Addressing the knowledge gaps in two common age-related conditions, both associated with nutrition and physical activity: Alzheimer's Dementia and Sarcopenia

by

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Abstract

Background

Throughout the western world there is an aging demographic resulting in an increased prevalence of age-associated chronic disease, particularly Alzheimer's dementia [AD] and sarcopenia. Both conditions significantly impact independence. They also have other common effects on morbidity: increased length of hospitalisation; increased risk of malnutrition; increased risk of falls and fractures; decreased quality of life; and increased risk of institutionalized care. Both conditions also have no single diagnostic test, and require fulfillment of clinical diagnostic criteria. Diagnosis of AD relies on history, physical, and investigations to exclude other diagnoses. Diagnosis of sarcopenia requires muscle strength, mass, and function assessments. Tools to help in the diagnosis of AD are readily available (clinical cognitive tests), whereas tools to diagnose sarcopenia, particularly muscle mass assessment, require sophisticated diagnostics that are not readily available. However, unlike AD, treatment for sarcopenia has been well studied and validated. In AD despite extensive research, to date, there are limited therapeutic options, so the treatment becomes one of following the decline and planning for future disability.

These projects were undertaken to *address some of the clinical care gaps* in these two highly prevalent, interrelated, impactful, clinical conditions.

Methods

In AD, traditional approaches to therapy attempting to reduce amyloid load have proven ineffective. Current therapeutics provide some symptomatic relief for activities of daily living (ADLs) and behavior, at best, but do not modify the downward trajectory of the disease.

The first study (Chapter 3) evaluates the use of medium chain triglyceride (MCT) supplementation as a source of dietary ketones for brain energy. It looks at the ketogenic effect (β-hydroxybutyrate (BHB) levels) of multiple doses of MCT compared between participants with and without AD.

The second study (Chapter 4) evaluates the cognitive effect and safety of MCT versus olive oil supplementation in participants with established AD, in a randomised, double-blind, placebo-controlled, cross-over trial with an open label extension (15-month trial). Cognition is measured with standardised tools-Mini-Mental Status Examination, Montreal Cognitive Assessment, and Cognigram®.

The third study (Chapter 5) evaluates office-based, direct-to-consumer, bioimpedance scales as a means to easy clinical assessment of muscle and fat mass, and compares this to dual energy Xray absorptiometry (DXA) as the gold standard, in a group of community-dwelling older adults.

The fourth study (Chapter 6) evaluates the rate of sarcopenia in a cohort of independent, community-dwelling older adults, and the change in their sarcopenic status over 12-months. It also assesses involvement in a social group activity program on quality of life, and physical function.

Results

Study 1 showed that MCT is an equally effective nutritional source of ketones (beta-hydroxybutyrate, BHB) in participants with and without AD, identifying that AD per-se does not affect metabolism of supplemental MCT. *Study 2* showed no difference between MCT and placebo oil in the cross-over phase. Including the open-label phase showed exposure to a total of 11-months of continuous supplemental MCT, of at least 30ml daily, provided cognitive benefit

(stability or decreased decline) in participants with established AD, compared to those in whom the 11-months was interrupted by 4 months of placebo (olive) oil. Participants with a higher baseline cognitive score showed greater benefits. In *Study 3*, office-based direct-to-consumer bioimpedance scales were shown to be correlated with dual-energy Xray absorptiometry (DXA), and can reasonably be utilised for in-office assessment of muscle and fat mass, prompting further testing as indicated. *Study 4* showed that the rate of sarcopenia was slightly higher than anticipated (7.1%), but similar to the UK. The high rate of pre-sarcopenia (28.6%) was unexpected. The prevalence of obesity was highly variable depending on DXA and BIA body composition versus BMI criteria. Sarcopenic status did not appear to be correlated with cognitive scores, but was associated with activities of daily living function and quality of life, as reported in the literature. There was maintenance of function in the majority of participants over 12-months. The group social activity program (including both aerobic, resistance and balance exercises), despite its non-progressive, non-personalised nature, was shown to improve the balance component of the Tinetti score. Self-reported leisure activity was not found to be associated with sarcopenic status or Tinetti scores.

Conclusions

AD and sarcopenia are more prevalent with increasing age. These studies show that the MCT induced BHB response is unaffected by age or AD status, and that 11-months continuous consumption of MCT, may influence the AD disease course in participants with established AD. Community-based, direct-to-consumer BIA scales can improve office-based diagnostic ability for muscle mass and obesity. The rate of sarcopenia in independent community-dwelling older adults was higher than predicted, but remained relatively stable over 12-months.

Preface

This thesis is an original work by Angela G Juby. The research projects, of which this thesis is a part, received research ethics approval from the University of Alberta, Health Research Ethics Board.

Chapter 3 of this thesis has been published as: Juby AG, Brocks DR, Jay DA, Davis CMJ, Mager DR. Assessing the Impact of Factors that Influence the Ketogenic Response to Varying Doses of Medium Chain Triglyceride (MCT) Oil. *Journal of Prevention of Alzheimer's Disease* 2021; 8(1):19-28. doi:10.14283/jpad.2020.53.8(1): 19-28.

I was responsible for the design of the clinical trial, the funding, obtaining approval for MCT administration from Health Canada, ethics application from Health Research Ethics Board (HREB) at University of Alberta, clinical trial registration, and participant recruitment. DRB was also involved with the trial design. AGJ, CMJD and DAJ were responsible for administering and supervising consumption of the test MCT, performing the finger-stick testing, documenting any adverse events. Data entry was done by CMJD and DAJ. Statistical analysis was performed by DRB and DRM. Pharmacokinetic interpretation was performed by DRB. Data analysis and clinical interpretation was performed by AGJ, DRB and DRM. Manuscript was prepared by AGJ with input from DBR, and DRM. All the authors approved the final manuscript. I (AGJ) had primary responsibility for manuscript submission and revision, and was responsible for the final content.

Regulatory approval for MCT administration was from Health Canada, Canada Therapeutic Products Directorate approval No HC6-24-c218402, January 2019; HREB approval No. Pro 00087958, April 2019; ClinicalTrials.gov Identifier NCT04389983.

Chapter 4 of this thesis has been published as: Juby AG, Blackburn TE, Mager DR. Use of medium chain triglyceride (MCT) oil in participants with Alzheimer's dementia: A randomized, double-blind, placebo-controlled, crossover study, with an open-label extension. *Alzheimer's & Dementia: Translational Research & Clinical Interventions.* 2022;8(1):e12259.

I was responsible for the randomised-controlled, double-blind, crossover design of the clinical trial, applying and obtaining trial funding from the Glenrose Hospital Research Foundation,

funding for the DXA BC from MIC, funding for the Cognigram ®, obtaining approval for MCT administration from Health Canada, ethics application from Health Research Ethics Board (HREB) at University of Alberta, clinical trial registration, and participant recruitment. I was responsible for participant's screening, recruitment, and all their clinical evaluations. All cognitive testing was by TEB and AGJ. Blinded randomisation was performed by Joan Kravic (JK) (administrative support). Study appointment booking was done by JK and Debbie Smith (DS), (administrative support). JK and DS were responsible for bottling and labelling the MCT and placebo oil, and weighing and recording the returned investigational products for compliance evaluation for each participant in the 3 phases of the trial. AGJ and TEB documented any adverse events, and were responsible for addressing any clinical issues arising during the trial. DS performed the data entry. Statistical analysis was performed by DRM and AGJ. AGJ performed the data analysis and clinical interpretation. AGJ prepared the manuscript, with final approval from TEB and DRM. AGJ submitted the manuscript for publication, and was responsible for the revisions and final content prior to final publication.

Regulatory approval for MCT administration was from Health Canada, Canada Therapeutic Products Directorate approval No. HC6-24- c186660, September 2015; HREB approval No. Pro 00054165, November 2015. ClinicalTrials.gov Identifier NCT04396015.

Chapter 5 of this thesis has been submitted for publication as: Juby AG, Davis CMJ, Minimaana S, Mager DR. Addressing the main barrier to Sarcopenia identification: Utility of practical office-based assessment tools versus Dual Energy Xray Absorptiometry (DXA) Body Composition for identification of low muscle mass in older adults. *Canadian Geriatrics Journal,* 2022 (submitted).

I was responsible for the observational cohort design of the study, applying and obtaining trial funding from the Department of Medicine, Summer Student Research Program, funding for the DXA BC from MIC, and ethics application from Health Research Ethics Board (HREB) at University of Alberta. I was responsible for choosing the direct-to-consumer Ozeri® and Omron® BIA scales. I was responsible for participant screening, and all the clinical evaluations (including anthropometric evaluations). CMJD and SM performed all the BIA scale assessments and functional evaluations. Data entry was performed by CMJD, SM and Debbie Smith (DS). Data analysis was by AGJ, and statistical analysis was by DRM and AGJ. Data interpretation

was by AGJ and DRM. Manuscript preparation was by AGJ, with final approval by CMJD, SM and DRM. Manuscript submission was by AGJ, and once published, responsibility for the final manuscript is with AGJ.

Regulatory approval for this study was obtained from University of Alberta HREB No. Pro 00047132, July 2014.

Chapter 6 of this thesis has been submitted as: Juby AG, Davis CMJ, Minimaana S, Mager DR. Observational cohort study of healthy community dwelling older adults followed for 12-months to assess the impact of lifestyle on sarcopenic status. *Heliyon,* 2022 (submitted)

I was responsible for the observational cohort design of the study, applying and obtaining trial funding from the Department of Medicine, Summer Student Research Program. funding for the DXA BC from MIC, and ethics application from Health Research Ethics Board (HREB) at University of Alberta. I was responsible for participant screening, all the clinical evaluations (including anthropometric evaluations), and all the cognitive evaluations. CMJD and SM performed all the functional assessments. I was responsible for designing, and teaching the social activity class twice weekly for the 12-month study duration. Data entry was performed by CMJD, SM and Debbie Smith (DS). Data analysis was by AGJ, and statistical analysis was by DRM and AGJ. Data interpretation was by AGJ and DRM. Manuscript preparation was by AGJ, with final approval by CMJD, SM and DRM. Manuscript submission was by AGJ, and once published, primary responsibility for the final content is with AGJ.

Regulatory approval for this study was obtained from University of Alberta HREB No. Pro 00047132, July 2014.

Dedication

To my children

who have never questioned why their mother would be crazy enough to go back to University,

and have always ensured that I felt loved, and stayed grounded,

and make everything I do worthwhile.

You are my world.

Acknowledgements

I respectfully acknowledge that the work on this thesis took place on Treaty 6 territory; traditional lands of First Nations and Métis people.

Firstly, I would like to thank my preceptor, Dr Diana Mager, who was brave enough to take on a mature student with a busy clinical practice. For your enthusiasm for my research projects, and unending support and patience in navigating me through this PhD journey, I extend sincere thanks and gratitude. You will never know how heartwarming it was to meet a fellow Academic who had as much enthusiasm as I did for my areas of interest.

I would also like to thank my committee members, Dr Catherine Field and Dr Norman Boulé, for your invaluable feedback and support, and keeping me on focus! I have learned a lot from both of you that I know will also be of much future benefit. Thank you also to Dr Carla Prado, and Dr Catherine Chan for serving on my candidacy examination committee, and Dr Vera Mazurak for her role as chair. Thank you also to Dr Leah Gramlich and Dr Heather Keller for agreeing to be external examiners on my PhD defence, and to Dr Susan Gilmour for her role as chair.

Heartfelt thanks to all my participants and their families without whom the studies in this thesis would not have been possible. Your willingness and good humor in undergoing the numerous physical, cognitive and laboratory tests is much appreciated. A special thanks to my Alzheimer's dementia participants and their incredible caregivers – your strength in difficult times serves as a daily inspiration to me, and reminds me why I am a Geriatrician. To all the patients on three continents that I have had the pleasure to meet and learn from, who fed my desire to improve their care and lessen their suffering, and gave me joy when even a small part of that was possible. You are the ones who have inspired me to keep seeking out new solutions to provide ease to your dis-ease.

Grateful thanks is also extended to Ms Joan Kravic and Ms Debbie Smith for their administrative support and patience.

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imaging consultants (MIC, Edmonton) skilled radiology technologists gave their time to perform all the DXA body composition tests in this study.

Inspiration to be a lifelong learning has come from many sources in my life and it is appropriate that I should acknowledge them. Firstly, my parents who, when frustrated with my incessant childhood questions, sent me to look in books for the answers, which instilled a lifelong interest in research and discovery. My grade 7 teacher, Mrs. Margaret Mason, who was an exceptional teacher and encouraged my intellectual curiosity, and was the first person to say it was alright to ask "Why?" You supported my dreams of an academic career, and helped me believe it was possible. Professor James ("Jimmy") E. Thomas, Chair of the Department of Medicine at the Godfrey Huggins School of Medicine in Zimbabwe who was an exemplary clinician, and whom I hold up as the "gold standard" of clinical teaching. You always believed in my ability as a clinician, and continued to provide support and encouragement for my Academic career long after medical school was over, and we had both moved to the Northern hemisphere. My visits with you and your wife in your later years are very cherished memories, and I shall never forget your great sense of humor and your infectious laugh. My Grade 12 Mathematics Prize in Zimbabwe, which allowed me to purchase my very first medical textbook, a wonderful book on Histology by Dr C.R. Leeson and Dr T. S. Leeson. Little did I know at the time, I would end up working in the same Faculty of Medicine at the University of Alberta as Dr T.S. Leeson, completing the circle of life events that resulted in me being lucky enough to meet him.

To all the researchers around the world whose work I cited in this thesis. thank you for publishing your work to help educate and inspire others to continue to search for answers and strategies to improve the health of the global community.

"Intellectual growth should commence at birth and cease only at death"

Alberta Einstein

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List of Abbreviations

AACS: AcAc-CoA synthetase

Aβ: amyloid-beta

AbSPORU: Alberta Strategy for Patient Oriented Research Support Unit

AC-1202: MCT esterase (capsule)

AcAc: acetoacetate

ACC: acetyl-CoA carboxylase

ACLY: adenosine triphosphate citrate lyase

ACSS2: acetyl-CoA synthetase

AD: Alzheimer's dementia

ADAS CGIC: Alzheimer’s Disease Cooperative Study – Clinical Global impression of Change

ADAS Cog: Alzheimer’s Disease Assessment scale – Cognitive subscale

ADL: activities of daily living

ADP: Adenosine diphosphate

AgRP: agouti-related peptide

AHA: American Heart Association

α-KG: Alpha KetoGlutarate

aLM: appendicular lean muscle

AMP: adenosine monophosphate

AMPK: activated protein kinase

ANLS: astrocyte-to-neuron lactate shuttle

APOE: apolipoprotein E

ASCM: American College of Sports Medicine

ASM: appendicular skeletal muscle

ATP: Adenosine triphosphate

AUC: area under the curve

AWGS: Asian Working Group on Sarcopenia

β: beta

BADL: basic activities of daily living

BBB: blood brain barrier

BC: Body composition

BCAA: branched-chain amino acids

BDH1: D-β-hydroxybutyratedehydrogenase 1

BDNF: brain-derived neurotrophic factor

BHB: beta-hydroxybutyrate

BIA: bioimpedance assay

BMI: body mass index

BNT: Boston Naming Test

βOHB: beta-hydroxybutyrate

βox: beta-oxidation

BVMT: Brief Visual Memory Test

C: carbon

Ca2+: calcium

CACT: Carnitine-acylcarnitine translocase

CGA: Comprehensive Geriatric Assessment

ChEI: cholinesterase inhibitors

CI: citrate transporter

CIC: citrate/isocitrate carrier

CKD: chronic kidney disease

CoA: coenzyme A

CoA-SH: CoA sodium salt hydrate

CPT I: Carnitine palmitoyltransferase I

CPT II: Carnitine palmitoyltransferase II

CS: citrate synthase

cThiolase: cytoplasmic thiolase

COVID-19: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

CVD: cardiovascular disease

DASH: Dietary Approaches to Stop Hypertension diet

DB: double blind

DNL: de novo lipogenesis

DSS: Digital Symbol Substitution

DXA: dual-energy Xray absorptiometry

e−: electron

EAA: essential amino acid

EQ-5D: EuroQol 5D total

EQ-5D anxiety/depression: EuroQol 5D anxiety/depression

EQ-5D mobility: EuroQol 5D mobility

EQ-5D pain: EuroQol 5D pain

EQ-5D self care: EuroQol 5D self care

EQ-5D usual activities: EuroQol 5D usual activities

EQ-5D VAS: EuroQol 5D visual analogue scale

ETC: electron transport chain

EWGSOP: European Working Group on Sarcopenia in Older People

FACS: Fatty acyl-CoA synthetase

$FADH_2$: Flavin adenine dinucleotide

FATP: Fatty acid transporter protein

FDG-PET: Positron Emission Tomography with 18F-fluorodeoxyglucose

FFA: free fatty acid

Fru-6-P: fructose-6-phosphate

GABA: gamma-aminobutyric acid

GAD: glutamate decarboxylase

Gal-6-P: galactose-6-phosphate

GFs: growth factors

GLc: glucose

Glc-6-P: glucose-6-phosphate

Gln: glutamine

Glu: glutamate

GLUT1: glucose transporter 1

GLUT3: glucose transporter 3

Glyc: glycogen

GS: glutamine synthetase

GSK-3P: glycogen synthase kinase-3 phosphorylation

HBP: hexosamine biosynthetic pathway

HMD: beta-hydroxy-beta-methylbutyrate

HMGCL: 3-hydroxymethylglutaryl-CoA lyase;

HMGCS2: 3-hydroxymethylglutaryl-CoA synthase 2

HDL: high-density lipoprotein

HREB: Health research Ethics Board, University of Alberta

IADL: instrumental activities of daily living

AGF-1: Insulin growth factor-1

IKK: IκBα kinase

IL-6: interleukin-6

JNK: c-Jun N-terminal kinase

KDHC: ketoglutarate dehydrogenase complex activity

Lac: lactate

LCT: long chain triglycerides

LDL: low-density lipoprotein

LTP: long-term potentiation

MAL: malate

MCI: mild cognitive impairment

MCFA: medium chain fatty acid

MCP-1: monocyte chemoattractant proteion-1

MCT: medium chain triglyceride

MCT1/2: monocarboxylic acid transporters

ME: malate dehydrogenase

MIND: Mediterranean–DASH Intervention for Neurodegenerative Delay diet

mM: mmol/L (millimols per litre)

Mmass: muscle mass

MMSE: Mini Mental Status Examination

MoCA: Montreal Cognitive Assessment

MPC: mitochondrial pyruvate carrier

mThiolase: mitochondrial thiolase

n-3 PUFA: n-3 polyunsaturated fatty acids

NADH: Nicotinamide adenine dinucleotide

NALS: neuron-to-astrocyte lactate shuttle

NE: noradrenergic

NLRP3: nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain containing receptor 3

NMDA: N-methyl-D-aspartate

NMDARs: N-methyl-D-aspartate receptors

OAA: oxaloacetate

OC: Osteoporosis Canada

OLT: open-label trial

OP: Oxidative Phosphorylation

PAG: phosphate-activated glutaminase

PC: pyruvate carboxylase

PDH: pyruvate dehydrogenase

PDHC: pyruvate dehydrogenase complex

PEP: phosphoenolpyruvate

PGC-1a: peroxisome proliferator-activated receptor-γ coactivator 1α

PI-3λ: phosphoinositide-3 kinase

PKB/AKT: protein kinase B/Akt

POMC: pro-opiomelanocortin

PPP: Pentose phosphate pathway

pSer: phosphoserine

pTyr: phosphotyrosine

Pyr: pyruvate

QOL: quality of life

RCT: randomized controlled trial

RDA: recommended daily allowance

RDI: recommended dietary intake

RL/RI: Rappel Libre/Rappel Indice

RNA PKR: double-stranded ribonucleic acid-dependent protein kinase

ROS: reactive oxygen species

SCOT: succinyl-CoA:3-oxoacid-CoA transferase

SCWD: Society for Sarcopenia, Cachexia and Wasting Disorders

SF36: Short Form 36

SF36 COM: short form 36 combined

SF36 GH: short form 36 general health

SIRT 1: silent mating type information regulation 2 homolog sirtuin 1

SOD: superoxide dismutase

SPARC: secreted protein acidic and rich in cysteine

SPPB: Short Physical Performance Battery

SQ: square step test

Stroop: Stroop Colour Word Interference Task

T: tablespoon

Tau P: Tau phosphorylation

TCA: tricarboxylic acid cycle

TEN: 10-meter walk test

TG: triglyceride

TIN: Tinetti gait and balance scale

TNFα: tumor necrosis factor-α

TNFR: TNFα receptor

Trails: Trail making A, Trail making B

TrkB: tyrosine kinase B

TUG: Timed Up and Go

UCP 1: uncoupling protein-1

UDP-GlcNAc: uridine diphospho-N-acetylglucosamine

VEGF: vascular endothelial growth factor

VF: Verbal Fluency

VO_{2max}: vital capacity

V-PAL: Verbal Paired Association Learning test

WAIS-III: Wechsler Adult Intelligence Scale-3rd

WMS-R: Wechsler Memory Scale-Revised

List of Appendices

CHAPTER 1: BACKGROUND

1.1 Preface

The prevalence of sarcopenia and dementia increase with age. Patients with Alzheimer's dementia (AD) have been shown to have sarcopenia and dynapenia [Yazar and Yazar, 2019], and reduced lean mass has been reported even in patients with early AD [Burns et al, 2010]. Both sarcopenia and AD impact activities of daily living [ADL] function, but the presence of both compounds this impairment [Sugimoto et al, 2017].

In older adults with AD, there is an increased risk for malnutrition which further increases their risk of concomitant sarcopenia [Rodriguez et al, 2012]. The nutritional issues are not only related to reduced food intake and poor diet quality, but concomitant sarcopenia is also associated with lower levels of hemoglobin and 25 hydroxy vitamin D in females [Lee et al, 2020].

The method by which sarcopenia impacts cognitive impairment, or vice versa, is likely multifactorial. One study showed slow gait speed associated with cognitive impairment was primarily to be related to concomitant sarcopenia [Kim et al, 2016], although it is well established that AD per se slows gait speed [Cedervall et al, 2014]. A recent meta-analysis [Peng et al, 2020] looks at the complicated interaction between these two common conditions affecting older adults, and their similar impact on morbidity and mortality. The adjusted odds ratio (excluding studies deemed of fair quality) of having sarcopenia with cognitive impairment was reported as 1.87 (1.38; 2.54) [Peng et al, 2020].

1.2 Alzheimer's Disease/Dementia

1.2.1 Background

Alzheimer's disease (AD) was first described by a German neuropathologist Alois Alzheimer in 1906. Age ≥ 65 years is associated with an increased lifetime risk of AD, which is almost twice as high for women, 12.4% versus 6.2% in men. [Framingham study. Seshadri and Wolf, 1997].

It is one of the most common causes of dementia, with over 55 million people globally estimated to be living with AD in 2020, and 10 million new cases annually. It is the seventh leading cause of death. [WHO, 2021]. By 2030, the figure is being speculated to rise to 78 million. In 2019, the estimated total global societal cost of dementia was US$ 1.3 trillion. [WHO, 2021] It disproportionately affects women, with sixty-five percent of total deaths due to dementia in women, and women provide the majority of informal care for people living with dementia, accounting for 70% of carer hours [WHO, 2021].

AD is associated with progressive decline in memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. Consciousness is not affected. The impairment in cognitive function may be preceded by changes in mood, emotional control, behaviour, or motivation. As it is a clinical diagnosis, there are specific diagnostic criteria [American Psychiatric Association, 2013].

Pathologically, AD is characterized by the progressive accumulation of neuritic plaques of amyloid-beta (Aβ) and neurofibrillary tangles of hyperphosphorylated tau. Individuals with AD show impaired glucose utilization in the brain (using positron emission tomography with 18F-fluorodeoxyglucose (FDG-PET)), with a consistent pattern of reduction in the cerebral metabolic rate of glucose utilization in the hippocampus, posterior cingulate, precuneus, and prefrontal locations [Mosconi et al, 2007, 2009]. See **Figure 1.**

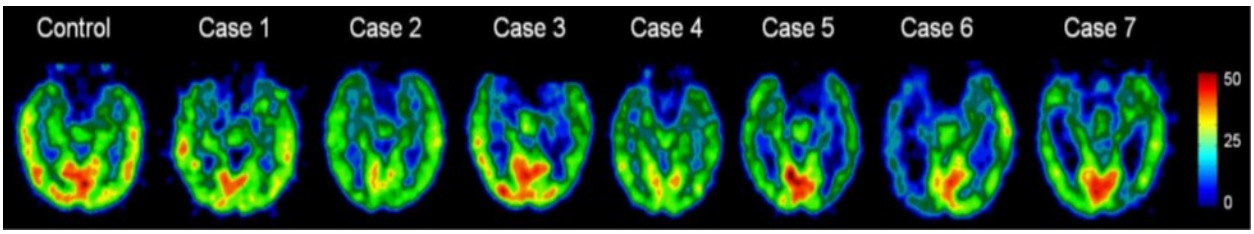

Figure 1: FDG-PET scans showing medial temporal lobe hypometabolism for each subject as compared to a healthy normal control. [Mosconi 2009] (Reproduced with permission).

The presence of insulin resistance in the brains of AD patients has also been demonstrated [Talbot et al, 2012], but there is no specific pharmacotherapy targeted at this currently. Licenced AD medications address neurochemical deficiencies related to neuronal loss, such as cholinesterase inhibitors (ChEI) and N-methyl-d-aspartate (NMDA) receptor antagonists, and are used in attempts to delay the progression of AD [Standbridge, 2004]. Cholinergic stabilization

with ChEI therapy implies neuroprotection, and a resultant slowing of disability and disease progression. The NMDA-receptor antagonist, memantine, may block neural excitotoxicity [Standbridge, 2004]. However, clinical results have not been as encouraging as hoped, prompting researchers to explore many other pharmacologic and nutritional options, as discussed in recent reviews [Srivastava et al, 2021; Noori et al, 2021; Ramesh et al, 2021]. In addition, the etiology of AD is still elusive [Adams, 2021].

1.2.2 Brain energy metabolism

The brain's preferred fuel is glucose, and it requires 140g of glucose daily (600kCal) [Cahill, 1970]. Although it only accounts for approximately 2% of the body weight, the brain consumes about 20% of the glucose-derived energy, thus it is the main consumer of glucose [Erbsloh et al, 1958]. It is well known that the cerebral metabolic rate of glucose is reduced in hippocampal and parietal regions, even many years before the onset of AD symptoms [Mosconi et al, 2010]. See **Figure 2**, where Pittsburgh Compound-B is used as a PET tracer for Aβ plaques, and FDG for glucose.

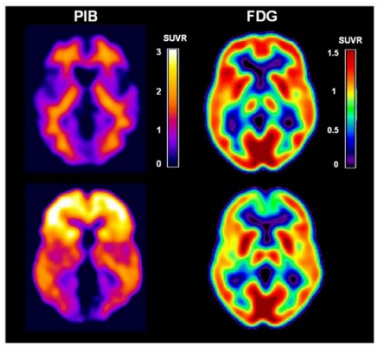

Figure 2: Pittsburgh Compound-B (PIB) and FDG-PET scans in two representative cognitively normal individuals: one at low risk for AD (top row: negative PIB and normal FDG uptake); and one at high risk for AD (bottom row: positive PIB and reduced FDG uptake) based on PET imaging findings. Cerebral-to-cerebellar PIB Standardized Uptake Value ratios (SUVR) are displayed for each modality using a colour coded scale. [Mosconi et al, 2010]. (Reproduced with permission).

Dependence on glucose as its preferred fuel is because of the tight junctions between brain endothelial cells in the blood-brain barrier (BBB), and its selective permeability for glucose in the adult brain. [Mergenthaler et al, 2013]. Although glucose cannot be replaced as an energy source, it can be supplemented by alternative substrates, such as pyruvate [Gonzalez et al, 2005],

lactate when blood levels of lactate are elevated during strenuous physical activity [Van Hall et al, 2009; Dienel, 2012], acetate [Rae et al, 2012] or ketones during prolonged starvation [Lutas and Yellen, 2013]. (See further discussion of ketones in 1.4). The BBB restricts entry of neuroactive compounds (e.g., glutamate, aspartate, glycine, D-serine) into the brain requiring these compounds to be synthesized from glucose *within* the brain. This is in sharp contrast with muscle and liver. Muscle and liver do not have the same tight junctions between their vascular endothelial cells, enabling them to metabolize glucose, monocarboxylic acids, fatty acids, amino acids, and ketone bodies [Mergenthaler et al, 2013].

In a steady-state, brain tissue glucose concentration is about 20% of that of plasma. Glucose enters the brain from the blood by crossing the BBB through glucose transporter 1 (GLUT1). Glucose and other metabolites (e.g. lactate) are then rapidly distributed through a highly coupled metabolic network of brain cells [Mergenthaler et al, 2013]. The predominant glucose transporter is GLUT1. Glucose transport capacity significantly exceeds demand, aided by the higher transport rate of another glucose transporter, glucose transporter 3 (GLUT3). This ensures that neurons have sufficient glucose supply under varying glucose levels and different activity states. [Dienel, 2019]. Some of the roles of glucose metabolism involve precursors for neurotransmitter synthesis, ATP synthesis to maintain ion gradients and cell function, synaptic activity, and autophagy control [Mergenthaler et al, 2013].

The role of glucose for brain function is summarised in **Figure 3** [Mergenthaler et al, 2013]. Of interest, because of its reliance on glucose, the brain can regulate systemic homeostasis and food intake through the vagal nerve and neuroendocrine signals (see (d) in Figure 3). Insulin and glucagon-like peptide-1 mediate metabolic signalling through brain insulin receptors, having a central as well as a peripheral effect on glucose metabolism.

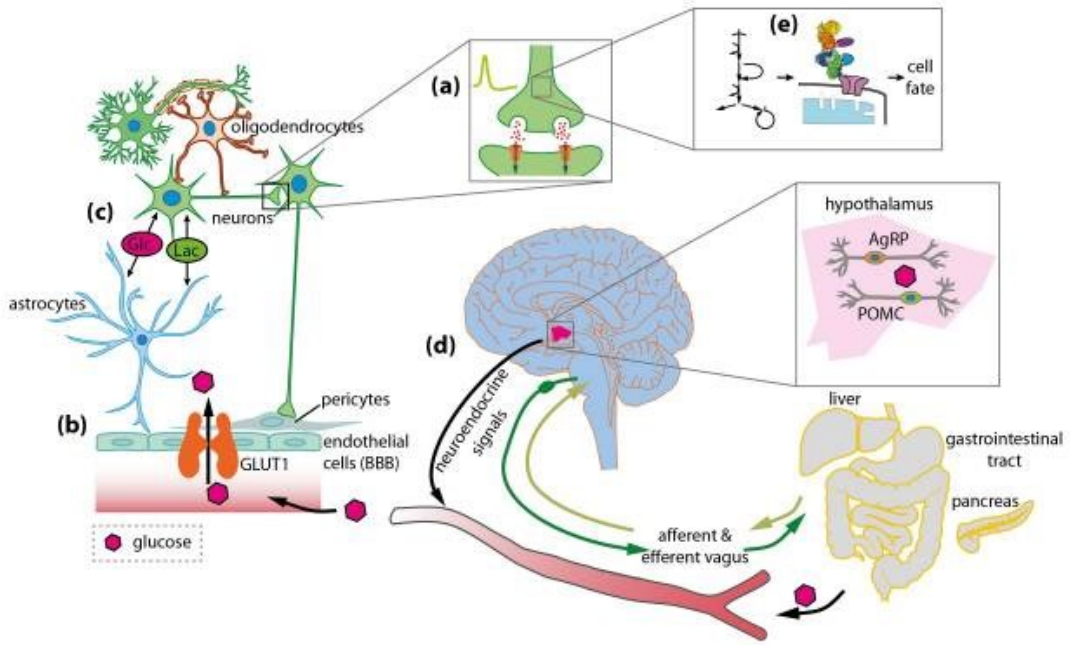

Figure 3: The role of glucose for brain function [Mergenthaler et al]. (Reproduced with permission).

Glucose-6-phosphate (Glc-6-P) is a precursor for glycogen, which is the brain's only energy reserve [Mergenthaler et al, 2013]. Glycogenolysis spares glucose for use by neurons, an important mechanism to prolong neuronal function during severe hypoglycaemia [Dienel, 2012]. Although both neurones and astrocytes are main consumers of glucose, their relative contributions to glucose utilisation remains controversial [Mergenthaler et al, 2013]. Increased blood lactate provides 'glucose sparing'. As a supplemental oxidative fuel lactate helps maintain availability of glucose for the glycolytic and pentose phosphate shunt pathways that provide critical functions for the brain [Mergenthaler et al, 2013]. In summary, there is a complex brain-body interaction geared towards ensuring glucose supply to the brain, peripheral nutrient uptake and utilisation, as well as feeding, in order to regulate glucose and energy homeostasis [Grayson et al, 2013].

A 2015 PET study showed that the regional *brain ketone metabolism was preserved in AD* patients (unlike regional glucose metabolism), and that there were large dissociations of the cerebral metabolic rate of glucose and ketones in the parietal, thalamus, and posterior cingulate

cortex [Croteau et al, 2018]. See **Figure 4**. This confirms the ability of the brain to still utilise ketones as an alternative energy source even in MCI and AD.

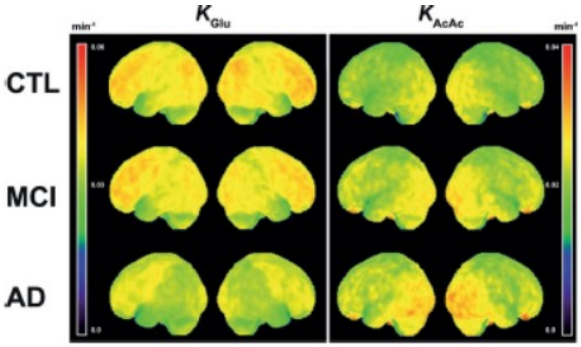

Figure 4: Preserved ketone metabolism in AD. Voxel-wise 3D surface projection of the brain uptake rate constant for glucose (KGlu; min−1) and acetoacetate (KAcAc; min−1) in healthy older controls (CTL; n = 24), mild cognitive impairment (MCI; n = 20) and early Alzheimer's disease (AD; n = 19) [Croteau et al, 2018]. (Reproduced with permission).

Mechanisms for impaired cerebral glucose metabolism

1. ***Impaired glucose transport***

Glucose utilization declines (up to 45%) in early AD, and is closely associated with the alteration of insulin signaling [Poddar er al, 2021]. In brain regions such as the hippocampus, there are many insulin receptors, and numerous studies have shown that insulin plays a crucial role in memory and learning processes [Craft et al. 1999; Park, 2001].

Insulin resistance (defined as decreased ability of insulin to exert it's influence on target tissues) is well described in diabetes and metabolic syndrome. What is less well known is ***cerebral insulin resistance***, associated with Alzheimer's dementia and Type 2 diabetes [Cholerton et al, 2013]. Insulin signalling is direct, via an insulin receptor, and indirect via neuromodulatory actions (such as NMDA receptors, and neurotransmitters). There are also fewer insulin receptors in the brain in insulin resistance. Lack of insulin in the brain *increases amyloid beta (Aβ) formation*, *decreases Aβ excretion* and *increases tau phosphorylation*, all of which are key pathological changes in the development and progression of AD [Busche and Hyman, 2020]. Insulin prevents the binding of Aβ to synapses, impeding its synaptotoxic effects [Cholerton et al, 2013]. Accumulation of Aβ further increases insulin resistance, and *synapse loss* which also increases function of the insulin degrading enzyme. This results in a vicious circle, positive

feedback loop. See **Figure 5** showing the summary of the cellular consequences of cerebral insulin resistance [Cholerton et al, 2013].

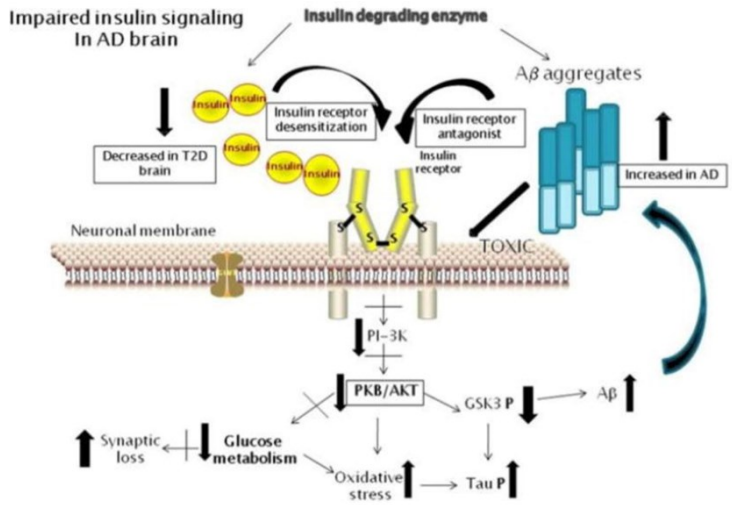

Figure 5: Consequences of insulin and Aβ interactions on reduced neuronal insulin receptor signaling and promoting Alzheimer's disease pathology [Cholerton et al, 2013].

Abbreviations: S: sensitisation; Aβ: amyloid β; PI-3λ: phosphoinositide-3 kinase; PKB/AKT: protein kinase B/Akt; GSK-3P: glycogen synthase kinase-3 phosphorylation; Tau P: Tau phosphorylation. (Reproduced with permission).

This abnormal insulin metabolism also *increases oxidative stress and neuroinflammation*, causing *decreased neurogenesis*. Increased fasting insulin levels can be measured in the serum in AD, even though the cerebral levels are low. Some authors refer to AD as "Type 3 diabetes" because of these metabolic changes [Leszek et al, 2017; Monte, 2019] See **Figure 6**.

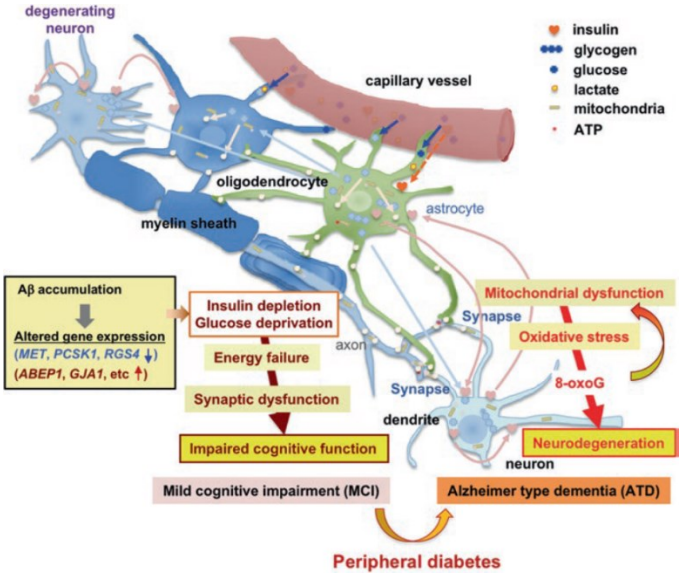

Figure 6: The full spectrum of Alzheimer's disease is rooted in metabolic derangements that drive type 3 diabetes [Monte, 2019]. (Reproduced with permission).

Figure 7 summarises central insulin resistance in normal versus AD brains [De Felice et al, 2014].

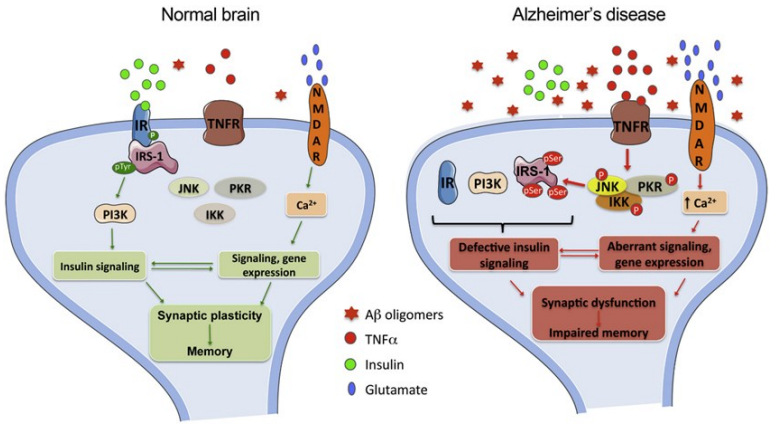

Figure 7: Impaired neuronal insulin signaling in Alzheimer's disease (AD).

Abbreviations: IR, insulin receptor; IRS-1, insulin receptor substrate 1; pSer, phosphoserine; PI3K, phosphoinositide 3-kinase; Ca^{2+}, calcium; pTyr, phosphotyrosine; NMDARs, N-methyl-D-aspartate receptors; TNFα, tumor necrosis factor-α; TNFR, TNFa receptor; JNK, c-Jun N-terminal kinase; RNA PKR, double-stranded ribonucleic acid-dependent protein kinase; IKK, IκBα kinase [De Felice et al, 2014] (Reproduced with permission).

2. ***Impaired central glycolysis***

There are three irreversible and rate-controlling steps of glycolysis, which are catalyzed by the enzymes hexokinase, phosphofructokinase and pyruvate kinase, yielding intermediate metabolites that can undergo conversion to serine, glycine and alanine. See **Figure 8** [An et al, 2018].

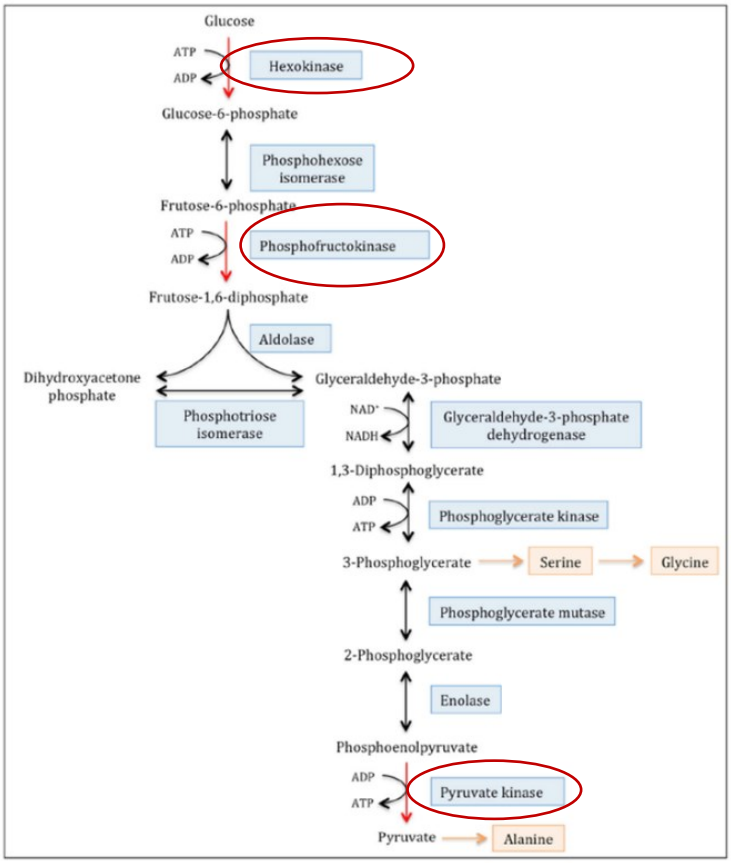

Figure 8: Glycolytic intermediates are biosynthetic precursors of the non-essential amino acids, serine, glycine and alanine. (Rate limiting enzymes circled in red). [Adapted from An et al, 2018]

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; NAD^+, nicotinamide adenine; NADH, nicotinamide adenine dinucleotide (Reproduced with permission).

Brain glucose regulation is critical in AD pathogenesis, and closely reflects severity and expression of AD. The activity of the three rate controlling enzymes is the lowest in clinical AD, when compared to asymptomatic AD and normal participants. An et al, speculate that this failure

of neuronal glucose utilization due to impaired glycolysis, is a fundamental feature of AD [An et al, 2018].

Impaired fat metabolism

Brains with AD have been found to have a higher incidence of an aberrant lipid metabolism with "adipose inclusions" or formation of "lipoid granules" [Foley, 2010].

Wahrle et al, showed evidence of direct regulation of brain cholesterol level on β-secretase activity and the production of Aβ [Wahrle et al, 2002]. Hypercholesterolemia is an early risk factor for the development of amyloid pathology, and there are several reports which indicate that in addition to cholesterol, other lipids (eg. isoprenoids, sphingolipids, and phospholipids) also play an important role in the production of Aβ [Hartmann and Kuchenbecker, 2007; Hannun and Obeid, 2008; Hooff et al, 2010].

Other impaired metabolites

Other metabolites are also found to have a correlation with the development and progression of AD. These include: 5-Hydroxyindoleacetic acid (5-HIAA), a major metabolite of serotonin (5-HT); Methionine, involved in one-carbon metabolism and methylation processes; Vanillylmandelic acid (VMA) or homovanillic acid (HVA), an end product of catecholamine metabolism, and; xanthosine, a metabolite of purine pathway. These metabolites are found to be significantly increased in AD [Kaddurah-Daouk et al, 2013].

Impaired metabolomics in normal versus AD is summarised in **Figure 9** [Poddar et al, 2021].

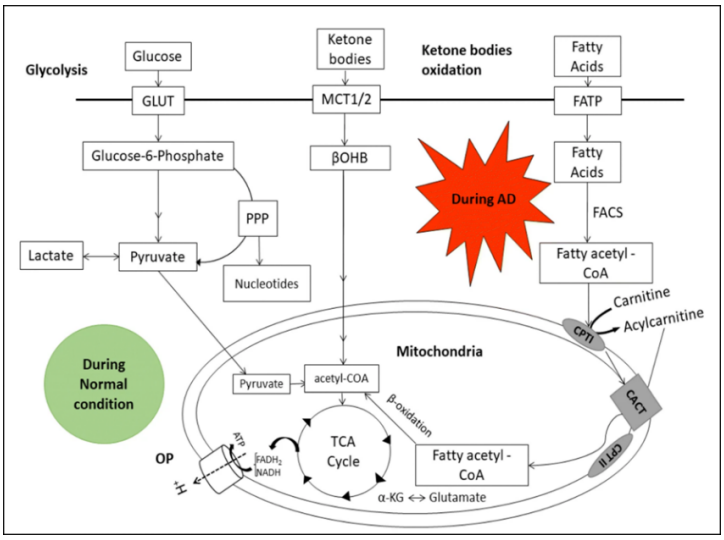

Figure 9: Changes in metabolomics in Alzheimer's disease (AD). [Podder et al, 2021]

Abbreviations: ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; CACT:Carnitine-acylcarnitine translocase; CoA: Co enzyme A; CPT I: Carnitine palmitoyltransferase I; CPT II:Carnitine palmitoyltransferase II; FACS: Fatty acyl-CoA synthetase; FADH2:Flavin adenine dinucleotide; FATP: Fatty acid transporter protein; GLUT: Glucose transporter; MCT1/2: Monocarboxylate transporters; NADH: Nicotinamide adenine dinucleotide; OP: Oxidative Phosphorylation PPP: Pentose phosphate pathway; TCA: Tri carboxylic acid cycle; α-KG: Alpha KetoGlutarate; βOHB: β-Hydroxybutyrate. (Reproduced with permission).

1.2.3 Alzheimer's disease and diet

The first methodologically robust study, the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), was a multidomain intervention consisting of diet, exercise, cognitive training, and vascular risk monitoring, and resulted in an improvement or maintenance of cognitive functioning in at-risk elderly people from the general population [Ngandu et al, 2015], but no volumetric changes on MRI compared to control [Stephen et al, 2019]. This 2-year intervention showed it was possible to reduce the risk of cognitive decline among the general elderly population, rather than solely with patients in a clinical setting.

Other studies attribute the diet alone as having a key role in delaying the progression of AD. Some have tested diets considered as heart-healthy, such as Dietary Approaches to Stop Hypertension diet (DASH), Mediterranean–DASH Intervention for Neurodegenerative Delay diet (MIND) [Morris et al, 2015], or the Mediterranean diet alone.

The inflammatory component of AD has been treated by supplementation with fatty acids, vitamins, flavonoids, polyphenols, probiotics, and reduction of dietary advanced glycation end products [Szczechowiak et al, 2019].

Ketogenic diets, which are protective in other neurological disorders, also seem to have great potential in the prevention and treatment of the AD [Broom et al, 2019], and will be discussed in more detail to follow. In humans with AD, few clinical trials have been carried out using ketogenic diets; instead, ketogenic agents have been used. In animal models of AD, the increase in ketone levels in the body through intermittent fasting or supplementation with ketone esters have shown promising results [Pawlosky et al, 2017].

The rationale for the role of ketones in mitigating AD is becoming more apparent. In addition to modifying cellular metabolism, ketones are therapeutic in protecting against production of the toxic Aβ plaques associated with AD [Van der Auwera et al, 2005] and may potentially reverse the Aβ neurotoxicity. (See further discussion in section 1.5). Modern diets high in carbohydrates (CHO) may potentially be contributing to increasing Alzheimer's incidence [Henderson, 2004]. Therefore, the ketogenic diet (including carbohydrate restriction) might be useful in the management of Alzheimer's disease [Broom et al, 2019]. Sweet cravings are common in individuals with AD [Mungas et al, 1990], and if this food preference develops early, dietary selections made by these individuals could further contribute to their risk of disease progression. In addition, this sweet craving could also make compliance with a low CHO ketogenic diet more difficult.

Providing ketones without need for strict diets is therefore a more viable option in the AD population.

1.3 Medium Chain Triglycerides (MCT)

1.3.1 Definition

Fats are an essential part of the human diet. Most dietary fats are composed of long chain fatty acids (containing >12 carbon (C) fatty acids), termed long chain triglycerides (LCT), such as fats found in dairy fat, meat fat and vegetable oils. Medium chain triglycerides (MCT), however

contain 6-12C fatty acids. Dietary sources include coconut and palm kernel oils, and breast milk, and usually constitute a small part of the human diet. On the other hand, for short chain fatty acids (SCFA) (<6C fatty acids), although found in the diet (dairy, breast milk), the most important source is bacterial fermentation of amylase resistant starch and non-starch polysaccharides in the gut [Schönfeld and Wojtczak, 2016]. The focus of this review will be on MCT because of its unique role in human health and its potential ketogenic effects that may be beneficial in optimizing cerebral energy metabolism. **Table 1** shows some examples of medium chain fatty acids (MCFA)

Table 1: Examples of medium chain fatty acids (MCFA), including their chemical structure. [Adapted from Schönfeld and Wojtczak, 2016].

Lipid number	Formula	Name		Appearance
		Common	Systematic	
C6:0	$CH_3(CH_2)_4COOH$	Caproic acid	Hexanoic acid	Oily liquid
C8:0	$CH_3(CH_2)_6COOH$	Caprylic acid	Octanoic acid	Oily liquid
C10:0	$CH_3(CH_2)_8COOH$	Capric acid	Decanoic acid	White crystals
C12:0	$CH_3(CH_2)_{10}COOH$	Lauric acid	Dodecanoic acid	White powder

1.3.2 Digestion and absorption

When consumed, unlike for LCT, MCT does not stimulate the release of cholecystokinin (CCK). This lack of CCK response is felt to be due to the shorter length of intestine exposed to MCFA (Hopman et al, 1984). Therefore, ingestion of MCT, as opposed to LCT, does not increase the release of pancreatic enzymes and bile salts. Neither may be necessary for the hydrolysis and absorption of MCT, consistent with their low molecular weight, shorter chain length and increased water solubility [Fernandes et al, 1962; Symersky et al, 2002].

Absorption into epithelial cells across the unstirred layer is greater, compared to LCT, because MCT has greater water solubility, and therefore can more readily cross the unstirred layer and transit across the epithelial cells, without the aid of bile salts [Bach and Babayan, 1982]. The small breakdown products of MCT digestion and initial epithelial cellular absorption, can be transported directly into portal venous system with no need for chylomicron formation [Bach and Babayan, 1982]. Therefore, MCTs stimulate less flow of lymph, and do not require lymphatic transport, unlike LCT. The transit time is faster with MCT than LCT (59 minutes versus 77 minutes) [Ledeboer et al, 1995], but there is individual variability. This potentially may reduce absorption of calcium, magnesium, and amino acids. Ketogenic diets in children with epilepsy have recently been shown to be detrimental to bone health [Hawkes and Levine, 2014; Simm et al, 2017]. In a recent rat study, octanoic acid (Caprylic acid, C8) affected bone mineralisation, but not decanoic acid (Capric acid, C10) [Jain et al, 2021].

1.3.3 Metabolism

MCFA cross the mitochondrial membranes of muscle and liver independently of the acylcarnitine transfer system, and thus are rapidly absorbed and a readily available energy source - as fast as glucose, with twice the caloric density of protein and carbohydrates (CHO) [Bach and Babayan, 1982]. Gram for gram, MCT versus LCT is 8.3 Cal/g versus 9 Cal/g, so there are 10% fewer calories with MCT. Rapid beta-oxidation occurs in the liver, with utilization as energy and little tendency to deposit as body fat [Bach and Babayan, 1982]. This rapid entry, and quick oxidation, produces ketone bodies which are then available to the brain through crossing the BBB [Lei at al, 2016].

See **Figure 10** for a summary of absorption and metabolism [Lei et al, 2016].

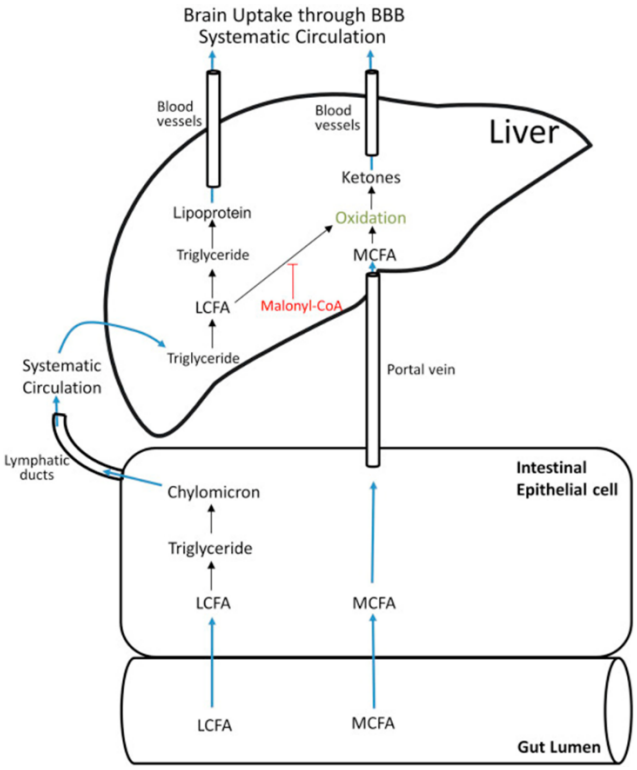

Figure 10: MCT absorption and metabolism.

Abbreviations: LCFA, long chain fatty acids; MCFA, medium chain fatty acids [Lei et al, 2016]. (Reproduced with permission by the publisher).

MCFA, unlike LCFA, do not require carnitine, or activated carnitine palmitotransferase 1 for entry into the mitochondria. By bypassing this step, there is accelerated β-oxidation to acetyl Co-A. The resultant acetyl-CoA is used either for ketogenesis or enters the tricarboxylic acid cycle for complete oxidation to water and carbon dioxide [Babyan, 1987]. Alternatively, via citrate metabolism, the acetyl-CoA can be transported to the cytoplasm and used for LCFA synthesis. [Kornacker and Ball, 1965]. See **Figure 11.**

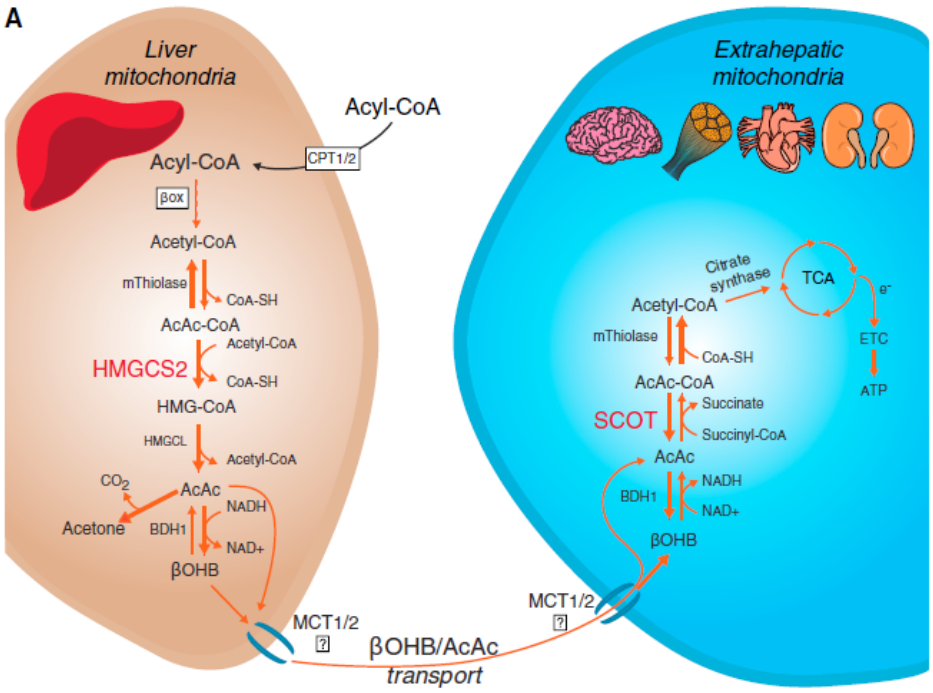

Figure 11: Ketone body metabolism (oxidative).

