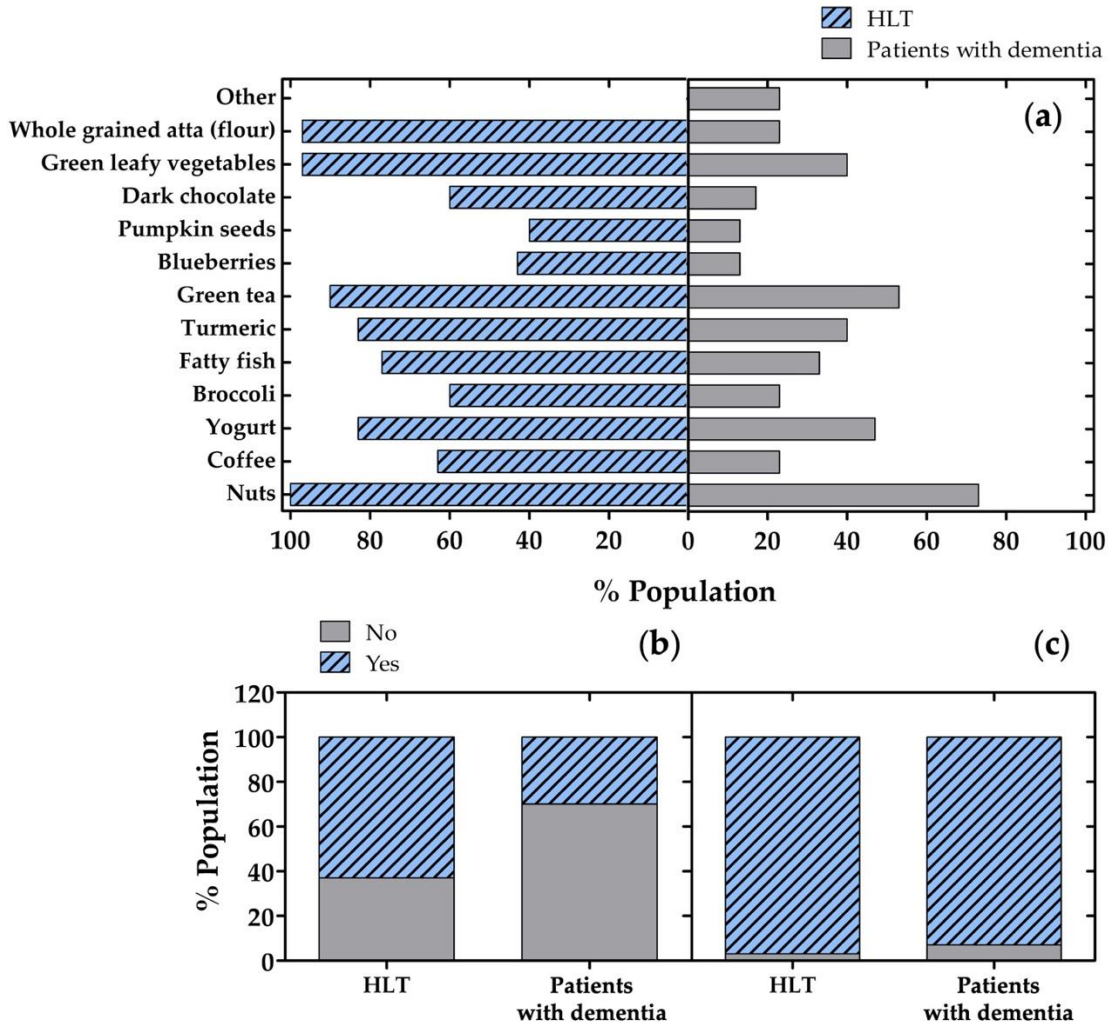


Figure 18. Dietary habits and consumption of neuroprotective foods among HLT and patients with dementia. (**a**) Percentage of participants reporting regular consumption of selected brain-healthy foods and ingredients. (**b**) Differences between HLT and patients with dementia in the consumption of additional foods specifically chosen to support brain function. (**c**) Awareness of the benefits of nutraceuticals contained in whole grains.

