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EFFECT OF 12 WEEKS FISH SUPPLEMENTATION OF AN ENHANCED USUAL DIET ON COGNITION OF RESOURCE LIMITED INDEPENDENTLY LIVING ELDERLY IN A RETIREMENT VILLAGE: A RANDOMISED CONTROLLED TRIAL

by

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DEDICATION

This thesis is dedicated to every person who is living with dementia or who is taking care of a loved one with dementia…

DECLARATION

I, Lizette Kühn, declare that the thesis, which I hereby submit for the degree PhD Dietetics at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other tertiary institution.

I declare that the foods for the study were sponsored by Lucky Star and Southern Oil. Shoprite Checkers contributed via vouchers (3 x R10 000) which were used to procure study food. Any study food which was in excess at the end of the study was donated to the consequent nutrition program at Allen Park Council for the Aged.

I declare that Allen Park Council for the Aged supported the study by reimbursing the psychometrist for the assessments conducted. In no way was Allen Park Council for the Aged or any of the abovementioned sponsors involved in the design of the study, the development of the protocol or the interpretation and reporting of the research.

Blood for biomarker testing was drawn by two phlebotomists from Ampath Laboratories free of charge. Analysis of blood was done by the Centre of Excellence in Nutrition from North West University free of charge.

ETHICS STATEMENT

I, Lizette Kühn, have obtained, for the research described in this work, the applicable research ethics approval **(542/2017)**. I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of ethics for researchers and the Policy guidelines for responsible research. Initial ethics approval was given on 8 February 2018. Approval for an amendment was granted on 16 July 2018. The final ethics report was signed off on 28 September 2020.

_____ _____ ___10/11/2020_____

Signed Date

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ABSTRACT

Introduction

The high prevalence and impact of dementia call for preventative measures, including application of an optimised diet. Omega 3 polyunsaturated fatty acids (PUFA) may influence the risk for developing dementia by supporting cardiovascular health and by decreasing inflammation. Research, particularly randomised controlled trials, studying a food-based approach that uses Omega 3 PUFA intake from foods such as fish to counteract dementia in low/middle income countries (LMIC), is lacking.

Aim

To determine the effect of supplementing diets of independently living, resource-limited elderly participants for 12 weeks with fish versus non-fish foods on cognition.

Methods

In a randomised controlled trial the usual diet of independently living elderly persons in a resource-limited retirement centre in urban South Africa was enhanced with context-appropriate foods i.e. canned baked beans, canola oil and peanut butter mimicking elements of the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) Intervention for Neurodegenerative Delay (MIND) diet. Additionally, the intervention group received canned pilchards and fish spread (equivalent to a calculated daily intake of 2.2g Omega 3 PUFA) weekly compared to canned meatballs and texturised soya protein (meat substitute) received by the control group. Cognition and level of functioning were measured before and after intervention with the Cognitive Abilities Screening Instrument (CASI) and the Lawton Instrumental Activities of Daily Living (IADL). Adherence was assessed by determining dietary intake with a study-specific food frequency questionnaire (FFQ) and red blood cell (RBC) PUFA biomarkers before and after the intervention. Data were analysed by non-parametric analysis of covariance (ANCOVA) with, and without, bootstrap imputation.

Results

Fifty seven (74% female, mean age: 72 ±7 years) elderly participants participated in this study. There was a significant post intervention difference (P=0.036) in the total CASI scores between the intervention and control groups, when the model was fitted with imputation and controlled for baseline scores. The predicted total CASI score of the intervention group was higher than

the score of the control group. Likewise the calculated dietary Omega 3 PUFA intake and red blood cell (RBC) Eicosapentaenoic acid (EPA) and Docosapentaenoic acid (DPA) content differed significantly between the intervention and control group after the intervention phase. The Lawton IADL presented similar results over the course of the study with limited variance.

Conclusion

Fish intake in the context of the MIND diet may exert a positive effect on cognition as the current study showed that fish can have a significant effect on the cognition of resource-limited elderly after 12 weeks of supplementation of an enhanced diet.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
AA	Arachidonic Acid
AD	Alzheimer's Disease
ALA	Alpha Linolenic Acid
ANCOVA	Analysis of covariance
APOE	Apo Lipoprotein E
ARCD	Age related cognitive decline
BALD	Basic activities of daily living
BHT	Butylated Hydroxytoluene
BL1	Baseline 1 Assessment
BL2	Baseline 2 Assessment
BMI	Body Mass Index
˚C	Degrees celcius
CASI	Cognitive Abilities Screening Instrument
CI	Confidence Interval
CHO	Carbohydrate
CIND	Cognitive impairment not dementia
CNS	Central nervous system
CRIBSA	Cardiovascular Risk in Black South Africans
/day	Per day
DASH	Dietary Approach to Systolic Hypertension
DHA	Docosahexaenoic acid
DM	Diabetes Mellitus
DNA	Dexorybonucleic acid
DPA	Docosapentaenoic acid
DRS	Dementia Rating Scale
EDTA	Ethylenediamine tetraacetic acid
EPA	Eicosapentaenoic acid
et al.	Et alia
FINGER	Finnish Geriatric Intervention Study to prevent Cognitive impairment and Disability
FFQ	Food frequency questionnaire
g	Gram
G	Centrafugal force
GI	Glycaemic Index
HB	Haemoglobin
HIC	High income countries
IADL	Instrumental Activities of Daily Living

ID	Identification
i.e.	Id est (that is)
Kg	Kilogram
LCPUFA	Long chain polyunsaturated fatty acids
LMIC	Low/middle income countries
m^2	Square metre
MAPT	Multidomain Alzheimer Preventive Trial
max	Maximum
MCI	Mild cognitive impairment
MCC	Medicine Control Council
MCT	Medium chain triglyserides
mg	Miligram
MIND	Mediterranean-DASH Intervention for Neurodegenerative Delay
mL	Mililitre
MMSE	Mini Mental State Examination
MS	Multiple Sclerosis
MUFA	Monounsaturated fatty acid
N	Sample size
NaCl	Sodium chloride
NWU	North West University
Omega 3 LCPUFA	Omega 3 Long chain polyunsaturated fatty acid
Omega 3 PUFA	Omega 3 Polyunsaturated fatty acid
Omega 6 AA	Omega 6 Arachidonic acid
OPAL	Older People and Omega 3 long-chain polyunsaturated fatty acid
PD	Parkinson's Disease
PI	Post Intervention Assessment
Pmol/L	Picomoles per litre
PUFA	Polyunsaturated fatty acid
R	Rand (South Afican currency)
RBC	Red blood cell
RDA	Recommended dietary allowance
REC	Research Ethics Committee
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SD	Standard Deviation
SFA	Saturated fatty acids
SU.FOL.OM3	Supplementation with folate, vitamin B6 and B12 and/or Omega 3 fatty acids
SU.VI.MAX	Supplementation en Vitamines et. Mineraux Antioxydants
T	Table spoon

TE	Total energy
µL	Micro litre
USA	United States of America
US $	United States Dollar
/week	Per week
Wk	Week

CHAPTER 1
INTRODUCTION

1.1 Background to the study

The effect of diet on cognition is a phenomenon that attracts growing interest these days in both the scientific world and general public. A possible explanation for this tendency is that nowadays most human beings have an extended lifespan and, therefore, a longer period of time during which humans must be self-sufficient. The increasing prevalence of dementia globally may also fuel this interest in the relationship between diet and cognition.

Dementia is a disease that increases in numbers yearly. Management through medication is possible to an extent, but to date, no cure exists.[1] For this reason the emphasis is even greater on preventative approaches, such as improving lifestyles and diets. Globally 50 million people are living with dementia. Estimations project that this number will have increased to 152 million by 2050, especially in LMIC where two thirds of individuals with dementia reside.[2] According to the World Alzheimer Report of 2016 (based on 2015 statistics), the South African population had grown to nearly 55 million people of whom 4.4 million were aged 60 years or older. Approximately ten percent of the 4.4 million were older than the age of 80 years. It was estimated that nearly 186 000 people in South Africa live with dementia and 75% of them are women. The expectation is that this number may rise to 275 000 in 2030.[3]

Apart from the impact that this neurodegenerative condition has on the individual and his/her immediate surroundings, the effect on the global economy is of concern. According to the Lancet Expert Committee (2020), the effect of dementia on individuals and the global economy is one trillion United States (US) dollars per year.[2] Currently nearly all the studies related to the prevention of dementia are from high income countries (HIC). This is problematic as risks, lifestyle modifications and interventions that are possible and affordable in HIC, may differ from those in LMIC.[2]

Research for the 2016 World Alzheimer Report indicated a very low awareness of the prevalence and impact of dementia among South Africans. Even general practitioners often considered dementia as a normal consequence of aging and had very little understanding of care and treatment options. Priorities listed for the improvement of dementia care and treatment in South Africa include aspects such as creating awareness, being more prepared and promoting research.[3] The current study aimed to address the

above mentioned factors by investigating whether practical, affordable changes to the usual diet of elderly persons could positively affect cognition.

Dementia is a disease with many aetiological factors of which lifestyle is one of the modifiable factors. Diet as part of lifestyle warrants more research as a possible preventative strategy, but cost and food availability need to be regarded as essential factors when dietary changes are considered. Many HIC including America, Australia and some European countries have declared dementia a public health priority and are researching diet as a preventative approach to the problem.[2] According to estimations 9.2 million people can be saved from developing dementia by 2050 if the onset of the disease is delayed by one year.[4]

As dementia develops over the course of life, the question also arises as to what age a change in diet can still impact on cognition. Years of cumulative neuropathology have already occurred by the time Alzheimer's Disease (AD) is clinically evident. The question arises if nutritional factors can still exert a neuroprotective influence in later life.[5]

A 2015 systematic review by Van de Rest et al. supported nutrition as an important modifiable risk factor in the preventative strategy against dementia.[4] Studies on the role of nutrition in cognition among elderly people began in the 1980s with growing interest as time went by. In 1997 the possible role of diet in the development of AD was highlighted.[6] This was followed by an exponential growth in the number of studies published on nutrition and cognition in the older person. Initially studies were designed as cross-sectional, cohort and longitudinal studies,[6] but as they indicated associations between nutrition and cognition, the need for randomised controlled trials on the subject arose.

As mentioned previously, the majority of research studies are carried out in HIC. [2] For the LMIC where resources are limited, conducting research, especially randomised controlled trials is more challenging. Between May 2016 and August 2020, the Scopus, PubMed, Medline and Science Direct databases were searched, but no publication of any study regarding nutrition (including use of supplements) and cognition in the older person (with the specific aim to prevent cognitive decline) could be found in the African or South African context.

1.2 Problem statement

The high prevalence of dementia in LMIC and the possible underestimation in South Africa[2,7] served as the main motivation for the study. Cognitive decline (especially dementia) poses a large burden for the social and economic infrastructure of a country. There is an urgent need for preventative measures as dementia imposes a huge burden on care giving institutions, care giving and resource utilization.[8] Intervention studies are essential to examine affordable and feasible lifestyle changes which can reduce cognitive decline or even promote cognition among the South African elderly persons. It was estimated that by delaying the disease for five years the global prevalence and cost could be reduced by half.[9]

Omega 3 PUFA is of special interest as a number of intervention studies from HIC indicated promising effects of Omega 3 PUFA on cognition.[10-13] Furthermore affordable dietary sources of Omega 3 PUFA are readily available in the form of canned fish or fish spread in South Africa. The availability and affordability of the canned fish and fish spread support Omega 3 PUFA intake through a food-based rather than a supplement-based approach. A food-based approach needs to be viewed in context of the total diet as there is a synergistic working between nutrients.[14] The American Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet[15] is probably the most practical food-based approach to support cognition with the aim of preventing dementia. Not only is the MIND diet quantified into specific servings for the relevant cognitive supporting food components, its scoring system supports assessment of any person's usual diet in terms of these cognitive supporting food components. Therefore although the MIND diet originated in a HIC, modification thereof by substituting food components with nutritionally similar, but context relevant and affordable foods, may lead to a feasible version which can be applied in LMIC.

1.3 Research aim and question

The study aimed to examine the effect of a food-based intervention - with a focus on fish intake - for 12 weeks in a resource-limited group of South African elderly people. Their usual diets were supplemented with affordable foods in an effort to mimic the cognitive enhancing characteristics of the more expensive Mediterranean approach, which is

included in the MIND diet.[15] The focus was on fish intake because as mentioned before, a number of intervention studies from HIC indicated promising effects of Omega 3 PUFA on cognition.[10-13] Therefore further differentiation between an intervention and control group was used, where the intervention group received canned fish and fish paste (test foods) and the control group received canned meatballs and texturised soya protein (control foods) for the period of 12 weeks. Cognition and changes in cognition were measured by the Cognitive Abilities Screening Instrument (CASI)[16] whereas level of functioning and the change in this skill, were measured by the Lawton Instrumental Activities of Daily Living (IADL).[17] Hence, the research question read: What is the effect of 12 weeks of fish supplementation of an enhanced usual diet on the cognition of independently living elderly persons?

1.3 Null hypothesis and objectives

Null hypothesis

There will be no change in the cognition of the elderly person as measured by the CASI score when their enhanced usual diet is supplemented with fish providing about 2.2g Omega 3 PUFA per day for 12 weeks.

Primary objectives

Against the backdrop of the explained aim the following objectives were set:

- To determine the change in cognition (as indicated by a CASI score) and level of functioning (as indicated by a Lawton IADL score) in both the intervention group (receiving fish as part of an enhanced usual diet) and the control group (receiving an enhanced usual diet without fish supplementation).
- To compare the change in cognition (as indicated by a mean CASI score) and level of functioning (as indicated by a mean Lawton IADL score) between the intervention and control group.

Secondary objectives

- To determine the change in diet (as indicated by a modified MIND diet score) in both the intervention group (receiving fish as part of an enhanced usual diet) and the control group (receiving an enhanced usual diet without fish supplementation).

- To compare the change in diet (as indicated by a mean modified MIND diet score) between the intervention and control groups.
- To determine the change in red blood cell (RBC) polyunsaturated fat (PUFA) composition (Omega 3 PUFA, Eicosapentaenoic Acid (EPA), Docosapentaenoic Acid (DPA), Docosahexaenoic Acid (DHA), Omega 6 PUFA) in both the intervention group (receiving fish as part of an enhanced usual diet) and the control group (receiving an enhanced usual diet without fish supplementation).
- To compare the change in RBC PUFA composition (Omega 3 PUFA, EPA, DPA, DHA, Omega 6 PUFA) between the intervention and control groups.

In addition to the above, the secondary objectives would allow for the assessment of the extent of adherence to the study diets.

1.4 Assumptions and delimitations

The assumptions and delimitations of the study are presented in Table 1.

Table 1: Assumptions and delimitations

Assumptions	Delimitations
1. Participants consumed the study food themselves and did not exchange food with each other.	1. Both the intervention and control groups received an enhanced diet which possibly influenced the magnitude of the effect of the Omega 3 PUFA-rich fish.
2. A period of 12 weeks is long enough for the fish (high in Omega 3 PUFA) to affect cognition.[11]	2. The focus was on a food-based approach of supplementing Omega 3 PUFA, monounsaturated fatty acids (MUFA) and plant protein components of the MIND diet only, not on the other dietary components of the MIND diet..
3. A change in total CASI score by five points indicated a clinical significance. Refer to 3.3.2	3. Cognition pertaining only to the domains and total score of the CASI, was determined.
4. Nutritional information on the labels of study foods were accurately indicated by manufacturers.	4. Only one retirement village was included.

1.5 Definition of key concepts

Table 2: Definition of key concepts

Key concept	Theoretical definition	Conceptualised definition
Adherence	The tenacity required to execute and maintain lifestyle changes (including diet) which correspond with recommendations from a health care provider/researcher. [18,19]	The study participants' commitment to the intake of study food and their honesty not to exchange study foods between the two groups. Adherence was assessed by determining by dietary intake with a study-specific food frequency questionnaire (FFQ) and RBC PUFA biomarkers before and after the intervention phase.
Age related cognitive decline	Also known as age associated cognitive decline. It is defined as "normal (non-pathological, normative, usual) cognitive aging". The extent of this experience differs between individuals.[20]	Same as theoretical definition
Alzheimer's disease (AD)	It is a neurodegenerative disease which is initially characterised by short-term memory impairment and executive dysfunction. As the disease progresses it may lead to deficits influencing the brain as a whole leading to total incapacity.[4]	Same as theoretical definition
Cognition	Cognition is a summary term which describes an individual's thought processes while interacting with other humans and the environment and includes any and all processes by which this individual becomes aware of his/her situation, needs, goals, and required actions. The individual then uses this information to implement problem solving strategies for optimal living. Cognition includes further aspects such as: perceiving, thinking, knowing, reasoning, remembering, analysing, planning, paying attention, generating and synthesising ideas, creating, judging, being aware, and having insight.[20-22]	Same as theoretical definition; however reflected in the current study by the total score of the CASI and its underlying domains.
Compliance	The extent to which a patient/participant acts in accordance with the prescriber/researcher's recommendations or yields to a request.[18]	The study participants' commitment to collect the study foods weekly. Compliance was monitored by recording participant numbers when they collected food.
Control foods	No theoretical definition.	The canned meatballs and texturised soya protein supplied to the control group during the intervention phase.

Key concept	Theoretical definition	Conceptualised definition
Control group	The control group consists of study participants which are similar to the intervention group in all aspects that may affect the outcome of the study except for the intervention/exposure under investigation.[23]	The group of participants receiving the foods to enhance usual diet and the control foods during the intervention phase.
Dementia	"Dementia is a descriptive term indicating an observable decline in mental abilities. It is an acquired clinical syndrome characterised by deterioration of mental functioning in its cognitive, emotional and conative aspects."[21]	Same as theoretical definition
Foods to enhance usual diet	No theoretical definition	Foods supplied weekly during the intervention phase to both the intervention and the control group with the aim to enhance their usual diets. These foods aimed to mimic aspects of the MIND diet in terms of its fatty acid and plant protein components. MUFA intake was enhanced by peanut butter and canola oil. The plant protein intake was enhanced by canned baked beans. Refer to Table 8 in Chapter 3 for specific MUFA and plant protein content.
Fish	Fish defined in the context of the MIND[15]diet: Tuna sandwich, fresh fish as a main dish; not fried fish cakes, sticks, or sandwiches.	Oily fish rich in Omega 3 LCPUFA: Canned pilchards and fish paste Refer to Table 8 in Chapter 3 for specific Omega 3 PUFA content.
Intervention group	The participants in a research study who receive the study treatment or intervention.[24]	The group of study participants who received test foods (fish and fish spread) in addition to their enhanced diet.
Intervention phase	No theoretical definition	The 12 weeks during which all the study participants received study foods to enhance their usual diet.
Level of functioning	Level of functioning refers to an individual's ability to perform self-care, self-maintenance and physical activity. In other words the individual's ability to perform activities of daily living.[25]	Ability to perform activities of daily living as assessed by the Lawton IADL instrument.
Mild cognitive impairment:	"…syndrome defined as cognitive decline greater than expected for an individual's age and education level, but that does not interfere notably with activities of daily life."[26]	Same as theoretical definition

Key concept	Theoretical definition	Conceptualised definition
Study foods	No theoretical definition	All the foods supplied to the intervention and control groups during the intervention phase. They included the foods to enhance the usual diet of all the participants as well as the test foods (fish and fish paste) for the intervention group and the control foods (canned meatballs and texturised soya protein) for the control group.
Test foods	No theoretical definition	The canned fish and fish paste supplied to the intervention group during the intervention phase.
Usual diet	An individual's typical/habitual pattern of eating and drinking. This might exert both beneficial and detrimental influences.[27]	Same as theoretical definition

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The aim of this literature review is to provide insight into the relationship between cognition and diet. At first the focus is on general concepts such as defining cognition and establishing its link to nutrition. The review refers broadly to some dietary avenues related to the subject of cognition and diet explored by research. This is followed by a more in depth discussion on the Mediterranean Diet, MIND diet and Omega 3 PUFA.

2.2 Cognition and cognitive decline

Cognition is a summary term which describes an individual's thought processes while interacting with other humans and the environment and includes any and all processes by which this individual becomes aware of his/her situation, needs, goals, and required actions. The individual uses this information to implement problem solving strategies for optimal living. Cognition includes further aspects such as: perceiving, thinking, knowing, reasoning, remembering, analysing, planning, paying attention, generating and synthesising ideas, creating, judging, being aware, and having insight.[20-22]

Neurodegenerative processes can occur in the aging brain due to multiple factors, including oxidative stress and inflammatory processes. These detrimental processes can lead to damage of the cellular structures of the brain and contribute to neurodegenerative diseases.[28] The effect of oxidative stress on the brain is best explained by the free radical theory. All cells in the body (including neuronal cells) are exposed to free radical induced cell damage on the macromolecular level as endogenous antioxidants become less effective to counteract the effects of these unstable molecules.[29,30] The mitochondria are especially prone to oxidative damage as mitochondria consume 90% of intracellular oxygen for the generation of energy. Mitochondria are therefore both producers and targets of reactive oxygen species (ROS). Although mitochondrial damage occurs as a normal result of aging, the extent thereof (for example how much the mitochondrial deoxyribonucleic acid (DNA) is impacted) and the region of the brain affected most may result in a neurodegenerative disorder. Reactive microglia are the other major sources of ROS as they continue to signal ongoing inflammation in the degenerative areas. As is the case with the mitochondria, oxidation occurs when the endogenous antioxidant systems lose their efficiency to counteract oxidative damage. In conclusion it seems as if the functional decrease in the aging brain is caused by accumulated oxidation of mitochondrial DNA, lipids and protein (protein leading to an increase in reactive nitrogen

species (RNS)).[29,30] Long term oxidative exposure also leads to damage of the lipid profile of all cell membranes with a significant decline in the concentration of PUFA.[30]

Aging is, therefore, the primary risk factor for the development of neurodegenerative disease, but neurodegenerative disease is not an inevitable result of aging.[31] A decline in memory and cognitive function is a normal consequence of aging. Memory loss is, however, a health concern. Prevalence estimates in 2010 indicated that 22.2% of Americans (5.4 million) had Mild Cognitive Impairment (MCI), of which 12% would develop into dementia per year.[10] Dementia as an umbrella term to describe a clinically observable progressive cognitive decline characterised by deterioration of mental functioning in its cognitive, emotional and conative aspects.[21,32] The classification of dementia involves the recognition of its presence and the diagnosis of the underlying cause.[32,33] AD is the leading cause of dementia, followed by vascular dementia.[33]

AD is a neurodegenerative disease, which is initially characterised by short-term memory impairment and executive dysfunction. As the disease progresses it may cause deficits throughout the brain leading to total incapacity.[34] AD is a chronic disease and develops over the course of life. It is a disease that develops slowly and causes changes in the brain long before any changes in one's behaviour or memory are detected. In the majority of cases, it manifests and is diagnosed above the age of 65 years. Research has shown that there is no difference in the age of manifestation between the two genders.[35]

The brain destruction in AD is mainly caused by four major processes, namely:

- formation of extracellular amyloid plaques between neurons,
- the aggregation of intracellular neurofibrillary tangles due to excessively phosphorylated tau protein,
- inflammation
- and neurodegeneration or cell death.[36,37]

Theories involving various risk factors for the development of AD have been proposed including culture, diet, lifestyle, socioeconomic status, genetics and head injury. Age and chronic inflammation reactions are the two most widely accepted risk factors.[38] As the development of AD is associated with a genetic-environmental interaction the life-course approach may be applicable to the aetiology of AD. The life-course approach is based

on the identification of “vulnerable periods”.[39] An individual is at greatest risk of damage if exposed to a putative risk factor (alone or in combination with others) during these periods. These periods pertain mostly to the intrauterine phase and early childhood development. Childhood socioeconomic status, mental ability and education, as well as midlife occupation and lifestyle have also been associated with cognitive function in later life. Exposure to risk factors can interrelate – exposure to one can lead to a cascade of subsequent exposures to others.[39]

2.3 Nutrition and cognition

Various avenues regarding nutrition and cognition have been explored. Initially studies, mainly designed as prospective cohorts, were focused on single nutrients and bio-active compounds.[6] Over time, interest in a more natural dietary approach (mimicking real life) has grown. Focus shifted from single nutrients to multi-nutrient and food-based approaches. These types of approaches incorporate the complexity of diet and the possible synergy and interaction between nutrients.[4] See Figure 1. A systematic review of randomised controlled trials by Canevelli et al. in 2016 emphasised the need for randomised, placebo-controlled trials to support the epidemiological evidence that proposes the possibility that cognitive decline can be prevented by diet or individual dietary components. Special attention should be paid to duration of follow-up, as well as the clinical meaningfulness of different neuropsychological scores.[40]

For the purpose of this study, the literature review is focused mainly on the food-based approach and the effect of Omega 3 PUFA within the context of the whole diet. For the sake of comprehensiveness and because these nutrients are of interest in the MIND diet, the B-vitamins and vitamins C and E (as antioxidants) are mentioned as examples of the food-based approach and broadly discussed.

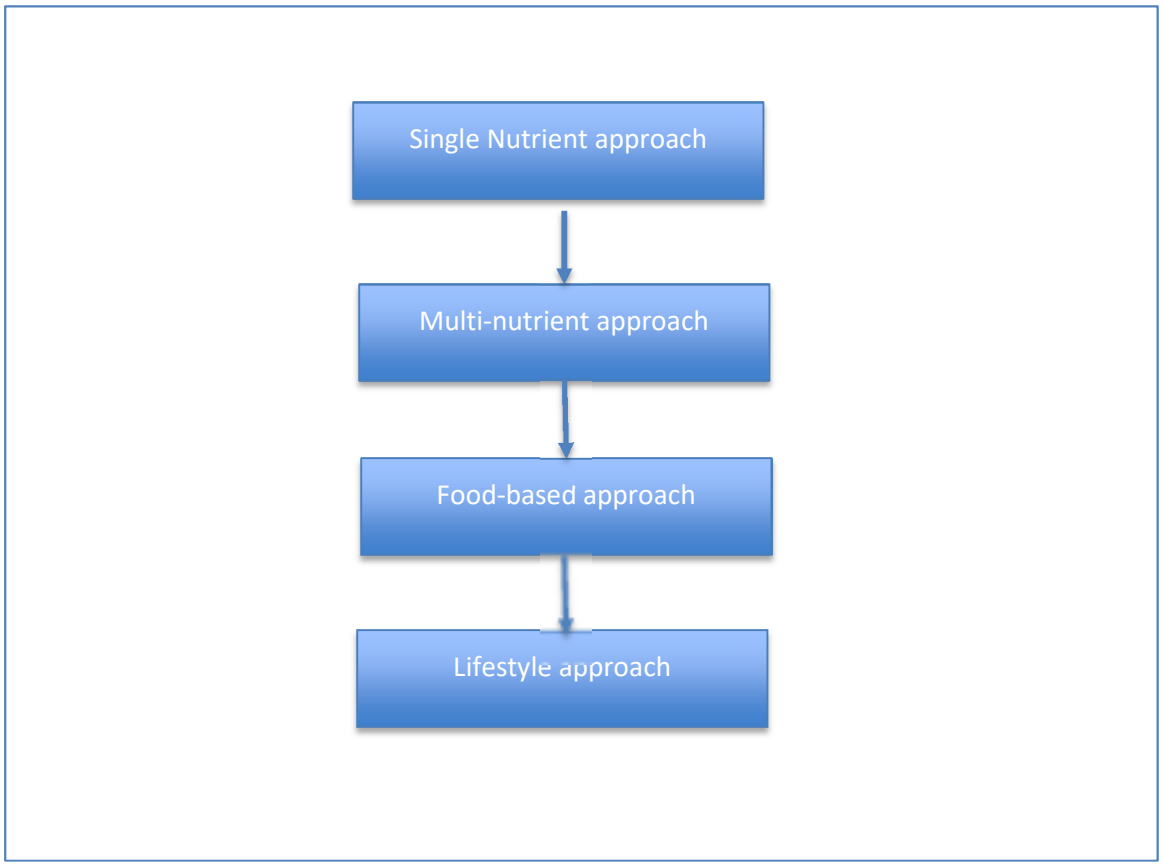

Figure 1: Progressive focus of nutrition-cognition research

2.3.1 Relationship between cardiovascular health, insulin function, inflammation and cognition

There is a direct association between cardio-metabolic disorders such as the metabolic syndrome, impaired glucose tolerance and diabetes, and the risk of cognitive decline, especially of memory, executive functioning, information processing speed, attention and overall intellectual function.[11] The association is worth mentioning because theoretically any dietary intervention aimed at addressing one of the former conditions may potentially impact on cognition.[2]

Insulin, a growth hormone, has a potent effect on the brain. Initially it was erroneously believed that insulin is only found in peripheral blood and not in the brain. Insulin is, however, transported across the blood brain barrier and into the central nervous system (CNS), where it reacts with many receptors on astrocytes and neurons causing synaptogenesis and synaptic remodelling.[41] An acute rise in insulin may be beneficial to cognition, but prolonged peripheral hyperinsulinaemia may lead to a suboptimal uptake by the brain as the insulin receptors at the blood brain barrier downregulate the process.[41]

The metabolic syndrome or insulin resistance syndrome is characterised by peripheral insulin elevations, reduced activity of insulin and reduced levels of insulin in the brain. It is associated with age-related memory impairment and AD.[42] Clinical and epidemiological studies reported an increased risk for the development of AD in Type 2 diabetics or people with hyperinsulinaemia.[43-45] AD and Type 2 diabetes share several molecular processes that underlie the degenerative developments.[41]

Because of the above-mentioned similarities in molecular processes, the issue of blood glucose regulation and support of cognition with the help of low Glycaemic Index foods (GI), has become a subject of interest in the field of nutrition-cognition interaction. Theoretically raising blood glucose levels to a normal range may promote cognitive function because glucose is the brain's main source of energy.[46] Low GI foods may minimise food-induced cognitive decrements as the insulin response is more gradual. So far, research findings are still inconclusive.[46,47]

Obesity defined as a Body Mass Index (BMI) of 30 kilogram per square metre (kg/m^2) or higher, is associated with many medical conditions, particularly cardiovascular disease and Type 2 Diabetes Mellitus (DM).[48] Growing evidence suggests that there is an association between obesity and adverse neurocognitive outcomes.[48] Although the exact mechanism remains unknown, there is a relationship between BMI and cognitive performance. It is possible that obesity may contribute to cognitive decline through metabolic, inflammatory and neuronal pathways.[49] There is no evidence to support an age-related interaction. An elevated BMI is associated with many pathological changes in physiology, which might negatively impact on cognitive function. The relationship works both ways i.e. that persons with impaired executive function are more at risk of obesity. Executive functioning exerts a direct influence on the ability to maintain energy balance, impulse control, self-monitoring and goal directed behaviour.[39] A study by Sriram et al. (2002) concluded that obesity may be viewed as an independent risk factor for AD as it is associated with both temporal lobe atrophy and white matter disease in older adults.[50]

Cholesterol plays an influential role in promoting the production of amyloid beta protein and the possible progression of AD.[51] Evidence reported an association between the pathology of cholesterol metabolism, type 2 (DM), Apo Lipoprotein E (APOE) and the

metabolism amyloid protein precursor. It has been suggested that changes to diet and lifestyle might reduce the risk of AD.[52] A 2006 review by Panza et al. also supported the possible interaction between cholesterol levels and APOE genotype to affect AD progression.[52] It can be concluded from the literature that the risk for cognitive decline can be partially reduced by addressing the metabolic syndrome and its related diseases.

From the above it is clear that neuro-inflammation is implicated in AD. Nutrition can possibly play an important role by modulating the immune system. Several nutrients or bioactive compounds may affect inflammation.[53] Polyphenols, unsaturated fats and antioxidant vitamins are a few noteworthy examples. They are the backbone of the MIND diet (discussed in more detail in paragraph 2.3.3).

2.3.2 Single and multi-nutrient approaches

2.3.2.1 B Vitamins

The B vitamins are a group of eight water-soluble vitamins, which are essential to human health and have closely related roles on the cellular level. They are involved as co-enzymes in many anabolic and catabolic reactions and play a very important role in the physiology of the brain in terms of energy production, DNA/RNA synthesis, methylation and the synthesis of neurochemical and signalling molecules.[54]

As high homocysteine levels are viewed as a risk factor for AD, research on B vitamins and cognition is focused mainly on the vitamins involved in homocysteine metabolism, namely folate or folic acid (the synthetic version), vitamin B12 and vitamin B6.[54]

Aisen et al. (2008) designed a multi-centre randomised double-blind controlled trial to determine the effect of combination supplementation with vitamins B12, B6 and folic acid on the cognition of subjects with mild to moderate AD. Over an eighteen month period, the vitamin supplement regimen was effective in reducing homocysteine levels, but had no effect on change in cognition.[55] The *Supplementation with folate, vitamins B6 and B12 and/or Omega 3 fatty acids* (SU.FOL.OM3) trial, published in 2011, is one of the interventions that assessed the effect of folate and vitamin B6 on cognition of subjects with cardiovascular risk factors.[56] Refer to paragraph 2.4.2 for a more detailed discussion of the trial.

Interestingly, blood levels of the homocysteine-lowering B vitamins together with vitamin D and Omega 3 long chain polyunsaturated fatty acids (LCPUFA) were used as parameters in a blood-based nutritional risk index to explain cognitive trajectories over a three year period in the Multidomain Alzheimer Preventive Trial (MAPT).[14] By using a nutritional index derived from concentrations of specific nutrients or their metabolites in the blood, an objective assessment can be made regarding diet quality and its link to cognitive decline. This approach supports the theory that interaction and synergy occur between different nutrients.[14] Studies on single B vitamins or B complexes (refer to the following paragraphs) have been executed, but results remain controversial.