Abbreviations: Abbreviations: AcAc, acetoacetate; ATP, adenosine triphosphate; BDH1, D-β-hydroxybutyrate dehydrogenase 1; βOHB, β-hydroxybutyrate; βox, β-oxidation; CoA, coenzyme A; CoA-SH, CoA sodium salt hydrate; CPT, carnitine palmitoyltransferase; e−, electron; CS, citrate synthase; ETC, electron transport chain; HMGCL, 3-hydroxymethylglutaryl-CoA lyase; HMGCS2, 3-hydroxymethylglutaryl-CoA synthase 2; mThiolase, mitochondrial thiolase; SCOT, succinyl-CoA:3-oxoacid-CoAtransferase; TCA, tricarboxylic acid.CoA-SH; mitochondrial HMG-CoA synthase (mHS) and HMG-CoA Lyase (HL)MPC, mitochondrial pyruvate carrier; CPT1/2, carnitine palmitoyltransferase 1/2; PEP, phosphoenolpyruvate; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; ME, malate dehydrogenase; OAA, oxaloacetate; MAL, malate; TCA, tricarboxylic acid cycle; CS, citrate synthase; CIC, citrate/isocitrate carrier; AcAc, acetoacetate; βOHB, beta hydroxybutyrate; MCT1/2, monocarboxylate transporters 1/2; ACLY, adenosine triphosphate citrate lyase; ACC, acetyl-CoA carboxylase; ACSS2, acetoacetyl-CoA synthetase 2; mThiolase, mitochondrial thiolase; cThiolase, cytosolic thiolase. [Puchalska andCrawford,2017]. (Reproduced with permission).

Different MCFA have been shown to have differential effects in the generation of plasma ketones. In an 8-hour metabolic study, St. Pierre and colleagues showed the ketogenic effect was

three-times higher with C8 versus C10, and six-times higher versus C12. They also demonstrated this was not related to the plasma lipid levels which were equivocal [St-Pierre, et al, 2019].

A. Effect on satiety

MCT increases satiety. Several mechanisms are proposed for this: pre-absorptive effect, due to a bad taste when broken down by lingual lipase causing less consumption [Bach and Babayan, 1982]; increased production of ketones which suppress hunger [Krotkiewski, 2001] ; hyperinsulinaemic response [Opara et al, 1994]; no second peak in serum triglycerides (as happens in LCTs), leading to more satiety with the initial meal [Van Wymelbeke et al, 1998]; and, greater rise in the post prandial peptide tyrosine-tyrosine (YY) [St Onge et al, 2014].

B. Effect on insulin metabolism

MCT administration can increase insulin-mediated glucose metabolism in people with diabetes, as well as normo-triglyceridemic and hyper-triglyceridemic non-diabetics [Eckel et al, 1992]. The mechanism of this improved insulin-mediated glucose metabolism is unclear, but it persists for at least 12-hours after the MCT ingestion, without any effect on serum glucose, and reduction of prandial glycemic excursions was also identified. [Eckel et al, 1992]. This effect is postulated to be via increased insulin secretion and/or increased insulin sensitivity of peripheral tissues and/or liver. [Wylie and Leveille, 1973; Yeh and Zee, 1976].

C. Effect on serum lipids

MCTs are saturated fats so are assumed to increase cardiovascular risk factors and body weight [Cunnane et al, 2016]. The effects on lipid profile theoretically should be minimal because MCFAs are not incorporated into hepatic lipid synthesis [Bach and Babayan, 1982] and there is a decrease in intestinal absorption of cholesterol, as well as a slowing of cholesterol synthesis from acetyl-CoA. But this remains controversial, with *increase* total cholesterol (11%), LDL cholesterol (12%), VLDL cholesterol (32%) LDL/HDL ratio (12%) plasma total triacylglycerol (22%) reported by some authors [Tholstrup et al, 2004]. Based on their study, Eckel and colleagues felt there were no safety concerns with regard to ketosis in short term administration of MCT to diabetics, because of a lack of a significant BHB response in diabetics (unchanged versus 164% in controls). However, they did note a mild hypertriglyceridemia in their diabetic

participants [Eckel et al, 1992]. These effects seem to be negated if MCT is combined with beneficial oils such as fish oil and olive oil ("functional oils") [St Onge et al, 2003].

However, other studies have shown dietary MCT *reduced* serum triglycerides, glucose and insulin concentrations [Wilson et al, 1983]. In others, the effect on lipid profiles was *neutral* [Mumme et, 2015].

D. Effect on body composition

This remains controversial [Andrew et al, 2012]. Some studies showed *reduced* visceral and subcutaneous fat accumulation in a rat study, which was greater when combined with exercise [Ooyama et al, 2008]. Hypertriglyceridemic individuals showed reduced weight, BMI, waist circumference, hip circumference, waist/hip ratio, body fat and subcutaneous fat with MCT doses of 1.7-24g/day, compared to control individuals on LCT supplementation [Xue et al, 2009]. Increased lipolysis with increased brown adipose activity has been reported in obese mice fed a high fat diet that included MCT [Zhang et al, 2015]. There are also dose dependent effects, with higher doses stimulating more energy expenditure, even though lower doses (probably below 60g/day) may be enough for cardio-metabolic improvements [Bhavsar and St Onge, 2016]. In a meta-analysis of thirteen trials comparing MCT to LCT consumption, within the limitations of the trials reported, the authors concluded that MCT supplementation could induce modest reductions in body weight (0.23-0.8 kg over 10 weeks) and composition. [Mumme et al, 2015].

Generation of ketones as a rapid energy source, may also spare oxidation of branched chain amino-acids and reduce skeletal protein catabolism [Babayan, 1987]. The recent meta-analysis by Mumme and colleagues, however, did not provide enough data for evaluating the effect of MCT on fat:lean mass [Mumme et al, 2015].

E. Effect on inflammation

Synergistic effects of MCT with other fats is still poorly understood, but animal studies and early human studies with MCT and fish oil, versus fish oil alone, showed added benefits of the MCT component [St Onge et al, 2008]. MCT increases n-3 PUFA uptake and decreases inflammation. [Carpentier et al, 2010].

The net effects of MCFA mediated signalling is summarised in **Figure 12** [Huang et al, 2021].

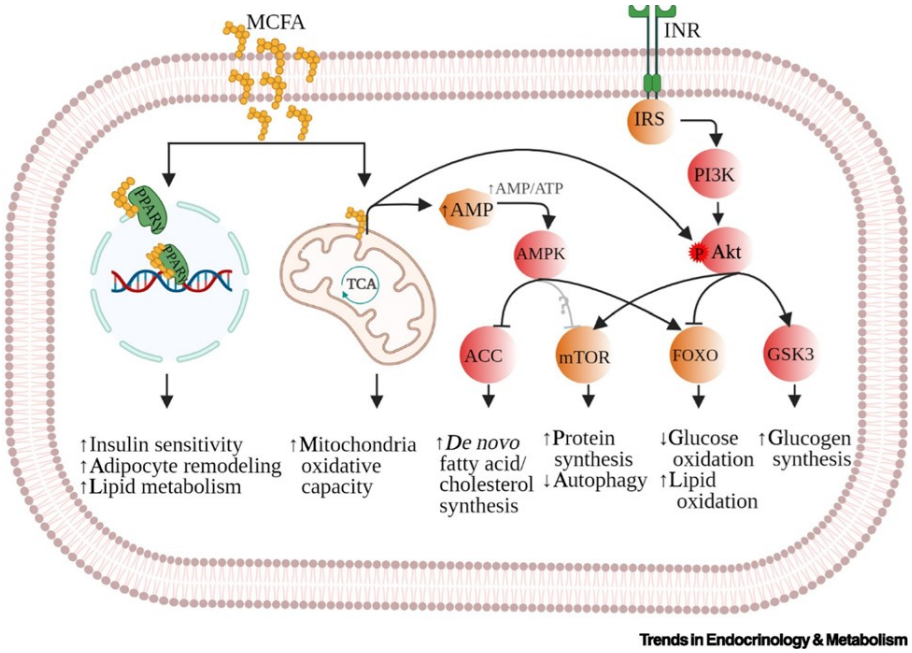

Figure 12: Summary of MCT cellular signalling. Schematic of Medium-Chain Fatty Acid (MCFA)-Mediated Signaling Pathways via Intracellular Receptors and Key Second Messenger Molecules.

Key and abbreviations: ↑, increase; ↓, decrease; ACC, 1-aminocyclopropane-1-carboxylic acid; Akt, protein kinase B; AMPK, AMP-dependent kinase; GSK3, glycogen synthase kinase 3; FOXO, forkhead box O; INR, insulin receptor; IRS, insulin receptor substrate; mTOR, the mammalian target of rapamycin; p, phosphorylation; PI3K, phosphatidylinositol-3-kinase; TCA, tricarboxylic acid cycle. [Huang et al, 2021]. (Reproduced with permission).

F. Effect on cognition

MCT has also been shown to increase ghrelin levels by its role in increasing acylated ghrelin. [Kojima et al, 1999; Yoshimura et al, 2018; Nishiri et al, 2012], and is discussed in more detail in Section 1.6.7 C (1). Regardless of its acylation status, ghrelin has been shown to promote neuronal cell survival by multiple signalling pathways, and have anti-epileptic properties [Frago et al, 2011]. Involvement of the ghrelin system in central nervous system disease, however, is unknown. [Frago et al, 2011].

Other cognitive effects, specifically of ketone bodies, are discussed in further detail in Section 1.4.2: Neurological effects of ketone bodies.

G. Effect on GI tract

Clinically this is one of the most important issues as it requires titration of dietary MCT supplementation, and limits the total dose tolerated.

Diarrhoea

Possible mechanisms include: the high osmotic pressure of MCT oils, due to rapid hydrolysis resulting in the formation of osmotically active fatty acids [St Onge et al, 2008]; impaired hydrolysis of MCT due to lack of stimulation of pancreatic enzyme secretion by cholecystokinin (CCK) [Hopman et al, 1984]; and, direct effects on increasing GI tract mobility resulting in decreased (accelerated) small-bowel transit time that was shown to be CKK independent [Ledeboer et al, 1995]. There is also considerable individual variability in GI side effects such as abdominal cramping and diarrhoea [Juby et al 2020].

1.4 Ketone Bodies (Ketones)

Although glucose is the main fuel for brain function, under certain circumstances, such as prolonged fasting, ketones can replace glucose as the main fuel, and provide 50-60% of the brain energy needs [Cahill, 2006]. Even if glucose availability is acutely reduced, by experimental hypoglycemia, ketone infusion or MCFA ingestion preserves cognitive function [Veneman et al, 1994; Page et al, 2009].

1.4.1 Generation of ketone bodies

Ketogenesis within hepatic mitochondria is the primary source of circulating ketones [Puchalska and Crawford 2017]. See Figure 11. Ketogenesis is mediated by two enzymes, mitochondrial HMG-CoA synthase (mHS) and HMG-CoA Lyase (HL). mHS is expressed in the greatest level

in the liver, and the rate of ketogenesis is substrate dependent [Fukao et al, 2004]. HL is expressed in most tissues, but it's activity is likely most important during fasting [Cullingford, 2004].

There are three ketone bodies: β-hydroxybutyrate (BHB); acetoacetate (AcAc); and acetone, which are water-soluble molecules produced from excess fatty acid metabolism. BHB is the most abundant ketone (70%) in the body [Newman and Verdin, 2017]. **Figure 13** shows their chemical structure.

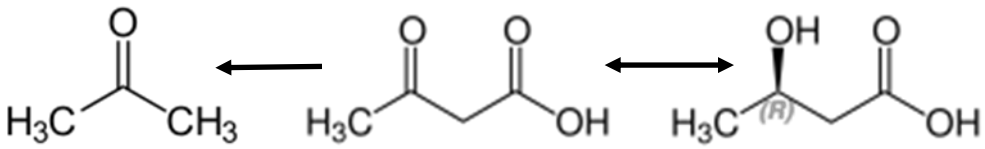

Figure 13: Ketone body chemical structure, conversion and interconversion [Adapted from Berg, 2019].

Ketone body production is inhibited by insulin, and enhanced by glucagon. They usually supply approximately 5% of total energy expenditure. But, they can be a significant source of energy metabolism (approximately 20%), and are *endogenously generated* in numerous physiological conditions including: the neonatal period; pregnancy; long-term starvation; short term fasting; or low carbohydrate high fat diets [Balasse and Fery, 1989]. Apart from serving as energy fuels for extrahepatic tissues such as the brain, heart, or skeletal muscle, ketone bodies also play pivotal roles as signaling mediators, drivers of protein posttranslational modification, and modulators of inflammation or oxidative stress [Puchalska and Crawford, 2021]. Not surprisingly, therefore, they are being investigated for their role in cell metabolism, homeostasis, and signaling under a wide variety of physiological and pathological states [de Cabo and Mattson, 2019].

The ketone bodies synthesised in hepatocytes from fatty acid β-oxidation, generate BHB. This ketone body can be measured in serum, and is used as a marker for the degree of ketone generation [St.-Pierre et al, 2019]. Although most of the BHB used for cognitive function is from hepatocytes, BHB can also be synthesised endogenously by astrocytes, which are the only brain

cells capable of oxidising fatty acids [Edmond et al, 1987]. This BHB serves as another source of fuel for the other brain cells [Achanta and Rae, 2016].

BHB needs to be converted to acetoacetate (AcAc) before it can enter mitochondrial energy generating biochemical pathways [Puchalska and Crawford, 2021]. BHB provides negative feedback to adipose tissue to limit lipolysis. Although, in states of diabetic ketoacidosis, this appears to be bypassed, and may be related to impaired insulin sensitivity, as insulin sensitivity is key for normal ketogenesis [Balasse and Fery, 1989; Green and Bishop, 2019]. Acetone is a volatile ketone, and clinically can be smelt in the breath of people with pathological ketosis, such as diabetic ketoacidosis. Ketone bodies are the primary alternative fuel (to glucose) for the brain, as circulating free fatty acids have limited ability to fuel neurons [Puchalska and Crawford, 2021]. Endogenous production of ketone bodies is regulated by AMP-activated protein kinase (AMPK) activity, and AMPK-activated ketogenesis in astrocytes is stimulated by both hypoxia and hypoglycaemia [Takahashi, 2020].

Ketone metabolism is summarised in **Figure 14.** [Puchalska and Crawford, 2021].

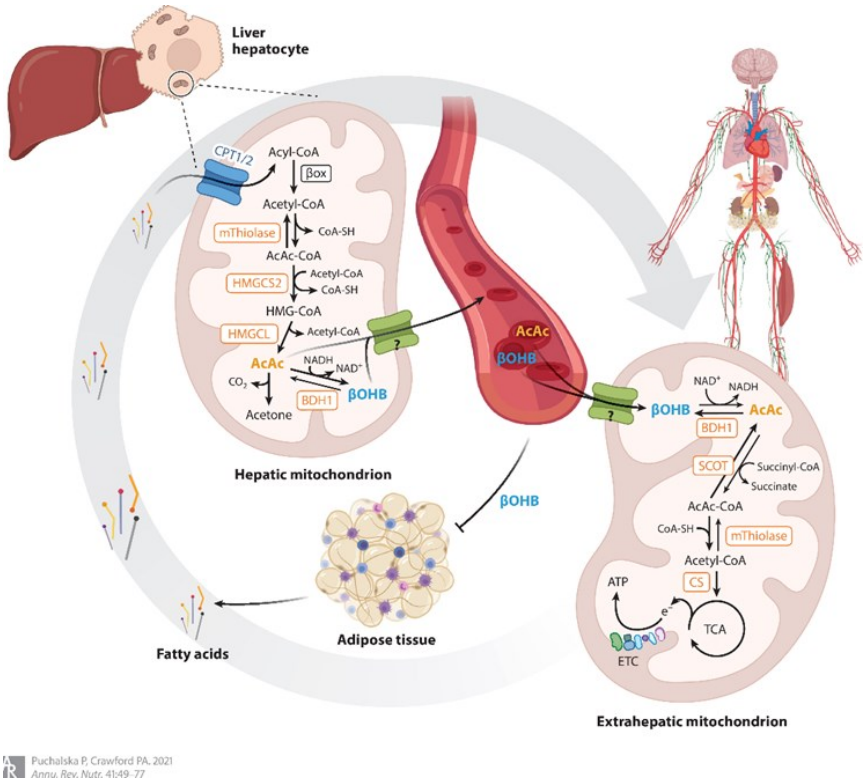

Figure 14: Ketone body physiology and turnover. [Puchalska and Crawford, 2021].

Abbreviations: AcAc, acetoacetate; ATP, adenosine triphosphate; BDH1, D-β-hydroxybutyratedehydrogenase 1; βOHB, β-hydroxybutyrate; βox, β-oxidation; CoA, coenzyme A; CoA-SH, CoA sodium salt hydrate; CPT, carnitine palmitoyltransferase; e−, electron; CS, citrate synthase; ETC, electron transport chain; HMGCL, 3-hydroxymethylglutaryl-CoA lyase; HMGCS2, 3-hydroxymethylglutaryl-CoA synthase 2; mThiolase, mitochondrial thiolase; SCOT, succinyl-CoA:3-oxoacid-CoA transferase; TCA, tricarboxylic acid.

Figure adapted from images created with BioRender.com. (Reproduced with permission)

Ketones can also be generated from exogenous consumption of MCT, as discussed previously in Section 1.3.3. MCFA is one substrate for the generation of ketone bodies. Ketones can also be generated from catabolism of amino acids, especially leucine, and the relative contribution depends on the physiological state, eg. prolonged starvation or diabetes versus high-protein diet or intermittent fasting [Thomas et al, 1982]. The focus in this thesis discussion will be on MCFA as this is the most relevant to the research being presented.

1.4.2 Disposition of ketone bodies

Ketone bodies are disposed of by uptake into extrahepatic tissues [Balasse and Fery, 1989], with a small amount excreted in the urine. The ketolytic pathway occurs in extrahepatic tissues, via two reversible reactions that mediate the activation of free AcAc to AcAcCoA and the creation of AcCoA for energy production by *terminal oxidative* metabolism [Fukao et al, 2004]. See **Figure 14**. Ketones can also be metabolised in *non-oxidative (anabolic) pathways* such as cholesterogenesis and de-novo lipid synthesis. See **Figure 15.** [Puchalska and Crawford, 2021]. Terminal oxidation capacity is tenfold higher than anabolic pathways [Puchalska and Crawford, 2021].

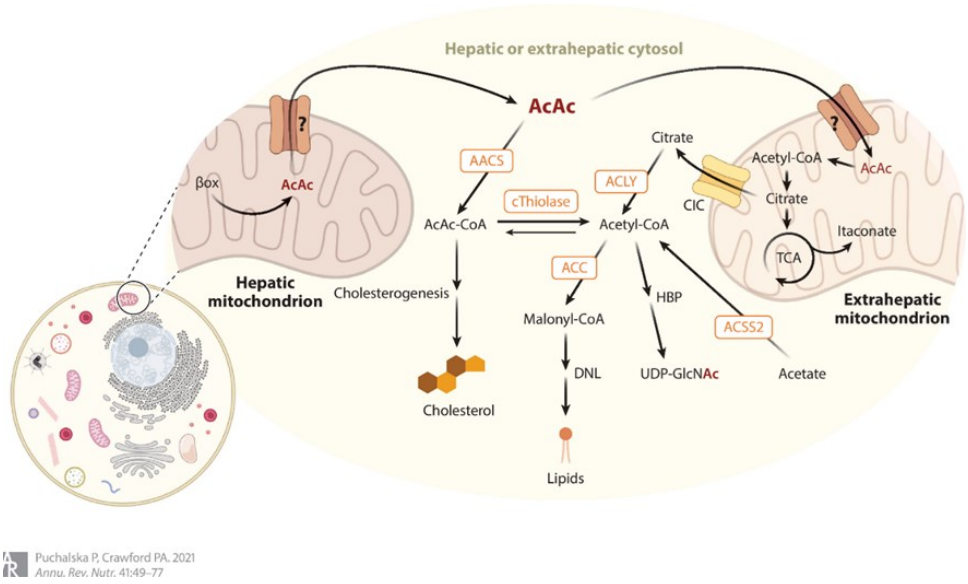

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Figure 15: Ketone body metabolic fate beyond terminal oxidation [Puchalska and Crawford, 2021].

Abbreviations: AACS, AcAc-CoA synthetase; AcAc, acetoacetate; ACC, acetyl-CoA carboxylase; ACLY, adenosine triphosphate citrate lyase; ACSS2, acetyl-CoA synthetase; βox, β-oxidation; CIC, citrate transporter; CoA, coenzyme A; cThiolase, cytoplasmic thiolase; DNL, de novo lipogenesis; HBP, hexosamine biosynthetic pathway; TCA, tricarboxylic acid; UDP-GlcNAc, uridine diphospho-N-acetylglucosamine.

Figure adapted from images created with BioRender.com. (Reproduced with permission.)

The usual level of total ketones (acetoacetate plus BHB) after an overnight fast are very small, approximately 0.1-0.4 mM, or 30-60g/24-hours. With fasting, there is a rapid rise of FFA and ketone bodies, increasing rapidly to a new steady state after 5 days, to 7-10 mM [Balasse and Fery, 1989]. After ingestion of varying doses of MCT, BHB levels rise from a baseline of 0.0-0.3mM to up to 1.7mM, depending on the MCT administered [St-Pierre, et al, 2019]. The effect of older age is less clear, as reports of variation of ketogenesis or circulating ketones in aging mice and humans have been inconsistent [London et al, 1986]. The impact of age and dementia status on ketogenesis remains largely unknown in humans, but is examined within this thesis.

By mass, the myocardium is the highest ketone body consumer. Cardiomyocytes preferentially oxidise ketone bodies over fatty acids or glucose. Because succinyl CoA transferase is not expressed in hepatocytes, the liver cannot utilise the ketones it generates.

1.4.3 Neurological effects of ketone bodies

A. Glucose-sparing

Ketone bodies are not only able to save glucose, but also support brain metabolism during energy crises without prior adaptations from fasting [Xin et al, 2018]. This saves protein degradation, which would otherwise be the main source of gluconeogenesis [Henderson, 2008].

B. Neuroprotection

Ketone bodies have been shown to have *neuroprotective effects* through two main mechanisms: improved mitochondrial function, and regulation of gene expression [Maalouf et al, 2009]. The improved mitochondrial function leads to reduced reactive oxygen species (ROS), and increased energy output. Effects on gene expression result in decreased activity of pro-apoptotic factors, and increased levels of neuroprotective factors such as neurotrophins [Maalouf et al, 2009]. Oxidative stress is characterized by an accumulation of ROS, due to overproduction or diminished elimination [Puchalska and Crawford, 2017]. Ketone bodies may regulate ROS balance through direct and indirect pathways, but the exact mechanism is still not clear [Maalouf et al, 2009].

Sirtuins are a large and diverse family of enzymes that regulate gene expression and multiple cellular responses, some of which affect lifespan and cognition/neuroplasticity (Sirtuin 1 (SIRT1), and silent information regulator 2 (SIRT2)) [Michán et al, 2010], and attenuation of accumulation of Aβ (SIRT3) [Brenmoehl and Hoeflich, 2013]. Their function is *upregulated* by BHB.

Another important group of genes *inhibited* by BHB are class I histone deacetylases (HDACs) [Newman and Verdin, 2017], and inhibition of HDAC has been shown to improve cognition in mice [Fischer et al, 2007]. In addition, regulation of the secretion of neurotransmitters such as gamma aminobutyric acid (GABA), glutamate, serotonin, dopamine, and brain-derived neurotrophic factor (BDNF) also occurs [Puchalska and Crawford, 2017; Jensen et al, 2020]. Another anti-inflammatory effect, other than the effect on SIRT1, is inhibition of potassium

efflux, thereby inhibiting the activation of the nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain containing receptor 3 (NLRP3) inflammasome [Youm et al, 2015].

Direct application of BHB protected cultured hippocampal neurons against Aβ toxicity [Kashiwaya et al, 2000].

Several of the signaling functions of BHB broadly regulate longevity and diseases of aging pathways, most prominently HDAC inhibition and inflammasome inhibition [Newman and Verdin, 2017]. This section only briefly highlights some of the extensive neurophysiological roles of BHB, but even the experts concede that "deeper mechanistic understanding of the downstream effects of BHB signals and improved systems for the targeted delivery of BHB for both experimental and therapeutic goals" is needed [Newman and Verdin, 2017].

C. Decrease insulin resistance

Some of the CNS roles of insulin include: feeding control; neuronal survival; neurogenesis; memory function; and brain aging [Zhao and Alkon, 2001; Kullmann et al, 2016], and insulin receptors are prevalent in the parts of the brain associated with memory function, such as the hippocampus and cortex [Nistico et al, 2012]. Insulin resistance is known to be a factor in AD [Griffith et al, 2018; Arnold et al, 2018]. This brain insulin resistance has been shown to aggravate toxic Aβ production and tau-hyperphosphorylation in several studies [Son et al, 2012; Devi et al, 2012; Kim et al, 2009; Puig et al, 2012].

The metabolism of ketone bodies mitigates some of the negative CNS effects of hyperglycemia [Sato et al, 1995], thereby *improving insulin sensitivity and attenuating insulin resistance* [Tadif et al, 2001; Wutz et al, 2012]. In the brain, Aβ oligomers promote insulin resistance in hippocampal neurons in the diabetic brain [Zhao and Townsend, 2009], an effect which can be mitigated by BHB [Chung et al, 2022].

In addition, they may also mitigate the negative effects of hyperglycemia on neurotransmitters, such as GABA and glutamate [van Bussel et al, 2016], and serotonin (a trigger for insulin resistance) [Gingrich et al, 2001], through their effects discussed above. Diabetes promotes mitochondrial dysfunction, increasing the risk for neuronal degeneration [Calabrese et al, 2001] and ROS [Nishikawa et al, 2000; Stafstrom and Rho, 2012].

Therefore, trials showing clinical cognitive benefits may not only be due to the acute effect of the ketone supplementation as an alternative BHB fuel source.

1.5 Medium Chain Triglycerides (MCT) in AD and MCI

1.5.1 Biochemical rationale

To summarise thus far: MCTs, the main constituents of coconut and palm kernel oils, are absorbed and metabolized via medium-chain fatty acids into ketone bodies. Usually, the main energy substrate for the brain is glucose. However, under certain circumstances, such as extended fasting or a very high-fat ketogenic diet, the liver produces ketone bodies as an energy substrate for extrahepatic tissues including the brain [Cunnane et al, 2011]. When acetyl CoA production from β-oxidized fatty acids exceeds the capacity of the tricarboxylic acid cycle, the excess acetyl CoA can condense into ketones. This process happens predominantly, but not exclusively, in the liver [Cunnane et al, 2011]. BHB and AcAc enter the brain via the BBB, and are oxidized in astrocytes as an alternative fuel under ketogenic conditions [Mosconi et al, 2007; Laybaert et al, 2007].

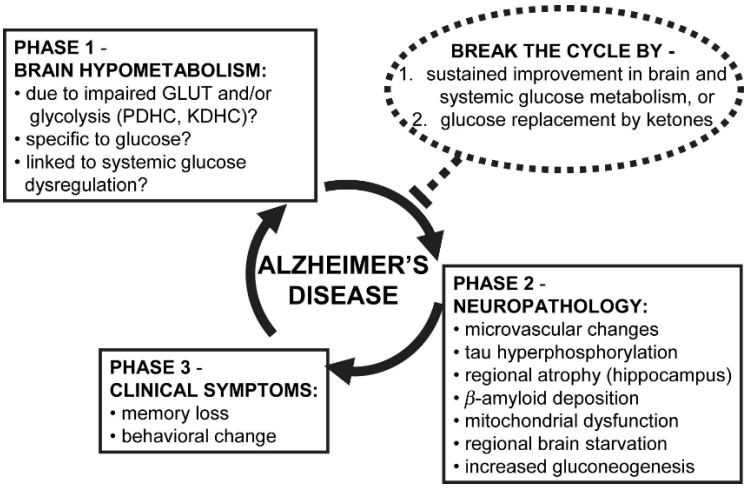

Figure 16: Schematic overview of the concept that brain hypometabolism (phase 1) contributes to the neuropathology underlying Alzheimer's disease (AD) (phase 2), leading to the clinical symptoms of AD (phase 3).

Abbreviations: GLUT, glucose transport; PDHC, pyruvate dehydrogenase complex; KDHC, ketoglutarate dehydrogenase complex activity. [Cunnane et al, 2011] (Reproduced with permission).

The brain consumes 20% of the body's resting energy expenditure, and these needs can be sustained by ketone bodies [Cahill, 2006]. The rationale for ketone supplementation to support brain health originates from the benefits of starvation or ketogenic diets for seizure disorders [Sondhi et al, 2020]. Neuroinflammation is a common feature in neurodegenerative disease and may promote energy crisis [Lacourt et al, 2018], and this can be mitigated by ketones as previously discussed in Section 1.4.2. The effects on feeding behavior, energy expenditure, mood and behavior, and neuroprotection have all been observed, and are summarised in **Figure 17**. [Puchalska and Crawford, 2021].

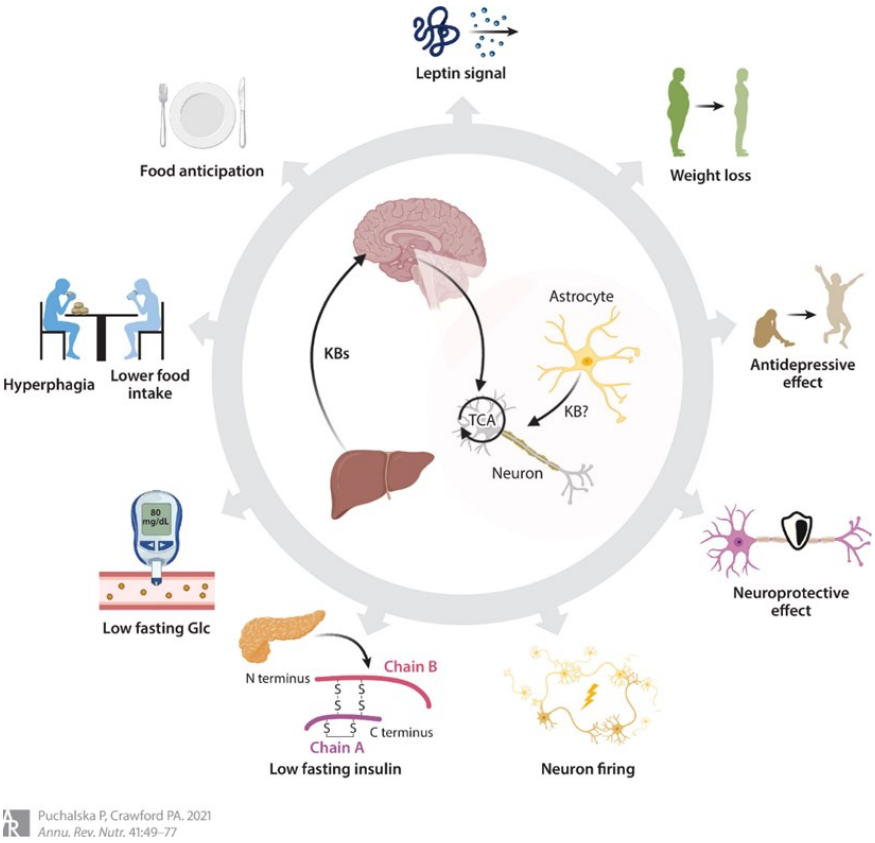

Figure 17: Integrative role of ketones in the nervous system.

Abbreviations: Glc, glucose; KB, ketone body; TCA, tricarboxylic acid. [Puchalska and Crawford, 2021]. Figure adapted from images created with BioRender.com. (Reproduced with permission).

Several studies have indicated that **ketogenic diets** have neuroprotective effects in the brain and may improve cognitive function. Further discussion of ketogenic diets in beyond the scope of this thesis, but for a full review see Paoli et al, 2014.

This thesis is focused on MCT supplementation. Nonetheless, ketosis can also be achieved through **ingestible ketone body precursors** such as BHB salts or ketone esters. However, concerns have been raised over the high sodium load and liver health issues with these formulations [Puchalska and Crawford, 2021].

For **MCTs**, there are interesting metabolic differences between C8 and C10 MCFA. When provided alone, C8 and C10 metabolism was comparable, but when provided together C10 was preferred over C8 as a metabolic substrate [Andersen et al, 2021]. St-Pierre and colleagues have also shown differing ketone body production between C8, C10, C12 or a mix of C8C10 [St-Pierre et al, 2019]. This suggests the intriguing possibility that different formulations of MCT may have different clinical effects [Andersen at al, 2021].

Figure 18 below summarises the discussion thus far, and the vicious circle created by this CNS energy loss.

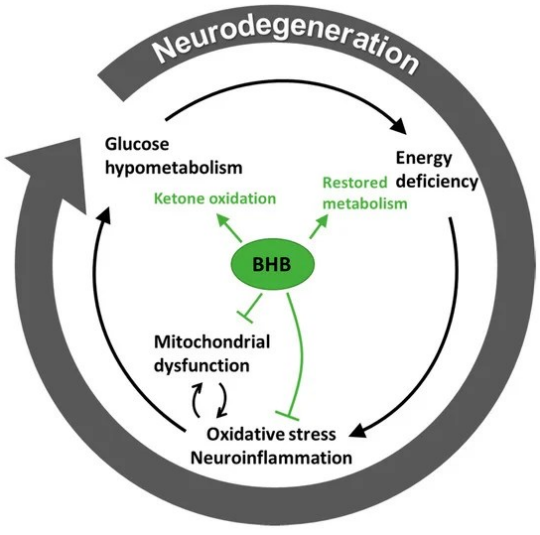

Figure 18: Vicious circle of energy crisis in neurodegenerative disease.

The proposed effects of beta-hydroxybutyrate (BHB) on disease mechanisms are illustrated in green, demonstrating an inhibition of oxidative stress, neuroinflammation and mitochondrial dysfunction together with a facilitated ketone oxidation, which results in at least a partially restored metabolism [Jensen et al, 2020]. (Reproduced with permission).

1.5.2 Clinical evidence for MCT on cognition to date

Even in Alzheimer's dementia (AD) when cerebral glucose metabolism is impaired (as previously discussed), cerebral ketone uptake is preserved. [Croteau et al, 2018]. (See previous **Figure 4)**. Ketones provide an alternative metabolic precursor to glucose in the brain, and ketogenic diets may potentially reduce amyloid plaques and their associated neurotoxicity. In contrast, modern diets high in carbohydrates (CHO) may be contributing to the increasing incidence of AD. The ketogenic diet (including CHO restriction) might therefore be useful in the management of AD [Broom et al, 2019]. However, as already highlighted, sweet cravings are common in individuals with AD [Mungas et al, 1990], and whether this food preference contributes to their risk of developing the disease, or its progression, this dietary preference makes complying with a long-term low CHO ketogenic diet that much more challenging.

Most of the laboratory research on the neuroprotective effects of ketones has been with ketogenic diets/calorie restriction, with the net result being an increase in circulating BHB [Mattson and Wan, 2005]. The rationale for the role of supplemental ketones in mitigating AD is becoming more apparent, given the challenge of compliance with diets. The physiology and biochemistry summarised in section 1.5.1, further supports the potential therapeutic benefit of increasing ketone bodies as a management strategy in AD [Kashiwaya et al, 2000]. In addition, in mouse models of AD, ketone bodies were reported to show a cognition-sparing property and to reduce Aβ and tau pathology [Van der Auwera et al, 2005; Kashiwaya et al, 2013].

A study reported that older adults with mild cognitive impairment (MCI) exhibited ketosis and showed superior verbal memory performance after receiving a very-low-carbohydrate diet for 6 weeks, compared to their performance after receiving a high-carbohydrate diet for the same period [Krikorian et al, 2021]. Exogenous ketone bodies may have beneficial effects on cognitive outcomes in both established AD and MCI, although the findings of previous studies are

sometimes conflicting [Reger et al,2004; Henderson et al, 2009; Ohnuma et al, 2016]. Reger and colleagues initially reported that a single intake of an MCT drink (NeoBEE®) containing 40 ml of MCT (mainly caprylic (C8) triglycerides) significantly facilitated performance on the AD Assessment Scale-Cognitive Subscale (ADAS-cog) among participants without the ε4 allele of apolipoprotein E (APOE), but not among those who carried the ε4 allele [Reger eta l, 2004]. The ε4 allele of APOE is known to be associated with increased vascular risk factors, as well as an increased risk for sporadic AD. Twenty-five percent of the population carry at least one ε4 allele. Those homozygous for APOE4 are 15-times more likely to develop AD [Hunsberger et al, 2019]. Heterozygotes are also at increased risk, accelerating onset of AD by 2-5 years compared to 5-10 years for homozygotes [Corder et al, 1993]. Thereafter, Reger and colleagues ran a larger-scale randomized controlled trial of the MCT compound known as AC-1202 (20 g of caprylic (C8) triglycerides per day) in mild-to-moderate AD patients. They observed significantly beneficial effects at day 45, but not day 90 in the total patient series. However, the effects remained at day 90 among the participants who did not carry the ε4 allele [Hendersen et al, 2009].

Ohnuma and colleagues performed an open label trial using Axona®, containing 20 g of caprylic (C8) triglycerides for 3 months in mild-to-moderate AD patients, but they failed to find significant improvement on any cognitive functions compared to the baseline. However, they observed that some APOE4-negative AD participants, with a baseline Mini-Mental State Examination (MMSE) score ≥14/30, showed improvement in their MMSE and ADAS scores [Ohnuma et al, 2016]. When the same research group re-analyzed their study's data, they found that the AD participants with a baseline MMSE score ≥15/30 showed significant improvement of memory and orientation [Kimoto et al, 2017].

Using yet another MCT preparation, Ota and colleagues reported that a single dose of an MCT-based ketogenic formula (Ketonformula®) had cognition-enhancing effects on working memory, visual attention, and task switching in *non-demented* elderly individuals [Ota et al, 2016]. They went on to examine the possible effect single and chronic (12-weeks) administrations of their formula on cognitive function in participants with mild-to-moderate AD. The chronic consumption was shown to be more beneficial than a single dose, and at 8 weeks the participants showed significant improvement in their immediate and delayed logical memory tests compared

to their baseline scores. At 12 weeks they also showed significant improvements in the digit-symbol coding test and immediate logical memory test compared to the baseline. [Ota et al, 2019].

Table 2 summarises the MCI and AD MCT case reports and clinical trials to date, and shows the different MCT agent/diet used, outcome measures, duration and inclusion criteria. [Reger et al, 2004; Hendersen et al, 2009; Hendersen and Poirier, 2011; Krikorian et al, 2021; Maynard and Gelblum, 2013; Newport et al, 2015; Farah, 2014; Rebello et al, 2015; Yang et al, 2015; Ohnuma et al, 2016; Taylor et al, 2018; Chan et al, 2017; Fortier et al, 2019; Ota et al, 2019].

Table 2: Showing published studies of MCT treatment in MCI and AD to date.

Study	Design	Participants (number)	Intervention, Duration	Cognitive outcome measured	Results
Reger et al (2004)	DB, RCT	AD or MCI (20)	MCT oil 40g/visit, 2 visits	ADAS Cog MMSE Stroop	+1.5/70 Only in APOE4-
Hendersen et al (2009)	DB, RCT	AD (mild-mod) (152)	AC-1202 (C8) 20g/d, x 3 months	MMSE ADAS Cog ADCS-CGIC	+1.9-5.7/70. Better in APOE4-*
Hendersen, Poirier (2011)	DB, RCT	AD (mild-mod) (120)	AC-1202 20g/d, X 3 months	ADAS Cog, pharmaco-genetic	Higher BHB, higher ADAS Cog in APOE4-*
Krikorian et al (2012)	RCT	MCI (23)	High CHO vs low CHO diet X 6 weeks	Trail B, V-PAL	Improved V-PAL (p=0.01)
Maynard, Gelblum (2013)	Case series	Mild-mod AD (55)	MCT (C8) 20g/d X 18.8 ±9.2 months	MMSE	MMSE stable
Newport et al (2015)	Case study	Mod-sev AD (1)	Ketone monester 28g/tid, X 75 days	MMSE, ADL	MMSE +8/30, ADAS Cog +6/70
Farah (2014)	Case report	Mild AD (1)	MCT (C8) 20g/d X 3 months	MMSE MoCA	MMSE +5/30, MoCA +4/30, FDG PET no change
Rebello et al (2015)	Pilot DB, RCT	MCI (4)	MCT oil 56g/d, X 6 months	ADAS cog	+ve trend ADAS Cog

Yang et al (2015)	RCT	AD in LTC (44)	Coconut oil (C8:C10 5:1) 40ml/d X 3 weeks	MEC-WOLF	Improved score*
Ohnuma et al. (2016)	1 arm	Mod-sev AD (20)	MCT (C8 50%) 20g/d X 3months	MMSE ADAS Cog	Trend to improve MMSE in APO E4 negative
Taylor et al (2017)	Feasibility OLT (1 arm)	Mild-mod AD (10)	Ketogenic diet (+MCT C8:C10 5:3) 22.5-45ml/d X 3 months	MMSE, ADAS Cog	MMSE +1/30, ADAS Cog + 4.1/70*
Chan et al (2107)	RCT	Mild/mod/severe AD (40)	Coconut oil 60ml/d X 6 months	MMSE, CDT	No benefit (high dropout due to diarrhea)
Fortier et al (2019)	DB, RCT	MCI (52)	kMCT drink (C8 60%) 30g/d (15g bid) X 6 months	Brain ketone metabolism, MMSE, MoCA, RL/RI-16, BVMT, TMT, Stroop, VF, DSS, BNT	Increased brain CMR (211%) MMSE, MoCA unchanged. + BNT
Ota et al A (2019)	RCT	Mild-mod AD [20]	MCT (C8:C10 3:1) 20g/d X 2 days	WAIS III, WMS-R, Stroop, TMT	No sig. changes
Ota et al B (2019)	1 arm	Mild-mod AD (19)	MCT (C8:C10 3:1) 20g/d X 3 months	WAIS III, WMS-R, Stroop, TMT	Improved WAIS-III, WMS-R, Stroop*

*** Statistically significant result.**

Abbreviations: DB (double blind), RCT (randomized controlled trial), AD (Alzheimer's Disease), MCI (mild cognitive impairment), ADAS Cog (Alzheimer's Disease Assessment scale – Cognitive subscale), MMSE (Mini Mental status Examination), Stroop (Stroop Colour Word Interference Task) AC-1202 (MCT capsule), ADAS CGIC (Alzheimer's Disease Cooperative Study – Clinical Global impression of Change), MEC-WOLF (Spanish version of MMSE), OLT (open-label trial), T (tablespoon), RL/RI (Rappel Libre/Rappel Indice), BVMT (Brief Visual Memory Test), TMT (Trail making A, Trail making B), VF (Verbal Fluency), DSS (Digital Symbol Substitution), BNT (Boston Naming Test), V-PAL (Verbal Paired Association Learning test), CMR (cerebral metabolic rate), WAIS-III (Wechsler Adult Intelligence Scale-3rd), WMS-R (Wechsler Memory Scale-Revised).

1.5.3 Quality of clinical trials to date

In a recent meta-analysis of the trials of MCT supplementation in AD by Averignos and colleagues, although they concluded that MCTs can induce mild ketosis and may improve cognition in patients with mild cognitive impairment and Alzheimer's disease, they did *express concerns about the quality* of the trials [Averginos et al, 2020].

In order to assess for potential bias, they used two strategies. Firstly, the Newcastle Ottawa Scale (NOS), a quality assessment tool for non-randomized trials [Wells et al, 2000], that they adapted to assess the included non-randomized studies. The NOS tool originally examines the domains of selection, comparability and outcome for quality and gives ideally four, two and three points respectively for each domain (with maximum score 9/9). Secondly, for the randomized controlled trials, they assessed risk of bias based on study design with the tool from the Cochrane collaboration [Higgins et al, 2011], and if any domain was scored as high risk, they characterised the whole study as having high risk of bias.

All the non-randomised controlled trials were deemed good quality (4/6-6/6), but had a high risk of bias as there were no comparators [Maynard and Gelblum, 2013; Farah et al, 2014; Newport et al, 2015; Ohnuma et al, 2015; Taylor et al, 2017; Ota et al B 2019]. Some were case studies/series and therefore selection bias was a concern [Maynard and Gelblum, 2013; Farah et al, 2014; Newport et al, 2015].

For the randomised controlled trials, only one had a *low risk* of bias [Rebello et al, 2015], however they had only 6 participants enrolled, with only 4 participants completing the study, and only 2 of these were on MCT supplementation. One of the dropouts was due to GI issues, and the other to non-compliance.

Three were at *high risk* for bias [Henderson et al, 2009; Chan et al, 2017; Fortier et al, 2019] primarily because of incomplete data due to attrition. The study by Henderson and colleagues had additional potential for bias as the study was sponsored by the MCT product (AC-1202) manufacturer, all the data was collected and analysed by the company, who also wrote the paper, and all the authors had financial interests in the company. Attrition was also high with 38 dropouts in the intervention arm (the majority due to side effects 20/38) and 14 in the placebo (4/14 due to adverse events). Three of the other trials were deemed *unknown*, due to missing

information [Reger et al, 2006; Yang et al, 2015; Ota et al A, 2019]. They concluded that the risk of bias of existing studies necessitates future trials [Averginos et al, 2020].

1.6 Sarcopenia

1.6.1 Background

The term **sarcopenia** is derived from Greek sarx "flesh" and penia "loss", and refers to a reduction of muscle mass and function, most commonly associated with increasing age, defined as being <2 standard deviations (SDs) of appendicular skeletal muscle mass (ASM, kg) per height squared (m^2) below the mean of a young reference group [Rosenburg,1989]. It is felt to be a normal part of ageing, distinguishing it from cachexia, ie. muscle loss caused by inflammatory disease, starvation, and malignancy [Roubenoff et al, 1997]. Some authors believe low grade chronic inflammation and increased protein degradation may also apply in age-related sarcopenia [Waters et al, 2003]. Sarcopenia may best be viewed as an organ failure (muscle insufficiency) and is usually chronic, but can develop acutely (for example, during hospitalisation) [Cruz-Jentoft et al, 2010].

1.6.2 Diagnostic criteria

Diagnosis was initially based predominantly on *loss of muscle mass* [Rosenburg, 1989]. However, it became apparent that muscle strength and physical function were equally important, and the European Working Group On Sarcopenia in Older People (EWGSOP) consensus group developed diagnostic guidelines in 2010 [Cruz-Jentoft et al, 2010], designed to create more standardisation in the diagnosis. These guidelines were subsequently revised in 2019 [Cruz-Jentoft et al, 2019]. The revision made the most important diagnostic criteria to be *low muscle strength* over low muscle quantity (mass), and created sub-categories of *probable sarcopenia, sarcopenia* and *severe sarcopenia* based on physical performance in addition. See **Table 3.**

Table 3: EWGSOP operational definition of sarcopenia [adapted from Cruz-Jentoft et al, 2019].

Probable sarcopenia is identified by Criterion 1. **Sarcopenia** is confirmed by addition of Criterion 2. **Severe sarcopenia** If Criteria 1, 2 and 3 are all met
(1) Low muscle strength (2) Low muscle quantity or quality (3) Low physical performance

Cut points for the diagnosis of low muscle strength, physical function and low muscle mass were based on the available literature, and for some categories differed between men and women. Options for assessing *muscle strength* include: grip strength and chair stand. For *muscle quantity* options are dual energy Xray absorptiometry (DXA), bioimpedance assay (BIA), or computerised tomography body composition, and for *muscle function*, gait speed, Short Physical Performance Battery (SPPB), or Timed Up and Go (TUG). Cut-offs for BIA come from the Asian working group (AWG) [Chen et al, 2020], and are reported as total muscle mass (kg) per height squared (m^2).

More recently, the AWG has suggested separate cut-off algorithms for community versus hospital settings [Chen et al, 2020], and have also recommended the chair stand test over grip strength, given its greater reproducibility [Nishimura et al, 2017]. Additionally, *five-time chair stand time* has been proposed as a surrogate for gait speed in sarcopenia diagnosis. A five-time chair stand time cut-off of 11.6 seconds (s) corresponded to a walking speed of 1.0 m/s. The AWGS 2019 recommends ≥ 12 s as the cut-off for low physical performance [Nishimura et al, 2017]. **Table 4** summarises the current recommended cut-offs from the two consensus groups.

Table 4: Sarcopenia cut-off points [adapted from Cruz-Jentoft et al, 2019; Chen et al, 2020; Nishimura et al, 2017].

	Cut points for men	Cut points for women
Low muscle strength		
Grip strength	<27 kg	<16 kg
Chair stand	>15 s for five rises	>15 s for five rises
Low muscle mass		
DXA: ASM	<20 kg	<15 kg
DXA: ASM/height^2	<7.0 kg/m^2	<5.5 kg/m^2
BIA: mm/height^2	<7.0 kg/m^2	<5.7 kg/m^2
Low muscle function		
Gait speed	≤ 0.8 m/s	≤ 0.8 m/s
SPPB	≤ 8 point score	≤ 8 point score
TUG	≥ 20 s	≥ 20 s
400m walk test	Unable or ≥ 6 mins	Unable or ≥ 6 mins
Chair stand (≡ gait speed)	>12 s for five rises	>12 s for five rises

Abbreviations: DXA: dual energy Xray absorptiometry; ASM: appendicular skeletal muscle; BIA: bioimpedance assay; mm: muscle mass; SPPB: short physical performance battery; TUG: timed up and go; kg: kilogram; m: metres; s: seconds.

Recognition of the impact of concomitant obesity has resulted in addition of another category, *sarcopenic obesity* [Baumgartner, 2006], with obesity being defined as a *body fat of >25% in men and >35% in women* [Roubenoff,2004]. (Further discussion to follow in Section 1.7).

1.6.3 Screening questionnaire

To improve clinical detection of sarcopenia, a screening questionnaire has been developed and validated - the SARC-F tool [Malmstrom and Morley, 2013]. SARC-F scores ≥ 4 showed high sensitivity (90-94%) but low specificity (14-21%) for sarcopenia [Ida et al, 2018], and has been validated in multiple languages. See **Table 5** below for details of the questionnaire, and the scoring system.

Table 5: SARC-F questionnaire (includes scoring) [Bauer et al, 2019]. (Reproduced with permission).

Component	Question	Scoring
Strength	How much difficulty do you have in lifting and carrying 10 pounds?	None = 0 Some = 1 A lot or unable = 2
Assistance in walking	How much difficulty do you have walking across a room?	None = 0 Some = 1 A lot, use aids, or unable = 2
Rise from a chair	How much difficulty do you have transferring from a chair or bed?	None = 0 Some = 1 A lot or unable without help = 2
Climb stairs	How much difficulty do you have climbing a flight of 10 stairs?	None = 0 Some = 1 A lot or unable = 2
Falls	How many times have you fallen in the past year?	None = 0 1 – 3 falls = 1 ≥ 4 falls = 2

Scores ≥ 4 has a specificity of 14-21% and sensitivity of 90-94% for sarcopenia.

The role for this tool in case finding for sarcopenia is shown in the algorithm in **Figure 19,** as recommended in the 2019 guidelines. The steps of the pathway are represented as Find-Assess-Confirm-Severity (F-A-C-S). They remind users to consider other reasons for low muscle strength, such as depression, stroke, balance disorders, and peripheral vascular disorders [Cruz-Jentoft et al, 2019].

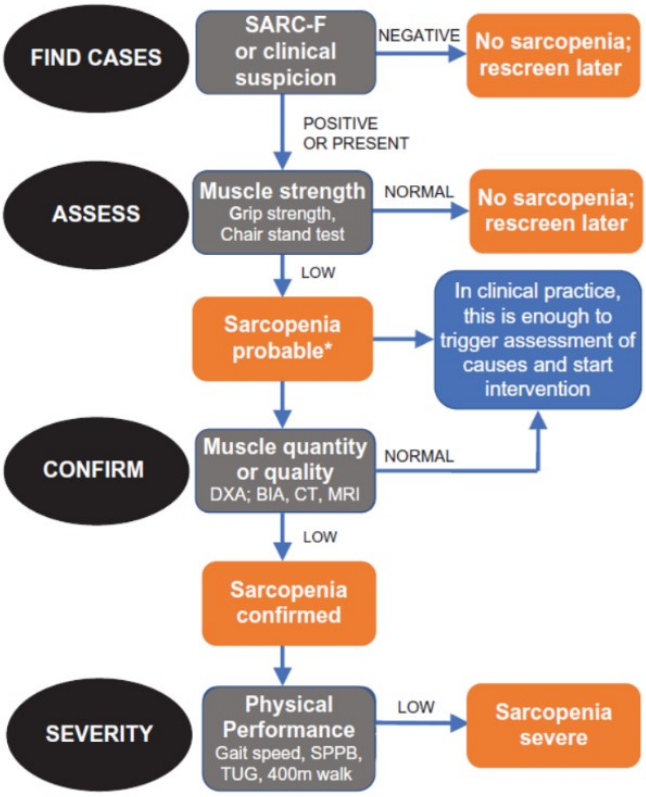

Figure 19: EWGSOP2 algorithm for sarcopenia case finding [Cruz-Jentoft et al, 2019]. (Reproduced with permission).

1.6.4 Sarcopenia Prevalence

This varies among the population being studied, the diagnostic criteria being used, and the method of measuring muscle mass (dual energy X ray absorptiometry (DXA) or bioimpedance essay (BIA)).

Using EWGSOP criteria, a UK study of community dwelling older adults, reported a prevalence of 4.6% in men and 7.9% in women [Patel et al, 2013], compared to 36.5% in the USA [Brown et al, 2016], and 3.9-13.6% in Taiwan [IC et al, 2014]. Also in Taiwan, Chien and colleagues reported a prevalence of 18.6% in women and 23.6% in men using both MRI and BIA, but using Asian-specific BIA cut-offs [Chien et al, 2008]. In Japan reported prevalence ranged from 2.5-28% in men and 2.3-11.7% in women (based on DXA), and 7.1- 98% in men and 19.8-88% in women, using different BIA cut-offs [Kim et al, 2016], highlighting the need for specific population-based cut-offs, especially for BIA. An East Asian study in 2019 also reported a

higher prevalence of sarcopenia in community dwelling men (19.2%) than women (8.6%) [Du et al, 2019]. The male sarcopenic participants also had a higher odds ratio for osteoporosis (4.21) and dyslipidemia (4.15) than non-sarcopenic men. For women the sarcopenic participants had a 1.12 fold higher risk for osteoporosis and 4.21 fold higher risk of hyperglycemia than their non-sarcopenic peers. Sarcopenic obesity was also found to be higher in men than women in this population [Du et al, 2019].

Using data from the Canadian Longitudinal Study on Aging, with the EWGSOP revised criteria Purcell and colleagues [Purcell et al, 2020] reported a prevalence for low muscle mass (pre-sarcopenia) of 5.8% in males and 8.2% in females, but sarcopenia only in 0.2% and severe sarcopenia in 1.2%. However, using the International Working Group on Sarcopenia (IWGS) criteria in their population, the sarcopenia prevalence is reported as 6.7%. The IWGS criteria differ in that the gait speed cut-off is higher (<1.0m/s versus <0.8m/s) as is the grip strength for both sexes, accounting for the higher prevalence with this tool. The prevalence of obesity (defined by BMI) was 18.6-32.2% depending on the age group [Purcell at al, 2021].

A systematic review of 15 prevalence studies, concluded that taking into account regional and age-related variations, the prevalence in community-dwelling populations is 1 to 29% [Cruz-Jentoft et al, 2014]. In a 2017 meta-analysis the overall prevalence of sarcopenia was reported as 10% in both men and women, although there was *considerable heterogeneity in the studies*, and variations depending on the DXA or BIA cut-off used, and the population studied, despite the studies all using sex specific cut-offs, as per the guidelines [Shafiee et al, 2017]. In a more recent review, Mayhew and colleagues report prevalence's from 9.9 to 40.4% [Mayhew et al, 2019]. Even studies using different definitions on the same population, have found that sarcopenia estimates vary up to 40% by definition [Bijlsma et al, 2013]. Cruz-Jentoft stated that "this heterogeneity in published estimates of sarcopenia prevalence may be influenced by multiple factors such as the age and sex distribution of the population, and the methods and cut-points used to measure muscle mass and muscle function to define sarcopenia" [Cruz-Jentoft et al, 2014]. Mayhew summarised it well stating that the best definition of sarcopenia will be "the one with the strongest association with the health outcomes relevant to sarcopenia" [Mayhew et al, 2019]. Once the definition is established, more precision will be needed at choosing study populations and outcomes.

In Alzheimer's disease the prevalence of sarcopenia is higher in all stages of the disease when compared to non-demented people (36-60% in AD versus 11-13%). One study found higher age, lower BMI, and low MMSE scores were all associated with sarcopenia [Ogawa et al, 2018].

1.6.5 Consequences of Sarcopenia

Loss of >40% baseline lean mass is considered to be associated with a significantly increased risk for mortality [Roubenoff et al, 1997]. Muscle strength is important for mobility, so sarcopenia is, not surprisingly, associated with an increased prevalence of falls and self reported physical disability -independent of ethnicity, age, obesity, income, or health behaviors [Baumgartner et al, 1998]. Women with sarcopenia were at 3.6 times higher, and men 4.1 times higher, risk for developing disability than those with normal muscle mass [Baumgartner et al, 1998]. Sarcopenia is also associated with an increased risk of overall frailty.

Furthermore, some studies have also suggested a sex difference in *sarcopenia progression*. One showed that knee and hip pain may directly contribute to the progression of sarcopenia and increased falls risk in older women, but found no such association between pain and sarcopenia indicators in men [Scott et al, 2012].

In addition, sarcopenia per se may be associated with a higher risk for cognitive decline and dementia [Beeri et al, 2021].

Figure 20 summarises the risks, and shows a conceptual framework for sarcopenia as proposed by Curtis and colleagues [Curtis et al, 2015].

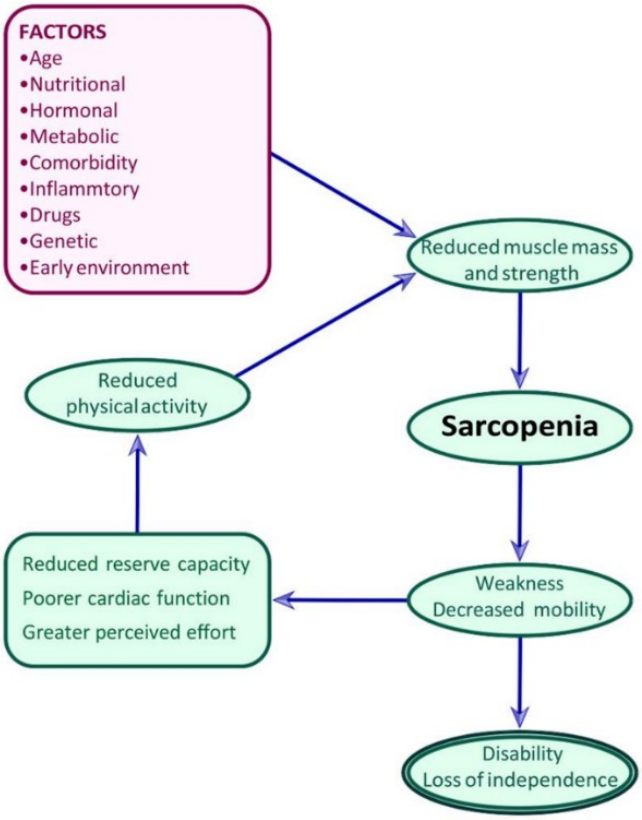

Figure 20: Sarcopenia: a conceptual framework [Curtis et al, 2015]. (Reproduced with permission).