Attribution of the causes of dementia

Figure 19a shows that a large majority of HLT participants (71 %) and a similarly high proportion of people with dementia (68 %) believe that “certain vitamin deficiencies” are a primary cause of dementia, far more than other potential causes such as side-effects of medications, brain infections/tumors, or emotional problems. This indicates that both laypersons and patients often attribute dementia to modifiable nutritional factors. Such intuitions are not unfounded: growing scientific evidence supports a link between low vitamin status and cognitive decline. For instance, a 2023 meta-analysis found that deficiency of Vitamin D deficiency is associated with a ~1.42-fold increased risk of dementia and a ~1.57-fold increased risk of Alzheimer's disease (X.-X. Zhang et al. 2024). Similarly, a 2022 meta-analysis of 95 studies with over 46,000 participants

showed that supplementation with B vitamins (especially folate) may slow cognitive decline and reduce the risk of incident dementia among older adults without dementia (Z. Wang et al. 2022). Moreover, clinical research has demonstrated that treating Vitamin B12 deficiency in patients with mild cognitive impairment can lead to measurable improvements in cognition (Jatoi et al. 2020; Rosenberg 2024). In addition, neuroimaging data indicate that B12 deficiency is associated with brain atrophy (e.g., hippocampal shrinkage), a structural correlate of dementia (Ueno et al. 2025). Therefore, the high frequency with which participants cite vitamin deficiencies as a cause of dementia appears broadly consistent with current scientific understanding of modifiable risk factors, though not all vitamin deficiencies lead to dementia, and supplementing vitamins does not guarantee prevention.

Sense of community and social belonging

Figure 19b shows a stark difference between HLT participants and patients with dementia in their reported sense of belonging to the local community: 87% of HLT describe their sense of belonging as "*very strong*", whereas only 17% of patients with dementia do so, with 43% of patients rating it "*somewhat strong*" and 40% "*somewhat weak*". This suggests that people living with dementia often experience a significantly diminished feeling of social belonging compared to cognitively healthy individuals, a finding that may reflect the well-documented risk of social isolation, diminished social participation, and reduced opportunities for engagement experienced by this population. This pattern resonates with recent scientific literature. For example, a 2023 study reported that participation in community-based social initiatives like *Dementia Cafés* gave people with dementia and their caregivers an enhanced sense of belonging and purpose, an important boost to their social health and wellbeing (Innes et al. 2022). Similarly, a 2024 ecological momentary assessment of social interaction in long-term care showed that many people with dementia spend large portions of the day without any social contact: in the sample, no interaction occurred during ∼72% of observation periods (Gebhard et al. 2024). Moreover, a recent meta-analysis demonstrated that feelings of loneliness and social isolation are highly prevalent among individuals with mild cognitive impairment or dementia, pointing to social disconnectedness as a common, serious problem (Hajek e König 2025). These findings help explain why so few patients in your sample report a "*very strong*" sense of community: their disease often disrupts social networks, reduces opportunities for meaningful interaction, and undermines feelings of inclusion and belonging. At the same time, the positive impact of community-based support structures (like dementia cafés, peer-support groups, inclusive neighbourhood efforts) highlights that strengthening social belonging is not only desirable but potentially meaningful for well-being and care.

