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# The Combined Effects of Physical Exercise and Cognitive Training on Gait Speed and Primary Motor Cortex Metabolism in Individuals with Mild Cognitive Impairment: A 1H-MRS Analysis

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# Abstract

Mild cognitive impairment (MCI) is a transitional stage before dementia. Altered gait in MCI has been associated with progression to dementia. Using magnetic resonance spectroscopy, a relationship between primary motor cortex (M1) neurochemistry and dual task gait speed has been reported in MCI. Interventional research suggests exercise, cognitive training, and vitamin D supplementation may benefit MCI, yet the combined effect of these treatments on gait speed and M1 metabolism is unknown. Participants with MCI (N=75) were assigned to one of five intervention arms and dual task cost on gait speed and M1 metabolism was assessed before and five months after intervention. Cognitive training paired with exercise increased M1 N-acetyl aspartate (NAA)/creatine concentrations compared to physical exercise alone. Additionally, those with greater changes in dual task cost on gait speed showed greater decreases in NAA and choline, further establishing a relationship between gait speed and M1 function in MCI.



# Keywords

Cognitive training, dual task, gait speed, magnetic resonance spectroscopy, metabolism, mild cognitive impairment, N-acetyl aspartate, physical exercise, primary motor cortex, vitamin D


# Summary for Lay Audience

Mild cognitive impairment (MCI) is considered a transitional state between healthy aging and dementia. Unlike dementia, some patients with MCI are responsive and stabilize after incorporating regular exercise, cognitive training, and vitamin D supplementation. Therefore, our understanding of how to delay the onset of dementia is vital to the outcome of a patient's disease progression. Slow walking speed in MCI has been associated with a significantly higher risk of progressing to dementia. Further, when patients are asked to perform a secondary cognitive task while walking (dual task), those with slower gait were more likely to have abnormal primary motor cortex (M1)

metabolism, the region responsible for initiating leg movements. The current study examined whether five months of combined exercise, cognitive training, and vitamin D supplementation could increase dual task speed and improve M1 metabolism in people with MCI. We also wanted to know whether changes in dual task speed were associated with changes in M1 metabolite levels after five months. Before and after the trial, we used magnetic resonance spectroscopy, a non-invasive imaging tool, to assess M1 metabolite levels. We also measured the cognitive cost of the dual task on gait speed by taking the percentage difference in speed between dual task- and normal walking. Greater dual task cost is associated with greater gait impairment, greater cognitive impairment, and a higher risk of progressing to dementia. Five-month changes in M1 metabolite levels and dual task cost were assessed in 75 participants with MCI that were assigned to one of five treatment arms varying in combinations of physical exercise, cognitive training, and vitamin D regimens. Cognitive training combined with physical exercise showed improved M1 neuron function over time compared to participants who only did physical exercise. Additionally, we found that individuals with increased dual task cost on gait speed over time were also more likely to show declines in M1 neuron function. Our findings provide evidence of metabolic benefits in M1 following combined physical and cognitive training. This study also corroborates previously documented relationships between dual task gait speed and the metabolite profile of M1 in people with MCI.

# Co-Authorship Statement

For the manuscript in Chapter 2, Jack Elkas is the first author, and Dr. Robert Bartha; Dr. Frederico Pieruccini-Faria; Dr. Manuel Montero-Odasso; Dr. Guangyong Zou; Dr. Amer Burham; Dr. Mark Speechley; Dr. Quincy J. Almeida; Dr. Teresa Liu-Ambrose; Dr. Laura E. Middleton; Dr. Richard Camicioli; Dr. Nick W. Bray; Dr. Karen Z.H. Li; Dr. Sarah Fraser; Dr. Nicolas Berryman; Dr. Maxime Lussier; Dr. Kevin J. Shoemaker; Dr. Surim Son; Dr. Louis Bherer; and the Canadian Gait and Cognition Network, are co-authors given their contribution to facilitating the data collection and completion of the SYNERGIC trial. Jack Elkas did not aid with data collection, though was primarily responsible for all data compilation, analysis, and interpretation in Chapter 2.