1.6.6 Pathogenesis of Sarcopenia

With ageing there is not just loss of muscle fibers and mass, but physiological changes. These include muscle composition, aerobic capacity and metabolism, fatty infiltration, insulin resistance, fibrosis and neural loss of alpha motor neurons, muscle cell recruitment and apoptosis and all play a role in the impact of aging [Baumgartner, 2006]. In addition, aging is associated with *"anabolic resistance"* that can influence the development of sarcopenia. This is described as a blunted response to essential amino acid (EAA) feeding when combined with an age-related decreased insulin secretion, thereby reducing the anticatabolic effect from carbohydrate and protein intake [Cuthburtson et al, 2005]. As a consequence, a greater amount of protein or EAA is required to promote muscle protein synthesis, and prevent muscle protein breakdown [Wilkes et al, 2009; Moore et al, 2015] with ageing. Hormonal changes in insulin, estrogens, androgens, growth hormone, prolactin, thyroid, Vitamin D and parathyroid hormone, catecholamines and

corticosteroids are also involved in the etiology and pathogenesis of sarcopenia. However, controversy persists regarding their respective roles and effects on skeletal muscle in adulthood and old age [Rolland et al, 2000].

Increased pro-inflammatory cytokines associated with aging per-se may contribute to the development of sarcopenia, independent of other chronic diseases [Roubenoff et al, 1998]. Evidence suggests that myocyte apoptosis is a basic mechanism underlying sarcopenia [Dupont-Versteegden, 2005], and muscle biopsies of older persons show differences associated with apoptosis compared with younger people [Giresi et al, 2005]. Apoptosis may represent a common final mechanism for muscle loss in sarcopenia, but multiple agents and etiologic pathways may also lead to this mechanism.

Early reports of *mitochondrial decline* in aging found a progressive 5% reduction per decade in mitochondrial DNA abundance and ATP production rate, leading to the hypothesis that mitochondrial DNA abundance may drive the aging process in skeletal muscle [Johnson et al, 2013]. Results suggest that sarcopenic muscle demonstrates more susceptibility to stress, such as reactive oxygen species (ROS). This age-related increase in oxidative stress is mediated by increases in ROS, and is one process that, if not matched by the activity of endogenous antioxidants, could cause mitochondrial dysfunction by damaging cellular lipids, proteins, and nucleic acids [Johnson et al, 2013].

Sarcopenia and poor physical performance in the elderly are also associated with *birth weight* in both men and women, independent of adult weight and height. (Low birth weight <2.5kg (5lb 8oz)) [Cutland et al, 2017]. A 1 kg increase in birth weight corresponded in men to a 4.1 kg (95% CI: 3.1, 5.1), and in women to a 2.9 kg (2.1, 3.6) increase in lean mass in adulthood [Ylihärsilä et al, 2007]. The implication is that this intrauterine growth deficit decreases the number of muscle fibers. Low birth weight also predicted a higher proportion of fat compared to lean mass, within a given body mass index (BMI). This suggests that conditions of very early life may additionally program risk for both sarcopenia and sarcopenic obesity in old age [Sayer et al, 2004, Ylihärsilä et al, 2007].

Lifestyle behaviors such as physical inactivity, smoking, poor diet, medications, comorbidities, genetics, early environment, as well as age-related changes in hormones and cytokine levels are additional important risk factors, and are summarised in **Figure 21** [Bauer et al, 2019].

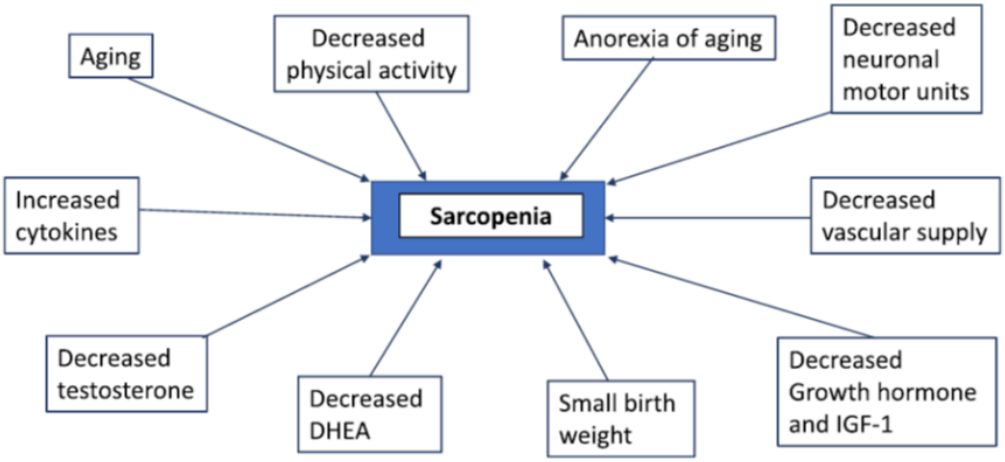

Figure 21: factors involved in pathogenesis of primary (age related) sarcopenia [Bauer et al, 2019]. (Reproduced with permission). Abbreviations: IGF-1, insulin-like growth factor-1; DHEA, dehydroepiandrosterone.

1.6.7 Interventions in sarcopenia

A. Prevention of sarcopenia

Reduce inactivity/prevent unnecessary bedrest

Inactivity is an important contributor to loss of muscle mass and strength, at any age [Kortebein et al, 2007]. Bed rest decreases muscle strength first, before loss of muscle mass [Heymsfield et al, 1997]. This contributes to a potentially vicious cycle in bed-bound older adults.

Physical activity-aerobic and resistance

Physical activity's impact on *preventing sarcopenia* depends on the type of activity (aerobic versus resistance activity).

a. ***Aerobic activities*** (walking, running, cycling or swimming) increase maximal oxygen consumption (VO^2max), *improve muscle quality* (muscle strength ÷ muscle mass), improve neuromuscular adaptation, and improve muscle function. They are associated with decreased morbidity and mortality, independent of body fat [Coggan et al, 1992]. Aerobic exercise does not contribute as much to muscle hypertrophy as resistance exercises, but it stimulates muscle protein synthesis [Shefield-Moore et al, 2004], satellite cell activation, and increased muscle fibre area [Coggan et al, 1992; Charifi et al, 2003]. Aerobic exercises also reduce body fat, including intramuscular fat, which is important for improving the functional role of muscle relative to body weight [Rolland et al, 2008].
b. ***Resistance training (RT),*** such as weightlifting, increases myofibrillar muscle protein synthesis [Yarasheski et al, 1999; Hasten et al, 2000], muscle mass, and strength, even in the frail elderly [Jozsi et al, 1999; Welle et al, 1995; Fiatarone et al, 1994, Yarasheski et al, 1993; Ivey et al, 2000; Cress et al, 1999; Hikida et al, 2000; Hagerman et al, 2000]. Muscular strength alone is independently associated with functional ability in the elderly [Hurley and Roth, 2000]. Adults who do not perform regular RT lose approximately 0.46 kg of muscle annually from the fifth decade onwards [Nelson et al, 1994]. Furthermore, by age 80 years, they experience a 50% reduction in type 2 muscle fibers, the fibers responsible for high levels of strength [Hurley and Roth, 2000].

In contrast to aerobic exercises, *muscle mass, strength, and muscle quality* (strength adjusted for muscle mass), reportedly improve significantly with resistance training in older people [Friedman et al, 1985]. Strength gains result from a combination of improved muscle mass and quality, and neuronal adaptation. Adaptations to RT is *sex dependent*, as summarised in a recent meta-analysis [Jones et al, 2021]. Jones and colleagues report that following RT, older males gain more *absolute* upper and lower-body strength than older females, who gain more *relative* lower-body strength than older males. Older males gain more *absolute* muscle size than older females, but there are no sex differences in changes in *relative* muscle size or upper-body strength in older adults. Finally, they also conclude that older males may benefit from higher intensity programs, whereas older females may benefit from higher weekly repetitions (volume) [Jones et al, 2021]. Their study highlights the important distinction between absolute and relative

(corrected for BMI) muscle mass gain, which should be clearly stated in future studies that may need to consider sex-specific interventions.

Aerobic exercise training does not improve muscular force production in the elderly. Muscular strength may become more limiting for activities of daily living than cardiovascular function in frail elderly individuals, so RT may be the training modality of choice for some segments of the elderly population [Pendergast et al, 1993].

Nonetheless, sarcopenia is still observed in master athletes who maintain resistance training activities throughout their lifetime [Trappe, 2001]. Mitochondrial dysfunction underlies age-related muscle changes [Johnson et al, 2013], and are summarised in **Figure 22.** This model suggests that decreased protein turnover with age leads to the accumulation of damaged proteins with decreased function.

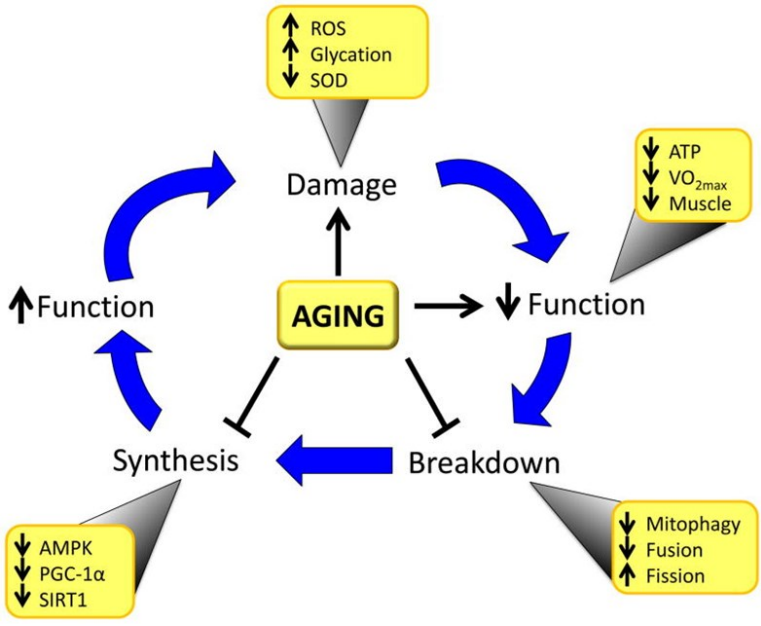

Figure 22: Age associated changes in muscle mitochondrial function [Johnson et al, 2013].

Abbreviations: ROS: reactive oxygen species; SOD: superoxide dismutase; ATP: adenosine triphosphate; VO_{2max}: vital capacity; AMPK: AMP activated protein kinase; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator 1α; SIRT 1: silent mating type

information regulation 2 homolog sirtuin 1. (Reproduced with permission from the publisher).

c. ***Leisure physical activity*** is not enough to prevent the age-related decline in muscle mass [Raguso et al, 2006], but aerobic and resistance activities can improve balance, fatigue, pain, cardiovascular risk factors, and appetite.

Thus, promoting an active lifestyle can prevent the functional effects of sarcopenia, but resistance training is the best approach to prevent sarcopenia. Although, both training modalities contribute to the maintenance and improvement of muscle mass and strength in the elderly [Rolland et al, 2008].

Avoid weight loss after age of 70 years

In elderly populations, any form of weight loss in thin, normal, overweight, and obese elderly results in a loss of muscle mass and increased rate of death [Morley, 2003]. Weight loss should be avoided after the seventh decade of life, especially if it results in a reduction of the BMI with no corresponding reduction in waist circumference [Heiat et al, 2001, Elia, 2001].

Nutrition - birth size and lifelong

Prevention of sarcopenia should occur throughout life. Specific exposures at critical development periods may have a major impact on the risk of sarcopenia in old age [Sayer et al, 2014]. In addition to low birth weight causing decreased total muscle mass, it has also been associated with reduced grip strength in both sexes, even when adjusting for adult height and weight [Sayer et al, 2004]

Protein intake

Epidemiological studies correlate higher dietary protein intake with higher bone mineral density [Promislow et al, 2002; Kerstetter et al, 2000; Rapuri et al, 2003], slower rate of bone loss [Hannan and Chida, 2009], and *muscle mass* [Scott et al, 2010]. Those failing to meet the Recommended Dietary Intake (RDI) for protein had significantly lower DXA appendicular lean muscle (ALM) mass at baseline (−0.81kg 95% confidence interval (CI)=−1.54 to −0.08; P=.03) and follow-up (−0.79 kg 95% CI=−1.42 to −0.17; P=.01) after adjustment for age, sex, energy

intake, and physical activity. There were no differences between these groups for muscle strength [Scott et al, 2010]. Protein intake was not only significantly positively associated with ALM, but was also a positive predictor of change in ALM over 2.6 years in their cohort [Scott et al, 2010].

Leucine is a high proportion of the branched-chain amino acid (BCAA) found in whey protein, and is reported to be one of the crucial factors for the stimulation of muscle protein synthesis, according to some researchers [Luiking et al, 2014; Kramer et al, 2015]. One epidemiological study also showed a positive association between higher dietary protein intake and fewer health problems in older women [Vellas et al, 1997]. A prospective metabolic study, providing the recommended daily allowance (RDA) for protein (0.8g/kg/day) and enough dietary energy to maintain body weight, showed loss of skeletal muscle (mid-thigh muscle area), suggesting the RDA may be marginally inadequate for older adults to prevent muscle loss [Campbell et al, 2001].

Other nutrients

Antioxidants are possible mediators of sarcopenia, given the catabolic effect of oxidative stress on skeletal muscle [Weindruch, 1995]. Diets high in antioxidants may therefore be important for ameliorating the progression of sarcopenia because of their neutralizing effects on free radicals [Weindruch, 1995]. Lower plasma concentrations *of carotenoids* and *alpha-tocopherol (vitamin E)* are associated with poorer grip, hip, and knee strength [Semba et al, 2003]. The Hertfordshire Cohort Study also reported that *selenium, beta-carotene,* and *vitamin C* were positively associated with grip strength [Robinson et al, 2008]. Unfortunately, direct measures of muscle mass in these population-based studies are rare. However, in a Tasmanian study using a Food Frequency Questionnaire they showed, in addition to protein, significant positive associations at baseline were observed between ALM and *fibre, calcium, iron*, *magnesium, niacin, niacin equivalents, phosphorous, potassium, riboflavin, and zinc* for muscle mass and rate of muscle loss (but not strength) [Scott et al, 2010]. The significant associations continued over the two years between ALM and *cholesterol, folate, and vitamin C,* but not for fibre, niacin or riboflavin [Scott et al, 2010].

A positive correlation has been shown to exist between ***Vitamin D*** serum 25(OH)D concentration and muscle function. Low levels (<75nM) are associated with an increased risk for

falls, and fractures, that can be reduced with supplementation [Bischoff-Ferrari et al, 2004]. Low levels are also associated with sarcopenia [Visser et al, 2003], and Vitamin D supplementation has been shown to increase muscle strength, more so in those with lower levels at baseline [Beaudart et al, 2014]. Recent evidence indicated that Vitamin D can also stimulate protein synthesis, and Vitamin D receptors in skeletal muscle play an important role in muscle hypertrophy [Bass et al, 2020]. Vitamin D may *improve muscle strength and muscle mass* and so should be useful for the prevention of sarcopenia. However, Vitamin D supplementation does not always improve muscle function. In a meta-analysis of 16 randomized, controlled trials investigating the effects of Vitamin D supplementation on muscle function in postmenopausal women, Vitamin D supplementation did not improve grip strength or back muscle strength [Tabrizi et al, 2019].

A *significant negative association* was also observed between ALM and *saturated fat* intake at baseline, and *starch* and *retinol* at follow up [Scott et al, 2010]. The negative association they also reported between energy-adjusted retinol intake (adjusted for protein) and change in ALM was novel, although serum retinols have been linked to increased fracture risk in men [Michaëlsson et al, 2003]. The main dietary sources of retinoids are fish, liver, fortified foods, and dairy products. Only a small proportion of carotenoids from vegetables and fruits is also converted to retinol.

The mechanisms by which these nutrients are associated with muscle synthesis is likely multifactorial and beyond the scope of this chapter, but these nutrients may mediate age-related hormonal or immunological changes that restrict skeletal muscle anabolism [Roubenoff and Castaneda, 2001].This data supports a role for more than just dietary protein in the prevention of sarcopenia.

B. Treatment of sarcopenia

Considerable evidence suggests that **sarcopenia is a reversible cause of disability** and could benefit from intervention, especially at the early stage of sarcopenia [Guralnik et al, 2001].

For sarcopenia, improving muscle strength or muscle power is more relevant clinically for the outcomes of disability and mobility, than increasing muscle mass. However, increasing muscle mass is more important for other outcomes such as protein stores and thermogenesis [Rolland et al, 2008].

(1) Resistance exercise and resistance training

Resistance exercise is any exercise that causes the muscles to contract against an external resistance, such as dumbbells, rubber exercise tubing, own body weight, bricks, bottles of water, or any other object that causes the muscles to contract. In contrast, ***resistance training (RT)*** is a progressive strengthening program, defined by proper exercise instruction (e.g., technique, breathing, correct use of equipment), goal setting (so the program can target specific areas of interest), a method of evaluation of training progress toward training goals, the correct prescription of the acute program variables, and the inclusion of specific methods of progression targeting particular areas of muscular fitness. It is important that resistance training should be supervised by qualified professionals for the prevention of injury, and for maximizing the health and performance benefits [Kraemer and Ratamess, 2004]. It causes microscopic damage/tears to the muscle cells, which are quickly repaired, to help the muscles regenerate and grow stronger [Burton et al, 2017]. Only 8.7% of older adults (>75 years of age) in the United States participate in muscle-strengthening activities as part of their leisure time, with barriers cited as: safety; fear; health concerns; pain; fatigue; and lack of social support.

The American Heart Association (AHA) and the American College of Sports Medicine (ASCM) encourage older adults to engage in moderate-intensity cardiorespiratory exercise training for ≥30 minutes per day on ≥5 days per week for a total of ≥150 minutes per week, vigorous-intensity cardiorespiratory exercise training for ≥20 minutes per day on ≥3 day per week (≥75 minutes per week), or a combination of moderate- and vigorous-intensity exercise to achieve a total energy expenditure of ≥500-1000 MET-minutes per week [Garber et al 2011]. (A MET is the ratio of the rate of energy expended during an activity to the rate of energy expended at rest, and MET-minutes are calculated from MET level x time in minutes, where 1 MET is sitting and approximately 7 METs is jogging). On 2-3 days per week, adults are also recommended to perform resistance exercises for each of the major muscle groups, and neuromotor exercises

involving balance, agility, and coordination [Garber et al 2011]. **Table 6** summarises the comparative physiological effects of aerobic and resistance training [Braith and Stewart, 2006].

Table 6: Comparison of effects of aerobic training to resistance training on health and fitness variables [Braith and Stewart, 2006]. (Reproduced with permission).

Variable	Aerobic Exercise	Resistance Exercise
Bone mineral density	↑	↑ ↑ ↑
Body composition		
Fat mass	↓ ↓	↓
Muscle mass	↔	↑ ↑
Strength	↔	↑ ↑ ↑
Glucose metabolism		
Insulin response to glucose challenge	↓ ↓	↓ ↓
Basal insulin levels	↓	↓
Insulin sensitivity	↑ ↑	↑ ↑
Serum lipids		
High-density lipoprotein	↑↔	↑↔
Low-density lipoprotein	↓↔	↓↔
Resting heart rate	↓ ↓	↔
Blood pressure at rest		
Systolic	↓ ↓	↓
Diastolic	↓ ↓	↓
Physical endurance	↑ ↑ ↑	↑ ↑
Basal metabolism	↑	↑ ↑

↑ indicates increased; ↓, decreased; and ↔, negligible effect.

No pharmacological or behavioral intervention to reverse sarcopenia has proven to be as efficacious as resistance training [Rolland et al, 2008]. Frail older people can gain muscle strength and function into their 9th and 10th decades of life, as shown in resistance-training studies [Serra-Rexach et al, 2011]. Despite use of different resistance interventions, a recent meta-analysis concluded it was a "highly effective preventive strategy to delay and attenuate the negative effects of sarcopenia and frailty in both early and late stages" [Talar et al, 2021].

Figure 23 shows a hypothetical model of how declining mitochondrial DNA copy number in aging skeletal muscle may relate to sarcopenia, and the role of exercise training on reversing sarcopenia [Johnson et al, 2021].

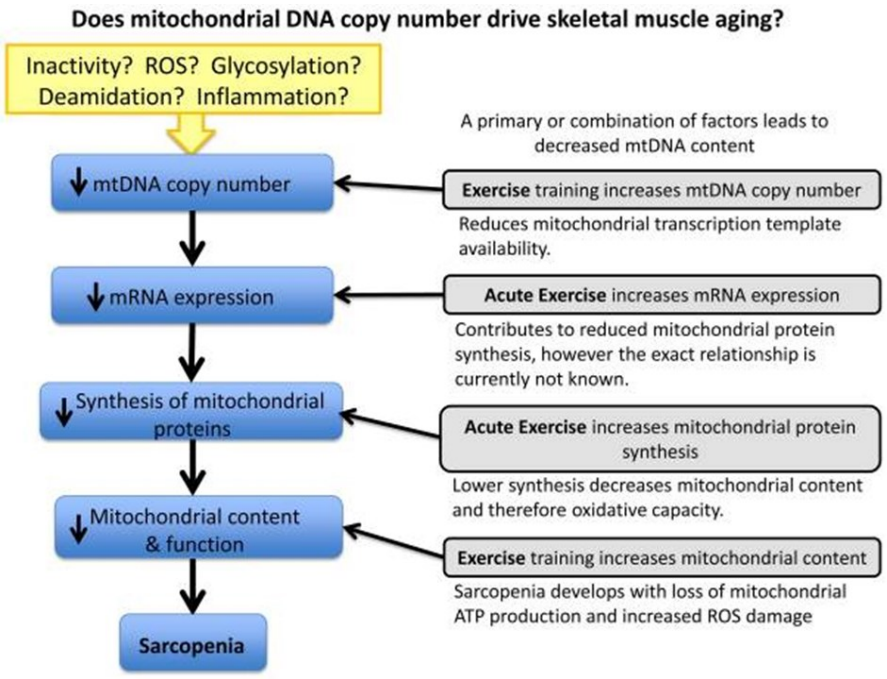

Figure 23: The role of declining mitochondrial DNA copy number on skeletal muscle aging [Johnson et al 2021]. (Reproduced with permission).

There were clear sex difference in effects of RT in a recent review [Jones et al, 2021]. **Table 7** summarises the key points from their study [Jones et al, 2021].

Table 7: Sex differences for Resistance Training effects: key points [Jones et al, 2021]. (Reproduced with permission).

Following resistance training, older males gain more absolute upper and lower-body strength than older females.
Older females gain more relative lower-body strength than older males.
Older males gain more absolute muscle size than older females.

There are no sex differences in changes in relative muscle size or upper-body strength in older adults.
Older males may benefit from higher intensity programs, whereas older females may benefit from higher weekly repetitions (volume).

For people with sarcopenia as defined by EWGSOP 2010 or AWGS 2014, exercise alone effectively increased appendicular skeletal muscle (ASM) and gait speed. The Society for Sarcopenia, Cachexia and Wasting Disorders (SCWD) recommend resistance exercise should be prescribed for any older person suspected of having sarcopenia, both for secondary prevention and/or treatment [Bauer et al, 2019].

The specificity of exercise training (endurance vs. strength) on the phenotype of muscle physiology in relatively younger people is well known. However, the role that both play in reversing sarcopenia in individuals over 80 years of age remains to be determined. Additionally, how the muscle selectively responds to a specific exercise program, and whether very advanced age modifies the response remains to be explored [Johnson et al, 2021].

A suggested RT prescription is shown in **Table 8.** [Braith and Stewart, 2006].

Table 8: Summary of guidelines for Resistance Training for disease prevention [Braith and Stewart, 2006]. (Reproduced with permission).

Exercise mode	Resistance exercise consists of weight lifting. Machines are preferred for safety and ease of use; hand-held weights, barbells, and elastic bands can also be used.
No. of exercises	8 to 10 exercises covering the major muscle groups; chest, shoulders, arms, back, abdomen, thigh, lower legs
Intensity	Resistance (weight) is set at 30% to 40% of 1 repetition maximum for upper body and 50% to 60% for lower-body exercises. One repetition maximum is the highest weight lifted 1 time. If testing is not available, use a weight that can be lifted for 8 to 10 repetitions; increase weight when 15 repetitions can be done easily.
Duration	Resistance training consisting of a single set of 8 to 10 exercises takes about 20 minutes
Frequency	Resistance exercise should be done at least twice per week.

Precautions	Risk/benefit ratio of resistance exercise is very favorable. Contraindications to resistance training are the same as those for aerobic exercise. Treatment for systolic BP>160 mm Hg or diastolic BP>100 mm Hg should be initiated before starting any type of exercise program. Avoid extended breath-holding to minimize exaggerated BP response.

Resistance training also has a role specifically in patients with Alzheimer's dementia *to preserve ADL function and potentially mitigate neuroinflammation, and is discussed in the following section on Alzheimer's disease. (See section 1.8.2).*

(2) Protein supplementation

Many healthy older adults fail to eat enough dietary protein, and this is exacerbated by sickness or disability. These changes may limit activities, further decreasing adequate food consumption, and increasing protein and energy deficits. Three factors influence protein use in older individuals: inadequate intake of protein (e.g. anorexia or appetite loss, gastrointestinal disturbances); reduced ability to use available protein (e.g. insulin resistance, protein anabolic resistance, high splanchnic extraction (first-pass effect), immobility), and; a greater need for protein (eg. inflammatory disease, increased oxidative modification of proteins) [Bauer et al, 2013]. The net result of this is functional loss, as summarised in **Figure 24** [Bauer et al, 2013].

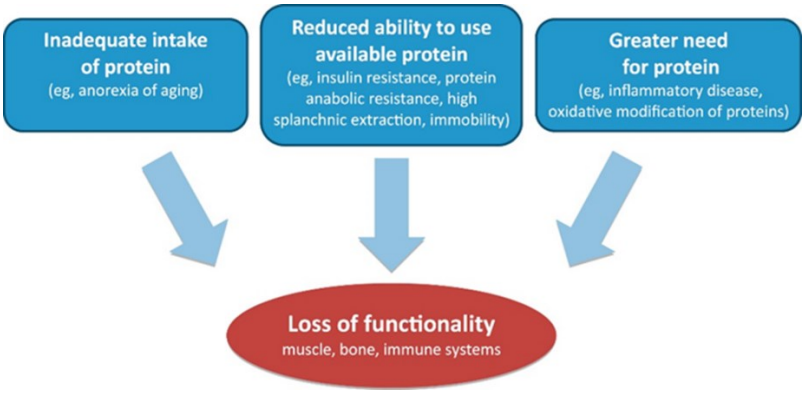

Figure 24: Aging-related causes of protein shortfall. Such protein deficits have adverse consequences, including impairment of muscular, skeletal, and immune function [Bauer et al, 2013]. (Reproduced with permission).

Strategies to overcome *anabolic resistance* (lower sensitivity to nutrients and hormones, as discussed in section 1.6.6) aim to improve postprandial anabolic signaling or sensitivity to nutrients. These include **providing sufficient protein/amino acid** intake to maximize muscle protein anabolism and/or using exercise to improve sensitivity to nutrients and hormones (particularly insulin) [Timmerman et al, 2012; Fujit et al, 2007; Cermak et al, 2012]. **Figure 25** summarises these interdependent features [Bauer et al, 2013].

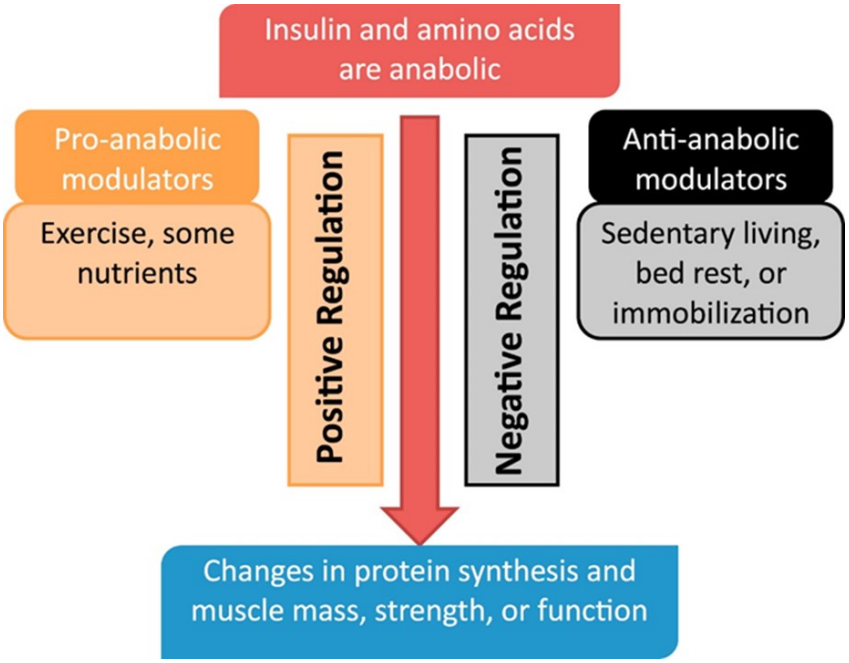

Figure 25: Anabolic effects of insulin and amino acids on protein synthesis are enhanced by physical activity and some nutrients and are impaired by sedentary lifestyle, bed rest, or immobilization [Bauer et al. 2013]. (Reproduced with permission).

The PROT-AGE international study group consensus [Bauer et al, 2013] recommendations for optimal dietary protein intake in older people is a daily intake of 1.0 to 1.2 grams protein per kilogram of body weight per day (13-16% of total calories), with the higher level being for older

adults who are exercising [Bauer et al, 2013]. However, they do recommend caution in those with severe kidney disease who are not on dialysis, as they may need to limit their protein. In otherwise healthy older adults, there is no evidence that high-protein diets cause kidney damage [Surdykowski et al, 2010; Gaffney-Stomberg et al, 2009]. Their recommendations are summarised in **Table 9** [Bauer et al, 2013].

Table 9: Protein Recommendations in Acute and Chronic Diseases [Bauer et al, 2013 (PROT-AGE)]. (Reproduced with permission).

PROT-AGE recommendations for protein levels in geriatric patients with specific acute or chronic diseases

- The amount of additional dietary protein or supplemental protein needed depends on the disease, its severity, the patient's nutritional status prior to disease, as well as the disease impact on the patient's nutritional status.
- Most older adults who have an acute or chronic disease need more dietary protein (ie, 1.2–1.5 g/kg BW/d); people with severe illness or injury or with marked malnutrition may need as much as 2.0 g/kg BW/d.
- Older people with severe kidney disease (ie, estimated glomerular filtration rate [GFR] < 30 mL/min/1.73m^2) who are not on dialysis are an exception to the high-protein rule; these individuals need to limit protein intake.

Type of protein

An extensive review of protein is beyond the scope of this thesis. An important reference on the type of protein is Watford and Wu, 2018 ; the digestibility of proteins are discussed by Schaafsma, 2012, Pillai and Kurpad, 2012, Dangin et al, 2001 and Pennings et al, 2011 Huang et al, 2020, ; and the source of protein (animal versus plant) discussed by Pannemans et al, 1998, Luiking et al,2011 Yuzbashian et al, 2015, Lew et al, 2017, Joshi et al, 2021, Knight et al, 2003, and Azadbakht et al, 2009.

Tieland and colleagues showed improved muscle strength and physical function when frail older people were given supplemental protein daily (milk concentrate) (15 g at breakfast, 15 g at lunch) for 24-weeks [Tieland et al, 2012]. Interestingly, this improvement occurred in the

absence of measurable changes in muscle mass, suggesting that protein feeding alone may improve muscle strength and function more readily than muscle mass.

L-leucine (branch chain amino acid (BAAA)) supplementation is known to enhance protein synthesis in humans [Wang and Proud, 2006]. Increased BCAA in the diet could therefore be an additional strategy in sarcopenia [Robinson et al, 2015]. Katsanos evaluated the acute effects of leucine supplementation *without exercise* and reported that a higher level of leucine ingestion was needed among older versus younger adults to promote postprandial muscle protein synthesis (1.7 vs 2.8 g) [Katsanos, 2006]. This ties in with the concept of *anabolic resistance* discussed earlier [Cuthbertson et al, 2005]. The leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) has also been shown to improve muscle mass and preserve muscle strength in older adults [Bearet al, 2019].

Meta-analyses of 12 RCTs (not exclusively using AWGS 2014 or EWGSOP 2010 criteria), showed variable effects of nutrition alone on muscle mass, strength and gait speed [Yoshimura et al, 2017]. They reported that interventions focused on the intake of essential (indispensable) amino acids may improve knee extension in patients with sarcopenia. Although the combined interventions improved sarcopenia in the RCTs reviewed, longer-term effects beyond 3-months were uncertain [Yoshimura et al, 2017].

(3) Resistance exercise plus protein supplementation

The *anabolic effects of insulin and amino acids on protein synthesis are enhanced by physical activity* and some nutrients (Omega-3 fatty acids, Vitamin D) and are impaired by sedentary lifestyle, bed rest, or immobilization. (See previous **Figure 25**) [Bauer et al, PROT-AGE 2013].

A *protein rich diet (1-1.5g/kg/day) in combination with RT* has been shown to be *even more efficacious*, particularly with **leucine**. In young and old people alike, protein ingestion together with exercise training increased synthesis of skeletal muscle [Yang et al, 2012]. The quality of the protein consumed may influence the synthetic response. High-leucine–containing and rapidly digested whey proteins showed an advantage over isolated casein and soy proteins, as already discussed [Yang et al, 2012; Pennings et al, 2011]. A 13-week intervention of a Vitamin D and leucine-enriched whey protein oral nutritional supplement resulted in improvements in muscle mass and lower-extremity function among sarcopenic older adults [Bauer et al, PROVIDE

2015]. Other studies have also shown added benefits of combining RT and leucine supplementation [Liao et al, 2017; Funderburk et al, 2020]. Doses of protein were 17-40 g/day, correcting or overcoming protein intake deficiency.

In a 24-week study of community-living women in Japan, those taking whey protein after resistance exercise had increased muscle mass, grip strength and gait speed compared to those in either single intervention arm [Mori and Tokuda, 2018]. Another study of community-living men and women randomized them to either 12-weeks of exercise, exercise plus HMB-enriched supplement, or a wait-listed control group [Takeuchi et al, 2019]. They found there was no effect on the primary outcome of gait speed, but both intervention groups had improved leg extension and five-time chair stand performance, which persisted for 12-weeks after the intervention ended. Leg muscle mass and ASM only increased in the exercise plus nutritional supplementation group, however, unfortunately, the increase in muscle mass had disappeared by 24-weeks [Takeuchi et al, 2019].

At present, it is not clear whether **creatine** or **HMB** can enhance exercise responses in older people, in the same way as these agents have been shown to do in younger people [Rowlands and Thomson, 2009]. Creatine is an endogenously produced metabolite, but is also a popular dietary supplement for enhancement of high intensity exercise [Gualano et al, 2016]. Of note, creatine is of no benefit without concomitant resistance training [Dolan et al, 2019]. Candow and colleagues recently reviewed the evidence and potential role of creatine in ageing muscle and bone, concluding that the "accumulating evidence indicates that creatine supplementation, with and without resistance training, has possible anti-sarcopenic and anti-dynapenic effects" [Candow et al, 2019].

When combining exercise-protein therapy, the *timing of protein or amino acid* intake relative to exercise is central to muscle anabolism. Exercise enhances muscle protein synthesis by sensitizing muscle to insulin- or amino acid-mediated anabolic actions, an effect that appears to peak in the first 3-hours after exercise [Tang and Phillips, 2009], and may persist 18 to 24 hours after an exercise bout [Burd et al, 2011]. This suggests that protein should be consumed close after exercise (or physical therapy) to take advantage of its sensitizing effect, although the actual time window requires further study for both proteins and CHO [Aragon and Schoenfeld, 2013].

C. Investigational Treatments for Sarcopenia

(1) Dietary

There is increased research interest in using dietary modification with the addition of ***medium chain triglycerides (MCT)*** for sarcopenia treatment. Details of MCT metabolism have already been discussed earlier in this chapter (see section 1.3), as well as their role in the provision of cerebral ketones in AD. In sarcopenia MCT is postulated to work by *activation of ghrelin* (an orexigenic peptide). Octanoic acid (C8:0) specifically acylates ghrelin, [Kojima et al, 1999], which binds the growth hormone secretagogue receptor 1a (GHS-R1a) regulating the release of growth hormone, appetite and food intake, gastric acid secretion, gastric motility, glucose homeostasis and adiposity [Lemarie et al, 2018; Delporte, 2013]. Because ghrelin can only bind GHS-R1a in its acetylated form the octanoyl moiety appears crucial for its functions. Dietary caprylic acid and ghrelin O-acyltransferase (GOAT) enzyme activity regulate octanoylated ghrelin production, circulating concentration and functions [Lemarie et al, 2018]. Supplementation with MCTs increased the concentration of acyl-ghrelin (active form) but does not affect that of desacyl-ghrelin (inactive form) in serum or plasma [Kawai et al, 2017; Yoshimura et al, 2017]. Growth hormone and acyl-ghrelin concentrations were significantly lower in older adults compared with young adults, suggesting that supplementation with MCTs might be potentially more effective in older adults [Nass et al, 2014].

Abe and colleagues studied ***MCT in combination with cholecalciferol or leucine*** [Abe et al, 2016, 2017]. The ***MCT and leucine*** group at 3-months showed improvement in grip strength, walking speed, 10-s leg open and close test, and peak expiratory flow, compared to the leucine alone group [Abe et al, 2016, 2017]. They then repeated their study with an MCT only group [Abe et al, 2019]. At 3-months, participants in the MCT group had a 48.1% increase in 10-s leg open and close test performance compared with long chain triglycerides, and a 27.8% increase in a 30-s repetitive saliva swallowing test [Abe et al, 2019]. Their increases were greater at 3-months than at 1½-months after MCT initiation and were attenuated at the end of a 1½-month washout period, suggesting that a 3-month intervention may be required to obtain substantial effects from MCTs and that the effects are reversible. The timing of the 6g MCT supplement at breakfast or supper did not appear to affect its efficacy [Abe et al, 2021].

Medium chain triglyceride supplementation, at a dose of 6g/day for 1½ months has been shown to increase muscle mass and function, as well as cognition in frail older adults in Japan [Abe et al, 2022]. The same group also studied long term care residents with ***MCT plus leucine (1.2g) and Vitamin D*** versus long chain triglycerides (LCT) plus leucine (40% leucine in 3g essential amino acids) and Vitamin D (800IU) for 3-months.[Abe et al, 2016]. They showed improved grip strength, leg extension strength, walking speed, and peak expiratory flow rate in the MCT group compared to the LCT group. They speculated that MCTs might increase energy expenditure via the upregulation of mitochondrial oxidative capacity in muscle, or by enhancing cognitive function (which was not seen in the LCT group). However, they did not measure muscle mass, other than using anthropometric measures, so had no information on muscle quality.

Vitamin D supplementation between 700 and 800IU per day reduces risk of hip fracture (and any non vertebral fracture) in community-dwelling and nursing home elderly [Bischoff-Ferrari et al, 2005] and the risk of falls [Bischoff-Ferrari et al, 2004]. The underlying mechanism may be the increased muscle strength. Vitamin D deficiency is reported to cause histological muscle atrophy, predominantly of type II fibers [Janssen et al, 2002]. Whether Vitamin D prevents sarcopenia remains to be proven, but the relationship of Vitamin D and calcium on muscle mass and function in the elderly is an ongoing important area for research. In elderly individuals aged 60 to 80 years, Vitamin D has been reported to reduce intramyocellular lipid accumulation in combination with treadmill aerobic training [Thomas et al, 2019]. 1,25-Dihydroxyvitamin D (1,25(OH)2D) may also have beneficial effects on skeletal muscle by regulating mitochondrial function, with 1,25(OH)2D supplementation improving the function of mitochondrial oxidative phosphorylation in the skeletal muscle in Vitamin D deficient adults [Sinha et al, 2013]. A meta-analysis showed increased muscle strength, but not muscle mass. Effects were greater for those starting off Vitamin D insufficient [Beaudart et al, 2014].

In a recent meta-analysis, looking at sarcopenia specifically, ***Vitamin D plus protein supplementation*** *increased muscle strength* in patients with sarcopenia [Gkekas et al, 2021]. But again, there was no evidence of improvement in muscle mass and performance by Vitamin D plus protein in patients with sarcopenia. The authors concluded that more research is needed to

clarify the optimal dose, mode of administration, and duration of vitamin D and/or protein supplementation on sarcopenia indices [Beaudart et al, 2014; Gkekas et al, 2021].

***Calorie restriction**,* by its effect on decreasing apoptosis via decreased tumour necrosis factor alpha (TNFα) is also under investigation [Philips and Leeuwenburgh, 2005]. A recent review felt the risk-benefit ratio in adults ≥65 years, even those with obesity, was still unclear [Normandin et al, 2015].

(2) Pharmacologic

Selective androgen receptor modulators (SARMS*)** are partially effective in increasing muscle mass, but not strength or function [Rooks and Roubenoff, 2019]. ***Testosterone and other anabolic steroids have only a modest effect on muscle mass and strength, but have significant side effects precluding their usefulness [Sakuma and Yamaguchi, 2012]. ***Growth hormone*** supplementation does not increase muscle mass or strength, and may increase the risk of malignancies [Pollak, 2000].

Early trials suggest that ***myostatin neutralizing antibody*** or ***activin IIB receptor blockade*** may significantly increase aspects of functional performance, but the potential clinical benefits remain uncertain [Rooks and Roubenoff, 2019]. Newer pharmacological agents, including ***angiotensin 2 converting enzyme (ACE)*** inhibitors, are being investigated because activation of the renin-angiotensin-aldosterone system may be involved in the progression of sarcopenia [Carter et al, 2005], but currently should not be used to treat sarcopenia [Bauer et al, 2019].

1.6.8 Measuring treatment outcomes

Available evidence suggests that ***exercise plus protein improves muscle strength and function, with variable effects on muscle mass***. However, there is uncertainty about the desired treatment outcome indicators for intervention: whether the goal should be changing sarcopenia status (reversal from sarcopenic to non-sarcopenic); or changing the individual components (muscle mass, strength and physical performance) that define sarcopenia to above their respective cut-offs. Furthermore, the outcomes desired in community versus hospital settings may differ. Last

but not least, it would be relevant to include patient-related outcome measures, such as general or specific quality of life measures, or self-rated health [Beaudart et al, 2015; Kaushal et al, 2018].

1.6.9 Summary of sarcopenia

Improved understanding and treatment of sarcopenia would have a dramatic impact on improving the health and quality of life for older adults, reducing the associated morbidity, and disability, as well as stabilizing rising health care costs. A comprehensive approach to sarcopenia requires a **multi-disciplinary approach, including exercise and nutrition specialists.** An important clinical endpoint should be the prevention of mobility disability, along with reducing, stopping, or reversing the loss of muscle mass, muscle strength or muscle quality.

Rolland and colleagues suggest, "a well-balanced diet, along with adequate amounts of essential minerals, fatty acids, and amino acids, together with an active and healthy lifestyle with regular periods of aerobic and resistance training would go a long way toward reducing the prevalence of sarcopenia and other chronic diseases in future elderly generations" [Rolland et al, 2008].

1.7 Sarcopenia obesity overlap: sarcopenic obesity

Many definitions of sarcopenic obesity have been proposed, but a clear and decisive definition is still lacking. The negative effects of sarcopenia and obesity are additive [Zamboni et al, 2008; Roubenoff, 2004]. Links between loss of muscle mass and adipose tissue dysfunction associated with aging suggest common etiologic factors. **Figure 26** summarises this complex interaction [Zamboni et al, 2008].

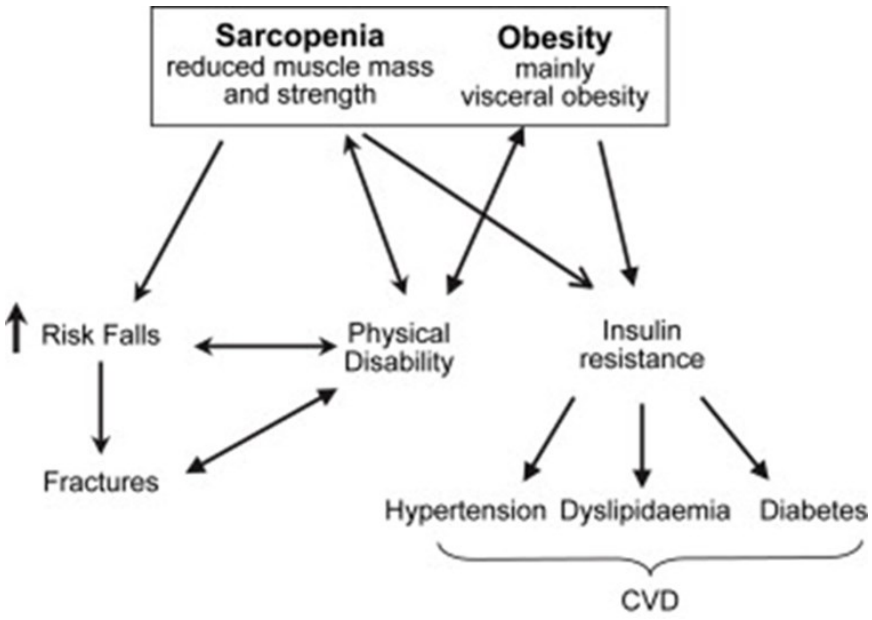

Figure 26: Possible consequences of sarcopenic obesity in elderly.

Abbreviations- CVD: cardiovascular disease [Zamboni et al, 2008]. (Reproduced with permission).

Irisin, a peptide produced by myocytes during exercise, may control fat gain by eliciting browning response in white fat, causing its transdifferentiation into brite (brown-in-white) adipose tissue. [Zamboni, Gattazzo et al, 2019]. A reduction of physical activity with aging may determine a decline in the production of irisin by the muscle, which leads to an increase in fat mass, and thus to sarcopenic obesity. As a result of the increased inflammatory profile, insulin resistance occurs and promotes muscle catabolism [Zamboni, Rubele et al, 2019]. The evidence is also increasing for cross-talk between myocytes and adipocytes through insulin resistance and inflammation further exacerbating both conditions. See summary **Figure 27**. [Zamboni, Rubele et al, 2019].

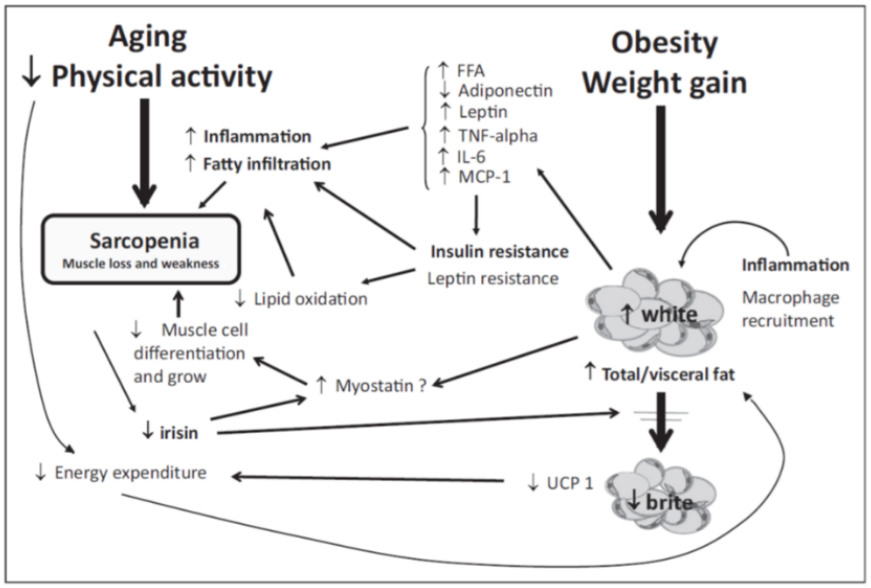

Figure 27: Cross talk between adipocyte and myocyte in older age: a mechanism leading to sarcopenic obesity. (Main steps are given in bold).

Abbreviations: FFA: free fatty acids; TNF: tumour necrosis factor; IL-6: interleukin-6; MCP-1: monocyte chemoattractant proteion-1; UCP 1: uncoupling protein-1 [Zamboni, Rubele et al, 2019]. (Reproduced with permission).

1.8 Sarcopenia and Alzheimer's Dementia (AD) overlap

Throughout the western world, with the aging demographic, there is an increased prevalence of age associated chronic disease, particularly AD and sarcopenia. Sarcopenia prevalence varies from 0.9% to 85.4% in the geriatric population based on different measuring tools and cut-off values for muscle mass and function, and the population being studied. Sarcopenia is associated with an increased risk of cognitive impairment independent of the study population, the sarcopenia definition, or the cognitive impairment definition [Peng et al, 2020].

Both conditions significantly impact function and independence. They also have other effects on morbidity in common: increased length of hospitalisation, increased risk of malnutrition, increased risk of falls and fractures, decreased quality of life, increased risk of institutionalized

care [Baumgartner et al, 1998; Roubenhoff, 2001]. Both also increase the risk of mortality [Chang et al, 2016].

Sarcopenia and AD have clearly defined definitions, but ***both require clinical assessment*** to make the diagnosis. There is no single diagnostic test for either condition.

Both conditions also do not have specific disease-modifying pharmacotherapeutic agents. AD has medications such as acetylcholinesterase inhibitors and NMDA receptor inhibitors [Husna et al, 2020], but these provide mild symptomatic benefit at best, and do not have any significant disease modifying activity. In the case of sarcopenia, agents are under investigation [Morley 2020], but there are no pharmaceutical agents that have been shown, to date, to impact disease outcome or course.

1.8.1 Lifestyle interventions

A. Impact on Alzheimer's Dementia of the lifestyle changes investigated for sarcopenia management.

(1) Epidemiological evidence

Around 35% of dementia is attributable to a combination of nine risk factors: low education level, midlife hypertension, midlife obesity, hearing loss, later-life depression, diabetes, smoking, social isolation, and low physical activity [Livingston et al 2017].

Both AD and sarcopenia have been shown to be impacted by lifestyle -nutrition and exercise. In the case of AD, the data is primarily epidemiological, where it has been shown that older adults consuming a Mediterranean style diet are at lower risk for developing AD [Singh B et al, 2014]. In certain areas of the world, known as the "blue zones", the common feature in diets are an abundance of whole foods with a vegetable predominance [Poulain and Herm, 2021]. Other factors in common in these areas have included ongoing physical activity, and social interaction with a sense of purpose [Legrand et al, 2019]. Data for treatment of established AD with diet and nutrition is less robust and more limited, likely related to the heterogeneity of people with a clinical diagnosis of AD.

In the case of sarcopenia, there is less clear epidemiological data, but more prospective data suggesting the benefits of nutrition and physical exercise [Bauer et al,2013]. Given the nature of the diagnosis of sarcopenia, it is difficult to do retrospective population-based studies in the same way as those are possible in adults with AD. However, reports of the prevalence of sarcopenia vary greatly among the population being evaluated (community versus institutionalized versus hospitalized) [Rolland et al, 2008; Sayer et al, 2004]. No one common feature has been identified in populations with a reported lower sarcopenia prevalence.

(2) Biochemical basis

Sarcopenia leads to the imbalance in secretion of myokines from skeletal muscle, and results in impaired blood vessel homeostasis. These impaired myokines and carotid atherosclerosis can also contribute to cognitive dysfunction. [Jo et al, 2022]. See **Figure 28.**

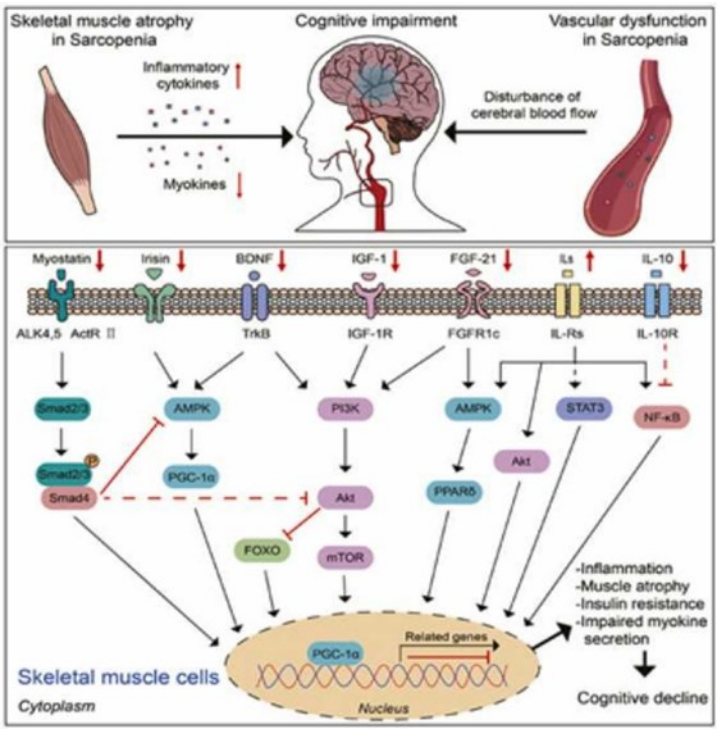

Figure 28: Cognitive impairment caused by imbalanced myokine secretion and vascular dysfunction.

Abbreviations: IL-6:interleukin-6; IL-10: interleukin-10; BDNF: brain-derived neurotrophic factor; SPARC: secreted protein acidic and rich in cysteine; AMPK: activated protein kinase [Jo et al, 2022]. (Reproduced from with permission).

(3) Research/Prospective evidence

Prospective studies have shown that **protein intake** appears to be key for sarcopenia prevention and treatment, particularly L leucine [Liao et al, 2017; Luiking et al, 2014; Kramer et al, 2015], as discussed previously. However, there is less robust data for protein supplementation in AD, for AD prevention or treatment. A common feature may be disturbance of the microbiome. Skeletal muscle and the development of sarcopenia are influenced by *gut microbiota*, which in turn is affected by whey protein and RT [Zhang et al, 2020; Mailing et al, 2019; Strasser et al 2021]. Gut microbiota may be a key factor for whey protein and/or RT in age-related sarcopenia.

In AD, state-of-the-art metabolomics studies demonstrate that altered *BCAA* metabolism accompanies AD development [Larsson and Markus, 2017]. Lower plasma valine levels are correlated with accelerated cognitive decline, and, conversely, an increase in valine concentration is associated with reduced risk of Alzheimer's disease. Further discussion can be found on animal models [Polis and Samson, 2020], CNS role of BCAA in animals [Fernstrom, 2005], and in BCAA use in severe traumatic brain injury[Aquilani et al, 2005]. So far, for AD, the studies are only in mouse models, and the results are controversial because of elevated phosphorylated tau in those fed leucine [Li et al, 2018]. This supports genetic data showing a raised plasma isoleucine level, (but not valine or leucine), is positively associated with AD [Larsson and Markus, 2017].

Combination intervention makes scientific sense for addressing both cognition and sarcopenia. In a Japanese study [Abe et al, 2016, 2017], 38 elderly nursing home residents (11 men and 27 women with a mean age of 86.6 years) were enrolled in a 3-month randomized, controlled, single-blind, parallel group trial. The participants were randomly allocated to three groups. The first group received a daily L-leucine (1.2 g) and cholecalciferol (20 mg)–enriched supplement with 6 g medium-chain triglycerides (LD + MCT); the second group received the same leucine and cholecalciferol–enriched supplement with 6 g long-chain triglycerides (LD + LCT); and the third group did not receive any supplements (control) [Abe et al, 2016, 2017]. Outcomes for both cognition and muscle function improved in the intervention arms versus control, with the *greatest benefit in participants receiving LD and MCT.*

B. Impact on Alzheimer's Dementia of the exercise interventions investigated for sarcopenia management.

The following facts regarding **exercise** are well established: Low levels of physical activity are a risk factor associated with Alzheimer's disease; older adults who exercise are more likely to maintain cognitive function; exercise modulates amyloid β turnover, inflammation, synthesis, and release of neurotrophins, and cerebral blood flow [De la Rosa et al, 2020]. More recently, it has been found that engaging in physical activity and lowering vascular risk may have additive protective effects on delaying the progression of AD [Livingston et al, 2020]. Greater physical activity and lower vascular risk independently attenuated the negative association of Aβ burden with cognitive decline and neurodegeneration in asymptomatic individuals [Sun et al, 2018].

In addition, exercise, particularly **resistance exercise**, has been shown to be the most beneficial in producing measurable changes in sarcopenic indices [Yoshimura et al, 2017], and can impact AD [De la Rosa et al, 2020]. Sixteen weeks participation in a resistance training protocol (three sets of 20 repetitions in five exercises) resulted in significant improvement in ADL function (house mobility, climbing stairs, standing up from the floor and putting on socks) in a group of participants with mild AD (average MMSE 18.4 ± 4.3) [Garuffi et al, 2013]. The authors however, added the caveat that the battery of tests used, although specifically designed for the elderly, have not been validated for patients with AD, and it was necessary to adjust the evaluation procedure in their study with respect to the direction and guidance of patients during testing [Garuffi et al, 2013]. They did not show any improvement in aerobic capacity (800m walk test), unlike some other researchers [Santana et al, 2008].

In a less cognitively impaired group, the SMART study group, [Mavros et al, 2017] did however show that **high-intensity progressive RT** resulted in significant improvements in cognitive function, muscle strength, and aerobic capacity in older adults with MCI. These studies taken together, suggest strength gains, but not aerobic capacity changes, likely mediated the cognitive benefits of progressive RT.