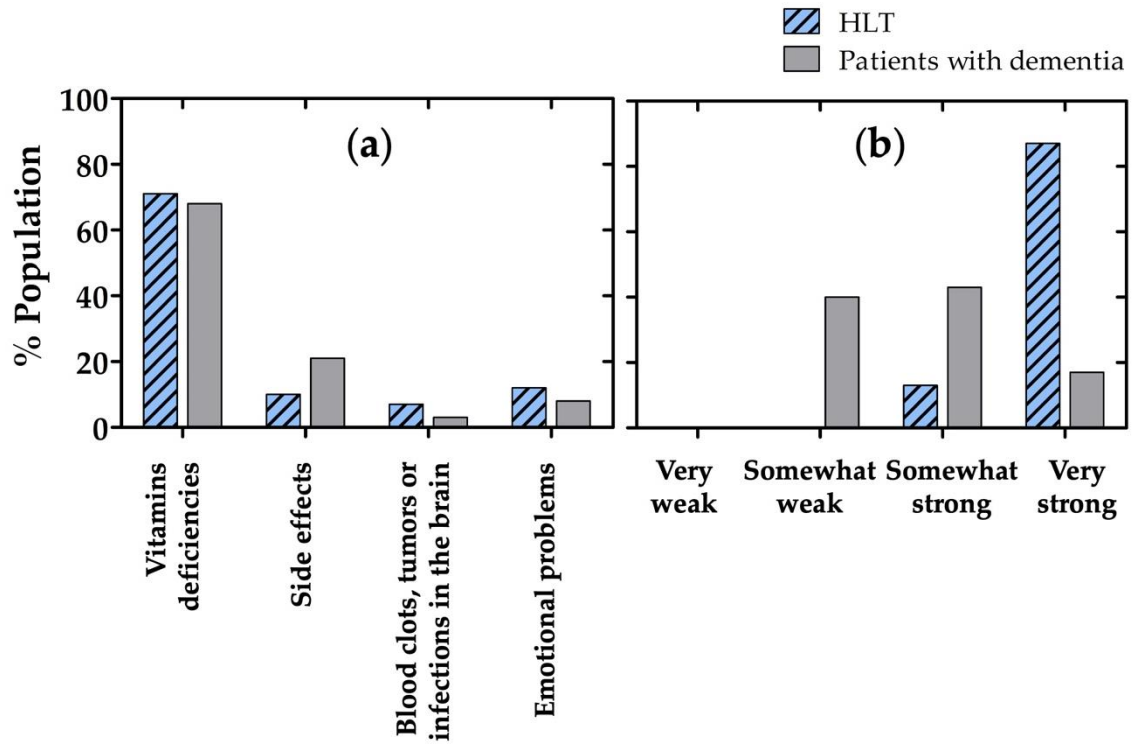


Figure 19. (**a**) Percentage of respondents identifying potential causes of memory problems. (**b**) Differences between HLT participants and patients with dementia in their sense of belonging to the local community.

Discussion

This cross-sectional study provides insight into the potential role of dietary whole wheat flour intake in Pakistan as a modifiable factor relevant to long-term dementia prevention. Although causality cannot be inferred, the findings should be interpreted within the growing body of evidence linking whole grains, dietary fiber, and bioactive compounds to mechanisms involved in cognitive aging and neurodegeneration.

Whole wheat flour is a rich source of dietary fiber, B-group vitamins, minerals, and phenolic compounds, which are largely removed during refining. Previous nutritional analyses have demonstrated that whole grains contain antioxidant and anti-inflammatory components capable of modulating oxidative stress and systemic inflammation, both of which are central to dementia pathophysiology (Fan et al. 2020; Zannini et al. 2022). These mechanisms are particularly relevant given the established role of chronic low-grade inflammation and oxidative damage in neuronal dysfunction and synaptic loss (Burg et al. 2013).

Furthermore, dietary fiber from whole grains contributes to improved metabolic regulation, including glycemic control and lipid profiles, which are known risk modifiers for vascular dementia and Alzheimer's disease (Călinoiu e Vodnar 2018; Dysken et al. 2014). The association

between metabolic disorders and cognitive decline has been consistently reported, supporting the biological plausibility of dietary interventions targeting staple foods.

Recent evidence suggests that whole grain intake may influence brain health through modulation of the gut–brain axis. Whole wheat-derived fibers and polyphenols can alter gut microbiota composition, leading to increased production of short-chain fatty acids with neuroprotective and anti-inflammatory properties (Guo et al. 2024; Shen et al. 2025). Dysbiosis and intestinal permeability have been increasingly implicated in neurodegenerative processes, reinforcing the relevance of dietary patterns rich in unrefined plant foods.

In addition, experimental and toxicological studies indicate that whole grain phytochemicals may mitigate neurotoxicity and hormonal dysregulation linked to cognitive impairment (Ganamurali et al. 2025; Dong et al. 2023). These findings support the hypothesis that long-term consumption of whole wheat flour could contribute to neuroprotection through multiple converging biological pathways.

While randomized controlled trials directly assessing whole wheat flour and dementia outcomes remain limited, observational and interventional evidence supports the role of plant-based, fiber-rich diets in reducing cognitive decline. Reviews and systematic analyses indicate that diets emphasizing whole grains are associated with better cognitive performance and reduced dementia incidence, although heterogeneity and methodological limitations persist (Ashley et al. 2021; X. Liu et al. 2023; Farina et al. 2017). Recent clinical and pharmacological perspectives also highlight nutrition as a key component of multidomain dementia prevention strategies (K. Wang e Zhou 2023; Abdelgawad et al. 2025).

In Pakistan, wheat flour constitutes a primary caloric source, making the distinction between refined and whole wheat flour particularly important at a population level. The widespread consumption of refined wheat may contribute to micronutrient insufficiencies and metabolic risk, whereas greater reliance on whole wheat flour could offer a feasible, culturally acceptable intervention. Food science research supports the nutritional superiority of whole wheat products and emphasizes the need for improved processing and consumer awareness to enhance acceptability and health impact (A. Liu et al. 2025; Zou et al. 2021).