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*I dedicate this work to my late grandfather ~*

*Thomas "Peter" Barron*

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# List of Abbreviations

| | |
|---|---|
| $^{1}H$ | proton |
| AD | Alzheimer's Disease |
| aMCI | amnestic mild cognitive impairment |
| ANCOVA | analysis of covariance |
| BAT | balance and toning |
| cDT | counting by 1's dual task |
| cDTC | counting by 1's dual task cost |
| CHESS | chemical shift saturation selection |
| Cho | choline |
| Cr | creatine |
| CT | cognitive training |
| DT | dual task |
| DTC | dual task cost |
| ECC | eddy current correction |
| M1 | primary motor cortex |
| MCI | mild cognitive impairment |
| MMSE | Mini-Mental State Examination |
| MNI | Montreal Neurologic Institute |
| MoCA | Montreal Cognitive Assessment tool |
| MP-RAGE | magnetization prepared rapid gradient echo |
| MRS | magnetic resonance spectroscopy |
| Myo | myo-inositol |
| NAA | N-acetyl-L-aspartate |
| naMCI | non-amnestic mild cognitive impairment |
| nDT | naming animals dual task |
| nDTC | naming animals dual task cost |
| PE | physical exercise |
| PRESS | point resolved spectroscopy |

| | |
|---|---|
| pVitD | placebo vitamin D |
| QUALITY | quantification improvement by converting lineshapes to the Lorentzian type |
| QUECC | combined QUALITY deconvolution and eddy current correction |
| RF | radiofrequency |
| sCT | sham cognitive training |
| sDT | serial sevens dual task |
| sDTC | serial sevens dual task cost |
| SNR | signal-to-noise ratio |
| ST | single task/usual gait |
| T0 | baseline timepoint (pre-intervention) |
| T5 | five-month timepoint (post-intervention) |
| TE | echo time |
| TR | repetition Time |
| VitD | vitamin D |

# Chapter 1

# 1 Introduction

## 1.1 Dementia & Mild Cognitive Impairment

Neurodegenerative diseases leading to dementia increase in prevalence in the elderly. In Canada, 1 in 9 people over the age of 65 have dementia, affecting nearly 600,000 individuals [1]. The symptoms of dementia manifest as progressive declines in executive function, memory, language, sensory processing, and motor function [1]. The clinical presentation of symptoms is specific to the type of dementia [2]. In late stages of disease, patients become less independent, which not only places a physical, emotional, and financial burden on relatives, but also puts a strain on the healthcare system. Although recent trials of immunotherapies have shown some benefits in Alzheimer's disease (AD), there are currently very few effective therapeutic agents for any neurodegenerative disease [3], [4]. Consequently, the costs associated with supporting people with dementia in Canada in 2016 was $10.4 billion (CAD) and is estimated to increase to $16.6 billion by 2031 [1].

Within the neuropathological study of dementia exist a myriad of factors that contribute to the patient's symptoms and class of disease. Alzheimer's disease (AD), for instance, accounts for 60-80% of dementia cases, and is characterized primarily by beta-amyloid plaques and neurofibrillary tangles [1], [5]. Research in the last several decades has identified other factors contributing to AD pathology such as increased oxidative stress, inflammation, as well as genetic predispositions [6]–[8]. As of 2023, no pharmacological cures exist for dementia, and existing symptomatic treatment options are only sometimes effective for a limited duration and are often accompanied by unwanted side effects [9], [10]. As a progressive disease, mild symptoms that are present early on gradually worsen over time. Studies have found that early identification and intervention can sometimes slow progression [11], [12].

Mild cognitive impairment (MCI) is considered a transitional state between cognitively healthy aging and dementia (**Figure 1.1**). MCI is characterized by cognitive decline that is greater than what is expected for an individual's age and education level, yet not compromising an individual's independence [2]. It is estimated that 15-20% of individuals over the age of 65 live with MCI, equating to nearly 13 million people in North America [13]. Due to the aging of the population, MCI is becoming increasingly prevalent where the number of North Americans with MCI is expected to reach 19 million by 2040 [13], [14]. Clinically, MCI is a critical stage in the continuum of cognitive decline as individuals with MCI are at ten times higher risk of progressing to dementia. One third of these patients will stabilize and remain at the MCI stage and some may even revert to typical cognitive aging [15]–[18].