(1) Physical exercise for <u>prevention</u> of Alzheimer's disease

A meta-analysis that included 16 studies with more than 160,000 participants found a 45% reduction in the risk of developing AD with regular physical activity (hazard ratio = 0.55, 95% confidence interval: 0.36−0.84, p = 0.006) [Hamer and Chida, 2009]. Exercise regulates learning, neurogenesis and angiogenesis through growth factor cascades. Insulin growth factor-1 (IGF-1), brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) derived from central and peripheral sources act in concert to modulate exercise-dependent effects on the brain. See summary in **Figure 29** [Cotman et al, 2007].

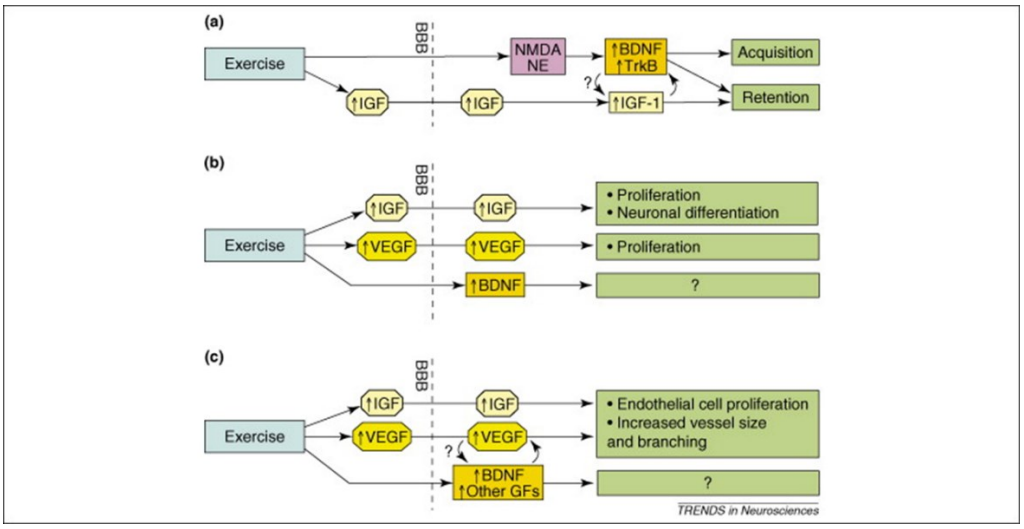

Figure 29: Exercise regulates learning, neurogenesis, and angiogenesis through growth factor cascades.

Abbreviations: Insulin growth factor-1 (IGF-1); brain-derived neurotrophic factor (BDNF); and vascular endothelial growth factor (VEGF); N-methyl-D-aspartate (NMDA) receptor; noradrenergic (NE) system; long-term potentiation (LTP); blood–brain barrier (BBB); receptor for BDNF, tyrosine kinase B (TrkB); growth factors (GFs) [Cotman et al, 2007]. (Reproduced with permission).

The positive impact of long-term exercise training, by delaying the onset of physiologic memory loss suggests the effectiveness of **exercise as a preventive strategy** against age-related memory loss and neurodegeneration [De la Rosa et al, 2020].

But even older-age onset exercise interventions have also shown positive results in the delay of brain aging. A ground-breaking paper, published in 2011, found that 1 year of **moderate-**

intensity exercise (40 min duration, 3 days/week) *increased the size of the hippocampus*, as well as increasing spatial memory, in healthy older individuals [Erikson et al, 2011]. The *anatomic changes induced by aerobic exercise* have also been confirmed by other research groups. A six-month exercise program (60 min duration, 3 days/week) was sufficient to increase both the grey and white matter in the anterior cingulate cortex, as measured by magnetic resonance imaging, in older, cognitively healthy participants [Colcombe et al, 2006].

Longer aerobic exercise training protocols (3 years' duration) in sedentary older women showed improvements in reaction time, motor function, and cognitive processing speed [Rikli and Edwards, 1991]. This indicates that exercise is effective in reversing, or at least slowing, the age-related declines in motor performance and cognitive processing speed [Rikli and Edwards, 1991].

However, there is some contradictory evidence showing that aerobic exercise interventions do not always induce improvements in cognitive function in older adults (60−80 years) [Blumenthal et al, 2019; Hill et al, 1993; Panton et al, 1990]. These contradictory results may be explained by the training duration, frequency, and/or intensity of the interventions.

The **impact of resistance exercise (RT) on cognitive function** has gained interest in the past few years. The neuroprotective effects of resistance training in cognitive impairment are not well characterized. But one animal study has shown short-term resistance training improved cognitive function in 3xTg mice, and conferred beneficial effects on neuroinflammation, amyloid and tau pathology, as well as synaptic plasticity [Liu et al, 2020]. The problem remains though, that lack of resistance training protocols in animal models of AD is a huge gap [De Sousa et al, 2021].

A meta-analysis of 24 studies, investigated the effects of *weightlifting* on different cognitive outcomes in older individuals [Landrigan et al, 2020]. RT showed positive effects on measurements of cognitive impairment and executive function. However, RT had no effect on working memory measurements, with the caveat that high heterogeneity was observed, and future research will need to determine why the effects are so variable [Landrigan et al, 2020].

We can therefore conclude that exercise programs with components of both aerobic and resistance training, of moderate intensity, and lasting at least 45 min per session, on as many

days of the week as possible, are likely beneficial in terms of cognitive function in older healthy adults, and may prevent dementia [Orgeta et al, 2019].

(2) Physical exercise for <u>treatment</u> of Mild Cognitive Impairment (MCI)

Improvements in executive functions, memory, and cognitive tests in individuals with MCI who engaged in **aerobic exercise** programs [Zheng et al, 2016; Scherder et al, 2005], an **aerobic and resistance exercise** program [Baker et al, 2010], aerobic exercise and Dietary Approach to Stop hypertension (DASH) diet [Blumenthal et al, 2019], and leisure time physical activity [Wueve et al, 2004], have been reported.

Nagamatsu and colleagues compared the difference between 6-months of progressive aerobic (AT) and **resistance training** in older women (70-80 years) with MCI, compared to a control group of MCI women who did only balance and tone training (BAT) [Nagamatsu et al, 2012]. They showed 6-months of twice-weekly RT improved selective attention/conflict resolution, associative memory, and regional patterns of functional brain plasticity, compared with twice-weekly BAT exercises. In contrast, 6-months of twice-weekly AT only improved physical function, showing that RT can benefit multiple cognitive domains in those at risk for dementia [Nagamatsu et al, 2012]. This was despite exercise compliance being low in their study, suggesting that these results are likely conservative estimates of the efficacy of RT on cognition and functional plasticity.

Yoon and colleagues showed elastic band-based high-speed power training, as compared with low-speed strength training, was more efficient in older women with MCI in improving their cognitive function, physical performance, and muscle strength [Yoon et al, 2017]. Another randomized, double-blind trial in a 100 people with MCI (55-86 years of age) showed that 6-months of RT improved memory, attention, and executive functions [Singh et al 2014]. Moreover, these benefits persisted 12-months after the end of the intervention period. Electroencephalogram (EEG) changes were also evaluated with RT and showed positive changes [Hong et al, 2018].

Theses intervention therefore have the potential to prevent the progression of MCI to Alzheimer's disease.

(3) Physical exercise for <u>treatment</u> of Alzheimer's dementia

In individuals diagnosed with AD, and, in general, at early stages of the disease, **aerobic exercise** alone, or accompanied with cognitive stimulation, induces improvements in some aspects of brain function [Coelho et al, 2009; Friedman and Tappen, 1991; Rolland et al, 2000; Öhman et al, 2016; Heyn et al, 2004; Yu et al, 2006; Vreugdenhil et al, 2012; Panza et al, 2018; Morris et al, 2017; Palleschi et al, 1996].

Fewer studies have been published on the effects of **resistance training (RT)** in people with AD [Vital et al, 2016; Garuffi et al, 2013]. Comparing RT to a social gathering group, there was no significant differences on cognition in AD patients, but it was a low intensity RT protocol [Vital 2012]. Although RT improved agility, lower limb strength, balance and flexibility in AD patients, there was no measurable effect on cognition in a 16-week study [Garuffi et al, 2013].

The potential impact of RT on lipid profile and homocysteine in patients with Alzheimer's disease produced no effect [Vital et al, 2016], although studies in non-AD participants have shown reduction in homocysteine, and improved lipid profiles. The issue may be that the RT may not have reached the appropriate intensity, and the sample group was small (14 participants) [Fahlman et al, 2002]. It can be argued that the studies in established AD may need to be longer to demonstrate both physical and cognitive benefits.

1.8.2 Summary of sarcopenia and AD overlap

The recent Dementia prevention, intervention, and care: 2020 report of the Lancet Commission states that "it is never too early and never too late in the life course for dementia prevention" [Livingston et al, 2020], as shown in **Figure 30**. They identified **twelve modifiable risk factors**: less education; hypertension; hearing impairment; smoking; obesity; depression; *physical*

inactivity; diabetes; low social contact; excessive alcohol consumption; traumatic brain injury; and air pollution. These account for 40% of worldwide dementias,

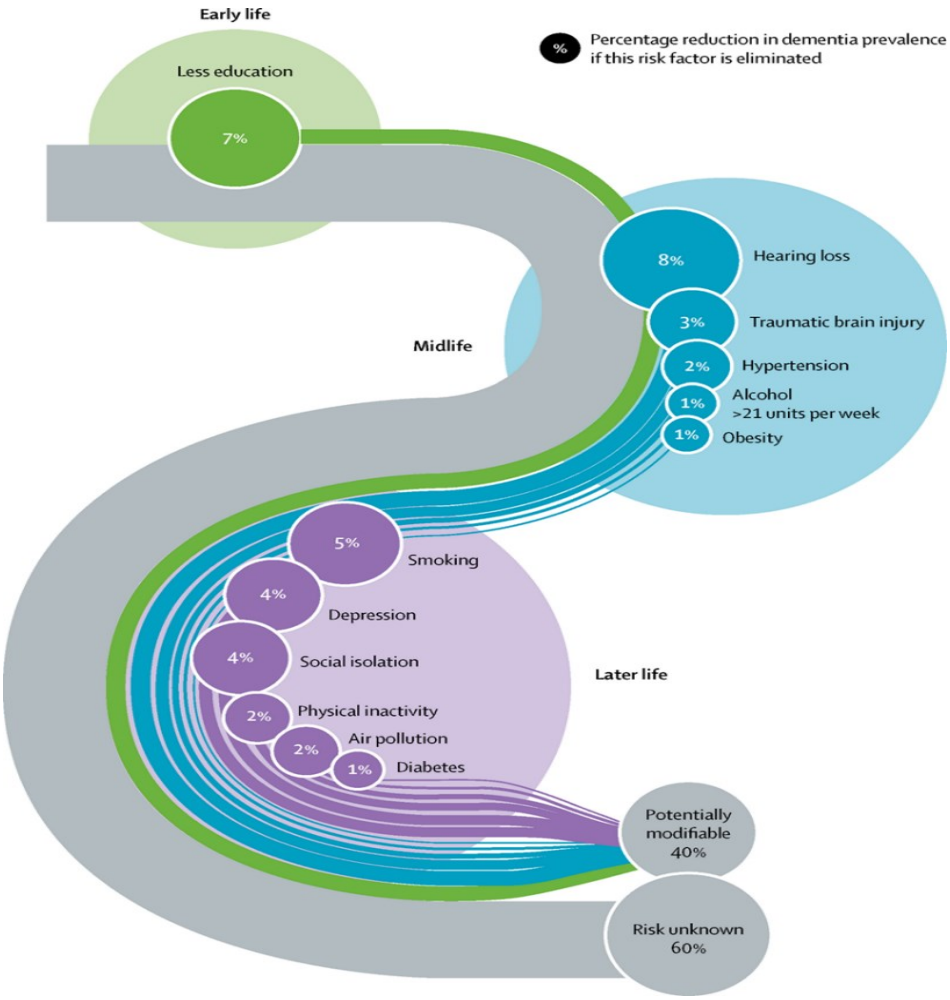

Figure 30. Population attributable fraction of potentially modifiable risk factors for dementia [Livingston et al, 2020]. (Reproduced with permission).

1.9 Research Gaps in the current literature

Having discussed the key information around each topic, this section summarises some of the most important gaps in the research literature pertaining to AD, the use of MCT supplementation, and sarcopenia.

1.9.1 Alzheimer's Dementia

The most important clinical gap is a strategy to effectively prevent and delay progression of AD. From a basic science perspective developing a complete understanding of the neurobiology may serve to identify novel treatment options.

1.9.2 Medium Chain Triglyceride (MCT) supplementation

Given that the use of MCT supplementation for disease management is relatively new there are more clinical gaps. The effect of underlying disease and age on MCT metabolism needs to be further elucidated. The role of ketone replacement in MCI and AD has had some limited study, but other neurological diseases: Parkinson's; non-Alzheimer's dementias; post-viral cognitive decline; could also potentially benefit. In addition, as already highlighted in Section 1.5.3, there are potential biases in the published studies of MCT supplementation in MCI/AD that need to be addressed in future studies.

The role of ketone replacement in sarcopenia is intriguing, as discussed earlier in this chapter, and also warrants further investigation.

The effects of ketone supplementation on mitochondrial dysfunction, per se, raise further exciting possibilities for the potential role of ketones in age-related disease prevention (including AD).

And finally, there are still some practical strategies that need to be studied such as ways to improve tolerance and dosage of MCT supplementation, including ways to increase ketosis potentially by combining diet and/or exercise with MCT supplementation, as well as long-term safety.

1.9.3 Sarcopenia

Clinically the need is for practical steps to improve screening, diagnosis, and management in general medical clinics. Once the diagnosis is made, then addressing practical strategies for management of sarcopenia can follow. This includes translating evidence-based RT to non-

research settings. As with AD, prevention is preferrable to treatment of established disease, so more longer-term trials are required to look at aspects related to prevention of the development of sarcopenia, as well as further understanding of the multifactorial aetiological risk factors.

1.10 Rationale for the thesis Research Projects

Based on this literature background four studies were carried out, in an attempt to address some of the many knowledge gaps discussed in this chapter.

CHAPTER 2: RESEARCH PLAN

THESIS TITLE: Addressing the knowledge gaps in two common age-related conditions, both associated with nutrition and physical activity: Alzheimer's Dementia and Sarcopenia.

2.1 Rationale

Ageing is associated with an increased risk for chronic diseases, morbidity, and mortality. Age ≥ 65 years is also associated with an increased lifetime risk of Alzheimer's dementia (AD), which is almost twice as high for women (6.2% in men and 12.4% in women). [Framingham study. Seshadri et al 1997]. In AD, physiological changes affect cerebral energy with changes in cerebral glucose uptake [Mosconi at al, 2007, Cholerton et al 2013], and metabolism, as summarised in a recent review by Poddar and colleagues [Poddar et al 2021], that can begin years or decades prior to the onset of AD symptoms. [Cholerton et al, 2013]. In addition, any available cerebral glucose is not as readily metabolised for adenosine triphosphate (ATP) synthesis through glycolysis, because of the decreased enzymatic activity of the three rate-controlling irreversible steps of glycolysis [An et al, 2018]. The result is one of several causes for mitochondrial dysfunction in AD [Eckert et al 2003; Cadonic et al 2015, Van Giau et al 2018]. Several mitochondrial functions have been shown to be affected in AD. These include diminished glucose metabolism, mitochondrial enzymatic failure, and increased ROS production (before Aβ and tau tangles have begun forming) [Esteves et al, 2009].

Physiologically, lack of available cerebral glucose in states of starvation, is addressed by the generation of ketones (acetoacetate, B-hydroxybutyrate and acetone) by the liver for extrahepatic energy, including the brain [Balasse et al, 1989, Henderson 2008]. When available, the brains of those with AD can still utilise cerebral ketones in the same way as in the non-AD state [Croteau et al, 2018] thereby affording the opportunity to treat changes in cognition by supplying ketones

[Broom et al. 2019]. Other than starvation, ketones can be generated from ketogenic diets or with provision of dietary ketones via medium chain triglyceride (MCT) supplementation. Ketogenic diets have been investigated in numerous neurological disorders, including AD, and the data is summarised in two reviews by Paoli, Broom, and colleagues [Paoli et al, 2014, Broom et al 2018]. AD patients frequently undergo changes in food preference toward sweet, carbohydrate-rich foods [Wolf-Klein at al 1991, Keene et al 1997, Mungas et al 1990], which makes compliance with a ketogenic diet difficult. Given theses challenges, MCT supplementation appears to be a reasonable alternative. Some benefit has been shown in short clinical trials in mild cognitive impairment or very early AD. Henderson and colleagues investigated a ketone ester (AC-1202) for 90 days, and showed some cognitive benefits [Henderson et al 2009]. Safety of MCT has been reported in numerous animal studies, and in short term human studies (60-90 days) [Traul et al, 2000]. However, Xu and colleagues reported elevated total cholesterol and HDL cholesterol in their cross-over study with canola versus MCT (17.3g/day) for a total of 30 days in each arm [Xu et al 2020].

Major gaps in the literature regarding MCT and AD, include: studies with low potential for bias; the lack of studies > 90 days duration; impact of MCT oil supplementation on cognition in more advanced AD; and the long-term safety on the serum cholesterol profile.

Another important morbidity with aging is sarcopenia, defined as reduction of muscle mass and function [Rosenburg 1989]. For sarcopenia, the biggest clinical challenge remains the identification of patients in the community [Morley 2020]. The challenge is the availability of easy-to-use tools to assess muscle mass in the ambulatory setting, given that the recommended gold standards are MRI or DXA body composition assessments.

Inactivity is known to contribute to loss of muscle mass and strength [Kortebein et al 2007, Lee et al 2007] implying that physical activity should be protective, but studies have suggested that the amount of protection depends on the type of physical activity. [Rolland et al 2008]. Improvement in muscle mass and strength has been shown in well controlled studies using closely prescribed and supervised resistance training (RT) [Friedman et al 1985, Joszi et al 1999, Welle et al 1995, Fiatarone et al 1994, Yarasheki et al 1999, Ivey et al 2000, Cress et al 1999, Hikida et al 2000, Hagerman et al 2000]. However, the implementation of financially

sustainable, ongoing RT programs, outside of research studies remains, as does the need for development of community programming specifically to treat this condition.

Leisure physical activity is not enough to prevent decline in muscle mass associated with aging [Raguso et al 2006] and there is limited evidence on the impact of leisure activity on established sarcopenia. However, leisure activity may prevent the functional effects of sarcopenia by improving balance, fatigue, pain, cardiovascular risk, and appetite [Rolland et al 2008].

In the case of AD, physical inactivity is also a documented risk factor for the development of dementia [WHO 2019, Alty et al 2020]. There is clear epidemiological data [De La Rosa et al 2020, Livingstone et al 2017] and prospective population studies [Blondell et at al meta-analysis 2014] suggesting regular physical/leisure activity delays/and or prevents the development of cognitive decline and dementia. Data on the benefits for improvement in cognition in established AD is more limited, but has been suggested in MCI, and seems to favour aerobic exercise [Farina et al 2013, Zheng et al 2016].

Despite there being some common pathophysiologic mechanisms in these two, age-associated, overlapping conditions, AD and sarcopenia, the research and clinical care gaps generate different clinical questions. To evaluate and address them requires a disease-specific approach.

2.2 Objectives and Hypotheses

The overall objectives of this research is to address some of the knowledge gaps in two common age-related conditions both impacted by nutrition and physical activity: Alzheimer's Dementia (AD) and Sarcopenia.

2.2.1 ***With respect to AD***, intervention with MCT oil has a scientifically valid, theoretical benefit. It has been shown to provide some clinical improvement in patients with early AD or mild cognitive impairment (MCI). Its effect in moderate to severe AD has not been studied, nor have there been any long-term (>3months) studies on the safety and tolerability of MCT in AD. In addition, to date, MCT supplementation in AD has been based on the unstudied assumption that MCT ketone generation is the same in AD as in healthy adults.

1.The **first objective** is to study MCT supplementation in AD and non-AD participants. This was further divided into sub-objectives and hypotheses:

a. The first sub-objective is to evaluate the ketogenic response to MCT supplementation in those with and without AD. We hypothesised that the ketogenic effect (as measured by serum beta hydroxybutyrate (BHB)levels) of MCT supplementation is comparable between those with and without AD.

b. The second sub-objective is to determine whether there is a BHB dose-response to different doses of supplemental MCT. We hypothesised that there is a linear dose-dependent effect between the amount of MCT ingested and serum BHB levels.

c. The third sub-objective is to determine the relationship between BHB levels and reported side-effects. We hypothesised that the higher the measured BHB level the greater the chance of clinical side effects, predominantly gastrointestinal.

d. The fourth sub-objective is to determine factors that may affect the absolute BHB level. We hypothesised that the BHB MCT maximum achieved level may be reduced by baseline breakfast, BMI, or body composition.

Reported in **Chapter 3**, this is the first study to confirm the ketogenic (BHB) response is not affected by AD status, and the first study to identify the importance of concomitant diet and body composition on dose dependent BHB responses.

It is published as: Juby AG, Brocks DR, Jay DA, Davis CM, Mager DR. Assessing the Impact of Factors that Influence the Ketogenic Response to Varying Doses of Medium Chain Triglyceride (MCT) Oil. The Journal of Prevention of Alzheimer's Disease. 2021 Jan;8(1):19-28.

2. The **second objective** was to evaluate the association of MCT supplementation with cognitive changes in participants with established AD (mild-moderate stage) over a 15-month period, with particular effort being made to reduce some of the biases highlighted in previous studies. To this end there are several sub-objectives and hypotheses that were studied.

a. The first sub-objective was to compare the cognitive effect of MCT supplementation compared to placebo oil supplementation in a double-blind randomised, cross-over design study, over an 8-month period We hypothesised that MCT supplementation would stabilise or improve

cognitive function compared to placebo oil, as measured by changes in objective cognitive scores.

In order to address the potential biases and gaps in the studies reported in the literature to-date, several issues were addressed in the study design: participants were randomised; allocation was concealed from the investigators; both participants and investigators were blinded as to the intervention; and outcomes were included that were not tester dependent.

b. The second sub-objective was to determine the cognitive effect of an additional 7-months of open-label MCT supplementation on cognitive function. We hypothesised that longer duration of MCT supplementation would provide continued stabilisation of cognitive function or further cognitive improvement.

c. The third sub-objective was to evaluate the safety and tolerability of long-term exposure to supplemental MCT. We hypothesised that MCT supplementation would have no negative impact in terms of body composition, blood glucose, serum triglycerides, serum cholesterol, and GI side-effects.

This is reported in **Chapter 4.** It is the first study to include participants with moderate AD, to use the Cognigram® computerised cognitive testing in this setting, and the longest clinical AD trial to-date of MCT supplementation, and the longest trial evaluating the safety profile of MCT in this setting.

It is published as: Juby AG, Blackburn TE, Mager DR. Use of medium chain triglyceride (MCT) oil in subjects with Alzheimer's disease: A randomized, double-blind, placebo-controlled, crossover study, with an open-label extension. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2022;8(1):e12259.

2.2.2 ***With respect to sarcopenia***, the major gap in clinical practice is the office-based assessment of muscle mass. Although there is now consensus on the definition of primary sarcopenia, body composition diagnostic tools are not readily available in a clinical setting. Given the prevalence of the condition and the important comorbidities, timely diagnosis of this

condition is vital. Few studies evaluate change in sarcopenic status over time, and whether leisure activity has any impact in higher functioning Seniors.

3. The **third objective** of this thesis is to evaluate the use of two readily available, practical, office-based direct-to-consumer bioimpedance (BIA) scales for the objective evaluation of muscle mass, compared to dual energy Xray absorptiometry (DXA) body composition. This main objective was divided into five sub-objectives and hypotheses.

a. The first sub-objective was to evaluate the diagnostic predictive value of two office-based (non-research) direct-to-consumer BIA scales for assessing muscle mass compared to DXA body composition. We hypothesised that there would be good correlation between the BIA scales and DXA for assessing low muscle mass using published (EWGSOP) [Cruz-Jentoft et al, 2019] diagnostic cut-offs.

b. The second sub-objective was to compare the diagnostic predicative value of the two office-based, direct-to-consumer BIA scales for low muscle mass. We hypothesised that the BIA scale using the additional hand grip input would have a better diagnostic predictive value for DXA derived low muscle mass compared to the BIA scale using only foot input.

c. The third sub-objective was to compare the percentage fat body composition measured by DXA and the two office-based, direct-to-consumer scales. We hypothesised that the BIA scales would have high correlation with DXA percentage fat.

d. The fourth sub-objective was to evaluate the rate of obesity in this independently functioning community-dwelling cohort. We hypothesised the prevalence of obesity (as defined by DXA EWGSOP cut-offs) would be lower than age-based population averages (28%) [Canadian Community Health Survey, combined annual files 2017–2018; Purcell at al, 2021].

e. The fifth sub-objective was to compare the diagnosis of obesity using EWGSOP cut-offs comparing DXA and the BIA scales. We hypothesised that there would be good correlation between the participants identified by DXA and BIA percentage fat cut-offs.

Reported in **Chapter 5,** this study is the first study to evaluate the utility of office-based direct-to-consumer BIA scale muscle mass and percentage fat assessment compared to DXA body

composition, to attempt to address the major care-gap for office-based diagnosis of low muscle mass and awareness of sarcopenic obesity.

The study is submitted as: Juby AG, Davis CMJ, Minimaana S, Mager DR. Addressing the main barrier to Sarcopenia identification: Utility of practical office-based direct-to-consumer bioimpedance assay (BIA) assessment tools versus Dual Energy Xray Absorptiometry (DXA) Body Composition for identification of low muscle mass in older adults. (submitted 2022)

4. The **fourth and final objective** of this thesis was to determine the rate of sarcopenia in an independent living community-dwelling cohort using DXA body composition, and to follow this cohort over 12-months to assess for change in sarcopenic status, and the association of leisure physical activity on sarcopenic status. This was divided into six sub-objectives:

a. The first sub-objective was to assess the rate of sarcopenia in a cohort of independent community-dwelling older adults using the EWGSOP cut-offs for DXA muscle mass assessment [Cruz-Jentoft et al, 2019], and standardised tests for muscle strength and function. We hypothesised that the rate of sarcopenia would be low (<5%) in this highly functioning cohort.

b. The second sub-objective was to observe these participants for 12-months and measure their muscle mass and function over time, to assess for change, and factors such as obesity, Vitamin D status, serum albumin and protein, that may have predicted this change. We hypothesised that in this group of highly functioning older adults there would not be significant decline in their sarcopenic status over 12-months.

c. The third sub-objective was to evaluate the rate of obesity in this independently functioning community-dwelling cohort at baseline and over 12-months. We hypothesised the prevalence of obesity (as defined by BMI) would be lower than age-based population averages (28%) [Canadian Community Health Survey, combined annual files 2017–2018; Purcell at al, 2021], and would not change significantly over the 12-months.

d. The fourth sub-objective was to record the attendance at a supervised group social activity program, and determine if attendance was associated with the participant's sarcopenic status over the 12-month period. We hypothesised, that the group activity program would not be associated with changes in sarcopenic status.

e. The fifth sub-objective was to determine if the level of self-reported leisure activity was associated with baseline sarcopenic status, or change, over 12-months. We hypothesised that those reporting more leisure-based activity would have a better sarcopenic status at baseline.

f. The sixth sub-objective was to evaluate the quality of life of participants and to determine the association with sarcopenic status, self-reported leisure activity, or group social activity class attendance. We hypothesised that those with less sarcopenia, more self -reported leisure activity, and those attending the group social activity class, would have a higher self-reported quality of life.

Chapter 6 reports the first study done in an independent, community-dwelling older adult cohort in Edmonton, and reports on the final objectives of this thesis, and has been submitted for publication as: Juby AG, Davis CMJ, Minimaana S, Mager DR. Observational cohort study of healthy community-dwelling older adults followed for 12-months to assess the impact of lifestyle on sarcopenic status. (submitted 2022).

CHAPTER 3

Assessing the impact of factors that influence the ketogenic response to varying doses of Medium Chain Triglyceride (MCT) oil.

Juby AG, Brocks DR, Jay DA, Davis CM, Mager DR. Assessing the Impact of Factors that Influence the Ketogenic Response to Varying Doses of Medium Chain Triglyceride (MCT) Oil. The Journal of Prevention of Alzheimer's Disease. 2021 Jan;8(1):19-28.

3.1 Abstract

Objectives, Design, Setting

The ketogenic effect of medium chain triglyceride (MCT) oil offers potential for Alzheimer's disease prevention and treatment. Limited literature suggests a linear B-hyroxybutyrate (BHB) response to increasing MCT doses. This pharmacokinetic study evaluates factors affecting BHB response from MCT supplementation in three participant groups.

Participants

Healthy adults without cognitive deficits <65years, similarly healthy adults ≥ 65years, and those with Alzheimer's Disease were assessed.

Intervention

Different doses (0g,14g, 28g, 42g) of MCT oil (99.3% C8:0) were administered, followed by fasting during the study period.

Measurements

BHB measured by finger prick sampling hourly for 5-hours after ingestion. Each participant attended four different days for each ascending dose. Data was also collected on body composition, BMI, waist/hip ratio, grip strength, gait speed, nutrient content of pre-study breakfast, and side effects.

Results

Twenty-five participants: eight healthy; average age of 44yr (25-61), nine healthy; 79yr (65-90), and eight with AD; 78.6yr (57-86) respectively. Compiled data showed the expected linear dose response relationship. No group differences, with baseline corrected area under the blood vs. time curve (r^2=0.98) and maximum concentrations (r^2=0.97). However, there was notable individual variability in maximum BHB response (42g dose: 0.4 -2.1mM), and time to reach maximum BHB response both, within and between individuals. Variability was unrelated to age, sex, sarcopenic or AD status. Visceral fat, BMI, waist/hip ratio and pretest meal CHO and protein content were each associated with the BHB response ($p<0.001$).

Conclusion

There was a large inter-individual variability, with phenotype effects identified. This highlights challenges in interpreting clinical responses to MCT intake.

3.2 Introduction

Nutritionally induced ketosis (either by diet and/or MCT oil or esters) is being increasingly studied for the prevention and treatment of AD. The rationale is based on research that clearly identifies cerebral glucose hypometabolism in pre-symptomatic and symptomatic AD. This impaired metabolism and blood flow has been shown to be correctable with supplying the brain with ketones-through direct infusion [Hasselbalch et al, 1996] or via a ketogenic agent [Torosyan et al, 2018]. This knowledge creates the opportunity for avenues of investigation for new approaches in the prevention and management of AD [Cunnane et al, 2016; Vandenbergh et al, 2020]. Extensive discussion of this topic is beyond the scope of this study, but is covered in a recent review [Chatterjee et al, 2020].

Ketones (acetone, acetoacetate and betahydroxybutyrate (BHB)) are produced endogenously under conditions of reduced glucose availability, primarily from Beta-oxidation of fatty acids [McPherson et al, 2012]. Therapeutic levels of BHB (for treatment of medical conditions such as epilepsy and obesity) require a very low calorie, or very low carbohydrate (CHO), ketogenic diet to be maintained long term. A very low-calorie ketogenic diet, requires strict medical supervision and is unsustainable in the long term because of compliance and biochemical side effects [Caprio et al, 2019]. A very low CHO, ketogenic, iso caloric diet is more sustainable

[Manninen et al, 2004]. Other methods of nutritional ketone generation such as MCT oil supplementation may serve as possible solutions, either alone or as an adjunct to ketogenic diets.

MCTs (commonly produced from coconut or palm oil), with a chain length of 6-10 carbons, are metabolized differently from other triglycerides (short and long chain), and result in ketone production, even in the face of adequate blood glucose. Gastric and pancreatic lipases hydrolyse MCT into medium chain fatty acids (MCFA) enabling rapid absorption from the gut, where the majority is transported in the portal vein to the liver, with rapid diffusion into hepatocytes [Ramirez et al, 2001; Marten et al, 2006]. MCFAs have a high propensity for oxidation, behaving more like glucose than fat in oxidative pathways [Marten et al, 2006]. The resultant increased acetyl-Co A in the liver leads to ketogenesis and ketone release into the circulation and therefore immediate energy production, without adipose tissue deposition [Bach et al, 1996]. Because ketones generated in the liver cannot be utilized by the liver for energy, all therefore flow from the liver to extra-hepatic tissues as fuel [Bach et al, 1996].

MCT in the form of oil or tablet esters, is increasingly being used to promote ketogenesis by members of the public. Its purported benefits are for weight loss, increased energy, and cognitive enhancement [Bach et al, 1996; Corchesne-Loyer et al, 2013; Hall et al, 2016; Taylor et al,2018; Henderson et al, 2009]. MCT intake however can be associated with gastrointestinal side effects which can decrease tolerability and the sustainability of their ketogenic effect [Dubois et al, 2007].

In previous studies evaluating ketogenesis for various clinical effects [Hall et al, 2016; Henderson et al, 2009], pooled BHB response has been measured with little comment on potential confounding factors such as age, BMI, or body composition.

This study seeks to further our understanding of factors affecting BHB response to MCT oil by evaluating the impact of: Age - by using three groups of adults of differing age; Dose – by giving each participant three incremental doses of MCT oil and measuring their individual serum BHB ketone response; Phenotype - by assessing the association of variables such as muscle mass and function, and body composition on BHB response; Tolerance – by detailed monitoring for side

effects at different doses for each individual; Disease state - by including healthy elderly and young, and a group with AD to provide comparative data these subgroups.
This information will enable more meaningful design and interpretation of future clinical trials in all areas of possible therapeutic ketone use (eg. seizures, weight management), but especially for the prevention and management of an increasing number of the population with AD for which there are currently only limited treatment options.

3.3 Methods

3.3.1 Participants

This is a single-centre, open-label, dose response study in three groups of adults: healthy adults under 65 years, healthy adults 65 years and older, and adults over 50 years of age with confirmed Alzheimer's disease. The study was carried out in Edmonton, Alberta, Canada.

Alzheimer's participants were included to assess their tolerance and BHB response to the MCT oil, to see if it differed from healthy adults of a similar age. They had a diagnosis of probable dementia of the Alzheimer's type (mild to moderate severity) based on the revised National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [Dubois et al, 2007] and Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [APA DSMIV, 2000] criteria, of at least 12-months duration.

Exclusion criteria included consumption of a ketogenic diet, prior use of MCT oil or esters (within 6-months), or a diagnosis of diabetes mellitus.

3.3.2 Study design and intervention

Each participant's individual response to varying doses of MCT (Bulletproof Brain Octane®) oil was measured. Participants attended for four different study days. Each day they arrived having eaten breakfast. Details of the participant's pre-study meal were recorded, and the nutritional components (CHO, protein, fat, fiber) and calories calculated at the study completion, using

Cronometer® software. In the case of AD participants, breakfast details were confirmed by their caregivers. Participants were asked to eat a similar pre-study breakfast on each study day, the nutritional content of which was documented. This was consumed 60-90 minutes prior to the study start.

All participants were given a 150ml standardized fruit drink (containing 17.4g carbohydrate, and 1.2g protein), the nutritional content of which was taken into account when evaluating the total baseline nutrients per participant. On study Day 1 the fruit drink consumed contained 0ml (0g) MCT oil, Day 2 it was mechanically stirred with 15ml (=14g) MCT oil, Day 3 with 30ml (=28g), and Day 4 with 45ml (=42g). The ascending dose direction was chosen for several reasons. Most notably, to minimize the length of time that is needed for washout. Another is the possibility that higher doses cause some sort of physiological change that would affect the measures with lower doses. Finally, ethically, because 42g doses have not been extensively studied when administered as a single dose, it was important to ensure the individual tolerability (especially GI side effects [Courchesne-Loyer et al, 2013]) of lower doses first.

The oil and fruit juice were mixed with a handheld battery-operated milk frother immediately prior to consumption, to improve miscibility of the MCT in the juice. The consumption of the MCT oil drink was directly supervised by the investigators and was consumed in a single drink of 150ml fruit juice and oil, in 1-3 swallows (0.5-1 minute). After their study drink, the participants were only allowed to consume water, and underwent finger-stick testing as discussed in detail in Section 3.3.3 to follow.

The study doses of 14g, 28g and 42g were chosen based on several published studies. One showed a ketogenic response with doses over 10g of their MCT product [Courchesne-Loyer et al, 2017]. Their dose maximum was 30g as their previous research has shown that a minimum daily dose of 30g MCT is required to generate adequate brain ketone uptake to theoretically replace, in part, the deficit in brain glucose uptake and/or use in AD [Courchesne-Loyer et al, 2013]. Another showed the value of higher doses resulting in a greater ketogenic effect, although there appeared to be a plateau effect between 75-100ml doses of their MCT product,

however they were measuring acetone and not BHB [Freund and Weinsier, 1966]. Therefore, also testing a dose above 30g for ketogenic response and tolerance was important.

Neither participants nor investigators were blinded to their dose as this was an efficacy and safety study examining incremental MCT dosing for tolerance. In addition, BHB response was assessed using incremental area under the curve, including correction for baseline BHB levels. All participants were occupied with sedentary activities (puzzles, board games, movies, reading, socializing) between their hourly testing for the study duration, on each study day.

Each participant was also evaluated for weight, estimated muscle mass (Omron® Full Body Sensor Body Composition and Monitor Scale, Omron® Healthcare Co, Ltd, China), BMI, waist/hip circumference. Although AD participants were included, cognitive function was not a study outcome given the short study design, but the information in these participants is applicable to longer AD studies where cognitive outcomes are more feasible [Juby et al, 2022].

3.3.3 Measurements

At baseline and hourly for 5-hours (h) thereafter, participants had measurements of blood BHB by finger-prick testing for blood glucose and BHB done using the standardized protocol for the FreeStyle Precision Neo Blood Glucose and Ketone Monitoring System (Manufactured by Abbott Diabetes Care, Canada) [Abbott Laboratories 2020]. The tests strips used were Precision Blood β-ketone and Precision Blood Glucose strips, and used in accordance with the manufacturer's instructions. The lower limit of quantification for BHB using this test is 0.1mM. Values were reported numerically. Values below 0.1mM were registered as 0.0mM on the device. These BHB ketostrips have been validated in previous studies for specificity, sensitivity, accuracy, and precision. [Guerci et al, 2003; Forrow et al, 2005] with good accuracy (r=0.97) compared to whole blood BHB levels, and precision (%CV) no more than 3.1-3.8%. Random participants had a repeat BHB analysis from different finger prick sites at the same time for test re-test validity. The glucometers were all checked for accuracy daily, using the manufacturer's standardized solutions, and met the standardization criteria.

As the test oil (Bulletproof Brain Octane®) is an over-the-counter product and not a pharmaceutical product, it was felt to be important for the validity of this study for the reported nutrient content of the MCT test oil to be independently verified in an unrelated research laboratory. All the study oil came from the same lot number (1901279039, expiration 01/22) as that tested in the independent laboratory. The manufacturer stated 15ml contains 14g of MCT, but does not specify the oil specific gravity.

Side effects were recorded as per standard Health Canada protocols and reporting. They were classified as mild, moderate, or severe, based on pre-specified criteria [Government of Canada NHP]. Side effects were actively solicited at each hour of testing, as well as being self-reported. Any symptoms were classified and recorded.

3.3.4 Ethics and Registration

This study was approved by the Health Products and Food Branch, of Health Canada, (HC6-24-c186660) and the local University of Alberta Health Research Ethics Board (Pro 000087958), with Clinical trial registration: ClinicalTrials.gov Identifier NCT04389983.

Procedures followed were also in accordance with the Helsinki Declaration of 1975 as revised in 1983. All participants (or their caregivers) had read an Information sheet (mailed prior to the first study visit). At the start of the study all signed a Consent Form if they were cognitively able to understand the study process. For those with cognitive impairment, an Assent Form was signed by the participant and their legal representative. All AD participants in this study were accompanied by their caregivers throughout the study duration. These documents were reviewed and approved by the local Research Ethics Board as well as Health Canada.

3.3.5 Statistical Analysis

1. Pharmacokinetic analysis

The BHB blood concentration versus time data after administration of MCT oil were analyzed using noncompartmental pharmacokinetic methods. The maximum blood concentrations (C_{max})

and the time to attainment of C_{max} (t_{max}) from each participant were obtained from inspection of the raw data. The area under the blood concentration vs. time curve for each participant, from the time of dosing to the last measured concentration, were calculated using the linear trapezoidal rule [Gagnon and Peterson, 1998] All values were corrected from baseline responses.

The baseline-corrected AUC (BC AUC) after MCT oil were determined by subtracting the $AUC_{0\text{-}5h}$ after the 0 g MCT dose (representing the endogenous BHB concentrations) from the $AUC_{0\text{-}5h}$ after the MCT oil. The baseline-corrected C_{max} (BC C_{max}) was also determined by subtracting from each sampling time measurement after MCT oil, the corresponding measure of BHB in the same patient after no MCT oil was administered. The time at which the BC C_{max} occurred (BC t_{max}) was also denoted.

This study was examining an endogenous compound (BHB). There is natural variability in the measure of a single sample due to a number of factors including error in the assay itself, and diurnal variation. Therefore, a repeated measures approach was not used as this could lead to significant errors due to variations in a single value at one time of the day. AUC baseline was used instead, as this takes into account all natural variability from multiple samples drawn over a period of time, and provides a much better estimate of baseline concentrations in the absence of the treatment. Multiple concentrations were measured to arrive at either a C_{max} or an AUC value.

2. Statistics

Power calculations showed a requirement for a sample size of 8-12 participants per group to allow adequate statistical precision to notice a difference of 0.1mM in BHB for a p value of 0.05 [Courchesne-Loyer, 2013; St-Pierre et al, 2019].

Continuous variables were described with mean values and standard deviations for variables demonstrating normal distributions and as medians (interquartile ranges) for variables demonstrating non-parametric distributions. The Shapiro Wilk test was used to determine normality for outcomes variables [Shapiro and Wilk, 1965]. For descriptive statistics, analysis of

variance with post-hoc t-tests (for parametric variables) were used to analyze differences in BMI, body composition, waist/hip ratio, gait speed and dietary macronutrients between participants, and between groups (with Bonferroni corrections) [Dunn, 1961]. Relationships between MCT dose and measures of systemic exposure (C_{max} and AUC) of the BHB were assessed by linear regression. To assess intra-participant increases in exposure between each dose, mean differences in C_{max} and AUC for each participant were determined and the 95% confidence intervals calculated and assessed. Statistical tests were two-tailed with significance set at $p<0.05$.

3.4 Results

Forty-three people expressed interest in participating in the study (9 in the young group (<65years), 17 in ≥ 65-year group, and 17 with Alzheimer dementia (AD). One was excluded in the young group (unable to make all the days), 8 in the ≥65-year group (other activities on research days) and 9 in the AD group (caregivers felt the study days were too long for them). See **Figure 1**.

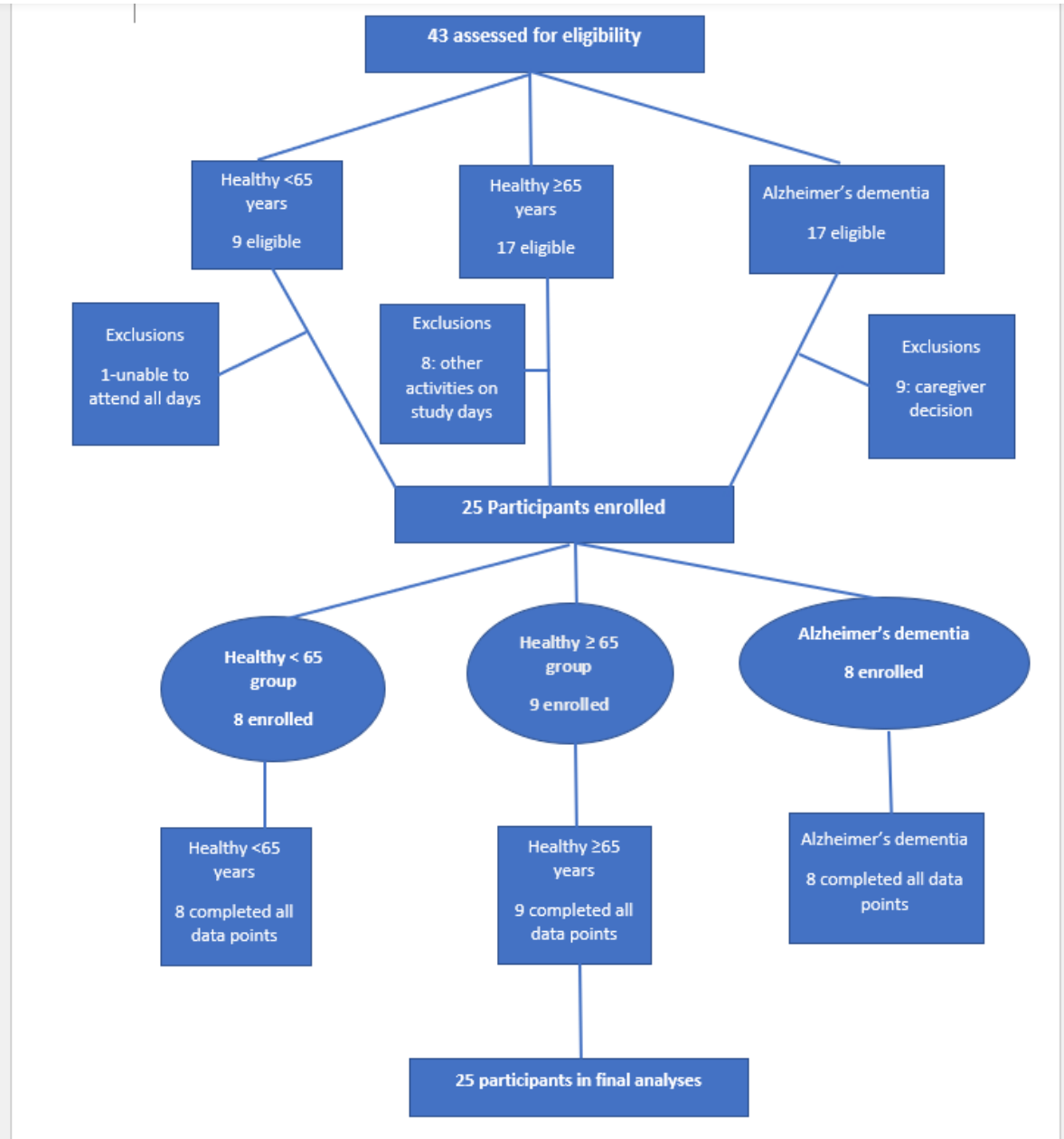

Figure 1: Participant screening and follow up

Twenty-five participants were enrolled, and all completed all 4 days and all 4 doses of the study drink. Eight (young group), nine (healthy ≥ 65-year group) and eight (AD) participants were in the three groups, with an average age of 44 years (25-61), 79 years (65-90) and 78.6 years (57-86) respectively. By chance, all the AD participants whose caregivers wanted them to participate were male. See **Table 1** for details.

Table 1: Study participant demographics.

Group	Healthy <65	Healthy ≥ 65	Alzheimer's Disease
Number	8	9	8
Female/Male	6/2	8/1	0/8
Age	49.5 [39-50][a]	80 [77-82][b]	74 [70-77][b]
BMI	27.4 [20-35]	23 [23-31]	26.4 [24.5-27.8]
Waist/hip ratio	0.81 [0.74-0.92]	0.92 [0.81-1.98]	0.98 [0.91-1.03]

Data is expressed as median [interquartile range].
Variables with different superscripts are statistically significant at p<0.015 (accounting for multiple comparisons).

Participants attended consecutive days for each increased dose. Research days were Monday, Thursday, and Friday, so based on the participant's availability the maximum time between study visits was 7 days, with an average of 2 days, and a minimum of 1 day.

Detailed nutritional analysis of reported pre-study breakfast each day confirmed that participants had the same breakfast, as requested. Daily breakfast calorie intake ranged from 166-1545 kcal (average 485.4kcal), 4.5-138 g carbohydrate (CHO) (average 49.4g), 14-56.7g protein (average 24.4g) and 2.0-46.1g fat (average 19.1g) There were no significant differences in energy and fat intake noted *within each participant* for their daily breakfast over the four days of the study. See **Table 2** for details.

Table 2: Nutrient content of pre-study breakfast meal.

Variable Name	Healthy < 65	Healthy ≥65	Alzheimer's dementia (AD)	p value
Kcal/d	**Median [interquartile range]**	**Median [interquartile range]**	**Median [interquartile range]**	
Energy (kcal)	433 [442-622]	433 [352-484]	514 [253-454]	0.73
CHO (g/d)	35.1 [36.8-55.4][a]	47.1 [38.8-59.1][a,b]	57.7 [43.4-62.7][b]	0.31
% CHO	37.3 [29.5-41.5]	40.2 [37.9-57.7]	39.5 [35.8-49.3]	0.21
Protein (g/d)	28.2 [24.5-36.6]	15.1 [15.3-22.8]	21.6 [19.4-29.3]	0.89
% Protein	21.4 [19.8-29.4]	15.3 [13.5-30.1]	16.2 [15.3-25.0]	0.32
Fat (g/d)	20.2 [14.9-27.9]	15.7 [13.3-18.9]	15.9 [14.9-25.3]	0.70
% Fat	32.1 [27.5-40.3]	35.2 [37.9-57.7]	34.4 [26.1-39.5]	0.79
Fiber g/d	13.6 [9.9-15.7]	5.3 [5.3-9.7]	11 [12.8-22.9]	0.23

***different superscripts denotes statistically significant difference between groups: [a]healthy <65 versus healthy ≥65; or [b]all healthy versus AD, at p<0.015 (multiple comparisons using Bonferroni correction).**
Note pairwise comparisons < and > 65 healthy[a], >65 healthy vs AD, < 65 yrs healthy vs AD. (Abbreviations: CHO, carbohydrate.)

CHO intake only differed between the two healthy groups, and between them and the AD participants. There was no difference in CHO intake at baseline between the healthy >65-year group and those with AD. Percentage CHO content of the breakfasts however were comparable. The standardized study fruit drink contained an extra 17.4g of CHO for each participant. There was no statistically significant difference between the under and over 65-year healthy groups, and the healthy groups compared to the AD group, for breakfast protein, fat and fiber intake. The F test of homogeneity did not show any differences for fat (p=0.91), protein (p=0.99) or CHO (p=0.92) showing there was no significant intrasubject variability between intake for any of the three macronutrients (absolute amount).

The MCT test oil (Bulletproof Brain Octane®) was verified as 99.3% C8, 0.6% C10 and 0.1% C12:0 by an independent lipid research laboratory (Dr Vera Mazurak's Lipid Research Laboratory, University of Alberta).

Baseline BHB levels were in the range expected for non-fasting adults on a non-ketogenic diet (0.0-0.3 mM). Individual responses in BHB level varied at each dose, and the pattern of individual response also varied between and within individuals for each dose. See **Figure 2**.

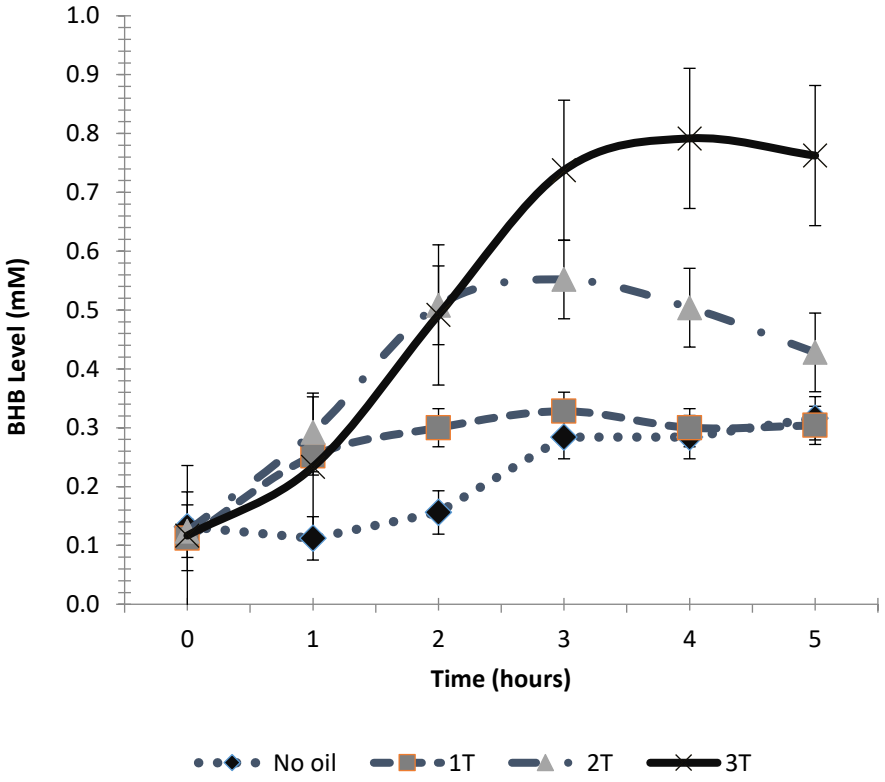

Figure 2: Mean±CI (confidence interval) in absolute β-hydroxybutyrate (BHB) levels (mM) with each MCT dose, for all participants (1T: one tablespoon (15ml, 14g); 2T: two tablespoons (30ml, 28g); 3T: three tablespoons (45ml, 42g)).

Only three participants were on statins (low dose): one healthy elderly; and two AD participants. They did not appear to have any blunting of response, with all three reaching peak BHB level of 0.8-1.1mM with the highest dose, which was within the average for the entire group.

Endogenously produced BHB was detected in all participants and a full AUC was calculable in all the participants. The median t_{max} of BHB without MCT oil was 3h after the sampling was started in the participants. As the dose of MCT oil was introduced and increased, there were associated increases in the mean measures of AUC and C_{max}. (**Table 3**). Considerable inter-participant variability was apparent in the measures of overall exposure in the absence of MCT oil; this variability generally dropped as the dose of MCT oil increased.

Table 3. Summary of pharmacokinetic exposure data (mean±SD). Observed and baseline-corrected (BC) values are shown. Variables are mean +/- standard deviation. (AUC: area under the blood concentration time curve; h hour; C_{max}: maximum blood concentration; t_{max}: time of C_{max}; BC: baseline corrected)

Dose, g	$AUC_{0\text{-}5h}$, mM×h	C_{max} mM	t_{max}, h	BC $AUC_{0\text{-}5h}$ mM×h	BC C_{max} mM	BC t_{max}, h
0	1.06±0.64	0.35±0.25	3	-	-	-
14	1.39±0.58	0.48±0.24	2	0.30±0.46	0.29±0.22	2
28	2.13±0.69	0.75±0.20	3	1.07±0.72	0.52±0.21	3
42	2.82±1.18	1.05±0.42	4	1.76±1.18	0.77±0.40	4

To ascertain how much the dose of MCT oil contributed to the BHB levels, baseline correction of the AUC and C_{max} were undertaken by subtracting the baseline (starting) values from concentrations (to obtain C_{max}) or baseline AUC from AUC after MCT oil. When this was done, increases in exposure with increases in MCT oil doses were still apparent, again with notable inter-participant variability. Despite the inter-individual variability, compiled pharmocokinetic evaluation of pooled data produced overall a highly linear response between the mean measures of C_{max} and $AUC_{0\text{-}5h}$ (raw and baseline-corrected) and the administered dose (See **Figure 3**).

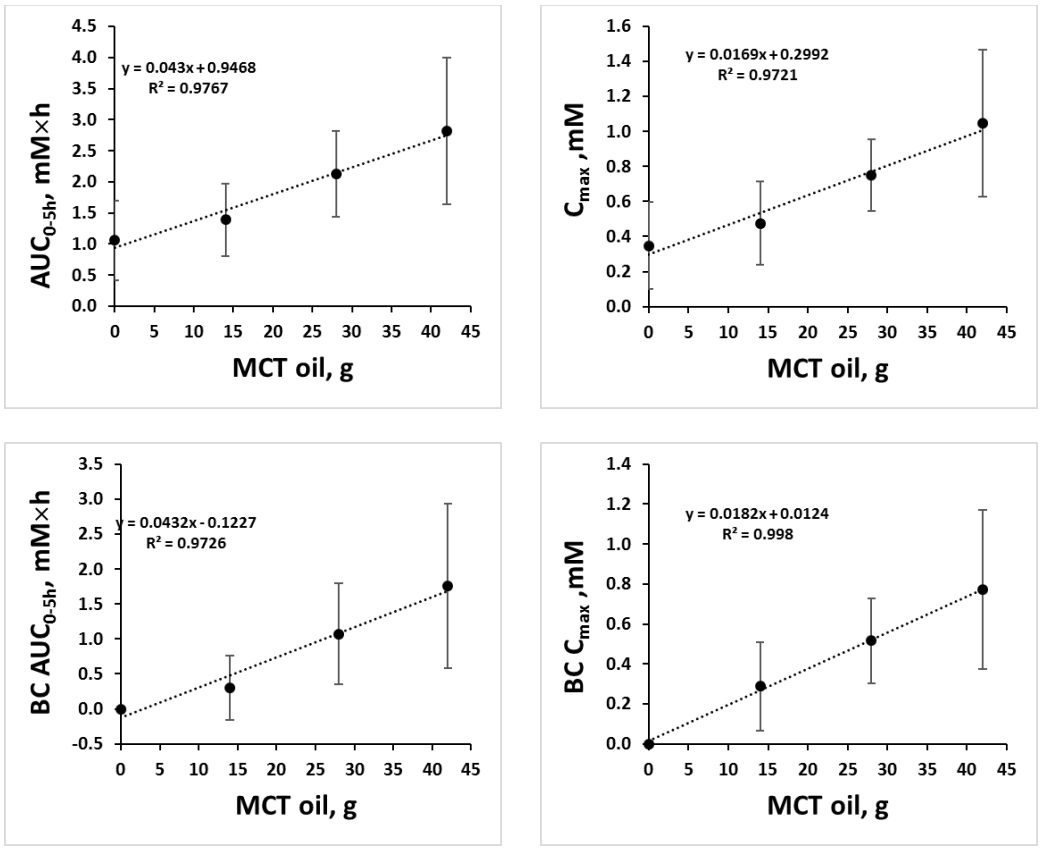

Figure 3: Mean±SD exposure data plotted versus the dose of MCT oil. (AUC: Area Under Curve; C_{max}: maximum B-Hydroxybutyrate response)

To better understand the nature of the increase exposure with increases in the dose of MCT oil, the mean intra-participant increase in concentrations between discreet doses was calculated along with the 95% confidence intervals of those differences (**Table 4**). For every dose increment it was apparent that there were significant increases in exposure in the participant. It was also apparent that between the first to third doses the increases in exposure were nearly the same, thus indicating overall dose proportionality within individuals.