Vitamin B12 is of special interest when the association between cognition and the B vitamins is assessed and this vitamin is often the subject of reviews.[57,58] In the review by McCaddon (2013) the high prevalence of vitamin B12 deficiencies especially in the elderly is emphasised.[58] Severe vitamin B12 deficiency manifests in a neuropathological syndrome. This syndrome can present with anaemia, polyneuropathy, subacute combined degeneration of the spinal cord and neuropsychiatric problems such as dementia. Subjects with low vitamin B12 levels can also present with poor memory performance.[59] A deficiency is associated with cognitive impairment, dementia, AD, Parkinson's Disease (PD) and multiple sclerosis (MS) (conditions which are all associated with chronic neuro-inflammation and oxidative stress).[58]

Folate, another B vitamin of interest, is found in leafy vegetables, fruits, mushrooms and animal protein. The term folate is used for the natural form, the synthetic version is termed folic acid.[60] Supplementation with folic acid may mask a vitamin B12 deficiency. Morris et al. (2012) analysed the data from the Framingham Heart Study and identified a specific range (187-256.8 picomole per lite (pmol/L)) for plasma vitamin B12 levels to predict cognitive decline. It was also stated that high plasma folate levels or supplemental use of folic acid correlate with vitamin B12 levels being low and within this specific range.[61]

As published in 2014, results from the Women's Health Initiative Memory Study (sample (N) = 7 030) with a follow-up of five years also supported the finding that folate intake below the Recommended Dietary Allowance (RDA) may increase risk for MCI or probable dementia in later life, but levels exceeding the RDA for folate are not recommended.[62] This observational study and as well as another in 2016 by Horvart (N =4 166), concluded

that there is no unequivocal support for the importance of vitamins B12 and folate in cognitive function.[63] Research on single B vitamins may be limited in scope as the focus should rather be on the whole group of B vitamins which complement each other and are essential for optimal physiological and neurological functioning.[54]

A systematic review in 2013 by Health Quality Ontario concluded that there is:

- Low quality evidence to support the association between elevated plasma homocysteine (as by-product of B vitamins) and onset of dementia.
- Moderate quality evidence (suboptimal duration of follow-up): treatment with vitamin B12 supplementation does not significantly change cognitive function.
- Low to moderate quality of evidence: treatment with vitamin B12 and folate in patients with MCI may slow the rate of brain atrophy.[57]

A 2015 systematic review and meta-analysis of cohort studies by Cao et al. supported the protective effect of vitamin B intake associated with risk of dementia.[64] This review was contradicted by a 2020 review of randomised controlled trials which found no supportive evidence for the use of oral vitamin B supplementation in the preventative strategy against cognitive decline.[65]

2.3.2.2 Antioxidants

As oxidative stress is a contributor to cognitive decline and the development of dementia, it is critical to study dietary antioxidants as a preventative measure.[66]

Oxidative stress can be defined as “a highly oxidized environment within cells that forces these cells into a highly activated state due to loss of control of their regulatory systems.”[67] In other words, oxidative stress pertains to the imbalance between production and detoxification of ROS and RNS.[68] Key features of AD such as metabolic, mitochondrial, and cell cycle abnormalities are associated with oxidative stress.[67] Oxidative stress causes mitochondrial decay which in turn contributes to neurodegenerative disease. Refer to paragraph 2.2. One type of mitochondrial decay is the oxidative damage to main mitochondrial enzymes. As a result these enzymes do not function properly because no proper binding to substrates can take place.[69] Evidence suggests that the oxidative damage of beta-amyloid peptide is hydrogen peroxide mediated.[70] Increasing evidence indicates that beta-amyloid and tau aggregation are a compensatory response to

underlying oxidative stress. Therefore, removal of the proteinaceous accumulations may worsen the condition by increasing oxidative damage. Because of the former changes, the inflammatory cascade is initiated.[36] All neurons do not respond to this stress in the same way. Some neurons are selectively more vulnerable and may first exhibit decline in function or apoptosis.[68]

Antioxidants can play a protective role in AD by preventing oxidative stress that causes neuronal damage.[71] An antioxidant is a substance that protects other substances against oxidation by being oxidized itself.[72] Various vitamins, minerals and bioactive compounds can act as antioxidants. Because the focus of this literature review is not on antioxidants as such, only vitamins E and C, and polyphenols will be briefly discussed. These three antioxidants occur abundantly in the MIND diet.[15]

Vitamin E is a fat-soluble vitamin, which exists in different forms. These forms differ in terms of biological activity. Alpha-tocopherol, a powerful biological antioxidant, is the most active form found in the body.[72] It is the major lipophilic antioxidant in the brain and it has been suggested that it delays the development of AD.[73] A 2004 review of the use of tocopherol in the prevention and treatment of AD and other neurodegenerative disease, found that the dietary and supplemental forms may differ in efficacy, but that there was no conclusive evidence to support prescription of tocopherol for the prevention or treatment of AD.[74] In 2000, the Cochrane Collaboration stated conclusively that there is not sufficient evidence to support the efficacy of vitamin E in preventing or treating AD or MCI.[75] Almost twenty years later, this point of view is still supported by an updated Cochrane review and another review published by Browne et al. as the evidence about the use of Vitamin E as a preventative strategy is inconclusive. Theoretically the argument in favour of Vitamin E has merit and discrepancies in research may be caused by different factors ranging from different methodologies used to determine effectiveness to individual genetic predisposition in responsiveness to supplementation.[76,77]

Similarly, evidence suggests that vitamin C exerts a protective effect on the development of AD because of its anti-oxidant properties. The evidence is, however, insufficient and public recommendations cannot be made.[78] In a cross-sectional study published in 2019 by Travica et al., plasma levels of vitamin C were significantly associated with specific

cognitive functions but supplementation above adequate intake through food did not seem to affect cognition.[79]

Fruit and vegetables contain powerful anti-oxidants named polyphenols. High concentrations of polyphenols (in fruit and vegetable juice) possess stronger neuroprotection against hydrogen peroxide than anti-oxidant vitamins. Intake of fruit and vegetable juice with high polyphenol content may play an important role in delaying the onset of AD.[70]

A systematic review by Crichton et al. in 2013 considered the epidemiological and longitudinal evidence of the association between the habitual intake of antioxidants (vitamins C and E, flavonoids and carotenoids) and cognitive function and/or dementia. The findings did not consistently support an association between habitual antioxidant intake and better cognition or reduced risk for dementia.[80] Another 2016 review and meta-analysis of cohort studies supported the view that more studies are needed to support the protective effect of antioxidants (vitamins C and E and flavonoids) against dementia.[64]

It is evident from the literature that antioxidants may have a protective effect against cognitive decline, but that more research is essential to clarify recommendations on whether they should be food or supplement based, what the effective dosage would be and in which combination they should be used. As with vitamins E and C, randomised controlled trials are warranted to obtain conclusive evidence.[81]

Novel antioxidative supplements (combining different antioxidants with other nutrients to specifically target one pathology path of AD), are one of the current avenues that are being explored for use as preventative and treatment options in AD. Promising results published in a 2020 review support more research on the matter.[82]

2.3.3 Food-based (whole diet) and multi-nutrient approaches

Whole diet or multi- nutrient approaches may prove to have more robust effects on cognition due to the synergy between nutrients.[14]

The *Prevention co Dieta Mediterranea* (PREDIMED) trial focused on the relationship between diet, vascular health and cognition. It provides evidence that the Mediterranean dietary pattern may reduce the risk of dementia.[83] The Mediterranean diet has been identified as the diet of choice for cognitive support,[4,64] as well as the reduction of cardiovascular disease, as it is hypothesised that the Mediterranean diet exerts a protective effect through its antioxidant and anti-inflammatory properties.[84]

A meta-analysis of nine prospective cohort studies (N=34 136) indicated a 21% lower risk of developing cognitive disorders for those who primarily complied with the Mediterranean Diet for up to twelve years of follow-up.[84] The Mediterranean diet describes the traditional eating habits of people in Crete, Southern Italy and surrounding Mediterranean countries and is predominantly plant-based. It is characterised by a high consumption of fruits, vegetables, legumes and cereals, a moderate consumption of fish and wine and a low consumption of meat and dairy products (i.e. sources of saturated fat).[53]

The most unique property of the Mediterranean diet is probably the large contribution that olives and olive oil make to the daily energy intake.[85] Olives and olive oil are high in monounsaturated fat, and so are nuts. A large intervention study (N = 522) by Martinez-Lapiscina et al. (2013) found significant improvements in cognitive performance in elderly subjects with a high vascular risk when they consumed a Mediterranean diet containing 1L of extra virgin olive oil per week or a Mediterranean diet containing 30g of nuts per day. The duration of the nutritional intervention was 6.5 years. The control group who consumed a general, healthy diet low in fat, did not display any significant improvements in cognition.[83] Another positive property of olives is their high concentration of biophenols which serve as potent antioxidants.[28]

The Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet is a combination of the Mediterranean diet and the Dietary Approach to Systolic Hypertension (DASH) diet to which specific brain supportive foods have been added.[15] Both the Mediterranean and DASH diets protect against cardiovascular factors that can adversely affect brain health, but they lack dietary components that specifically support brain health. The MIND diet emphasises dietary components and number of servings or meals linked to neuroprotection and dementia prevention.[15] Evidence from epidemiological studies

and animal models was used for the development of this unique approach. It differs from the original Mediterranean diet in the following ways: green leafy vegetables are in a category of their own; except for berries, fruit is not included; fish intake is measured on a weekly (not a daily) scale, because it appears likely that fish consumption two to three times a week might be neuroprotective.[84] In addition, the foods that may possibly be detrimental to cognition, such as snacks, processed dairy products, soft drinks and processed meat are not incorporated into the Mediterranean diet scoring system. The MIND diet accommodates these in its scoring system by monitoring consumption of pastries and sweets, butter and margarine, as well as fast fried foods.[84] It is important to include these foods as dietary patterns associated with AD tend to contain high intakes of meat, butter, full fat dairy products and refined sugar.[28,86] Animal studies have indicated increased cerebral oxidative stress and metabolic disturbances in amyloid precursor protein after administration of a typical Western diet for four weeks.[28] A comparison of the DASH-[87], Mediterranean diet[88] and the MIND[15] diet is presented below in table format.

Table 3: Comparison of the DASH-[87], Mediterranean-[88] and MIND[15] diet servings and scoring

DASH[87]		**Mediterranean Diet**[88]		**MIND**[15]	
DASH components	Max score	Mediterranean diet components	Max score	MIND components	Max score
Total grains >=7/d	1	Non-refined Grains >4/d	5	Whole Grains >=3/d	1
Vegetables >=4/d	1	Vegetables >4/d	5	Green Leafy >=6/wk	1
		Potatoes >2/d	5	Other Vegetables >=1/d	1
Fruits >=4/d	1	Fruits >3/d	5	Berries >=2/wk	1
Dairy >=2/d	1	Full-fat Dairy =<10/wk	5		
Meat, poultry and fish =<2/d	1	Red meat =<1/wk	5	Red Meats and products <4/wk	1
		Fish >6/wk	5	Fish >=1/wk	1
		Poultry =<3/wk	5	Poultry >=2/wk	1

DASH[87]		**Mediterranean Diet**[88]		**MIND**[15]	
Nuts, seeds & legumes >=4/wk	1	Legumes, nuts & beans >6/wk	5	Beans >3/wk	1
				Nuts >5/wk	1
				Fast/fried food <1/wk	1
Total fat =<27% of Total Energy	1				
Saturated fat =<6% of Total Energy	1				
		Olive oil >=1/d	5	Olive oil primary oil	1
				Butter, margarine <1 T/d	1
				Cheese <1/wk	1
Sweets =<5/wk	1			Pastries, sweets <5/wk	1
Sodium =<2400 mg/d	1				
		Alcohol <300 mL/d but >0	5	Alcohol/wine 1/d	1
Total DASH Score	10	Total Mediterranean Diet Score	55	Total MIND Score	15

Abbreviations: d – day, DASH-Dietary Approach to Systolic Hypertension, max – maximum, mg – milligrams, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay, T – tablespoon, wk – week

Table 3 also reflects the scoring of the three diets. Fish intake plays a prominent role in all three of the above-mentioned diets. Although the use of all three diets for the prevention of cognitive decline is supported by epidemiological evidence, their relevance in cognition and brain health still needs to be supported by well-designed intervention studies. The MIND diet in particular attracts interest, as modest adherence may play a substantial role in the prevention of AD whereas only the highest adherence concordance to the DASH and Mediterranean diets was associated with AD prevention.[15]

Although promising, the literature needs to be seen in context. It appeared as if the protective effect of the MIND diet had not been tested or compared with the Mediterranean Diet outside the United Stated of America (USA) before 2019, when an

Australian longitudinal cohort study compared the effects of the MIND and Mediterranean diets.[84] The researchers concluded that the cognitively protective effect of the MIND diet can be generalised to populations in Australia. This is relevant because the effect the diet has on cognition is also influenced by between country variation in food supply and other dietary factors. Studies of the MIND diet in other populations and geographic locations (especially in developing countries) are required to evaluate its protective effects.[84]

2.3.4 Multi-domain interventions

Although multi-domain interventions are not the focus of this study, information in this regard is included in the review for the sake of comprehensiveness and to provide insight into the specific dietary components that were studied. In a multi-domain intervention, various domains such as diet, physical activity and psychological wellbeing are targeted at the same time. The interventions could be effective in strategies to prevent dementia. However, they may be burdensome and not universally acceptable to researchers and participants.[89]

Two of the best known interventions are probably the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) and the MAPT trials. In the FINGER trial, a 2 year multi-domain intervention of diet, cognitive training and exercise, the nutrition intervention focused on two recommendations to achieve a dietary intake of 2.5 – 3g of PUFA daily: the use of vegetable/rapeseed oil instead of butter and consumption of at least two portions of fish per week. Cognitive function increased with elevated fish consumption.[90] The results of the trial indicate that multi-domain interventions can maintain or improve cognition.[90]

The MAPT trial was a 36 months, multicentre, randomised, placebo controlled trial with a four arm design. The sample consisted of community dwelling older adults (70 years or older) at risk of dementia. Subjective memory complaint was one of the inclusion criteria. The primary outcome was a change in baseline composite cognitive score after the intervention period. The first group received Omega 3 LCPUFA supplementation, the second group received a multi-domain intervention (including nutritional and exercise counselling, as well as cognitive training), group three received both the Omega 3 LCPUFA and the multi-domain intervention and group four was the placebo group.[91]

Results showed no significant difference over a 3-year period in cognitive decline between any of the intervention and control groups.[92]

2.3.5 Epigenetics

Genetic predisposition is a risk factor for cognitive decline and the development of dementia. Epigenetics is a relatively new concept, which will be explored to see whether it can also be used as a preventative measure to combat cognitive decline. It can be defined as: "heritable changes in gene expression that are, unlike mutations, not attributable to alterations in sequence of DNA."[93] Research supports an interplay between epigenetic patterns and environmental factors, including diet. Diet induces epigenetic alterations, which may have profound effects on risk of health and disease.[94] Epigenetics is a field of research, which yields promising results in regard to combatting cognitive decline and further exploration and research are a priority, but as it is not one of the focus points of this study it will not be discussed.

2.4 Omega 3 Polyunsaturated fatty acids (PUFA) and cognition

The relationship between dietary fat intake and brain health is a subject of great interest for researchers and very relevant in both HIC and LMIC. In a 2014 systematic review of several prospective studies a relationship between intakes of saturated and trans fat and cognitive decline was identified.[27] A 2016 meta-analysis of cohort studies focused on dietary patterns and risk of dementia. The results supported the protective effect of both MUFA and PUFA in cognitive decline.[64] For the purpose of this review the focus will be on the intake of Omega 3 PUFA only.

Omega 3 PUFA is the compound name used for a family of PUFA characterised by the last double bond between the third and fourth carbon. The LCPUFA Omega 3 fatty acids which are essential to the body and which need to be supplied by the diet as synthesis is not efficient in humans are Eicosapentaenoic Acid (EPA), Docosapentaenoic Acid (DPA) and Docosahexaenoic Acid (DHA).[95]

As the human brain is predominantly composed of lipids (especially DHA),[96] the relation between cognition and Omega 3 PUFA became a research focus point. The role of Omega 3 PUFA in cognition can be explained by different mechanisms, many of which address several key metabolic risk factors for cardiovascular disease e.g. lowering blood

pressure and triglycerides, reducing inflammatory markers, improving glucose metabolism and improving insulin sensitivity.[13,47] Omega 3 PUFA also exert an influence on the anti-apoptotic pathways.[47] Omega 3 PUFA's effect on gut microbiota may also be neuroprotective . A possible risk factor that contributes to neurodegenerative disease is described in the gut-brain axis hypothesis.[97] Prolonged stress, an unbalanced diet and the use of medication may lead to altered microbiota (dysbiosis) in the gut, which in turn may result in increased intestinal permeability and a leaky gut.[53] This is relevant because clinical evidence suggests that the development of many neurodegenerative diseases may be related to gut microbiota, which modulate the activity of the central nervous system.[98] A study by Watson et al. (2017) found a significant positive change in microbiota after eight weeks of supplementation with a 4g EPA and DHA combination.[98]

The same positive results were obtained in another small trial, the Canola Oil Multi-Centre intervention trial (N=25) where participants with at least one metabolic risk factor were exposed to one of five different unsaturated oil blends. Both the conventional canola oil and the DHA enriched high oleic canola oil (3.5g DHA) showed an increase in so-called beneficial bacteria.[99]

As mentioned before, DHA is the predominant Omega 3 LCPUFA found in the brain. 20% of the brain's dry weight consists of Omega 3 LCPUFA of which DHA comprises 90%.[100,101] It affects neurological function as it is a key component in the anatomical and physiological structure of the brain. Not only does it support the membrane integrity of the neurons, it also influences neurotransmission by playing a role in the functioning of membrane receptors and synaptic plasticity. DHA can modify the production of neurotransmitters and brain peptides.[101] DHA is well known for its anti-inflammatory properties and exerts significant protection against neuro-inflammation.[100] DHA accumulates in the brain at different rates over the lifespan, the most rapid stages being gestation and early infancy.[101] DHA deficiencies may increase with normal aging.[102]

A randomised, double-blind, placebo controlled intervention study (N=485), published in 2010, assessed the effects of DHA administration on cognition in healthy older adults with age related cognitive decline (ARCD). Their usual diet was supplemented with 900mg of oral DHA daily for 24 weeks or they received a matching placebo. The researchers found

an improvement in learning and memory, which support the use of DHA as a beneficial supplement to support cognitive health with aging.[10]

The research on the use of Omega 3 LCPUFA (combination of EPA and DHA) or EPA and DHA, respectively, as support for cognition, is inconclusive in terms of combination and dosage. To date, no specific dose of EPA or DHA that will support cognition has been specified although DHA plays a prominent role in the anatomy and the physiology of the brain. EPA also has strong anti-inflammatory properties and is ultimately converted to DHA.[103] Because of this interaction, intervention studies usually focus on a combination of EPA and DHA instead of only one of these compounds. The anti-inflammatory effect of EPA occurs with intakes of between 1.35 – 2.7g/day and that of total Omega 3 PUFA with intakes of 2g/day.[95]

The research about the effect of Omega 3 PUFA on cognition is controversial. This is due to many reasons including sample size, duration of intervention[104] and type of measuring instrument used to determine the change in cognition. The *Older people and omega 3 long chain polyunsaturated fatty acids* (OPAL) study is one of the studies that could not reject the null hypothesis of no effect of fish oil supplementation on cognitive function in cognitively healthy older people. The baseline data supported the hypothesis that higher fish consumption is associated with better cognitive function in later life. However, after a two year intervention where 867 cognitively healthy subjects received either Omega 3 LCPUFA (200mg EPA 500mg DHA) or olive oil capsules, no significant difference between the intervention or control groups was detected.[9]

A cross-sectional study by Phillips et al. (2012), assessed the Omega 3 PUFA intake and status (blood serum levels) in cognitively healthy older adults and older adults living with either MCI or AD. This comparison found Omega 3 PUFA intake, plasma DHA and plasma EPA levels all positive predictors of memory functioning.[105] "Omega 3 fatty acids are also regarded as capable of reducing the risk of dementia and cognitive decline."[105] This statement is supported by a 2012 intervention study, which found significant improvements in working memory when healthy older subjects received a supplement containing 3g of Omega 3 PUFA on a daily base for five weeks.[11] The *Older people, Omega 3 and cognitive health* (EPOCH) trial, a randomised, double-blind controlled trial studied the effect of Omega 3 LCPUFA (600mg EPA and 1720mg DHA) on cognition of

a group of elders (N=391) from Adelaide.[106] The intervention group daily received an Omega 3 LCPUFA capsule for 18 months. The control group received a capsule containing olive oil instead. It was found that daily supplementation of DHA-rich fish oil did not improve or maintain cognitive function.[107]

A randomised controlled trial by Konagai et al. (2013) compared the effects of krill oil, sardine oil and medium chain triglyceride (MCT) oil on the cerebral cortex. Both the krill and sardine oil which are good sources of Omega 3 LCPUFA activated the function of the cerebral cortex.[108] A trial by Van de Rest where the two intervention groups received either 1800mg of a EPA and DHA combination or 400mg of a EPA and DHA combination showed no effect on cognition when compared to the control group who received high oleic sunflower oil.[109] Jaremka et al. (2014) used a secondary analysis of the data from a parent trial (that assessed the anti-inflammatory properties of Omega 3 PUFA supplementation) to examine the difference in effect between different dosages of Omega 3 PUFA supplementation (1.25g and 2.5g) daily on cognition and loneliness. The only cognitive test that showed a significant difference between the control and intervention groups, was the verbal episodic memory score test. The control group had a poorer score after supplementation compared to both intervention groups.[13]

The question also arose whether the effect of Omega 3 LCPUFA supplementation on cognition is related to the current Omega 3 LCPUFA status of the participant. A study by Hooper et al. (2017) explored the effect of daily Omega 3 LCPUFA supplementation (800mg DHA, 225mg EPA) on the cognition of individuals with subjective memory complaints and a low Omega 3 Index. (The Omega 3 Index is calculated by adding up the percentages of DHA and EPA, and expressing the sum as a percentage of total RBC fatty acids. A low Omega 3 Index in this particular study was defined as having a percentage in the lowest quartile of all the participants, below or equal to 4.83%). The results of the study supported a possible beneficial effect of 36 months of Omega 3 PUFA supplementation on executive functioning at risk for dementia with a low Omega 3 Index.[91]

The Omega 3 Index was also studied in the study by Van der Wurff et al. (2019) where Dutch adolescents received krill oil supplements for a year to determine its effect on depression and self-esteem. Adolescents with a lower Omega 3 Index (the cut-off in this

particular study was less than 5%) were allowed to participate. Although no significant relationships between the supplementation, the Omega 3 Index or the outcomes were found, this type of investigation is worth considering for future studies.[110]

Similarly it can be argued that the Omega 6/Omega 3 ratio can also impact on cognition. The typical diet in a HIC has a high content of Omega 6 PUFA which is regarded as having an adverse effect on cognition by depleting DHA in the brain. A lower ratio has been shown to predict better cognitive function.[111] This is a very relevant point, but it will not be pursued further in this literature review and trial.

2.4.1 Omega 3 PUFA: supplement or fish?

As the majority of studies were making use of dietary supplements to ensure the desired level of intake, more studies are needed on the effect that Omega 3 fatty acids obtained through fish intake will have on cognition.[64] The amount of fish per week people eat, is questionable. The researchers involved with developing the MIND diet claim that consumption of one fish meal per week is adequate to lower the risk of dementia, whereas the Mediterranean and DASH diets support a higher number of portions per week.[15] Three large observational studies regarding cognition and fish intake showed promising results:

The results of a large prospective cohort (N=3718) of subjects from the Chicago Health and Aging project supported the potential benefit of fish consumption in reducing cognitive decline in the elderly. The findings indicated a reduction in decline by 10 – 30% per year when one or more fish meals per week were consumed.[112] The study focused on consumption of fish and not on Omega 3 fatty acid in isolation. There was no consistent association with Omega 3 fatty acids, but effect estimates tended in the direction of slower decline.[112] The Hordaland Health Study (N=2031), which examined the cross-sectional relationship between dietary intake of fish and cognitive performance, found that consumers of fish or fish products present with better cognitive function than non-consumers, but that the effect is dependent on dose and type of fish or fish product.[113] The subjects with a mean daily intake of fish and fish products above 10g/day had significantly better test scores and better cognitive performance compared to those with an intake of less than 10g/day – the maximum effect was observed at 75g/day. Although sea food in general exerted a positive effect on cognition, most of the cognitive functions

were influenced by fish intake. The effect was more pronounced with non-processed lean and fatty fish.[113] As discussed elsewhere, baseline data from the OPAL study supported the hypothesis that higher fish consumption is associated with better cognitive function in later life, although factors such as socioeconomic status or health behaviour may have acted as confounders. The need for randomised controlled trials to elaborate on the role of Omega 3 fatty acid in cognition in later life of a healthy population, were emphasised.[114]

A randomised controlled trial in preschool children in Germany (N=205), showed promising results when their usual diets were supplemented with Atlantic salmon three times per week for sixteen weeks. Not only did those receiving fish improve in some aspects of the neurocognitive battery, the EPA and DHA in their phospholipids and glycerol increased.[115] Similarly a trial in Norway (N=218) investigated the effect of an increased intake of EPA- and DHA-rich mackerel and herring on the cognition of four- to six-year-olds. Both the intervention and control group received three hot lunches per week. The lunches differed in protein source, the intervention group received 50-80g of fish while the control group received meat. The intervention group presented with a significant increase of RBC Omega 3 LCPUFA and when adjusted for dietary compliance, a higher score in some cognitive domains.[116]

The well-known Doetinchem Cohort (N = 2612) study, assessed diet (with the focus on fish intake) and cognition at baseline and at a five year follow-up. No consistent association between fatty fish consumption and cognitive decline was observed. However, higher n-3 PUFA (especially alpha-linolenic acid (ALA)), was associated with slower cognitive decline.[117]

2.4.2 Omega 3 PUFA in combination with other nutrients

Strike et al. (2016) executed a pilot study (N = 27) where they studied the effect of Omega 3 LCPUFA (160 mg EPA, 1000mg DHA) in combination with other nutrients and bioactive compounds on the cognition of postmenopausal women. Ginkgo Biloba, phosphatidylserine, tocopherol, folic acid and vitamin B12 were included in the supplement. The researchers made use of a computerised battery of tests to measure cognitive function and found a significant effect on the following cognitive functions: motor screening, memory, mobility and habitual walking speed. Significant differences in two of four cognitive tests between the intervention and control group were detected.[12]

The SU.FOL.OM3 trial (N=1748) assessed the effect of Omega 3 supplementation (EPA:DHA 2:1) in combination with B vitamins on the cognition of French adults with a history of cardiovascular disease. They found no significant difference between the intervention and placebo groups when cognitive function was compared. There was evidence supporting disease- and age-specific cognitive effects. Although the trial used a large sample and had a long duration of intervention (four years), the measurement of cognitive status took place via a structured phone interview.[56] Omega 3 in combination with alpha linolenic acid (ALA) has also been studied, but the target population consisted of subjects who already had probable dementia as screened by the Mini Mental State Examination and Clinical Dementia Rating[118] and for that reason a more comprehensive discussion on the study is not included in this review.

During the SU.VI.MAX (Supplementation with Antioxidant Vitamins and Minerals) trial, repeated 24hr recalls were used to determine fatty acid intake. The results of this study pointed towards the possibility that Omega 3 LCPUFA might be more beneficial for cognition in individuals with an adequate antioxidant status. It once again emphasised the synergy between nutrients.[119]

A study by Barberger – Gateau (2011) assessed whether the protective effect of the Mediterranean diet in relation to cognitive decline might be mediated by Omega 3 PUFA. The data of the French Three City study (N = 1050), was analysed and it was concluded that higher Omega 3 levels in the blood might partly enhance the protective effect that the Mediterranean diet exerts against cognitive decline.[120]

2.4.3 Dietary intake of MUFA and Omega 3 PUFA in the South African context

Dietary sources of unsaturated fatty acids (MUFA and PUFA) are found abundantly in South Africa, but are not necessarily included in the diet consumed by the whole population due to high cost. MUFA sources, in particular, can be expensive as they are mainly provided by olives, olive oil, nuts and avocado pear. More affordable sources would be canola oil, peanuts and peanut butter. The main affordable food sources of omega 3 PUFA available in South Africa are canned fish (such as pilchards or sardines) or fish paste – a spread developed by mincing fish and adding water. Omega 3 PUFA content of canned pilchards and fish spread may range between 1400mg/100g and

3300mg/100g. [121,122] Other sources include the more expensive oily fish such as salmon and mackerel. Processed fish products, for example fish fingers or fish cakes, also contain Omega 3 PUFA. Plant sources include canola- or flaxseed oil.

2.4.4 Cost of a brain supportive diet

Cost is a determining factor when assessing the feasibility and success of any diet. Research may support the use of certain foods for their medicinal or health properties, but if they are not affordable for the individual, the chances are good that they will not be integrated in the person's daily diet. The same applies for the citizens of a specific country, the recommended foods need to be easily available in that country and be offered at a reasonable price to its citizens. A systematic review completed by Saulle et al. in 2013, focused on the cost and cost- effectiveness of the Mediterranean diet and concluded that the possibility exists that people are moving away from this dietary approach because of cost implications.[123] Other barriers to changing one's usual diet successfully to the Mediterranean approach may include: cultural beliefs, palatability, food access, time for food shopping and preparation and environment.[124] It should be noted that the studies included in this review were mainly conducted HIC, raising the concern that the situation may even be worse in resource restricted countries. The motivation behind this study is to assess the effect of an affordable, enhanced usual diet on the cognition of elderly people living South Africa, identified components from the MIND diet (derived from the Mediterranean diet) will be substituted with more affordable options of South African foods as reflected in Table 4.

Table 4: Comparison of cost and nutritional composition between specific expensive MIND diet food components and affordable South African options

Specific expensive MIND diet food components available in South Africa			**Affordable alternative options available in South Africa**		
Component	Analysis[a]	Cost[b,c]	Component	Analysis	Cost
Olive oil	MUFA: 70g/100g - 73.7g/100g	R60.00 ($3.5) per 750ml	**Canola oil** as source of MUFA and Omega 3 PUFA	MUFA: 50g/100g - 58.9g/100g Omega 3 PUFA: 9062mg/100g	R30.00 ($1.8) per750mL
Nuts	MUFA: 23g/100g- 33g/100g	Ranges between R40 ($2.4) to R50 ($3) per 100g.	**Peanut butter** as source of MUFA	MUFA: 20.42g/100g	R7.00 (less than $1) per 100g

Specific expensive MIND diet food components available in South Africa			Affordable alternative options available in South Africa		
Component	Analysis[a]	Cost[b,c]	Component	Analysis	Cost
Fish (not specified)	***Frozen hake*** Omega 3 PUFA: 295mg/100g	R10.75 per 100g (Less than $1 per 100g)	**Fish rich in Omega 3** PUFA and preserved (do not need to be refrigerated until opened)	***Canned pilchards*** Omega 3 PUFA: 1 625mg/100g MUFA: 2g/100g	R5 per 100g (Less than $1 per 100g)
Fish (not specified) continues	***Canned tuna*** Omega3 PUFA: 390mg/100g-496mg/100g	R11.75 per 100g (Less than $1 per 100g)	Fish rich in Omega 3 continues	***Fish spread*** Omega 3 PUFA: 3 280mg/100g MUFA: 2.6g/100g	R17.65 per 100g ($1 per 100g)
	Fresh salmon Omega 3 PUFA: 1520mg/100g ***Canned salmon*** Omega 3 PUFA: 3 135mg/100g	R50.00 per 100g ($3 per 100g)			
	Fresh mackerel Omega 3 PUFA: 9610mg/100g ***Canned mackerel*** Omega 3 PUFA: 2 000mg/100g	R35.00 per 100g ($2 per 100g) R25.00 per 100g (Less than $2 per 100g)			
Legumes (not specified)	***Canned beans*** Fibre: 8.1g/100g Protein: 6.4g/100g	R11 per can (Less than $1 per 100g)	Canned beans as source of fibre and protein.	***Canned beans*** Fibre: 8.1g/100g Protein: 6.4g/100g	R11 per can (Less than $1 per 100g)
The nutritional values displayed in this table are based on the nutrition composition tables as they appeared on the labels of the products (2017). Costs and exchange rate (US $1 = R15.69) as available in October 2020.					

Abbreviations: MUFA – Monounsaturated Fatty Acid, PUFA – Polyunsaturated Fatty Acid

2.5 Measuring cognition and determining the relationship with diet

Scientific examination of the relationship between nutrition and cognition is challenging and identification of the most appropriate cognitive test (assessing specific behavioural tasks) is essential. For these results to be accurate, it is crucial that the correct behavioural tasks are chosen to detect the effect of food constituents.[125] A complete assessment by a neuropsychological battery of tests is superior to the use of screening tools, but in most research studies the latter are used due to financial constraints and the large amount of time required for individual assessments.[126] Considering the most applicable screening tool for a healthy study population is challenging in two ways: the tool should be suitable to detect change in people whose cognitive function is not yet on the level of impairment and it should be suited for the particular level of education and literacy of the subjects.[127] Other factors to consider, include: length and ease of administration, burden on the participants and generalisability to the broad population.[126] A review by Paddick et al. (2017) found the Cognitive Abilities Screening Instrument (CASI) and the Mini Mental State Examination (MMSE), the instruments that had more than one good quality when used to study an illiterate/low educational level study population.[127] Both of these tools can be used in populations with normal cognitive function. The CASI was developed for quantitative research purposes.[16] Pilot studies conducted in Japan and America support the cross-cultural applicability, usefulness in dementia screening, monitoring of disease progression, and profiling of cognitive impairment.[16] The CASI produces scores out of 100 and creates a profile of nine neurocognitive domains: An MMSE score can be extracted from the CASI and is used as an inclusion/exclusion criterion in studies to create homogeneity in the study population.[16,64]

Level of functioning is related to cognition and the more cognitive function declines, the more functions are inhibited.[128] Level of functioning can be assessed as an additional measurement to monitor cognition[25] and usually pertains to activities of daily living. Depending on the level of impairment, instruments can either assess basic activities of daily living (BALD) such as bathing, dressing, etc. or instrumental activities of daily living (IADL) for example shopping, driving and managing own finances.[128]

2.6 Conclusion

Dementia is a global problem and diet as cost effective strategy in the prevention thereof needs to be researched. As nutrients work together in synergy and because of the practicality thereof, the food-based approach should be the focus of more research studies. The Mediterranean diet may currently be the diet of choice for cognitive support, but the feasibility thereof in LMIC still needs to be researched with additional randomised controlled trials. The MIND diet (which was developed by combining the Mediterranean and DASH diets) may be a practical tool to assess and guide intake once it has been adjusted for resource-restricted study populations. Fish as an important component of both the Mediterranean and MIND diets may be a good source of Omega 3 LCPUFA which has shown great potential in cohort studies and randomised controlled trials as a protective factor against cognitive decline. Canned fish, an affordable source of Omega 3 LCPUFA in South African may be an effective cognitive supportive food if consumed within the context of the modified MIND diet.