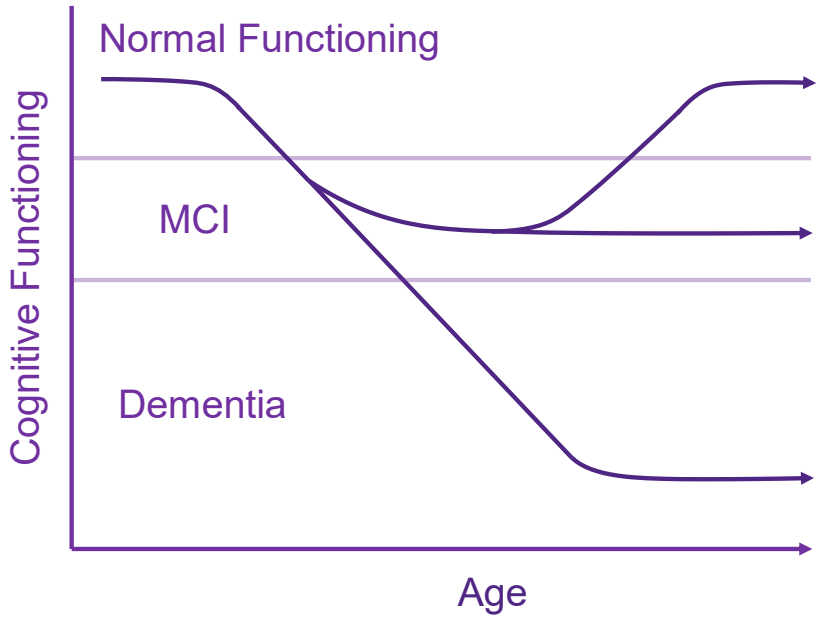


**Figure 1.1** Trajectories of cognitive decline from mild cognitive impairment (MCI) over time.

The etiology of MCI influences the type of dementia a patient experiences [2]. MCI can be further categorized into either amnestic (aMCI) or non-amnestic MCI (naMCI). aMCI is diagnosed based on whether a patient exhibits symptoms of memory impairment, whereas naMCI is diagnosed with any cognitive deficits apart from memory impairment.

The associated symptoms of memory in aMCI are empirically in line with progressing to Alzheimer's Disease (AD), whereas naMCI patients are more likely to progress to other neurodegenerative diseases such as Parkinson's Disease, Fronto-temporal Dementia, Lewy Body Disease, and others [2].

## 1.2 Interventions During the MCI phase

Since there is currently no cure for dementia, early intervention is crucial to optimize the long-term well-being of people with cognitive impairment [4], [9], [11], [12]. Only a few pharmacological agents have shown potential to reduce the severity and rate of cognitive decline, however, most of these drugs come with unwanted side-effects and are not effective in all patient types and provide only temporary benefit [4], [9].

Remediation strategies to prolong the onset of dementia in patients with MCI are primarily focused on eliminating unhealthy lifestyle habits such as poor diet and sleep hygiene, while promoting beneficial habits such as regular physical exercise and cognitively stimulating activities [9].

### 1.2.1 Physical Exercise

Physical exercise (PE) has been shown to be effective in reducing the rate of cognitive decline and improving the overall health of people with MCI [19]–[22]. The benefits of PE are dependent on the mode, duration, and intensity. Aerobic exercises can improve cardiovascular health and muscular tone, whereas resistance training, such as the use of weights, can help increase muscle strength [23]. Although it is recommended that people with MCI incorporate a balance of both aerobic and resistance training, there is still little research on the gained effect of combining them [20], [24]. Several mechanisms underlie the beneficial effects of PE on cognitive health, including increased neurogenesis, enhanced synaptic plasticity, improved cardiovascular health, increased cerebral blood flow, and reduced inflammation [25], [26]. These neurobiological effects can translate into cognitive benefits for those with MCI, including preservation of memory, attention, executive functioning, and processing speed [21].

### 1.2.2 Cognitive Training

Cognitive training (CT) comprises of structured activities designed to stimulate and challenge cognitive processes, such as memory, attention, language, and problem-solving. Studies have shown that CT can enhance cognitive functioning and slow down cognitive decline in individuals with MCI [27], [28]. The underlying benefit of CT relates to increasing neuroplasticity, which refers to the brain's ability to reorganize and form new neural connections [29]. By providing targeted cognitive challenges, CT promotes the formation of new neural pathways and strengthens existing ones. This process leads to improvements in cognitive functions, particularly in domains targeted by the exercises [27], [29]. Additionally, in line with the cognitive reserve hypothesis, CT may enhance compensatory strategies and improve the efficiency of cognitive processes, mitigating the impact of cognitive impairment on daily activities due to pathology [27].

### 1.2.3 Vitamin D

Vitamin D (VitD) is a fat-soluble vitamin that is most commonly known to maintain musculoskeletal health and is involved in regulating the absorption of calcium in the body [30], [31]. VitD exists in several forms, but the main types are Vitamin D2 (ergocalciferol) or D3 (cholecalciferol) [31]. The differences between D2 and D3 like mainly in their origin and potency, as D2 is primarily derived from plant sources, whereas animal based D3 can be produced endogenously in the skin upon exposure to sunlight and deemed to be more potent than D2 [31], [32]. VitD deficiency has been associated with an increased risk of cognitive decline in MCI and dementia [30], [33]–[36]. VitD receptors are widely distributed throughout the brain, including regions involved in memory and cognition [37], [38]. VitD exhibits anti-inflammatory and neuroprotective properties against oxidative stress by increasing the expression of antioxidants and detoxifying enzymes [35], [36], [39]–[41]. Additionally, VitD contributes to synaptic plasticity and neurotransmission [42]. However, the literature regarding whether regular VitD supplementation can improve cognition and reduce the risk of cognitive decline is inconsistent [37], [41], [43], [44].