Table 4: Mean intra-individual differences between doses in exposure error between doses with 95% confidence intervals shown in parentheses. All differences between pairs of the rising doses were significantly different at p<0.05. (AUC: area under the blood concentration time curve; h hour; C_{max}: maximum blood BHB)

Differences	$AUC_{0\text{-}5h}$ mM×h	C_{max} mM	BC $AUC_{0\text{-}5h}$ mM×h	BC C_{max} mM
14 g - 0 g	0.33 (0.14, 0.52)	0.13 (0.03, 0.23)	-	-
28 g - 14 g	0.74 (0.5, 0.99)	0.27 (0.19, 0.36)	0.74 (0.5, 0.99)	0.23 (0.12, 0.34)
42 g - 28 g	0.69 (0.31, 1.07)	0.30 (0.16, 0.44)	0.69 (0.31, 1.07)	0.26 (0.12, 0.39)

When assessing factors that may be responsible for this individual variation, the BHB level was found to be unrelated to: AD status; age; sex; and body weight. BIA estimated percentage muscle, did not appear to affect BHB responses. However, for BMI and body composition, increasing BMI, and increasing waist/hip circumference were all associated with decreased BHB serum levels 2-3h post MCT ingestion ($p<0.001$), and this was also seen in the AUC responses for all the doses.

Apolipoprotein E4 status was not evaluated as this data was only available for eight (32%) of participants, and only two of these had at least one APOE4 allele. Therefore, there was not enough power in this study to assess for any APOE4 effect.

Increasing CHO and protein in the pre-study breakfast meal was associated with lower levels of serum BHB at 2-5h ($p=0.002$) at all dosing levels. These effects were also apparent in the AUC response, at different MCT doses, at all time points ($p=0.008$). Although the study drink contained CHO, this was identical for all participants and did not affect the baseline BHB as that was measured immediately before the study drink was consumed. There was no apparent effect of total kcal, or fiber.

There were no serious adverse events. Four participants (16%) experienced “influenza-like” symptoms (headache, diaphoresis, fatigue) with either 28g or 42g MCT oil, or both, lasting approximately 2h. Blood glucose measurements taken at the time of these symptoms were in the normal range. None of these symptoms were reported by the participants to be severe enough for study discontinuation, and those experiencing adverse events at 28g had no concerns about

returning for the 42g test dose study day. Eighteen (72%) experienced some minor gastrointestinal discomfort, not necessarily related to the measured blood BHB level. Twenty-eight % of participants experienced no side effects. In all cases, all the symptoms resolved completely within minutes of eating the post study meal. See **Table 5.**

Table 5: Adverse events (AE) (Some participants reported several side effects). (g: gram, n: number of participants).

MCT Dose (g)	**0**	**14**	**28**	**42**
Total AE (n)	**0**	**3**	**13**	**13**
Abdo cramp/indigestion	0	1	5	7
Nausea	0	0	6	3
Burping/bloating	0	0	1	1
Diarrhea	0	0	1	1
Fatigue	0	1	0	2
Dizziness	0	0	1	2
Headache	0	1	3	3
Brain fog	0	0	2	1
Sweating/clamminess	0	0	2	1

3.5 Discussion

MCT oil led to therapeutic BHB levels in many of our participants. In healthy individuals BHB is generally less than 0.1-0.5mM. Under physiological low glucose conditions, ketones rarely reached over 3mM, but can reach 5-8mM in adults after prolonged (one week) fasting [Owen et al, 1967; Cahill and Veech, 2003]. Levels of 4-6mM stimulate increases in insulin secretion resulting in a reduced production and increased urinary excretion of ketones. Only in dysregulatory conditions such as diabetic ketoacidosis can levels reach 10-20mM [Cahill and Veech, 2003]. Nutritional ketosis with a ketogenic diet alone can be difficult to maintain, especially in adults with cognitive impairment. Even in a group of highly motivated parents of children with intractable seizures, 33% discontinued their ketogenic diet (4:1 fat:CHO and

protein) within 12-months because of side effects or lack of seizure benefit [Buchhalter et al, 2017]. However, they did reach BHB levels up to 5mM in some participants. In addition, ketogenic diets vary in their nutritional content. In one study in adults, of a ketogenic diet (1.8:1 fat: CHO and protein) alone (without additional MCT oil) their highest level of BHB was 0.7 +/- 0.62mM [Urbain and Bertz, 2016]. To address this, researchers have combined ketogenic diets with very low calories [Caprio et al, 2019] or low carbohydrate intake, or have added MCT oil to a ketogenic diet. [Corchesne-Loyer et al, 2013; Urbain and Bertz, 2016; Volek et al, 2019; Hallberg et al, 2019; Phinney et al, 1983]. With these interventions, BHB levels ranged from >0.7mM to >2.0mM. In this current study, with the highest MCT dose (42g), the highest individual level was 2.1mM.

Assuming non-saturable absorption mechanisms, a linear relationship may be expected between dose and blood measures for MCT, as shown by Courchesne-Loyer and colleagues. They performed a dose-response study, and found the BHB response of C8:0 MCT was more linear than C10:0. They did not comment on any individual variability in their 10 healthy study participants, or their BMI [Courchesne-Loyer et al, 2017]. Although all the participants in the previously mentioned study were fasting at baseline, with only the study agent and a standard breakfast (2 slices of toast and jelly) at the start of the study, we observed a similar linear pattern for varying doses in the current study, despite participants consuming a different breakfast from one-another (see Figure 3). In addition, the MCT oil in this study was a predominantly C8 MCT oil.

Although there was this linear dose response in our compiled data (shown in Tables 3 and 4, and Figure 3), the marked individual variability in pattern of response, degree of response, and side effects was somewhat unexpected. Not only did individuals have a unique pattern of BHB response to the MCT dose, this pattern was not necessarily the same with subsequent doses. Some participants showed evidence of multiple peaks in their BHB blood concentration versus time profiles. This may have been due to factors such as variability in absorption secondary to gastrointestinal mixing, or presence of food components, or the delay attributed to the lymphatic pathway of absorption [Brocks and Davies, 2018]. This information has important clinical

relevance. In the "real" world people do not have standardized meals, so knowing how a potential therapeutic agent behaves in this setting is of value.

3.5.1 Covariate analysis

A covariate analysis was performed to possibly predict a "good" BHB responder from an "average" or "non" responder. Looking at the influence of the pre-study breakfast, there seemed to be an effect of the baseline CHO and protein content of the meal, with higher levels reducing the BHB response. In addition, the significant association of BMI and waist/hip circumference with reduced BHB response, suggests that insulin sensitivity may play a role in the response of an individual to MCT-induced nutritional ketosis. Fasting insulin was not measured, but visceral adiposity (as suggested by increased waist/hip circumference in this study) is associated with an increased risk of metabolic syndrome, one component of which is insulin resistance [Griffith et al, 2010].

There was no impact of age or cognitive health observed here. AD participants were included because of our ongoing interest in the role of MCT oil as a source of nutritional ketones for influencing cognitive function. AD is associated with cerebral insulin resistance and perhaps also peripheral insulin resistance [Willette et al, 2015]. Ongoing research is looking at MCT induced nutritional ketosis as a possible cognitive enhancer in AD participants [McPherson and McEneny, 2012; Taylor et al, 2018; Henderson et al, 2009], so it is an important group in whom to assess their tolerance and response to the ketogenic effects of MCT. There was no statistical signal suggesting the AD group responded any differently to the healthy groups with respect to BHB response or tolerance. Cognitive change after the MCT administration was not assessed, as that was not an objective in this study. Other authors have suggested cognitive benefits with MCT oil or with ketone monoesters [Ramirez et al, 2001; Marten et al 2006]. Discussion of this data is beyond the scope of this pharmacokinetic paper, but covered in several recent reviews [Cunnane et al, 2016; McDonald and Cervenka, 2018].

3.5.2 Pharmacokinetics

We are aware of only two other published specific pharmacokinetic studies, and both evaluate *ketone esters* rather than MCT oil as a source of nutritional ketosis. One study, by Clarke and colleagues, involving a synthetic ketone monoester, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate, was performed in 54 healthy adults between 18-45 years [Clarke et al, 2012]. In 36 participants the kinetics of this monoester were evaluated after administration of doses of 0.42, 1.07, and 2.14g/kg body weight for 5 consecutive days. Additionally, 18 participants were evaluated in a single dose (0.14, 0.357, or 0.714g/kg) pharmacokinetic analysis, where each participant only had one test dose. Their participants, unlike in this study, were fasting at baseline. For their three doses, the C_{max} of BHB was 0.28, 1.00 and 3.30mM respectively. The t_{max} ranged from 1.5 to 2.5h. They did not comment on individual BHB responses with any of the doses, only the pooled response, nor was there mention of baseline-correction to account for endogenous BHB production. They assessed BMI (but not body composition or waist/hip ratio) but did not comment on its relationship to BHB. The use of a synthetic ketone may also have produced different pharmacokinetics than MCT oil.

In the second study, Shivva and colleagues used the same ketone monoester in their subsequent single dose study with five different doses in 37 young healthy volunteers, followed for 5-7h post dose [Shivva et al, 2016]. Only total percentage fat mass was reported, and the same method of BHB measurement was used as in the current study. Their objective was to develop a pharmacokinetic prediction model, including accounting for endogenous ketone production and the other nutrients in their study drink. They found that lean body weight and sex affected the BHB response, contrary to the present study. In their conclusion they stated the "pharmacokinetics of BHB is complicated" and encouraged more research.

Although these agents differ from the ketogenic agent used in this study, it does provide some comparable data on C_{max} and some information on factors affecting the dose response. In addition, investigators in this field are evaluating ketone esters [Henderson et al, 2009] as possible therapies for AD given that their tablet/powdered form is more convenient than liquid MCT. However, this ketogenic tablet has a bitter taste that may limit adherence [Soto-Mota et al, 2020].

3.5.3 Ketogenic response

The first dose-response study following MCT ingestion was reported by Freund and Weinsier, who measured acetone concentrations in expiratory air following different dose of MCT, which rapidly fell with sucrose administration [Freund and Weinsier, 1966]. Interestingly, they also included participants with insulin-dependent diabetes (three), showing an acetone response 2.5 times greater than the nondiabetic subjects (four). Their test dose was 25ml of emulsified MCT (74.7% C8:0) administered after a 12-hour fast, increased in one subject to 100ml. GI side-effects occurred in 3/7 subjects. Acetone concentrations decreased progressively, proportional to ingestion of increasing doses of sucrose. The anti-ketotic effect of carbohydrates could be due to increased ketolysis or decreased ketogenesis or both. Based on this study they concluded there is a *dynamic equilibrium* between hepatic production and peripheral utilization of ketones [Freund and Weinsier, 1966].

The present study also found that a higher CHO intake breakfast prior to the study start diminished the ketone response, in this case the ketone being BHB and not acetone. The CHO intake between participants for their baseline breakfast meal varied from 166-1545 kcal (average 485.4kcal), and 4.5-138 g CHO (average 49.4g) However, the CHO did not vary between study days for individuals. The biggest variability between baseline CHO intake was between the young healthy group and the two older adult groups. There was no difference between the adults ≥65 and those with AD (see Table 2). This CHO difference may have influenced the maximum BHB level obtained but this impact most likely would have been consistent across the study days for each study participant.

In a feasibility study for a ketogenic diet in AD, Taylor et al [Taylor et al, 2018] administered 1.5-3 tablespoons of MCT oil (17.5-42g) daily with food to their participant's, as well as asking them to consume a ketogenic diet. Only 60-80% of participants were able to consume their targeted MCT dose because of gastrointestinal side effects, and there was an increase in average monthly BHB from baseline. Although AUC was unreported, there was significant individual variation in BHB levels (0-1.6 mM) reached at the maximum time point, which occurred in the first month of their study. With the significant individual variation in MCT dose taken, this variable response is hard to interpret. Their study was daily MCT over three months making

compliance more challenging. This differs from the present study design where compliance was 100% because it was only a single dose on each day, meaning participants were able to consume exactly the same dose as one another, making between group and individual comparisons possible. Nonetheless, the current study also found BHB response to vary considerably between individuals.

Freemantle et al [Freemantle et al, 2009] examined three age groups of healthy adults, provided with a low CHO (3g), ketogenic (110g fat) diet with MCT oil. They measured serum BHB hourly, and breath acetone and plasma insulin over 6h. BHB levels rose from 0.1mM to 1.3mM with peak BHB response at 2-4h post dose. Plasma insulin peaked at 1-2h with no reported difference between the groups. The insulin level was not correlated with individual BHB response, but there were higher plasma glucose (but not BHB or insulin) and lipids in the elderly group compared to younger participants.

Vandenberghe and colleagues compared different combinations of MCT oils (coconut, tricaprylin (C8), and tricaprin (C10)) to assess the ketogenic potential [Vandenberghe et al, 2017]. Their tricaprylin product (95%C8) most closely resembles the MCT oil used in the current study. They looked at total ketones (BHB plus acetoacetate) making the actual levels achieved difficult to compare. However, they did find their C8 oil significantly increased plasma ketones by 2.88 ± 1.9 mM above baseline when given with a meal, and increased it further when an additional dose was given on an empty stomach. It was the most ketogenic of the oils in their study. Unfortunately, they did not discuss side effects in their test participants.

Different MCT formulations, including an emulsified formulation, have been investigated in young healthy (mean age 31-years) to examine their influence on BHB response [Courchesne-Loyer et al, 2017]. Their MCT was a mix of 60% C8:0 and 40% C10:0, rather than the 99.3% C8:0 oil in this study. Their participants were fasting at baseline, and then consumed a standardized breakfast with their test drink. They were followed for 4h post dose, as opposed to the 5h follow-up in this study. Their data showed emulsification of the MCT drink increased BHB response, with less diarrhea, and a stronger correlation with C8:0, (but not C10:0) serum

levels, and ketogenic response. They did not mention body composition or BMI in their participants [Courchesne-Loyer et al, 2017]

Adding exercise to MCT is another strategy to increase the ketogenic response [Vandenberghe et al, 2019]. In a five-day study the authors evaluated 15g twice daily of their MCT oil (55%C8 35% C10) and 30-mins daily of aerobic exercise. This combination resulted in 69% higher plasma ketone levels (and AUC nearly doubled) in their normoglycemic participants, than either intervention alone. The absolute level achieved cannot be compared to this study as theirs was for total ketones. Unfortunately, there was no report on adverse events.

3.5.4 Side effects

Side effects (nausea, vomiting, bloating, abdominal cramps, diarrhea) with MCT oil ingestion are well documented [Marten et al, 2006], and limit the amount of MCT oil that can be ingested at any one time, usually to 25-30g [Marten et al, 2006]. Somewhat surprising, in this study there was no clear relationship between gastrointestinal side effects and dose. For participants reporting abdominal pain, headache, and diaphoresis at the 28g dose, they did not necessarily experience them (or any side effects) with the higher dose, even in the face of a higher serum BHB. Furthermore, some participants with higher serum BHB than those experiencing symptoms, were asymptomatic. There appeared to be a lack of correlation between the level of BHB and report of side effects, both within and between individuals. In this study, the overall tolerance with the 42g dose was greater than anticipated from the literature [Marten et al,2006]. The gastrointestinal side effects were expected. The brain fog, dizziness, headaches and diaphoresis reported by some participants was somewhat unexpected. Blood glucose assessment done at the time of the symptoms showed that these were not related to low or high blood glucose. Courchesne and colleagues only reported it in one of their participants, at the un-emulsified 20g dose [Courchesne-Loyer et al, 2017]. Ketones have been reported to increase cerebral blood flow equally in healthy and AD participants [Hasselbalch et al, 1996; Torosyan et al, 2018] but unfortunately neither of these studies reported data on adverse events in their participants. APO E4 negative participants had higher cerebral blood flow. In this study there was no APO E4 data on all the participants. The cognitive side effects were reported by non-AD

participants, except for one AD participant, who interestingly was known to be APO E4 negative.

Keto-adaptation [Corchesne-Loyer et al, 2013; Phinney et al, 1983] is unlikely to be the cause of the inconsistent side-effects between doses, given the single dose and short duration design of this study. Tolerance has been shown to occur with gradual dose titration, so most authors (us included) design longer studies to include a titration period [Juby et al, 2020; Courchesne-Loyer et al, 2013]. Of most interest for investigators, is that the clinical ketosis symptoms/side effects did not seem to be a good guide of the participant's current BHB level.

3.6 New data

This study differs from these previously published studies in that it collected an AUC of exposure that included an AUC after a 0g dose, which allowed accurate correction for baseline AUC, as opposed to just relying on a zero time point, resulting in more robust AUC estimates.

In addition, other studies have not reported BMI or waist/hip ratio, which this study suggests are important factors affecting the BHB response to MCT oil supplementation. There may therefore need to be consideration of higher doses of MCT in studies involving participants with elevated BMI or increased waist/hip ratio, in order to elicit the desired BHB level.

The individual response variability identified highlights the difficulty in interpreting pooled data, and subsequently extrapolating it to individuals with the assumption of achieving the same response. The apparent lack of association between absolute BHB level and side effects, and the varied individual tolerance shown, differs from other published studies which have implied more side-effects with higher MCT doses and/or BHB levels [Courchesne-Loyer et al, 2017].

3.7 Limitations

Although the sample size appears small, a priori testing illustrated that a minimum of eight participants per group was sufficient to detect differences in the primary outcomes.

All participants were Caucasian Canadians, possibly limiting generalizable to other demographics. All the AD participants were male, but as no overall sex differences in BHB responses were identified, that may not negate the applicability of the data to female AD participants. In addition, Courchesne-Loyer and colleagues found no sex differences in their study [Courchesne-Loyer et al. 2013]. Participants were un-blinded to their MCT dose, although this is unlikely to have influenced blood concentration measures, and did not appear to influence reported adverse events. The only disease state included was AD (without DM), preventing safety assessment in DM.

Although study duration was only 5h, the majority of participants had a peak in BHB response around 3h, suggesting that a longer duration was not critical. Extending the evaluation duration could have been complicated by the production of fasting-related endogenous ketones (a response which we did start to see, even at hour 5, in some participants). Research suggesting 8-hour follow-up was based on a two 10g dose protocol [Vandenberghe et al, 2020]. Our study design prevented comment on longer duration MCT intake with respect to tolerance, and modified BHB response. We did not measure acetoacetate levels. We acknowledge that the ratio of acetoacetate and BHB can vary with different MCT products and is affected by additional oils such as coconut [Vandenberghe et al, 2017]. But, in the case of >95% pure C8 oil (as in this study) these authors showed that the acetoacetate/BHB ratio was similar to their control oil. Urbain and colleagues showed the course of blood and urine ketones to be very similar [Urbain and Bertz, 2016] so we feel confident that the measured serum BHB level alone is a reflection of total body ketone production, and pattern of ketone response, but acknowledge that the actual BHB level may under-reflect the total body ketone level. We did not measure fasting insulin or HOMA IR in this study, but in the study by Courchesne-Loyer and colleagues they showed no change in plasma glucose or insulin in their population [Courchesne-Loyer et al, 2017].

The goal of the study was not to assess adverse events specifically based on dose, but adverse events in general. We do acknowledge that the serial dosing may have masked some adverse events, but the serial dosing was necessary as previous studies have suggested that single doses over 15g may be more difficult to tolerate [Courchesne-Loyer et al, 2013]. Ethically, we did not want to expose participants to the 42g dose if they had significant side effects at the 28g dose. In

addition, we know from our previous experience, that symptoms resolve immediately with CHO consumption. This has also been shown in studies evaluating timing of MCT around meals [Vandenberghe et al, 2020]. As previously mentioned, it is unlikely that the serial dosing led to development of tolerance as this has been shown to take longer to occur, and with multiple daily doses [Courchesne-Loyer et al, 2013]. The lack of washout between doses has already been discussed, and is felt unlikely to have influenced this study given the AUC baseline correction design.

3.8 Strengths

All participants completed all doses of the study. There was a large age range (from 25-90 years) and AD participants were included. Parameters of body composition were evaluated, and these were ultimately found to be of importance in the BHB response. As discussed, other kinetics studies have not included or analyzed these variables. In addition, there was correction for baseline BHB production ensuring that the measured BHB response was due to the consumed MCT oil.

3.9 Conclusions

This pharmacokinetic study with supplementary MCT oil looks at individual and group responses. It shows the expected linear dose-response in ketone concentrations in the pooled data, which is not impacted by age, sex, fat free or total fat mass, or AD status. However, it shows there is a marked individual response in the BHB level achieved with varying MCT doses, which is influenced by BMI and waist/hip ratio. Individual tolerance to elevated BHB levels is variable and, unexpectedly, was not dependent on the absolute BHB level. Future studies, assessing response in clinical outcomes to defined MCT doses (for example, AD and cognition) will need to take these important variables into consideration, in order to accurately design the studies and interpret the results.

CHAPTER 4

Use of medium chain triglyceride (MCT) oil in subjects with Alzheimer's disease: A randomized, double-blind, placebo-controlled, crossover study, with an open-label extension

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ClinicalTrials.gov Identifier NCT04396015.

4.1 Abstract

Introduction

Alzheimer's dementia (AD) is the most common form of dementia, with approximately 6.5 million cases in the USA currently. Cerebral glucose and insulin action is impaired in AD, andketones have been shown to provide alternative cerebral energy. Medium chain triglyceride (MCT) oil is a nutritional source of ketones, and has been shown to have some benefit in short-term trials, particularly in mild cognitive impairment. MCT for established AD has been less well studied. The purpose of this study was to examine whether MCT supplementation provides any benefit on cognition in those participants with probable mild-moderate AD?

Methods

This is a 6-month randomized, double-blind, placebo-controlled, crossover study, with a 6-month open-label extension in probable mild-moderate AD participants, on stable medications. The MCT dose was 42 g/day, or the maximum tolerated. Cognition was assessed with Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and the Cognigram®. Total study duration was 15-months to allow for a 1-month oil titration (from 14-42g) at

baseline, at the crossover (month 4 of the study), and crossover completion/start of the open label phase (month 8 of the study).

Results

There were twenty participants enrolled, with an average age of 72.6 years, and 70% had a university level of education. 45% were women. Their baseline MMSE score was 22.6/30 (10–29); MoCA was 15.6/30 (4–27); baseline Cognigram® Part 1: 65–106, and Part 2: 48–107. Ten were randomised into each arm of the crossover phases. The MCT oil used was 99.3% C8, and the average MCT oil consumption was 2 tablespoons/day (28g). Higher doses were limited by side-effects or compliance. There was no statistically significant difference between placebo and MCT for the crossover phase of the study. However, from start to end of the study, there was stabilisation or improvement of MMSE in 80% or participants. Longer continuous MCT exposure and age > 73, were associated with a higher final Cognigram® 1 ($p < .001$) score.

Discussion

This is the longest duration MCT AD study to date. Eighty percent of participants had stabilization or improvement as measured by the MMSE. An improvement in Cognigram®1 scores were seen in those with 9-months continual MCT oil (i.e. those whose started with placebo oil). Further trials are required with strategies to increase amount of MCT consumed, and look at more homogenous participants to reduce the confounders of age, and educational level.

4.2 Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting one in four people over the age of 80. Worldwide prevalence is estimated at 50 million cases [Alzheimer's Society, 2019] with cases expected to double every 20 years [Prince et al, 2013]. The number of cases in the USA alone is currently estimated at 6.5 million, and projected to rise to 13.8 million in 2060 without a medical breakthrough to prevent, slow or cure the disease [Alzheimer's Association,

2022]. Current pharmacotherapy provides symptomatic relief at best, and does not affect the disease trajectory [Eleti, 2016]. No new pharmaceutical agents have reached the Canadian market since 1997. In 2020, Cummings and colleagues identified 121 agents as being in clinical trials for AD treatment, with 65 in Phase 3 trials [Cummings et al, 2020]. Only three of these agents are nutritional: gingko biloba; icosapent ethyl (a purified form of omega-3 fatty acid EPA); and tricaprilin (12.5g caprylic triglyceride as a powder).

The brain preferentially uses glucose as it's fuel, using 120 to 130 g/day of glucose [Owen et al, 1967]. At rest, it uses 16% of the body's total O_2 consumption, despite representing only 2.0% to 2.3% of adult body weight. In conditions of low carbohydrate intake or fasting, the body uses ketones (acetoacetate and beta hydroxybutyrate [BHB]) as an alternative energy source to glucose. Ketones are normally generated in fasting states from beta-oxidation of adipose stores to maintain cerebral function. In long-term fasting, ketones can supply > 60% of the brain's energy requirements [Owen et al, 1967; Drenick et al, 1972], and are preferentially taken up by the brain over glucose [Hasselbach et al, 1996; Corchesne-Loyer 2017]. This occurs in cognitively normal younger and older adults, as well as in those with mild cognitive impairment (MCI) and AD [Nugent et al, 2011; Croteau et al, 2018].

Ketones can also be induced with a very low carbohydrate high fat (VLCHF) diet [Taylor et al, 2018]. Medium chain triglyceride (MCT) oil has the potential to produce a nutritional source of ketones for an alternative brain fuel to glucose [Fernando et al, 2015; Lei et al, 2016; Newport et al, 2015; Taylor et al, 2018; Rebello et al, 2015; Reger et al, 2004], or by the consumption of MCT oil or esterases in freeze-dried form [Hendersen et al, 2009]. Hendersen and colleagues found their MCT formulation (AC-1202) was able to elevate serum ketones, even in the presence of dietary carbohydrates, and they didn't control for diet in their study [Hendersen et al, 2009]. Long-term compliance with fasting or VLCHF and LCHF diet regimes is challenging and requires strict medical supervision [Caprio et al, 2019]. Hence, the potential advantage of nutritional ketone sources (MCT) over these restrictive diets. In addition, dietary preferences change in AD with a predilection for sweet foods [Mungas et al,1990; Keene et al, 1997], adding another challenge to low carbohydrate diets. Our recent study showed a clear dose-dependent effect on ketone (BHB) generation with varying doses of MCT supplementation, and was found to be equivalent in young, elderly, and AD participants [Juby et al, 2021].

In AD, the brain is unable to use glucose normally [Hoyer et al, 1992; Cunnane et al, 2011], causing hypofunction of 20% to 40% in key areas of the brain responsible for the symptoms in AD. This hypometabolism can be demonstrated with fluorodeoxyglucose positron emission tomography scanning [Mosconi et al, 2007] even in preclinical AD. It results from several factors: an abnormality of cerebral glucose receptors [Laybaert et al, 2007], cerebral insulin resistance [Cholerton et al, 2013], and abnormal cerebral glucose metabolism [An et al, 2018]. Hence, MCT oil may provide benefit as a source of readily available alternative energy, in the form of ketones.

MCTs are commonly produced from coconut or palm oil, and are a saturated oil source. They have a chain length of 6-10 carbons, are metabolized differently from other triglycerides (short and long chain), and result in ketone production, even in the face of adequate blood glucose [Marten et al, 2006]. Gastric and pancreatic lipases hydrolyse MCT into medium chain fatty acids (MCFA) enabling rapid absorption from the gut, where the majority is transported in the portal vein to the liver, with rapid diffusion into hepatocytes [Ramirez et al, 2001; Marten et al, 2006]. MCFAs have a high propensity for oxidation, behaving more like glucose than fat in oxidative pathways [Marten et al, 2006]. The resultant increased acetyl-Co A in the liver leads to ketogenesis and ketone release into the circulation, and therefore immediate energy production, without adipose tissue deposition [Bach et al, 1996]. Because ketones generated in the liver cannot be used by the liver for energy, all therefore flow from the liver to extra-hepatic tissues as fuel [Bach et al, 1996]. Given their origin from saturated fat sources (palm and coconut oil), there is potential concern for effects on the lipid profile or body composition in adults consuming the oil regularly [Cater et al, 1997].

In humans, ketone infusions have been shown to reduce hormonal responses to acute hypoglycemia and improve cognitive functioning [Veneman et al, 1994]. They also increase cerebral blood flow [Croteau et al, 2018]. This has had a clinical benefit in conditions such as intractable epilepsy [Vining et al, 1998]. However, the data in cognitive impairment is mixed [Newport et al, 2015; Rebello et al, 2015; Hendersen et al, 2009; Reger et al, 2004; Veech, 2000; Krikorian et al, 2012; Lange et al, 2017; Fortier et al, 2019], continuing the speculation about their potential role in aging and AD [Cunnane et al, 2016]. The few clinical studies that have

been done are only in participants with MCI or early AD, and are of short duration—a few weeks or months.

The purpose of this study was to use MCT oil supplementation to address some of the gaps in the current literature, including its effect on cognition in participants with mild to moderate AD, the effect of longer duration (> 6 months), and the tolerance and safety (weight, lipid profile) with longer duration therapy. To this end a placebo-controlled trial of at least 6-months duration is needed.

Based on previous studies suggesting improved cognitive function with provision of nutritional ketones for MCT consumption in earlier stages of AD, we hypothesized that intermittent elevation of serum BHB, provided by regular ingestion of MCT oil, would result in stabilization or improvement of cognitive function in participants with mild–moderate AD, without significant cardiovascular safety concerns.

4.3 Methods

4.3.1 *Design*

This study is a randomized, double-blind, placebo-controlled, convenience sample, crossover study, with an open-label MCT arm extension, in participants with probable AD (see **Figure 1**). ClinicalTrials.gov Identifier NCT04396015. Each crossover arm includes a 1-month dose titration followed by 3-months of therapy. The open-label phase includes a 1-month titration then 6-months of therapy, for a total study duration of 15-months. It was carried out at a single tertiary care hospital outpatient site in Edmonton, Alberta, Canada, with one principal investigator (PI) and one research nurse.

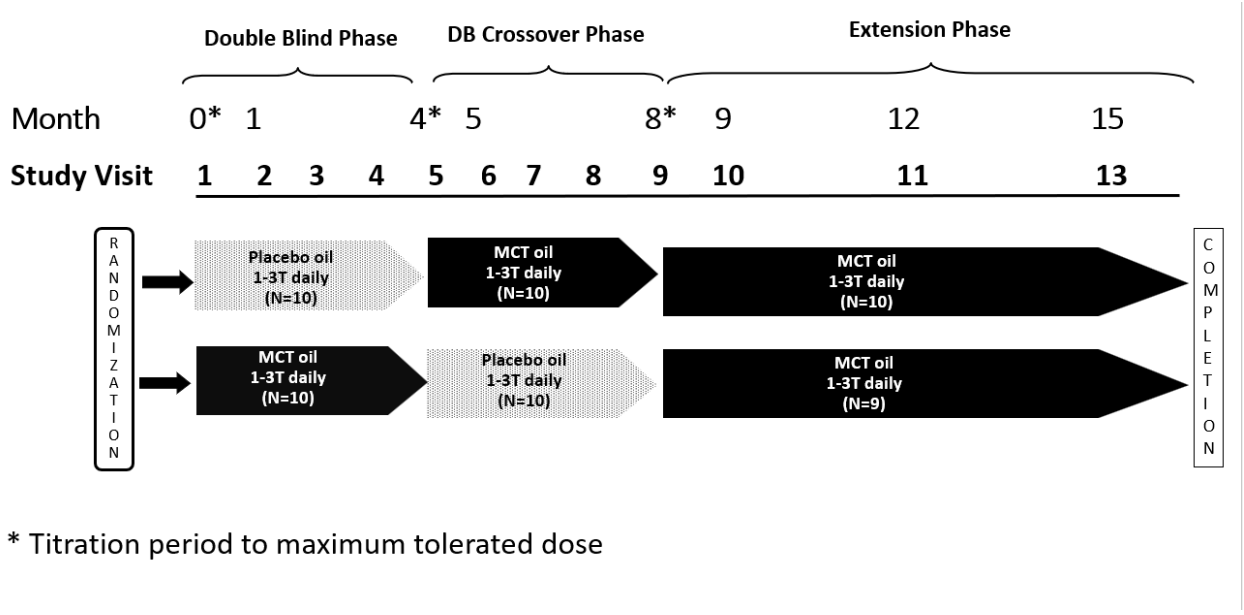


Figure 1: Study design

There is no washout between phases. The effects of one dose on BHB levels lasts less than 6 hours after consumption (as shown in the study discussed in Chapter 3) negating the need for a washout period between the cross-over phases. Hence also the need for repeated oil titrations in each phase of the study to improve the maximum tolerated dose.
As the effect size was unknown, a convenience sample of 10 participants per group was chosen, with the cross-over design also serving to increase power with a smaller sample size.

4.3.2 *Ethics*

This study was approved by the Health Products and Food Branch of Health Canada (HC6-24-C186660) and the local University of Alberta Health Research Ethics Board (Pro 000054165). Procedures followed were also in accordance with the Declaration of Helsinki Declaration as revised in 1983. All participants provided informed consent or assent.

4.3.3 *Participants*

Participants were community dwelling, with a diagnosis of probable dementia of the Alzheimer's type (mild to moderate), using standard criteria. AD diagnosis was based on the revised National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [Dubois et al, 2007] and Diagnostic and

Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [American Psychiatric Association, DSM-IV, 2020] criteria, of at least 12-months duration.

Recruitment was by way of recruitment posters (approved by the local HREB) in the waiting rooms and in the offices of geriatricians and neurologists in the Edmonton area, and at the Edmonton Alzheimer's Society. This minimized any potential selection bias related to recruitment.

Participants could continue AD medications (acetyl cholinesterase inhibitors [AChEI] and/or memantine), antidepressants, and antipsychotics as long as the doses were stable for at least 3-months prior to enrollment, and were required to remain stable for the duration of the study. All comorbidities needed to be stable. A baseline Mini-Mental Status Examination (MMSE) [Folstein and Folstein, 1975] score between 10 and 29 was required. Participants had to have at least one consistent caregiver who lived with them, was able to give a good clinical history about the participants functional and cognitive status, would be willing to dispense and supervise the test oil consumption, complete the oil consumption diary, monitor for side-effects, bring the participants to all the study visits, take them for laboratory and imaging tests as required, and be able to answer the questionnaires at each visit.. Given the unknown effect size, a convenience sample of 20 participants was enrolled.

Exclusion criteria included age < 50 years; non-English speaking; allergy to coconut or MCT oil; swallowing issues; unstable medical conditions or malignancy; diabetes; history of alcohol abuse; known liver or renal disease; major depression; clinically significantly abnormal B12, lipid profile, hepatic, or renal function tests at screening; and high cardiovascular risk.

4.3.4 *Protocol*

Comprehensive Geriatric Assessment, medication documentation, and MMSE [Folstein and Folstein, 1975] score evaluated for eligibility. Eligible participants who chose to participate signed an ethically approved consent or assent form, depending on their cognitive ability. Randomization was done using a randomization sequence (Randomization.com) with 1:1 allocation and two blocks, by a study administrator (JK) who was not involved in any of the clinical assessments or in data analysis.

Cognitive tests: Further baseline cognitive testing included **Montreal Cognitive Assessment (MoCA)** [Nasreddine et al, 2005] and the computer based **Cognigram®** [Mielke et al, 2015]. This is a validated computer-based cognitive test that assesses processing speed, attention, visual learning, and working memory. It can be repeated frequently as there is no learning component. It is independent of education, which is very important, particularly in groups with low or high education (as in this study). The cognitive tests are discussed in more detail in the next few paragraphs.

Justification for cognitive tools: **MMSE** and **MoCA** were included as these tests are the ones most clinicians are familiar with, and therefore makes the understanding and clinical relevance of the study results that much greater. Although the Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) is considered the gold standard cognitive test in AD pharmaceutical trials, it is not clinically practical (it takes 30-45 minutes to complete). And although there may be statistically significant changes in the score (given it is a 70-point scale) [Burns et al, 1999] this often does not translate to any clinically perceivable/relevant difference [Schrag et al, 2012].

The **MMSE** test (see **Appendix Figure 1**), is used primarily for screening for dementia [Folstein and Folstein, 1975], but has been adopted and is also used for ongoing cognitive evaluation. It can be administered in 5–10 minutes. The MMSE measures several cognitive areas: orientation (10 points), word registration (3 points), calculation (5 points), word recall (3 points), language and visual construction (9 points) [Folstein and Folstein, 1975]. Total score is 30 points, with abnormal cut-off being age and education dependent (≤18-29) [Crum et al, 1993]. The MMSE has a learning component.

The **MoCA** test (see Version 1 in **Appendix Figure 2**) was developed as a screen for mild cognitive impairment (MCI) as it is a more difficult cognitive test. In practice it is also used in AD patients, and for ongoing evaluation of both MCI and AD. It has 3 versions to minimise the learning component. In this study the Versions 1-3 were used sequentially. It can be administered in 15 minutes. The MoCA test domains include: visuospatial/executive (5 points); naming (3 points); attention (6 points); language (2 points); abstraction (2 points), delayed recall (5 points), and orientation (6 points). If the education level is ≤ 12 years, then 1 point is added to the score. The total score is 30, with an abnormal score of ≤26 [Nasreddine et al, 2005].

The **Cognigram®** test (see more information in **Appendix Figure 3**) was included because of its validity for repeated frequent use (with no learning component), lack of dependence on tester, standardization of administration given it is a computer-based test, acceptance of participants (it is a card game–based test), and its ability to detect small changes over time [Mielke et al, 2015], unlike the MMSE and MoCA, which are primarily validated for screening and not ongoing evaluation (although this is how they are used in clinical practice). The Cognigram ® is also *language and education independent*. It requires no computer skills, and requires simply pushing a "yes" or "no" button. Administration takes 15 minutes. For each cognitive domain there is a short test session before the actual test to remind participants of the instructions. The domains include: joker cards for detection; a different joker card for identification of colours; regular playing cards for the one card learning test; and regular cards for the one back test.

Other tests: The **Euroqol EQ-5D** quality of life questionnaire [Brooks et al, 2003] was administered at baseline and every study visit. This is a validated general quality of life questionnaire, and is not disease-specific.

Apolipoprotein E (*APOE*) ε4 status was evaluated with saliva testing.

Oil diary: Caregivers completed a study oil intake diary, and returned all unused study oil at each visit. All oil containers were returned at each visit, and weighed to double-check compliance.

Safety monitoring: included monthly bloodwork including evaluation of lipid status. At every visit, weight, waist/hip circumference, body mass index (BMI) and bioimpedance assay body composition status (using Omron® HBF-510 Full body sensor body composition direct-to-consumer scale), blood pressure and heart rate, gastrointestinal (GI) and cardiac symptom screening, and current medication update were done. Baseline and end-of study dual-energy X-ray absorptiometry (DXA) bone density and body composition was completed.

4.3.5 *Outcome measures*

Primary outcome measures were: Cognigram® tests (1 and 2), MMSE, and MoCA.

Secondary outcomes were: effect of *APOE* ε 4 status on cognitive response; effect of MCT on trajectory of cognitive decline in MMSE and MoCA (using estimated average decline as defined by Doody and colleagues [Doody et al, 2001]; quality of life (QOL) in all phases of the study; and BHB level over the study.

Serum BHB testing was done on a fasting morning sample, prior to the morning (breakfast) dose of MCT.

Safety measures included lipid profile fasting insulin, homocysteine, BMI, body fat composition (DXA), and clinical adverse events.

4.3.6 *Intervention*

Test MCT oil was Bulletproof Brain Octane ® MCT oil (NPN 80057199) and placebo oil was olive oil (Hermes Olive Pomace oil ®).

Bulletproof MCT oil is clear, colorless, and tasteless, and was chosen because of the Health Canada (HC) requirement for a Drug Identification Number (DIN).

Placebo oil (pomace olive oil) was chosen for its minimal taste and color.

People with AD have impaired smell and taste sensation, so if there was any potential smell or taste, they would not be aware of this [Velayudhan et al, 2015; Steinbach et al, 2010]. No participants had used MCT oil before, so did not have any prior information on its appearance. In addition, the oil was administered in dark colored plastic spoons to prevent either the caregiver or the subject being aware of the oil colour. Finally, the participants/caregivers never had both the MCT and placebo oil at the same time, so would not have been able to compare one oil to the other. All left over oil was returned at each study visit, so other than in the open-label phase, both participants and caregivers would have been blinded to the oil randomisation.

Study test oil was provided in solid white BPA-free plastic 500ml bottles so that neither the clinical study team or the participants could see the oil colour, and it was labelled only with the participant's study information (as per Health Canada requirements). Bottles were filled by study administrators (JK and DS) who were unaware of any of the cognitive test results, and who were aware to not disclose the oil allocation to participants or investigators, to preserve the study

integrity. Both oils appeared clear in the containers, but to further avoid any awareness of colour, the oil was dispensed in a standardized 1-tablespoon (15ml) measuring spoon made of dark orange BPA-free plastic (Trudeau Maison® measuring spoons). Neither oil has any smell, with the placebo oil having minimal taste. These components are shown in **Appendix Figure 4.** Participants, their caregivers, and investigators were therefore blinded to the contents.

Instructions were to consume the oil with a meal, to improve dose tolerance (with the understanding that this may affect the maximal BHB response). The first month of the study was a week of study oil dosed at 15 mL once daily, increasing every week to three times daily by week three, if clinically tolerated, or to the maximum tolerated dose. This dose was continued for the study arm (4-months in total). The protocol was then repeated when the crossover occurred (4-months in total), and a similar titration schedule was followed for the open-label extension (7-months in total). Titration was to maximum tolerated dose (or 3 T [45 mL] daily) for each stage (see **Figure 1**).

As both oils are over-the-counter products, for the validity of this study, the ingredients in both oils were independently verified by an independent lipid research laboratory (Dr Vera Mazurak's Lipid Research laboratory, University of Alberta).

4.3.7 *Compliance*

At each study visit, unfinished and empty bottles were returned. These were weighed, and intake dairies reviewed, to allow calculation of the amount of oil consumed.

4.3.8 *Study visits*

Study visits were at screening/baseline (time-point 0), then monthly after the titration month for the double blind randomised cross-over phases of the study (0-8), at baseline for the open-label phase (month 9) and then at month 12 and 15 which was the final visit. MMSE and MoCA were done at baseline, end of phase 1, end of phase 2, start of open-label phase (after 1 month titration) and end of the study. Different versions of the MoCA test (versions 1, 2, or 3) [Nasreddine et al, 2005] were used sequentially at each assessment to minimize any learning effect. Cognigram ® was done at baseline and every visit. The Cognigram® was done monthly, as this has no learning component.

The cognitive testing was done under the same conditions at each visit, either by the PI or the research nurse, to ensure reliability and consistency in administration of the cognitive tests.

At the end of each study visit the participants picked up their new supply of oil.

4.3.9 *Adverse events*

Health Canada approval for MCT supplementation required exclusion of participants with concomitant diabetes, and regulated the ongoing evaluation of serum lipids.

Adverse events (AE), recorded as per standard HC protocols and reporting, were enquired for at each study visit, and at telephone contacts with the study nurse between visits, or spontaneously reported by participants or caregivers. They were classified as mild, moderate, or severe based on pre-specified criteria [Government of Canada NHP]. Serious adverse events (SAE) were defined as death; life threatening event; hospitalization—initial or prolonged; disability; or medically important event. Any adverse events was also classified as: “Related” to study intervention = events identified in regulatory documents such as Investigator Brochure or product monograph and occurring within expected frequency estimates, or those identified in research ethics board submission and Letter of Information to participants; or “Unrelated” to study intervention = the result of the natural progression of the person's disease/illness and/or state of health; or “Unexpected (Unanticipated)” = events not identified in Investigator's Brochure, Product Monograph, or local protocol or occurring with more than expected frequency.

4.3.10 *Statistical analysis*

Data analysis was completed using the SAS 9.0 statistical software (version 9.4; SAS Institute Inc.). P-values ≤ 0.05 were indicative of statistical significance. Data were expressed as mean ± standard deviation (SD) variable for parametric variables, or median and interquartile range (IQR) for non-parametric variables unless otherwise specified. The Shapiro-Wilk test was conducted to assess the normality of distribution.

The cross-over part of the study was analysed using the standard statistical analysis for crossover study method described by Jones and Kenward [Jones and Kenward, 2003], with analysis of sequences AB and BA (with A being MCT and B being placebo). In addition, repeated measures

analysis of variance (for each treatment phase placebo vs MCT oil supplementation, and Open label) was performed to assess the effects of MCT supplementation on primary outcomes (Cognigram® 1/Cognigram® 2, MMSE, and MoCA tests) and secondary outcomes (serum BHB, safety) over the entire course of the study. This included the potential for time-treatment interactions.

To address the potential for inter-subject variation between each treatment, the difference between each subject between the treatment effects were also calculated and the differences assessed using the appropriate tests (t-test for parametric, Mann Whitney for non-parametric). Analysis of covariance was performed to adjust for any variables influencing these outcomes (baseline MMSE, MOCA, Cognigram® 1/Cognigram® 2, age, sex). To assess for the potential differences in intrasubject variability between treatment allocations, the F-test of Homogenity was performed. Chi-square tests were used to measure differences in categorical data. Univariate and multivariate analyses were conducted to assess potential relationships between MCT supplementation and primary outcomes.

To attempt to evaluate whether the intervention affected the usual downward trajectory of MMSE in AD, participants were evaluated using data published by Doody and colleagues [Doody, Massman, et al, 2001]. They reported what the expected decline would be *based on baseline MMSE* as follows: Slow, with initial MMSE 24 = 0–1.9 pts/year; Interim, with initial MMSE = 18 2–4.9/year; or Rapid, with initial MMSE 14 > = 5/year.

4.4 Results

4.4.1 *Participants*

Referrals were screened by AGJ to ensure they met the inclusion criteria, and then study information was provided to them, and they were invited to join the study. From January to June 2016, 46 adults were screened, and 26 were excluded for the reasons shown in the flow diagram (see **Figure 2).** The majority were excluded by themselves (8 declined to participate) or because they did not fulfill the NINCDS-ADRDA criteria for probable AD (6 participants).

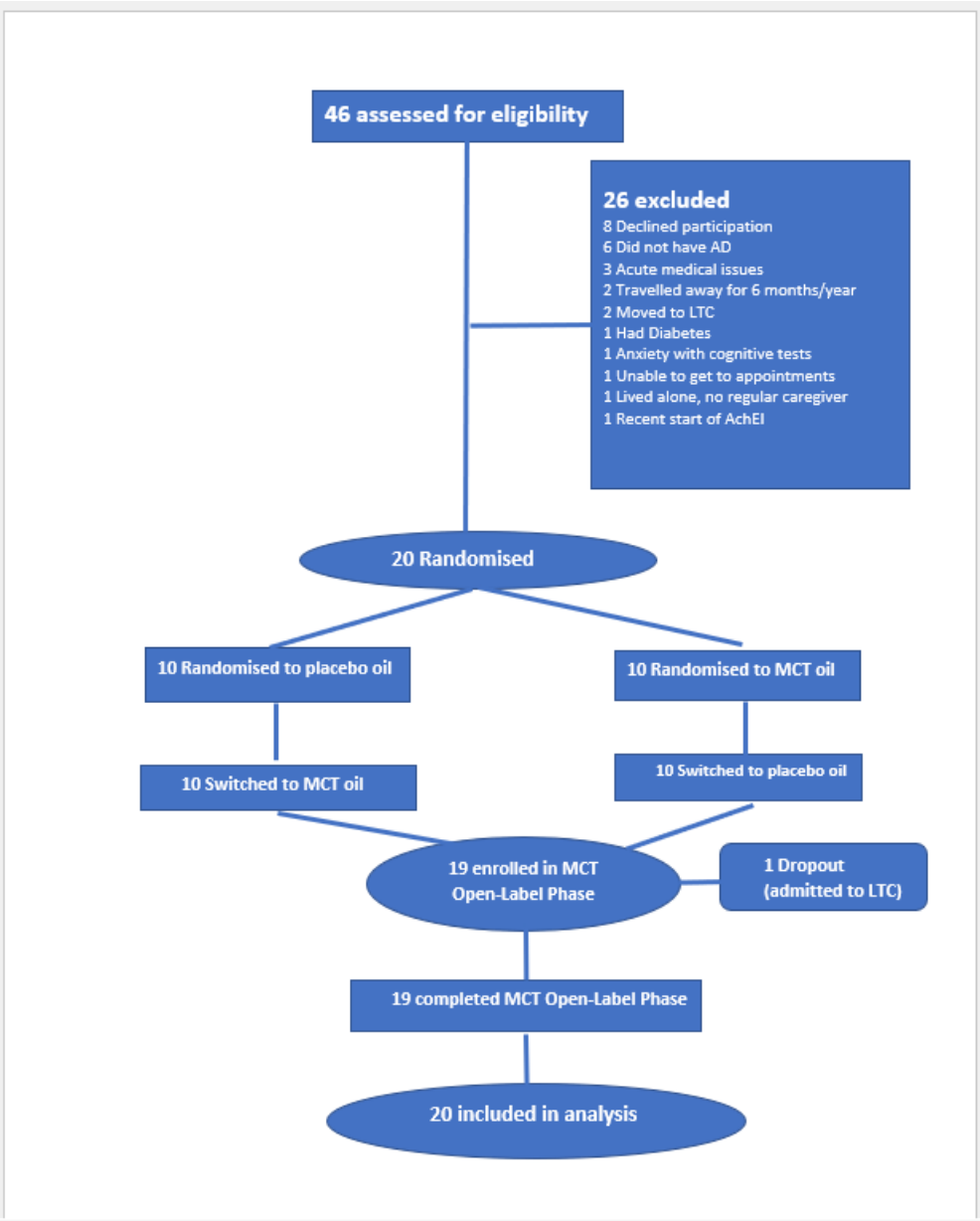


Figure 2: Participant screening and study flow

The twenty participants included in the study after screening were from the following referral sources: Geriatricians 7 (includes 2 from AGJ); Alzheimer's Society 5; Neurologists 3; Caregivers 3; Family Doctors 2. All twenty participated in the double-blind phase, and 19 in the open-label extension. One participant dropped out of the open-label extension due to caregiver

stress requiring long-term care admission (participant referred by a Family Physician). The remaining 19 participants completed the total study duration (15-months).

4.4.2 *Demographic and anthropometric data*

Table 1 shows baseline demographics of the two arms. There were no statistically significant differences in demographic and anthropometric data. The MCT start group had a higher prevalence of APOE ε4 positivity, but this difference was not statistically significant.

TABLE 1. Baseline demographics in groups assigned to Placebo oil or MCT oil start

Variable	Placebo (n = 10)	MCT oil (n = 10)	*P*-values
Median [interquartile range]			
Age (years)	79 [69.6-80.9]	68.1 [60-76]	.67
Sex (M:F)	4:6	7:3	.89
Height (cm)	165 [155-169]	165 [162-181]	.73
Weight (kg)	83.6 [70-88]	74.8 [61-83]	.82
BMI (kg/m2)	27 [26.6-30.3]	23 [22-26]	.07
Level of education (% college/university)	50.0	90.0	.49
APOE ε4 status	**N = 10**	**N = 9**	.27
Absent (%)	6 (60%)	3 (33%)	.25
Heterozygote (%)	2 (20%)	5 (56%)	.11
Homozygote (%)	2 (20%)	1 (11%)	.60

Variable	Placebo (n = 10)	MCT oil (n = 10)	*P*-values
AChEI use (%)	6 (60%)	8 (80%)	.35
Memantine use (%)	2 (20%)	2 (20%)	.82
Mean ± SD			
Baseline MMSE	23.8 ± 4.7	22.8 ± 6.4	.79
Baseline MoCA	18.6 ± 6.5	16.7 ± 8.3	.67
Baseline Cog 1	90.2 ± 15.9	76.7 ± 21.6	0.12
Baseline Cog 2	84.9 ± 13.1	80.8 ± 24.3	.43

Note: Data represents mean ± SD or median (interquartile range) unless otherwise specified. P values <0.05 represent statistical significance. Abbreviations: *APOE*, apolipoprotein E; AChEI: acetyl cholinesterase inhibitor; BMI, body mass index; MCT, medium chain triglyceride; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; Cog 1: Cognigram ® 1; Cog 2: Cognigram®2

No differences in baseline MMSE scores were noted between patients above and below the median age, but the older patients had higher BMI and WC than the younger patients. There were no differences between groups in baseline cognitive tests.

Level of education (LOE) was high in this study group, with only 2 participants having a LOE less than Grade 12. Of the remaining 18, 4 had post secondary education, and 14 had a University Degree. The level of education was higher in the MCT start group (Group 2, 8/10 University and 2 post-secondary LOE) but this was not statistically significant.

4.4.3 *Study oil analysis*

All the MCT oil and olive oil came from the same lot number (1507075254, expiration 07/17; 16036E15, expiration 02/18, respectively) as that tested in the independent lipid research laboratory. Bulletproof Brain Octane ® MCT oil was verified as **99.3% C8**, 0.6% C10, and 0.1% C12:0. The placebo oil was 72.2% C18:1, 11.3% C18:2 (83.5% oleic acid), 12% C16:0, confirming it as a long chain, predominantly oleic oil.

4.4.4 *MCT oil consumption*

The F-test of homogeneity was used to test whether there were differences in variability within and between subjects over the study phases. There was no difference between the two groups ($p > .05$), indicating no difference in intake whether on the placebo oil or MCT oil. (See **Table 2**). The mean oil consumption was 1.94 tablespoons (29.1ml), so practically speaking, the average oil consumption was *2 tablespoons (30ml) daily*.

Daily intake of 3 tablespoons seemed to be limited mainly by compliance (lunchtime dose often forgotten by caregiver). However, in some participants tolerance (predominantly GI related, such as abdominal pain or diarrhea) was an issue with more than 2 tablespoons daily.

Table 2: Oil consumption (in tablespoons) in study phase and participant group.

Study Phases		Number of participants	Mean	Standard deviation	Variance
Phase 1	Group 1 (Placebo oil)	10	1.94	0.46	0.21
	Group 2 (MCT oil)	10	1.71	0.65	0.43
Phase 2 (after crossover)	Group 1 (MCT oil)	10	1.46	0.63	0.40
	Group 2 (Placebo oil)	10	1.90	0.51	0.26

Open label	Groups 1+2 (MCT oil)	19	1.89	0.39	0.15

P >0.05 in all Phases

4.4.5 *Outcomes*

Primary outcomes

Baseline

There was no statistically significant difference for MMSE, MoCA, Cognigram ® Part 1 and 2 between Group 1 and Group 2 at the start of the trial, and at the end of each phase of the double-blind crossover, or at the start of the open-label phase. (see **Table 3**).

Table 3. Cognitive Scores in each phase of the study

	Double-blind placebo-controlled Phase 1			**D-B placebo-controlled Phase 2 (crossover)**			**Open label MCT extension Phase 3**			**Open label MCT extension Phase 3**
	Baseline: Visit 0			**Baseline: Visit 5**			**Baseline: Visit 9**			**Final: Visit 13**
Variable	***Group 1 (Placebo start)***	***Group2 (MCT start)***	***P***	***Group1 (crossed over to MCT)***	***Group 2 (crossed over to placebo)***	***P***	***Group 1 (Placebo start)***	***Group 2 (MCT start)***	***P***	***Complete Group (Group 1 + Group 2)***
MMSE	23.8 ± 4.7	22.8 ± 6.4	.75	23.7 ± 4.8	21 ±6.9	.44	23.4 ±5.5	20.1±7.7	.06	20.6 ±7.8

	Double-blind placebo-controlled Phase 1 Baseline: Visit 0			D-B placebo-controlled Phase 2 (crossover) Baseline: Visit 5			Open label MCT extension Phase 3 Baseline: Visit 9			Open label MCT extension Phase 3 Final: Visit 13
Variable	***Group 1 (Placebo start)***	***Group2 (MCT start)***	***P***	***Group1 (crossed over to MCT)***	***Group 2 (crossed over to placebo)***	***P***	***Group 1 (Placebo start)***	***Group 2 (MCT start)***	***P***	***Complete Group (Group 1 + Group 2)***
MoCA	18.6 ± 6.5	16.4 ±8.3	.68	19.7 ±5.4	14.8 ±7.9	.12	17.1 ±5.1	16 ±7.3	.5	17.1 ± 5.7
Cog 1	90.2 ±15.9	76.7 ±21.6	.12	91.3 ±13.7	79.2 ± 22.9	.17	89.8 ±19.3	80.5±21.2	.09	87.4 ± 16.4
Cog 2	84.9 ±13.1	80.8 ±24.3	.62	84.4 ±12.9	73.1 ±27.7	.24	88.7 ± 13.5	83.8±21.9	.3	85.4 ± 12.9

Values are mean ± SD. P values for post-hoc multi-pair wise correction and Boneferroni correction is a p<0.013. (Group 1, refers to group starting study in placebo arm, Group 2, refers to group starting in MCT arm). Abbreviations: MCT, medium chain triglyceride; MMSE, Mini Mental Status Examination; MoCA, Montreal Cognitive Assessment; Cog 1, Cognigram® Part 1; Cog 2, Cognigram® Part 2.

Footnote: **For the cognitive tests, all participants completed all the MMSE tests. Two participants were unable to complete all the MoCA tests, one competing 4/5 and the other 2/5 (due to excessive anxiety with the testing). Both participants were in the moderate AD stage at enrollment. For the Cognigram®, the same two participants plus two others, also in the moderate stage at baseline, had difficulty with Cognigram ® Part 2. This resulted in missing 4/100 data points for MoCA, 27/180 for Cognigram® Part 1 and 28/180 for Cognigram® Part 2.**

Cross-over analysis

Statistical analysis for this crossover study [Jones and Kenward, 2003] showed no statistically significant differences between Groups 1 and 2 in the crossover phase of the study for MMSE. MoCA and Cognigrams®. Because of the absence of a washout period, Group 1 and Group 2 were also analyzed in the first phase of the study to check for any carryover effects (Wilcoxon rank test), and again there was no statistically significant difference.

Baseline versus study completion

When evaluating the start to end of the study, there was also no difference between MMSE and MoCA in the open label phase. however, there was a **statistically significant difference in Cognigram® 1** (attention and psychomotor function) scores between the two groups at the study completion (after the additional 7-months open-label MCT oil), $p = 0.003$, in those who has started with the placebo oil (i.e., participants who had 11-months of uninterrupted MCT oil consumption vs. those interrupted by 4-months of placebo oil). However, there was no statistically significant difference in Cognigram® 2 scores between the two groups.

Over the entire study the average was stable, but there was individual variation in response.