CHAPTER 3
METHODOLOGY

3.1 Introduction

This chapter aims to clarify the methods essential for the execution of this randomised controlled trial. It is important to note that there is reference to an intervention group (definition: the group who received fish and fish spread in addition to their enhanced diet) and an intervention phase (definition: the twelve weeks during which all study participants received study foods to enhance their usual diet). The study consisted of two phases (no-intervention and intervention phase), divided by three different assessments in time (Baseline 1 assessment (BL1), Baseline 2 assessment (BL2) and Post Intervention (PI) assessment) and two groups (an intervention and a control group).

3.2 Study design

The study was designed as a randomised controlled trial with two groups, an intervention and a control group. Overall the duration of the study was 30 weeks (April – November 2018): 12 weeks without any intervention (no food was supplied, participants continued with their usual diet), 12 weeks with an intervention (supplemental foods were handed out on a weekly base) and 8 weeks for baseline assessments, the post intervention assessment and communication to participants about logistics. Two baseline assessments were executed, one (BL1) before the no-intervention period and another one (BL2) before the three month intervention period. A PI assessment was done after the second three month period. See Figure 2. The motivation behind the no-intervention phase was to determine the cognitive change that might happen over 12 weeks in the particular study population if there is no intervention of any kind.

The following diagram provides an overview of the timeline and design of the study.

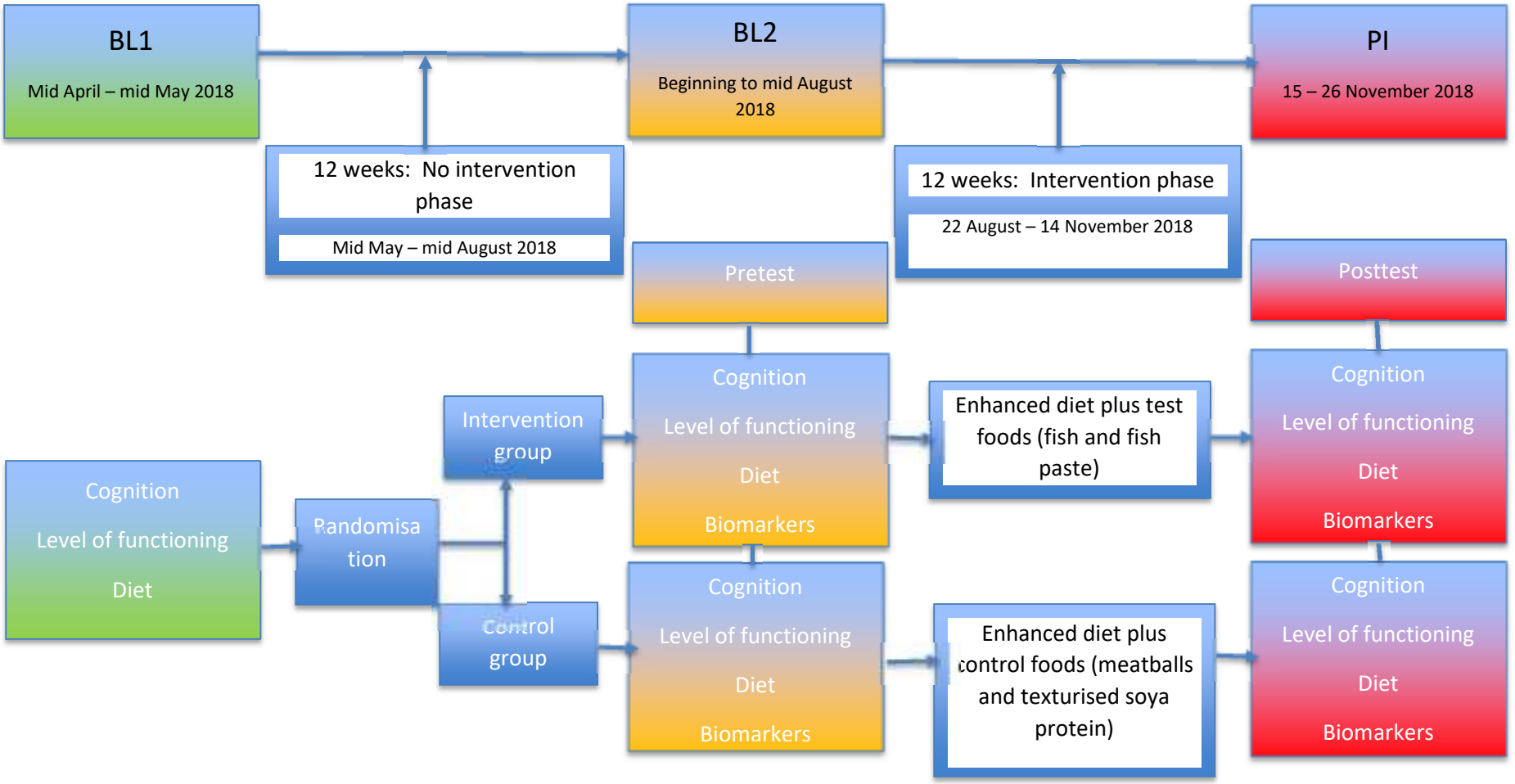

Figure 2: Study design and timeline

Abbreviations: BL1 – Baseline 1 Assessment, BL2 – Baseline 2 Assessment, PI – Post Intervention Assessment

As summarised in Table 5, the following assessments were conducted over the course of the study, therefore at BL1 – before the no-intervention phase, BL2 – immediately before the intervention phase and PI – after the intervention phase.

The assessments which were conducted, included the CASI (cognition), Lawton IADL (level of functioning) and a food frequency questionnaire (extracted information was used to score the modified MIND diet and to calculate Omega 3 PUFA intake). The testing of biomarkers in all those willing to be tested were included at BL2 and PI. Red blood cell fatty acids (RBC PUFA, -MUFA, and -saturated fatty acids (SFA) were biochemically analysed. The focus was specifically on total Omega 3 PUFA; LCPUFA (EPA, DPA, DHA) and Omega 6 Arachidonic acid (AA) (to determine if the Omega 6 PUFA was replaced by the Omega 3 PUFA). As part of the analysis, certain micro nutrients (vitamin A, iron) and anti-inflammatory markers were also tested. (For the purpose of this study only the results of the fatty acid analyses will be reported – total Omega 3 PUFA, EPA, DPA, DHA and Omega 6 AA).

Table 5: Summary of assessments of core constructs at different points in time

Construct measured	Reason	Measuring instruments / parameters	By whom	When	Details
Cognition	Primary outcome	CASI	Psychometrist (blinded) trained by a psychiatrist	BL 1 BL 2 PI	Refer to paragraph 3.8.1.2 for details on CASI.
Function	Primary Outcome	Lawton (IADL)	Psychometrist (blinded) trained by a psychiatrist	BL 1 BL 2 PI	Refer to paragraph 3.8.1.3 for details on Lawton's IADL.
Diet	Secondary outcome To monitor adherence to intervention and to make comparison possible, also for estimation of Omega 3 PUFA intake	Adjusted FFQ (scored modified MIND diet)	Researcher	BL 1 BL 2 PI	Refer to paragraph 3.8.1.4 for details on compilation of FFQ.
Biomarkers	Secondary outcome To monitor adherence to the diet	RBC: Omega 3 PUFA ALA EPA DHA DPA Omega 6 AA	Venipuncture by a phlebotomist and analysis by the Centre of Excellence for Nutrition at the North-West University	BL 2 PI	Refer to paragraph 3.8.1.5 for details on the management and analysis of biomarkers.
The gathering of all data and administration of measuring tools took place at a central point on the premises of the retirement village					

Abbreviations: AA – Arachidonic Acid, ALA – Alpha Linolenic Acid, BL1 – Baseline 1 Assessment, BL2 – Baseline Assessment, CASI – Cognitive Abilities Screening Instrument, DHA – Docosahexaenoic Acid, DPA – Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, FFQ – Food Frequency Questionnaire, IADL – Instrumental Activities of Daily Living, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay, PI – Post Intervention Assessment, PUFA – Polyunsaturated fatty acid, RBC – Red Blood Cell

3.3 Study setting

The study took place at a retirement village (residents above the age of 59 years and including all races) in Kempton Park, Gauteng Province, urban South Africa. The village consists of 236 apartments (mostly bachelor which has one area for living, sleeping and preparation of food with a separate bathroom, or one bedroom) where independent living residents (individually or as couples) care for themselves and a 50 bed care centre where frail elderly are cared for. The residents living independently have the option to apply for very basic homebased care service, if required. These residents are responsible for their own food preparation, but are allowed to dine at the dining hall of the community centre, either at own cost or by using meal coupons provided to them by the social worker as part of social support. The whole community is resource-restricted especially in terms of financial income.

3.4 Study population

The study population was the independent living residents who were still able to care for themselves, who rarely dined at the dining hall and had a monthly income of R3500 ($223) per person, or less at the start of the study. The focus was on the independently living residents for two reasons, namely: they were still able to prepare their own food and to incorporate the study foods into their diets and in addition, their cognition and level of functioning were assumed to be on a higher level than many of those in the care centre who were reportedly already diagnosed with MCI or dementia.

3.5 Sample

3.5.1 Sample size considerations

The sample size was calculated in consultation with a biostatistician and by using nQuery8.[129] Cognitive function was measured with the CASI, which uses a scale of 0 to 100. Based on data from an American cohort (no other comparative values were available at the onset of the study) in similar socio-economic conditions, it was expected that the study population would score between 70 to 90 points on the CASI.[130] (P. Becker, personal communication, 19 June 2017). It was assumed that a mean change of at least 5 points id est (i.e.) 25% (of 20 points when 70 is subtracted from 90) could be regarded as clinically relevant for the intervention (enhanced diet with fish) group. (P. Becker, personal communication, 19 June 2017) Furthermore, a recommendation regarding the inclusion of fish in the diet would be justified when the improvement in the intervention

group was at least twice that of the control (enhanced diet without fish) group. The maximum change in CASI score was unlikely to exceed 15 points, hence a conservative standard deviation of 2.5 (15 divided by 6) was assumed. (P. Becker, personal communication, 19 June 2017). Since change from BL2 was assessed, a standard deviation (SD) of 3.54 (square root of 2 multiplied by 2.5) points was used. A sample size of 44 participants per group would have 90% power to detect the difference based on a two-sided t-test at the 0.05 level of significance. Note that one-sided testing, i.e. superiority of intervention, would have required a sample size of 26 participants per group. However a sample size of 44 was aimed for.

3.5.2 Sampling method

All members of the study population (124 people) were invited by letter to attend an information meeting regarding the study. The meeting was held at the central hall of the retirement village under the leadership of the researcher. Various smaller group meetings were held following the initial meeting for those who could not attend the first meeting. Basic background information to the study was given and the consent forms, screening forms and numbers (study identification numbers) were explained and handed to potential participants. Refer to Annexure A1, page 146 and Annexure B1, page 157. The potential participants were asked to complete the consent and screening forms on their own and return them to the social worker (full time employed at the retirement village) before a specified date if they wished to be included in the study. Those who gave informed consent were included in the sample and kept in the study for as long as they preferred irrespective of whether they complied with the inclusion or exclusion criteria. This was done for the following ethical reasons: to lend food support without obligating anyone to participate and not to embarrass anyone who did not qualify for the study. The exclusion criteria were applied before data analysis to obtain the analytic sample. Those individuals who were allergic to, or not willing to eat the foods, were excluded at BL2.

BL1 formed part of the screening process and to allow for a larger sample size recruitment continued until BL2. The screening form was handed to participants irrespective to whether they entered at BL1 and BL2. Any participant who received an MMSE score below 22 at either BL1 or BL2 was excluded from data analysis. Refer to Table 6 for inclusion and exclusion criteria.

Table 6: Inclusion and exclusion criteria for the analytic sample

Inclusion	Exclusion
• 59 years and older (the specific age was chosen to include all those in the study population – the youngest person was 59 years - and to support the sample size.) • Those participants who gave informed consent. • Any person with a monthly income of R3500 or less • Independent living in the retirement village • Rarely dining at the main dining room	• Sensory impairment that would influence administering of assessments – this was indicated on the screening form or assessed by the psychometrist at the BL1 assessment. • Using a specific psychiatric medication or anti-depressant for less than 3 months. • Allergic or not willing to eat any of the intervention foods. • MMSE score < 22 (This was included to maintain a homogenous sample, as far as possible, in terms of cognitive ability).

3.6 Randomisation into intervention and control groups

Randomisation took place after the no-intervention phase just before the BL2 assessment. The participants were randomised by household into 2 groups. The households were numbered. At first all the single membered households were randomly divided into two groups by an independent person, who was not related to the study or the retirement village. A random number table was used for this purpose. The same procedure was followed for the double membered households. With the double membered households the two people of the household were allocated to the same group receiving a double ration of food. Both groups received foods to enhance their usual diet. In addition to the basic enhancement, the two groups received additional foods which differed in omega 3 LCPUFA content and were responsible for the real exposure of the intervention group. Refer to Table 7 for differentiation between study foods for the two groups. The independent person (who allocated the participants to groups) did not know which study foods each group would receive.

3.7 Intervention phase

3.7.1 Intervention versus control group

Both groups collected the supplemental foods weekly which they used to enhance their usual diet. The study foods that were offered to the participants to enhance their diet were the same (baked beans, peanut butter and canola oil) for both groups. However the additional study foods differed in the real exposure to the fatty acid content, the

intervention group received the test foods (fish and fish paste) and the control group received the control foods (food with a similar protein content but without the fatty acids). Table 7 provides more detail on the study foods offered and the differentiation between the intervention and control groups. Tables 8 and 9 present the nutrient analysis of all the supplemental foods as indicated by the manufacturer on the labels. The nutritional values of the foods of the intervention and control groups were compared to attempt to ensure similar exposure in nutrient intake. The only major difference was planned to be the Omega 3 PUFA intake, which for the intervention group was estimated at 2.2g day. This intake was calculated by adding the total Omega 3 PUFA that the intervention group received as part of the real exposure per week and dividing it by seven days. (Refer to Table 9, relevant values typed in red) The 2.2g was thus in addition to their usual intake. Calculation: 15.9g divided by 7 days equals 2.2g.

Table 7: Differentiation between study foods for the intervention and control groups

	Study foods for the intervention group	**Study foods for the control group**	**When these foods were offered**
Basic enhancement of usual diet with (same study foods for both groups)	2 x 410g of baked beans		Weekly
	1 x 400g tub of smooth peanut butter		Weeks 1,4,7,10 (four times during the intervention)
	1 x 750ml canola oil		Weeks 1,5,9 (three times during the intervention)
Real exposure in addition to enhancement (different additional study foods for both groups – test foods for the intervention group and control foods for the control group)	2 x 410g cans of pilchards in tomato sauce 1 x 85g tub of fish spread	2 x 410g cans of meatballs (beef-chicken combination) 1 x 200g texturised soya protein	Weekly

Table 8: Total energy (kJ/100g) and nutrient analysis (g/100g) as displayed on the label of study foods in June 2017[a]

Study foods	Intervention Group									Control Group								
	TE (kJ)	Prot (g)	CHO (g)	Fat Total (g)	MUFA (g)	PUFA Total (g)	PUFA Ω3 (g)	PUFA Ω 6 (g)	SFA (g)	TE (kJ)	Prot (g)	CHO g)	Fat Total (g)	MUFA (g)	PUFA Total (g)	PUFA Ω3 (g)	PUFA Ω6 (g)	SFA (g)
	Foods for enhancement of usual diet									**Foods for enhancement of usual diet**								
Baked beans (KOO)	340	4.90	17	1.40	0.0	0.90			0.30	340	4.90	17.00	1.40	0.0	0.90			0.30
Canola oil (B-well)	3362			90.90	58.90	25.80	9.06	16.77	6.20	3362			90.90	58.90	25.80	9.06	16.77	6.20
Peanut butter (Yum-Yum)	2299	20.30	28	44.10	21.10	13.40			7.50	2299	20.30	28.00	44.10	21.10	13.40			7.50
Subtotal (composition of study foods for enhancement only)	6001	25.20	45.00	136.4	80.00	40.10	9.06	16.77	14.00	6001	25.20	45.00	136.4	80.00	40.10	9.06	16.77	14.00
	Test foods									**Control foods**								
Pilchards (Lucky Star)	438	17.00	2.00	5.10	1.20	1.80	1.63		2.00									
Fish spread (Redro)	571	16.00	2.00	6.90	2.60	3.60	3.28		3.00									
Meatballs (Bull Brand)										312	6.70	5.80	2.30					0.10
Texturised soya protein (dry values)(Imana)										1307	24.40	51.80	8.30	2.30	1.30			4.60
Subtotal (composition of study foods for exposure and control)	1009	33.00	4.00	12.00	3.80	5.40	4.90	16.77	5.00	1619	31.10	57.60	10.60	2.30	1.30			4.60
TOTAL	7010	58.20	49.00	104.30	83.80	45.40	13.96	16.77	19.00	7620	56.30	99.60	102.90	82.30	41.40	9.06	16.77	18.60

[a] The empty cells .represent an absence of nutritional values on the manufacturers' labels
Abbreviations: CHO – Carbohydrate, MUFA – Monounsaturated fatty acid, Prot – Protein, PUFA - Polyunsaturated fatty acid, TE – Total Energy, SFA – Saturated fatty acids

Table 9: Total energy (kJ) and nutrient analysis in grams per container/s of all study foods indicated as a weekly average[a,b]

Study foods	Intervention Group									Control Group								
	TE (kJ)	Prot (g)	CHO (g)	Fat Total (g)	MUFA (g)	PUFA Total (g)	PUFA Ω3 (g)	PUFA Ω 6 (g)	SFA (g)	TE (kJ)	Prot (g)	CHO g)	Fat Total (g)	MUFA (g)	PUFA Total (g)	PUFA Ω3 (g)	PUFA Ω6 (g)	SFA (g)
	Foods for enhancement of usual diet									**Foods for enhancement of usual diet**								
Baked beans (2x410g)	2788	40.2	139.4	11.5	0.0	7.4			2.5	2788	40.2	139.4	11.5	0.0	7.4			2.5
Canola oil (1x187.5ml)	6304			170.5	110.5	48.4	17.00	31.5	11.6	6304			170.5	110.5	48.4	17.00	31.5	11.6
Peanut butter (1x133g)	3065	27.1	37.3	58.8	28.1	17.9	17.9		10	3065	27.1	37.3	58.8	28.1	17.9	17.9		10
Subtotal	**12157**	**67.3**	**176.7**	**240.8**	**138.6**	**73.7**	**34.9**	**31.5**	**24.1**	**12157**	**67.3**	**176.7**	**240.8**	**138.6**	**73.7**	**34.9**	**31.5**	**24.1**
	Test foods									**Control foods**								
Pilchards (2x400g)	3504	136	16	40.8	9.6	14.4	13		16									
Fish spread (1x85g)	485	13.6	1.7	5.9	2.2	3.1	2.9		2.6									
Meatballs (2x400g)										2496	53.6	46.4	18.4					0.8
Texturised soya protein (1x200g)										2614	48.8	103.6	16.6	4.6	2.6			9.2
Subtotal	**3989**	**149.6**	**17.7**	**46.7**	**11.8**	**17.5**	**15.9**		**18.6**	**5110**	**102.4**	**150**	**35**	**4.6**	**2.6**			**9.2**
TOTAL	**16146**	**216.9**	**194.4**	**287.5**	**150.4**	**91.2**	**50.8**	**31.5**	**42.7**	**17267**	**169.7**	**326.7**	**275.8**	**143.2**	**76.3**	**34.9**	**31.5**	**33.3**

[a]Not all the study foods were offered weekly, refer to Table 8 for a breakdown of when specific foods were offered.
[b] The empty cells .represent an absence of nutritional values on the manufacturers' labels
Abbreviations: CHO – Carbohydrate, MUFA – Monounsaturated fatty acid, Prot – Protein, PUFA - Polyunsaturated fatty acid, SFA – Saturated fatty acid, TE – Total Energy

3.7.2 Distribution of foods

Participants received their weekly provision from the researcher on a Wednesday at a storage room on the grounds of the retirement village. The intervention group (referred to as Group 1 by the researcher and participants) collected food during the early morning and the control group (referred to as Group 2 by the researcher and participants) collected food during the late morning. Although the participants were offered the specified study foods, most preferred not to take all the foods every week as the amounts were perceived to be excessive. They indicated which foods they would take and this was recorded by the researcher. The planned dates and times for study food collection were given to participants during small group meetings (6 people at a time) held during the month prior to the intervention. Each participant received a handout containing his/her study number and the above mentioned information. They were asked to bring along the handouts whenever they came for collection or assessments. The instructions for record keeping of study food consumption and the return of empty containers were explained to the participants in person at their first collection. At each visit the participants received a personal blank record form for the following week. (Refer to Annexure B5, page 171) The participants were requested to return this form together with the empty containers each week when they came and collected their food. As an incentive a lucky draw was held three times during the intervention phase. Unless the participants returned at least the form or the container, they were not eligible to enter the monthly lucky draw, but they still received food.

3.8 Data collection

3.8.1 Measuring instruments and their administration

3.8.1.1 Demographic and background information: screening tool

(Annexure B1, page 156)

The screening tool requested information on demographic and health background and was completed before the onset of the study by the resident him-/herself. The questionnaire was available in Afrikaans and English. The information from the questionnaire was interpreted by the researcher and used to determine whether a participant met the inclusion criteria for the study. The information related to the exclusion criteria was only applied before data analysis to determine the analytical sample. Refer to paragraph 3.5.2 for more detail.

3.8.1.2 Cognition: Cognitive Abilities Screening Instrument (CASI)
(Annexure B2, page 160)

The CASI is a quantitative research tool that combines specific items from three established cognitive screening tools (Hasegawa Dementia Screening Scale, Mini-Mental State Examination, Modified Mini-Mental State Examination) and adds additional ones.[16] CASI can be used on people with, and without dementia, and could potentially be used to monitor disease progression or treatment response.[130] It takes about 15-20 minutes to administer the CASI. CASI scores from 0-100 to give an overall score for cognition, but can be divided into nine cognitive domains: attention, concentration, orientation, short-term memory, long-term memory, language abilities, visual construction, list-generating fluency, abstraction and judgement[16] By scoring these nine cognitive domains a neuropsychological profile is created which reportedly correlates well (values not documented) with disease specific profiles derived from lengthier and standardised tests and experimental investigations.[16] The overall CASI score was used as the primary outcome to monitor cognitive change in the present study. Due to its cross-cultural applicability, the CASI is available in English, Japanese, Chinese, Spanish and Vietnamese.[130] It was not available in Afrikaans, which is the mother tongue of the participants. The CASI, therefore, had to be administered in either Afrikaans (majority) or English. The CASI had to be translated and as with translation of a measuring instrument faced two major challenges namely to translate the instrument in such a way that it is psychometrically sound and efficient and effective for a research setting.[131] The CASI was translated into Afrikaans by a content specialist (psychiatrist) through forward and backward translation which is according to Tsang (2017) an essential guideline for translating a measuring instrument.[131] The translated CASI was checked by another psychiatrist who also used it for a number of patients consulted in practice to determine its practicality. No changes were made to the content of the instrument, but with each assessment (BL1, BL2 and PI) different items were used to test short-term memory. For example at BL1 participants were shown a comb, key and pen and then later in the assessment asked to recall these items. At BL2 the previous items were replaced with other items, such as a toothbrush, watch and pencil. With each assessment items were of similar nature and of everyday household use. This was done in an effort to prevent a learned response.

Apart from being the main outcome, the CASI was also used as a screening tool at BL1 and BL2: a MMSE score was generated from it and the data of anyone with a score below 22 was excluded from analysis. This was done to improve homogeneity of the sample. (The general cut-off score of 24[132] was lowered in an attempt to not exclude too many participants from the already small sample). It was administered and interpreted (coded) by a psychometrist trained by a psychiatrist who was blinded to the intervention. During BL1 the assessment was done by a qualified psychometrist with an honours degree in psychology. This person had to withdraw due to personal reasons and was replaced by an industrial psychologist (also a qualified psychometrist) at BL2 and PI. Participants met the psychometrist for one-on-one sessions at the retirement village's community centre where she had a private office/room for interviews.

3.8.1.3 Level of functioning: Lawton Instrumental Activities of Daily Living (IADL) (Annexure B3, page 162)

This is a tool to assess the instrumental activities of daily living and includes aspects on: telephone use, shopping, food preparation, housekeeping, laundry, mode of transport, responsibility for own medication and ability to handle finance.[17] The above mentioned aspects are scored according to a participant's ability to independently or partially independently perform an activity and adds up to a score out of 8.[17] The Lawton IADL was chosen over the basic activities of daily living (BADL) instrument, as it was assumed that it would be more sensitive to detect earlier cognitive decline, because the neuropsychological processing capacity required to support the former test is more complex.[133] These specific functions are thus an indication of a higher level of cognitive function. The Lawton was available in English, therefore it was translated by the researcher into Afrikaans and checked to see if translation had been accurate by a psychiatrist. This instrument was administered to the participants by the same psychometrist that administered the CASI.

3.8.1.4 Diet: Modified MIND diet focused food frequency questionnaire (Annexure B4, p 163)

As no validated population specific (or even similar) food frequency questionnaire (FFQ) or other tool to assess relevant usual dietary intake could be found, the researcher developed a study specific FFQ. The intake reference period was set at

one month and a standardised method of administration was used in the dietary intake interview. Based on informational observation, the foods which were included, were specifically those that were generally known to be consumed by the study population. The FFQ did not focus on total food intake, but rather on total intake of foods related to a modified MIND diet.

Because the MIND diet originated in the USA, some of the dietary components, which were evaluated, were not available to and/or affordable for the population of this study. (Refer to Table 4 in Chapter 2). These components were replaced with South African options that are more affordable, but have comparable nutritional values in terms of the nutrients of interest (the cognitive supporting nutrients i.e.the monounsaturated fat), hence the reference to a modified MIND diet. The instrument was administered by the researcher who is a registered dietitian.

The following table compares the original MIND diet with the modified MIND diet – only the modified components were highlighted.

Table 10: Original MIND diet versus modified MIND diet

Original MIND diet[15]	Modified MIND diet	
	Components that stayed the same	**Components that changed**
Whole Grains >=3 servings/d	Whole Grains >=3 servings/d	
Green Leafy Vegetables >=6 servings/wk (Kale, collards, greens; spinach; lettuce/tossed salad)		Green Leafy Vegetables >=6 servings/wk **(As for MIND diet plus cabbage and broccoli)**
Other Vegetables >=1 serving/d (Green/red peppers, squash, cooked carrots, raw carrots, broccoli, celery, potatoes, peas or lima beans, tomatoes, tomato sauce, string beans, beets, corn, zucchini/summer squash/eggplant, coleslaw, potato salad.)		Other Vegetables >= 1 serving/d **(As for MIND diet plus all types of pumpkin and sweet potato)**
Berries >=2 servings/wk Strawberries		Berries >= 2 servings/wk **(As for MIND diet plus red/purple/black grapes)**
Red Meats and products < 4 servings/wk	Red Meats and products < 4 meals/wk	
Fish >=1 meal/wk Tuna sandwich, fresh fish as main dish; not fried fish cakes, sticks, or sandwiches		Fish >= 1 meal/wk **(As for MIND diet plus canned pilchards, Anchovette fish paste, fish cakes and fingers which were not fried)**
Poultry >=2 meals/wk	Poultry >= 2 meals/wk	

Original MIND diet[15]	**Modified MIND diet**	
	Components that stayed the same	**Components that changed**
Beans >3 meals/wk Beans, lentils, soybeans.		Beans > 3 meals/wk **(As for MIND diet plus texturised soya protein)**
Nuts >5 servings/wk		Nuts > 5 servings/wk **(As for MIND diet plus ground nuts and peanut butter)**
Fast/fried food <1 serving/wk	Fast/fried food <1 serving/wk	
Olive Oil primary oil		**Canola Oil primary oil**
Butter, margarine <1 T/d	Butter, margarine <1 T/d	
Cheese <1 serving/wk	Cheese <1 serving/wk	
Pastries, sweets <5 servings/wk	Pastries, sweets < 5 servings/wk	
Alcohol/wine 1 glass/d	Alcohol/wine 1 glass/d	

Abbreviations: d – day, wk - week, T - tablespoon

Serving sizes as specified by the original MIND diet.

To support the accuracy of the dietary information collected by the FFQ, the following portion size estimation and food description aids were used:

- Standardised bean bags to determine portion sizes especially for meat, starchy foods and vegetables. Two beige-coloured, round bags of each of the following volumes were presented: 500mL, 375mL, 250mL, 125 mL, 62.5 mL and 30mL.
- Photographs indicating types of margarine/oil, canned fish, cheese for recognition of products.
- Life size photographs indicating thickness of spread on bread and size of grape serving for portion size.
- Different sizes (5mL, 10mL and 12.5mL) of household spoons to estimate sugar intake.

3.8.1.5 Biomarkers: RBC Omega 3 LCPUFA, EPA, DPA, DHA and Omega 6 AA

Biomarkers were tested in all those participants who consented for blood tests. These tests were carried out with the main aim of monitoring adherence to the diet. The change in biomarkers was not one of the primary outcomes of the study and, therefore, the focus was on the RBC Omega 3 LCPUFA (EPA, DPA, DHA) and Omega 6 AA only. Omega 6 AA was included to determine whether the Omega 3 LCPUFA replaced the Omega 6 AA within the cells. The other markers that formed part of the Quansys analysis,[134] were analysed by the laboratory, but were not statistically analysed and not included in the results or discussion of this study. The testing of biomarkers is summarised in Table 11 and discussed in subsequent paragraphs.

Table 11: Testing of biomarkers

Test	Specific biomarkers
RBC fatty acids	• polyunsaturated, Omega 3 and Omega 6 fatty acids • monounsaturated fatty acids • saturated fatty acids
Quansys Q-Plex Micronutrient Analysis[134] (these factors could act as confounders for RBC Omega 3 status)	• Ferritin • Soluble transferrin receptor • Retinol binding protein • C-reactive protein • Alpha 1-acid glycoprotein • Thyroglobulin • Histidine- rich protein 2

Test	Specific biomarkers
HemoCue point of care testing to screen for possible iron deficiency	• Haemoglobin

Abbreviations: RBC – red blood cell

Blood was drawn by two phlebotomists of an accredited chemical pathology laboratory (Ampath Accreditation number, M006E). All the materials were provided by the Centre of Excellence for Nutrition at the North-West University (NWU). Participants came to the clinic on the premises of the retirement village. All the blood (2-4mL per participant) for BL2 was drawn during one morning and the process was repeated for the PI bloods. Participants did not have to be fasting. After drawing the blood, the phlebotomists immediately handed it to laboratory technicians from NWU who adhered to the protocols specific for the testing of the biomarker in question. (Methods are stipulated in order of execution).

- *HemoCue Haemoglobin test (Hb)*

An Hb log sheet, with designated areas for high and low control values was compiled before commencement of the study. The HemoCue Hb 201+ analyser provides quick and accurate Hb results. Good quality control was adhered to and a high and low control were done before analysis of participant samples. The control values were compared with the control ranges. The control values were documented on the Hb log sheet. The ethylenediaminetetraacetic acid (EDTA) blood tube was mixed well, and then the HB of the participant was determined. The Hb value was then documented next to the participant's identification (ID) on the Hb Log sheet. Hb levels below 12g/dL were considered low.

- *Plasma for Micronutrient Analysis (Quansys)*

After the HB was determined, the 2-4mL EDTA blood tubes were centrifuged at 2000G for 10 minutes. The plasma was removed and aliquoted into 2x 500µL labelled microtubes. All the remaining plasma and Buffy Coat were then removed and safely discarded into a biohazardous waste container. The plasma samples were transported on dry ice and upon arrival at the laboratory, were stored at -80˚C until analysis. Vitamin A, iron and anti-inflammatory status may act as confounding factors

for Omega 3 PUFA status. As the RBC Omega 3 PUFA content was not the primary outcome of the study further methods for analysis are not discussed, but can be viewed in Annexure C, page 174.)

- *Sample Handling and storage of Omega 3 PUFA samples (RBC)*

After the plasma was removed from the RBC, to store the RBC for the Omega 3 PUFA analysis, the EDTA whole blood was washed as follows: RBCs were washed twice with 0.15 mol/L sodium chloride (NaCl) (saline solution) and centrifuged at 2000G X for 10 minutes. The washed RBC sample was aliquoted into 1x 500 µL labelled microtube. The RBC samples were transported on dry ice and upon arrival at the laboratory, they were stored at -80˚C until analysis.

- *Method of Omega 3 PUFA analysis*

Lipids were extracted from each lipid pool with chloroform:methanol (2:1, v:v; containing 0.01% Butylated Hydroxytoluene (BHT) using a modification of the method of Folch et al.[135] The lipid extracts were concentrated and the neutral lipids separated from the phospholipids by Thin-Layer Chromatography (TLC) (Silica gel 60 plates, 10 3 20 centimetre (cm), Merck) and eluted with diethyl ether:petroleum ether:acetic acid (30:90:1, v:v:v). The lipid band containing phospholipids was removed from the TLC plate and transmethylated with methanol:sulphuric acid (95:5, v:v) at 70°C for 2 h to yield fatty acid methyl esters (FAME). The resulting FAME were extracted with water and hexane. The organic layer was evaporated and redissolved in hexane. FAMEs were analysed with an Agilent Technologies 7890A gas chromatograph system equipped with an Agilent Technologies 7000B triple quad mass selective detector (Agilent Technologies). The gas chromatography separation of FAMEs was carried out on a HP88 capillary column (100 m x 0.25 mm x 0.20 micrometre (µm); Agilent) by using helium as the carrier gas at a flow rate of 2.2 mL/min. The gas chromatography injector was maintained at a temperature of 270 °C, and the mass spectrometry source at 250 °C. The injection volume of the sample solution was 1 µL by using a split ratio of 1:80. The oven temperature was programmed to rise from 50 °C to 170 °C at 30 °C /min, then from 170 ºC to 215 ºC at 2 ºC/min, and lastly at 4 °C/min to 230 °C. After that the temperature was held isothermally at 230 ºC for 7 min. The total analysis time was 38.25 min. Mass spectrometry was carried out in positive impact multiple reaction monitoring mode, with at least two transitions per compound.