### 1.2.4 Synergistic Effects

The combined effects of PE, CT, and VitD supplementation on mediating functional decline in MCI is not well understood and has not been studied extensively. Pairing physical and cognitive exercises has shown positive effects on cognition, activities of daily living and mood in older adults and people with MCI and dementia [45]. Vitamin D supplementation has synergistic effects when combined with physical exercise in rat models of dementia, and can enhance attention, executive function, and processing speed when paired with aerobic exercise in elderly women [35], [36], [46]. Additionally, providing individuals with MCI physical exercise, cognitive training, and vitamin D supplements increased functional brain connectivity when compared to controls; though this combined effect did not differ when compared to physical exercise alone [47]. The potential synergistic effects of combining physical and cognitive exercises with vitamin D supplementation in people with MCI requires further investigation on other aspects of brain health to help clinicians determine the most effective intervention strategy.

## 1.3 Gait Measurements

Gait is a term related to the pattern of a person's walking performance. To measure gait, an individual walks along an electronic mat embedded with pressure sensors that sample at high frequencies. The electric signals detected by the pressure sensors from a footprint (**Figure 1.2**) are sent to a computer with custom software to calculate the exact location of each footprint. Using cartesian coordinates, the distance between ipsi- and contralateral footfalls, measured at the center of each heel, provides spatial gait metrics; and the time elapsed between sequential foot landings provides temporal gait metrics. As such, these mats effectively detect gait impairment with high spatiotemporal resolution [48], [49].