Figure 3 shows the actual change in MMSE over the study for all the participants. Using data reported by Doody [Doody, Massman, et al, 2001], the average expected MMSE decline is shown in black, depending on the MMSE at the start.

79% of participants showed either less decline than expected or a stabilisation in their MMSE scores. In 21% (four participants), there was a decline in MMSE. These participants did not differ statistically from the other participants in baseline parameters. Three of the four decliners were on AChEI therapy, and all were homozygous or heterozygous for the APOE ε4 allele.

Those with a *higher baseline MMSE* (earlier in the clinical presentation of the disease) were more likely to have a stable or improved MMSE ($p = .04$). Those having an MMSE 25 to 29 at the start being more likely to decline less than expected. *MCT starters* had a trend toward greater declines in MMSE scores than placebo starters ($p = .06$).

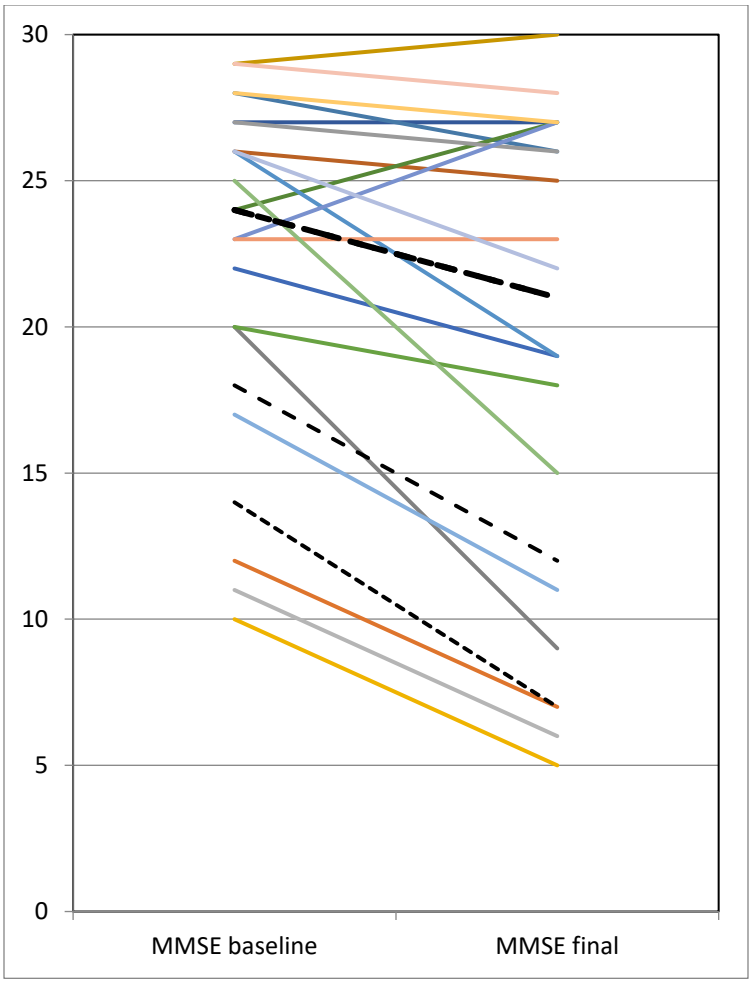


Figure 3: Graph showing change from baseline to final Mini-Mental State Examination (MMSE; 15-months for 19 participants, 8-months for one). Black lines represent theoretical decline anticipated, trajectory depending on starting MMSE level [Doody, Massman et al, 2001].

Age appeared to be associated with stabilization and/or increases in MMSE scores. Fewer participants under the median age of 73 years showed MMSE stabilisation or improvement: 3/10 versus 8/9 over the median age of 73, p = .009.

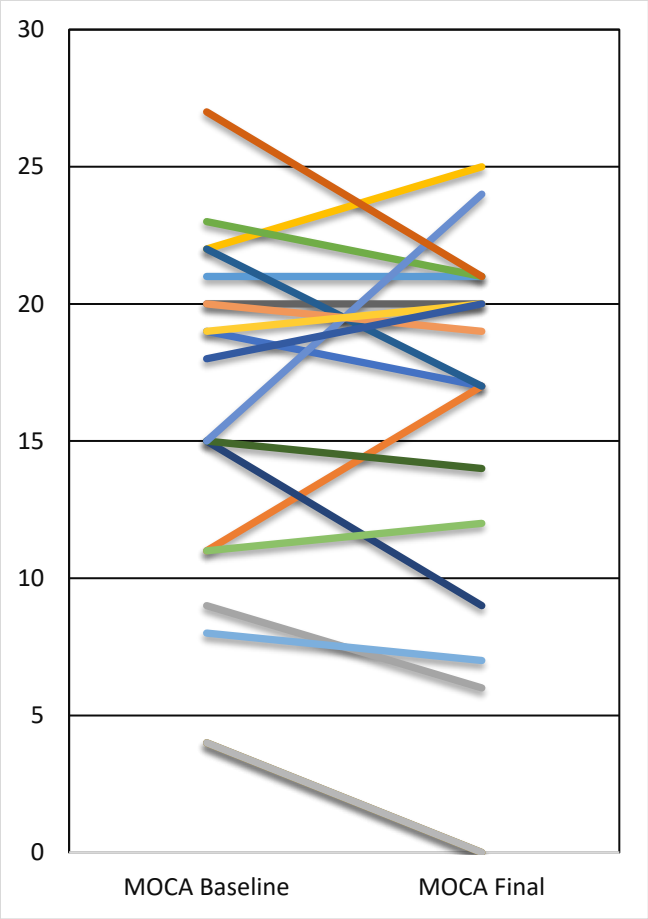


Figure 4: Graph showing change in Montreal Cognitive Assessment (MoCA) from baseline to final (15-months for 19 participants, 8-months for one)

Overall, there was also stability or improvement in MoCA scores (shown in **Figure 4**) in 8/17 participants. Given that it is a more cognitively demanding test than the MMSE, it is likely that there would have been a decline in this score over 15-months for all participants. However, unlike for MMSE, there is no data on the expected decline trajectory for this test, so some of the decliners may also have declined less than expected.

See **Appendix** for additional figures of individual changes in MMSE at each phase of the study, grouped by randomisation. (**Appendix Figure 5: 1 A, B: First double-blind phase; 2 A, B: Second double-blind phase (crossover); 3 A, B: Overall change in MMSE during double-blind crossover phases).**

Secondary outcomes

(i) *Serum BHB*

These levels were measured in a fasting state, before the morning test oil consumption, so are a reflection of background BHB level. To see if consistent intake of MCT oil (over 7-11 months) had any long-term effect, the fasting BHB at baseline was compared to that at the end of the study. Average fasting baseline BHB was 0.2 mmol/L (0–0.4), and at study completion was 0.2 mmol/L (0.1–0.9), suggesting no long-term change in baseline BHB production despite exposure to MCT supplementation. This result also confirmed no significant dietary pattern changes (toward a ketogenic diet) during the study.

Cognitive testing was done consistently over the study course, in the morning, usually 2-4 hours after breakfast and the participant's dose of MCT or placebo oil.

(ii) *Apolipoprotein ε4 status*

There was no measurable effect of APOE ε4 status on the response to MCT oil.

(iii) *Quality of Life*

Perceived QOL reported by participants was high throughout the study. The EQ-5D-5L average visual analogue scale score was 84.4/100 with a range from 40 to 100. There was no difference between baseline (average 85.1) and study completion (average 85.9).

The caregivers reported high satisfaction in being involved in a research study. They appreciated the extra clinical support and the ready availability of the study nurse.

4.4.6 *Safety*

Lipid values were examined at baseline and at study completion, as per Health Canada requirements for safety evaluation. There was no statistically significant change in lipids (total cholesterol [TC], low density lipoprotein [LDL], triglyceride [TG]) in either group (on or off MCT oil; $P \geq .05$). **Table 4** shows lipid results from baseline and after the 15-months of oil

supplementation. A weak association was noted for lower LDL and TC in those consuming higher doses of MCT oil.

Table 4. Lipid Profile safety evaluation

Variable (mmol/L)*	Baseline (n = 20)	Study completion (n = 19)	*P*-values
Total cholesterol	5.1 (4.5-5.6)	5.4 (4.6-6.0)	.47
Triglyceride	0.9 (0.8-1.2)	1 (0.9-1.3)	.10
HDL	1.7 (1.9-2.2)	1.6 (1.5-1.9)	.52
LDL	2.8 (2.5-3.3)	2.9 (2.4-3.5)	.72
Ratio Cholesterol/HDL	3.1 (2.7-3.3)	3.4 (2.0-4.1)	.15

***Values are expressed as medians (interquartile range). Baseline and study completion lab values all normally distributed. Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein.**

Reference ranges (mmol/L): Total cholesterol <4.15; Triglyceride 0.0-1.7; HDL >1.15; LDL 0.0-3.4; Ratio Cholesterol/HDL-<4.0.

DXA body composition showed no significant change in percent body fat in both study groups from baseline to the end of the study.

In addition, there was no impact on glycemic control (HbA1c and fasting insulin), p = 0.06, during the study.

4.4.7 *Adverse events*

Thirty-nine mild to moderate adverse events were reported in 17 (85%) participants. Of these, 25 (64%) were GI and included vomiting, diarrhea, or abdominal cramping, felt to be related to the study oil. In all cases the participants were taking MCT oil when they reported their GI symptoms. In no cases was this severe enough to require study discontinuation, and symptoms resolved with a dose decrease. Their average oil consumption did not change whether on the placebo or MCT oil.

There were two unrelated serious adverse events (SAE). Both SAE were hospitalizations unrelated to the study intervention. The first was for a total abdominal hysterectomy for dysfunctional uterine bleeding (no malignancy identified), and the second was for orthopedic surgery after a fall and shoulder fracture dislocation.

4.4.8 *Concomitant medications*

None of the participants changed AD medications or started an antipsychotic agent during the double-blind study period. One participant had AChEI treatment discontinued due to bradycardia, in the open-label phase. There were no dosage increases for memantine or AchEI treatment as all participants were on maximum doses at the study start.

4.4.9 *Dropouts*

There was one dropout (in the open-label stage) due to admission to long-term care resulting in loss of direct caregiver supervision of oil consumption, and inability to attend study visits.

4.5 Discussion

The crossover study design enabled all participants to be exposed to both placebo and MCT oil, and the main purpose was to separate treatment effects from period (timing) effects. Cross-over analysis did not reveal any difference between the two groups, suggesting no obvious treatment effect. The lack of measurable cross-over effect may also have been because there was no washout between the two crossover arms. However, there is no clinical data to suggest that

MCT has any benefit (other than theoretically) after direct consumption is discontinued. Only ongoing MCT consumption can provide sustained elevation of ketone levels, as was shown in the study reported in Chapter 3, where the BHB level started to drop within 6-hours even after the highest dose, which is more than the participants in this study were able to take over the course of a day.

The natural history of Alzheimer's dementia is cognitive decline overtime. The rate of this decline however is variable, and the trajectory is not necessarily linear [Salmon et al, 1990; Morris et al, 1993]. Factors such as age of onset [Jacobs et al, 1994], neuropsychiatric features [Chui et al, 1994; Stern et al, 1997], initial MMSE score [Doody, Messman et al], and baseline education level [Amieva et al, 2014], have all been shown to influence the AD trajectory. Higher baseline education, in particular, tends to lead to a more rapid course for the dementia. This is felt to be based on the principle of "cognitive reserve" [Stern, 2012; Xu et al, 2015]. Those with a higher level of education have a higher cognitive reserve, ie. they can lose significant cognitive function before it becomes clinically apparent. However, once there are clinical features of dementia there has already been significant cognitive loss, leading to the more rapid deterioration [Amieva et al, 2014]. The participants in this study had a very high baseline education level and so would have been anticipated to have had a measurable decline over the 15-months. However, this was not the case, with the majority of participants showing stabilisation of MMSE, MoCA and Cognigram®. One could also argue, that stabilisation in this case was due to AchEI therapy, but clinical trials have shown only modest benefit with these medications, and there remains a downward trajectory in cognition [Doody, Geldmacher et al, 2001]. Although there was no statistically significant difference between the groups, overall, by chance, the group starting with MCT (Group 2) had a higher level of education, so would have been expected to decline more rapidly than their peers in Group 1. Nonetheless, based on the Doody and colleagues 2001 classification [Doody, Messman, 2001], 79% of participants did not decline at the expected rate based on their baseline MMSE score (see **Figure 3**). However, caution is required when interpreting this data, as with any historic comparables, because of unaddressed variables such as age, sex, diet, lifestyle, and cholinesterase inhibitor treatment.

If the theoretical effect of ketones (BHB) is to modify amyloid plaque formation, then there may well have been carryover effects that may have accounted for the lack of any obvious difference

between the two crossover arms. As discussed by Wellek and Blettner [Wellek and Blettner, 2012], to mitigate any analysis error from potential carryover effects only the first trial period should be analysed. In this study, when the placebo and MCT group were analyzed separately (phase one only), there remained no difference between the two groups, suggesting no carry-over effect. There are potentially a number of explanations for this lack of difference: the duration of each crossover phase was not long enough; the dose of MCT tolerated by participants was not high enough to produce a measurable difference from the placebo; the Alzheimer's disease was more advanced and therefore cognition was less likely to benefit from MCT supplementation; the study was under-powered to detect any difference; or, the MCT oil had no effect.

In addition, when evaluating the difference in final outcome between those who started with the placebo oil versus those who started with the MCT oil in the crossover phase (Group 2), those who had MCT oil continuously (including the one-month titration between ending the crossover and starting the open label phase) showed improvement that was statistically significantly different in their Cognigram® Part 1 scores when compared to those who had a break of four months between their two MCT supplementation periods. This difference was not due to differences in oil consumption in the two Groups, as shown in **Table 2**. This suggests there may have been some impact of the continuous MCT consumption.

Ketones provide energy to support cerebral function in the face of abnormal glucose cerebral metabolism [Xin et al, 2018]. An unknown is whether the energy provided by the ketones allows for cognitive function at that time only, or whether it has any lasting, clinically meaningful neuroprotective effects. The results of the present study raise the intriguing possibility of a longer duration, neuroprotective effect. Published studies suggest other roles of MCT as an anti-inflammatory agent [Pinto et al, 2018; Daulatzai et al, 2017] or a neuroprotective agent [Kashiwaya et al, 2000]. As AD is known to be related to cerebral inflammation, this may be one factor for ketone-induced neuroprotection [Pinto et al, 2018]. There is also evidence that ketones can reverse amyloid accumulation already present in cell cultures [Kashiwaya et al, 2000]. BHB was shown to provide neuroprotection from the proteolytic fragment of the β-chain of the amyloid precursor protein, AB1-42, with increased cell survival and neurite number compared to controls [Kashiwaya et al, 2000].

This study has also been able to show longer term safety of MCT supplementation, with no significant changes in laboratory parameters over the 11-month exposure to MCT supplementation, particularly with respect to serum lipids and body composition.

Although previous studies have been shorter and in less cognitively impaired groups, they have all shown a statistically significant cognitive change [Taylor et al, 2018; Hendersen et al, 2009; Hendersen and Poirier, 2011], or a trend towards one [Newport at el, 2015; Rebello et al, 2015; Reger et al, 2004; Krikorian et al, 2012; Fortier et al, 2019]. None of the previous studies had a cross-over design, or were as long in duration (15-months) as the present study, or included moderate AD participants. All suggested a positive cognitive signal/result, with most using either ADAS-Cog [Rosen et al, 1984], or MMSE. This study chose to use cognitive tests that are more accessible and applicable to clinicians in daily practice. Although the ADAS-Cog is considered the gold standard for clinical dementia drug trials, it is not used in clinical settings, as it is time-consuming to administer, and changes of up to 4 points may be statistically significant, but are not usually clinically relevant, and depend on the duration of the study [Rockwood et al, 2009].

Baseline MMSE could be an important factor during this study, suggesting the provision of alternative brain energy as ketones may be more beneficial earlier in the course of the disease. Other authors have also suspected this. Hence, of the few clinical studies done, 50% have been in MCI. Positron emission tomography (PET) cerebral glucose studies have clearly shown a progression in impaired cerebral glucose metabolism proportional to the clinical stage of the disease [Daulatzai, 2017]. Unlike the published data [Henedersen et al, 2009; Reger et al, 2004; Hendersen and Poirier, 2011] (including some smaller than this study), we did not show any apparent effect of APOE ε4 status on the response to MCT oil, although fewer participants in the placebo start group were homozygous or heterozygous for APOE ε4 (4/10 versus 6/9).

Previous data, and the present study, raise the question as to whether there should be MCT dose adjustments or additive dietary changes in those with more advanced disease. Given the intolerance reported with higher MCT oil supplementation, however, it is unlikely that this can be done purely by increasing the MCT intake. One option would be a combination of a ketogenic diet and MCT. However, clinicians caring for patients with AD know the challenges involved with food intake, given the change in taste and food preferences as the disease progresses

[Murphy et al]. It is practically unlikely that a moderate AD person is going to comply with a ketogenic diet. This challenge was reported by Taylor and colleagues in their study in moderate AD [Taylor et al, 2018]. There was a 33% dropout in their diet/MCT combination study because of caregiver burden, suggesting a lack of long-term feasibility. Their data also showed a marked individual variability in BHB response to their intervention. We also previously demonstrated a marked individual response of BHB levels, affected not only by amount of MCT consumed, but also by concomitant carbohydrate intake, and phenotype [Juby et al, 2021]. These variables may be an added reason why, with similar interventions, there may be an individual response to cognitive outcomes, but not necessarily a group effect.

Dietary changes are a challenge, even in those with MCI. Krikorian and colleagues used a very low carbohydrate diet alone (rather than the high fat diet used by Taylor et al, or the MCT supplement alone used in this study) to induce ketosis [Krikorian et al, 2012]. They showed a trend to improved cognitive function in those on the diet. Yet, despite their intervention group reporting better perceived health and function, only one participant (of 12) was planning to continue the lifestyle change long term. Adding exercise to supplemental MCT is another theoretical possibility to raise absolute BHB levels, but compliance with aerobic and/or resistance exercise in AD participants may be as challenging as diet compliance given that many have issues with gait and balance, in addition to cognitive issues, already affecting their ADL function.

4.6 Assessment of bias

In order to address the potential for biases highlighted in the recent meta-analysis of the randomised studies of MCT and MCI or AD reported in the literature to-date [Averginos et al, 2020], several measures were taken in this study and are summarised in **Table 5**. Bias evaluation was done using the Cochrane collaboration's tool [Higgins et al, 2011], as used by Averignos and colleagues [Averginos et al, 2020].

Table 5: Assessment of this randomised controlled trial bias using Cochrane Collaboration's tool for assessing risk of bias [adapted from Higgins and Altman, 2008] [Higgins et al, 2011].

Bias domain	Source of bias	Support for judgment	Review authors' judgment (assess as low, unclear or high risk of bias)
Selection bias	Random sequence generation	1.Randomisation done by a team member who was not involved in clinical evaluations using block randomisation. 2. Participants from multiple referral sources	Low
	Allocation concealment	Participants were booked for their first visit, and on arrival allocated a study number that, based on the randomisation results, put them initially into the placebo or MCT arm. This allocation was not known to the participants, caregivers or other study personnel. The investigational product was provided in a concealed form (solid white plastic bottle) at the end of each visit (after all the testing had been done).	Low
Performance bias	Blinding of participants and personnel*	1.The study personnel performing the evaluations were blinded to the oil allocation. They never even saw the bag of oil bottles. These were provided at the end of the visit, and collected at the beginning of each subsequent visit before the clinical evaluation. 2.The caregivers never saw both oils side-by-side and had no experience with the appearance of MCT oil at baseline, so even if they felt the oil had some colour this did not provide them with any clue as to the group allocation. 3. Caregivers, even if they had suspicions of the oil allocation -from side effects or cognitive effects- were discouraged from voicing these opinions to the clinicians. (According to JK and DS who were responsible for the oil provision, the caregivers' guesses were often incorrect!)	Low for investigators, low-moderate for participants (only applicable in cross-over phase)
Detection bias	Blinding of outcome assessment*	1.Group allocation remained blinded until the study completion, including the open-label phase. Fortunately, there were no severe adverse events requiring un-blinding of any of the participants during the trial. 2. statistical analysis was done by study personnel not involved in any way with the original data collection. Outcomes were evaluated anonymously based on study number only.	Low-moderate
Attrition bias	Incomplete outcome data*	All participants completed the randomised cross-over phases of the study. 1.There was no missing data for MMSE, 2. Missing data points for MoCA 4/100, Cognigram ® part 1 27/ 180 and part 2 28/180. 3. All caregiver questionnaires were completed. 4. All required laboratory testing was completed. 5. No data was excluded from the analysis.	Low
Reporting bias	Selective reporting	All the data collected was reported, including all the pre-specified outcomes for cognition MMSE, MoCA and Cognigram® and all the laboratory safety parameters.	Low
Other bias	Anything else, ideally prespecified	Concern may arise from bias due to the clinical role of AGJ as a geriatrician, potentially selecting and enrolling only participants from	Low

Bias domain	Source of bias	Support for judgment	Review authors' judgment (assess as low, unclear or high risk of bias)
		her practice. This was not the case, as there were only 2 participants from the clinical practice of AGJ.	

***Assessments should be made for each main outcome or class of outcomes.**

4.7 Strengths

This study is currently the longest duration (15-months) prospective MCT AD study, and the only study including participants with moderate-stage AD. It has a low risk of bias (See details in 4.6).

The study shows that regular daily intake of MCT is feasible long-term for both participants and caregivers, and was safe, with no adverse effects on serum lipid profiles, body fat composition, or glucose metabolism. It also suggests that longer continuous intake of MCT oil may provide greater cognitive benefit. Using a validated computerized tool (Cognigram®) was useful for frequent evaluations, and acceptable in AD participants who found it less stressful than typical cognitive testing. It was also a tester-independent evaluation reducing any risk for rater confounders.

4.8 Limitations

Limitations of the study include: small sample size; wide spectrum of ages and AD disease state; lack of continuous BHB monitoring so preventing assessment of the maximum BHB level achieved; and, difficulty reaching maximum (42 g) dose due to side effects or missing the lunchtime dose, highlighting the long-term practicality of a liquid, three times daily formulation. Additionally, Cognigram® requires preservation of baseline coordination and, at least in our study, was not useful in those with MMSE scores < 10/30. There was no washout between each phase of the study which may be considered a limitation, but only if it is shown in future the

MCT can have persistent benefits even when serum BHB is not elevated. Use of olive oil as the placebo oil in this study may also be considered a limitation. Current research suggests that consumption of olive oil as part of a *long-term* Mediterranean diet has been shown to *prevent* dementia, but not impact dementia once it is established [Coelho-Júnior et al, 2021]. A recent cohort study from Japan also showed improved MoCA and delayed recall in elderly participants reporting consuming more oleic acid in their diets [Sakurai et al, 2021].

4.9 Conclusions

The cross-over phase of the study did not show any measurable effect on MMSE, MoCA, or Cognigram®.

Over the entire15-month study there seemed to be an effect on the attention and psychomotor domains of Cognigram® (Cognigram® Part 1) in those with a more continuous MCT oil consumption. This result is difficult to interpret given the limited published studies in AD using the Cognigram®, but it certainly raises food-for-thought as to what cognitive component in Part 1 may have led to this result. Overall, cognitive function appeared to be more stable than would be anticipated over the total study duration especially in those with a higher baseline MMSE, and was independent of age and APO ε4 status.

In terms of safety, there was no effect on body weight, composition, or serum lipids with 11-months of MCT oil and 4-months of placebo (olive) oil consumption. MCT supplementation at three tablespoons daily (42 g) was difficult for some to tolerate, due to GI side effects.

Given the paucity of new therapeutic options for AD treatment, it is increasingly urgent to explore other therapeutic options. The expanding basic science and clinical research highlighting the physiological and clinical basis for efficacy of MCT supplementation, raises an intriguing and hopeful new therapeutic addition for AD treatment. Further studies are needed to look at strategies to improve MCT tolerance and dose, and studies with more homogenous AD populations may help minimise potential confounders in this complex disease.

CHAPTER 5

Addressing the main barrier to Sarcopenia identification: Utility of practical office-based assessment tools versus Dual Energy Xray Absorptiometry (DXA) Body Composition for identification of low muscle mass in older adults.

Juby AG, Davis CMJ, Minimaana S, Mager DR. Addressing the main barrier to Sarcopenia identification: Utility of practical office-based assessment tools versus Dual Energy Xray Absorptiometry (DXA) Body Composition for identification of low muscle mass in Seniors. 2022 (submitted to a peer-review journal)

5.1 Abstract

Background

Sarcopenia is associated with increased morbidity and mortality. In busy clinics, consideration of sarcopenia can be overlooked, especially in those with obesity. Diagnostic criteria for sarcopenia are published. Diagnosis includes muscle mass and muscle function assessments. Muscle function can be readily assessed (grip strength, chair stand test). However, muscle mass is assessed by dual energy Xray absorptiometry (DXA) Body Composition (BC) or other costly tools, which may not be readily available.

This study aims to evaluate muscle mass using inexpensive direct-to-consumer, office-based bioimpedance assay (BIA) scales compared to DXA.

Methods

Body composition was evaluated by DXA and two direct-to-consumer, office-based, BIA scales (Ozeri® and Omron®), differing by the latter including both feet and hand sensors. Participants are independent, community-dwelling Seniors who are in a prospective 12-month observational study. The two evaluations (BIA versus DXA) were done independently, and the results were not known to those performing either evaluation. The European Working Group on Sarcopenia in Older People (EWGSOP) DXA or BIA low muscle mass diagnostic cut-offs were used to

classify participants as having low or normal muscle mass. All the analyses were done at study completion.

Results

Fifty participants, 11 males and 39 females were enrolled. The average age was 75.8 years, ranging from 67-90 years. Forty-two completed the DXA evaluation. Using the EWGSOP criteria, of appendicular skeletal muscle/height^2 for DXA, 15 participants had low muscle mass. Using BIA generated muscle mass/height^2 cut-offs, low muscle mass was identified in 7 participants with Ozeri®, and 27 with Omron®. The negative predictive value for low muscle mass versus DXA (as the gold standard) for the Ozeri® scale was 73.3% and for the Omron® scale was 92.8%. Sensitivity and specificity for Ozeri® were 40% and 92.6% respectively,and for the Omron® scale was 93.3% and 48% respectively. Eighty-one percent were obese based on DXA body fat cut-offs. Using Bland Altman correlation, there was good correlation between both BIA scales and DXA for body fat estimates.

Discussion

The Omron® direct-to-consumer BIA scale captured all participants with low muscle mass identified by DXA, plus others on the DXA low muscle mass cut-off borderline. The Ozeri® scale was more specific than the Omron® scale, but it missed identifying some participants shown to have low muscle mass with DXA. With the high prevalence of obesity an objective measure of muscle mass is required. The Omron® scale correlated well with DXA for percentage fat assessment.

Conclusions

A low cost, readily available, direct-to-consumer scale, especially with hand sensors (Omron®), provides important information on muscle and fat mass. This can prompt additional investigations and/or interventions for low muscle mass.

5.2 Background

Low muscle mass is known to be associated with a significant increase in morbidity (eg. reduced activities of daily living (ADL) function) [Baumgartner et al, 1998] and increased mortality

[Roubenoff, 2001]. Low muscle mass, in combination, with decreased performance in physical tasks (gait speed, grip strength, chair stands) has a greater impact on morbidity and mortality [Cruz-Jentoft et al, 2010]. This condition is defined as *Sarcopenia*, and consensus groups have developed cut-offs that can be used to categorize patients as: Pre-sarcopenia (low muscle mass alone); probable sarcopenia (low muscle strength alone); Sarcopenia (low muscle strength + low muscle quantity or quality); or severe sarcopenia (low muscle strength, low muscle quantity/quality and low physical performance) [Cruz-Jentoft et al, 2010]. This categorisation has important implications for prognosis. An added complication is the increasing prevalence of obesity. *Sarcopenic obesity* has a cumulative effect on complications [Roubenoff, 2004]. A recent systematic review highlighted the ongoing challenges, and stated that current research does "not allow definitive conclusions on the prevalence and relevance of sarcopenic obesity from a clinical and functional standpoint" [Donini et al, 2020].

Sarcopenia can be screened for in a population using the *SARC- F tool* [Malmstrom and Morley, 2013], and is highly sensitive, but poorly specific at identifying people with sarcopenia that have poor outcomes [Woo et al, 2014; Gomes et al, 2020, Malmstrom et al, 2016]. It is based on self reporting (*S*trength, *A*ssistance walking, *R*ising from a chair, *C*limbing stairs and *F*alls), so can be used by all healthcare professionals, and also completed by the patient themselves.

Standardized definitions of sarcopenia, based on Dual Energy Xray Absorptiometry (DXA) body composition (BC) evaluation of muscle mass, or bioimpedance (BIA) evaluations, are needed to ensure both a sensitive and specific diagnosis of sarcopenia [Satoshi et al, 2018]. In many circumstances neither of these diagnostic tools are accessible - either because of availability, or cost. This significantly limits the ability of clinicians, to make an objective assessment of muscle mass as part of the routine clinical evaluation. In somebody with low body weight, decreased muscle mass is usually visually apparent, raising clinical suspicion for sarcopenia. However, in obese people, low muscle mass can easily be missed, as they appear outwardly robust.

Like many other diseases, sarcopenia is initially asymptomatic [Beaudart et al, 2017]. Therefore, early diagnosis and subsequent intervention are essential. This requires awareness among health-care professionals of the condition. In a recent study describing the current knowledge and practice regarding sarcopenia in a group of health-care professionals in Australia and New Zealand, only 14.7% identified sarcopenia as a disease [Yeung et al, 2020]. At baseline, 12%

reported making sarcopenia diagnosis part of their practice, and even after an educational program, this number only increased to 14.3% [Yeung et al, 2020]. Barriers to diagnosing and treating sarcopenia in this cohort of Australian and New Zealand health-care professionals were also reported. *Lack of diagnostic tools* was reported to be the main reason for not diagnosing sarcopenia. Others included it not being their role to diagnose sarcopenia, and inappropriate definitions being applied (eg. European Society for Parenteral and Enteral Nutrition malnutrition definition, or frailty scales) [Yeung et al, 2020]. These findings are in line with a previous study, which reported that the *availability of diagnostic tools was the most often-reported barrier* to implementation of diagnostic criteria among Dutch health-care professionals [Reijnierse et al, 2017].

Bioelectrical Impedance (BIA) relies of the fact that muscle, blood vessels and bones have a high water content that conducts electricity easily. Body fat is tissue that has little electrical conductivity. The scales send an extremely weak, undetectable, electrical current of 50 kHz or less and 500 μA through the body to determine relative percentage of muscle, bone, and fat. BIA equipment does not measure muscle mass directly, but instead derives an estimate of muscle mass based on this whole-body electrical conductivity. Early BIA systems used fat-free mass prediction equations developed using traditional two-compartment reference methods such as underwater weighing or total body water. Now BIA devices use a conversion equation that is calibrated with a reference of DXA, MRI or CT-measured lean mass in a specific population [Yamada et al, 2017; Gonzalez and Heymsfield, 2017].

In a recent sarcopenia review, the author concludes, “there is a pressing need to provide better diagnosis, diagnostics, prevention, and individualized health care” in sarcopenia [Papadopoulou, 2020].

This study objective was therefore to look at two types of practical, affordable, readily available, direct-to-consumer BIA BC scales, and compare their diagnostic ability for both low muscle mass, and obesity, to the less available current standard of DXA BC. There are no previous publications of studies using these particular BIA scales.

5.3 Methods

Community-dwelling Seniors participating in a 12-month observational study in Edmonton Alberta, Canada, were invited to participate. Inclusion criteria were: age ≥65 years; English speaking; independent mobility (with or without walking aids); and stable chronic medical conditions. Those with hip or knee arthroplasties were permitted. Exclusion criteria included: pacemaker or other implanted device; unstable medical conditions; stable chronic congestive heart failure; any other cause of peripheral oedema; unable to stand for 5 minutes without a walking aid and arms extended. Ethics approval was obtained through the University of Alberta Health Research Ethics Board (Pro00047132).

5.3.1 Study protocol

Body composition assessment was evaluated with the two BIA scales (*Ozeri® and Omron®*) in their baseline study evaluation. Ozeri® and Omron® BIA BC was done first thing in the morning, wearing light-wight indoor clothing. Participants were asked to have breakfast at least two hours prior to their study visit, with no extra fluids prior to the evaluation. All measurements were done with the devices on a hard, flat linoleum floor. Participants had bare feet. For both scales, participants were instructed to stand up straight, look straight ahead, barefoot with each foot over both sensors, weight evenly distributed, and with no bent knees. For the Ozeri® arms are by the side. For the Omron® scale the arms are also held out straight, raised horizontally to 90^{o}, with the display facing upwards. (See **Appendix Figure 6**)

Within the next 2 weeks participants had a *DXA body composition* evaluation. DXA BC evaluations were done in a standardized way by trained radiology technologists at a Medical Imaging Consultants site in Edmonton. Repeat DXA evaluations were done at the same site as the initial test.

Anthropometric data was evaluated included *waist and hip measurements, arm and calf circumferences* per standard protocol. *Skin fold thickness* was measured using skinfold calipers in millimetres (mm) at 3 body sites: scapula, anterior pelvis, and triceps as per instruction manual [Wallace C. Donoghue, Creative Health Products, Ann Arbor, Michigan, Thirty-Sixth printing August, 2012]. Percentage fat was calculated using age and sex specific population charts based on total 3-site mm measurements.

Height measurement, was done both at the study visit (using standard professional medical-grade equipment (see below), and at the time of the DXA BC, using a wall attached stadiometer.

5.3.2 Study equipment details

The BIA scales chosen were for convenience, availability, and cost. The Ozeri® and Omron® BIA scale equations used to calculate the reported parameters are considered proprietary, and so are not currently available.

A. The **Ozeri ® Touch Total Body Scale** (China) combines advanced algorithms (considered proprietary, so not specified) with BIA incorporating a person's age, height, sex, and weight, for its measurements. Height, age, and sex are entered into the scale. It has 4 high precision GX sensors, with a maximum weight of 200kg (440lbs), and is safe for use in those with a pacemaker. It reports weight, percentage fat, percentage "muscle", percentage "bone", and hydration (percentage water). Approximate purchase cost is $72-$95. See **Figure 1.**

Figure 1: Ozeri® Touch Total body scale.

B. Omron HBF-510® Full Body Sensor Body Composition Monitor and Scale manufactured for Omron Healthcare Co. Ltd [Shiokoji Horikawa, Shimogyo-ku, Kyoto 600-8530 Japan]. Unlike other body composition monitors that rely on foot-to-foot measurements, Omron's monitor measures the whole body (arm to foot), with 8-sensors (four feet and 4 grip sensors). Omron's algorithm focuses on the Bioelectrical Impedance Method as well as height, weight, age, and gender. Height, age, and sex are entered into the scale. It reports weight, percentage body fat, body mass index (BMI), percentage "skeletal muscle" and percentage "visceral fat" (estimated as a relative

value and not an absolute value). The Omron® Full Body Sensor Body Composition Monitor and Scale differs from the Ozeri® in that it takes measurements from both hands and feet, so theoretically reduces the impact of diurnal influenced water movement on the body composition results.

Maximum weight is 150kg (330 lb) and height 1.68m (6.5 feet), and use with a pacemaker or other implanted device is not recommended. Approximate purchase cost is $132-152. See **Figure 2**.

Figure 2: Omron® HBF-510 Full Body Sensor Body Composition Monitor and Scale

DXA body composition: Hologic® Discovery DXA, Bedford MA/ USA, was used and tests performed by DXA-trained radiology technologists from Medical Imaging Consultants Diagnostic Imaging, Edmonton, Canada. The dose of DXA ionising radiation is similar to normal background radiation received over one day at sea level [Shepherd et al, 2017]. Participants lay on the DXA table and were positioned according to standard protocol with their feet internally rotated and secured in a device. The whole body is scanned to measure whole body bone mass and soft tissue composition [Laskey, 1996]. The DXA software measures the following parameters: bone mineral content (BMC) in grams (g), projected bone area (AREA) in cm^2, bone mineral density (BMD = BMC/AREA) in g/cm^2, headless BMC (SubBMC) in g, headless area (SubAREA) in cm^2, headless BMD (SubBMD) in g/cm^2, lean soft tissue mass (LSTM) in g, fat mass (FM) in g, total mass (TM = LSTM + BMC + FM) in g, and percent fat (PFAT = FM/TM x 100). Additional parameters are derived from these standard regions,

including appendicular lean soft tissue mass (ALSTM = arms soft tissue mass + legs soft tissue mass), android percent fat (AndroidPFAT), gynoid percent fat (GynoidPFAT), and android and gynoid percent fat ratio (A/G ratio) [Shepherd et al, 2012]. ALST is a fat and bone mineral-free component that includes muscle and other components such as skin, tendons, and connective tissues [Fuller et al, 1992]. Skeletal muscle (SM) constitutes the largest fraction of ALST (approximately 74%) [Heymsfield et al, 1990], but with aging, SM decreases when other components such as connective tissue and the lean portion of adipose tissue increase, so DXA ASM models adjust for age and sex [Kim et al,2002].

Appendicular skeletal muscle mass (ASM) and percentage fat are the only DXA parameters reported in this study. ASM has been shown to accurately quantify skeletal muscle mass in vivo [Heymsfield et al, 1990], and has been validated against Magnetic Resonance Imaging [Kim et al, 2002]. Because muscle mass is correlated with body size, once ASM has been calculated as the sum of upper and lower limb ASLT, it is then adjusted for the height of the individual and reported as ***ASM/height^2,*** which is the parameter used by EWGSOP to identify normal versus low muscle mass. Appropriate corrections were made for the presence of a hip and/or knee arthroplasty when calculating total ASM.

Skinfold calipers: Creative Health Slim Guide 696251 Skinfold Caliper, Michigan, USA.

Height assessment: Seca® wall-mounted stadiometer.

5.3.3 Study cut-offs used

For **DXA BC**, low muscle mass was defined as ***ASM/height^2 ≤ 7.0 kg/m^2 in males and ≤ 5.5 kg/m^2 in females.*** The EWGSOP consensus group defined these cut-offs as valid cut-offs associated with clinical outcomes [Cruz-Jentoft et al, 2019]. Using similar, but not identical cut-offs, Bischoff-Ferrari and colleagues compared nine different definitions of sarcopenia varying by threshold values for appendicular lean mass index (ALMI = ASM/height^2) combined with different strength measures. She showed the sarcopenia definition cut-offs by Baumgartner [Baumgartner et al, 1998] of ALMI < 7.26 kg/m^2 (male) and 5.45 kg/m^2 (female), gave the best prevalence and probability of falls in a prospective study of community dwelling males and females [Bischoff-Ferrari et al, 2015].

For **BIA BC,** Ozeri® and Omron® derived muscle mass percentage was converted to kilograms (kg). The BIA-predicted skeletal muscle mass (SM) equation (SM/height^2) was then calculated. The cut-offs used were based on -2 standard deviations below the mean of young adults, ***males: <8.87 kg/m^2; females <6.42 kg/m^2*** as recommended by the EWGSOP and Asian Working Group, validated in older European and Asian populations [Cruz-Jentoft et al, 2010; Chien et al, 2008; Janssen et al, 2000; Yu et al, 2016].

Obesity was defined as a body fat composition of ***>25% in males, and >35% in females*** [Cruz-Jentoft et al, 2019] for both DXA and BIA scales.

5.3.4 Statistical analysis

Data analysis was completed using SAS 9.0 statistical software (SAS, Version 9.4; SAS 124 Institute Inc., USA). Data was expressed as mean ± SD for variables showing normal distributions and/or median [interquartile range] for non-parametric variables. The Shapiro-Wilk test was conducted to assess the normality of distribution. Bland Altman was used to assess agreement between Ozeri ®, Omron ® and DXA derived values for % fat mass. Pearson correlations were also performed, and the Phi coefficient of correction was applied for comparisons between reported low muscle mass [Guilford, 1941]. Sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) were determined to evaluate the performance of the surrogate muscle mass measures for correctly identifying low muscle mass in older adults, using DXA as the reference method [Florkowski, 2008]. A difference with a p value < 0.05 was considered significant.

5.4 Results

Of the 50 participants enrolled in the study, 11 were male and 39 female. All were independent of basic activities of daily living at baseline, and most instrumental activities (some needed assistance with driving, finances). **Table 1** shows the demographic data of the participants.

Table 1: Baseline demographic data by sex. (Data are mean ± SD and/or median (interquartile range), where * denotes statistical significance.

Variable name	Male (n=11)	Female (n=39)	P value
Age (years)	78.9 ± 5.1	74.9 ± 4.6	0.02*
Weight (kg)	76.1 ± 12.2	72.7 ± 12.6	0.09
Height (m)	173 ± 7.0	159 ± 5.4	<0.00*
BMI	28.9 ± 4.8	27.8 ± 5.4	0.53
Waist to hip	1.00 ± 0.06	0.84 ± 0.08	0.001*
% Fat (skinfold)	28.9 ± 5.8	41.3 ± 5.9	<0.001*
% Fat (DXA)	30.8 ± 5.2	40.7 ± 6.8	0.004*

Prior to DXA BC evaluation, 8 dropped out (3 males): 6 no longer being interested after visit one; 1 due to caregiver responsibilities; and 1 due to declining physical health. Forty-two participants completed the DXA body composition.

By EWGSOP diagnostic criteria (ASM/height^2) with DXA, 15 (5 males, 10 females) were classified as low muscle mass.

Using BIA cut-offs [Chien et al, 2008] (muscle mass/height^2) with Ozeri® 7 (4 males, 3 females), and Omron® 27 (7 males and 20 females) had low muscle mass. See **Figure 3.**

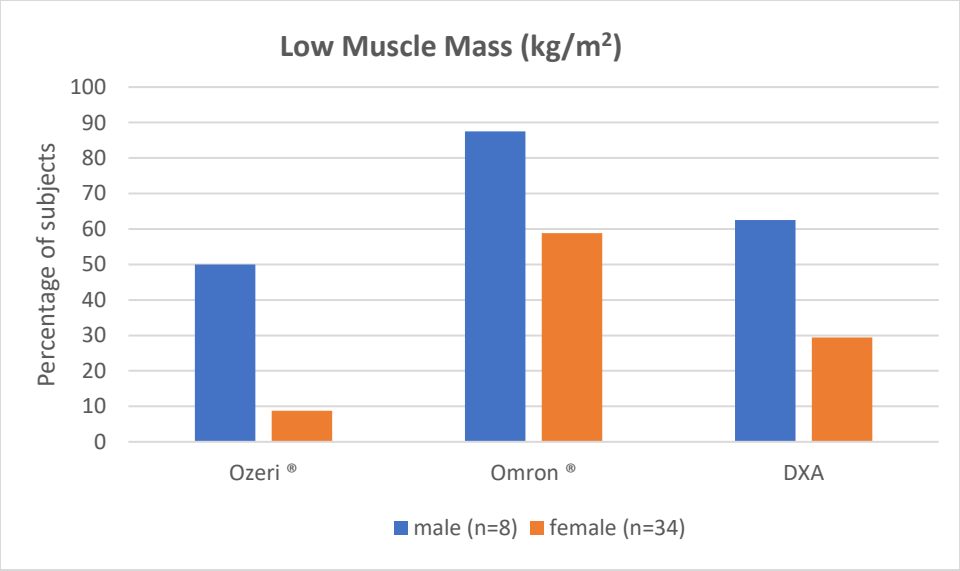


Figure 3: Comparisons between DXA and BIA scales (Ozeri ® and Omron®) for identification of low muscle mass.

Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) for the diagnosis of low muscle mass with the BIA scales versus DXA BC are shown in **Table 2**.

Table 2: Percentage of diagnostic specificity, sensitivity, and predicative value of BIA scales Ozeri ® and Omron ® versus DXA body composition for low muscle mass.

	Specificity (%)	Sensitivity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)	Positive Likelihood ratio	False omission rate (1-NPV)
Ozeri ®	92.6	40	75.2	73.3	73.8	5.4	26.7
Omron®	48.2	93.3	50.3	92.8	64.3	1.8	7.2

The Phi coefficient of correlation between DXA and BIA scales for low muscle mass is shown in **Tables 3: A and B.** Ozeri ® versus DXA R^2 0.16, and Omron ® R^2 0.18. The Phi value comparing Ozeri ® and Omron ® was 0.343, R^2 0.12, p=0.026. Phi values >0.310, for this number of participants, may be considered significant associations, with those >0.407 very significant [Guilford, 1941]. But, other references would call this correlation weak, with strong being 0.7-1.0

The Cohen's kappa is a statistical coefficient that represents the degree of accuracy and reliability in a statistical classification. It measures the agreement between two raters (judges) who each classify items into mutually exclusive categories [Landis, JR & Koch, GG, 1977] The % agreement using this analysis between DXA diagnosis of low muscle mass and Ozeri® is 73.8% with κ 0.36, and Omron® 64.3% with κ 0.34, both suggesting fair agreement.

Table 3A: showing correlation between diagnostic cut-offs for low muscle mass between DXA Body composition and Ozeri ® and Omron® BIA scales (* denotes statistical significance)

	Phi coefficient of correlation	P value
Ozeri® low muscle mass	0.398	.01*
Omron ® low muscle mass	0.422	.006*

Table 3B: Correlation between diagnostic cut-offs for low muscle mass between Ozeri ® and Omron® BIA scales (* denotes statistical significance)

	Phi coefficient of correlation	P value
Ozeri® vs Omron®	0.343	.026*

Obesity, based on DXA body fat cut-offs, was present in 81% of the participants. The comparison between 3-site skinfold measurement, Ozeri®, Omron® and DXA for percentage body fat diagnostic cut-offs for obesity, is shown in **Figure 4.**

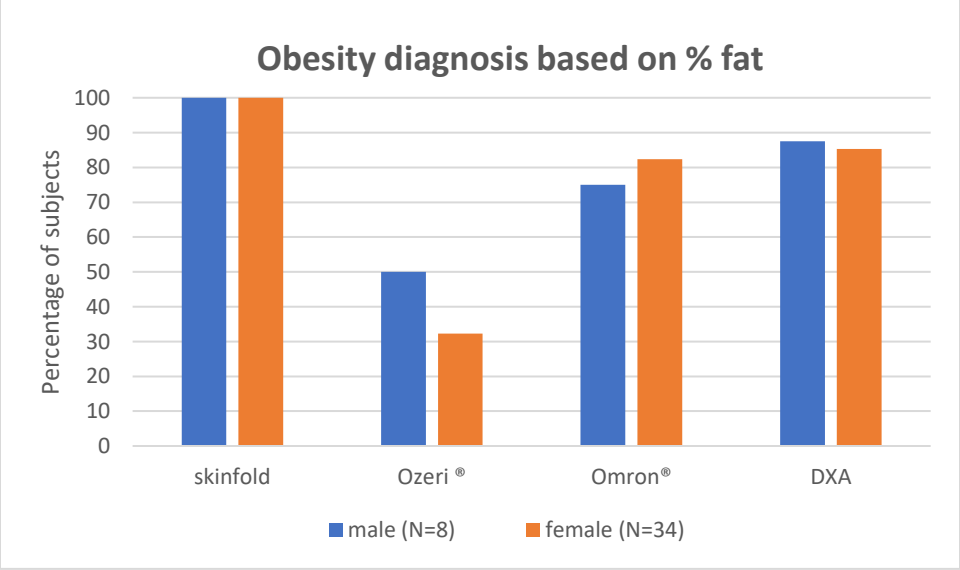
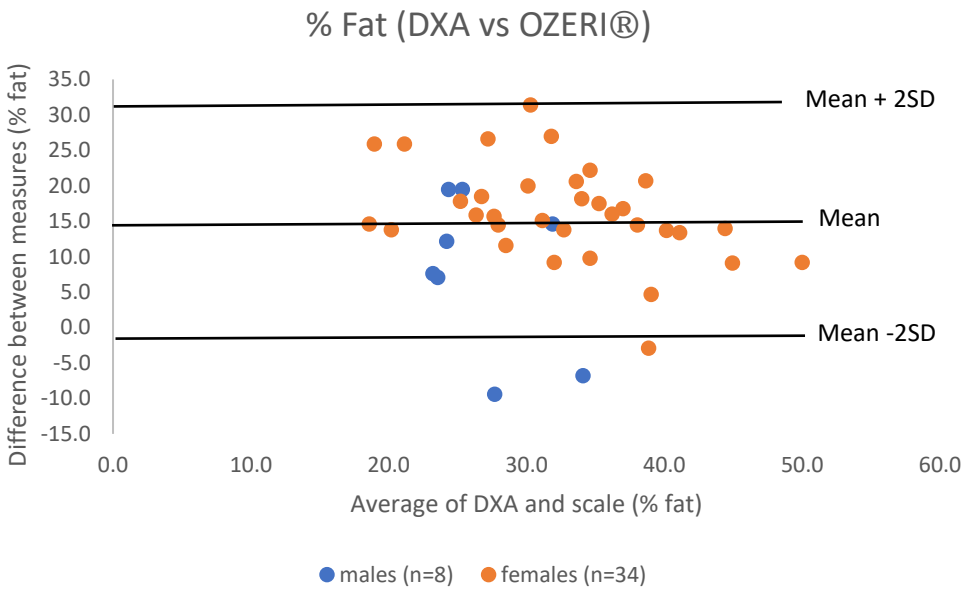


Figure 4: Comparison between 3-site skinfold measurement, BIA scales (Ozeri ® and Omron®), and DXA for the diagnosis of obesity

There was good agreement between DXA BC and BIA scales for % fat cut-offs, but the agreement was better for the Omron® scale. See **Figures 5 A and B** for Bland Altman graphs with Ozeri ® and Omron®.

(A)



(B)

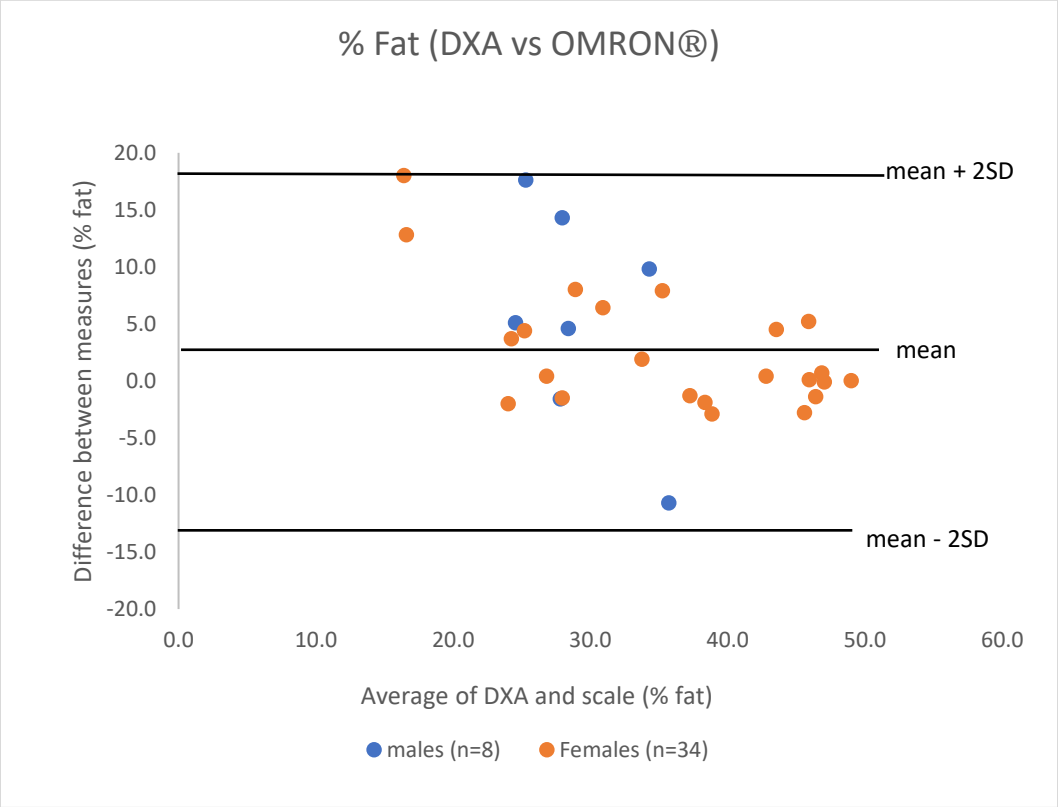


Figure 5: Bland Altman calculation comparing DXA Body Fat composition and, (A) Ozeri® BIA scale, and (B) Omron ® BIA scale.

5.5 Discussion

Sarcopenia is characterised by early decreased muscle size, followed by reduced muscle quality with increased intramuscular fat, fibrosis, altered muscle metabolism, oxidative stress, and degeneration of the neuromuscular junction, all of which result in progressive loss of muscle function [Ryall et al, 2008]. The definition of sarcopenia with cut-offs for low muscle mass is now well delineated by several consensus groups, yet clinical assessment remains limited, particularly in community settings [Bauer et al, 2019].

Comparing DXA derived muscle mass and BIA derived muscle mass is felt to be valid [Sergi et al, 2017], and has been done in other older adult populations, and BIA has been found to be a valid surrogate when DXA, MRI or CT are not practical [Yamada et al, 2017]. Although the latter are more accurate methods of assessing low muscle mass, they need specially trained users, are expensive, require a large amount of time needed to perform the test (MRI), and potentially have adverse events such as radiation exposure (CT and DXA), hence the interest in BIA as a more practical and portable alternative.

There are no other studies in the literature, to our knowledge, using these particular direct-to-consumer BIA tools. Even when using sophisticated BIA devices (Akern ® BIA single frequency device) different participants were identified as sarcopenic versus non-sarcopenic when compared to DXA BC [Reiss et al, 2016]. Using the same BIA cut-offs as this study, misclassification of 1 out of 6 participants (false positives) occurred. The agreement for classifying participants as having normal or reduced muscle mass was, at best, 80% depending on the BIA cut-offs used [Reiss et al, 2016]. This misclassification has also occurred when using even more sophisticated BIA tools (ImpediMed DF50 single-frequency device) in a research laboratory setting [Bosaeus et al, 2014]. The published misclassification is similar to that suggested in this study when using the Omron® BIA scale, which also overestimated low muscle mass, but is unlike the Ozeri® which underestimated low muscle mass. The issues with different BIA devices is that the various BIA prediction models for fat free mass differ according to the characteristics of the sample in which they have been derived and validated. Ideally a BIA device needs to be validated in the specific age and ethnicity being investigated [Sergi et al, 2017]. The BIA devices (Ozeri ® and Omron®) in this study both required input of the participants' sex, and there were noticeable differences between males and females. However, the number of males in this study was small, and so this sex-specific data needs to be interpreted with caution.

A high sensitivity test refers to a better ability to correctly identify individuals with low muscle mass, while a high specificity test is important to correctly identify people without low muscle mass [Florkowski, 2008]. Therefore, an ideal test needs to be both highly specific, and highly sensitive. In this study, the Ozeri ® scale was found to have a high specificity for low muscle mass (92.6%) but low sensitivity (40%), versus the Omron® scale having low specificity (48.2%), but high sensitivity (93.3%). Clinically the latter would be more useful as fewer cases

would be missed (negative predictive value 92.8%). The phi coefficient is a measure of the association between two dichotomous variables, and in this study the values suggested a slightly higher level of correlation between Omron® and DXA muscle mass assessment, than Ozeri® and DXA, although both were only considered fair. This suggests, at least in the population studied, that the Omron® device was more representative of the information provided on muscle mass by DXA. The specificity and sensitivity of the Omron® are very similar to that for the sarcopenia screening tool (SARC-F) which are 14-21% and 90-94% respectively [Ida et al, 2018], so the Omron® is at least as reliable as the SARC-F in the population evaluated in this study.

For assessing the absolute percentage fat measures (as shown in the Bland Altman graphs), the Omron® again performed better then the Ozeri® when compared to DXA percentage fat assessment.

The availability of an objective, in-office tool, which can be utilised by all healthcare professionals, is likely to enhance the consideration for, and identification of, low muscle mass. Research in every disease has shown that once a test is shown to be abnormal, this is more likely to prompt further appropriate investigations and management [Majumdar et al, 2014]. Early recognition and intervention is key to improving outcomes, so should be a "routine part of healthcare visits" in Seniors [Dhillon and Hasni, 2017; Morley et al, 2014]. Until this early recognition and awareness happens, it seems unlikely that educational interventions alone will have much impact [Yeung et al, 2020].

5.6 Limitations

All participants were Caucasians, living in one city, this may affect the applicability of the results to other populations. The Taiwanese sex-specific low muscle mass cut-offs were used, as these are the ones recommended by the EWGSOP consensus group, and have been used by other research groups in non-Asian populations [Graf et al, 2017]. The study group was predominantly female. Because of the small number of males, there was insufficient power to detect sex

differences for the sensitivity and specificity of the BIA scales. In addition, the BIA algorithms used by the Ozeri ® and Omron® BIA scales to calculate the different components of body composition are unknown, as these are considered proprietary and are not disclosed.

5.8 Conclusions

The challenge of assessing muscle mass remains given the recommended standard of DXA, MRI or CT body composition**.** This study attempts to address that limitation by providing data to show that direct-to-consumer BIA BC scales can provide rapid, useful clinical information on both muscle and fat mass, and raise the suspicion of sarcopenia, particularly in patients with concomitant obesity. The presence of an in-office scale may serve to remind practitioners of the importance of objective muscle mass assessment. The Ozeri® scale had high specificity but low sensitivity, and the Omron® scale low specificity but high sensitivity, for low muscle mass when compared to DXA BC. Both scales have only fair correlation with DXA, but the Omron® has similar sensitivity and specificity to a validated, recommended sarcopenia screening tool, the SARC-F.

CHAPTER 6

Observational cohort study of healthy community-dwelling older adults followed for 12-months to assess the association of lifestyle on sarcopenic status.

Juby AG, Davis CMJ, Minimaana S, Mager DR. Observational cohort study of healthy community-dwelling older adults followed for 12-months to assess the association of lifestyle on sarcopenic status. 2022 (submitted to a peer-review journal)

6.1 Abstract

Background: The rate of sarcopenia varies depending on the cohort evaluated. Most studies have been done in "vulnerable" populations. Very few studies have been done on successfully aging community-dwelling older adults (Seniors), and few have been done in a Canadian population

Objectives*:* To prospectively evaluate the sarcopenic status of healthy community-dwelling older Canadian adults; whether this changed over 12-months; and the association with self reported leisure activity.