The principal limitation of this study is its cross-sectional design, which prevents assessment of temporal relationships between whole wheat flour intake and cognitive outcomes. Dietary intake was self-reported and may be subject to recall bias, and cognitive status was not assessed longitudinally. Moreover, dementia is a multifactorial condition influenced by genetics, education, physical activity, and socioeconomic factors that could not be fully accounted for.

Future research should prioritize prospective cohort studies and randomized dietary interventions in South Asian populations, incorporating standardized cognitive assessments and

biomarkers of inflammation, oxidative stress, and gut microbiota composition. Such studies would help clarify whether long-term substitution of refined wheat with whole wheat flour can meaningfully contribute to dementia prevention.

In conclusion, this study adds to the emerging evidence that whole wheat flour intake may be relevant to long-term brain health through metabolic, inflammatory, and gut-mediated mechanisms. Given the central role of wheat in the Pakistani diet, promoting whole wheat flour consumption represents a potentially impactful and scalable strategy within broader dementia prevention frameworks.

CONCLUSION

In conclusion, this thesis underscores the importance of integrated metabolic and nutraceutical strategies in the prevention of cognitive decline. The research findings demonstrate the significant potential of peripheral biomarkers and dietary interventions as tools for early risk stratification and personalized preventive measures. By emphasizing the role of tailored approaches, this work lays the foundation for future research aimed at translating these insights into practical applications within clinical settings and public health initiatives. Moving forward, it is essential to explore the mechanisms underlying these interventions and to develop evidence-based guidelines that can inform therapeutic strategies and policy-making in the fight against cognitive decline.

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APPENDIX A

Shifa Tameer-e-Millat University

شفا تعمیرِ ملّت یونیورسٹی

Focus group interview to determine whether the bioactive compounds in whole wheat flour have an impact on the prevention or delaying of dementia in the old age.

Patient's Particulars				
Name	Age	Weight	Height	BMI

Demographic Background					
Gender	Marital Status	Education	Income	City	Profession

1. Do you have any comorbidities/disease?

_____

2. Describe the type of diet you have been consuming throughout your life (prior to the diagnosis of dementia)

__Breaskfast_____
__Lunch_____
__Diner_____
__Teatime/snack_____

3. Specify the frequency of outdoor meals you've been consuming throughout your life span?

_____

4. What noticeable symptoms of dementia do you have?
    1. Language problems
    2. Increasing confusion
    3. Personality or behavior
    4. Reduced concentration
    5. Loss of ability to do everyday tasks
    6. Apathy and withdrawal or depression

7. Not recognizing friends and family
8. Problems with planning and decision-making
9. Not remembering where they live or where they are
10. Memory problems, particularly remembering recent events
11. None of above
12. Other ____

5. When were you diagnosed with dementia?

_____

6. What stage of dementia do you have?
   1. No cognitive decline
   2. Very mild cognitive decline
   3. Mild cognitive decline
   4. Moderate cognitive decline
   5. Moderately severe cognitive decline
   6. Severe cognitive decline
   7. Very severe cognitive decline

7. What do you think is the cause of dementia?
   1. Certain vitamins deficiencies
   2. Side effects of certain medicine
   3. Blood clots, tumors or infections in the brain
   4. Emotional problems such as stress, anxiety or depression

8. How would you describe your sense of belonging to your local community?
   1. Very weak
   2. Somewhat weak
   3. Somewhat strong
   4. Very strong

9. Does dementia have any impact on your dietary routine or feeding practices?
   1. Overeat or even develop an insatiable (unstoppable) appetite
   2. Forget to eat or drink not recognize when they are hungry, thirsty or full.
   3. Swallowing difficulties (called dysphagia), leading to weight loss, malnutrition or dehydration
   4. Other _____

10. What dietary approaches such as, Mediterranean/DASH Diet, have you been following after diagnosis or knowing about dementia to control or slow down its progression?
    (MIND diet: more vegetables, especially green leafy vegetables; berries over other fruit; whole grains; beans; nuts; one or more weekly servings of fish; and olive oil. Limiting servings of red meat, sweets, cheese, butter/margarine, and fast/fried food)

_____

11. Mark healthy food you consume on daily basis to support a healthy brain function.
    1. Nuts
    2. Coffee

3. Yogurt
4. Broccoli
5. Fatty fish
6. Turmeric
7. Green tea
8. Blueberries
9. Pumpkin seeds
10. Dark chocolate
11. Green Leafy Vegetables
12. Whole grained atta (flour)
13. Other _____

12. Were you physically active with an exercise routine throughout your life?

_____

13. What type of flour do you prefer for making chapati at home? And since how long have you been consuming it?

_____

14. Why do you prefer this specific type of Flour?
    1. Commercial availability_____
    2. Economical_____
    3. Appetizing (attractive color, appearance?)_____
    4. Health Benefits (high fiber/nutraceuticals/ vitamins)_____
    5. Other _____

15. Have you heard about the benefits of nutraceuticals in whole wheat? Or been recommended by any physician?

_____

16. Have you been using any multivitamin for your brain health/mental performance?

_____

17. Have you been consuming any other specific food for maintaining a healthy brain function? and since how long?

_____

18. Is there anything else you would like to add? Anything we haven't covered?

_____

……*Thank You*.....