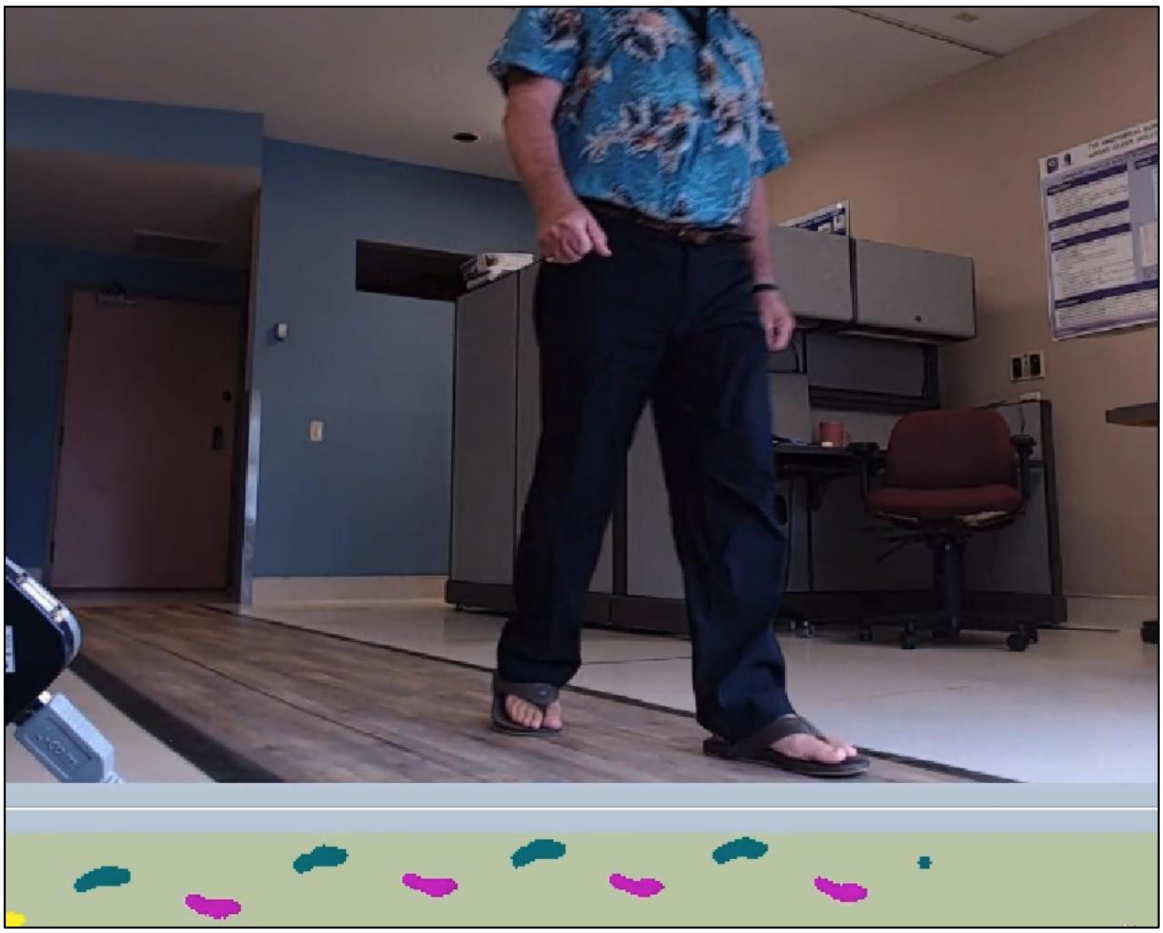

**Figure 1.2** Electronic gait mat with a subject's footsteps recorded live.

### 1.3.1 Gait Impairment in MCI

Gait impairment is common in the elderly and in over 30% of people with MCI [50], [51]. Importantly, slow gait speed is predictive of cognitive decline and symptoms of slow gait can manifest up to a decade before the first cognitive symptoms of MCI [52]–[55]. In longitudinal studies of healthy older individuals, slow gait was strongly associated with progression to MCI and subsequently dementia [53], [55].

#### 1.3.1.1 Dual Task Paradigms

The use of dual task (DT) paradigms has become widely used in MCI and dementia as a tool to assess disease severity and the likelihood that someone will progress to dementia [56]–[58]. During a DT, a secondary cognitive task is completed in parallel to a motor task such as walking. The research facilitator does not instruct participants to prioritize

walking or the second task. The secondary task serves as a cognitive stressor to increase the demand on attentional networks. Individuals with more severe pathology will show greater decrements in velocity and variability in their gait compared to regular single tasked (ST) walking. This phenomenon occurs because of the brain's limited compensatory ability following a functional impairment in gait-associated regions [59]. Typically, individuals with a gait impairment will allocate attentional resources to correct for deficits in gait performance. However, when the cognitive load required to walk and complete a secondary task exceeds the person's cognitive capacity, the individual will show a performance decline in either walking, the cognitive task, or both [58], [59]. The relative difference in gait parameters between the single task (ST) and DT is represented as the DT cost (DTC) (**Equation 1**), which indicates the cognitive cost of the DT on walking [59]. Additionally, each DT is specific to the cognitive domain of interest. For instance, a mental arithmetic task such as counting back from sevens (serial sevens) engages the pre-frontal cortex for working memory, while verbal fluency tasks that require participants to name animals use semantic memory and thus target frontoparietal regions typically affected in MCI and dementia [60]. The application of using different DTs to measure various domains of cognition has helped establish early motor signatures that differentiate cognitively healthy individuals from those that will progress to MCI [53], as well as distinguishing people who have aMCI from naMCI [61]. Thus, the link between brain pathology and gait impairment may be specific to the DT and subtype of MCI [57], [61], [62].

$$\mathrm{DTC} = [\mathrm{Speed}_{ST} - \mathrm{Speed}_{DT}]/\mathrm{Speed}_{ST} \times 100 \quad (1)$$

$\mathrm{Speed}_{ST}$ and $\mathrm{Speed}_{DT}$ are the participant's gait speed for the ST and DT, respectively.

## 1.4 Primary Motor Cortex & Gait

"The neural mechanisms underlying gait execution are intricate, encompassing both motor and sensory processes [63]. The primary motor cortex (M1) plays a pivotal role in voluntary movements, housing upper motor neurons that transmit efferent signals to brainstem locomotor regions important for posture control and rhythm [63], as well as to specialized circuits in the spinal cord referred to as central pattern generators. These

generators consist of interneurons and lower motor neurons, which project to muscles, initiating muscle contractions [63] (see **Figure 1.3**). Once initiated by M1, central pattern generators generate rhythmic patterns of motor activity, coordinating muscle contraction and relaxation during walking. These patterns persist until subsequent adjustments are needed. During the execution of muscle contractions by the central pattern generators in the spinal cord, M1 receives sensory feedback signals from both central and peripheral sources. This sensory input contributes to the refinement of motor commands. Consequently, M1 acts as a crucial integrator by coordinating voluntary gait and damage within M1 can lead to varying degrees of gait impairment [64].”