Quantification of FAMEs was performed with Masshunter (B.06.00). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMEs (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of fatty acids (% weight for weight (w/w)) were calculated by taking the concentration of a given fatty acid derivative as a percentage of the total concentration of all fatty acids identified in the sample.

3.8.2 Testing of measuring instruments

None of the instruments was validated for the study population. Refer to paragraphs 3.8.1.1 to 3.8.1.3. Testing of the instruments in a similar population took place as soon as ethical clearance was obtained. Ten people from the same retirement village, but not part of the study population, were asked whether they would be willing to undergo the assessments. Nine agreed and were assessed. Although not planned, three of the nine people joined the study from BL2 onwards. In an effort to increase the sample size their inclusion was allowed as they had only been exposed to the measuring instruments once (hence the same exposure as the other participants who had undergone BL1 assessment). Initially these three participants did not comply with the inclusion criteria, but their circumstances changed and with the commencement of BL2, they complied. No changes were made to the instruments after testing. Test data were analysed to determine whether proposed statistical models would be appropriate. The duration of the administration during the test was used to determine time slots necessary for individual assessments at BL1.

The testing of instruments served as part of the training for the psychometrists who administered the assessments. Psychometrists performing the cognitive and functionality assessments received training from a psychiatrist. Refer to Table 12 for a summary of the administration of measuring instruments.

Table 12: Administration of measuring instruments

Measuring instruments / parameters	Administered	When	Venue	Individual or group interviews	Sequence of assessments at a specific point in time
Demographic and background information	Self administered	Before onset of study	At the participants' homes	Individual	Not applicable
CASI	Psychometrist	BL1 BL2 PI	Private office at the community centre of the retirement village	Individual	Either before or after the dietary assessment. (Determined by available time slots) After the Lawton
Lawton	Psychometrist	BL1 BL2 PI	Private office at the community centre of the retirement village	Individual	Either before or after the dietary assessment. (Determined by available time slots) Before the CASI
FFQ	Researcher (dietitian)	BL1 BL2 PI	Private office at the community centre of the retirement village (separate from the psychometris)	Individual or couple interviews (one FFQ filled out per individual)	Either before or after the Lawton and CASI. (Determined by available time slots)
Biomarkers (RBC Omega 3 PUFA, EPA, DPA, DHA and Omega 6 AA)	Phlebotomist	BL2 PI	Clinic on premises of retirement village	Individual	Separate day from the other assessments. During the course of the morning. Not fasting.

Abbreviations: AA – Arachidonic Acid, BL1 – Baseline 1 Assessment, BL2 – Baseline 2 Assessment, CASI – Cognitive Abilities Screening Instrument, DHA – Docosahexaenoic Acid, DPA – Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, FFQ – Food Frequency Questionnaire, PI – Post Intervention Assessment, RBC – Red Blood Cell, PUFA – Polyunsaturated Fatty Acids

3.8.3 Compliance and adherence

For the current study compliance was defined as the participants' commitment to collect study foods. Compliance was monitored by recording the participant's number each time he/she collected food. Personal record keeping of the intake of study foods on the provided personal record sheet (Annexure B5, page 171) and the handing in of empty containers were used to promote both compliance and adherence. All participants were requested to indicate their intake of the study foods in terms of

frequency and amount on a personal record which they handed in each week with the collection of their food supply for the following week. Those returning their containers and/or record sheets were entered into a monthly draw (twice during the three month intervention). The prizes (two per month – one for the intervention group and one for the control group) included treats such as bubble bath, soap and coffee mugs, which were sponsored by the researcher. None of the prizes exceeded the value of R200 ($12).

Adherence, conceptually defined as the study participants' commitment to the intake of study food and their honesty not to exchange study foods between the two groups, was assessed by determining by dietary intake with the study-specific FFQ (Annexure B4, page 163) and by testing biomarkers (RBC-total Omega 3 LCPUFA, -EPA, -DHA and –DPA and Omega 6 AA) before and after the intervention phase. Refer to paragraphs 3.8.1.4 and 3.8.1.5. In an effort to further promote adherence, a meeting with all the participants was held at week nine of the intervention. It was arranged that a psychiatrist (specialising in geriatrics) address them in general and answer non-study related questions. At the same meeting, the researcher addressed the participants to answer questions they may have about the study. Throughout the study, participants were requested to bring recipes containing the study foods they received when they come and collect study foods. This was done to keep the participants interested and motivated. These recipes will be combined in a recipe book (to be published by the retirement village).

3.9 Data management and analysis

3.9.1 Data management and cleaning

3.9.1.1 Cognition and level of functioning: CASI and Lawton

The psychometrist was responsible for the management of the data on cognition and functionality as collected by the CASI and Lawton measuring instruments. An Excel spreadsheet with formulae was created for each participant at the different times of assessments, hence a participant who underwent all three assessments BL1, BL2 and PI had three different spreadsheets. Crude scores per question on the CASI and Lawton instruments were recorded on the score spreadsheet. The final score (for the instrument and its underlying domains) was calculated by the programmed spreadsheet. This final score was double checked by the psychometrist and

researcher before it was read into the comprehensive (merged) datasheet which was imported into the statistical software programme for the statistical analysis. The final datasheet was also rechecked before the statistical analysis was performed.

3.9.1.2 Diet: FFQ related to MIND diet and calculated Omega 3 PUFA intake

A similar process as for cognition and level of functioning was followed for the calculation of the MIND diet score (as defined in Chapter 2) and its underlying components, by the researcher. The MIND diet specific data from the FFQ were fed into a programmed spreadsheet for each individual at each assessment. The spreadsheet generated a final score (again for the instrument in total and its components) which was copied to the comprehensive (merged) datasheet used for the statistical analysis. Data on each individual spreadsheet was rechecked for accuracy by the researcher. The same was done for the comprehensive (merged) datasheet.Information derived from the FFQ was also used for calculating average Omega 3 PUFA intake for each participant at BL1, BL2 and PI. The main food sources of Omega 3 PUFA used for the calculation were fish and fish products. Refer to Tables 8 and 9.

The same individual spreadsheet used for the calculation of the MIND diet score was used for calculating the Omega 3 PUFA intake. The spreadsheet was programmed with values of Omega 3 PUFA content (total Omega 3 LCPUFA, ALA, EPA, DPA, DHA) and the focus was mainly on fish and fish products and some additional foods (canola oil, margarine and peanut butter). For the intervention foods (foods supplied by the researcher, namely pilchards, fish spread and canola oil) the Omega 3 PUFA content as presented on the container was used. The accuracy of these values was confirmed by the manufacturers. For any other fish, fish products, peanut butter and margarine, the values from the USDA databases were used.[122] The USDA databases were chosen above the FoodFinder3 (2002) v1.1.3 program,[136] which is based on the South African food composition database, because the South African database lacked comprehensive data on fatty acid content at the time of the study. The total Omega 3 LCPUFA was calculated by adding up the various totals for EPA, DPA and DHA.

The following table (Table 13) gives a breakdown of the nutrient value per 100g used for the calculation. The total Omega 3 PUFA values may differ slightly from the values

in Table 8. The reason for this is that the values in Table 8 are as specified by the composition table used by the manufacturer, whereas the values in Table 13 were calculated as the sum of EPA, DPA, and DHA. This was done because many of the foods in the participants' usual diets (not the study foods) were calculated by making use of the USDA database which did not contain total Omega 3 PUFA values, but only the individual EPA, DPA, DHA values. For consistency, the same route was followed for the supplemental study foods, namely adding the EPA-, DPA- and DHA-values up to calculate total Omega 3 PUFA intake.

Table 13: Nutritional information used for estimation of Omega 3 PUFA content per 100g[a]

Food	Source of content information	ALA (mg)	EPA (mg)	DPA (mg)	DHA (mg)	Total Omega 3 LCPUFA (sum of EPA, DPA, DHA) (mg)
Pilchards	Label and manufacturer	-	963	-	398	1361
Fish spread	Label & (manufacturer)	-	558	115	871	1544
Hake (Frozen)	USDA (NDB_No: 15033)	-	40	10	90	140
Fish fingers/cakes (commercial – mainly prepared from Hake)	USDA (NDB_No: 15027)	240	50	-	90	140
Tuna	USDA (NDB_No: 15121)	-	30	-	200	230
Canola oil	Label: (manufacturer)	8808	-	-	-	-
Margarine	USDA (NDB_No: 04128)	2200	-	-	-	-
Nuts	USDA (NDB_No: 12137)	80	10	-	-	-

[a] Values not available in the database or on product label are indicated by an (-).
Abbreviations: ALA – Alpha Linolenic Acid, DHA -Docosahexaenoic Acid, DPA – Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, USDA NDB – United States Department of Agriculture National Database[122]

3.9.1.3 Biomarkers

Biomarkers were analysed by the micronutrient laboratory of the Centre of Excellence for Nutrition at the NWU for fatty acids and biomarkers using Quansys analysis and the Haemocue test. RBC fatty acid values were provided in an Excel spreadsheet by the laboratory, which performed the analysis. All fatty acid values (including PUFA, MUFA and SFA) were included, but only Omega 3 PUFA and 6 PUFA results will be reported in this thesis. Each participant's values was presented as Omega 3 PUFA of different chain lengths including total Omega 3 PUFA, EPA, DPA, DHA. The results were presented as the percentage of composition meaning that it is a percentage of the total fatty acids in the RBC membrane.

3.9.2 Statistical analysis

STATA 15[137] was used for data analysis. Two-sided testing was done at the 0.05 level of significance. All the data from participants who did not follow through from assessment BL1 to BL2 were excluded. The data from the participants who entered the study at the BL2 assessment was included. Because all the participants at BL1, who did not follow through to BL2 (those that were not randomised) were excluded, the data at BL1 could also be presented in terms of intervention and control groups. Introducing new participants at BL2 is conservative in the sense that they were not influenced by having been part of the study from the beginning, i.e. since BL1. New participants were introduced at BL2 to append the sample size.

3.9.2.1 Descriptive statistics

Descriptive statistics were used to describe the sample in terms of demographic information. Data summaries of CASI total and Lawton scores at BL1, BL2 and PI per study group were reported as mean values, standard deviations and 95% confidence intervals. Because the Lawton seemed to produce similar results over the different assessments, it was only included in the descriptive statistics and not used for further inferential statistics. Scores of the nine domains from the CASI were also analysed to detect if there was any significant difference between them. The different domains of the CASI showed little variance and were only included in the descriptive statistics. The total MIND diet score and the differences in MIND diet categories (dietary characteristics) between the two groups at the different assessments were compared. Characteristics between the intervention and control group at BL2 were compared by

making use of a two-sample t test with equal variances. This was done to evaluate whether randomisation was successful. Percentage change in scores (CASI, MMSE, MIND) from BL1 to BL2 was calculated by dividing the difference in score between BL1 and BL2 with the score at BL2.

3.9.2.2 Inferential statistics

Intervention and control groups were compared with respect to outcome variables (CASI score, MMSE score, MIND diet score, Omega 3 PUFA intake, RBC Omega 3 PUFA) using non-parametric regression (non-parametric ANCOVA) with bootstrap estimation and the BL2 values of the relevant outcome variables, education category and Omega 3 FA supplementation as covariates. This analysis was also done when last observations (BL2) were carried forward (i.e. with imputation) in instances where PI data were missing. For comprehensiveness, values with and without imputation are presented in the chapter on results. The differences in MIND diet categories (dietary characteristics) between the two groups at the different assessments over time were compared by using Fisher's exact test to indicate significant differences.

3.10 Ethical and legal considerations

As with any randomised controlled trial, the ethical implications were considered with the utmost caution. Not only was the study population viewed as a vulnerable group because of their age and socioeconomic situation, but also because cognitive assessments which could provide sensitive data were performed. All these factors were incorporated in the development of the protocol and the study at all times aimed to benefit the study population. The four principles of bioethics as discussed on the European Alzheimer Association's website served as the principal guidelines for the study.[138] The four principles were incorporated into the study.

3.10.1 Autonomy

Autonomy can be defined as the respect that a researcher has for a participant's view about participation and also the role that the participant is allowed to play in decision making.[138] Autonomy was supported in the current study by supplying potential participants and enrolled participants with sufficient information to make informed choices about their participation in the study. Information meetings were held with the study population before the commencement of the study and written informed consent

(available in Afrikaans and English) was obtained before inclusion in the study. (Refer to Annexure A1, page 146). Throughout the study, communication was supported by means of group meetings or written handouts to inform participants about the logistics of the study. Participants were supplied with the researcher's telephone number and had many individual contact sessions with her during the assessments (BL1, BL2 and PI) and the intervention phase, during which where their questions (if any) were also addressed. As stated in the consent letter, participation was voluntary and participants were able to withdraw from the study at any stage without giving a reason and without any consequences. It was also explained to them that even if they withdrew, they could still collect foods for the duration of the study. Participants had the choice of taking foods offered to them weekly, no pressure was exerted on them to take foods if they did not want to. The participants' privacy was also respected in terms of confidentiality. Questionnaires only made provision for identifying numbers and were kept anonymous. The researcher and psychometrist were the only people that had a list linking the participants to their numbers. All physical and electronic data were safely stored with the researcher for the duration of the study. After completion of the study, storage will be provided by the Department of Human Nutrition for fifteen years.

3.10.2 Beneficence and non-maleficence

Beneficence pertains to balancing the effects of treatment (or intervention) against the risks and costs involved, whereas non-maleficence is focused on avoiding the causation of harm.[138] To ensure that both of these principles were taken into account, the protocol (including the ethical considerations and the retirement village's written approval, Annexure A2, page 151), was defended at two committee meetings (first at the Department of Human Nutrition and secondly at the School of Health Care Sciences, University of Pretoria (UP)). After passing the scrutiny of these committees, the protocol was submitted to the Research Ethics Committee (REC) of the Faculty of Health Sciences, UP) who approved the study **(542/2017)**. (Refer to Annexure A3, page 153). The REC gave approval only after the Medicine Control Council of South Africa (MCC) (Refer to Annexure A4, page 155) had also worked through the protocol and indicated that their approval was not necessary because the study was food based. An amendment was approved by the REC to include testing for more biomarkers (vitamin A, iron and inflammatory markers) even if not included in the present study. The study was registered on the National Health Trial register **(DOH-**

27-0618-6026). Furthermore, participants benefited from the study not only by receiving food, but being referred for follow-up by the relevant person if such a need was identified. All participants with a final score of 22 or lower on the MMSE were confidentially referred to the social worker who was responsible for referral to a state psychiatrist. The same procedure was followed for those participants who had low haemoglobin levels. Participants with low levels indicating a possible iron deficiency, were referred to a clinic via the social worker. Should any participant experience an adverse effect (which no one did) it would be reported the REC.

3.10.3 Justice

Justice is defined as the moral obligation to act on the basis of fair adjudication between competing claims.[138] In health ethics, justice can be divided in three categories: fair distribution of scarce resources, respect for people's rights and respect for morally acceptable laws.[139] It is believed that the current study honoured the principles of justice by respecting participants in both groups unconditionally. There was no discrimination between the two groups. The diets of both groups were enhanced with cognitive supporting foods. The only difference was in the real exposure where the intervention group received fish and fish spread and the control group received foods comparable in terms of protein and palatable acceptability. All the people from the study population who were not eligible/willing to take part in the study, or those that had withdrawn, could still collect food every week for the duration of the study. The only condition was that they show interest in the study by returning the consent form, either giving consent or withholding it. The study population was a vulnerable group that was already getting social support via donations and the poverty relief fund through the social worker. This support continued throughout the study. The findings of the study were used in the implementation of a nutrition programme at the retirement village. Many of the food products/donations from the study were still available and donated to the nutrition programme which followed the study. Lastly, prizes used for the Lucky Draws were sponsored by the researcher – they were not food related and each prize did not exceed the value of R200 ($12). Participants were allowed to enter if they brought back their control sheet and empty containers. The Lucky Draws took place on a monthly basis.

CHAPTER 4
RESULTS

4.1 Introduction

In this chapter the results of this randomised controlled trial are presented. It includes information on the following: the final sample size, the flow (changes over time) of participants in the study, the statistical sample, randomisation as well as the presentation and interpretation of all the data collected throughout the study. Data were presented according to the objectives i.e. cognition and level of functioning, diet and biomarkers.

4.2 Final sample size

The final size of the sample at PI was 57 (Intervention n = 31 Control n = 26). Had the SD of the CASI score been the assumed 3.54 and the t-test one sided, the power would have been 83.59% with the current sample.

4.3 Flow of participants, compliance and statistical sample

As discussed in Chapter 3, paragraph 3.9.2 all the data from participants who did not follow through from the BL1 assessment to the BL2 assessment were excluded. (Thus N = 53 at BL1) and the data from the participants who only entered the study at the BL2 assessment, were included. Because all the participants at BL1 who did not follow through to BL2 (those that were not randomised) were excluded, the data at BL1 can also be presented in terms of the intervention and the control group. The twelve people that entered at BL2 were randomly assigned to the intervention and control group (six new members to each group). One participant did not undergo a cognitive assessment at BL2 and another participant did not undergo a dietary assessment at BL2 due to not being available for the assessments, but both of them had BL1 assessments. The data for these two missed assessments were excluded. (Thus N = 65 at BL2, but 64 observations were used where analysis applied). Eight participants dropped out of the study between BL2 and PI. (Thus N = 57 at PI). Refer to Figure 3 to explain the flow of participants. Of the fourteen people that exited the study between BL1 and BL2, three people passed away, one was diagnosed with dementia, one fell ill (a condition not related to the study) and nine withdrew due to personal reasons. Between BL2 and PI another eight people exited the study of which two people passed away and six people withdrew for personal reasons without explanation.

Throughout the intervention phase compliance was measured by recording the participant's study number when he/she came for the weekly food. The return of the personal record forms (Annexure) and empty containers was also recorded even though it was not used to measure compliance. The intervention group seemed to have had better compliance than the control group as an average of 86% participants attended weekly collections versus 79% of the control group. The return of containers and personal record forms were also better for the intervention group where 70% of participants returned either the personal record form or the containers. An average of 61% of participants form the control group returned the abovementioned.

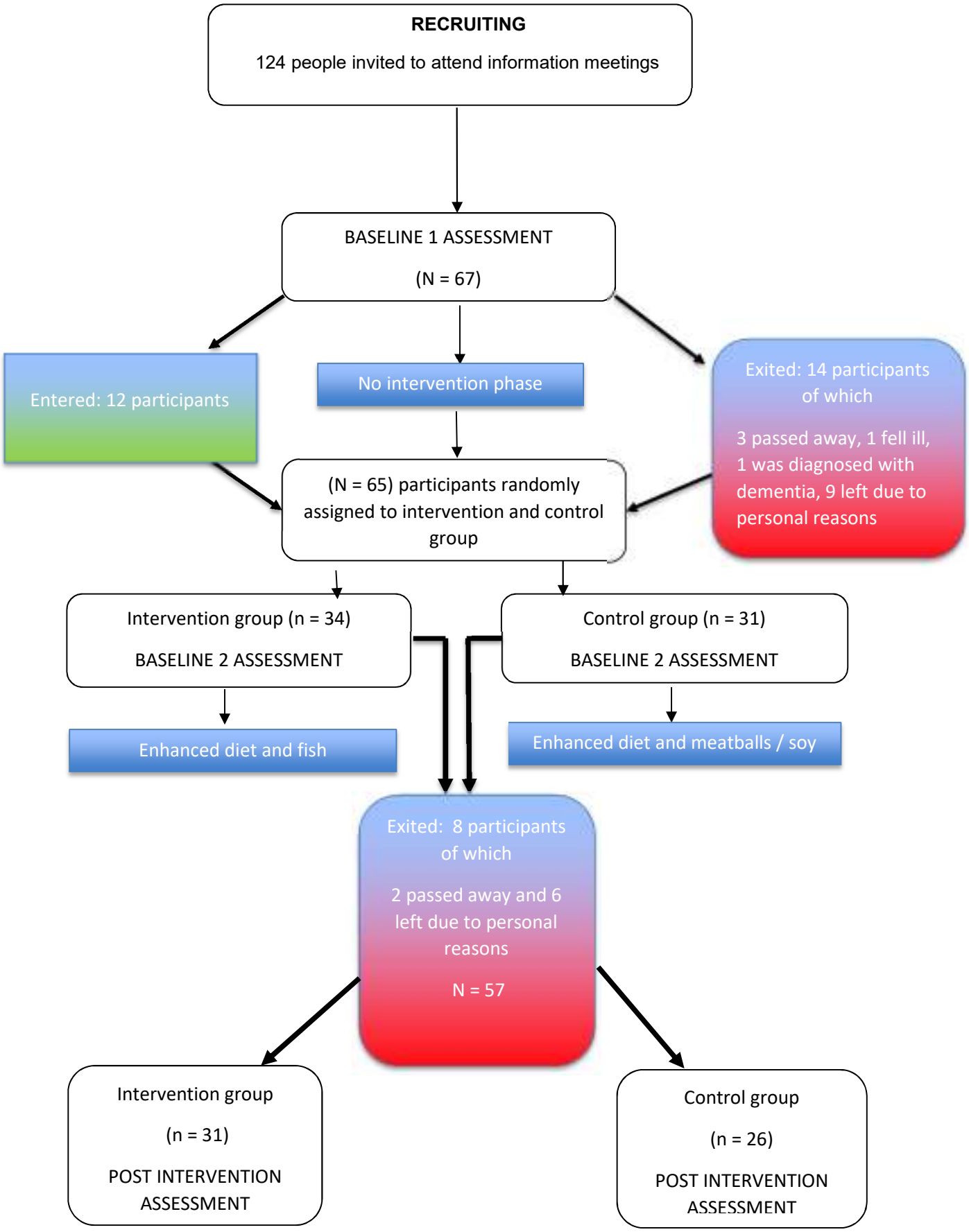

Figure 3: Flow of participants through study

4.4 Description of sample

4.4.1 Demographic information

Table 14 presents demographic data of the intervention and the control group at the three different assessments over time. The two groups (intervention and control group) presented with similar demographic characteristics. At BL2 there was no significant difference between the intervention and the control group in any of the demographic characteristics.

The gender distribution in the two groups stayed the same with the female participants forming the majority of more than 73%. In regard to age, the distribution between the two groups was also quite similar over the study. Throughout the study, the participants in the lower education category were a slightly greater number than the higher education category, with the exception of the control group at BL2, but this difference was not significant. The intervention and control groups compare well in regard to distribution of education category. The same applies to smoking, more than 77% of the participants did not smoke and the distribution between the two groups was similar. The majority of participants did not use Omega 3 PUFA supplementation and all of them had been on their prescribed medication for longer than three months. There was no significant difference between the two groups in gender, education category, smoking and Omega 3 PUFA supplementation before the onset of the intervention.

4.4.2 Exposure and outcome characteristics at BL2 assessment

Table 15 compares the intervention and control groups at the BL2 assessment in terms of cognition, dietary characteristics (MIND diet score and Omega 3 PUFA intake) and RBC LCPUFA composition. There was no significant difference between the two groups in regard to any of the characteristics.

Table 14: Demographic information at different assessments over time

Components	Baseline 1 Assessment (N = 53)		Baseline 2 Assessment (N = 65)			Post Intervention (N = 57)	
	Intervention group (n = 27)	Control group (n = 26)	Intervention group (n = 34)	Control group (n = 31)	P-value[a,b]	Intervention group (n = 31)	Control group (n = 26)
Gender							
Male, n (%)	7 (25.9)	7 (26.9)	9 (26.5)	8 (25.8)	1.00	8 (25.8)	7 (26.9)
Female, n (%)	20 (74.1)	19 (73.1)	25 (73.5)	23 (74.2)		23 (74.2)	19 (73.1)
Age, mean (SD)	71.78 (4.5)	73.69 (5.8)	70.94 (4.8)	73.58 (6.5)	0.07	70.97 (5.0)	74.08 (5.5)
Education Category, n (%)							
Gr8_10	17 (63.0)	15 (57.7)	21 (61.8)	15 (48.4)	0.32	18 (58.0)	14 (53.9)
Post Gr 10	10 (37.0)	11 (42.3)	13 (38.2)	16 (51.6)		13 (41.9)	12 (46.2)
Smoking, n (%)							
Yes	4 (14.8)	6 (23.1)	7 (20.6)	7 (22.6)	1.00	6 (19.4)	4 (15.4)
No	23 (85.2)	20 (76.9)	27 (79.4)	24 (77.4)		25 (80.7)	22 (84.6)
Omega 3 PUFA supplementation; n(%)							
Yes	5 (18.5)	2 (7.7)	6 (17.7)	4 (12.9)	0.43	5(16.1)	3 (11.5)
No	22 (81.5)	24 (92.3)	28 (82.4)	27 (87.1)		26 (83.9)	23 (88.5)
Using chronic medication for longer than 3 months, n (%+)	27 (100)	26 (100)	34 (100)	31 (100)	-	31 (100)	26 (100)

[a]P-value to indicate comparability before onset of intervention, [b]P-value for age determined by two sided t-test, P-value for other characteristics determined by Fisher's Exact test

Table 15: Comparison of cognitive, dietary and biochemical characteristics for the intervention and control groups at BL2

	Characteristics	Intervention group (N = 33)[a]			Control group (N = 31)			P-value[b]
		Number of obser-vations[c]	Mean (SD) / Median (IR)[d]	95% CI	Number of obser-vations[c]	Mean, SD / Median (IR)[d]	95% CI	
Cognition	Total CASI (maximum score: 100)	33	91.64 (5.10)	89.83; 93.45	31	90.68 (4.61)	88.98; 92.37	0.43
	MMSE (maximum score: 30)	33	27.67 (2.01)	26.95; 28.28	31	27.39 (2.09)	26.62; 28.15	0.59
Dietary components	MIND diet (maximum score: 15)	33	8.0 (1.27)	7.5; 8.4	31	8.0 (1.48)	7.4; 8.5	0.97
Omega 3 PUFA intake per day (mg)[d]	Total Omega 3 PUFA	33	613 (438; 876)	--	31	532 (344; 873)	-	0.58
	EPA intake	33	112 (41; 273)	-	31	135 (17; 266)	-	0.90
	DPA intake	33	1 (0; 3)	-	31	1 (0; 3)	-	0.74
	DHA intake	33	128 (57; 208)	-	31	140 (55; 199)	-	0.88
	ALA intake	33	283 (220; 503)	-	31	220 (220; 660)	-	0.71

	Characteristics	Intervention group (N = 33)[a]			Control group (N = 31)			P-value[b]
		Number of obser-vations[c]	Mean (SD) / Median (IR)[d]	95% CI	Number of obser-vations[c]	Mean, SD / Median (IR)[d]	95% CI	
RBC Omega 3 PUFA (percentage composition of RBC fatty acids)	Total Omega 3 LCPUFA	30	5.76 (1.53)	5.28; 6.39	26	5.84 (1.37)	5.28; 6.40	0.84
	RBC EPA	30	0.27 (0.15)	0.22; 0.33	26	0.24 (0.10)	0.21; 0.28	0.40
	RBC DPA	30	1.55 (0.33)	1.43; 1.67	26	1.43 (0.32)	1.31; 1.56	0.19
	RBC DHA	30	3.86 (1.24)	3.39; 4.32	26	4.12 (1.08)	3.68; 4.55	0.41
	RBC ALA	30	0.04 (0.01)	0.03; 0.04	26	0.03 (0.01)	0.03; 0.04	0.26
RBC Omega 6 LCPUFA (percentage composition of RBC fatty acids)	Total Omega 6 LCPUFA	30	32.93 (3.38)	31.67; 34.19	26	32.17 (2.92)	30.99; 33.35	0.37
	RBC AA	30	15.90 (2.72)	14.89; 16.92	26	15.21 (2.60)	14.15; 16.26	0.33
	RBC Omega 6:Omega 3	30	6.15 (1.83)	5.47; 6.83	26	5.87 (1.31)	5.34; 6.40	0.52

[a] Data missing for 1 cognitive and 1 dietary assessment at BL2
[b] P-value determined by a double sided t-test
[c] Difference in N – only those who agreed to biomarker testing, have RBC values for fatty acids
[d] For Omega 3 PUFA intake only the median and interquartile range were reported, P-value determined by Wilcoxon rank-sum test
Abbreviations: AA – Arachidonic Acid, ALA – Alpha Linolenic Acid, CASI – Cognitive Abilities Screening Instrument, DHA - Docosahexaenoic Acid, DPA - Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay, MMSE – Mini Mental State Examination, RBC – Red Blood Cell

4.5 Cognition and level of functioning as primary outcomes

4.5.1 Cognition at different assessments over time

Table 16A presents cognitive characteristics of the intervention versus the control group as assessed by the mean, SD and 95% CI intervals at different assessments over time.

Although the intervention group presented with a slightly higher total CASI score at BL1, the same trend can be observed in both groups over the three different assessments namely an increase in score over time with the largest increase occurring between BL1 and BL2. It is also important to note that the SD ranges between 5.10 and 5.91 for the intervention group and for the control group between 4.61 and 6.78. At all three assessments the CASI score for the intervention group was higher than for the control group with a difference between the two groups of 1.48 points at BL1, 0.95 points at BL2 and 2.27 points at PI. However none of these differences were significant. The difference in PI CASI scores between the intervention and control groups was observed after non-parametric ANCOVA with imputation was performed. Refer to paragraph 4.5.3 and Table 16D.

When the different domains of the CASI were assessed, only one domain indicated a significant difference (P = 0.02) between the intervention and control groups, namely the visual construction domain at the PI assessment.

The other domain scores seemed to be very similar at the three assessments over time with only slight differences at certain points, which - although not statistically significant - could be indicative of a trend.

Attention in both groups increased from BL1 to BL2 and dropped again slightly between BL2 and PI. The concentration score for the intervention group was lower at BL2 than at BL1 but stayed the same for the control group. For both groups there was an increase once again at the PI assessment. The orientation domain follows a similar pattern to that of the attention domain, namely an increase for both groups between BL1 and BL2, and then a decrease to PI for the intervention group. The control group stayed the same between BL2 and PI. Long-term memory showed an increase over the course of the study for the intervention group, whereas the control group showed

a decrease between BL1 and BL2 and then an increase again between BL2 and PI. Short-term memory also showed an increase over the course of the study for the intervention group, while the control group results decreased between BL2 and PI. The language and visual construction domains followed the same pattern as the short-term memory, namely an increase for the intervention group over the course of the study, but a slight decrease for the control group between BL2 and PI.

The list-generating fluency domain showed an upward curve for both groups over the three different assessments, the same tendency applied for the abstraction and judgement domain. The intervention group presented with a higher score compared to the control group in all nine domains at the PI assessment. No further statistics were done on the domain scores, as they were so similar.

The MMSE score was calculated by scoring particular questions of the CASI. Although the differences are very slight, the score of both groups increased over the course of the study with the intervention group always scoring slightly higher than the control group. The difference was not significant (P =0.25). As the MMSE score is generated by using relevant subsections of the CASI only, even though the CASI indicated significant change over time and differences between the two groups, the MMSE did not.

Table 16A: Cognitive characteristics at different assessments over time

Components (maximum score)	Baseline 1 Assessment (N = 53)			Baseline 2 Assessment (N=64)[a]			Post Intervention (N=57)		
	Intervention group (n = 27) Mean (SD)	Control group (n = 26) Mean (SD)	P-value[b]	Intervention group (n = 33) Mean (SD)	Control group (n = 31) Mean (SD)	P-value[b]	Intervention group (n = 3 1) Mean (SD)	Control group (n=26) Mean (SD)	P-value[b]
Total CASI (100)	87.56 (5.91)	86.08 (6.78)	0.40	91.64 (5.10)	90.68 (4.61)	0.43	94.13 (5.03)	91.65 (6.66)	0.12
Attention (8)	7.63 (0.74)	7.77 (0.51)	0.43	7.97 (0.17)	8.00 (0.00)	0.34	7.94 (0.25)	7.81 (0.49)	0.21
Concentration (10)	7.67 (2.08)	7.58 (2.35)	0.88	7.42 (2.17)	7.58 (2.35)	0.78	8.10 (2.36)	7.96 (2.41)	0.83
Orientation (18)	17.67 (0.68)	17.46 (1.24)	0.46	17.88 (0.55)	17.58 (1.06)	0.16	17.74 (1.09)	17.58 (0.76)	0.52
Long-term memory (10)	9.15 (1.17)	9.42 (0.90)	0.34	9.52 (0.87)	9.16 (1.13)	0.16	9.87 (0.50)	9.69 (0.74)	0.28
Short-term memory (12)	10.42 (1.60)	10.41 (1.86)	0.97	11.24 (0.75)	11.36 (0.61)	0.51	11.32 (1.05)	11.12 (1.11)	0.47
Language (10)	9.60 (0.57)	9.42 (0.90)	0.42	9.97 (0.17)	10.00 (0.00)	0.34	10.00 (0.00)	9.89 (0.43)	0.14
Visual construction (10)	7.22 (2.23)	7.16 (1.93)	0.85	8.15 (1.66)	7.65 (1.98)	0.27	8.81 (1.54)	7.54 (2.42)	0.02*
List-generating fluency (10)	8.93 (1.49)	8.15 (1.76)	0.09	9.33 (1.29)	8.74 (1.60)	0.11	9.32 (1.28)	9.19 (1.27)	0.70
Abstraction and judgement (12)	9.30 (1.38)	8.73 (1.97)	0.23	10.15 (1.66)	10.61 (1.73)	0.28	11.03 (1.70)	10.89 (1.45)	0.73
MMSE (30)	26.33 (2.27)	26.27 (2.63)	0.92	27.67 (2.01)	27.39 (2.09)	0.59	28.16 (1.99)	27.46 (2.57)	0.25

[a]Data missing for 1 cognitive assessment at BL2, [b]P-value determined by a two-sided t-test, *Significant value
Abbreviations: CASI – Cognitive Abilities Screening Instrument, MMSE – Mini Mental State Examination

4.5.2 Cognition: difference between BL1 and BL2

Table 16B-D provides further comparison between BL1 and BL2 assessments in terms of cognition as calculated by a double sided paired t-test. Only those participants that followed through from BL1 to BL2 and completed both cognitive and dietary assessments were included in the analysis, thus N = 52. The total CASI score increased significantly (P= 0.000) between BL1 and BL2 by 4.42 points, thus by 5.09% for the group as a whole. The same applies for the MMSE score which improved significantly (P = 0.000) between BL1 and BL2 by 1.23 points, thus by 4.68%. Table 16C presents the same results differentiated per group.