Methods: Community-dwelling older adults were invited to participate in a 12-month observational study. All were independent of all basic activities of daily living at baseline, and most instrumental activities (some needed assistance with driving, finances). They were assessed for physical function (TUG, SPPB, gait speed, Tinetti, grip strength), muscle mass (DXA, arm and calf circumference), body fat (skinfold, DXA), reported daily exercise (aerobic, resistance), and laboratory parameters.

Results: Of 50 participants, 11 were male and 39 female. Average age was 75.8 (67-90) years, and BMI was 28 (18.8-39.2). Average MMSE and MoCA cognitive scores were 28.1(20-30) and 24.8 (14-30) respectively. Eight participants dropped out prior to their first DXA test. Of the remaining 42, 17 participants (5 male) fulfilled the EWGSOP revised criteria for probable, pre-

sarcopenia, or sarcopenia, giving a rate of total sarcopenia of 40.5% in this community-dwelling sample. The majority were pre-sarcopenic (28.6%), and sarcopenia was present only in 7.1%. The total sarcopenia group had a lower BMI (25.6 ± 5.1 versus 29 ± 5, p=0.01), less body fat by skinfold measurement (36.4 ± 6.5 versus 39.3 ± 8.1, p=0.01) and lower mid-calf (35.6 ± 3.2 versus 37.6 ± 3.4, p=0.04) and mid-arm (29.1 ± 2.5 versus 31.9 ± 3.5, p=0.02) circumferences when compared to their non-sarcopenic peers. After 12-months, 39 participants remained in the study. Of these, the sarcopenic status of 7 improved, 10 declined, with the remaining majority not changing. There were no statistically significant differences in baseline laboratory parameters between the groups, including 25(OH)D status. But, of the status decliners, 40% had suboptimal 25(OH)D at baseline. Some participants attended a social group activity class. Those with sarcopenia attending the class had improvements in balance and total Tinetti scores, compared to those not attending, or those not in the total sarcopenia group.

Conclusions: The rate of sarcopenia was 7.1%, but the total rate of pre, probable and sarcopenia in this highly functioning, community-dwelling older adult cohort was 40.5%, In the majority (75%), there was either no change, or an improvement, in their sarcopenic status over 12-months. There was no association identified with leisure activity.

6.2 Background

Like many other diseases, sarcopenia is asymptomatic in its initial stage [Beaudart et al, 2017]. The prevalence of sarcopenia reported in the literature varies according to the population being studied. Community-dwelling prevalence is anywhere from 2.5% to 36.5% depending on the diagnostic criteria used, and the population studied. [Patel et al, 2013; Brown et al, 2016; IC et al,2014; Kim et al, 2015]. The varied definitions have resulted in confusion among community practitioners not specialising in sarcopenia. They may therefore have adopted a rather nihilistic approach to making the diagnosis of sarcopenia, despite being aware of clear evidence of the significant effect on morbidity and mortality [Brown et al, 2016]. Diagnostic tools have recently been updated to focus on muscle function over muscle mass and strength [Cruz-Jentoft et al,

2019]. This helps with their practical applicability allowing use of the chair stand test for both muscle strength and function [Nishimura et al, 2017].

Community-dwelling individuals are usually more physically active and have a better dietary regimen [Grammatikopoulou et al, 2007; Papadopoulou et al, 2003], and should have less sarcopenia. Nursing home residents report more sedentary activities, were less likely to report being currently physically active, and were also more likely to be malnourished [Senior et al, 2015; Dodds et al, 2017]. Alcohol consumption is often less apparent in elderly women, yet is an added risk for sarcopenia [Yoo et al, 2017]. Community-dwelling individuals are more likely to be sarcopenic in situations where they are less physically active and lack good nutritional status [Yu et al, 2014; Neto et al, 2016; Beaudart et al, 2015; Yoshida et al, 2014; Legrand et al, 2013].

Physical exercise, especially including resistance training has been shown to be the most beneficial intervention in prevention of sarcopenia [Nelson et al, 1999] and preventing its progression [Friedman and Trappen, 1991]. Ideally, exercise training should include both aerobic and resistance exercises to improve muscle mass and strength. [Rolland et al, 2008]. But studies have also shown that resistance training alone [Jones et al, 2021], and aerobic exercise alone can improve muscle mass and strength, in addition to it's cardiorespiratory and metabolic benefit [Crane et al, 2013].

Exercise programs in clinical trials are usually supervised by trained exercise therapists, are intensive and personalised, which makes them hard to replicate in a non-research setting. General leisure activity is felt *not* to be sufficient to prevent decline in muscle mass [Raguso et al, 2006]. This was also found by Rojer and colleagues, who noted an association with self-reported physical activity and muscle strength, but not muscle mass [Rojer et al, 2018]. However, this has not been evaluated in a Canadian community dwelling population.

6.3 Objectives

The primary objective was to evaluate the sarcopenic status of a group of independently functioning community-dwelling older adults, followed over 12-months, to evaluate whether their sarcopenic status changed over the 12-months.

Secondary objectives were to evaluate at baseline and over 12-months: cognitive performance; physical performance; laboratory parameters; quality of life: rate of obesity; the amount of self-reported exercise over 12-months; attendance at an optional group social activity program over 12-months.

6.4 Methods

6.4.1 Participants

This is a 12-month prospective, observational, cohort study, of community-dwelling older adults (aged over 65-years) residing in the greater Edmonton area in Alberta, Canada. Participants were recruited by direct contact, posters in the clinic, and by advertisements through Seniors community organizations. *Inclusion criteria* were: age ≥65 years; English speaking; independent mobility (with or without walking aids); and stable chronic medical conditions (eg, diabetes, hypertension, hypothyroidism, depression). *Exclusion criteria* included: a pacemaker or implanted device; chronic peripheral oedema; and unstable medical conditions.

Ethics approval was obtained through the University of Alberta Health Research Ethics Board (Pro00047132).

6.4.2 Study protocol

Participants had *evaluations at baseline, 6-months, and 12-months*, at the same site, by the same testers.

Each study visit included:

Comprehensive Geriatric Assessment and *cognitive evaluations (Mini-mental status examination (MMSE)* [Folstein and Folstein, 1975] and *Montreal Cognitive Assessment (MoCA)* [Nasreddine et al, 2005]).

Grip strength assessment using an Almedic® dynamometer per standard protocol [Roberts et al, 2011; Iley et al, 2014].

Physical function with *Timed Up and Go (TUG)* [Podsiadlo and Richardson, 1991], *Short Physical Performance Battery (SPPB)* [Pavasini et al, 2016]), *4-square step test* [Langford, 2015] and *gait speed (10m walk test)* [Graham et al, 2008], and *Tinetti Gait and Balance scale* [Tinetti et al, 1986].

Height measurement using a medical-grade Seca® stadiometer.

Waist and hip circumference to enable calculation of waist/hip ratio using a medical grade measuring tape.

Arm and calf circumference (cm) by standard protocol following the published guidelines [Geneva: WHO, 1995; Santos et al, 2019].

Body fat (mm) by *3-site skinfold thickness* (using Creative health products, Michigan, skinfold calipers) following standard protocols [Wagner and Heyward, 1999] at all visits. Skinfold thickness was measured at subscapular, suprailiac and triceps, and the total thickness in mm combined. Using standardised charts (based on the Jackson Pollock equations), corrected for age and sex, percentage body fat was estimated [Wagner and Heyward, 1999]. *DXA body composition percentage fat* was assessed only at baseline and 12-months.

Nutrition risk using the NSI checklist "Determine Your Nutritional Health" [Barrocoas et al, 1995].

Quality of life using Euroqol-5D validated questionnaire [Balestroni et al, 2012].

Physical Activity record to report average daily exercise routines. This included type, length of time, and frequency of exercise. Based on the reports it was classified as aerobic (eg. Walking, golf, pickle ball), resistance (eg. Weightlifting or resistance band exercises) or both (eg. Yoga). If they also attended the study group activity class this was included in their total exercise time. The few with short term memory issues were aided by their caregiver to complete this task.

Laboratory parameters (See **Table 5** for details). The laboratory work was performed as part of routine clinical care and was analyzed in the Laboratories of Alberta Health Services according to standard methodology.

At baseline and 12-months only

Muscle mass by dual energy X-ray absorptiometry (DXA) (Hologic® Discovery DXA, Bedford MA/ USA) body composition (only at baseline and 12-months). Tests performed by DXA-trained radiology technologists from Medical Imaging Consultants Diagnostic Imaging, Edmonton, Canada.

6.4.3 Seniors Group Activity Class (optional)

Participants continued their normal activities, but were also invited to participate in a twice weekly 60-minute group activity program if they so chose. This consisted of a ≈5-minute warm-up (general movement to music), 10 to 15-minutes balance training (such as: toe-to toe walk forwards and backwards; one-legged toe curls and toe lifts; yoga "tree" pose; and yoga "warrior 3" pose), ≈10-minutes of aerobic exercises (high intensity interval exercises, 2 x 2-minute bursts), ≈15-minutes of resistance band exercises (such as: biceps curls; triceps extension; shoulder abduction; latissimus dorsi pull-downs; upper back/posture exercises; leg extension and flexion; and leg lifts), ≈10-minutes stretching (upper and lower body), posture, and ≈10-minutes relaxation (mindfulness or meditation), and breathing (yoga-based) activities.

Any transportation costs incurred to attend the classes were reimbursed, to ensure that cost was not an impediment if they wished to attend. Group activity class attendance was documented.

At the conclusion of the study, all participants (even those who never attended the activity class) were provided with a video that included: the total 60-minute activity class; 15-20 minute videos of specific aerobic, resistance, stretching and relaxation components. They also received a manual with all the exercises explained, including some general health and nutritional advice. (Cover images shown in **Appendix Figure 7**).

6.4.4 Study cut-offs and definitions

For **DXA**, **low muscle mass** was defined as calculated appendicular lean muscle mass ÷ height^2 (ALM/ht^2) as ≤ 7.0 kg/m^2 in males and ≤ 5.5 kg/m^2 in females [Cruz-Jentoft et al, 2019].

Obesity was defined as a *body fat composition* of >25% in males, and >35% in females [Cruz-Jentoft et al, 2019] for DXA and total skinfold thickness. For *BMI*, obesity was defined as BMI >30.

Physical performance evaluation was using gait speed. Based on European Working Group on Sarcopenia in Older People (EWGSOP) defined low gait speed as a cut-offs of ***gait speed*** <0.8m/s [Cruz-Jentoft et al, 2019].

Physical strength was assessed using ***grip strength*** EWGSOP2 defined cut-offs of *<16kg (females) and <27kg (males)* for low grip strength [Cruz-Jentoft et al, 2019].

"Sarcopenic group" included all participants classified by EWGSOP2 guidelines as *pre-sarcopenia (low muscle mass only), probable sarcopenia, sarcopenia,* or *severe sarcopenia.* (See **Table 1**). Those with just low gait speed were classified as "slow", and as there is no classification for them under the EWGSOP2 criteria, and were included in the "non sarcopenic" group.

Table 1: Definition of Sarcopenia categories (based on EWGSOP 2018 consensus [Cruz-Jentoft et al, 2019] plus "slow classification).

	Low muscle strength	**Low muscle mass (quantity) or quality**	**Low physical performance**	**Obesity**
Pre-sarcopenia	0	√	0	
Probable sarcopenia	√	0	0	
Sarcopenia	√	√	0	0
Severe sarcopenia	√	√	√	0

Sarcopenic obesity	√	√	√ or 0	√
"Slow"	0	0	√	

(√ denotes present, 0 denotes absent).

6.4.5 Statistical analysis

Data analysis was done by another member of the team who was not involved in the data collection, who had no knowledge of the individual participants, or their attendance at the classes, to prevent any bias during the analysis.

Data analysis was completed using SAS 9.0 statistical software (SAS, Version 9.4; SAS 124 Institute Inc., USA). Data was expressed as mean ± SD for variables showing normal distributions and/or median [interquartile range] for non-parametric variables The Shapiro-Wilk test was conducted to assess the normality of distribution. At baseline, t-tests (parametric) and/or Mann Whitney tests (non-parametric variables) were done to determine differences in anthropometric, demographic and laboratory variables between sarcopenic vs non-sarcopenic participants. Paired t-tests (parametric variables) and Wilcoxon tests (non-parametric) were performed to determine differences between baseline and 12-month time points for anthropometric, demographic, laboratory, functional measures, and body composition. In addition, univariate and multi-variate correlations were performed to determine associations between physical activity and outcomes of interest (body composition, functional tests). Analysis of co-variance for potential confounding variables (sex, age) was used where needed. Chi-square tests (or when necessary, Fisher Exact test) were used to measure differences in categorical data. A difference with a p value < .05 was considered significant.

6.5 Results

6.5.1 Participants

Fifty-seven participants expressed interest in the study. Seven were unable to participate as they were "snowbirds" and would miss the six-month visit. Of 50 participants enrolled, 11 were male and 39 female. All were independent of basic activities of daily living at baseline, and most instrumental activities (some needed assistance with driving, finances). **Table 2** shows their baseline information.

Table 2: Baseline demographic data by sex. Data are mean ± SD and/or median (interquartile range), where * denotes statistical significance.

Variable Name	Male (n=11)	Female (n=39)	P value
Age (years)	78.9 ± 5.1	74.9 ± 4.6	0.02*
MMSE MoCA	27± 2.8 22.3 ± 4.2	28 ±2.4 25.6 ± 4.4	0.10 0.04*
Weight (kg)	76.1 ±12.2	72.7 ± 12.6	0.09
Height (m)	173 ± 7.0	159± 5.4	<0.00*
BMI	28.9 ± 4.8	27.8 ±5.4	0.53
Waist to hip	1.00 ± 0.06	0.84 ± 0.08	0.001*
% Fat (skinfold)	28.9 ±5.8	41.3±5.9	<0.001*
% Fat (DXA)	30.8 ± 5.2	40.7 ± 6.8	0.004*
Gait speed (m/s) Grip strength	1.5 ± 0.9 31.4 ± 6.7	1.4 ± 0.3 20.7 ± 5.2	0.53 <0.001*

Eight dropped out (3 males): 6 no longer being interested after visit one; 1 due to caregiver responsibilities; and 1 due to declining physical health. Of the remaining 42 participants, the average age was 75.8 (67-90) years, and BMI was 28 (18.8-39.2). Average MMSE cognitive scores were 28.4/30 (20-30) (normal adjusted score 22-26/30). For the MoCA, the average was 25.7/30 (14-30), normal score ≥26/30.

Forty-two completed the DXA body composition at baseline (8 males and 34 females).

6.5.2 Sarcopenic status at baseline

Participants were classified as *normal, slow* (defined by low physical performance only), pre-*sarcopenic, sarcopenic* or *severely sarcopenic* as per EWGSOP2 criteria (see **Table 1**).

Using DXA cut-offs (ALM/ht^2), 17 had low muscle mass at baseline. These participants (5 males and 12 females) were in the "total sarcopenia group", fulfilling the criteria for sarcopenia which included all the stages from pre-sarcopenic to sarcopenic. There were no severe sarcopenic participants at baseline, and 2 females classified as slow.

Twenty-three were classified as normal (54.8%), 3 males and 20 females.

As a cohort, and not a cross-sectional study, sarcopenia presence or absence is reported as rates and percentages, and not prevalence. The percentage of participants in the *"total sarcopenia group" (probable + pre + sarcopenic + severe* sarcopenic) was **40.5%** of whom **28.6%** had *pre-sarcopenia*. The number with *sarcopenia only* (sarcopenic + severe sarcopenic) was **7.1%.** See **Figure 1.**

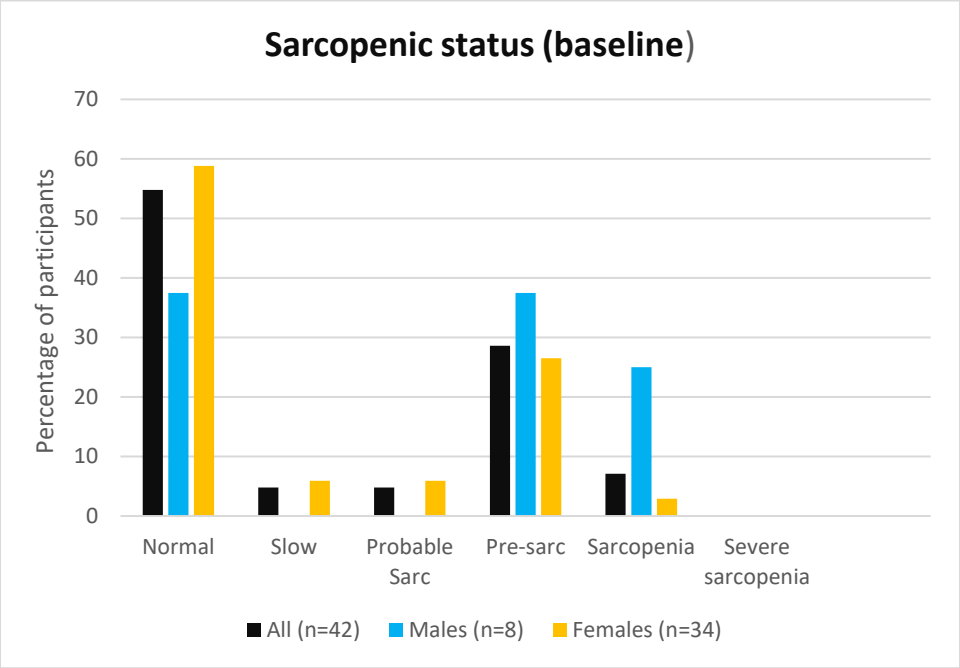


Figure 1: Sarcopenic status at baseline.

Table 3 shows the *baseline* demographic features between the total sarcopenia and non-sarcopenia participants.

Table 3: Anthropometric and Demographic data at baseline.

Variable Name	Total Sarcopenia group (includes probable, pre-, and sarcopenic) (n=17)	Non-Sarcopenia group (includes "slow" and normal) (n=25)	P value
Sex (M/F)	5/12	3/22	0.82
Age (years)	76.6 ± 4.3	75 ± 5.2	0.13
Weight (kg)	64.8 ±11.9	72.7 ± 12/6	0.02
Height (cm)	162 ± 7.3	163 ± 8.2	0.31
BMI	25.6 ± 5.1	29 ± 5	0.01*
Waist to hip ratio	0.85 ± 0.1	0.89 ± 0.1	0.45
Fat (skinfold) (%)	36.4 ± 6.5	39.3 ± 8.1	0.01*
Fat (DXA) (%)	38 ± 6.4	39.2 ± 8.4	0.29
Calf circumference (cm)	35.6 ±3.2	37.6 ± 3.4	0.04*
Arm circumference (cm)	29.1 ± 2.5	31.9 ± 3.5	0.02*
Total skinfold (mm)	74.7 ± 26.2	95.7 ± 38.9	0.006*

(*denotes statistical significance)

Sarcopenia group status was not associated with baseline cognitive function, MMSE (p=0.47) and MoCA (p=0.78), or IADL (p=0.09). The sarcopenia group had a *lower BMI* (25.6 ± 5.1 versus 29 ± 5, p=0.01), *less body fat* by skinfold measurement (36.4 ± 6.5 versus 39.3 ± 8.1, p=0.01), and *lower calf* (35.6 ± 3.2 versus 37.6 ± 3.4, p=0.04) and *arm* (29.1 ± 2.5 versus 31.9 ± 3.5, p=0.02) *circumferences* when compared to their non-sarcopenia peers. See **Tables 3 and 4.**

Table 4: Baseline cognitive, functional, and quality of life status.

Variable	Total Sarcopenia group (includes probable, pre-, and sarcopenic) (n=17)	Non-Sarcopenia group (includes "slow" and normal) (n=25)	P-value
Cognition			
MMSE **	28.4 ± 1.9	28 ± 2.7	0.35
MOCA ##	24.5 ± 2.9	24.9 ± 5.2	0.44
Function			
BADL	1.0	5.7 ± 5.8	0.57
IADL	8 ± 4.2	16.3 ± 11,8	0.09
SPPB Chair stand (sec)	11.8 ± 4.6	12.9 ± 6.1	0.73
SPPB 8 feet walk (sec)	3.4 ±1	2.9 ± 0.9	0.29

SPPB 8 feetWalk score	3 ± 0.9	3.6 ± 0.7	0.01*
TUG (sec)	12 ±5.8	10 ± 4.1	0.09
TEN (average) (sec)	5.6 ±2.1	4.6 ±1.4	0.02*
TIN-Balance	14.8± 2.3	15.3 ± 1.5	0.09
TIN-GAIT	10.7± 2.2	11.1± 1.4	0.62
TIN-Total	25.5±4.3	26.3 ± 2.7	0.19
Hand grip 1 (kg)	20.7 ± 6.6	24.4 ± 7.2	0.07
Hand grip 2 (kg)	21.4± 8.7	22.4 ± 6.7	0.11
SQ1 (sec)	11.5 ±4.1	11.1 ± 2.9	0.46
SQ2 (sec)	10.9±3.6	10.4 ± 2.1	0.16
Quality of Life			
EQ 5D VAS	76.8 ±18.5	85.7 ± 10.1	0.06
EQ 5D mobility	1.9 ±1.0	1.8 ± 1.0	0.38
EQ5D self care	1.3 ± 0.6	1 ± 0	0.07
EQ5D usual activities	1.9 ± 1.1	1.3 ± 0.6	0.05
EQ5D pain	1.9 ± 0.7	2.1 ± 0.7	0.51
EQ5D anxiety/depression	1.6 ±1.0	1.3 ± 0.5	0.18

(*denotes statistical significance, ** MMSE: normal range ≥22/30, ##MoCA: normal range ≥ 26/30).

Abbreviations: MMSE: Mini Mental Status Examination; MoCA: Montreal Cognitive Assessment; BADL: Basic Activities of Daily Living; IADL: Instrumental Activities of Daily Living; SPPB: Short Physical Performance Battery; SPPB walk score: gait ordinal score for 8 feetwalk; TUG: Timed Up and Go test; TEN: 10 Meter Walk test; TIN: Tinetti Gait and Balance scale; SQ: Square Step test; EQ-5D VAS: EuroQol 5D Visual Analogue Scale; EQ-5D mobility : EuroQol 5D mobility score; EQ-5D self care: EuroQol 5D self care score; EQ-5D usual activities: EuroQol 5D usual activities score; EQ-5D pain: EuroQol 5D pain score; EQ-5D anxiety/depression: EuroQol 5D anxiety/depression score.

There were no statistically significant differences in baseline laboratory parameters between the two groups, including 25(OH)D status. See **Table 5.**

Table 5: Laboratory parameters in Sarcopenia versus Non sarcopenia groups.

Variable Name	Total Sarcopenia group (includes probable, pre-, and sarcopenic) (n=17)	Non-Sarcopenia group (includes "slow" and normal) (n=25)	P value
25(OH) vitamin D (nM)	101 ± 26	90 ± 29	0.27
Vitamin B12 (pmol/L)	454 ± 226	358 ± 203	0.19
Total protein (g/L)	67.8 ± 4.1	67 ± 4.4	0.81

Albumin (g/L)	42.2 ± 2.2	40.9 ± 2.7	0.10
CRP (mg/L)	3.1 ± 5.5	2.9 ± 3.3	0.99
GGT (U/L)	35.2 ± 53.9	32.8 ± 33.9	0.49
TSH (mU/L)	2.8 ± 0.8	2.2± 1.7	0.20
Cholesterol (total) (mmol/L)	5.6 ± 0.7	5 ± 1.1	0.38
Triglyceride (mmol/L)	1.3 ± 0.5	1.2 ± 0.6	0.58
HDL (mmol/L)	1.8 ± 0.4	1.7 ± 0.5	0.46
LDL (mmol/L)	3.1 ± 0.6	2.8 ± 0.90	0.58
Creatinine (umol/L)	72 ± 20	69 ± 18	0.22
GFR (ml/min/1.73m^2)	74 ± 15	78 ± 11	0.19
Homocysteine (umol/L)	11.5 ± 3.2	11.4 ± 3.6	0.45
Fasting insulin (pmol/L)	52 ± 39	59 ± 25	0.32
Hemoglobin A1C (%)	5.7 ± 0.3	5.7 ± 0.5	0.81

(Normal reference ranges: 25(OH)D 80-200 nM; B12>150pmol/L; total protein 64-84g/L; albumin 35-50g/L; CRP<8.0 mg/L; GGT <70U/L; TSH 0.2-4.0 mU/L; cholesterol <6.2mmol/L; triglyceride <1.7 mmol/L; HDL >0.9mmol/L; creatinine 50-115umol/L; GFR >59ml/min; homocysteine <12.1 umol/L; fasting insulin 35-140 pmol/L; Hemoglobin A1c 4.3-6.1%)

6.5.3 Participants status/dropouts at 12-months

At baseline there were 42 participants enrolled in the study. During the study there were 3 dropouts, all females, who were unable to complete the final visit, leaving *39 participants at 12-months.*

Reasons for dropouts were poor health in 2, and generalised pain in 1. For the 3 dropouts 2 were normal, and one pre-sarcopenic at baseline.

6.5.4 Sarcopenic status at 12-months

At 12-months 39 participants completed their second DXA BC test (8 males and 31 females).

The sarcopenic status evaluated at 12-months in all participants is shown in **Figure 2**, comparing baseline status in all, to 12-month evaluations.

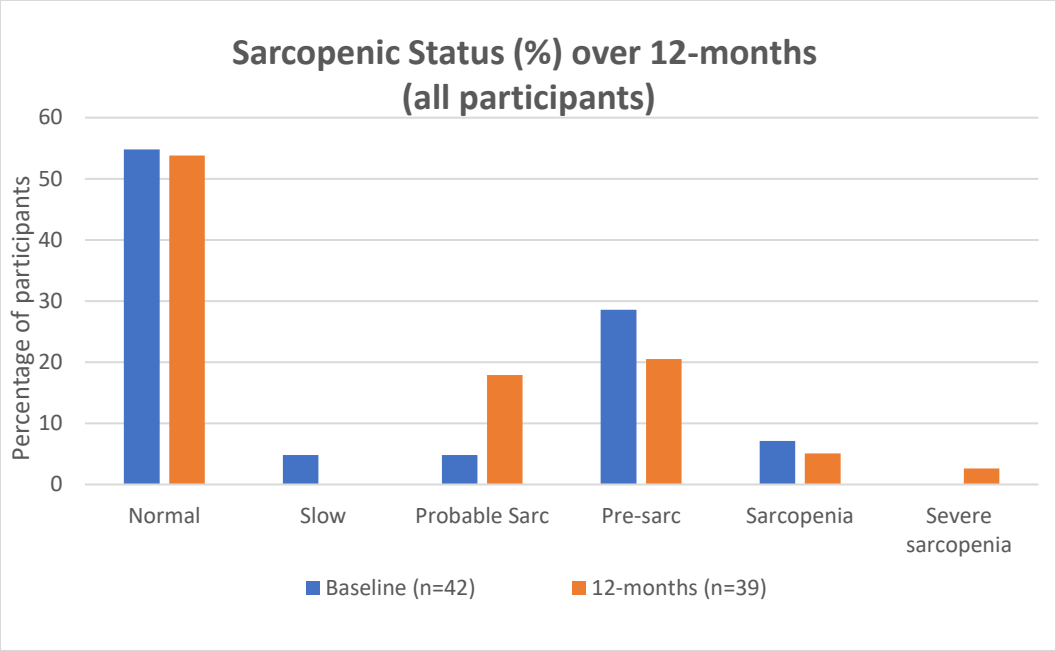


Figure 2: Baseline versus 12-month sarcopenic status (percentage of all participants).

For 54% of participants, there was no change in their sarcopenic status. However, 29.7% did have a decline, and 6% improved. See **Figure 3.**

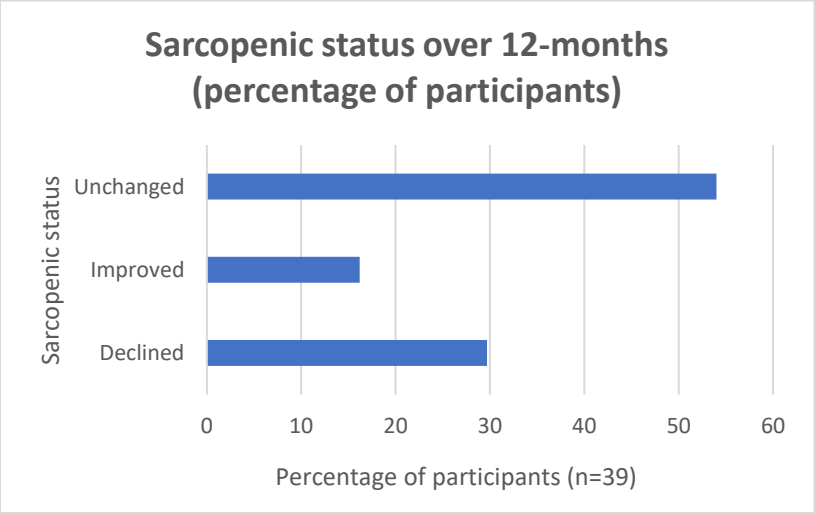


Figure 3: Sarcopenic status in participants at 12-months compared to baseline.

For those whose sarcopenic status ***declined*** (4 males and 7 females), 7 moved from normal to probable because of a decline in their grip strength, 2 moved from normal to pre-sarcopenia (due to a decline in muscle mass), 1 moved from pre-sarcopenia to sarcopenia (due to a decline in grip strength), and 1 moved from sarcopenia to severe sarcopenia due to decreased gait speed. The EQ5D VAS scale score did not reflect this decline in sarcopenic status. 82% of decliners had concomitant obesity (defined by DXA percentage body fat).

For the 6 participants (1male, 5 females), whose sarcopenic status ***improved***, 1 moved from probable to normal, 3 from pre-sarcopenic to normal, 1 from sarcopenia to pre-sarcopenia, and 1 from "slow" to normal. The EQ5D VAS scale score did not reflect this change in sarcopenic status, with only 3 participants showing increased EQ5D VAS, 2 remaining unchanged and 1 declining. 83% of those with improving sarcopenic status had concomitant obesity (defined by DXA percentage body fat).

This descriptive data does not seem to indicate any trend to predict change in sarcopenic status. The number of participants whose status changed is insufficient for statistical analysis.

6.5.5 Change over 12-months in cognitive function and quality of life

There were no significant changes in MMSE and MoCA over the study period, and this was consistent when comparing those participants in the sarcopenia or non-sarcopenia groups at 12-months, and those participants attending or not attending the group activity program. There was also no statistically significant change in quality of life between the total sarcopenia group and the non-sarcopenia participants, as measured by the EuroQol-5D tool. See **Table 6**.

Table 6: Change in MMSE, MoCA, and EQ5D VAS from baseline to 12-months depending on sarcopenic status and attendance at group activity program.

	Total Sarcopenia group (includes probable, pre-, and sarcopenic) (n=18)	**Non-Sarcopenia group (includes "slow" and normal) (n=21)**	**p**
% change in MMSE	0.05 ± 4.6 (0)	1.35 ± 3.5 (0)	0.33
% change in MoCA	4.6 ± 14.6 (4)	-10.04 ± 7.5 (-3.4)	0.13

% change in EQ5DVAS (6-12 months)	-1.93 ± 28.9 (0)	8.7 ± 25.4 (3.4)	0.23
	Attendance at group activity program (n=17)	**No attendance at group activity program (n=22)**	
% change in MMSE	0.89 ± 3.5 (0)	0.75 ± 4.5 (0)	0.84
% change in MoCA	2.4 ± 12.3 (0)	0.46 ± 10.7 (-3.5)	0.91
% change in EQ5DVAS (6-12 months)	0.7 ±12.5 (0)	6.6 ± 34.7 (3.4)	0.52

(Median in parentheses).

Abbreviations: MMSE, Mini Mental Statue Examination; MoCA, Montreal Cognitive Assessment; EQ5DVAS, EuroQol-5D visual analogue scale.

6.5.6 Obesity status

Using ***BMI >30 kg/m^2,*** at baseline, **36%** of participants (n=50) were obese. Of those who had a DXA (n=42) ***7.5%*** had concomitant pre, probable or severe sarcopenia, fulfilling the criteria for *sarcopenic obesity*. At 12-months, 26% of remaining participants (n=39) had obesity, with ***7.7%*** having *sarcopenic obesity.*

However, using ***DXA body composition*** percentage fat criteria (>25% fat in males, and >35% fat in females), **80.9%** of participants (n=42) had obesity. Using these DXA percentage fat cut-offs, at baseline ***64.7%*** participants had concomitant obesity in the total sarcopenia group, and 17.6% in the sarcopenia only group. At 12-months ***72.2%*** in the total sarcopenia group, had concomitant obesity, and 11.1 % having sarcopenia and severe sarcopenia. See **Table 7.**

Table 7: Rate of obesity using BMI and DXA percentage fat.

	BMI >30	**DXA % fat (>25% men, >35% women)**
BASELINE	(N = 50)	(N = 42)
All participants	36%	80.9%
Sarcopenic obesity (pre+probable+sarcopenia)	7.5%	64.7%
12-MONTHS	(N= 39)	(N = 39)
All participants	26%	79.5%
Sarcopenic obesity (pre+probable+sarcopenia)	7.7%	72.2%

Abbreviations: N: number of participants; DXA: Dual energy X-ray absorptiometry; BMI: body mass index (kg/m^2).

6.5.7 Nutritional status

The "Determine Your Nutritional Health" checklist (NSI checklist) was used to screen for malnutrition risk [Barrocas et al, 1995]. This consists of ten items, weighted with a numerical score, scores range from 0-21 with a score of ≥6 considered at high nutritional risk, 3-5 moderate risk, and 0-2 classified as "good". It is equivalent to the MNA [Beck et al, 1998]. There was no significant difference in scores between those in the total sarcopenia group versus those in the non-sarcopenia group: mean score 2.36 ± 1.86 versus 3.23 ± 2.42, p >.05. There were 23% participants in the total sarcopenia group (pre and probable) and 7% in the non-sarcopenia group having scores ≥6, and 23% in the sarcopenia group in the moderate range with 30% in the non-sarcopenic group.

6.5.8 Quality of life

For quality-of-life parameters, in the EQ5D tool questions a higher score indicates worsening of symptoms, but for the visual analogue scale (VAS) scale question, a higher score indicates improved impression of health. There was an overall *increase in EQ5D self reported pain* score (p=0.02) over the duration of the study, and there was no difference between total sarcopenic and non-sarcopenic individuals for this parameter (p=0.21). In contrast, there was a *decrease in EQ5D Self Care scores* over the 12-months (suggesting better function) specifically in the total sarcopenia group participants, and it did appear to be related to group activity program participation in the sarcopenic participants only (p=0.04). In addition, there was a trend towards an increase in EQ5D VAS scores (p=0.09) in all participants, with non-sarcopenia group participants having higher scores overall than total sarcopenia group participants (p=0.03).

6.5.9 Group social activity program participation

The 60-minute group activity program was offered up to twice weekly. Fifty classes were available during the study (affected by space and instructor availability). Thirty-three participants attended at least 1 class. Off these, 16 only attended between 1 and 7 classes (average 3). Seventeen participants (42.5%) attended the group activity class regularly and so were the group that was evaluated. Of those 17 participants, the average number of classes attended was 21.2 ±9.35 (maximum 42, minimum 12). Class attendance in the 17 regular attendees was associated with increased age (p=0.03), but was not affected by the sarcopenia status of the participants. See **Table 8** for baseline physical parameters.

Table 8: Baseline anthropometric and body composition characteristics of participants at study entry, grouped by attendance at the group social activity program.

Variable Name	No attendance 3M/21F	Attendance 5M/12F	P value
Age	74.3 ± 4.8	77.9 ± 5.0	0.03*
Weight (kg)	69.9 ± 2.5	67.8 ± 13.5	0.59
Height (cm)	160.4 ± 6.6	163.8 ± 9.0	0.17
Waist to hip ratio	0.86 ± 0.12	0.87 ± 0.08	0.86
BMI (kg/cm)	27.8 ± 5.5	26.2 ± 4.2	0.34
Calf circumference (cm)	36.5 ± 3.9	36.5 ± 2.3	0.96
Arm circumference (cm)	30.6 ± 3.7	30.3 ± 2.6	0.72
Total skinfold thickness (mm)	89.0 ± 36.3	82.3 ± 40.1	0.61
% Fat by different methods			
Skinfold thickness	38.7 ± 7.7	37.1 ± 8.8	0.52
DXA BC	39.2 ± 7.3	36.4 ± 6.6	0.13
Measures of lean body mass			
Appendicular Lean-muscle mass/height^2 (DXA)	6.2 ± 0.9	6.2 ± 1.0	0.96

(*denotes statistical significance)

At 12-months, in those attending the group activity program, there was a *statistically significant difference in their Tinetti Balance scale (p=0.04) and their Tinetti Total score (p=0.05)* when compared to those who did not attend the activity class, but only in those with sarcopenia at

baseline. See **Figure 4**. There was no change in the other balance scores evaluated (balance component of SPPB and 4-square step test).

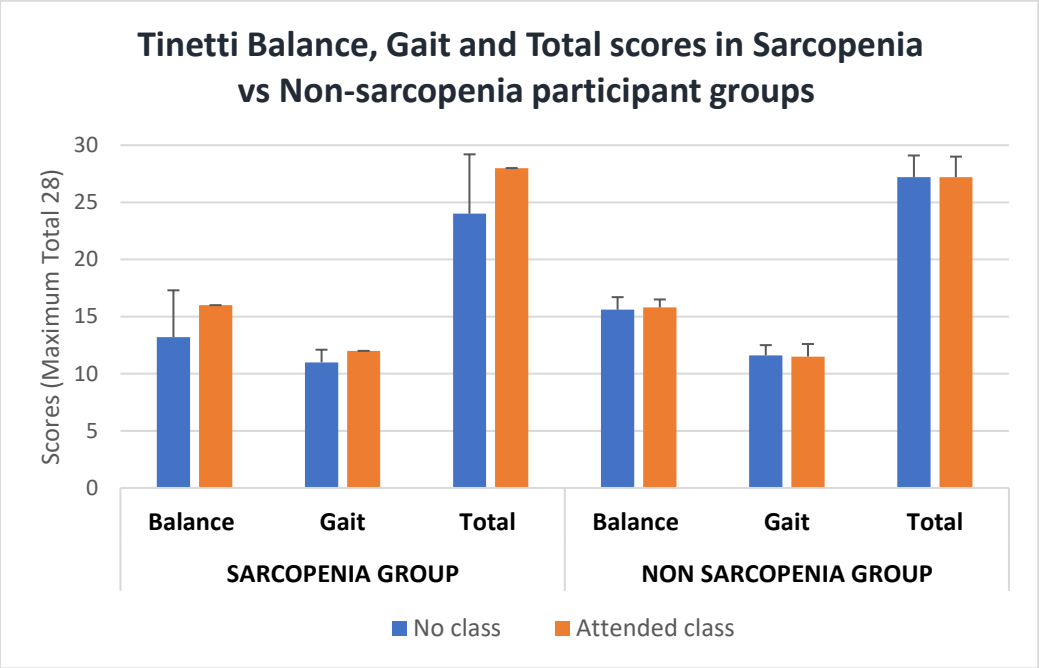


Figure 4: Tinetti Total, and Gait and Balance sub-scores in the non-sarcopenia and total sarcopenia groups, and attendance at the group social activity program.

Quality of life parameter, *EQ5D Self Care* score improved, but only in those in the total sarcopenia group, who attended the class (p=0.04). However, total *Short Physical Performance Battery* and *8 feet walk test time scores* increased in both total sarcopenia group and non-sarcopenia group participants attending the activity program (p=0.003 and 0.02 respectively). See **Table 9.**

Table 9: Baseline muscle strength and physical performance parameters in participants at study entry, grouped by attendance at the group social activity program.

Variable Name	No attendance 3M/21F	Attendance 5M/12F	P value
Short Physical Performance Battery			
SPPB (total score)	12.1 ± 3.9	11.7 ± 6.9	0.08
SPPB (# stands)	4.2 ± 1.9	4.9 ± 0.5	0.13

SPPB (side-side time)	10	10	NS
SPPB (side-side score)	2	2	NS
SPPB (semi-tandem time)	10	10	NS
SPPB (semi-tandem score)	2	2	NS
SPPB (tandem time)	8.7 ±3.0	9.4 ± 1.7	0.33
SPPB (tandem score)	1.7 ± 0.7	1.9 ± 0.3	NS
SPPB 8 feet walk (time)(s)	3.2 ± 1.0	2.8 ± 0.8	0.25
SPPB walk (total score)	3.3 ± 1.0	3.5 ± 0.7	0.36
Timed Walk and Other Tests			
TUG (s)	11.0 ± 5.1	9.0 ± 2.9	0.16
10 Meter walk test (1)(s)	5.2 ± 1.9	4.6 ± 1.3	0.25
10 Meter walk test (2)(s)	5.2 ± 2.1	4.5 ± 1.1	0.16
10 Meter walk test (3)(s)	4.9 ± 1.9	4.3 ± 1.1	0.31
10 Meter walk test (average) (s)	5.1 ± 1.9	4.5 ±1.2	0.23
Gait speed (m/s)	1.4 ± 0.7	1.4 ± 0.3	0.75
Square Test 1 (s)	11.5 ± 3.8	10.8 ± 2.8	0.52
Square Test 2 (s)	10.6 ± 2.9	10.0 ± 2.3	0.54
Tinetti Gait and Balance Test			
Balance	15.1 ± 1.9	15.5 ± 1.4	0.48
Gait	10.6 ± 2.0	11.6 ± 0.9	0.08
Total	25.7 ± 3.8	27.1± 2.1	0.21
Muscle Strength Tests			
Hand grip 1 (kg)	22.7 ± 8.1	23.2 ± 5.7	0.85
Hand grip 2 (kg)	20.6 ± 8.3	22.6 ± 5.9	0.41

Abbreviations: SPPB: Short Physical Performance Battery; TUG: Timed Up and Go test; s: seconds; M: males; F: females.

Overall, there were *no significant association between attendance at the group activity class* on measurable changes in weight (p=0.67), waist-hip ratio (p=0.31), DXA percentage fat (p=0.02) or BMI (p=0.58). Males and females attended at equivalent rates, although there were fewer males overall.

There was *no association with class attendance on the following laboratory parameters*, even when adjusting for sarcopenic status: GGT (p=0.29), CRP (p=0.95),albumin (p=0.27), B12 (p=0.39), 25(OH)D (P=0.90), total cholesterol (p=0.30), LDL (p=0.11), HDL (p=0.91), TG (p=0.55), total cholesterol/HDL ratio (p=0.6), total protein (p=0.39), TSH (p=0.08), creatinine (p=0.34), GFR (p=0.31), fasting insulin (p=0.24), hemoglobin A1C (p=0.52), and homocysteine (p=0.17).

There was also *no difference in cognitive scores or instrumental activities of daily living score* between those attending or not attending the group activity class. See **Table 10.**

Table 10: Baseline Laboratory, Cognitive, and ADL data grouped by attendance at the group social activity program.

Variable Name	No attendance 3M/21F	Attendance 5M/12F	P value
Laboratory Data			
Albumin (g/L)	42 ±1	42 ± 3	0.73
Total protein	67 ±3	67 ± 4	0.99
B12	415 ±194	446 ± 236	0.64
CRP	2.9 ± 4.5	3.1 ±3.7	0.89
GGT	34 ± 47	21 ± 11	0.39
TSH	2.2 ± 1.1	2.3 ± 1.3	0.65
Triglycerides	1.2 ± 0.8	1.1 ± 0.4	0.71
Total Cholesterol	5.3 ± 0.9	5.1 ± 0.8	0.62
HDL-cholesterol	1.9 ± 0.5	1.7 ± 0.5	0.38
LDL-cholesterol	2.9 ± 0.8	2.9 ± 0.6	0.88
Total Cholesterol Ratio	3.0 ± 1.0	3.2 ±0.9	0.51
Homocysteine	11.2 ±3.2	10.8 ± 1.9	0.58
Insulin	60 ± 35	47 ±22	0.19
Hba1c	5.7 ± 0.5	5.7 ±0.4	0.92
Cognition & ADL			
MMSE	28.8 ± 1.5	28.3 ±2.2	0.41
MOCA	26 ± 3	25 ± 3	0.31
IADL	5.7 ± 5	13.3 ± 11.2	0.34

Abbreviations: MMSE, Mini Mental Statue Examination; MoCA, Montreal Cognitive Assessment; ADL: Activities of Daily Living; IADL: Instrumental Activities of Daily Living; M: males; F: females.

(Normal reference ranges: 25(OH)D 80-200 nM; B12>150pmol/L; total protein 64-84g/L; albumin 35-50g/L; CRP<8.0 mg/L; GGT <70U/L; TSH 0.2-4.0 mU/L; cholesterol <6.2mmol/L; triglyceride <1.7mmol/L; HDL >0.9mmol/L; creatinine 50-115umol/L; GFR >59ml/min; homocysteine <12.1 umol/L; fasting insulin 35-140 pmol/L; Hemoglobin A1c 4.3-6.1%)

Attendance at the group activity class *did not appear to affect the change in sarcopenic status* over the 12-months.

6.5.10 Self-reported leisure activity level, 12-month evaluation.

Evaluation of self-reported leisure activity was done at 12-months by dividing the reported activities times into total duration: <30 minutes; 30-60 minutes; and > 60 minutes; and frequency > and < a median of 5 times/week. The average reported leisure activity time was 60 minutes (ranging from 0-360), 4 times weekly. Because the group social activity program appeared to impact Tinetti scores, this was evaluated looking at total self-reported leisure activity. There did not appear to be any effect on the Tinetti Gait and Balance scores based on self-reported leisure activity type and frequency, as opposed to those who attended the group activity class. See **Figure 5.**

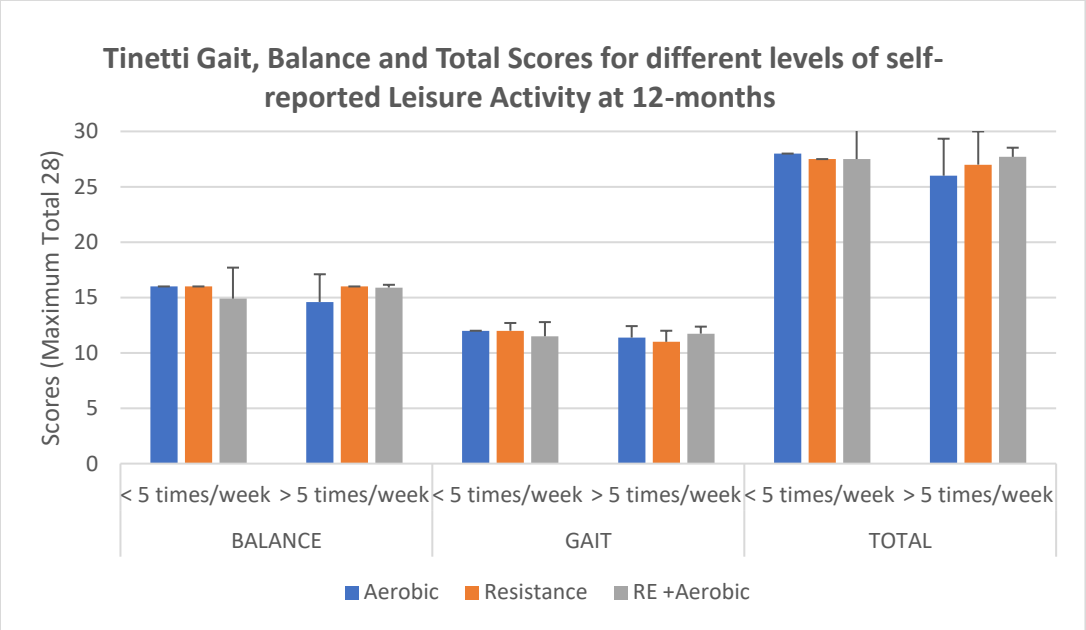


Figure 5: Association between self-reported leisure activity and Tinetti Total, Gait and Balance scores over 12-months.

6.6 Discussion

In the present study of a cohort of community-dwelling Canadian older adults, the percentage of participants in the *total sarcopenia group (probable + pre + sarcopenic + severe* sarcopenic)

was **40.5%,** of whom **28.6%** had *pre-sarcopenia*. The rate with *sarcopenia only* (sarcopenic + severe sarcopenic) was low at **7.1%.**

This study reported a total sarcopenia group to allow comparison with other published studies on similar populations, in other countries. Kim and colleagues followed a group of 538 community dwelling Japanese females for 4 years, average age 78 years. [Kim et al, 2015]. Using BIA for muscle mass assessment, they found a prevalence of total sarcopenia of 39.6%. Similar to this study, they included pre-sarcopenia, and severe sarcopenia in this number, but did not assess probable sarcopenia (low muscle strength alone). In the present study, if this group is excluded from the total sarcopenia group, then the percentage becomes 35.7%, very comparable to the Japanese group. As in this study, the majority of their total sarcopenia group were pre-sarcopenic (23.8%) with only 11.2% having established sarcopenia. In this study only 7.5% had established sarcopenia. This may be a reflection of the slightly younger age than the Japanese study, the higher average BMI (27 versus 22), and more self-reported leisure activity (67% reporting leisure activity 3 days/week versus 40%), reflecting a somewhat healthier older adult cohort and their use of BIA which tends to report more low muscle mass compared to DXA.

In a large community group (1890 participants) evaluated in the Hertfordshire, UK, the overall sarcopenia prevalence reported by Patel and colleagues, was 6.6% (for all participants) and 4.6% in males, and 7.9% in females [Patel et al, 2013]. With their data grouped in the same way as in this study, the total sarcopenia group (probable + pre + sarcopenic + severe sarcopenic) is 31.6% in their cohort [Patel et al, 2013]. Similar to this study (28.6%), the majority of their participants were in the pre-sarcopenia stage (25%). They did not report on probable sarcopenia. Their cut-offs for grip strength were higher (females <20kg versus <16kg, and males <30kg versus <27kg), therefore identifying more participants with low grip strength than the present study, and the average age of their participants was10 years younger than this cohort (67 years versus 75 years). In addition, in the majority of their participants they used a surrogate marker for muscle mass (skinfold-based fat-free mass) by calculating total mass and subtracting skinfold thickness calculated fat mass, although they did validate this against DXA in a subgroup of participants.

In contrast, Brown and colleagues, evaluated a large community-based cohort (4425 older adults 43.5% men and 56.5% women) in the USA, and reported a very high prevalence of 36.5% in their sarcopenia only group [Brown et al, 2016]. But their definition of sarcopenia was low gait

speed + low muscle mass, as opposed to the EWGSOP2 criteria of low strength (eg. grip) + low muscle mass. They also used BIA and not DXA to assess muscle mass. It is generally accepted that when using cut-off definitions with both BIA and DXA measures, BIA results in higher prevalence estimates than DXA [Mayhew et al, 2019]. They also did not assess grip strength, so could not identify any of their participants as probable or severe sarcopenia [Brown et al, 2016]. Instead, they used gait speed as a measure of muscle strength because of its correlation with lower limb muscle strength [Bohannon, 1997]. 24.4% had low muscle mass alone (pre-sarcopenia by EWGSOP2), which was similar to the present study, but of these, only 14% had normal gait and muscle mass, as opposed to the present study where 54% are in this category. Obesity as defined by BMI was 23.7%, and waist circumference was 57.9% in their cohort [Brown et al, 2016]. One objective of their study was to evaluate the impact of sarcopenia (based on their definition) on mortality. There was increased all cause mortality [hazard ratio 1.29 (95% confidence interval: 1.13–1.47); $P < 0.001$] in the sarcopenia group. Their sarcopenia prevalence is also higher than is reported in most other studies [Cruz-Jentoft et al, 2014].

Using data from the Canadian Longitudinal Study on Aging, with the EWGSOP2 criteria Purcell and colleagues [Purcell et al, 2020] reported a prevalence for low muscle mass (pre-sarcopenia) of 5.8% in males and 8.2% in females, but sarcopenia only in 0.2% and severe sarcopenia in 1.2%. This is *much lower than that reported in the present study*, *and in that reported by other authors* [Cruz-Jentoft et al, 2014], although all their participants were aged ≥ 65 years. However, using the International Working Group on Sarcopenia (IWGS) criteria in their population, the sarcopenia prevalence is 6.7%, similar to the rate found in this study. The IWGS criteria differ in that the gait speed cut-off is higher (<1.0m/s versus <0.8m/s) as is the grip strength for both sexes, accounting for a higher prevalence with this tool. Their prevalence of obesity (defined by BMI) was 18.6-32.2% depending on the age group [Purcell at al, 2020], similar to the present study (36%). Data from the Foundation for the National Institutes of Health (FNIH) project [Dam et al, 2014] identified lowers prevalence's of sarcopenia with the FNIH criteria than the EWGSOP2 criteria (1.3% versus 5.3% in males, and 2.3% versus 13.3% in females), however, their rates were similar to those seen in the present study with the EWGSOP2 criteria.

A systematic review of 15 prevalence studies, concluded that taking into account regional and age-related variations, the prevalence in community-dwelling populations is 1 to 29% [Cruz-

Jentoft et al, 2014]. The sarcopenia rate we report is more akin to that reported by Mayhew and colleagues. In a more recent review, they report prevalence's from 9.9 to 40.4% [Mayhew et al, 2019]. Even studies using different definitions on the same population, have found that sarcopenia estimates vary up to 40% by definition [Bijlsma et al, 2013]. Cruz-Jentoft stated that "this heterogeneity in published estimates of sarcopenia prevalence may be influenced by multiple factors such as the age and sex distribution of the population, and the methods and cut-points used to measure muscle mass and muscle function to define sarcopenia" [Cruz-Jentoft et al, 2014]. Mayhew summarised it well stating that the best definition of sarcopenia will be "the one with the strongest association with the health outcomes relevant to sarcopenia" [Mayhew and Raina, 2019]. Once the definition is established, more precision will be needed at choosing study populations and outcomes [Moore et al, 2020].

With regard to obesity, this studied identified a considerable difference between the diagnosis of obesity when using the BMI cut off (>30) versus DXA body composition cut-offs (>25% males, >35% females). DXA was able to detect 2.25 times more participants with obesity, And similarly more participants with sarcopenic obesity. Based on BMI, the percentage of sarcopenic obesity in total sarcopenia group in this study was only 7.5% at baseline and 7.7% at 12-months. However, using DXA criteria for obesity, the percentage of sarcopenic obesity was considerably higher at 64.7% and 72.2% respectively. In their review on sarcopenic obesity, Zamboni and colleagues highlight the importance of a clear and decisive definition for sarcopenic obesity [Zamboni et al, 2019]. Given the additive comorbidities of these two conditions it is important that the obesity component is not overlooked, which could be the case if only BMI criteria is used. To this point, Brown and colleagues reported no particular association of obesity with sarcopenia in their study, but this may have been a reflection of their definition of obesity [Brown et al, 2016]. There is a recent consensus statement from the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity (EASO) on sarcopenic obesity [Donini et al, 2022]. They define obesity screening by BMI or waist circumference with ethnicity-specific cut points, and confirmation using definitions recommended by Gallagher and colleagues [Gallagher et al, 2000]: 30% for males and 40% for females (higher than those recommended by EWGSOP used in the present research).

We found no association between sarcopenic status (total) and cognition, unlike suggestions from a meta-analysis by Peng and colleagues [Peng et al, 2020], where they suggested a 2.25 adjusted risk for cognitive impairment. But their meta-analysis included studies of specific disease states (eg renal failure, diabetes) and in-patients, memory clinic and long-term care residents, so did not focus only on community-dwelling older adults as in this study.

Despite the majority of participants in this study engaging in regular leisure time activities, this did not appear to mitigate their total sarcopenia status, as has been shown in other studies [Raguso et al, 2006]. Although the ideal for prevention and treatment of sarcopenia is a structured, progressive, supervised, personalised, resistance training program [Rolland et al, 2008], this is not feasible in many settings.

The social group activity program in this study was not anticipated to have any impact on sarcopenic status given its limited and non-progressive resistance training component. It was designed to be a "value-added" component for participants in the study. Community programs, although they exist, are often designed for less highly functioning older adults. The association of the social group activity program with improved Tinetti balance was unexpected and is encouraging. The activity class did contain specific balance exercises, and this may have accounted for the improved Tinetti balance scores, or this could be related to a Type 1 error, as, despite the improvement in Tinetti Balance scores, there was no measurable changes in the other balance scores evaluated (balance component of SPPB and 4-square step test) . The lack of change in sarcopenic status in the group attendees was not surprising since improvement in muscle strength needs a graded, personalised, increase in intensity, to be effective, and this was not part of the group activity program. Other parameters affected by balance, such as falls, were not captured, but there were no fragility fractures in any of the participants over the 12-months. Mills showed that a low-intensity aerobic exercise program in 20 elderly participants also showed an improved balance (22%), although this was not significant from the comparison group [Mills, 1994]. The balance scale she used was the Roberts Balance Scale which is also different from one used in the present study, and her study participants completed their program much more frequently (3 times/week). Although the ideal for prevention and treatment of sarcopenia is a structured, progressive, supervised, personalised, resistance training program [Rolland et al, 2008], this is not feasible in many settings.

Interestingly, older participants were the group activity class attenders, irrespective of their sarcopenic status, reinforcing what has been reported in the literature [Seguin et al, 2010]. One can speculate as to the reasons for this: no other regular leisure exercise/activities; more awareness of their need to improve/maintain their functional status; and/or loneliness. A recent study by Mays and colleagues reports that social isolation and loneliness were improved with a community-based group health class [Mays et al, 2021]. In the present study, in addition to the activity component, participants often arrived early, and stayed afterwards to socialise. There was also some informal education about healthy eating during this time, which evolved into many recipe exchanges over the 12-months of the exercise classes. As the group got to know one another better, laughter therapy was also included with a regular supply of jokes provided by the attendees. This group cohesion likely explained the reason for the consistent attendance at the group activity seen in this study [Spink and Carron, 1992]. In addition, perception of the exercise instructor as supportive, and the exercising as fun, have also been shown to endorse long term exercise persistence [Rodrigues et al, 2020]. All participants (including non group activity attendees) reported a relatively high QOL throughout the study, and this may have affected any demonstrable change associated with group activity attendance.