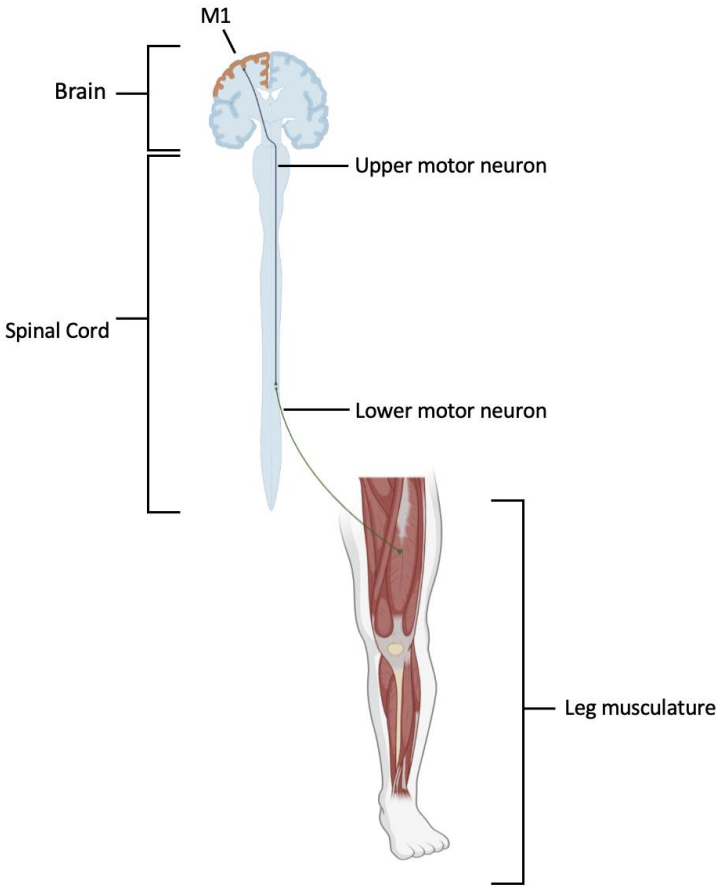


**Figure 1.3** Upper motor neuron projections from the primary motor cortex (M1) to lower motor neurons in the spinal cord that activate muscles in the leg. Created in part with Biorender.com.

One study found that inhibiting M1 using transcranial magnetic stimulation caused a reduction in muscle activation in the legs while walking [65]. Further, work has shown that M1 activation is directly proportional to the level of voluntary control when generating a physical movement, implying that more cognitive resources are being allocated [66]. As an individual consolidates a task through repetition, the cognitive load required is reduced as subcortical brain structures help supplement the movement being generated, thus reducing the level of M1 activity [66], [67]. Functional MRI studies have demonstrated this occurrence after participants were instructed to imagine walking. They found that M1 showed greater activity during complex walking compared to regular walking, whereas subcortical activity exhibited the opposite [66]. Consistent with this finding, Annweiler et al., found that the volume of M1 was directly associated with DT gait speed in individuals with MCI, indicating that those with reduced M1 volume exhibit slower DT speed [64].

## 1.5 Magnetic Resonance Spectroscopy

Proton magnetic resonance spectroscopy (MRS) is a technique that can non-invasively measure levels of specific metabolites within a defined voxel in the brain. Unlike magnetic resonance imaging (MRI) techniques, single voxel MRS does not use the magnetic gradients while acquiring the signal as there is no need for spatial encoding within the voxel [68]. Rather, the data collected using single voxel MRS techniques is a one-dimensional signal recorded as a function of time, which after applying a Fourier transform, produces a spectrum showing signals at various frequencies. Each peak in the frequency spectrum can be attributed one or more molecules that are associated with specific cellular metabolic processes. The amplitude of each peak in the spectrum is associated with the concentration of a specific metabolite within the tissue region of interest [68]. The molecules or metabolites visible in an in vivo brain spectrum are dependent on several factors including the strength of the scanner's magnetic field, the parameters of the pulse sequence used to collect the data, and the composition of the tissue. For a defined magnetic field strength and set of pulse sequence parameters, the number of visible peaks from each metabolite within the spectrum is determined by the intramolecular interactions between nuclei within each metabolite (peak splitting and

phase modulation), while the amplitude of a peak is determined by the number of nuclei [68].