Table 16B: Difference in cognition between BL1 and BL2 in group as a whole

Components (maximum score)	**Baseline 1 Assessment (N = 52)[a]**		**Baseline 2 Assessment (N=52)**		**P-value[b]**
	Mean (SD)	95% CI	Mean (SD)	95% CI	
Total CASI (100)	86.77 (6.38)	84.99; 88.55	91.19 (4.87)	89.84; 92.55	0.000*
Total MMSE (30)	26.27 (2.44)	25.59; 26.95	27.5 (2.11)	26.91; 28.09	0.000*

[a] Only the participants following through from BL1 to BL2 were included, hence the difference in "n" from Table 16A., [b] P-value determined by a double sided paired t-test. *Significant value
Abbreviations: CASI – Cognitive Abilities Screening Instrument, MMSE – Mini Mental State Examination

Table 16C: Difference in cognition between BL1 and BL2 by intervention and control groups

Component (maximum score)	**Intervention group (n = 26)[a]**					**Control group (n = 26)**				
	Baseline 1 Assessment		Baseline 2 Assessment		P-value[b]	Baseline 1 Assessment		Baseline 2 Assessment		P-value[b]
	Mean (SD)	95% CI	Mean (SD)	95% CI		Mean (SD)	95% CI	Mean (SD)	95% CI	
Total CASI (100)	87.46 (6.01)	85.03; 89.99	91.88 (5.01)	89.86; 93.91	<0.001*	86.08 (6.78)	83.34; 88.82	90.5 (4.73)	88.59; 92.41	<0.001*
Total MMSE (30)	26.27 (2.29)	25.34; 27.19	27.61 (2.06)	26.78; 28.45	0.01*	26.27 (2.63)	25.21; 27.33	27.38 (2.19)	26.5; 28.27	0.01*

[a] Only the participants following through from BL1 to BL2 were included, hence the difference in "n" from Table 16A. [b] P-value determined by a double sided paired t-test. *Significant value
Abbreviations: CASI – Cognitive Abilities Screening Instrument MMSE – Mini Mental State Examination

4.5.3 Cognition: effect of the intervention (change from BL2 to PI)

Table 16D presents the effects of the intervention. As discussed in Chapter 3, paragraph 3.9.2.2, non-parametric ANCOVA with bootstrap estimation and covariates was used for comparison of outcome variables (total CASI and MMSE scores). BL2 (not BL1) served as baseline before the intervention phase. This analysis was also done when last observations (BL2) were carried forward when PI data was missing (i.e. with imputation). For comprehensiveness, values with and without imputation are presented.

There is a significant difference (P = 0.036) of 2.3 points in the total CASI score between the intervention and the control groups at the PI assessment when the model was fitted with imputation. There was no significant difference without imputation. Refer to Figure 4 for a graphical representation of the effect of the intervention on total CASI scores in both groups. Both groups produced steep upward curves between BL1 and BL2, with the intervention group curve being slightly higher (but parallel) to the control group curve. For both groups the slope of the still upward-tending curves decreased between BL2 and PI, but the intervention group remained slightly higher, while the gap increased in width at PI.

The covariate, total CASI score at BL2, had a significant effect (P < 0.001). A similar significant tendency for this covariate was detected when the analysis was done without imputation (P < 0.001). Education category as a covariate was also significant (P < 0.005) with, and without imputation. Thus total CASI score at BL2 and education category would influence the total CASI score at PI significantly if not adjusted for. The estimated effect of Omega 3 PUFA supplementation was not significant in either of the analyses.

The MMSE showed no significant difference between the two groups when the regression was done with, or without imputation. There was a statistically significant increase in points for the whole sample from BL2 to PI, namely 0.47 points (1.66%) with a P-value of 0.001 when imputation was added and 0.40 points (1.3%) with a P-value of 0.021 without any imputation. Neither education category, nor Omega 3 PUFA supplementation had a significant effect as a covariate.

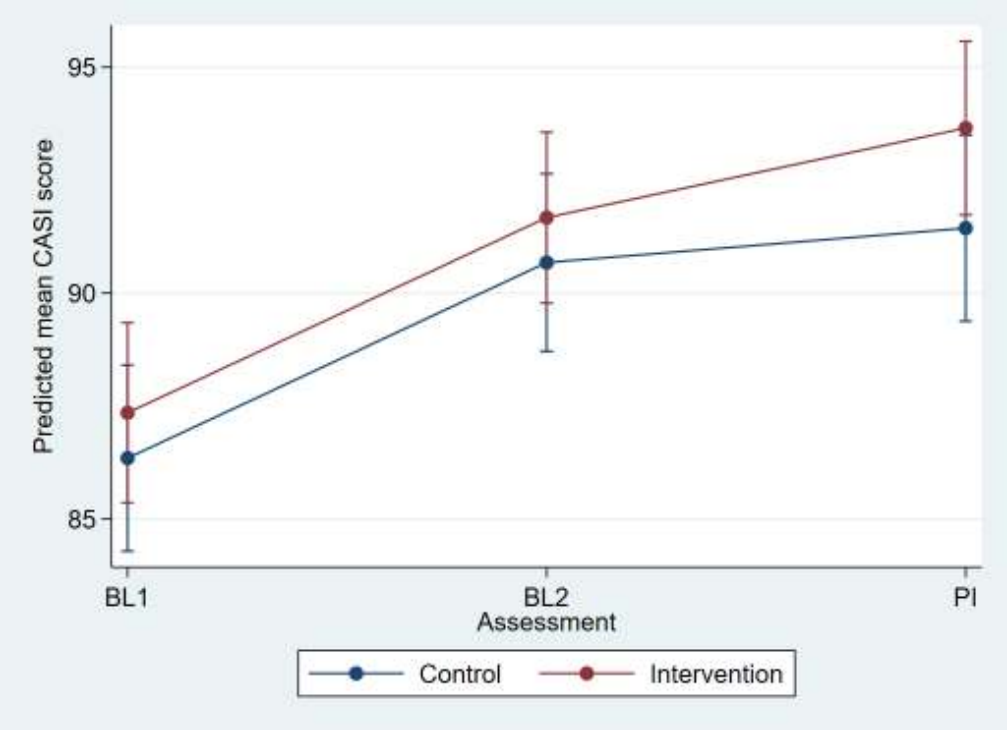

Figure 4: Predicted mean CASI score at BL1, BL2 and PI with imputation

Table 16D: CASI and MMSE of the intervention and control groups at PI as predicted by non-parametric ANCOVA with and without imputation

		Number of obser-vations[a]	Predicted Mean (95% CI)		Intervention vs Control		Covariates					
		Number	Intervention group	Control group	Estimated effect	P – value[b]	Estimated effect at BL2[c]	P – value[b]	Estimated effect of Education	P – value[b]	Estimated effect of n3 supplement intake	P – value[b]
Total CASI (100)	With imputation	64	93.36 (91.80; 95.10)	91.10 (89.23; 93.09)	2.26	0.04*	0.65	0.00*	2.95	0.00*	-0.54	0.63
	Without imputation	56	93.68 (91.93; 95.32)	91.54 (89.25; 93.66)	2.14	0.07	0.56	0.00*	3.18	0.00*	0.08	0.95
Total MMSE (30)	With imputation	64	28.17 (27.47; 28.85)	27.35 (26.57; 28.31)	0.47	0.15	0.47	0.00*	1.04	0.083	-0.56	0.56
	Without imputation	54	28.29 (27.60; 28.98)	27.37 (26.50; 28.53)	0.91	0.17	0.40	0.02*	0.35	0.577	-0.50	0.69

[a]Number of observations may differ due to missing data
[b]P-value was derived from non-parametric ANCOVA with covariates (score of relevant outcome variable at BL2, education category and Omega 3 PUFA supplement intake)
[c]Score of relevant outcome variable at BL2 served as covariate
*Significant value
Abbreviations: PI – Post Intervention Assessment, CASI – Cognitive Abilities Screening Instrument, BL2 – Baseline 2 Assessment, MMSE – Mini Mental State Examination

4.5.4 Level of functioning at different assessments over time

Table 17 presents characteristics of functioning at different assessments over time. The Lawton's scores showed very little variance over the course of time and between the two groups. For this reason it is only included in the descriptive statistics, no additional inferential statistics could be applied. Scores were high and very close to the maximum score.

Table 17: Characteristics of functioning at different assessments over time

Components (maximum score)	**Baseline 1 Assessment (N = 53)**		**Baseline 2 Assessment (N = 64)[a]**		**Post Intervention Assessment (N = 57)**	
	Intervention group (n = 27) Mean (SD)	Control group (n = 26) Mean (SD)	Intervention group (n = 33) Mean (SD)	Control group (n =3 1) Mean (SD)	Intervention group (n = 31) Mean (SD)	Control group (n = 26) Mean (SD)
FUNCTIONALITY Total Lawton (8)	7.56 (0.58)	7.50 (0.58)	7.74 (0.45)	7.81 (0.40)	7.77 (0.50)	7.81 (1.47)
Telephone use (1)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Does own shopping (1)	0.96 (0.19)	1.00 (0.00)	0.97 (0.17)	1.00 (0.00)	0.97 (0.18)	1.00 (0.00)
Preparing own food (1)	0.96 (0.19)	0.92 (0.27)	0.97 (0.17)	1.00 (0.00)	0.97 (0.18)	1.00 (0.00)
Does own housekeeping chores (1)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Does own laundry (1)	0.89 (0.32)	1.00 (0.00)	0.91 (0.29)	1.00 (0.00)	0.90 (0.30)	1.00 (0.00)
Responsible for own transportation (1)	0.74 (0.45)	0.62 (0.50)	0.91 (0.29)	0.74 (0.45)	0.94 (0.25)	0.81 (0.40)
Manages own medication (1)	1.00 (0.00)	0.96 (0.17)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Manages own finances (1)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)

[a] Data missing for 1 cognitive assessment at BL2

4.6 Diet as secondary outcome

4.6.1 Diet at different assessments over time

Table 18A presents dietary characteristics expressed in terms of the MIND diet and it's (scored) components at different assessments over time. Data are presented by comparing the scores of the intervention and control group and by using the Fisher's exact test to indicate significance in difference between categories (indicating how often a component is consumed). Categories versus components are discussed in Chapter 3, paragraph 3.8.1.4.

The MIND diet mean score out of 15 ranged between 7.76 and 8.53 points for the intervention group and between 6.98 and 9 points for the control group over the three assessments. Both groups showed an increase over the course of the study with the largest difference occurring between BL2 and PI. The only significant difference between the two groups was at BL1. The control group scored higher at the PI assessment than the intervention group, but the difference was not significant when only the descriptive statistics were applied. When the non-parametric ANCOVA was used (Refer to Table 18D and paragraph 4.6.3), a significant difference PI between the two groups was noted, before the imputation of values (P =0.04).

There was no significant difference between the distributions of the participants among the categories (0, 0.5 or 1) of intake of different dietary components (wholegrains, nuts etc.) at most of the assessments. Only three food components were identified as differing significantly between the intervention and control groups. At BL1 there was a significant difference between the two groups regarding legume intake, where the intervention group presented with a higher intake of legumes than the control group. Also at BL1 the intervention group presented with a significantly lower intake of sweets than the control group. After the intervention phase, the control group had a significantly higher poultry intake when compared to the intervention group.

Tendencies in intake of specific dietary components were identified.

In general the score for wholegrain intake tended to be lower, with the majority of participants scoring 0 or 0.5 at all three the assessments. This indicates an intake of less than one to two servings of wholegrains per day.

Overall, (for the intervention and control group at all three assessments) the scores reflect better on the butter/margarine intake where the majority of participants restricted their intake to less than 2 tablespoons per day. Throughout the study the control group had more than 50% of participants who achieved the recommendation of less than one tablespoon per day, at PI specifically 77% of the control group achieved the abovementioned recommendation compared to the 52% of the intervention group.

Throughout the study nut intake (which included peanuts and peanut butter) was scored at the 0.5 (middle) category for the majority of participants in both groups indicating that they had more than one serving per month but less than five servings per week. A shift between BL2 and PI also occurred, where the top category (equal to or more than 5 portions per week) had a higher adherence in both groups. Interestingly the intake was also higher at BL1 than BL2.

The tendency to consume more cheese than the recommendation of less than one serving per week was observed in both groups, where the majority of participants scored in the average (one to six servings per week) category. A positive change is detected at the PI assessment, where both groups had a higher achievement of the recommendation to restrict cheese intake, than at the other assessments.

Not many tendencies could be identified in the red meat intake. The majority of participants scored either 0.5 or 1 at all the assessments indicating that intake was lower or equal to between four and six servings per week. For poultry intake the control group scored significantly higher ($P = 0.01$) after the intervention phase.

Interestingly, fish intake was high for both groups throughout the study ranging between 76 and 90% adherence to optimal intake. At BL1 and BL2 the number of participants complying with equal to, or more than one serving per week, was very similar in both groups despite the fact that the control group had an extra participant at each assessment. After the intervention the numbers turned around with the intervention group having eight more participants than the control group who met the recommendation for fish intake. But the difference in distribution of participants between the different categories was not significant.

As noted before, there was a significant difference in legume intake between the two groups at BL1 assessments, where the intervention group had a tendency of ingesting more legumes than the control group. Throughout the rest of the study the intake seemed quite similar between the two groups, the majority of participants scored in the middle (0.5) category, indicating that they consume between one and three servings per week.

Throughout the study, adherence regarding intake of green leafy vegetable was not optimal, as the highest percentage of participants in both groups scored zero indicating an intake of less than two servings per week. The percentage of participants in both groups that scored one, indicating optimal adherence, ranged between 3.9% - 15%. The intervention group had slightly more people in the optimal compliance category than the control group at all three assessments. Better scores were obtained with the intake of other vegetables because the majority of the participants indicated that they consume one or more serving per day. The distributions of participants between different categories over assessments and between the two groups were similar.

As mentioned earlier, there was a significant difference in the intake of sweets between the two groups at BL1, where the intervention group consumed fewer sweets. Intake seemed to be similar between the two groups for the rest of the study with the highest percentage of participants obtaining the top score, thus indicating that they consumed fewer than five servings per week.

Food (either takeaway, restaurant or homemade) prepared in oil also showed a very similar score distribution between the two groups over the course of the study. The majority of participants scored one, indicating that they used less than one serving per week. At the PI assessment there was a shift from the top to the middle (0.5) category, indicating that participants started to use more oil for the preparation of food. The intake of canola/olive oil showed a steep upward trend in the curve, especially between BL2 and PI. There was no significant difference between the intakes of the two groups.

Throughout the study, alcohol (specifically wine) intake was very low for both groups (93 – 96% of participants indicated that they rarely drink wine and, therefore, scored zero).

The information in Table 15 also indicates that the intervention and control group obtained similar scores at BL2, indicating that randomisation in terms of the “usual diet” was successful.

Table 18A: Dietary intake (MIND Diet and its components) at different assessments over time

Components and their scoring	Baseline 1 Assessment (N = 53)			Baseline 2 Assessment (N = 64)[a]			Post Intervention (N = 57)		
	Intervention group (n = 27)	Control group (n = 26)	P-value[b,c]	Intervention group (n = 33)	Control group (n = 31)	P-value[b,c]	Intervention group (n = 31)	Control group (n = 26)	P-value[b,c]
MIND Diet									
Total score out of 15, mean (SD)	7.76 (1.40)	6.98 (1.47)	0.05*	7.96 (1.27)	7.97 (1.48)	0.97	8.53 (0.98)	9.00 (1.29)	0.13
Wholegrains, n (%)									
0: < 1 serving/day	11 (40.74)	9 (34.62)		14 (42.42)	15 (48.39)		11 (35.48)	11 (42.31)	
0.5: 1 – 2 servings/day	14 (51.85)	13 (50.00)	0.74	16 (48.48)	8 (25.81)	0.10	17 (54.84)	10 (38.46)	0.39
1: >= 3 servings/day	2 (7.41)	4 (15.38)		3 (9.09)	8 (25.81)		3 (9.68)	5 (19.23)	
Butter/margarine, n (%)									
0: > 2 tablespoons/day	6 (22.22)	7 (26.92)		8 (24.24)	9 (29.03)		8 (25.81)	3 (11.54)	
0.5: 1 – 2 tablespoons/day	6 (22.22)	6 (23.08)	0.94	9 (27.27)	4 (12.90)	0.38	7 (22.58)	3 (11.54)	0.18
1: < 1 tablespoon/day	15 (55.56)	13 (50.00)		16 (48.48)	18 (58.06)		16 (51.61)	20 (76.92)	
Nuts, n (%)									
0: <1 serving/month	2 (7.41)	5 (19.23)		3 (9.09)	6 (19.35)		2 (6.45)	2 (7.69)	
0.5: 1 serving/month - < 5 servings/week	18 (66.67)	18 (69.23)	0.24	24 (72.73)	24 (77.42)	0.10	19 (61.29)	19 (73.08)	0.57
1: >= 5 servings/week	7 (25.93)	3 (11.54)		6 (18.18)	1 (3.23)		10 (32.26)	5 (19.23)	
Cheese, n (%)									
0: >= 7 servings/week	4 (14.81)	1 (3.85)		1 (3.03)	3 (9.68)		0	1 (3.85)	
0.5: 1 – 6 servings/week	14 (51.85)	18 (69.23)	0.30	24 (72.73)	20 (64.52)	0.49	19 (61.29)	17 (65.38)	0.58

Components and their scoring	Baseline 1 Assessment (N = 53)			Baseline 2 Assessment (N = 64)[a]			Post Intervention (N = 57)		
	Intervention group (n = 27)	Control group (n = 26)	P-value[b,c]	Intervention group (n = 33)	Control group (n = 31)	P-value[b,c]	Intervention group (n = 31)	Control group (n = 26)	P-value[b,c]
1: < 1 serving/week	9 (33.33)	7 (26.92)		8 (24.24)	8 (25.81)		12 (38.71)	8 (30.77)	
Poultry, n (%)									
0: =< 1 serving/week	6 (22.22)	4 (15.38)		3 (9.09)	3 (9.38)		9 (29.03)	1 (3.85)	
0.5: 1 serving/week	5 (18.52)	7 (26.92)	0.70	7 (21.21)	4 (12.50)	0.71	3 (9.68)	1 (3.85)	0.01*
1: >= 2 servings/week	16 (59.26)	15 (57.69)		23 (69.70)	24 (77.42)		19 (61.29)	24 (92.31)	
Red meat, n (%)									
0: >= 7 servings/week	10 (37.04)	7 (26.92)		8 (24.24)	10 (32.26)		11 (35.48)	5 (19.23)	
0.5: 4 – 6 servings/week	6 (22.22)	6 (23.08)	0.73	15 (45.45)	7 (22.58)	0.14	9 (29.03)	11 (42.31)	0.38
1: < 4 servings/week	11 (40.74)	13 (50.00)		10 (30.30)	14 (45.16)		11 (35.48)	10 (38.46)	
Fish, n (%)									
0: rarely	3 (11.11)	1 (3.85)		3 (9.09)	2 (6.45)		2 (6.45)	4 (15.38)	
0.5: 1 – 3 servings/week	2 (7.41)	2 (7.69)	0.85	5 (15.15)	3 (9.68)	0.81	1 (3.23)	2 (7.69)	0.46
1: >= 1 serving/week	22 (81.48)	23 (88.46)		25 (75.76)	26 (83.87)		28 (90.32)	20 (76.92)	
Legumes, n (%)									
0: < 1 serving/week	5 (18.52)	16 (61.54)		7 (21.21)	11 (35.48)		5 (16.13)	4 (15.38)	
0.5: 1 – 3 servings/week	16 (59.26)	7 (26.92)	0.01*	18 (54.55)	13 (41.94)	0.47	16 (51.61)	15 (57.69)	0.93
1: >= 3 servings/week	6 (22.22)	3 (11.54)		8 (24.24)	7 (22.58)		10 (32.26)	7 (26.92)	

Components and their scoring	Baseline 1 Assessment (N = 53)			Baseline 2 Assessment (N = 64)[a]			Post Intervention (N = 57)		
	Intervention group (n = 27)	Control group (n = 26)	P-value[b,c]	Intervention group (n = 33)	Control group (n = 31)	P-value[b,c]	Intervention group (n = 31)	Control group (n = 26)	P-value[b,c]
Green leafy vegetables, n (%)									
0: =< 2 servings/week	15 (55.56)	17 (65.38)		19 (57.58)	22 (70.97)		17 (54.84)	14 (53.85)	
0.5: > 2 - < 6 servings/week	9 (33.33)	8 (30.77)	0.70	9 (27.27)	7 (22.58)	0.50	10 (32.26)	10 (38.46)	0.74
1: >= 6 servings/week	3 (11.11)	1 (3.85)		5 (15.15)	2 (6.45)		4 (12.9)	2 (7.69)	
Other Vegetables, n (%)									
0: < 5 servings/week	4 (14.81)	6 (23.08)		4 (12.12)	7 (22.58)		7 (22.58)	2 (7.69)	
0.5: 5 – 7 servings/week	6 (22.22)	7 (26.92)	0.65	4 (12.90)	5 (15.15)	0.56	4 (12.90)	2 (7.69)	0.25
1: >= 1 serving/day	17 (62.96)	13 (50.00)		20 (64.52)	24 (72.73)		20 (64.52)	22 (84.62)	
Berries, n (%)									
0: < 1 serving/week	25 (92.59)	25 (96.15)		33 (100.00)	30 (96.77)		27 (87.10)	24 (92.31)	
0.5: 1 serving/week	2 (7.41)	0	0.49	0	0	0.48	4 (12.90)	2 (7.69)	0.68
1: >= 2 servings/week	0	1 (3.85)		0	1 (3.23)		0	0	
Sweets, n (%)									
0: >= 7 servings/week	7 (25.93)	14 (53.85)		6 (18.18)	5 (16.13)		6 (19.35)	7 (26.92)	
0.5: 5 – 6 servings/week	0	1 (3.85)	0.04*	0	2 (6.45)	0.46	3 (7.02)	1 (3.85)	0.67
1: < 5 servings/week	20 (74.07)	11 (42.31)		27 (81.82)	24 (77.42)		22 (70.97)	18 (69.23)	

Components and their scoring	Baseline 1 Assessment (N = 53)			Baseline 2 Assessment (N = 64)[a]			Post Intervention (N = 57)		
	Intervention group (n = 27)	Control group (n = 26)	P-value[b,c]	Intervention group (n = 33)	Control group (n = 31)	P-value[b,c]	Intervention group (n = 31)	Control group (n = 26)	P-value[b,c]
Food prepared in oil, n (%)									
0: >= 4 servings/week	0	2 (7.69)		0	0		2 (6.45)	0	
0.5: 1 – 3 servings/week	8 (29.63)	6 (23.08)	0.48	12 (36.36)	7 (22.58)	0.28	14 (45.16)	11 (42.31)	0.57
1: < 1 serving/week	19 (70.37)	18 (69.23)		21 (63.64)	24 (77.42)		15 (48.39)	15 (57.69)	
Canola/olive oil, n (%)									
0: Not primary oil	18 (66.67)	20 (76.92)		19 (57.58)	14 (45.16)		1 (3.23)	1 (3.85)	
1: Primary oil	9 (33.33)	6 (23.08)	0.54	14 (42.42)	17 (54.84)	0.45	30 (96.77)	25 (96.15)	1.00
Wine, n (%)									
0: > 1 glass/day or never	26 (96.30)	25 (96.15)		30 (90.91)	29 (93.55)		29 (93.55)	25 (96.15)	
0.5: 1 glass/month – 6 glasses/week	1 (3.70)	0	1.00	3 (9.09)	1 (3.23)	0.61	1 (3.23)	0	1.00
1: 1 glass/day	0	1 (3.85)		0	1 (3.23)		1 (3.23)	1 (3.85)	

[a] Data missing for one dietary assessment at BL2. [b]P-value for MIND diet score derived from a double sample, double sided t-test, P-value from dietary components derived from Fisher's Exact test. *Significant value

Abbreviations: MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay

4.6.2 Diet: difference between BL1 and BL2 assessments

Table 18B provides further comparison between BL1 and BL2 assessments in terms of the MIND diet score and calculated Omega 3 PUFA intake. Only those participants who followed through from BL1 to BL2 and completed both cognitive and dietary assessments were included in the analysis, thus N = 52.

The MIND diet score improved slightly, but significantly (P = 0.010) by 0.55 points, which represents 7.47%. Total Omega 3 LCPUFA, EPA and ALA intake did not change significantly from BL1 to BL2. The intake of DPA and DHA decreased significantly between BL1 and BL2 with P-values of 0.001 and 0.018 respectively. The same results but differentiated between groups, are presented in Table 18C. The intervention group had a significantly different (P = 0.03) in DPA intake from BL1 to BL2, whereas the control group showed significant differences (P = 0.01; P = 0.01) for the MIND diet score and DPA intake.

Table 18B: Dietary intake (MIND diet and fatty acids) at BL1 and BL2

Dietary intake variables	**Baseline 1 Assessment (N = 52)**		**Baseline 2 Assessment (N = 52)**		**P-value[a]**
	Mean (SD)	95% CI	Mean (SD)	95% CI	
MIND diet (score out of 15)	7.36 (1.48)	6.94; 7.77	7.91 (1.39)	7.53; 8.30	0.01*
Total Omega 3 LCPUFA (mg)	800 (437)	678; 922	731 (465.)	602; 861	0.28
EPA (mg)	197 (200	142; 253	194 (253)	124; 265	0.93
DPA (mg)	2 (3)	2.; 3	1 (2)	1; 2	0.00*
DHA (mg)	2105 (158)	167.; 255	154 (18-)	118.; 191	0.02*
ALA (mg)	388 (385)	281; 500	383 (351)	285; 480	0.91

[a] P-value determined by double sided paired t-test. *Significant value
Abbreviations: ALA - Alpha Linolenic Acid, DHA - Docosahexaenoic Acid, DPA - Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay

Table 18C: Dietary intake at BL1 and BL 2 by intervention and control groups

Dietary intake variables	Intervention group (n = 26)[a]					Control group (n = 26)				
	Baseline 1		Baseline 2		P-value[a]	Baseline 1		Baseline 2		P-value[a]
	Mean (SD)	95% CI	Mean (SD)	95% CI		Mean (SD)	95% CI	Mean (SD)	95% CI	
MIND diet (score out of 15)	7.73 (1.42)	7.16; 8.30	8.08 (1.36)	7.53; 8.63	0.1	6.98 (1.47)	6.39; 7.58	7.75 (1.43)	7.17; 8.33	0.01*
Total Omega 3 LCPUFA (mg)	859 (446)	680; 1039	730 (438)	553; 907	0.23	741 (429)	567; 914	733 (498)	532; 934.87	0.91
EPA intake (mg) in milligrams	256 (252)	154; 357	230 (334)	95; 364	0.67	138 (104)	96; 180	159 (127)	108; 210	0.27
DPA intake (mg) in milligrams	3 (3)	2; 4	2 (2)	1; 3	0.03*	3 (2)	2; 4	2 (2)	1; 2	0.01*
DHA intake (mg) in milligrams	237 (167)	170; 304	166 (163)	100; 231	0.07	184 (148)	125; 244	143 (95)	105; 181	0.15
ALA intake (mg) in milligrams	364 (403)	201; 526	333 (245)	234; 432	0.68	413 (373)	262; 564	432 (431)	258; 607	0.77

[a]P-value determined by a double sided paired t-test. *Significant value
Abbreviations: ALA – Alpha Linolenic Acid, DHA - Docosahexaenoic Acid, DPA - Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay

4.6.3 Diet: effects of the intervention (change between BL2 and PI)

Tables 18D - E present the effects of the intervention. As discussed in Chapter 3, paragraph 3.9.2.2, the intervention and control groups were compared in respect to outcome variables (MIND, calculated Omega 3 PUFA intake, RBC Omega 3 PUFA) using non-parametric ANCOVA with bootstrap estimation and covariates. This analysis was also done when last observations (BL2) were carried forward when PI data was missing (i.e. with imputation). For comprehensiveness, values with, and without imputation, are presented.

Refer to Figure 5 for a graphical presentation of the predicted MIND diet score at different assessments over time.

The intervention group started with a slightly higher score than the control group at BL1, but then increased very slightly between BL1 and BL2, whereas the control group

that started with a lower score increased to a greater extent than the intervention group. Interestingly, the scores of the intervention and control groups intersect at BL2, from which point the control group keeps increasing with a steeper curve than the intervention group.

The MIND diet score did not differ significantly at PI between the two groups when values were imputed. Without imputation the control group scored significantly (P = 0.04) higher by 0.58 points (3.87%).

The covariate, MIND diet score at BL2 was significant (P < 0.001) with, and without imputed values, therefore, adjusting for MIND diet score at BL2 was necessary to prevent it from influencing the PI MIND diet score prediction. The covariates "education category" and "Omega 3 PUFA supplementation" did not have any significant effect.

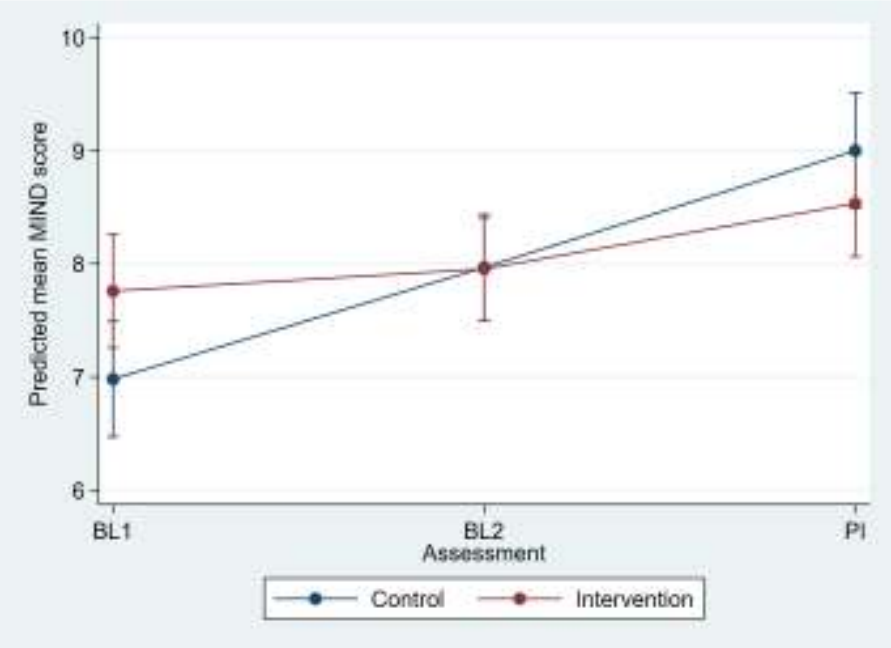

Figure 5: Predicted mean MIND diet score at BL1, BL2 and PI with imputation

Table 18D gives an overview of the PI Omega 3 PUFA intake as predicted by non-parametric ANCOVA. Total Omega 3 LCPUFA intake was significantly different (P<0.001) between the intervention and control groups at PI when regression was with, and without imputation, done. With imputation, the difference was 635.91mg, and without imputation it was 642.12mg.

The covariate, “total Omega 3 LCPUFA intake” at BL2 was significant (P<0.001), both with imputed values, and if no values were imputed (P=0.007). Neither education category, nor Omega 3 PUFA supplementation, had a significant effect when treated as covariates. Refer to Figure 6 for the predicted mean of total Omega 3 LCPUFA intake at different assessments over time. Although the intervention group had a slightly higher predicted mean of total Omega 3 LCPUFA intake, the two groups were quite similar at BL1, with less than a 100mg difference. At BL2 their intake seems to be the same, after which there is a steep upward increase in the curve for the intervention group to PI, where the intake is approximately twice that of the control group, which showed a smaller incline in the curve.