A study that evaluated the motivators and barriers to engaging in a nutrition and resistance program in the UK, cited self-perceived improved health, knowledge acquisition in nutrition and exercise, social well-being, professional support in a fun environment, and positive reported outcomes, were all motivators for engagement in the intervention [Dismore et al, 2020]. They also looked at continued activity after study completion, and showed peer encouragement, social bonds, and their retention, were the motivators to continuing engagement after study completion, especially in widowed women [Dismore et al, 2020]. No formal outcomes were evaluated in their MilkMAN pilot study, but participants did report a high level of satisfaction [Dismore et al, 2020]. Seguin and colleagues found the participant factors affecting long term adherence to strength training was higher age, higher lifetime physical activity, and better perceived health status. In addition, the exercise class leader's sports participation, and prior experience at leading programs, were also important factors reported by survey participants [Seguin et al, 2010].

In the present study, no activity classes were offered once the study was completed, but participants were encouraged to keep in touch, if they so chose, and were given the exercise

video and information booklet to allow them to continue the exercises at home. No formal follow up was done, but informal reports based on subsequent interactions with some of the participants subsequently, suggested that only 2 of the 17 more frequent exercise class attendees (a husband and wife) reported using the DVD consistently after the study completion, and they continue to do so. All the other participants reported never having used the material, despite reporting a high level of satisfaction with attending the class. The barriers reported to the PI were the loss of the peer support, educational component, and socialisation associated with attendance. Barriers reported by others to maintaining a post-study exercise activity included affordability, environmental factors, and concerns over negative health outcomes [Dismore et al, 2020].

The lack of association of sarcopenic status with general leisure activities may be a reflection that these were still less than the recommended levels, despite this being a group of independent community-dwelling older adults. The average leisure activity time (aerobic and resistance) reported was 60 minutes, 4 days/week. The American Heart Association (AHA) and the American College of Sports Medicine (ASCM) recommendations, however, are ≥30 minutes per day on ≥5 days per week for a total of ≥150 minutes per week for moderate-intensity aerobic exercise, and ≥20 minutes per day on ≥3 day per week (≥75 minutes per week) for vigorous-intensity activity [Garber et al 2011]. In addition, to counteract muscle loss and increase strength, resistance exercises are strongly recommended for 2-3 days per week for the major muscle groups, and to include balance, agility and coordination exercises [Garber et al 2011]. As there are few non-research community-based programs specifically for sarcopenia, utilising existing exercise classes may fill this gap. Many exercises programs for osteoporosis or fall prevention include the AHA recommended resistance, balance and agility exercises, such as International Osteoporosis Foundation [IOF], Osteoporosis Canada [OC], and the Otago Exercise program [Campbell et al, 1997] and are available free-of-charge on-line. Programs preferably should have a social component to encourage ongoing compliance [Dismore et al, 2020].

6.7 Limitations

This is a cohort study so is open to potential limitations/biases associated with the design. Attempts were made to minimise selection bias by general advertising, but all participants were Caucasians, and the group was predominantly female. The self-referred enrollment suggested they were motivated to maintain their health, and those remaining in the study may be the most motivated and/or the most healthy ("selective survivor bias") [Ramirez-Santana, 2018] although there were only 3 subject drop-outs). This may affect the applicability of the results to other populations.

The study group was predominantly female. Because of the small number of males, there was insufficient power to detect sex differences, however sex specific cut-offs were used in the assessment of sarcopenic status parameters. In addition, it is a very small cohort, compared to others published. However, the rates of sarcopenia reported are very comparable to Kim and colleagues (all female study) and Patel and colleagues (male and female participants) [Kim et al, 2015; Patel et al, 2013]. The numbers of participants improving or declining in their sarcopenic status were too small to be able to assess a particular risk factor for this change.

The apparent lack of association of leisure activity or social activity group attendance with sarcopenic status may reflect the challenge of a cohort design in conditions such as sarcopenia with long latency, suggesting the study duration was too short, and/or the numbers too small. The group activity class was not designed as an intervention, but a value-added for the participants. Although the classes included some resistance exercises, there was no formal structured resistance training program. Apart from those attending the exercise class where attendance was documented, the remainder of the leisure activity is self-reported and has the associated limitations inherent in self reporting. However, the participants were unaware of their sarcopenic status (as this was only analysed after study completion), or that the amount of exercise they did outside the classes was of any importance.

6.8 Conclusions

The rate of sarcopenia in this highly functioning community-dwelling cohort was low at 7.1%, but the rate of total sarcopenia (including pre and probable sarcopenia) was surprisingly high at

40.5%, of which 28.6% were pre-sarcopenic with concomitant obesity in 65-70% of those with low muscle mass. This suggests a window of opportunity to perhaps prevent progression to sarcopenia. Within the context of limitations already highlighted, leisure-related activity does not seem to be related to sarcopenic status, in this study. 12-month participation in group social activity program may have some measurable improvement in balance, more so in those in the total sarcopenia group, suggesting there may be some value for targeted programs in the future. The obesity rate was high, especially when using DXA percentage fat cut-offs, highlighting the need for obesity diagnostic criteria in addition to BMI. Further research in this field is undoubtedly needed. Although the current sarcopenia diagnostic criteria may not be without limitations, use of them will ensure comparability of results across studies and interventions.

CHAPTER 7: DISCUSSION AND INTEGRATION

7.1 Thesis objectives

The overall objectives of this thesis were to address some of the knowledge gaps in two common age-related conditions, both associated with nutrition and physical activity: Alzheimer's Dementia (AD) and Sarcopenia. For AD the objectives were to evaluate long-term medium chain triglyceride (MCT) supplementation and its association with cognitive function, having first confirmed that the presence of AD did not interfere with the ketogenic response to ingested MCT. For sarcopenia, the objectives were to assess diagnosis of low muscle mass with office-based direct-to-consumer BIA scales compared to DXA body composition, and to assess the rate of sarcopenia in the same group of community-dwelling older adults, and to follow their function and sarcopenic status over 12-months.

7.2 Summary of results

7.2.1 Objective 1: To study MCT supplementation in AD and non-AD participants

The first objective of this research was to evaluate the ketogenic response, using serum beta hydroxy butyrate levels (BHB), to different doses of MCT supplementation in participants with and without AD.

In **Chapter 3**, 25 participants - 8 young healthy, 9 healthy older adults, and 8 adults with AD, were shown to have a linear BHB dose response relationship to different doses (ranging from 0-42g) of MCT, which was not affected by age or AD status, Body composition, particularly visceral fat and BMI affected the maximal BHB response. This study therefore *confirmed our hypotheses (2.2.1 1a, 1b)* that generation of serum BHB was not associated with AD status, and that there was a clear dose response to MCT ingestion and BHB levels.

We *did not confirm all of hypothesis 2.2.1 1c.* One part stated that the higher the blood BHB level the greater the chance of side-effects. The study showed marked individual maximum BHB response to the same dose, as well as very different tolerances to the BHB elevation. Even in an individual participant there was not clear association with their reported side effects and their BHB level. However, we did *confirm the second part of the hypothesis* that the majority of the clinical side effects were gastrointestinal.

The *final hypothesis was confirmed (2.2.1 1d)* showing a measurable effect on maximum BHB dose-response related to the pre-study breakfast, and the participant's body composition (BMI and waist/hip ratio).

The ***contribution to the literature*** is significant given the ongoing interest in the use of MCT supplementation for many neurological conditions. It is the *first* kinetic study to measure Area Under the Curve (AUC) for exposure by correcting for baseline AUC, resulting in a more robust AUC estimate. It is the also the *first* true dose-response study for pure C8 MCT oil. This is the *first* study to concomitantly evaluate body composition parameters, and identify the importance of BMI and visceral adiposity in BHB response to ingested MCT. It is also *first* to highlight individual variability, and therefore the challenges of interpreting pooled data, as well as the challenge in assuming the same dose of MCT will produce an equivalent BHB response in different participants. In addition, it is the *first* study to show a clear *lack of association* between BHB level and side effects in different individuals. And finally, it is the *first* study confirming an equivalent BHB response in AD participants, allowing confidence for further studies including AD participants. This chapter has been published in the Journal of Prevention of Alzheimer's Disease [Juby et al, 2021].

7.2.2 Objective 2: Assess the cognitive effect of MCT supplementation in AD participants.

The second objective was to evaluate the cognitive effect of MCT supplementation in participants with established AD (mild-moderate stage) over a 15-month period, using a randomized, double-blind, placebo-controlled, cross-over design, with an open label extension, on both cognition, tolerance, and safety (lipid profile and body composition).

As reported in **Chapter 4**, twenty participants with established AD were enrolled in the double-blind placebo-controlled cross-over for 8-months, and 19 were followed in the open-label phase for the full 15-month study duration. There were no measurable cognitive differences in the cross-over phase (MCT versus placebo). When including the open-label phase, those who started in the placebo arm of the cross-over phase had a measurable improvement in Conigram® 1 scores and a trend towards stabilization or improvement of cognitive function as measured by MMSE, compared to those who started with MCT and had a placebo oil "break" before resuming MCT in the open-label phase. In addition, overall, there was less decline in all participant's MMSE score over the 15-months of the study in the majority of participants (80%), than was anticipated with the natural history of AD.

This study *did not confirm the hypothesis (2.2.1, 2a)* that MCT oil would have a measurable difference on cognitive function in the double-blind, placebo-controlled, crossover phase when compared to placebo oil. There were no measurable differences identified in this phase of the study. Possible reasons for this are discussed in detail in **Chapter 4 (4.4.10).**

The study *confirmed the hypothesis (2.2.1 2b)*, with those consuming higher doses of MCT over a consistent period of time showing at least a stabilization, and in some cases improvement, from their baseline cognitive function. It also confirmed that the benefits of dietary ketones are maximal in those with earlier disease, suggesting that there may be a requirement for a critical amount of remaining neuronal function for maximum benefit. Of interest, our study did not show any association between the response to MCT and apolipoprotein E4 status, unlike other studies in the literature. The study also *confirmed the hypothesis (2.2.1 2c)* that long-term consumption of MCT oil was safe with respect to body composition and serum lipids. It also showed that adults with AD were willing to consume MCT oil in its "native" form, and any inability to consume MCT was related to GI side effects and not to intolerance of the texture or taste.

Contributions to the literature are important to highlight. *No previously published studies* have had a cross-over design, or are as long in duration as this study, and few have included participants with moderate to severe AD. In addition, no other study has used a highly objective computer-based cognitive assessment tool, that is free of any learning component. This study adds significantly to the small, but growing, pool of research in this area. It shows people with more advanced AD are able to participate in a long-term study with a nutritional intervention,

and that it is not futile to continue to attempt to preserve as much cognitive function as possible, even in more advanced disease. This chapter is published in Alzheimer's & Dementia: Translational Research & Clinical Interventions [Juby et al, 2022].

7.2.3 Objective 3: Evaluate muscle mass in community-dwelling older adults with two office-based direct-to-consumer bioimpedance assay (BIA) scales compared to dual energy Xray absorptiometry (DXA).

The third objective was to evaluate muscle mass in community-dwelling older adults with two office-based direct-to-consumer bioimpedance assay (BIA) scales and compare these results to one of the accepted gold-standards for muscle mass assessment, dual energy Xray absorptiometry (DXA).

As reported in **Chapter 5,** this was a cohort study of 50 community-dwelling older adults. Forty-two participants completed the study, with 15 having low muscle mass by DXA BC. Using the recommended EWGSOP consensus defined BIA cut-offs, the two office-based BIA scales studied (Ozeri ® and Omron ®) were able to identify low muscle mass when compared to DXA BC, however with different sensitivity and specificity. The Ozeri ® scale had a specificity of 92.6% but a low sensitivity (40%), versus the Omron ® which had low specificity (48.2%) but high sensitivity at 93.3%. The Omron® not only identified all participants with a low muscle mass identified by DXA BC, but also those on the DXA BC low muscle mass cut-off borderline. In contrast the Ozeri® "mis-identified" some of the low muscle mass participants as normal. Both scales performed better on fat than muscle assessment when compared to DXA BC, although the Omron® scale again appeared to perform better.

The *first hypothesis (2.2.2 3a)* suggested there would be good correlation between the BIA scales and DXA BC for identifying low muscle mass. This *hypothesis was confirmed*, and, although the correlation coefficients were relatively low (0.39 for Ozeri® and 0.42 for Omron®) both reached statistical significance, and there may be differences between the scales in terms of clinical significance. Cohens kappa showed fair association between the BIA scales and DXA for low muscle mass identification. Of note, even research BIA tools have been reported to misclassify 1 out of 6 participants [Reiss et al, 2016] when compared to DXA BC. The *second hypothesis was*

confirmed (2.2.2 3b), that the BIA scale using the additional hand grip sensors (Omron®) would have a better diagnostic predictive value for DXA identified low muscle mass, compared to the BIA scale using only foot sensors (Ozeri®).

The *third and fifth hypotheses (2.2.2.3c and 3e)* regarding body fat assessment was *confirmed* with both scales showing good correlation with DXA BC percentage body fat, and obesity cut-offs, although again the Omron® BIA scale appeared to correlate better. We hypothesised that the prevalence of obesity would be low in this highly functioning community-dwelling cohort. This *hypothesis (2.2.2.3d) was not confirmed.* The prevalence of obesity defined by DXA EWGSOP body fat cut-offs was much higher than expected, with 81% categorised as obese.

The ***contributions to the literature*** by this study are predominantly practical. This study addresses one of the biggest challenges in the office-based clinical diagnosis of sarcopenia - the ability to accurately/objectively assess muscle mass. Using the Omron® BIA scale may over-diagnose the presence of low muscle mass, and therefore sarcopenia, but its sensitivity and specificity are equivalent to that of the SARC-F questionnaire. The treatment for low muscle mass is lifestyle modification with increased dietary protein and exercise including resistance exercises (preferably through a supervised resistance training program). So, the potential for harm in over-diagnosis is therefore low, as long as the information is provided in a clear and supportive way with a positive message about the lifestyle changes. It can prompt further evaluation with DXA BC if desired. But, if muscle mass is found to be normal with the Omron® BC scale, it prevents unnecessary further investigation as the chances of missing low muscle mass with the Omron®BC scale is very low. The lifestyle interventions have been shown to also benefit other common age-related diseases such as cardiovascular and bone health [Arciero et al, 2006; Agostini et al, 2018; Kemmler et al, 2020; Liao et al, 2017]. In addition, these interventions have also been shown to *prevent the development of sarcopenia*, so will provide benefit to the person even if they are actually only in the pre-sarcopenic stage [Rogeri et al, 2021]. Given the rising prevalence of obesity, there is now even more need for objective assessment of muscle mass in older adults who appear to be outwardly robust. Missing the concomitant presence of sarcopenia and obesity will lead to under-recognition and under-treatment of their additive comorbidities.

Using an office-based BIA tool will also enable clinical facilities in non-urban centres to objectively assess muscle mass, improving equity of clinical care. In Alberta, acquisition of DXA body composition requires out-of-pocket payment which is not accessible for all people. In addition, the results need to be interpreted by a knowledgeable practitioner, as the DXA BC providers make no data interpretation. Being able to have the BIA evaluation done as part of their routine clinical assessment will ensure that all will receive appropriate care and timely diagnosis, irrespective of their financial means.

This chapter has been submitted for publication, "Addressing the main barrier to Sarcopenia identification: Utility of practical office-based assessment tools versus Dual Energy Xray Absorptiometry (DXA) Body Composition for identification of low muscle mass in older adults" [Juby et al, submitted 2022].

7.2.4 Objective 4: Assess the rate of sarcopenia and determine the association with leisure physical activity.

To address this objective a prospective observational cohort study of independent living community-dwelling older adults, assessed at baseline, 6 and 12-months, was undertaken.

As reported in **Chapter 6,** fifty community-dwelling older adults were enrolled, and 39 completed the 12-month study.

The *hypothesis (2.2.2 4a)* of low prevalence (<5%) of sarcopenia in this highly functioning group of community-dwelling older adults was *not confirmed, as the rate was higher than predicted.* The rate, in our predominantly female cohort, was 7.1%, in keeping with quoted community-dwelling older adults reported from the UK (4.6% in males, 7.9% in females) [Patel et al, 2013] and slightly lower than Japanese females (11.2%) [Kim et al, 2015], but much lower than that reported from the United States (36%) [Brown et al, 2016]. It was also much higher than that reported in a retrospective evaluation of a cohort from the Canadian study of Longitudinal Health and Ageing (CLSHA) where the prevalence reported was 0.2% sarcopenia and 1.2% severe sarcopenia [Purcell et al, 2020].

What was also unexpected was the high level (40.5%) of "total sarcopenia" (probable + pre + sarcopenia), with most of this being pre-sarcopenia. What was reassuring, however, was that despite no specific intervention, and despite 28.6% having pre-sarcopenia, the majority of participants (54%) did not change their sarcopenic status over the 12-months, *confirming hypothesis 2.2.2 4b.*

Hypothesis 2.2.2 4c was not confirmed, with the rate of obesity being 36% using BMI cut-offs, and not <28% as hypothesised, although it did not change significantly over the 12-months. What we *did not hypothesise for, or anticipate*, was the large discrepancy in obesity diagnosis between BMI and DXA BC cut-offs. When using sex-specific DXA BC cut-offs, 81% (versus 36% with BMI) were categorized as obese. This highlights the importance of clearly defining the criteria used. It therefore also impacts diagnosis of sarcopenic obesity, changing the rate of total sarcopenia and obesity from 7.7% to 33.3% in this population.

The option of participating in a social group activity program once and/or twice a week, was also included in this study. *Hypothesis 2.2.2. 4d was confirmed* in that there was no change in sarcopenic status in those participating in the social activity group over the 12-months. Somewhat unexpectedly, those participants with baseline pre, probable or sarcopenia attending the group social activity program had a measurable improvement in their Tinetti balance score.

Hypothesis 2.2.2.4e was not confirmed as the level of self-reported leisure-based physical activity *did not* appear to be related to the presence of pre or established sarcopenia, at 12-months. Although this has also been shown in some other clinical trials, epidemiological studies would suggest that leisure physical activities may have a preventive role, hence we felt it was worth exploring again given that this was a different population group. The final *hypothesis (2.2.2 4f) was not confirmed.* All participants had a trend to improvement in their quality of life as measured by the EQ5D VAS scale over the 12-months and there was no statistically significant difference between those in the sarcopenia or non-sarcopenia groups, or those attending or not attending the social group activity program.

This study has ***contributed to the growing scientific literature*** highlighting the probable under-diagnosis of sarcopenia, even in apparently healthy population cohorts. Data from this study *further highlights the care gap*, reinforcing the much-published pleas of the need for formal evaluation of sarcopenic status in all older adults [Bauer and Morley, 2019]. The data on obesity

(and associated sarcopenic obesity) *adds to the literature about the importance of clearly defining the criteria for obesity* used in future studies, to allow accurate comparisons of sarcopenic obesity prevalence, and the impact of study interventions and outcomes. A recent consensus statement from the European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Association for the Study of Obesity (EASO) on sarcopenic obesity [Donini et al, 2022], defines obesity screening by BMI or waist circumference with ethnicity-specific cut points, and confirmation using definitions recommended by Gallagher and colleagues [Gallagher et al, 2000], at 30% for males and 40% for females (higher than those recommended by EWGSOP used in the present research). As there seemed to be some benefit from the group social activity program, this study further highlights the need for more community-based programs. Despite receiving a copy of the social group activity program in printed and video form, the majority of the group class participants did not continue with the activities, *adding to the literature highlighting the value of the social aspect* to promoting regular physical activity [Spink and Carron, 1992; Mays et al, 2021].

This chapter has been submitted for publication as "Observational cohort study of healthy community-dwelling older adults, followed for 12-months to assess the association of lifestyle on sarcopenic status" [Juby et al, submitted 2022].

7.3 Clinical Implication of studies

The dose response study [Juby et al, 2021] has several clinical and research implications. Although overall there was the expected linear dose-response, considerable individual variability was shown both in dose-response and side-effects. This thesis has also shown that it is simple, practical, and acceptable to AD participants, to measure BHB response to MCT supplementation with a finger-stick test. This should serve to also encourage future researchers to aim for a *target serum BHB level*, and to individualize their MCT supplementation based on the measured BHB response. This could allow for individual dosing rather than using a standardized formulation for all participants - which has been the case up until now, and may account for the variability of published results. Knowing the target BHB level reached will also facilitate more accurate

conclusions about therapeutic efficacy, or lack thereof. The main potential issue with targeting a BHB level, will be that there will be no way to blind participants or investigators to study group allocation if a placebo is also being utilised in the study design.

The effect of dietary carbohydrates on BHB response is previously well documented, and this study reinforces this information. It adds further information on *the importance of body composition on BHB response*, suggesting this should now be another variable to be considered/corrected for, in future studies. The study also showed that BHB responses did not appear to be affected by age, or AD status, which is helpful for those planning future trials.

Side effects will remain a clinical challenge with MCT consumption. Research from this thesis has shown that side effects are not always related to absolute BHB levels [Juby et al, 2021]. However, as shown by the longitudinal MCT AD study [Juby et al, 2022], participants are able to increase their maximum tolerated dose with titration over time. This has *implications in terms of the design of trials* to provide the most tolerable amount of MCT oil supplementation. Previous studies with MCT supplementation in participants with mild cognitive impairment or early AD [Rebello et al, 2015; Reger et al, 2004; Taylor et al 2018] have often added the MCT to a standardized nutritional supplement [Fortier et al, 2019], or looked at a formulation of MCT esterase that could be given in tablet form [Henderson et al, 2009, 2011; Newport et al 2014]. Although tablet formulations may seem an obvious solution, they may only be useful earlier in AD, as it is well known that as AD progresses, many patients not only become more resistant to taking medications, they additionally can develop swallowing problems associated with their dementia, precluding them from swallowing tablets whole [Parlak et al, 2021]. Studies in this thesis showed that AD participants can consume MCT in its "native" form, even for an extended period of time, suggesting that there is not a pre-requisite for sophisticated/expensive formulations.

The apparent lack of effect compared to placebo in the cross-over phase of the longitudinal AD study highlights the importance of *choice of placebo*, to ensure there are no unexpected effects from the placebo that may blunt any measurable effect from MCT. Mixing several oils, all at low dose, to make a placebo cocktail would potentially prevent there being a large enough quantity of a single oil to have unexpected therapeutic effects.

The long-term safety, confirmed in the MCT AD study in this thesis should provide reassurance to other researchers, and encourage extension of future studies for longer time frames. In a disease like AD with long latency and disease course, time is needed to assess the effect of MCT supplementation on disease progression, something which has always been a challenge with all AD studies [Thompson et al, 2012].

The two studies reported in sarcopenia *reinforce the importance of sarcopenia assessment* in all older adults. The high prevalence of both pre-sarcopenia and obesity in a highly functioning group of older adults highlights the potential for earlier intervention, before obvious clinical signs (such as loss of ADL function and independence) and complications (such as fractures) develop. Timely diagnosis will allow for timely interventions to potentially reduce progression of morbidity. Office based direct-to-consumer BIA scales may address the most commonly sited reason for lack of sarcopenia diagnosis, that is the lack of a diagnostic tool to objectively measure muscle mass in the clinic.

In addition, the sarcopenia studies show the *importance of clearly defining obesity*. This not only has implications for sarcopenic obesity, but also other chronic health conditions associated with obesity, such as diabetes and cardiovascular disease. Using BMI cut-offs in this study clearly under-reported the obesity rate, if it is assumed the DXA BC percentage fat is a more accurate reflection of obesity. The presence of an in-office direct-to-consumer BIA body composition scale can also address this issue, with an *accurate measure of percentage body fat*, with the BIA scales evaluated showing a high association with DXA body fat measurements.

The apparent lack of effect on sarcopenic status *of leisure activity* is not unique to the study reported in this thesis. Potential effects of leisure activity on cardiovascular parameters was not part of the study. The participants all had a high baseline level of cognitive function, precluding any conclusion about potential cognitive effects of leisure activity.

Those attending the group activity program were consistent over the 12-months, yet almost all did not use the post-study exercise video to continue the program at home. As in previous studies [Spink and Carron, 1992; Van Norman, 1998; Fraser and Spink, 2002], this thesis further reinforces the *value of socialisation and group cohesion* in a community-based activity program for ongoing compliance.

7.4 Future directions

Future research with the provision of dietary ketones is an exciting, emerging field in clinical medicine. Two of the studies in this thesis have reinforced the efficacy of MCT supplementation in elevating serum ketones even without dietary changes, and the demonstrated long-term safety in the AD study encourages the use of longer term MCT supplementation in other neurocognitive disorders where cerebral glucose is sub optimal. The implications for those with neurocognitive disorders is profound, should it be shown in the future that dietary ketones can influence the progression of diseases such as Alzheimer's or Parkinson's disease. Thinking of MCT supplementation more like a pharmaceutical agent, *BHB target,* rather than absolute MCT dose could become the goal for evaluating therapeutic efficacy. In addition, particularly if cognitive evaluations are going to be done more frequently based on a BHB target, evaluating the cognitive changes needs to be done with tools that can be administered in a non-operator dependent way, without a learning component, such as the Cognigram®. Because the measurable cognitive changes may be subtle, especially in early neurodegenerative states, it will also be important to use tools that are sensitive to change over a short period of time. Repeating studies in MCI and AD knowing these new facts may provide very different outcomes.

The recent COVID-19 pandemic has resulted in the emergence of a new neurocognitive disorder related to previous COVID-19 infection: long-COVID brain fog [Lopez-Leon et al, 2021]. Many of the symptoms are similar to the clinical neurocognitive changes seen with early Alzheimer's, including cerebral glucose hypometabolism, raising the intriguing possibility of using MCT supplementation for the management of these symptoms [Juby, Cunnane, et al, submitted 2022]. To that end, a research project has been designed to evaluate the impact of MCT supplementation on the neurocognitive symptoms related to long-COVID. By using research from this thesis, it is confirmed that younger adults have a similar BHB responds to older adults. This is important, because the neurocognitive disorders related to long-COVID unfortunately occur in a much younger demographic than AD (20- to 40-year-olds) [Lopez-Leon et al, 2021]. There is also increasing evidence that long-COVID can exacerbate AD symptoms in older adults with MCI or

AD at baseline [Ciaccio et al, 2021], suggesting a potential role for MCT supplementation in this long-COVID demographic too. Should the study show efficacy in the younger demographic being studied, this will then be translatable to the older adult population. Given the research and lessons learned from this thesis, BHB response will be measured, and based on BHB levels, MCT dosing will be individualized in this proposed long-COVID study - something which has not been done in any MCT clinical trials to date.

The mechanism of action of BHB on cognitive function can also be evaluated further with the more sophisticated neuroimaging modalities now available. Functional magnetic resonance imaging (MRI) [Buchbinder, 2016], and PET MRI [Ehman et al, 2017] imaging are becoming more available to clinicians and researchers. In combination with these new modalities and ongoing laboratory research, understanding of the potential neuroprotective effects of BHB will expand. Different formulations of MCT may be required to reach a certain target BHB level if higher doses are required, given the tolerance issue with the current formulations limiting significantly increasing the dose.

Up until now, sarcopenia knowledge has been focused within a group of well-informed researchers and clinicians. Just like osteoporosis, however, the care gap will continue unless all generalists caring for older adults have the necessary skills, tools, and knowledge, to make the diagnosis [Witham, 2019]. Akin to osteoporosis, sarcopenia does not fall under the umbrella of any particular medical subspecialty (an “orphan”) and similarly is at risk of being “lost” due to lack of ownership [Rothschild, 2021]. In osteoporosis this lack of ownership has resulted in the assumption that it is “someone else’s responsibility” to address bone health [Akessen and McGuigan, 2021]. As far as the diagnosis of sarcopenia goes, many of the same signals are appearing, and it is important that we do not make the same mistakes as have been made in osteoporosis, lest we also be looking at a similar ongoing treatment gap 24-years later [Juby and De Geus, 1998]. This thesis has shown that it is possible to *incorporate the diagnosis of sarcopenia into regular clinical practice.* As a start, including sarcopenia assessment in the Comprehensive Geriatric Assessment (CGA) currently being done could occur, with the only additional task required being the objective measure of muscle mass. Gait, balance and fall risks are already assessed, and instead of using the Timed-Up-and-Go test, the chair-stand test could

be substituted, providing information on gait, balance, and fall risk, as well as valuable additional information on muscle strength and gait speed.

Other care barriers in the management of sarcopenia include the fact that discussion of nutrition and exercise interventions is time consuming, and many practitioners also feel they do not have the necessary skills in these areas [Wynn et al, 2010]. By ignoring the diagnosis and practicing *therapeutic nihilism for sarcopenia*, clinicians can also ignore addressing these issues. This nihilism has been identified in older adults with malignancies, with a resulting reduced quality of life among the elderly with cancer, and their families [Biskup et al, 2020*]. Specific "exercise prescriptions"* have been shown to be more beneficial than general advice on increasing physical activity [Frémont et al, 2014]. For practitioners who do not have a comfort level with these prescriptions, or the resources from Allied Health professionals, using available online resources can address this need. Osteoporosis Canada [Osteoporosis Canada (OC)] has resources available on its website for practitioners that includes an exercise prescription, describing the frequency, intensity, and types of both aerobic and resistance exercises. There is also an online exercise prescription and referral tool available at www.exerciseismedicine.ca [Frémont et al, 2014]. In addition, the use of multidisciplinary teams can address knowledge gaps in clinicians.

Further to the challenge with sarcopenia management, is the absence of a specific pharmacologic intervention. Although admittedly, in osteoporosis, this has not had much impact on the care gap, with the only real impact being shown with Fracture Liaison Service's which address post-fracture care to prevent/reduce risk for future fracture [Akesson and McGuigan, 2021]. Without a "sentinel event" in sarcopenia, this type of model is a less practical option. In addition, this sentinel event (such as a fragility fracture in osteoporosis, or a myocardial infarction in cardiovascular disease), can be used to show the cost-effective benefit of interventions to prevent future events. However, for sarcopenia, there is no single outcome, making cost-effective studies difficult to do, and therefore costs of community interventions more difficult to justify. Falls are not a good surrogate for sarcopenia [Dalal et al, 2022], given their multi factorial aetiology. This highlights *the need for pleiotropic interventions*, that is, those providing beneficial actions across multiple organ systems -such as bone, cardiovascular, muscular, nutritional, and mental health. Numerous resources already exist in terms of patient information/education, and online exercise videos in the field of osteoporosis. Given the common comorbidities between osteoporosis and

sarcopenia, and the common benefits of interventions such as increased dietary protein and resistance exercises, use of existing osteoporosis resources for sarcopenia prevention and management is cost and time effective [Osteoporosis Canada; International Osteoporosis Foundation]. The existing patient information handouts can particularly address care gaps in clinical settings where there is no opportunity for input from a multidisciplinary team that includes dieticians and physiotherapists (such as in rural settings), and where the majority of the care burden falls on a single provider-either a Family Physician or a Nurse Practitioner.

The aging demographic is going to place an increasing burden on the health care system, especially if there are not more aggressive approaches to disease prevention and lifestyle modification. Reliance on medications and medical intervention once diseases are established is not only costly, but significantly less effective. Published research supports that fact that patient-initiated enquiries to their health care providers prompts further evaluation [Johnson et al 2021]. *Empowering Seniors themselves* with research knowledge is a significant educational challenge. One strategy may be partnering with patient advocacy organisations such as the Alberta Strategy for Patient Oriented Research Support Unit (AbSPORU), or existing patient focused organisations, such as Osteoporosis Canada. Another approach being addressed is peer educators providing the peer education. To this end, I am a co-principal investigator in an ongoing clinical trial (Supporting Healthy Aging by Peer Education and Support (SHAPES) program), as the developer of the educational resources for osteoporosis and fracture prevention, that is then used by older adults to educate their peers. Preliminary analysis shows this to be highly effective and acceptable [Rajabali et al, unpublished].

There are also many overlaps between AD and sarcopenia, in terms of both etiology and lifestyle interventions. *Osteosarcopenia* is an emerging concept [Hassan and Duque, 2017]. There are increasing interactions reported between muscle, bone, and fat related to the biological role of myokines, osteokines, and adipokines [Kirk et al, 2020], and interactions between the microbiome, osteosarcopenia, and dementia [Ticinesi et al, 2109]. Common interventions are exercise (aerobic and resistance) plus a balanced high-protein whole-food diet [Bischoff, 2016].

7.5 Conclusions

On the face of it, there would not appear to be much in common between sarcopenia and Alzheimer's disease, but both sarcopenia and Alzheimer's disease have significant impact on the quality of life and independence of older adults. This thesis summarises the significant crossover of lifestyle risks for both conditions, and the potential for lifestyle interventions, and dietary ketones, to influence both diseases. (See **Figure 7. 1**).

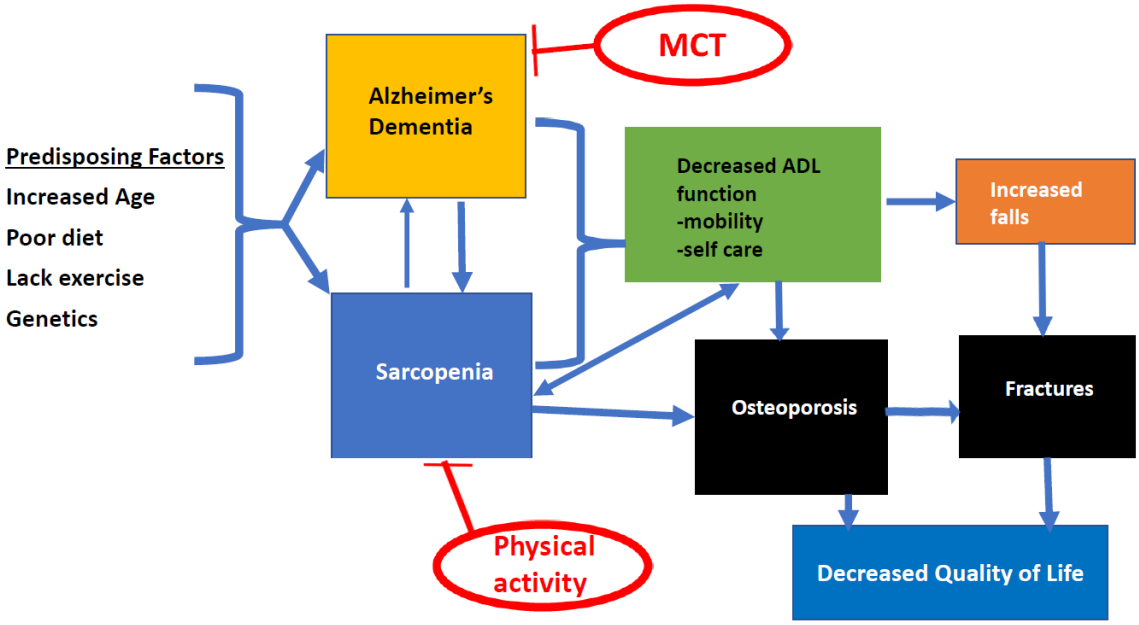


Figure 7.1: Summarizing the interactions between Alzheimer's dementia (AD) and Sarcopenia – etiology, outcomes, and interventions (in red).

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APPENDICES

Appendix Figure 1: Mini Mental Status Examination (MMSE). [Folstein and Folstein, 1975]

Standardized Mini-Mental State Examination (Folstein's)

Assessed By: _____ Date: _____

Level of Education: ☐ 0-4 yrs ☐ 5-8 yrs ☐ 9-12yrs ☐ College
If MMSE not completed, check appropriate box (explain in notes): ☐ Refused ☐ On Pass ☐ Language Barrier ☐ Other
Assess level of consciousness along a continuum.

Alert — Drowsy — Stupor — Coma

Instructions: I am going to ask you some questions and give you some problems to solve. Answer the best you can.

Orientation: Allow 10 seconds for each reply.	**Score**
1. "What year is it?" (exact answer only: taking last answer given)	/1
2. "What season is it? (within one week of season change either may be accepted)	/1
3. "What month is it? (On first or last day or month accept either month)	/1
4. "What is today's date?" (Accept previous or next day's date)	/1
5. "What day of the week is it?" (Exact answers only)	/1
6. "What country are we in?" (Exact answers only)	/1
7. "What province are we in?" (Exact answers only)	/1
8. "What town/city are we in?" (Exact answers only)	/1
9. "What is the street address or rural equivalent/building/facility?": (Exact answers only)	/1
10. "What room are we in: (in home) or "What floor of the building are we on?" facility (Exact answers only)	/1
Registration/Attention & Short Term Memory: Allow 20 seconds for each reply.	
11. "I am going to name three objects. After I have named all three objects, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes. "BALL, CAR, MAN". For repeat use: BELL, JAR, FAN, BULL, WAR, PAN. (If patient did not repeat all three, repeat until they are learned (Up to five times. Score first attempt only.)	/1 /1 /1
12. "Please spell the word WORLD". May repeat up to three times. If unable to spell, score zero. "Now spell it backwards, please." To score write client answer on right. Score is # of matches you can make without crossing lines. Starting at first letter and drawing lines in sequence. (Allow 30 seconds for reply.) See user's guide or serial 7's.)	/5
13. "Now what were the three objects, I asked you to remember?" (Allow 10 seconds for each reply. Score 1 point for each correct response regardless of order.)	/3
Language/Spatial Orientation & Coordination	
14. Point to pencil. "What is this called?" (accept pencil only)	/1
15. Point to watch. "What is this called?" (accept watch, wrist watch, or time piece only)	/1
16. I'd like you to repeat this phrase after me, "no ifs ands or buts". (Must repeat exactly to score one point.)	/1

D	L	R	O	W
93	86	79	72	65

17. Show CLOSE YOUR EYES. "Please read the words and then do what it says." (Allow 10 seconds. Repeat instructions up to three times if necessary. Score 1 point only	/1
18. Write any complete sentence on that piece of paper for me. The sentence must contain a subject, verb and object and make sense. Ignore spelling. Allow 30 seconds. Note handedness.	/1
19. "Copy this design please." (Allow up to one minute. Must produce four-sided figure between two five-sided figures to score a point.)	/1
20. "Take this paper in your right/left hand ( ), fold it in half with both hands ( ), and put the paper down on the floor ( )." (Score 1 point for each instruction correctly executed.)	/3
Score	**/30**
Adjusted Score	**/30**

*Please refer to SMMSE: A User's Guide for Guidelines on Adjusting Scores.

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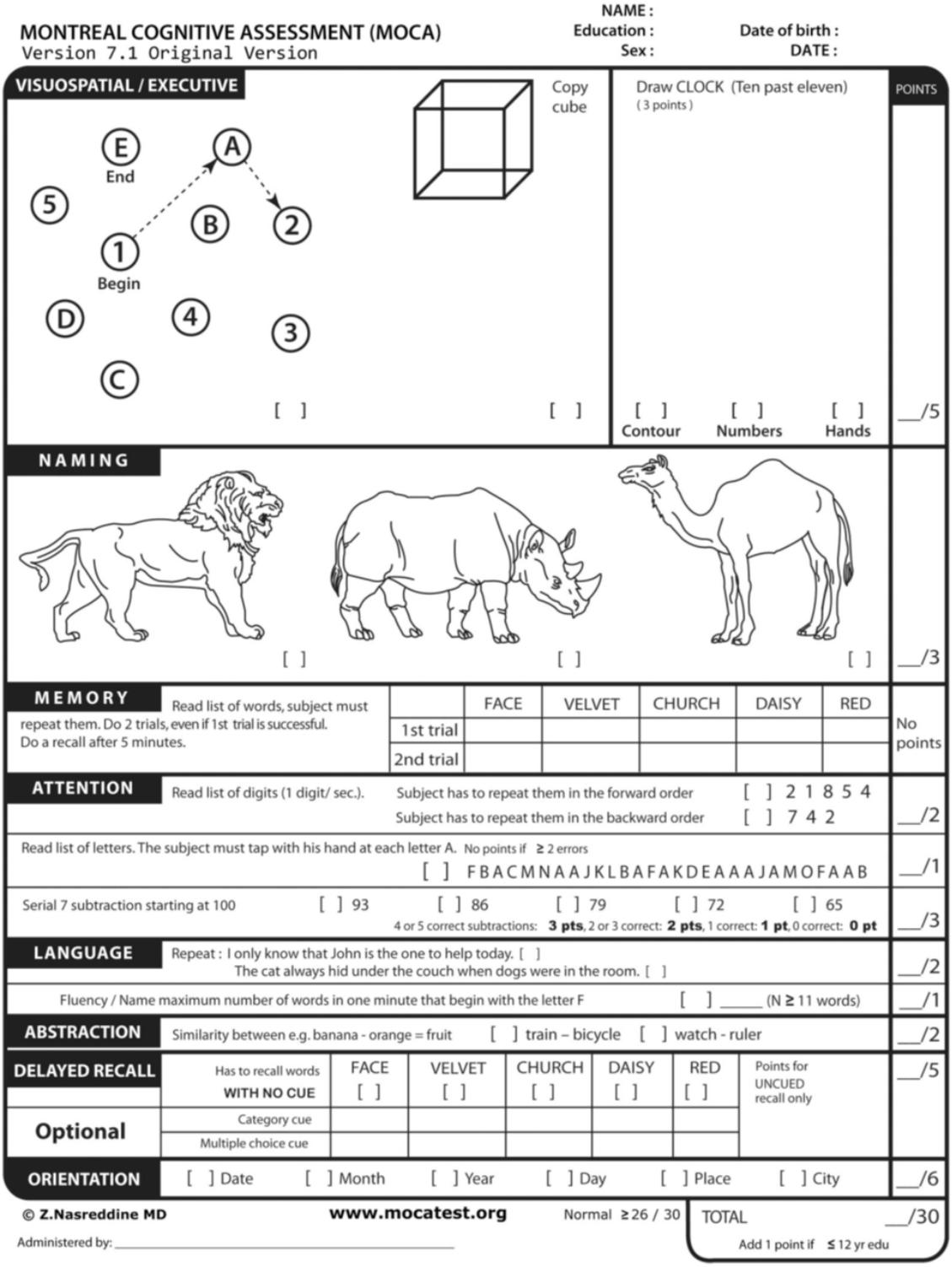
Appendix Figure 2: Montreal Cognitive Assessment (MoCA) version 1. [Nasreddine et al, 2005]



MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS

Copy cube

Draw CLOCK (Ten past eleven) (3 points)

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ]

[ ] Contour [ ] Numbers [ ] Hands ___/5

NAMING

[ ] [ ] [ ] ___/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2 ___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B ___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** ___/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ] ___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words) ___/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler ___/2

DELAYED RECALL — **Optional**

	FACE	VELVET	CHURCH	DAISY	RED	
Has to recall words WITH NO CUE	[ ]	[ ]	[ ]	[ ]	[ ]	Points for UNCUED recall only
Category cue						
Multiple choice cue						

___/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City ___/6

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30

TOTAL ___/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

Appendix Figure 3: Cognigram ® [Mielke et al, 2015]

Cogstate Brief Battery™

The Cognigram system utilizes the Cogstate Brief Battery, which has been used in over 1,000 research studies around the world and cited in hundreds of peer-reviewed journal publications. The battery consists of four tests, where a single playing card stimulus is presented in the center of the device screen. At the presentation of each playing card stimulus, the patient is required to respond either "YES" or "NO".

Detection Test

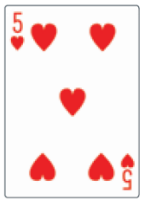
"Has the card turned over?"

Main Domain Measured
Psychomotor Function

Identification Test

"Is the card red?"

Main Domain Measured
Attention



One Card Learning Test

"Have you seen this card before?"

Main Domain Measured
Learning

One Back Test

"Is the card the same as the previous card?"

Main Domain Measured
Working Memory

See video on You tube: https://www.youtube.com/watch?v=9C5XR5xRbd0 for additional information.

The format used in this study was separate large (3 cm diameter) yes and no buttons, and the test was displayed on a desk top computer.

Appendix Figure 4: Test oil components

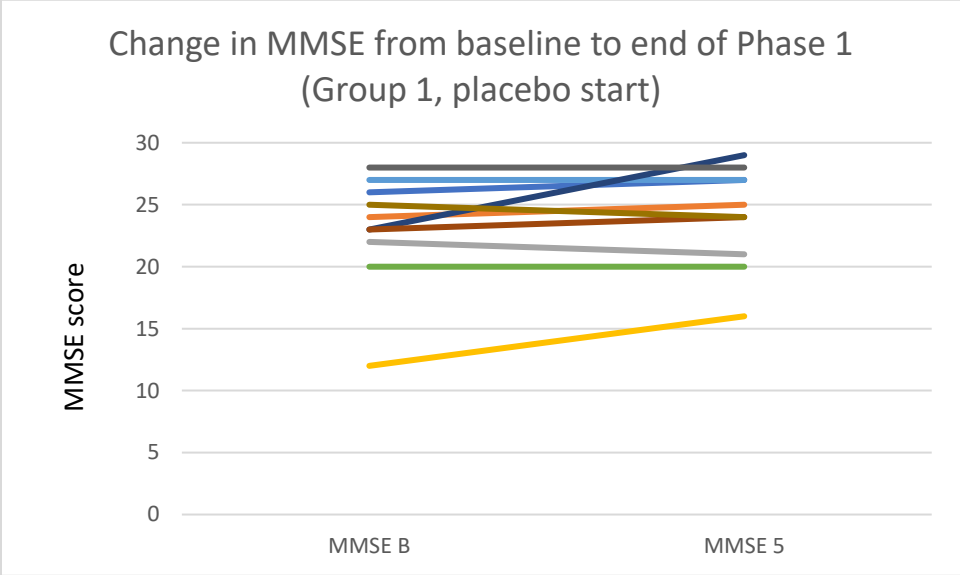
Richards Packaging, Pharmaceutical Grade 16 oz White Plastic Bottle

Trudeau Maison Measuring Spoons - Set of 5 (only Tablespoon (15ml) supplied to participants)

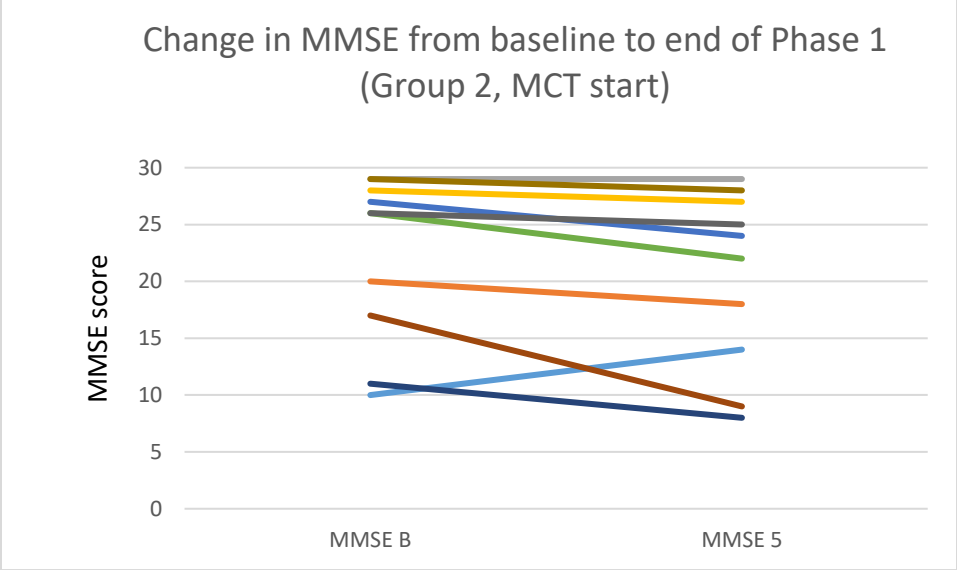
Appendix Figure 5: MMSE responses in individual participants grouped by Placebo (Group 1) or MCT (Group 2) start at different phases of the study.

1. **First double-blind phase**

A.



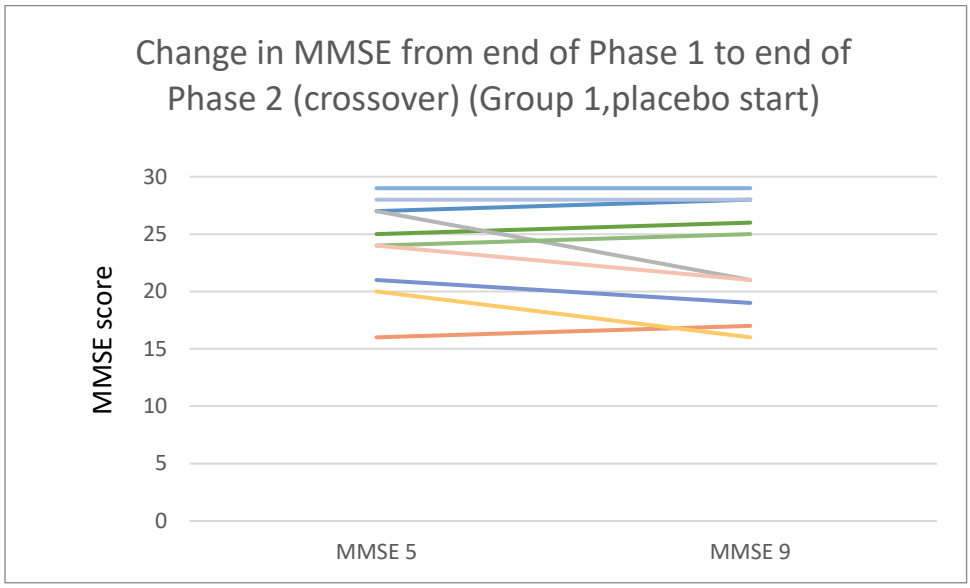
B.



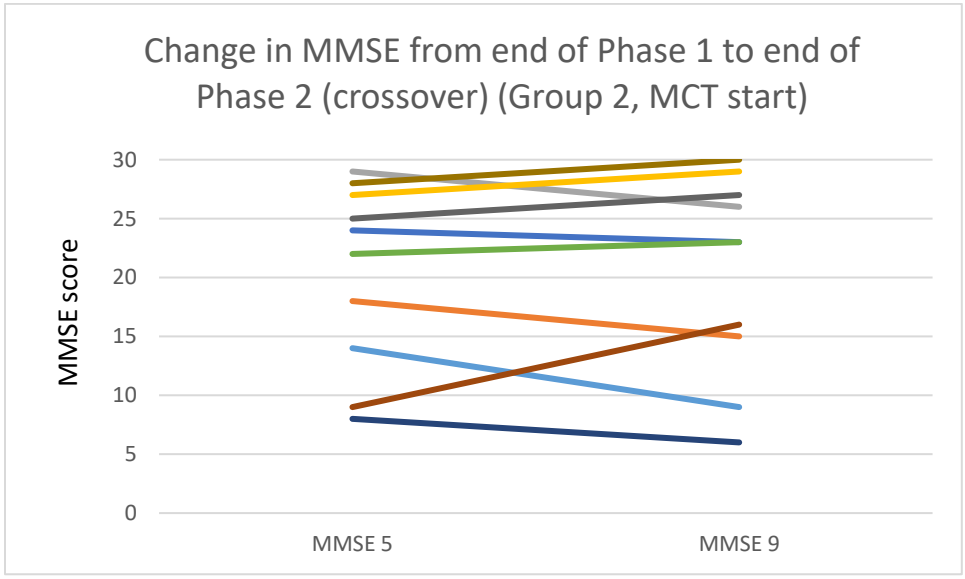
MMSE B; Baseline (visit 1); MMSE 5 (visit 5, end of phase 1)

2. **Second double-blind phase (crossover)**

A.



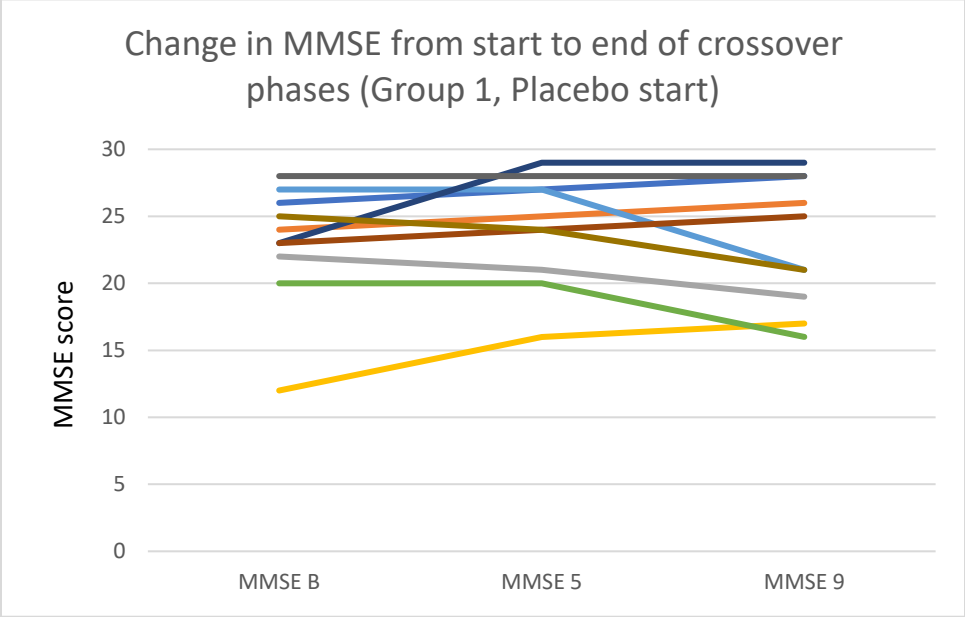
B.



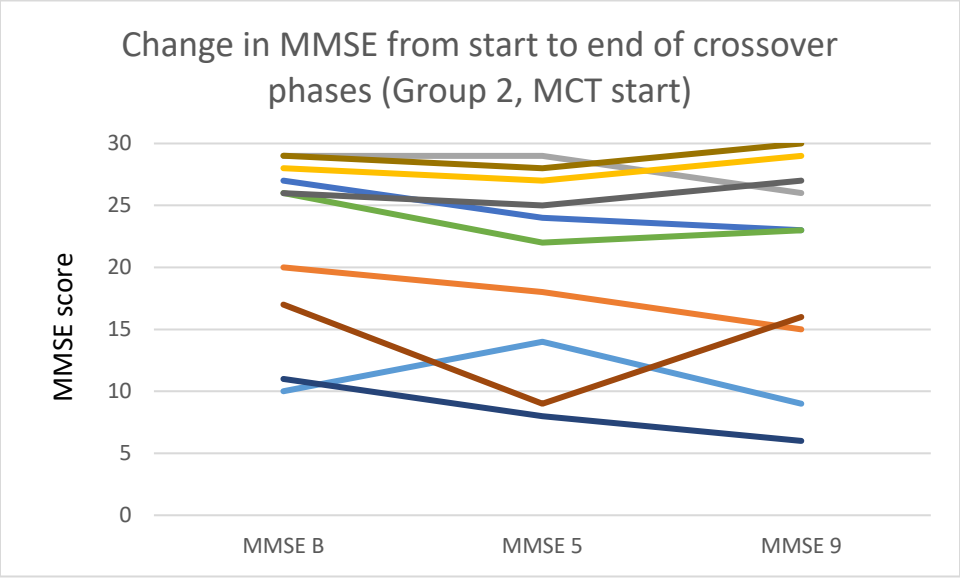
MMSE 5: (visit 5), end of phase 1; MMSE 9 (visit 9) end of phase 2

3. **Overall change in MMSE during double-blind crossover Phases**

A.

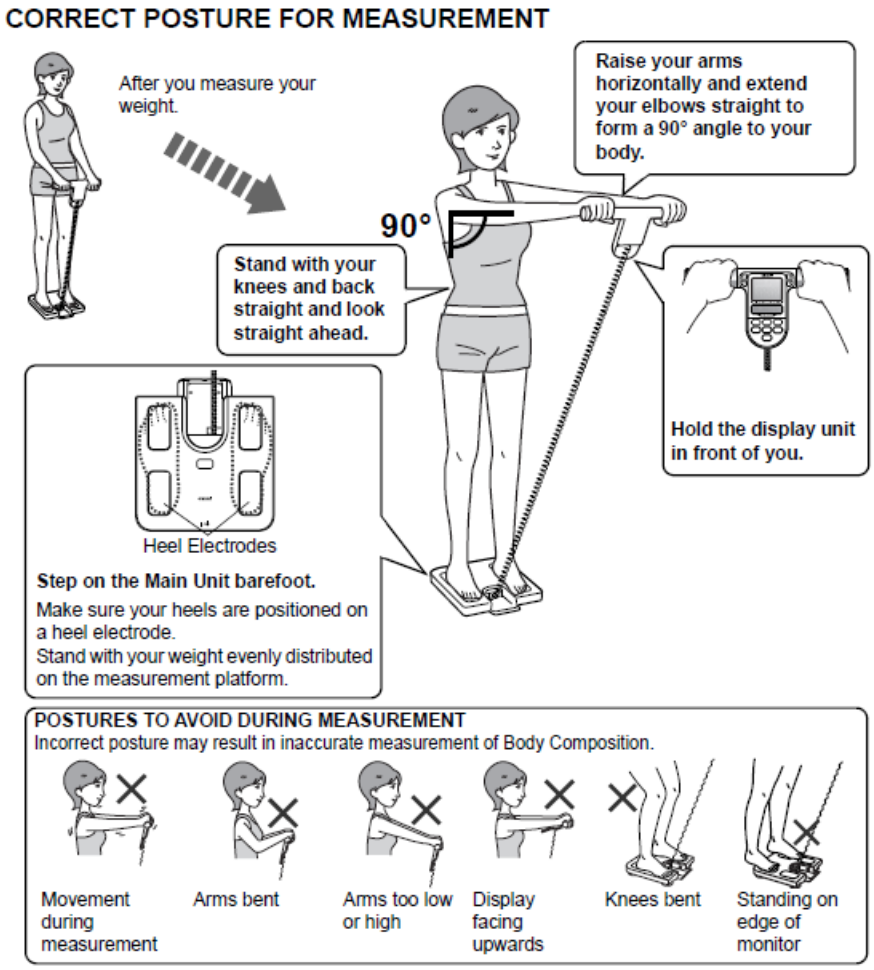


B.



MMSE B; Baseline (visit 1); MMSE 5: (visit 5), end of phase 1; MMSE 9 (visit 9) end of phase 2.

Appendix Figure 6: Correct positioning for Omron® BIA scale.



Appendix Figure 7: Participant exercise information DVD and manual