### 1.5.1 Magnetic Resonance Spectroscopy Acquisition

Any given hydrogen nucleus possesses unique spin properties that form resultant magnetic dipoles that point in all directions. When a nucleus is placed inside an MRI scanner and becomes subject to a large magnetic field, all nuclei begin to precess at the same frequency (Larmor frequency). Additionally, magnetic dipole moments have a greater tendency to align with the direction of the scanner's magnetic field creating a net positive magnetization [68]. Once the sample has reached thermal equilibrium a pulse sequence is then initiated, which is a set of instructions sent to the scanner that plays out a complex combination of radio frequency pulses and gradients to excite the hydrogen nuclei and refocus signal within a specific tissue region. The pulse sequence defines the type of data acquisition and the characteristics of the final spectrum. One of the most common MRS pulse sequences is called point resolved spectroscopy (PRESS) (**Figure 1.4**) [68]. A simplified diagram of the PRESS pulse sequence is illustrated below. This pulse sequence is comprised of three non-ionizing radiofrequency (RF) pulses each paired with a gradient (e.g in the x, y, or z plane). A voxel, which is a three-dimensional pixel, is formed at the intersection of all three of these planes. Each combination of RF pulse and applied gradient excites a specific plane. For each pair of RF pulse and applied gradient, the carrier frequency and bandwidth of the RF pulse combined with the gradient direction and amplitude determine the location, direction, and thickness of each plane and, therefore, the voxel location and size in a single dimension [68]. In the PRESS sequence (**Figure 1.4**), the net magnetization in the sample (that is precessing at the frequency of the applied RF pulse) is initially tipped 90º away from the z-direction (main magnetic field direction) by the first RF pulse (90º pulse). The second and third 180º RF pulses in the PRESS train of 90º-180º-180º pulses refocus the magnetization within the excited planes in the region that intersects with the initial excited plane. Only signal within the intersection of all three planes (**Figure 1.5**) is refocussed completely and forms an echo [68] that can be recorded. At the maximum intensity of the echo a readout period begins, and the signal is recorded as a function of time. This time-domain signal is often

called a free induction decay (FID). Applying a Fourier transform to this time domain signal, identifies the frequency components (**Figure 1.6**) and provides information regarding the amplitude of each component (e.g. concentration of metabolites) [68].

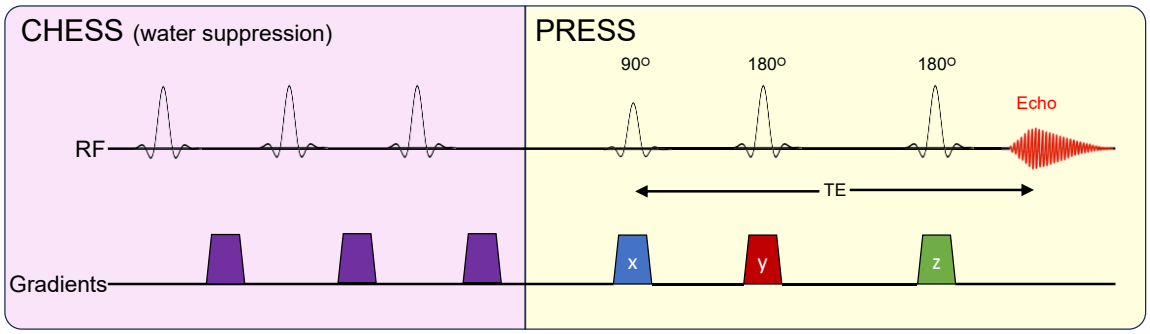


**Figure 1.4** Simplified Point Resolved Spectroscopy Sequence (PRESS) with a preparatory Chemical Shift Selective saturation sequence (CHESS) (see section 1.5.3.3 for details on CHESS).

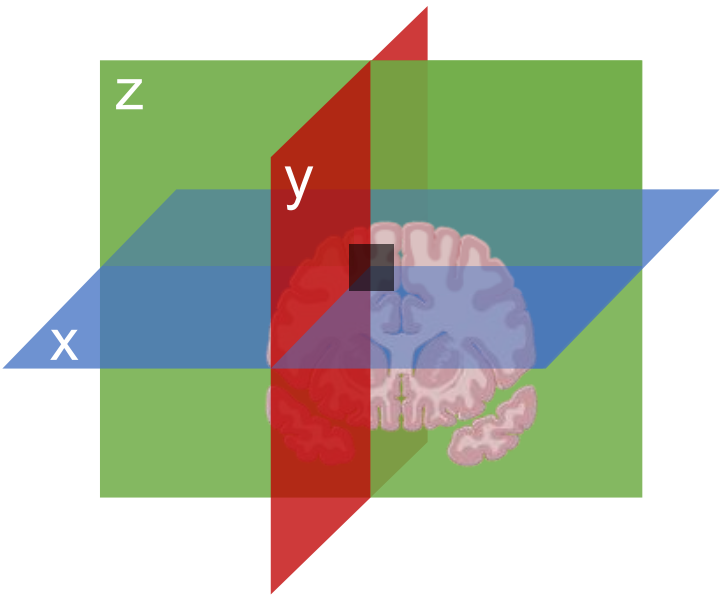


**Figure 1.5** Voxel localization to the primary motor cortex using a PRESS sequence. Slice thickness not to scale. The intersection of three planes defines a voxel (black) in which signal is refocussed and recorded. Created in part with Biorender.com.