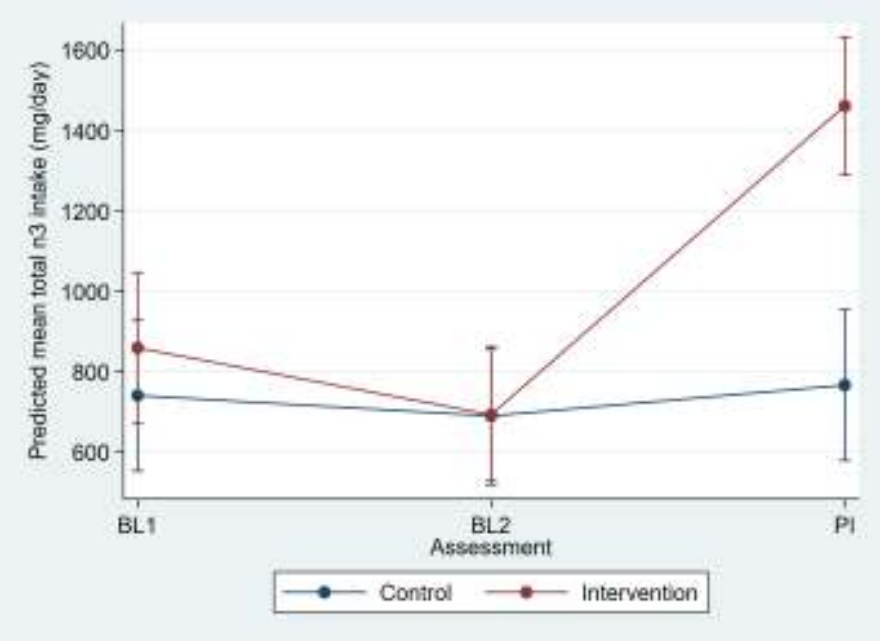

Figure 6: Predicted mean calculated total Omega 3 LCPUFA intake at BL1, BL2 and PI with imputation

The EPA intake followed a similar pattern with a significant difference (P<0.001) of 364.17mg (with imputation) and 385.02mg (without imputation) between the two groups at PI. EPA intake at BL2 had a significant effect (P = 0.014 and P = 0.023) with, and without imputation. Education category and Omega 3 PUFA supplementation had no significant effect. Refer to Figure 7 for change in predicted mean over the course of the study. Throughout the study the control group seemed to have had a lower predicted mean intake of EPA than the intervention group. The intervention group

started with a predicted mean intake above 200mg at BL1, this mean decreased slightly to 200mg at BL2, and increased rapidly to almost 500mg at PI. However, as mentioned before, only the PI assessment indicated a significant difference between the two groups.

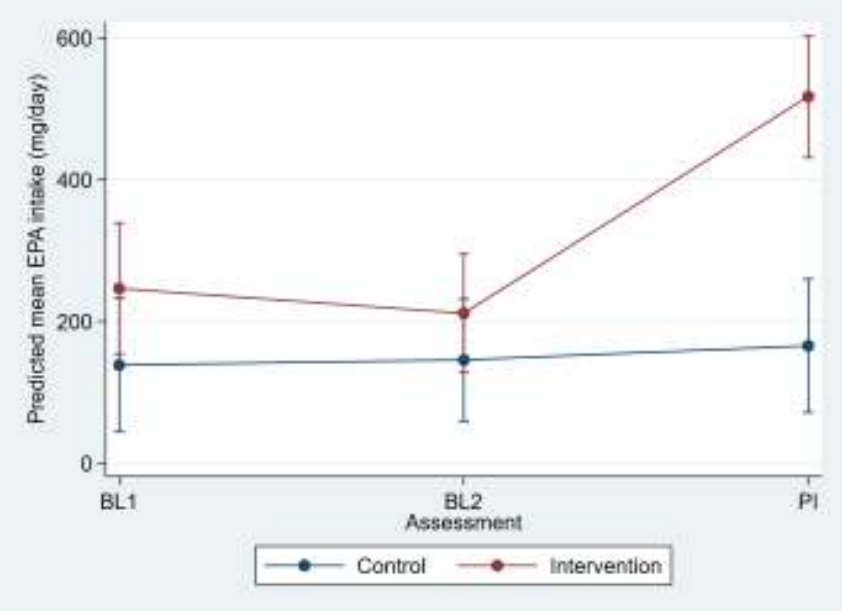

Figure 7: Predicted mean calculated EPA intake at BL1, BL2 and PI with imputation

At PI, DPA intake also presented with a significant difference of 3.13mg (P=0.004) between the intervention and control groups when values were imputed. Similarly there was a 3.65mg difference (P=0.001) without imputation. The covariates, DPA intake at BL2, education category and Omega 3 PUFA supplementation displayed no significant effect.

At PI, with and without imputed values DHA intake was significantly different between the two groups. With imputation the difference was 188.32mg (P<0.001) and without imputation the effect was 196.65mg (P<0.001). Intake of DHA at BL2 was significant as a covariate. None of the other covariates exerted a significant effect.

There was no significant difference in the ALA intake at any assessment between the two groups. Covariate, ALA intake at BL2, displayed a significant effect of 0.57mg (P=0.016).

Table 18D: Comparison of groups (intervention and control) in respect to MIND diet score and calculated Omega 3 PUFA intake at PI

Dietary intake variable	With/without imputation	Observations	Mean (95% CI)		Intervention vs Control		Covariates					
		Number [a] of observations]	Intervention group	Control group	Estimated effect	P – value[b]	Estimated effect (BL2)[c]	P – value[b]	Estimated effect (Education)	P – value[b]	Estimated effect (n3 supplements)	P – value[b]
Total MIND (15)	With imputation	64	8.44 (8.06; 8.76)	8.93 (8.51; 9.35)	-0.48	0.07	0.41	0.00*	-0.13	0.638	0.21	0.44
	Without imputation	56	8.47 (8.06; 8.86)	9.05 (8.57; 9.53)	-0.58	0.04*	0.40	0.00*	-0.11	0.705	0.36	0.15
Total Omega 3 LCPUFA intake (mg)	With imputation	62	1360 (1164; 1589)	724 (562; 896)	635.91	0.00*	0.88	0.00*	205.26	0.120	-84.81	0.65
	Without imputation	55	1416 (1213; 1702)	774 (605; 1009)	642.12	0.00*	0.75	0.01*	149.32	0.328	-42.63	0.87
EPA intake (mg)	With imputation	63	499 (377; 637)	134 (72; 204)	364.17	0.00*	0.74	0.01*	-56.22	0.360	3.89	0.97
	Without imputation	55	534 (402; 678)	149 (59; 223)	385.02	0.00*	0.73	0.02*	-91.81	0.176	58.99	0.64
DPA intake (mg)	With imputation	62	4 (3; 6)	1 (1; 2)	3.13	0.00*	-0.13	0.78	-0.63	0.448	0.24	0.86

Dietary intake variable	With/without imputation	Observations	Mean (95% CI)		Intervention vs Control		Covariates					
		Number [a] of observations]	Intervention group	Control group	Estimated effect	P – value[b]	Estimated effect (BL2)[c]	P – value[b]	Estimated effect (Education)	P – value[b]	Estimated effect (n3 supplements)	P – value[b]
	Without imputation	55	5 (3; 7.)	1 (0; 2)	3.65	0.00*	-0.40	0.50	-0.47	0.640	-0.05	0.98
DHA intake (mg)	With imputation	62	295 (243; 356)	107 (75; 135)	188.32	0.00*	0.52	0.02*	-26.49	0.377	15.58	0.75
	Without imputation	54	319 (249; 369)	122 (67; 137)	196.65	0.00*	0.29	0.22	-49.45	0.159	29.96	0.62
ALA intake (mg)	With imputation	62	554 (422; 715)	456 (318; 601)	98.01	0.35	0.57	0.02*	170.94	0.153	-128.60	0.25
	Without imputation	55	588 (437; 754)	498 (307; 712)	90.90	0.50	0.29	0.36	183.50	0.210	-170.50	0.22

[a] N may differ due to missing data or participants not taking in the nutrient
[b] P-value was derived from non-parametric ANCOVA with covariates (score of relevant outcome variable at BL2, education category and Omega 3 supplement intake)
[c] Baseline 2 value of relevant variable
*Significant value
Abbreviations: ALA – Alpha Linolenic Acid, DHA – Docosahexaenoic Acid, DPA - Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, MIND – Mediterranean-DASH Intervention for Neurodegenerative Delay

4.7 RBC Omega 3 PUFA as secondary outcome

4.7.1 RBC Omega 3 PUFA: effects of the intervention (change from BL2 to PI)

Table 18E summarises the PI values for RBC Omega 3 PUFA and Omega 6 AA as predicted by non-parametric ANCOVA with covariates (BL2 assessments for relevant variables, education category and Omega 3 PUFA supplementation). Fifteen participants did not consent for a blood sample to be taken.

There was no significant difference between the RBC Total Omega 3 LCPUFA content in the blood samples of the two groups PI. With imputation the covariate RBC Total Omega 3 LCPUFA content at BL2 was significant (P=0.047). Omega 3 PUFA supplementation as covariate was significant (P=0.033). Education category had no significant effect. All analyses done without imputation showed no significant effects. Refer to Figure 8 for pattern of predicted mean RBC Total Omega 3 LCPUFA at BL2 and PI.

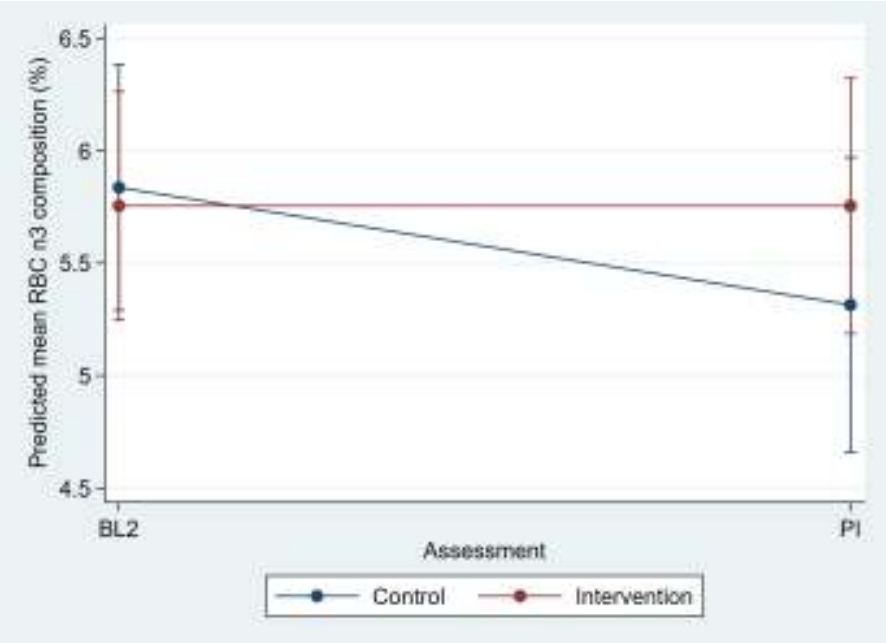

Figure 8: Predicted mean RBC total Omega 3 LCPUFA composition at BL2 and PI with imputation

Both with, and without imputation, the two groups differed in respect to mean RBC EPA content. With imputation there was a meaningful effect 0.11 (P=0.004) and without imputation 0.12 (P=0.001). In either case, the covariate RBC EPA content at

BL2 was also significant (P=<0.001), which confirms the necessity to adjust for baseline. Neither education category, nor Omega 3 PUFA supplementation showed a significant effect as covariates. Refer to Figure 9 for a graphical presentation of the change in RBC EPA content from BL2 to PI. The intervention group presented with a steep upward curve, whereas the slope of the control group curve was slightly lower.

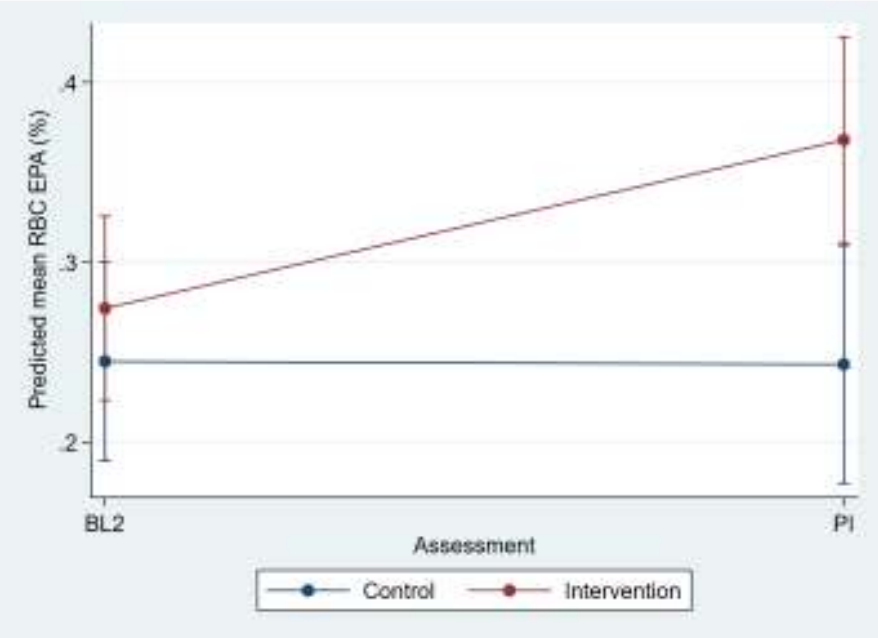

Figure 9: Predicted mean RBC EPA composition at BL2 and PI

The RBC DPA content followed a similar trend, namely a significant difference between the two groups with, and without imputation. When values were imputed, the estimated difference was 0.14 (P=0.013) and without imputation the difference was 0.18 (P=0.008). Covariate RBC DPA content at BL2 also had a significant effect (P = 0.000) with imputation and (P = 0.001) without imputation. The other covariates did not exert any significant effect.

The difference in RBC DHA between the intervention and control groups was not significant. When the regression was done with imputation, covariate RBC DHA content at BL2 had a significant effect. (P=0.013). Covariate Omega 3 PUFA supplementation was close to having a significant effect. (P-value=0.052). The rest of the analyses did not indicate any significant effects.

RBC ALA content was not significantly different between the two groups. The covariates (BL2, education category and Omega 3 FA supplementation) also did not exert any significant effect

Table 18E: Comparison of groups (intervention and control) in respect to RBC Omega 3 PUFA content at PI

Biomarker		Number of observations[a]	Mean (95% CI)		Intervention vs Control		Covariates					
			Intervention group	Control group	Estimated effect	P – value[b]	Estimated effect (BL2)[c]	P - value[b]	Estimated effect (Education)	P – value [b]	Estimated effect (n3 supplements)	P – value[b]
RBC Total Omega 3 LCPUFA % w/w	With imputation	56	5.91 (5.35; 6.35)	5.60 (4.97; 6.10)	0.32	0.330	0.36	0.047*	-0.47	0.151	0.94	0.033*
	Without imputation	41	5.73 (5.25; 6.37)	5.53 (4.59; 6.25)	0.20	0.697	0.14	0.666	-0.37	0.380	1.38	0.070
RBC EPA (% w/w)	With imputation	55	0.34 (0.27; 0.41)	0.24 (0.19; 0.26)	0.11	0.004*	0.89	0.000*	-0.04	0.325	-0.00	0.973
	Without imputation	42	0.37 (0.29; 0.44)	0.25 (0.18; 0.28)	0.12	0.011*	0.79	0.001*	-0.07	0.140	-0.01	0.882
RBC DPA (% w/w)	With imputation	56	1.44 (1.36; 1.53)	1.31 (1.24; 1.40)	0.14	0.013*	0.65	0.000*	-0.8	0.160	0.08	0.322
	Without imputation	41	1.43 (1.31; 1.50)	1.25 (1.14; 1.35)	0.18	0.008*	0.50	0.001*	-0.07	0.267	0.07	0.433
RBC DHA (% w/w)	With imputation	56	3.87 (3.48; 4.24)	3.74 (3.38; 4.15)	0.13	0.586	0.39	0.013*	-0.27	0.226	0.64	0.052
	Without imputation	42	3.63 (3.24; 4.16)	3.72 (3.19; 4.17)	-0.09	0.788	0.19	0.383	-0.23	0.438	0.80	0.076
RBC ALA (% w/w)	With imputation	56	0.05 (0.04; 0.06)	0.04 (0.04; 0.05)	0.01	0.196	0.47	0.072	-0.01	0.64	0.01	0.856
	Without imputation	42	0.06 (0.05; 0.07)	0.05 (0.04; 0.06)	0.01	0.173	0.34	0.296	-0.01	0.41	0.00	0.689

Biomarker		Number of observations[a]	Mean (95% CI)		Intervention vs Control		Covariates					
			Intervention group	Control group	Estimated effect	P – value[b]	Estimated effect (BL2)[c]	P - value[b]	Estimated effect (Education)	P – value [b]	Estimated effect (n3 supplements)	P – value[b]
RBC Total Omega 6 LCPUFA (% w/w)	With imputation	56	28.56 (27.83; 29.80)	30.53 (29.54; 31.53)	-1.97	0.007	0.30	0.096	0.83	0.376	-0.11	0899.
	Without imputation	42	28.36 (27.33; 29.15)	29.52 (28.74; 30.84)	-1.16	0.113	0.13	0.287	0.57	0.422	-0.25	0.785
RBC AA (% w/w)	With imputation	56	14.35 (13.81; 14.86)	14.87 (14.36; 15.42)	-0.52	0.156	0.09	0.302	-0.34	0.355	-0.23	0.686
	Without imputation	42	14.25 (13.71; 14.81)	15.08 (14.59; 15.65)	-0.84	0.050*	-0.03	0.672	0.14	0.722	-0.08	0.896
RBC Omega6: Omega 3	With imputation	55	5.12 (4.42; 5.62)	5.96 (5.30; 6.38)	-0.84	0.030*	0.45	0.003*	0.42	0.220	-0.92	0.049*
	Without imputation	42	5.05 (4.31; 5.39)	5.85 (5.19; 6.49)	-0.80	0.079	0.30	0.139	0.45	0.240	-1.00	0.070

[a] N may differ due to missing data. [b] P-value was derived from non-parametric ANCOVA with covariates (score of relevant outcome variable at BL2, education category and Omega 3 supplement intake). [c]Baseline 2 value of relevant variable. *Significant value Abbreviations: AA – Arachidonic Acid; ALA – Alpha Linolenic Acid, DHA – Docosahexaenoic Acid, DPA - Docosapentaenoic Acid, EPA – Eicosapentaenoic Acid, LCPUFA – Long Chain Polyunsaturated Fatty Acids, RBC – Red Blood Cell

4.8 Conclusion

The significant difference in predicted mean total CASI score PI between the two groups may be an indication of the positive effect that the fish supplementation had on the cognition of the intervention group. It seems as if both the intervention and control groups adhered sufficiently to their respective study foods, because significant differences in PI values for RBC EPA and DPA between the two groups were predicted by means of the non-parametric regression.

CHAPTER 5

DISCUSSION, RECOMMENDATIONS AND CONCLUSION

5.1 Introduction

Dementia is a global problem and research on preventative strategies is merited. Diet as part of the preventative strategy gained interest in many studies and evolved from the focus on a single nutrient approach to a multi-nutrient and even a whole food-based approach.[4] At the onset of this study very few articles focusing on dietary prevention of cognitive decline in LMIC could be found, which highlighted a gap in the research.[2] Hence the focus of this research, to assess the effect of Omega 3 PUFA-rich fish within the context of an enhanced usual diet on cognition in resource-limited elderly participants.

This chapter is structured to discuss the interplay between cognition and diet. The setting and design of the study, the sample, the strengths and limitations, the conclusion and recommendations are also discussed under separate headings.

5.2 Study design

Population based, parallel group randomised controlled trials are viewed as the gold standard for assessing the effectiveness of an intervention.[31] Although this would be the best potential design for the current study, it had its limitations. (Refer to paragraph 5.7.2) There should be a high homogeneity within the population to increase the likelihood of demonstrating the relationship between exposure and outcome.[31] As described in paragraph 5.2, monthly income was the main criterion used to identify the study population and not age as such. Although the age range was wide the mean ages of the intervention and control groups were similar. It was, however, attempted to keep the sample as homogenous as possible by using an MMSE cut-off score to ensure that the participants in the study had similar cognitive abilities. Exclusion criteria were also applied before data analysis to increase the homogeneity of the analytical sample. Another limitation of the design is that effectiveness is assessed by average treatment effect without considering individual specific characteristics which may have had an influence on treatment response.[31] A relevant example would be the possible difference in effect of the Omega 3 PUFA-rich fish in the participants with varying levels of Omega 3 PUFA status at the start of the study.

The study was designed as a food-based randomised controlled trial. It was essential for this trial to be food-based, although this created challenges on many levels. Food-based studies are scarce, but in the final analysis, humans eat foods, not nutrients and the

sustainability of the dietary intervention in question matters. As indicated in the title, the study focused on a resource restricted community. Consequently their financial resources would not allow them to continue with long term supplement use. As the study population was vulnerable in more than one way (they were older people and resource restricted), it was decided to enhance the diet for both groups, and make the fish component (and by extrapolation the Omega 3 PUFA intake) the variable that differed. Although this was the correct ethical route to follow, it possibly clouded the magnitude of the effect attributed to adding fish.

5.3 Sample

The initial sample size estimation was based on assumptions made from the data observations in a large USA cohort.[130] It was assumed that the data would have a normal distribution and that a SD of 3.54 for the total CASI score would apply. For data observed in this current (South African) study, the above mentioned assumptions did not hold true, the data did not follow a normal distribution and the SD exceeded 3.54. A non-parametric ANCOVA, in the format of nonparametric regression, was employed for the data analysis to account for the skewness of the data distribution. Despite the relatively small sample size, the ANCOVA detected a significant difference between cognition of the intervention and control groups. The smaller sample size could be attributed to the size of the study population which did not allow for a high attrition rate.

5.4 Cognition and function

As stated in Chapter 2, cognition can be defined as: “any and all processes by which a person becomes aware of his/her situation, needs, goals, and required actions, and uses this information to implement problem solving strategies for optimal living”[20] It includes aspects such as: perceiving, thinking, knowing, reasoning, remembering, analysing, planning, paying attention, generating and synthesising ideas, creating, judging, being aware, and having insight.[20] The change in cognition as reflected by the CASI score was the primary outcome of this study. This section on cognition is structured according to the following diagram. Refer to Figure 10.

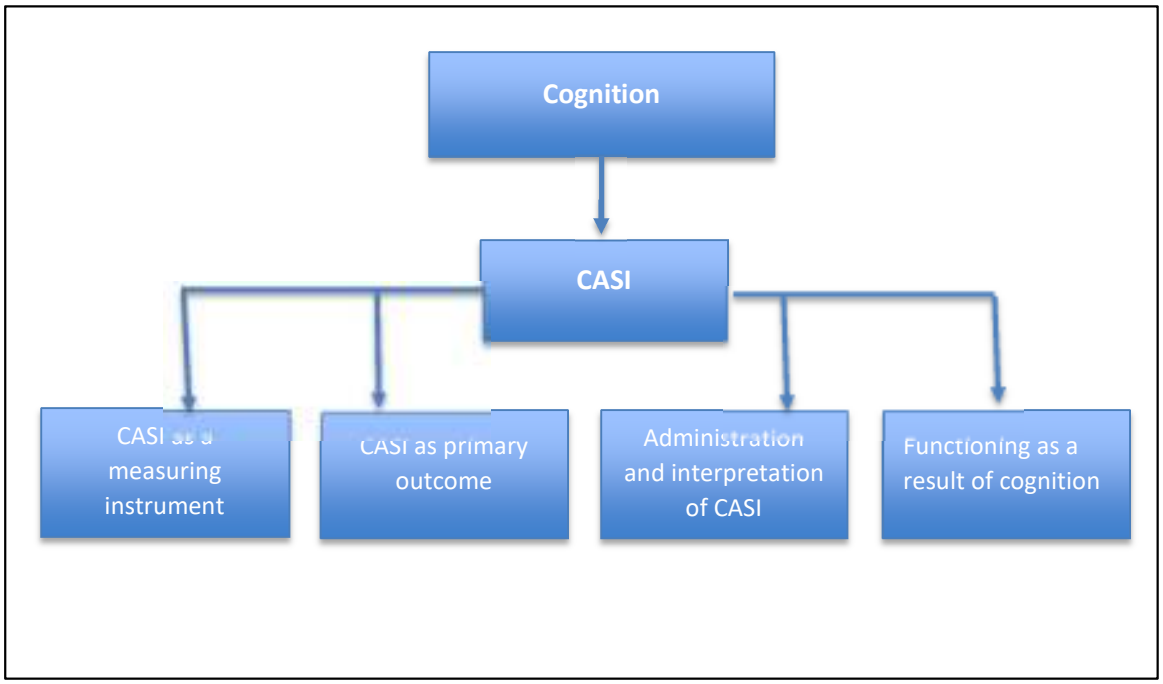

Figure 10: Structure for discussion of cognition

5.4.1 CASI as a measuring instrument

Different measuring instruments were considered as possible assessment tools for cognition. They ranged from a basic screening tool such as the MMSE to complex neuropsychological batteries that need to be executed by neuro- or clinical psychologists.[126] According to Palta et al. (2016) the following factors need to be considered before deciding on an appropriate measuring instrument for a research study: length and ease of administration, the extent to which the test will burden the participant, the psychometric properties and how generalisable results are. Cost constraints and availability of administrators may limit use of complex neuropsychological batteries in research studies.[126] Two other considerations specific to the current study were that the instrument should be suitable to detect change in people living with normal cognitive levels as well as in those who already have impairment. Secondly the instrument's use in people with a lower literacy level should be supported by the literature. All the above mentioned factors were taken into account when deciding on an instrument of choice for the current study.

Based on these criteria, the Cognitive Abilities Screening Instrument (CASI) was identified as the appropriate test to measure change in cognition for the purpose of this study. The CASI was *per se* developed for quantitative research purposes.[16] More detail on the

CASI is given in Chapter 3 under paragraph 3.8.1.2. If the three main characteristics of the CASI are summarised, it is evident why this test was used in the current study. The CASI can be used cross-culturally, it contains nine domains and the MMSE score can be directly extracted.[140] Both the CASI and the MMSE have been used in a number of good / fair quality studies in settings were educational levels and literacy were low.[127]

As is the case with all the cognitive screening instruments, the CASI is influenced by age and education.[16,130] Reference values based on data from a cohort of 2500 people in the USA for five year age categories were considered when the statistical analysis was planned.[130] Education category was treated as a covariate in the present study but did not show any significant effect until non-parametric ANCOVA with imputation was applied. As mentioned in Chapter 3, the CASI had to be translated into Afrikaans. This was done by a content specialist (psychiatrist). The translated CASI was reviewed by another psychiatrist who also tested the instrument on patients independent of the study to compare its practicality with other similar instruments used in practice.

No publication on the test-retest reliability of the CASI or minimal detectable change (MDC) in people without diagnosed dementia could be found. One paper had been published on a study of the abovementioned concepts in people living with dementia. In this particular study, 52 patients with dementia completed the CASI twice, separated by a two week interval. The test-retest reliability of the total score (as examined through the class correlation coefficient) was 0.97 which indicated excellent test-retest ability in persons living with dementia.[140]

As previously mentioned, an MMSE[141] score generated from the CASI formed part of the exclusion criteria of the study. The MMSE cut-off score was lowered from the general value of 24[132] to 22 to include only those participants with comparable levels of cognition at the start of the intervention. The value was lowered to accommodate the level of education of the participants. In a Southern Brazilian sample with a similar socioeconomic – and education level, the global cut-off was also lowered from 24 to 23 for those participants with middle education levels and 22 for those with lower education levels.[142] The adjustment of this value is supported by a Cochrane review which states that the cut-off score which defines “normal” cognitive function is usually set at 24, but that it could theoretically vary between 1 to 30. [132]

A prospective cohort of older Puerto Ricans (Boston Puerto Rican Health Study) also made use of lower MMSE scores to define the cognitive function in their sample. For those participants who did not complete high school or a similar education certificate the cut-off score was 21, for those who completed high school it was 23 and for those with tertiary education the score was 24. In the Boston Puerto Rican Health Study the cognition of the participants whom had a higher intake of Omega 3 LCPUFA and a higher RBC Omega 3 LCPUFA concentration was associated with improved executive function for a two year follow-up.[96]

5.4.2 CASI score as the primary outcome

A concern underlying the identification of an appropriate measuring instrument is always sensitivity and this also applied to the CASI. A question arose was concern that the instrument would not be sensitive enough to detect change in cognition after a relatively short 12 week intervention.

To determine the sensitivity of the CASI to distinguish between participants with dementia versus participants in the control group, as well as its sensitivity to detect change in cognition, data from four sites in America and Japan were pooled. It seems that the more sensitive items (cognitive characteristics) which were used to distinguish between participants with dementia versus participants in the control group, were the short term memory, temporal orientation and the ability to fluently generate a list, whereas attention, language abilities and long term memory of essential personal information were the least sensitive.[16] The results of these four sites in America and Japan, showed that the sensitivity and specificity to detect change in cognition ranged from 91-95% for sensitivity and 91-94% for specificity.[16] It was decided that the CASI was the most appropriate choice for the current study with the focus on time frame and cost involved.

It was expected that the study population would score from 70-90 points on CASI[130] (P. Becker, personal communication, 19 June 2017), but in reality they obtained higher scores than anticipated with a mean score of 87.18 (± 6.35) at BL1 and 91.16 (± 4.86) at BL2. The CASI score for both groups was higher at BL2 than expected, leaving smaller room for improvement during the intervention phase.

There was a notable increase in total CASI score between BL1 and BL2. It can be attributed to a few possible factors. The attention the participants received by being invited to participate, attending the information meeting and undergoing the BL1 and BL2 assessment, may have contributed to an overall increased sense of wellbeing and worth which could have had a positive impact on cognition. Participants were aware that the focus of the study was on cognition and that may have caused them to focus more and concentrate better. They might have made changes to their diet for the better just by being asked about specific brain supportive foods at BL1. Although the CASI was developed for epidemiological studies the time period between the two assessments was only 12 we`eks (3 months). It can be argued that participants could still remember the questions. In defence of the instrument it must be kept in mind, that it was developed to accommodate repeated assessments.[16]

As noted by the researchers in the Fins-Teens study a possible learning effect can occur if the same cognitive test is repeated, but such a learning effect will be equally distributed in both groups which makes detection of the intervention effect possible when the two groups are compared.[143] It is possible that participants could have been more at ease with the second and even third repetition.

Between BL2 and PI there was a smaller, but still statistically significant change in total CASI scores for both groups. Improvement in both groups was expected due the enhancement of the baseline diet. The effect of the MUFA in the canola oil and peanut butter and the plant protein in the legumes (the basic study foods for the overall enhancement) should not be underestimated, as it has been suggested in other studies, that MUFA may play a neuroprotective role.[64] The influence of diet is discussed more comprehensively in paragraph 5.6.

There was also a statistically significant difference of 2.3 points between the two groups, which suggests that the Omega 3 PUFA in the fish (received by the intervention group) may have exerted a statistically significant protective effect on cognition. In the initial protocol it was assumed that an overall (for both groups) 5 point change in CASI score would be clinically relevant and that the intervention group would have to score at least

2.5 points higher than the intervention group. As the baseline scores were higher than expected a 5 point change was unlikely.

A cognitive domain pertains to a typical approach to classify or characterise cognitive performance and it is originally related to the area of the brain in which these processes occur.[144] Disappointingly the scores for the nine domains were very similar at the different assessments and only one significant difference between groups could be detected. At PI there was a difference between the two groups for visual construction which entails the ability to copy or produce drawings of common objects. Visual construction comprises executive functioning, perceptual functioning and motor skills. Different cognitive domains need to be interpreted in context of each other, because they are interrelated, and no final conclusion about cognition can be drawn from a single domain only.[144] The fact that only the visual construction domain was influenced significantly cannot be used conclusively, but is may indicate the potential for further exploration in this specific domain. Because of the similarity in the domain scores no regression models could be applied to make any predictions.

5.4.3 Administration and interpretation of the CASI

A strength of the study is the fact that the CASI and Lawton were administered and interpreted by a psychometrist/psychologist, a professional who is trained in the administration of cognitive tests. It was unfortunate that the psychometrist who administered the assessments at BL1, had to withdraw and this also needs to be considered as a possible contributing factor to the big change in CASI scores between BL1 and BL2. Both psychometrists were, however, trained by the same psychiatrist in an effort to address interrater reliability.

5.4.4 Level of functioning as a result of cognition

Level of functioning can be influenced by many factors including impairment in cognition.[25,128] The instrument used for determining change in the level of functioning was the Lawton IADL which assesses more complex functions than a Basic Activities of Daily Living (BALD) tool. The instrument did not detect any change and limited descriptive statistics could be performed on the data. A possible explanation for the former is that the baseline cognitive scores were higher than anticipated and that the instrument was

not sensitive enough to pick up change in the specific sample. The Lawton scores were indicative of the high level of functioning that participants still possessed.

5.5 Diet

The dietary component of the study can be divided into two perspectives: the dietary exposure during the intervention phase (which includes the enhancement of the usual diet and the supplementation with Omega 3 PUFA-rich fish) and the dietary assessment that took place at BL1, BL2 and PI as part of establishing adherence. The additional discussion is structured according to the framework presented in Figure 11.