APPENDIX B

Shifa Tameer-e-Millat University

شفا تعمیرِ ملّت یونیورسٹی

A web-based survey study to check the impact of the type on the selection and consumption of wheat flour (desi-atta) among Pakistani people

Participants Particulars								
Name	**Age**	**Weight**	**Height**	**BMI**	**Gender**	**Marital Status**	**City**	**Profession**

Specify your Income group

1. <50000
2. >50000

Mark your education level

1. None
2. FA/FSC
3. BA/BSC
4. MA/MSC
5. Doctoral

Do you have any particular food allergies?

3. Yes _____
4. No

Please answer the following according to your particular eating habits?

1. How many times in a day do you eat?

1. One
2. Twice
3. Thrice

2. What wheat products do you commonly consume? (Select one or more)

1. Roti/Naan
2. Bread
3. Cakes/pastries
4. Cereal
5. Pasta

6. Cookies
7. Other _____specify

3. What type of wheat flour do you commonly use?

1. Whole grained flour (desi atta)
2. Mixed white and whole grained flour
3. All- purpose flour (maida)
4. Bread flour
5. Cake flour
6. Pastry flour
7. Other (specify)_____

4. Why do you prefer this specific type of Flour?
1. Economical
2. Commercial availability
3. Taste
4. Health Benefits
5. Appearance/Fine texture
6. Other _____

7. Where do you usually purchase wheat flour from?
1. Local market
2. Supermarket
3. Village
4. Online store
5. Other_____

6. How many times in a day do you eat chapati?
   1. Once
   2. Twice
   3. Thrice

7. How much wheat flour do you consume with a typical meal?
   1. One chapati
   2. Two chapati's
   3. Three chapati's

8. What size of chapati do you prefer?
   1. I prefer eating a 12 inches chapati
   2. I prefer eating a 6 inches small chapati

9. Do you consider factors like nutritional value of whole wheat over refine wheat while buying flour (desi atta)?

   1. Yes
   2. No

10. Do you considered price over nutritive value while buying flour (desi aata)?

    1. Yes
    2. No

11. Do you prefer white flour (refined wheat) over whole wheat flour for cooking roti due to its fine texture and good taste?
    1. Yes
    2. No

12. Have you been using any multivitamin for your brain health/mental performance?

    1. Yes
    2. No

13. Have you heard about nutraceuticals present in whole grained flour? Or been recommended by any physician?
    1. Yes
    2. No

14. Would you be willing to switch to whole wheat flour if recommended by a healthcare professional or nutritionist?
    1. Yes
    2. No
    3. Already using it

15. Have you been diagnosed with any of the following diseases?

    1. Hypertension (HTN)
    2. Cardiovascular Disease (CVD)
    3. Diabetes Mellitus (DM)
    4. Irritable Bowel Disease (IBS)
    5. Obesity
    6. Celiac disease
    7. Gluten intolerance
    8. Dementia
    9. None
    10. Other _____

16. If yes? Please specify the duration of the disease
    1. <10 years
    2. >10 years
    3. <20 years
    4. >20 years

17. Do you experience any of the symptoms mentioned below in daily routine?
    13. Language problems
    14. Increasing confusion

15. Reduced concentration
16. Personality or behavior issues
17. Loss of ability to do everyday tasks
18. Apathy and withdrawal or depression
19. Not recognizing friends and family
20. Problems with planning and decision-making
21. Not remembering where they live or where they are
22. Memory problems, particularly remembering recent events
23. None of above
24. Other _____

18. How would you rate your brain health?
    2. Bad
    1. Poor
    2. Average
    3. Good
    4. Excellent
19. What do you think is the cause of above-mentioned disease?
    3. Poor diet
    4. Consumption of white wheat flour
    5. Side effect of medication
    6. Age

20. Do you involve yourself in physical activity every day?
    1. Brisk walk
    2. Cleaning or gardening
    3. Cooking and washing up
    4. Gym
    5. None

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