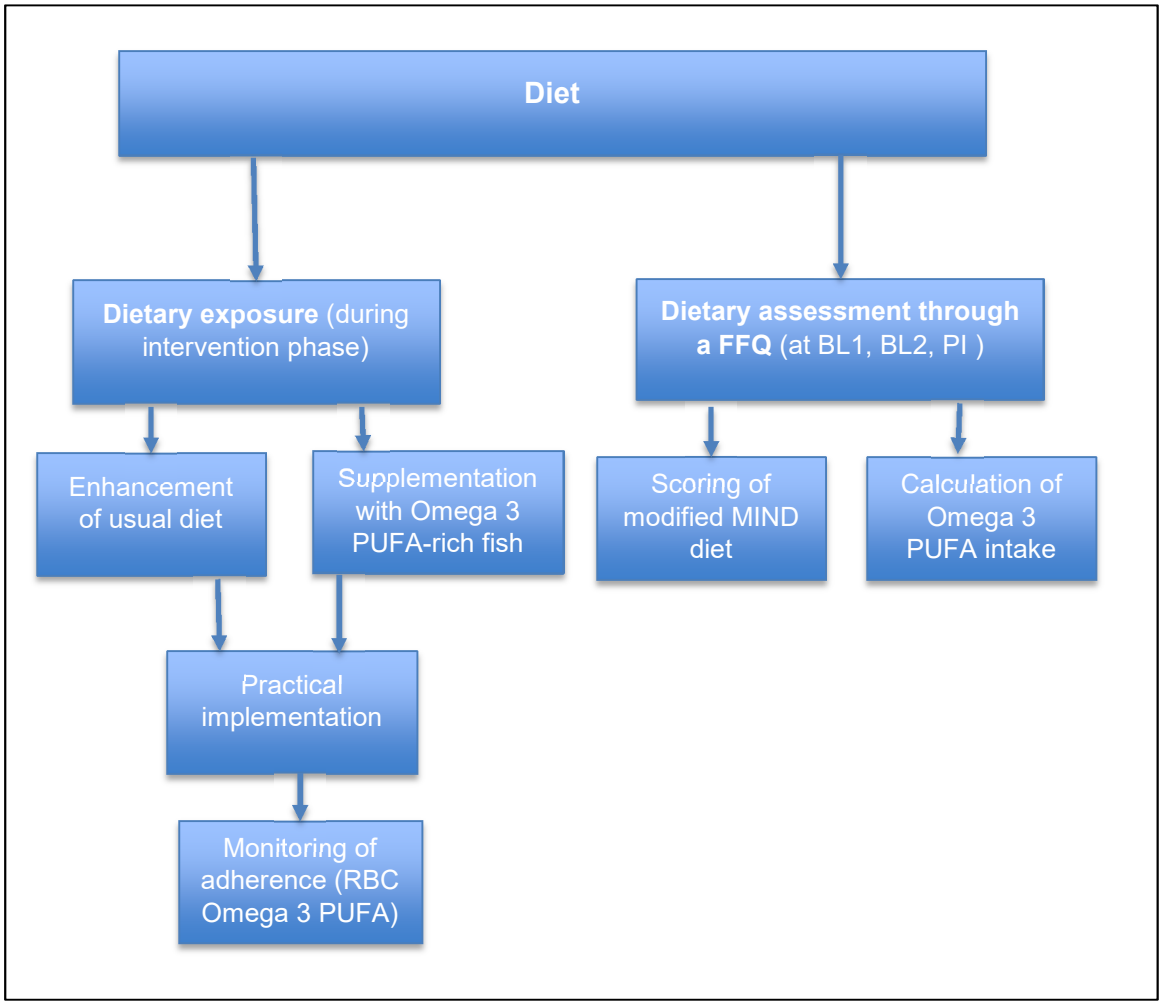

Figure 11: Structure for discussion of diet

5.5.1 Dietary exposure

5.5.1.1 Enhancement of usual diet

As noted in Chapter 2, the Mediterranean diet is the diet of choice for cognitive support. This recommendation poses challenges on many levels, such as cost of the diet, as well as adherence, if people are not used to including the foods characteristic of the Mediterranean diet in their usual eating pattern. The Medley study in Australia investigated the achievability of complying with the Mediterranean diet for 6 months in a group of older Australians. It was found that they were partially able to comply with the guidelines, but that culture and education still influenced their food choices. The predominant change was the increased use of olive oil[124]– changing to a different brand of oil is much more feasible than making big changes to a usual eating pattern. The former supports the inclusion of oil as part of the enhanced diet in the current study. Because the MIND diet (which is partially based on the Mediterranean diet) is specifically aimed at neurocognitive support, its components were used as guideline for the enhancement of the usual diet.

As discussed in Chapter 3, paragraph 3.7.1 the participants' usual diet was enhanced with canola oil, peanut butter and baked beans for both groups; canned pilchards and fish spread for the intervention group and canned meatballs and texturised soy protein for the control group. The other dietary components of the MIND diet were not supplemented due to a restricted budget, but intake was monitored with the aid of the modified MIND diet score.

An important component of both the Mediterranean and the MIND diets is olive oil which contains high amounts of biophenols and MUFA and has the potential to support cognitive function and may be used as a nonpharmacological strategy in the prevention of AD.[28] In the current study the olive oil was substituted with canola oil which also contains substantial amounts (Refer to Table 5 in Chapter 1) of MUFA, but is more affordable for the study population. The diets of both groups were enhanced with canola oil. The peanut butter (an affordable substitute for nuts) was another dietary source aimed at increasing MUFA consumption. The specific brand was chosen as a result of its total fat profile.(Refer to Table 10 in Chapter 3). The SFA was also taken into account and concentrations were kept as low as possible. This was done because research indicated a possible negative effect of high intakes of SFA on cognition.[27]

Another focus point of the enhancement was the supplementation of plant protein via canned beans (legumes). Not only did it address the challenge to provide sufficient protein, a deficiency of which is often associated with people with restricted financial resources,[145] but it also served as another cognitive supportive food. Elderly people especially those with restricted financial resources may be at risk of sarcopenia and protein energy malnutrition (PEM) because intake of animal protein sources tends to be low because of high costs.[145] In the current study, the same risk might apply, however no anthropometric data were collected. Legumes, a good source of affordable plant protein (of which the intake is promoted by the MIND diet), have been shown to exert neuroprotective effects.[146] As the diets of both groups were supplemented with legumes, the real exposure was determined by the presence or absence of fish as part of the study foods and is discussed comprehensively in paragraph 5.6.1.2 of this chapter. Because of the successful randomisation, the protective effect of the enhanced diet was expected to be equally distributed between the two groups, making comparison between the two groups regarding Omega 3 PUFA fish intake possible, even if it had an influence on the magnitude of the effect.

5.5.1.2 Supplementation with fish to obtain an intake of 2.2g Omega 3 PUFA per day

There are no international reference values (aimed specifically at cognitive support) for intake of Omega 3 PUFA and LCPUFA. This is evident from the wide range of recommendations used in intervention trials as discussed in Chapter 2. Therefore, the target intake for this study was based solely on amounts used in previous studies.

The initial target intake of Omega 3 PUFA intake of 2.2g/day was determined by identifying the range of Omega 3 PUFA amounts that seemed to have had an effect on cognition in similar studies.[4,9,13] This range was then compared to affordable food products which could deliver an amount of Omega 3 PUFA which was within the range. The duration of the intervention (12 weeks) was in line with similar studies.[4,142]

Few trials have been executed on the relation between change in cognition and supplementation of a usual diet with fish. Those that have been done, focused mainly on cognition in children and teenagers.[116,143,147,148] The relevance of these studies for comparison with the current study is questionable because of the differences in age

between the study populations. However due to the absence of published intervention studies in older populations, the former studies will be incorporated in the discussion. One trial focused on supplementing the usual diet of preschool children with Atlantic salmon.[115] (N = 205, 4-6 year olds). Although the target group was much younger than the current study population, it compares well with the current intervention in terms of portion size and duration, so that a change in cognition would be expected. For a period of sixteen weeks the children received either 50 – 150g salmon, or meat three times per week (hence 150 – 450g per week). In the current study the duration of the intervention was twelve weeks (it was therefore four weeks shorter), but the participants received 410 – 820g of fish per week. Refer to Table 11 in Chapter 3. In the children's trial there was a significant improvement in two out of eight scores of the Wechsler Preschool and Primary scale in the group of children receiving the fish. The plasma EPA and DHA of the fish group also increased significantly. The sixteen week period in the children's study was based on a recommendation by Stonehouse which found that brain fatty acid composition in non–human primates adapts within twelve weeks after increasing EPA and DHA intake, with detectable changes already occurring after one week.[147] In a German cohort of children an intake of 8g of fish per day (thus 56g per week) predicted a statistically significant probability that these participants would have a higher final mark in the subject of German language.[148]

A similar study in teenagers, the Fins-Teens study, supplemented the school lunch of teenagers three times per week with either Omega 3 LCPUFA supplements, or with meat, or with Omega 3 LCPUFA-rich fish (herring, salmon, mackerel: 80-100g servings). The duration of the intervention was twelve weeks and although there was a small beneficial effect of fatty fish compared to the other two groups, results were difficult to interpret due to low dietary compliance.[143] An interesting fact worth noting, is that adherence was also measured and where it measured 87% in the supplement group and 66% in the meat group, it was only 38% in the fish group. This highlights the possibility of food fatigue and also the challenging role that taste preference plays in food-based intervention trials.[143] In the 2018 published Fins-Kids Trial (N=232) which followed a similar design to the Fins-Teens study, significant cognitive improvement was seen only after adjusting for compliance. The trial had two arms, a fish and meat group and the lunch of pre-school children (aged 4 – 6 years) was supplemented with either Omega 3 PUFA-rich fish (50-80g) or meat three times per week for twelve weeks. Plate wastage per meal was

determined, hence the observation of low compliance.[116] In the current study, compliance and adherence were also of concern and these factors are discussed in more detail in paragraph 5.5.1.4.

The relationship between the Omega 3 PUFA-rich fish intake and cognition in the current study is also supported by the findings of a number of observational studies.[4,9,13] A small cohort study by Del Brutto et al. (2016) in a fishing community on the rural coast of Equador indicated a dose-dependent relationship between oily fish intake and cognitive performance especially in those who consumed fish as part of the Mediterranean Diet.[149] These findings were supported by a 2017 systematic review of prospective studies exploring the relationship between diet and the risk of cognitive decline, which found an inverse relation between fish intake and risk of developing dementia.[150]

5.5.1.3 Practical implementation

As noted in Chapter 3, the researcher offered specific foods to participants every week, instead of handing them out regardless of their needs and perceptions of amounts. This specific person-centred[151] approach was followed to maintain the good relationship between the researcher and participants in an effort to promote better adherence and compliance. Participants also appeared to be open and honest when they were questioned about their diets at the different assessments. Participants did not wish to take all the foods offered to them weekly. They perceived the amounts to be excessive. Therefore, specific foods were offered to them and they had the choice to take all, some or nothing. The foods and amounts were recorded by the researcher. Participants also recorded their intake on their record sheets and were asked to return the empty containers. The FFQ (as discussed under 5.5.2.1) administered at the assessments was used to report on final intake. Dietary intake (specifically in relation to the nutrients of interest) as assessed by the FFQ is believed to be an acceptable basis for comparing relevant consumption over time and between in the two groups.

5.5.1.4 Monitoring of compliance and adherence through testing of biomarkers

As stated in the previous section different taste preferences among participants are challenging in food-based intervention trials.[143] In the multi-domain Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) and Multidomain Alzheimer Preventive Trial (MAPT) (refer to Chapter 2, paragraph 2.3.4)

decreased compliance and adherence were noted as problem areas when interventions increase in complexity and intensity.[89] Therefore, in the present study, the intervention (handing out of foods) was approached in a person-centred manner.[151] This approach was followed to support compliance and to keep participants motivated to finish the trial. Food fatigue was also a very relevant concern in the study and much thought went into the monitoring of compliance and adherence. According to Chakrabarti (2014) compliance can be defined as: "the extent to which the patient matches the prescriber's recommendations."[18] In the present study the compliance was defined as the participants' commitment to come and collect the supplemental foods every week The participants valued the weekly personal contact with the researcher during which she asked participants about food preparation, interesting recipes and how they experienced the study.

Adherence is defined as: "the extent to which a person's behaviour, taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider."[18] In the present study it was defined as the participants' commitment to the intake of food and their honesty not to swop foods between the two groups. It was monitored by RBC fatty acid analysis immediately before (BL2) and after the intervention phase (PI).

The choice of erythrocytes or red blood cells (RBCs) as source biomarkers to monitor compliance in an Omega 3 PUFA intervention trial (whether food- or supplement based) is supported by research.[152,153] Not only do RBCs have a low biological variability, they are impervious to pre-analytical conditions (results are not dependent on the fasting state).[152,153] RBC membranes consist mainly of phospholipids and accurately reflect tissue fatty acid composition.[153] The RBC fatty acid concentrations offer a long-term (past three months) measure of Omega 3 PUFA exposure[96] and were for this reason chosen as adherence measure. In the present study the focus was on the change in the RBC EPA, DPA and DHA and whether the Omega 6 LCPUFA was replaced by the former.

As there was an increased concentration in RBC EPA (of which the fish used in this study is a concentrated source) and also a significant difference between the two groups in RBC EPA content. It can be assumed that the intervention group adhered to the fish supplementation. It is promising to observe a difference in Omega 3 LCPUFA. In the

Women's Health Initiative cohort (2017) (N = 6 706) higher RBC EPA and DHA levels were associated with a slower rate of cognitive decline over a 10 year period. The significantly lower Omega 6:Omega 3 ratio in the intervention group after only twelve weeks of intervention was an encouraging finding because it could possibly impact on the inflammatory profiles of the body and brain.[96] The difference in RBC PUFA PI values indicated that the control group did not eat fish (e.g. from the intervention group), but it did not necessarily mean they consumed the study foods.

5.5.2 Dietary assessment

5.5.2.1 Study specific FFQ

To examine the relationship between dietary intake and cognition is challenging when the diet in total is not controlled (supplied) by the intervention. To determine the effect of the intervention on cognition in the study, the usual diet at BL2 and the usual diet after the intervention (PI) should be within the same MIND diet score ranges except for the components directly influenced by the intervention such as the fish intake, nut intake, plant based protein intake and oil intake. A modified FFQ was used to assess dietary intake. Refer to Chapter 3, paragraph 3.8.3.4 for more detail on the compilation of the FFQ.

5.5.2.2 Scoring of the modified MIND diet

The MIND diet was developed by RUSH University in the USA.[15] As is the case with all the other studies on cognition and diet, application thereof in a LMIC such as South Africa had its challenges. South Africa, and more specifically the population which the study focused on, are resource-limited. Modifying the MIND diet to include more affordable foods which had a similar nutrient profile and theoretically would support cognition in the same way as the original MIND diet, was essential. Comparison of the findings of the current study with other South African studies was not possible as no other study on the MIND diet had been done in South Africa previously. There is also no relevant and recent national data in which elderly persons are well-represented, only data from fragmented studies.[154] Three studies could possibly be used for comparison, the study by Nel et al. (2002) which focused on the main food groups and average consumption per day in adults and children, the Cardiovascular Risk in Black South Africans (CRIBSA) study (2009) which focused on total energy distribution of macro nutrients in people above the age of

25 years and the PURE study (2017), a prospective cohort focussing on the association of fat and carbohydrate intake with cardiovascular disease and mortality in 18 countries. (Comparisons are made in the text per MIND diet component where applicable).

In the current study, the mean MIND diet scores of close to 7.5 out of 15 for both groups in all three assessments over time indicated a large potential for improvement. As with cognition, there was a significant increase in score for both groups in the non-intervention phase (P = 0.01). It is possible that participants adjusted their diets because of the awareness created by being exposed to the FFQ at BL1. Referring to Chapter 4, Figure 5, the two groups had similar scores at BL1 and intersected at BL2. The only significant difference between groups was seen at PI after the regression analysis (non-parametric ANCOVA) was done.

The control group ended up with a significantly higher MIND diet score than the intervention group at PI (P=0.04) indicating that their overall diet improved more throughout the intervention phase, than the diet of the participants in the intervention group. As the control group did not get fish supplementation, this result is rather unexpected. A possible explanation is that participants in the control group realised that the diet of the other group was superior because of the fish supplementation and that they compensated by being overly committed to the enhancement of their own usual diets. Another possibility is that the intervention group did not adhere fully to the diet. Theoretically the overall neuroprotective dietary component (excluding fish intake) as assessed by the modified MIND diet score is thus higher in the control group, which might have influenced the magnitude of the effect between the two groups.

When the individual dietary components were assessed, the only significant differences between the two groups were seen at BL1, where the intervention group had a lower intake of sweets and a higher intake of legumes, and at PI where the control group had a higher score for poultry intake. This can be attributed to the meatballs their diets were enhanced with, because the particular meatball used in the study was a combination of chicken and beef, and it was analysed as such.

Other differences between the two groups were observed, but these did not show any statistical significance.

Dietary components with the lowest score throughout the study, included wholegrains, healthy oil (low in SFA and Omega 6 LCPUFA), nuts, green leafy vegetables and red meat. The suboptimal intake of wholegrains can possibly be attributed to participants being set in their eating habits and finding it more convenient to eat processed grains, and not necessarily because of a dislike of wholegrains. The wholegrain intake corresponds with the low fibre intake as identified in a similar South African population in a cross-sectional study in 2001. The 2001 study identified other nutrients of concern, such as zinc and vitamin B6.[158] These particular nutrients are found in the food groups that are not consumed adequately by the current study population, indicating that this suboptimal intake may be a relatively widespread phenomenon and not only be restricted to the current study population only. The Dietary Intake of the Urban Black Population of Cape Town (CRIBSA) study,[156] was also a large cohort study which determined dietary intake in 19 – 64 year olds in 2009. The CRIBSA study additionally assessed change in dietary intake between 1990 and 2009. The findings also indicated a lower than recommended fibre intake in 1990 and in 2009. After twenty years there was still no significant change in fibre intake in the Capetonians of the CRIBSA study.[156]

Cost was tentatively a contributing factor to the high consumption of sunflower oil which is low in Omega 3 LCPUFA but high in Omega 6 LCPUFA. The same reason served as a motivation for the low intake of nuts. This is interesting, because they received social support from various companies and individuals from time to time to alleviate their financial situation. Just before the onset of the study (within the three months before BL1), each member of the study population received a container of nuts as part of a donation. This was incorporated into the data of BL1, but may be a skewed reflection of reality. A tendency identified by the CRIBSA study was that the PUFA intake increased between 1990 – 2009, namely both men and women in the particular study population consumed 10 -11.5% of their daily energy from PUFA.[156] The fact that consumers focus more on PUFA intake may work in favour of the implementation of the modified MIND diet, but careful consideration should be given to the Omega 3 to Omega 6 ratio.

Increased intake of fruit and vegetables is associated with reduced risk of cognitive impairment, but may be dependent on geographical region.[159] In the current study fruit and vegetables were not part of the intervention possibly due to the cost involved. Intakes of green leafy vegetables and berries were suboptimal in both groups. Circumstantial evidence suggests that participants eat the same foods out of habit and because their budget is planned around these foods. These findings are similar to the results from the CRIBSA study which also found fruit and vegetable intake to be less than the recommended amounts both in 1990 and 2009.[156]

The higher than expected intake of red meat by the participants in this current study, was also noteworthy because in South Africa red meat is usually more expensive than chicken. It is possible however, that this specific cultural group prefer red meat to chicken. It seems as if their intakes were mainly focused on the more affordable meat versions, such as minced meat and boerewors (processed minced meat prepared in a sausage).

When fish intake was assessed, both groups had relatively high scores throughout the study. At PI there was no significant difference in fish intake between the intervention and control groups. It is important to view this finding in perspective. The modified MIND diet scoring system may not be sensitive enough to detect differences in type of fish and frequency of intake more than one portion per week. However the control group possibly also scored well because the entire study population was familiar with the affordable canned fish (used in the intervention group) and was already including such fish in their diets. This fact probably also influenced the magnitude of the effect.

5.5.2.3 Calculation of Omega 3 PUFA intake

As discussed in Chapter 3, mean Omega 3 PUFA and -LCPUFA intakes were calculated based on the data (intake frequency, portion sizes and composition of fish and fish products) from the study specific FFQ at different assessments in time. This was done in an effort to determine whether Omega 3 PUFA intake approximated 2.2g/day as specified in the hypothesis. This approach had limitations: not all foods assessed by the FFQ containing Omega 3 PUFA were analysed (only the ones which are known as important sources e.g. fish and fish products), for some foods the manufacturer's labels were used (those foods that were handed out) and for others (consumed as part of the usual diet e.g. hake and tuna) the USDA Database[122] values were used, because Omega 3 PUFA

and -LCPUFA reference values were not available in the FoodFinder (v2019-07-01)[136] program used.

There was no significant difference between the two groups in Omega 3 PUFA and LCPUFA intake before the onset of the intervention phase. However, after the intervention, the intakes of the two groups differed significantly in relation to total Omega 3 LCPUFA, EPA, DPA and DHA. This may be an indication of the higher fish consumption in the intervention group. When the MIND diet score for fish intake was assessed independently, it seemed as if fish intake was similar in both groups at PI. However, the MIND diet score may not have been sensitive enough to detect the difference in portion size and the frequency of intake fish. The calculated Omega 3 LCPUFA showed that the intervention group consumed more fish than the control group during the intervention phase. At PI the intervention group's intake of total Omega 3 LCPUFA (1360mg) was approximately twice that of the control group (720mg) and dietary EPA (500mg) was four times higher than the value of the control group (130g). There was no significant difference in ALA consumption, which is indicative of the fact that the diet of both groups was enhanced with canola oil. When the intervention group's total Omega 3 LCPUFA (1360mg) and ALA (550mg) are added, the total Omega 3 PUFA intake is approximately 1900mg per day. Based on these calculations the conclusion can be made that the intervention group did not achieve the goal of ingesting 2.2g Omega 3 PUFA as specified in the hypothesis, but came close by achieving 86% of the planned intake.

5.6 Strengths and limitations

5.6.1 Strengths of the current study

The study was a real life, population based study. The intervention was also food- rather than supplement-based. The foods which were used to enhance the usual diet in both groups were well known to them, readily available and affordable which gave participants the opportunity to continue including them in their diet after the study ended. In other words the dietary intervention was sustainable. It also served as a starting point for the subsequent nutrition programme managed by the dietitian and resident social worker. Throughout the study, participants that withdrew were also eligible to receive study foods, but no participants wished to collect foods without being available for assessments.

The study design was another positive attribute of the study. It was a randomised controlled trial **(DOH-27-0618-6026)** and a first of its nature in South Africa. The study population (the elderly persons) was and is an under-researched group in South Africa. Because the study was conducted in a resource restricted community in a LMIC, it provided some valuable insights into the challenges researchers may face in similar situations dealing with this particular subject e.g. restricted funding, limited or no validated measuring instruments for the given study population and the feasibility of applying dietary guidelines from high income countries to less fortunate populations.

The results showed a notable change in cognition between BL1 and BL2, and it can be assumed that the Hawthorne effect (participants benefited simply by being part of the study) was partially responsible for the change. Being recognised as needed and by forming part of a group of identified individuals, may have positively impacted on their psychosocial wellbeing. Similar to the above mentioned enhancement, is the fact that participants with possible iron deficiencies (lower Hb values according to Haemocue testing) were identified and referred for follow-up. The participants identified with a risk for iron deficiency were provided with dietary advice on how to address a possible iron deficiency. Being identified and referred to a psychiatrist (via the social worker) might also be beneficial for those with a lower MMSE score.

In general the study was designed to support these participants on various levels (dietary, psychological, social) and was perceived to be a success in this regard. The closeness of the researcher to the participants and their weekly interaction can, however, be viewed as both a strength and a limitation. It might have supported trust, compliance and adherence, but it may also challenge replicability under other conditions.

5.6.2 Limitations of the current study

In the 2019 article by Soldevila-Domenech the unique challenges (cost, safety and sustainability for long term use) related to nutritional preventive intervention studies were highlighted.[31] This current study was no different and had a number of limitations. This section is structured according to type of limitation and source of bias that may have occurred.

5.6.2.1 Selection bias and sample size

Selection bias may have occurred as convenience sampling was used to compile the sample which consisted of volunteers from the study population. The small sample size was a serious limitation which had an influence on the power of the results. The main reason for the small sample size was related to the study population size of only 124 people and did not allow for a large attrition rate. The current attrition rate was 12%. Attrition occurred as a result of illness, death or personal reasons. It is believed that the researcher applied all possible measures to prevent attrition from happening. Refer to Chapter 3, paragraph 3.10.1 and Chapter 5, paragraph 5.6.1.4. Cost and logistical constraints did not allow the inclusion of more than one site

5.6.2.2 Limitations due to design

The motivation supporting the inclusion of the non-intervention phase was to assess what the normal change (change without any intervention) in cognition would be over twelve weeks. However including a non-intervention phase in the design was a limitation as participants seemed to have improved in cognition as a result of being part of a study. Refer to paragraph 5.6.2.5 for more details on the Hawthorne effect. The length of the intervention phase was also of concern and the question was asked if twelve weeks were long enough to have a measurable effect on cognition. The length of the intervention was motivated by the examples of other studies (as discussed in the Literature Review – Chapter 2) and could not have been longer since compliance and adherence were already challenged. Due to the cost involved foods used for the study were not chemically analysed by an outside laboratory. The researcher worked on the assumption that the label information as supplied by the manufacturer was correct and accurate. The USDA tables (not South African specific values) were used for analysis of study foods.

5.6.2.3 Instrument bias

Not one of the measuring instruments (CASI, Lawton, FFQ) were validated for the study population and could have resulted in instrument bias. This was addressed as far as possible by testing the instruments before the study on a similar population. (Refer to Chapter 3, paragraph 3.8.2) Another limitation was the fact that a screening tool and not a full neuropsychological battery was used for the assessment of cognition. The difficulty of identifying the proper measurement tool for cognition is discussed elsewhere. Refer to Chapter 1, paragraph 2.5.

5.6.2.4 Investigator bias

The possibility of investigator bias existed as the researcher was aware of the group allocation, responsible for the distribution of food and receiving the empty containers, conducting assessment interviews, analysing FFQs and entering data. The researcher did attempt to minimise investigator bias by conducting assessments strictly according to the questions of the FFQ. Once the FFQs were analysed and the data entered, only participant study numbers were used. Group allocation was done by an independent person. Refer to Chapter 3, paragraph 3.6. The group allocation numbers (group 1 for intervention group and group 2 for control group) were not indicated on the FFQs and entered onto the spreadsheet only after the dietary and cognitive assessment data had been entered. The psychometrist could also have contributed to investigator bias, but was blinded to the intervention and strictly asked questions as guided by the CASI and the Lawton measuring instruments. The fact that a different psychometrist was used at BL2 and PI than at BL1 was a limitation. Both of them were trained by the same psychiatrist to address this limitation as much as possible. The phlebotomists and the laboratory technicians (two additional sources of possible investigator bias) worked strictly on participant identification numbers and did not have access to group allocation numbers. Finally the biostatistician worked with participant identification numbers and group allocation numbers only. He was not informed of the details regarding the difference in dietary intake between the two groups.

5.6.2.5 Respondent bias

Various sources of respondent bias posed a risk. There was a high risk of cross contamination as total control over the actions of the two groups was impossible and they interacted with each other on a daily base. Although they had been asked not to exchange foods, this could have happened. The assumption was made that the subjects honestly and accurately reported on their intakes. The Hawthorne effect was of real concern as participants seemed to show improvement in cognition before the commencement of the intervention phase. However it was assumed that the Hawthorne effect, another source of respondent bias, may have occurred due to similarity of foods for the control and intervention groups. Refer to Table 8, Chapter 3 for the differentiation in study foods. As mentioned earlier, there was a risk of recall bias as the assessments were conducted with only an interval of three months separating them. The fact that the participants in the intervention group did not reach an intake of 2.2g Omega 3 PUFA per

day may also be a limitation. The recommendation of 2.2g was ranging in the higher levels of intake, specifically designed this way if intake is less.

5.6.2.6 Possible confounding factors

Trials with supplementation (whether food-based or not) may sometimes be disappointing because the type and dose of supplementation is based on the needs of the general population irrespective of the individual's nutritional status and needs e.g. a person with a lower Omega 3 PUFA status may respond differently to supplementation than someone with optimal stores.[14] There are factors which are known to alter an individual's response to the dose of supplementation such as the dose itself, bodyweight at baseline, gender, age, genetic factors, smoking and the dietary composition of the accompanying meal.[160] Insight into the interaction between nutrients may also be lacking.[14] For example, fish is a good source of Omega 3 PUFA, but is also rich in antioxidants and other vitamins which may promote neurovascular health through reduction of oxidative stress and chronic inflammation. It is challenging to try to identify the effect of a single dietary factor if the results may be due to a synergistic effect between different factors.[161,162] Hence the other nutritional qualities of fish may have acted as confounding factors in the current study.

Food interventions may also present with different effects when combined with other lifestyle factors such as level of activity.[163] Lifestyle could also be a possible confounder in research studies, as people who often consume fish and/or a Mediterranean diet may be more health conscious and their dietary intake may be associated with a healthier fat and salt profile.[162,164] Although the lifestyle of both groups in the current study seemed similar, lifestyle was not formally assessed which is a limitation of the study.

It is evident from the comprehensive list of limitations that there is great potential for improvement should the study ever be repeated. The following paragraphs therefore focus on recommendations, not only in terms of the research perspective,, but also in relation to the specific context where the study took place.

5.7 Conclusion

Considering that the majority of the research studies regarding dementia and its risk factors have been conducted in high HIC, there is a need for specific evidence on the impact of the risk factors in LMIC particularly in Africa.[2]

The 2020 Lancet commission report highlights the potential in LMIC for the prevention of dementia as an estimated 40% of cases may be due to modifiable risk factors.[2] Diet, especially the Mediterranean diet, is of interest.[2,3] Results of nutritional interventions are inconsistent.[31,84] However, the current study should be viewed as a stepping stone in the right direction, namely: creating awareness for the need of more research on cognition and diet in Africa. The change in CASI score over the course of the study (including the no-intervention phase) serves as motivation for future studies to focus not only on dietary interventions, but also on educational and psychological interventions as these may significantly impact on cognition even without any dietary changes. Similarly the modified MIND diet score can be used to guide dietary interventions or dietary education to motivate change of dietary habits in an elderly population. The study created awareness about the possible supportive role that diet (especially Omega 3 LCPUFA-rich fish) can play in a resource-restricted community in a LMIC. The significant difference in RBC EPA between the two groups after 12 weeks supports the intake of this relatively affordable type of fish in the particular study population.

The study also shows that dietary intervention later in life may still positively impact on cognition. Although the hypothesis that there would be no change in the cognition of the elderly as measured by the CASI score when their enhanced usual diet was supplemented with fish providing about 2.2g Omega 3 PUFA per day for 12 weeks could not be rejected because the fish intake (and hence the Omega 3 LCPUFA) was less than anticipated, a slight but significant change in the cognition of those who consumed the fish was observed. The current study showed that fish can have a significant effect on the cognition of resource-limited elderly after 12 weeks of supplementation of an enhanced diet.

5.8 Recommendations

5.8.1 Context specific practical recommendations

It is suggested that the retirement village used in this study (and similar ones in South Africa) promote awareness of the relationship between dementia and cognition through informative talks and newsletters. Another option is to inspire the gathering of residents with an interest in a specific condition such as dementia. By starting a health club (discussion group), that convenes monthly and is facilitated by a knowledgeable person,

people can be given an opportunity in a safe environment to ask questions, raise concerns and share experiences about a specific condition, such as dementia. Regarding nutritional support it is essential to continue the nutrition programme, but it is important that it should be approached in a multidisciplinary way to ensure effective management of resources.

Protein-energy undernutrition in elderly people is a relevant concern in the South African population.[165] A 2015 cross-sectional survey (N = 1008) by Naidoo et al. indicated that a possible 43.4% of the South African population aged 60 years and above might be considered at risk for malnutrition.[165] Malnutrition is related to a decline in general level of functioning and in itself may be a risk factor for the development of dementia and should be addressed.[166]

The mean MIND diet score for both groups indicated room for improvement. By continuing with the affordable enhancement (that formed part of the study foods) intake of MUFA and plant protein (two components of the MIND diet), are well supported. The addition of the canned fish, equal to one 410g can per week, may provide substantial support for the Omega 3 PUFA intake. The possibility of a vegetable garden for residents also needs to be discussed as higher vegetable intake will increase the modified MIND diet score. Focus should specifically be on intake of green leafy vegetables. This may work symbiotically with the fish intake to promote cognition.[120] Not only may a vegetable garden lend dietary support, it may increase functionality and as a result, quality of life. Some of the other components such as too high a red meat intake and the high intake of sugary foods can be addressed by informative health talks.

5.8.2 Future research considerations

A limiting factor in the current study was the lack of sufficient funding. The research was not funded and solely depended on donations by manufacturers, retailers and service providers (e.g. for drawing and analysis of blood). It is suggested that this study (or a study with a similar design and population), should be repeated with adequate funding in place. It may provide a more accurate insight into the challenges faced in a resource restricted community in a LMIC country. With proper funding the design could be strengthened in the following ways: allowing for a larger sample size in more than one setting, assessment of cognition through a comprehensive neuropsychological battery of

tests as administered by an expert, chemical analysis of study foods by an independent laboratory. A possibility for future research might be to consider using precision medicine where dietary interventions are tailored according to individual needs. As dementia is a heterogenous disease, precision medicine may be an evidence-based approach taking into account the inter-individual variability in response to treatment.[31]

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ANNEXURE A

Ethical Considerations

PARTICIPANT'S INFORMATION & INFORMED CONSENT DOCUMENT

TRIAL TITLE: Effect of 12 weeks of fish supplementation of an enhanced usual diet on cognition of resource limited independently living elderly in a retirement village: A randomised controlled trial

SPONSOR: Allen Park is one of the sponsors, the others are not confirmed yet.

Principal Investigators: Lizette Kühn

Institution: University of Pretoria

DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):

Daytime numbers: 072 514 0114

Afterhours: 072 514 0114

DATE AND TIME OF FIRST INFORMED CONSENT DISCUSSION:

23	**March**	**2018**
Dd	**Mm**	**Yy**

09:00
Time

Dear Resident

INTRODUCTION

You are **invited** to volunteer for a research study. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator. You should not agree to take part unless you are completely happy about all the procedures involved. In the best interests of your health, it is strongly recommended that you discuss with or inform your personal doctor of your possible participation in this study, wherever possible.

WHAT IS THE PURPOSE OF THE RESEARCH TRIAL?

The purpose of this trial is to determine whether the addition of certain foods to your usual diet may have an influence on your cognition (mental processes required for everyday living) and your functionality.

During the study you will receive foods to supplement your usual diet with on a weekly base. Participants will be randomly divided into two groups. This means that every household will be given a number. An outside person (that is somebody not involved in the study or in any way in the running of the retirement village) will draw numbers out of a hat to put the households of two people into one of the two groups and then will do the same for the single households. Both groups will receive foods, but the types of foods will differ slightly. The outside person will not know which foods each group will receive.

WHAT IS THE DURATION OF THIS TRIAL?

If you decide to take part you will be one of approximately 124 residents. The study will last for up to 7 (months): middle of April 2018 until middle of November 2018.

DESCRIPTION OF PROCEDURES

- Go through a screening process which will include the completion of a form asking about background but also health information. (Specific exclusion criteria will apply, for example being allergic to any of the foods that will be supplemented such as fish)
- If you pass the screening undergo assessments on your dietary intake by a dietitian and on your cognition and functionality by a psychometrist.
- These assessments will be in the form of questionnaires which will be completed by a psychometrist or dietitian and it will take at most 90 minutes.
- These assessments will be repeated 3 times and will take place in April, July/August and November 2018.
- Some people may be chosen to provide blood samples in order to determine the fat content of the blood. (These assessments will be repeated twice times over a three month period – thus July and August 2018). The people undergoing these blood tests will once again be selected by chance by an independent person. The reason for these tests is to determine whether you actually consumed the specific foods in each group adequately.
- In the period (August to November) you will be divided into one of two groups where you will accordingly receive supplemental foods for a 12 week period. You will have to collect these foods on a weekly base from the researcher on the Allen Park premises.
- Additional supporting services such as entering of lucky draws or attendance of group meetings (once per month) are available should you wish to participate.

HAS THE TRIAL RECEIVED ETHICAL APPROVAL?

This clinical trial Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 3563084 / 012 3563085 and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

WHAT ARE YOUR RIGHTS AS A PARTICIPANT IN THIS TRIAL?

Your participation in this trial is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other medical care. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest. If it is detected that you did not give an accurate history or did not follow the guidelines of the trial and the regulations of the trial facility, you may be withdrawn from the trial at any time.

MAY ANY OF THESE TRIAL PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

Venipunctures (i.e. drawing blood) which might be done as part of your assessment may pose the slight risk of discomfort. Drawing blood may result in a bruise at the puncture site, or less commonly

fainting or swelling of the vein, infection and bleeding from the site. Your protection is that the procedures are performed under sterile conditions by experienced personnel.

As people differ in sensitivity to certain foods, discomfort especially in the gut may occur e.g. a bloated 999999999999999999999999feeling or nausea. This will probably pass within a few days after adapting to the new dietary pattern, but you are at any stage welcome to withdraw from the study.

WHAT ARE THE BENEFITS TO YOU
By taking part in this trial you promote research regarding the relation between diet and cognition in South Africa.

WHAT ARE THE RISKS INVOLVED IN THIS TRIAL?
No medical risks known (if you are allergic to any of the study foods, you need to inform the investigator and be excluded from the study).

ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS TRIAL?
None

DISCONTINUATION OF TRIAL TREATMENT
You can discontinue the trial treatment at any stage without any explanation

INSURANCE AND FINANCIAL ARRANGEMENTS
The foods involved in this trial as well as all the assessments done, will be sponsored and you will not be expected to pay for it.

SOURCE OF ADDITIONAL INFORMATION
You are welcome to contact the principal investigator (Lizette Kühn) for the duration of this trial if you experience symptoms or problems, or if you have any questions. The 24 hour telephone number is 072 514 0114 through which you can reach her.

CONFIDENTIALITY
All information obtained during the course of this trial is strictly confidential. Data that may be reported in scientific journals will not include any information which identifies you as a patient in this trial.

INFORMED CONSENT
I hereby confirm that I have been informed by the investigator, Mrs. Lizette Kühn about the nature, conduct, benefits and risks of clinical trial. I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding the clinical trial.

I am aware that the results of the trial, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a trial report.

I may, at any stage, without prejudice, withdraw my consent and participation in the trial. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the trial.

Resident's name_____
(Please print)

Resident's signature_____ Date _____

I, Mrs. Lizette Kühn herewith confirm that the above resident has been informed fully about the nature, conduct and risks of the above trial.

Investigator's name_____
(Please print)

Investigator's signature_____Date _____

Witness's name_____
(Please print)

Witness's signature_____Date _____

VERBAL PATIENT INFORMED CONSENT (applicable when residents cannot read or write)

I, the undersigned, Mrs……, have read and have explained fully to the patient, named …….. and/or is/her relative, the patient information leaflet, which has indicated the nature and purpose of the trial in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the trial. The resident indicated that he/she understands that he/she will be free to withdraw from the trial at any time for any reason without an explanation.

I hereby certify that the resident has agreed to participate in this trial.

Resident's Name_____
(Please print)

Investigator's Name_____
(Please print)

Investigator's Signature _____ Date _____

Witness's Name _____
(Please print)

Witness's Signature _____Date_____

(Witness - sign that he/she has witnessed the process of informed consent)

AMENDMENT: PARTICIPANT'S INFORMATION & INFORMED CONSENT DOCUMENT

TRIAL TITLE: Effect of 12 weeks of fish supplementation of an enhanced usual diet on cognition of resource limited independently living elderly in a retirement village: A randomised controlled trial

Dear Resident

On page 2 of the Participant Informed Consent Document the following is stated:

- Some people may be chosen to provide blood samples in order to determine the fat content of the blood. (These assessments will be repeated twice times over a three month period – thus July/August and November 2018). The people undergoing these blood tests will once again be selected by chance by an independent person. The reason for these tests is to determine whether you actually consumed the specific foods in each group adequately.

The former paragraph is replaced by the following:

- Everybody is asked to provide blood samples in order to determine the fat and vitamin/mineral content of the blood. (These assessments will be repeated twice times over a three month period – thus July/August and November 2018). The reason for these tests is to determine whether you actually consumed the specific foods in each group adequately.

INFORMED CONSENT

I hereby confirm that I have been informed by the investigator, Mrs. Lizette Kühn about the nature of the amendment. I have also received, read and understood the above written information (Amendment to: Patient Information Leaflet and Informed Consent).

Resident's name_____
(Please print)
Resident's signature_____ Date _____

I, Mrs. Lizette Kühn herewith confirm that the above resident has been informed fully about the nature of the amendment.

Investigator's name_____
(Please print)
Investigator's signature_____Date _____

Witness's name_____
(Please print)
Witness's signature_____Date _____

Council for the Aged Kempton Park
Welfare no. 20/20008

23 Cactus Road
Allen Grove
Kempton Park 1619
P O Box 2844, Glen Balad, 1630
011 972 4220/4268
011 972 4279
E-mail: ontvangs@allenpark.co.za

29 August 2017

Tow whom it may concern:

Approval for PhD study at Allen Park Council for the Aged

We hereby give written permission that the dietetic study by researcher, Lizette Kühn, may be executed at Allen Park Council for the Aged. She has given us sufficient information about the research proposal and we feel comfortable about the transparency of the project. We are in contact with her on a regular base and should any query arise, we will address it to her.

You are welcome to contact us regarding the content of this letter.

Kind regards

A. van der Merwe
Chairperson

A. Naude
CEO

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

8/02/2018

Approval Certificate
New Application

Ethics Reference No: 542/2017

Title: EFFECT OF 12 WEEKS FISH SUPPLEMENTATION OF AN ENHANCED USUAL DIET ON COGNITION OF RESOURCE LIMITED INDEPENDENTLY LIVING ELDERLY IN A RETIREMENT VILLAGE: A RANDOMISED CONTROLLED TRIAL

Dear Ms Lizette L Kühn (Hanekom)

The **New Application** as supported by documents specified in your cover letter dated 8/02/2018 for your research received on the 8/02/2018, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 8/02/2018.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**542/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

012 356 3084 deepeka.behari@up.ac.za / fhsethics@up.ac.za http://www.up.ac.za/healthethics
Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4, Room 60, Gezina, Pretoria

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved 22/04/2014 and Expires 03/14/2020.

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Faculty of Health Sciences Research Ethics Committee

16/07/2018

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No.: 542/2017

Title: EFFECT OF 12 WEEKS FISH SUPPLEMENTATION OF AN ENHANCED USUAL DIET ON COGNITION OF RESOURCE LIMITED INDEPENDENTLY LIVING ELDERLY IN A RETIREMENT VILLAGE: A RANDOMISED CONTROLLED TRIAL

Dear Ms Lizette. L Kühn Human Nutrition

The **Amendment** as described in your documents specified in your cover letter dated 25/05/2018 received on 01/06/2018 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 11/07/2018.

Please note the following about your ethics amendment:
- Please remember to use your protocol number (**542/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committe may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics amendment is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed; PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762; OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2022.

08 September 2020

Acknowledgement Certificate
Research Completed

Ethics Reference No.: 542/2017
Title: EFFECT OF 12 WEEKS FISH SUPPLEMENTATION OF AN ENHANCED USUAL DIET ON COGNITION OF RESOURCE LIMITED INDEPENDENTLY LIVING ELDERLY IN A RETIREMENT VILLAGE: A RANDOMISED CONTROLLED TRIAL

Dear Mrs L Kuhn

The **Research Completed Report** as supported by documents received between 2020-08-24 and 2020-09-16 for your research, was acknowledged by the Faculty of Health Sciences Research Ethics Committee on 2020-09-16 as resolved by its quorate meeting.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

Research Ethics Committee
Room 4-65, Level 4, Tswaluvare Building
University of Pretoria, Private Bag x323
Tel +27 (0)12 356 3084
www.up.ac.za

Gmail Lizette Kuhn <lizettekuhn24@gmail.com>

Submission of protocol (Trial application_5026)

5 messages

Lizette Kuhn <lizettekuhn24@gmail.com> Wed, Jun 20, 2018 at 8:09 PM
To: Kedibone.Malatji@health.gov.za
Cc: Dorah Diale <dorah.diale@health.gov.za>, Friede Wenhold <Friede.Wenhold@up.ac.za>, Una MacIntyre <Una.MacIntyre@up.ac.za>, Carla Kotze <Carla_kotze@yahoo.com>

Dear Ms Malatji

(with cc to Dr. Dorah Diale and my supervisors: Prof. Friede Wenhold, Prof. Una MacIntyre and Dr. Carla Kotze)

Submission of protocol for food intervention study (Trial application: 5026)

I have been referred to you by Ms. Thozama Spotose after applying on the SANCTR site. If I understand correctly I need so supply you with more information on my study. Therefore attached please find my study proposal for your perusal. Regarding MCC approval – the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria has referred me to explore the necessity of approval by the MCC. (The correspondence with the MCC took place during the period of November 2017 – February 2018.) The MCC has reviewed my protocol and as the intervention entails the provision of general commercial food products to the designated intervention group it was decided that I do not need approval by the MCC. The final decision was communicated to me by Dr. Dorah Diale in February.

The timeline as presented in the proposal had to be adjusted because of logistical reasons and the intervention is proposed to start in August – middle November 2018.

I hope you find the information in order.

You are welcome to contact me if I can supply you with more information.

Kind regards

Lizette Kuhn

Research Proposal (MCC Lizette Kuhn).pdf
1965K

Dorah Diale <Dorah.Diale@health.gov.za> Thu, Jun 21, 2018 at 9:32 AM
To: lizettekuhn24@gmail.com, Kedibone Malatji <Kedibone.Malatji@health.gov.za>
Cc: Friede.Wenhold@up.ac.za, Una.MacIntyre@up.ac.za, Carla_kotze@yahoo.com

Dear Lizette and Thozama

Thank you for the email.
Lizette if nothing has changed following our previous discussion, Thozama may proceed with the registration.

Kind Regards
Dorah
>>> Lizette Kuhn <lizettekuhn24@gmail.com> 2018/06/20 8:09 PM >>>
[Quoted text hidden]

ANNEXURE B

Data Collection Instruments

RESEARCH SCREENING (BACKGROUND) FORM:

Participant number:_____

SECTION A:

Please mark the appropriate block with an "x".

1. Gender:
   - ☐ Male
   - ☐ Female

2. Age:
   - ☐ <65 yrs
   - ☐ 65 – 70 yrs
   - ☐ 71 – 75 yrs
   - ☐ 76 – 80 yrs
   - ☐ 81 – 85 yrs
   - ☐ 86 – 90 yrs
   - ☐ 91 – 95 yrs
   - ☐ 96 – 100 yrs
   - ☐ >100 yrs

3. What is your highest level of education?
   - ☐ Gr. 8 or lower (Standard 6 or lower)
   - ☐ Gr. 9 (Standard 7)
   - ☐ Gr. 10 (Standard 8)
   - ☐ Gr. 11 (Standard 9)
   - ☐ Gr. 12 (Standard 10)
   - ☐ After school training which requires Gr.12

4. Do you have any visual or hearing impairment that will influence the make it difficult for you to answer questions or to write?

5. ☐ Yes
   ☐ No

6. Please list any medical conditions that you are experiencing at present:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

7. Please list the medications that you are using and indicate whether you have been using them for three months or longer by making an “x” in the appropriate column?

Medication	Yes, I have been using it for 3 months or longer	No, I have not used it for 3 months

8. Do you smoke?
   - ☐ Yes
   - ☐ No

9. Do you use an Omega 3 (fish oil or flax seed oil supplement, thus a capsule containing oil)?
   - ☐ Yes
   - ☐ No

10. Are you allergic to any of the following products, please indicate?
    - ☐ Fish
    - ☐ Nuts
    - ☐ Peanut butter
    - ☐ Soya
    - ☐ Canola oil
    - ☐ Tinned meatballs
    - ☐ Tinned beans

    Other:_____

11. Are your willing to eat fish such as pilchards and fish paste more than twice a week for three months with your other foods?
    - ☐ Yes
    - ☐ No

CASI
Cognitive Abilities Screening Instrument
Version E-1.1

Name _____ ID No. [ ]

Date (mm-dd-yy) [ ] Examiner _____ [ ]

SEX ☐ 1 = male ☐ 2 = female

EDUCATION (years of schooling completed) [ ]
(code 99=Unk/Refused)

Testing start time (hr:min) ____:____

1. WHERE WERE YOU BORN?
City (Town/Village) [0 1]
State/Prefecture [0 1] → BPL
add above 2 scores then circle the answer → 2 1 0

2. WHEN WERE YOU BORN?
Year: Accurate 2; Missed by 1 - 3 years 1; Missed by > 3 years 0 → BYR
Month [0 1]
Date [0 1] → BDAY
add above 2 scores then circle the answer → 2 1 0

3. HOW OLD ARE YOU?
Accurate 2; Missed by 1 - 3 years 1; Missed by > 3 years 0 → AGE

4. HOW MANY MINUTES ARE THERE IN AN HOUR?
or HOW MANY DAYS ARE THERE IN A YEAR?
(score 2 if either question answered correctly) → MNT 2 0

5. IN WHICH DIRECTION DOES THE SUN SET?
(if confused, may provide 4 choices) → SUN 2 0

Select version #, then circle corresponding words in Question 8 and Question 22. → VRS# 1 2 3

6 a. I AM GOING TO SAY 3 WORDS FOR YOU TO REMEMBER. REPEAT THEM AFTER I HAVE SAID ALL THREE. → RGS1 3 2 1 0
☐ 1. SHIRT ____ BROWN ____ HONESTY ____
☐ 2. SHOES ____ BLACK ____ MODESTY ____
☐ 3. SOCKS ____ BLUE ____ CHARITY ____

b. If participant can't answer the first time, elaborate and repeat up to a total of 3 times. Score last performance. → RGS2 3 2 1 0

7. I SHALL SAY SOME NUMBERS, AND YOU REPEAT WHAT I SAY BACKWARDS. FOR EXAMPLE, IF I SAY 1-2, YOU SAY 2-1. OK? REMEMBER YOU REPEAT WHAT I SAY BACKWARDS.
(Rate: 1 digit/second)
1-2-3 (if unable, coach for 3-2-1, but score 0) → DBA 1 0
6-8-2 → DBB 2 0
(if score is 0 in both DBA and DBB, score DBC 0) → DBC 2 0
3-5-2-9

8. WHAT THREE WORDS DID I ASK YOU TO REMEMBER EARLIER? → RCTA
Spontaneous recall 1.5; After: "one word was something to wear" 1; After: "Was it SHOES, SHIRT, or SOCKS"? 0.5; Still incorrect 0

→ RCTB
Spontaneous recall 1.5; After: "one word was a color" 1; After: "Was it BLUE, BLACK, or BROWN"? 0.5; Still incorrect 0

→ RCTC
Spontaneous recall 1.5; After: "one word was a good personal quality" 1; After: "Was it HONESTY, CHARITY, or MODESTY"? 0.5; Still incorrect 0

Unless recall is perfect, give another reminder of the 3 words.

(For the first error only: score 0, but provide the correct answer. If subject asks examiner to repeat answer from previous step, provide the answer but score 0 at that step.)

9 a. FROM 100, TAKE AWAY 3, = HOW MANY? (97) → SUB3A 1 0

b. AND TAKE AWAY 3 FROM THAT EQUALS? (94) → SUB3B 1 0

(If a. and b. are both scored 0, score part c. 0)

c. Repeat "AND TAKE AWAY 3 AGAIN EQUALS?" three more times. 1 point each. → SUB3C 3 2 1 0
(91 88 85)

10. WHAT IS TODAY'S DATE? → YR
[YEAR]: Accurate 4; Missed by 1 year 2; Missed by 2-5 years 1; Missed >= 6 years 0 → MO
[MONTH]: Accurate or within 5 days 2; Missed by 1 month 1; Missed >= 2 months 0 → DATE
[DATE] (of the month): Accurate 3; Missed 1 or 2 days 2; Missed 3-5 days 1; Missed >=6 days 0

11. WHAT DAY OF THE WEEK IS TODAY? Accurate 1; Inaccurate 0 → DAY

12. WHAT SEASON ARE WE IN? Accurate within 1 month 1; Missed by > 1 month 0 → SSN
(may provide 4 choices if necessary)

13 a. WHAT _____ ARE WE IN?
State [0 2]
City/Town/Village [0 2] → SPA
add above 2 scores then circle the answer → 4 2 0

b. IS THIS PLACE A HOSPITAL (CLINIC), A STORE ( ), OR HOME? → SPB 1 0

14. WHAT ANIMALS HAVE 4 LEGS? TELL ME AS MANY AS YOU CAN. (30 sec.)
number of correct answers: 0 1 2 3 4 5 6 7 8 9 10 → ANML

15. AN ORANGE AND A BANANA ARE BOTH FRUIT.
(pause for 2 sec., then ask:)
(coach for correct answer if needed for "a." only)
a. AN ARM AND A LEG ARE BOTH ...?
Body parts, limbs, extremities 2; Long, bend, muscles, bones, etc 1; Incorrect; OK; tells difference 0
b. LAUGHING AND CRYING ARE BOTH ...?
Expressions of feelings/emotions 2; other correct answer 1; Incorrect; OK; tells difference 0
c. EATING AND SLEEPING ARE BOTH ...?
Necessary bodily functions 2; Other correct answer 1; Incorrect; OK; tells difference 0
add above 3 scores then circle the answer → SIM 6 5 4 3 2 1 0

15 a. WHAT ACTIONS WOULD YOU TAKE IF YOU SAW YOUR NEIGHBOR'S HOUSE CATCHING FIRE?
(prompt "WHAT ELSE MIGHT YOU DO?" once only, if necessary)
No. of appropriate actions: 0 1 2
b. WHAT ACTIONS WOULD YOU TAKE IF YOU LOST A BORROWED UMBRELLA?
1 point for each category of actions:
* Inform/Apologize
* Replace/Compensate 0 1 2
c. WHAT WOULD YOU DO IF YOU FOUND AN ENVELOPE THAT WAS SEALED, ADDRESSED AND HAD A NEW STAMP?
Mail 2; Try to locate the owner 1; Inappropriate action 0
add above 3 scores then circle the answer → JGMT 6 5 4 3 2 1 0

17 a. REPEAT EXACTLY WHAT I SAY:
"HE WOULD LIKE TO GO HOME."
Correct 2; 1 or 2 missed or wrong words 1; >= 3 missed or wrong words 0 → RPTA
(for each part of 17b, score 1 only if repeated exactly as given)
b. NOW REPEAT...
"THIS YELLOW CIRCLE [0 1]
IS HEAVIER THAN [0 1]
BLUE SQUARE" [0 1]
add above 3 scores then circle the answer → RPTB 3 2 1 0

Congitive Abilities Screening Instrument CASI E-1.1 Record form 3 of 4

18. PLEASE DO THIS: (Point to statement "RAISE YOUR HAND") — READ

Raises hand without prompting	1.5
Raises hand after prompting	1
Reads correctly, but does not raise hand	0.5
Neither reads nor obeys	0

19. LET ME HAVE A SAMPLE OF YOUR HANDWRITING.
PLEASE WRITE:
(HE) WOULD LIKE TO GO HOME. (1 min.)
(may dictate 1 word at a time if necessary) — WRITE

0 0.5 1 1.5 2 2.5

20. PLEASE COPY THIS: *(show pentagons - 1 minute)* — DRAW

	Left Pentagon	Right Pentagon
5 approx. equal sides	4	4
5 but un-equal (>2:1) sides	3	3
Any other enclosed figure	2	2
>= 2 lines but without closure	1	1
Less than 2 lines	0	0

Intersection:

4 cornered	2
Not 4-cornered enclosure	1
No enclosure	0

10 9 8 7 6 5 4 3 2 1 0

add above 3 scores then circle the answer →

(note: for question 21, do not repeat any part of the command)
(use non-dominant hand)

21. TAKE THIS PAPER WITH YOUR L (R) HAND [0 1] — CMD

FOLD IT IN HALF, AND [0 1]

HAND IT BACK TO ME. [0 1]

3 2 1 0

add above 3 scores then circle the answer →

22. WHAT THREE WORDS DID I ASK YOU TO REMEMBER EARLIER?

	RC2A	RC2B	RC2C
Spontaneous recall	1.5	1.5	1.5
After: "one word was something to wear"	1		
After "Was it SHOES, SHIRT, or SOCKS"?	0.5		
After: "one word was a color"		1	
After "Was it BLUE, BLACK, or BROWN"?		0.5	
After: "one word was a good personal quality"			1
After "Was it HONESTY, CHARITY, or MODESTY"?			0.5
Still incorrect	0	0	0

23. WHAT DO WE CALL THIS PART OF THE FACE/BODY? *(2 sec. each)* — BODY

FOREHEAD	[0 .3]	1.5
CHIN	[0 .3]	1.2
SHOULDER	[0 .3]	0.9
ELBOW	[0 .3]	0.6
WRIST	[0 .3]	0.3
add above 5 scores then circle the answer →		0

24. WHAT IS THIS? *(show one at a time, any order OK)*

OBJA

SPOON	[0 .3]	0.6
COIN	[0 .3]	0.3
add above 2 scores then circle the answer →		0

OBJB

TOOTHBRUSH	[0 .3]	0.9
KEY	[0 .3]	0.6
COMB	[0 .3]	0.3
add above 3 scores then circle the answer →		0

(Total number of objects either named spontaneously or repeated correctly after coaching.) 0 1 2 3 4 5 — RPNM

25. REMEMBER THESE 5 OBJECTS! *(Wait for 5 sec.; cover, then ask:)* — RCOBJ

WHAT 5 OBJECTS DID I JUST SHOW YOU?
(Any order is OK, circle the correct ones.)

SPOON COIN TOOTHBRUSH KEY COMB

3.0 2.4 1.8 1.2 0.6

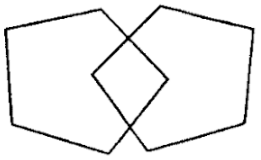
0.6 points each → 0

Finish time (hr:min) ____:____ Duration (minutes) [ ]

VALIDITY OF SCORE

Valid	1
Probably invalid: poor hearing	2
Probably invalid: poor eyesight	3
Probably invalid: impaired motor control	4
Probably invalid: language barrier	5
Probably invalid: impaired alertness or attentiveness	6
Probably invalid: significant physical or mental discomfort	7
Probably invalid: other reasons (specify):	8

RAISE YOUR HAND



CASI E-1.1 Record form 4 of 4

The Lawton Instrumental Activities of Daily Living Scale	
Scoring: For each category, circle the item description that most closely resembles the client's highest functional level (either 0 or 1).	
Lawton, M.P., & Brody, E.M. (1969). Assessment of older people: Self-maintaining and instrumental activities of daily living. The Gerontologist, 9(3), 179-186.	
A. Ability to Use Telephone 1. Operates telephone on own initiative; looks up and dials numbers..... 2. Dials a few well-known numbers..... 3. Answers telephone, but does not dial..... 4. Does not use telephone at all.....	 1 1 1 0
B. Shopping 1. Takes care of all shopping needs independently..... 2. Shops independently for small purchases..... 3. Needs to be accompanied on any shopping trip..... 4. Completely unable to shop.....	 1 0 0 0
C. Food Preparation 1. Plans, prepares, and serves adequate meals independently..... 2. Prepares adequate meals if supplied with ingredients..... 3. Heats and serves prepared meals or prepares meals but does not maintain adequate diet..... 4. Needs to have meals prepared and served.....	 1 0 0 0
D. Housekeeping 1. Maintains house alone with occasion assistance (heavy work)..... 2. Performs light daily tasks such as dishwashing, bed making..... 3. Performs light daily tasks, but cannot maintain acceptable level of cleanliness..... 4. Needs help with all home maintenance tasks..... ..... 5. Does not participate in any housekeeping tasks.....	 1 1 1 1 0
E. Laundry 1. Does personal laundry completely..... ..... 2. Launders small items, rinses socks, stockings, etc..... 3. All laundry must be done by others.....	 1 1 0
F. Mode of Transportation 1. Travels independently on public transportation or drives own car..... 2. Arranges own travel via taxi, but does not otherwise use public transportation..... 3. Travels on public transportation when assisted or accompanied by another..... 4. Travel limited to taxi or automobile with assistance of another..... 5. Does not travel at all.....	 1 1 1 0 0
G. Responsibility for Own Medications 1. Is responsible for taking medication in correct dosages at correct time..... 2. Takes responsibility if medication is prepared in advance in separate dosages..... 3. Is not capable of dispensing own medication.....	 1 0 0
H. Ability to Handle Finances 1. Manages financial matters independently (budgets, writes checks, pays rent and bills, goes to bank); collects and keeps track of income..... 2. Manages day-to-day purchases, but needs help with banking, major purchases, etc... 3. Incapable of handling money.....	 1 1 0

DIETARY INTAKE INTERVIEW

DATE: _____PARTICIPANT NUMBER_____

INTRODUCTION:

We want to find out what people living in a retirement village eat and drink. I will be asking you to think carefully about the food and drink you have consumed in the past month (from about middle March). I will go through a list of foods and ask you:

- If you eat the specific food.
- More information on some of the foods e.g. type or brand.
- The amount of food that you eat.
- How often you eat these foods.

To help you describe the type and amount of food you eat I will show you pictures and bean bags of different amounts of food.

There are no right or wrong answers.

Everything you tell me is confidential.

Do you want to ask anything now?

Are you ready to start?

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
Cooked porridge	Maize meal					
	Maltabella					
	Oats					
Cereals	Whole wheat					
Samp / Mielierice						
Rice	White rice					
	Brown rice					
	Basmati					
	Other:					
Pasta	Describe:					
Bread or equivalent:	Describe:					

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
Do you use a spread on your bread? Yes No **If no, continue with section on protein** **If yes, which spread do you use?**						
Margarine	Name and describe:					
Butter						
Peanut butter	Name and describe:					
Jam						
Fish paste	Name:					
Cheese	Describe:					
Other spreads	Describe:					
*You are very helpful, may I now ask about **chicken, fish, meat and other protein sources**?*						
Chicken	Portion with bone					
	Portion without bone					

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
	Fine					
Red meat/ Pork	Mince meat					
	Cubes of meat (stew)					
	Portions					
	Sausage					
Organ meat e.g. liver						
Processed meat	Describe:					
Pilchards / sardines	Prepared (fried)?					
Hake	Prepared (fried)?					
Fish cakes / fingers (bought)	Prepared (fried)?					
Tuna	Prepared (fried)?					
Other seafood?	Prepared (fried)?					

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
Eggs	Describe:					
Soya e.g. Imana						
Dried beans/ lentils						
Tinned beans/ lentils						
May we now discuss your fruit and vegetable intake?						
Cabbage						
Spinach / "Kale"						
Lettuce						
Other vegetables (including potato)						

	Description	Code / unit	Number of times eaten			
			Per day	**Per week**	**Per month**	**Seldom** **(less than 1x / mo)**
Berries	Describe:					
Fresh Fruit	Describe:					
*Do you use any **sauces** with your food?*						
Sauce	Describe:					
*May we continue and discuss your **snacks**?*						
Potato Crisps						
Peanuts						
Nuts	Describe:					
Sweets and chocolate	Describe:					

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
Biscuits and cake	Describe:					
Dessert	Describe:					
*Can we talk about your intake of **fast and fried** foods?*						
Fast / fried foods	Describe:					
Home fried foods	Describe:					
Which type of oil do you use **primarily** and how often?						
Sunflower						
Canola						
Olive						
Other	Describe:					
Alcohol and sugar						

	Description	Code / unit	Number of times eaten			
			Per day	Per week	Per month	Seldom (less than 1x / mo)
Alcohol	Describe, e.g. beer / wine					
Sugar	Describe:					
Any other food that you consume more than once per week which we have not discussed?						
Other food:						

PERSONAL RECORD: Group 1

Week:_____ Participant number:_____

Please indicate the amount of a specific food that you have eaten in the column of the appropriate day.

Indicate amount in terms of teaspoons for the fish spread, peanut butter and canola oil.

Indicate amount in terms of the tin (container) for the pilchards and baked beans.

Example:

Food	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Pilchards	½ tin		1 tin		½ tin		
Fish spread			4 teaspoons	2 teaspoons			
Baked beans		1 tin		1 tin			
Peanut butter	4 teaspoons				1 teaspoon		
Canola oil	1 teaspoon					5 teaspoons	

Food	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Pilchards							
Fish spread							
Baked beans							
Peanut butter							
Canola oil							

PERSONAL RECORD: Group 2

Week:_____ Participant number:_____

Please indicate the amount of a specific food that you have eaten in the column of the appropriate day.

Indicate amount in terms of teaspoons for the peanut butter and canola oil.

Indicate amount in terms of the tin (container) for the meatballs and baked beans.

Indicate amount in terms of tablespoons for soya.

Example:

Food	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Meatballs	½ tin		1 tin		½ tin		
Soya			4 teaspoons	5 teaspoons			
Baked beans		1 tin		1 tin			
Peanut butter	4 teaspoons				1 teaspoon		
Canola oil	1 teaspoon					5 teaspoons	

Food	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Meatballs							
Soya							
Baked beans							
Peanut butter							
Canola oil							

ANNEXURE C

Protocol for Quansys Analysis

Micronutrient status profile:

Quansys Q-Plex Micronutrient Analysis + HemoCue – Method description for scientific protocols
As compiled by Centre of Excellence for Nutrition at the North-West University

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The iron status indicators ferritin and transferrin receptor (TfR), the vitamin A status indicator retinol binding protein (RBP) and the iodine status indicator thyroglobulin (Tg) will be measured in heparin plasma using the Q-Plex™ Human Micronutrient Array (Quansys Bioscience, Utah, USA) at the micronutrient laboratory of the Centre of Excellence for Nutrition at the North-West University (Brindle et al., 2010).

The inflammation/infection markers C-reactive protein (CRP), alpha1-acid glycoprotein (AGP) and HRP2 (malaria marker; not relevant to this project) are also included in the analysis. The acute phase proteins AGP and CRP will be used to identify subjects with infection and inflammation, which could confound measures of iron (especially ferritin) and Vit A status.

The Q-Plex™ Human Micronutrient Array (7-plex) is a fully quantitative chemiluminescent assay allowing concurrent measurement of biomarkers used in nutritional assessment in heparinized plasma samples (min. 50 µL). Arrays will be analysed using Q-View Imager Pro. Raw data will be analysed using the Q-View software and compared to in-plate controls.

Haemoglobin concentrations will be measured on site using a portable Hb 201+ HemoCue system (HemoCue Angelholm, Sweden) in 20 µL of whole blood (capillary or venous [e.g. EDTA or heparin as anticoagulant]) to screen for anaemia.

Reference:

BRINDLE, E., FUJITA, M., SHOFER, J. & O'CONNOR, K. A. 2010. Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research. *J Immunol Methods,* 362**,** 112-20.

ANNEXURE D

Language editing and Turn-it-in receipt

P O Box 36405
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Tshwane, 0102
South Africa
04 November 2020

TO WHOM IT MAY CONCERN

I, Dr Ingrid Vivienne van Heerden, Id. No.: 440922-0021-082, Registered with SATI (Registration No.: 2522), herewith attest to reading, editing and correcting the language (English), of a Draft Version of a Ph.D. thesis entitled:

EFFECT OF 12 WEEKS OF FISH SUPPLEMENTATION OF AN ENHANCED USUAL DIET ON COGNITION OF RESOURCE-LIMITED INDEPENDENTLY LIVING ELDERLY PERSONS IN A RETIREMENT VILLAGE: A RANDOMISED CONTROLLED TRIAL
by
Lizette Kühn (née Hanekom)
Student number: 21011291
Doctoral thesis submitted in fulfilment of the requirements for the degree PhD Dietetics in the
Department of Human Nutrition
School of Health Care Sciences
Faculty of Health Sciences

I must, however, point out that I have not been tasked to read, edit, or correct the language (English) of the final version of this thesis, which I anticipate Lizette Kuhn will submit to the Internal and External Examiners at UP.

Dr. Ingrid Vivienne van Heerden (D.Sc. UP; M.Sc. (Dietetics), US; Hons. B.Sc. (Dietetics), US; Hons. B.Sc. (Psychology), UNISA)
Scientific Editor, Translator & Interpreter
Nutrition Consultant (ADSA (Ret), NSSA (Ret), SAAFost (Ret)

Signed in Tshwane on the 4th of November 2020
by

Dr I V van Heerden

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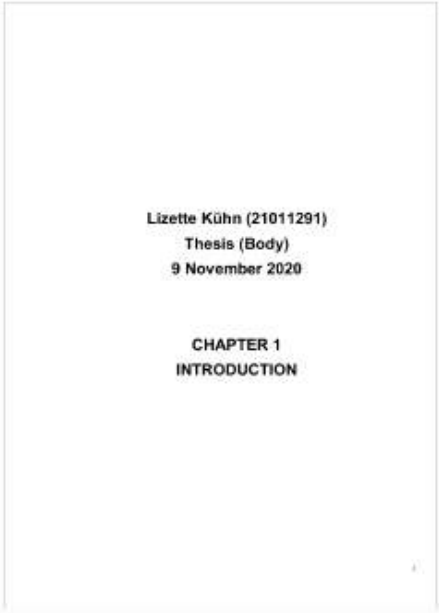
Page count:	127
Word count:	39,824
Character count:	198,298
Submission date:	09-Nov-2020 09:42PM (UTC+0200)
Submission ID:	1441112722



Lizette Kühn (21011291)
Thesis (Body)
9 November 2020

CHAPTER 1
INTRODUCTION