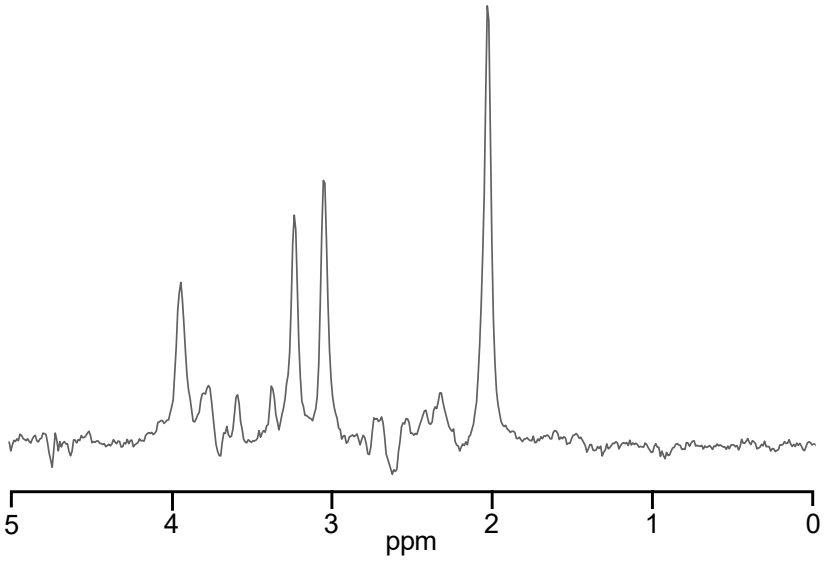


**Figure 1.6** Localized $^{1}$H-MR spectrum collected using a PRESS sequence at 3.0 T (TE/TR = 135ms/2000ms).

### 1.5.2 Metabolite Lineshapes

Each metabolite in the spectrum has a unique lineshape that is determined by the chemical structure of the molecule. This structure determines the chemical shift (position) and relative amplitudes of peaks in the spectrum. Nuclei that experience the same intramolecular forces as other bound nuclei are considered to be “chemically identical”, and therefore contribute signal to the same peak in a spectrum [68]. Importantly, the signal intensity of each peak in a spectrum is proportional to the number of chemically identical nuclei within the voxel. There are two other major factors that contribute to the spectral pattern of a metabolite. The first is the electronic shielding that the nucleus experiences. This shielding lessens the magnetic field experienced at the nucleus and thus decreases the precession frequency. A change in precession frequency corresponds to a shift in peak position in the spectrum (chemical shift). Within a spectrum, chemical shift (measured in parts per million or “ppm”) is visually represented by the difference in frequency between the measured nucleus and an empirical standard reference molecule

containing nuclei with high electronic shielding, divided by the Larmor frequency, and multiplied by $10^6$ [68]. Second is the through-bond interaction between nuclei with magnetic moments. This interaction is called J-coupling or J-splitting. A detailed description of this mechanism is beyond the scope of this thesis. But the consequence of this interaction is to split peaks into complicated patterns that alter the phase and amplitude of the resultant peaks in the spectrum. An example of chemical shift and J-coupling is provided in **Figure 1.7** for N-acetyl aspartate (NAA), where different chemical species of nuclei are represented as separate peaks or groups of peaks in the spectrum. This profile of peaks is specific to NAA. Each metabolite has its own "spectral signature" [68].

Considering that the relaxation properties of nuclei are different between molecules and within tissue types, the pulse sequence parameters influence the visibility of metabolites in a spectrum. The relaxation of a nucleus is characterized by the $T_1$ and $T_2$ relaxation time constants, which are specific to nuclei within each molecule. The $T_1$ relaxation time constant defines the time it takes for nuclei in the sample to magnetize and reach thermal equilibrium. The $T_2$ relaxation time constant defines the time for nuclei in the sample to exchange energy with each other and loose phase coherence [68]. When the time between the first RF pulse in a sequence to the beginning of the readout period, also known as the echo time (TE), is much shorter than the $T_2$ of a nucleus, a peak reflective of the number of nuclei in the molecule will be observed within the final spectrum. When the time for a pulse sequence to complete, also called the repetition time (TR), is shorter than the $T_1$ of a nucleus, successive applications of the pulse sequence will prevent the full recovery of magnetization and thus peaks within the spectrum will be lower in signal intensity. Therefore, most MRS pulse sequences are set with the TR to be greater than $T_1$ for the metabolites of interest (1500-2000ms) to allow for adequate signal recovery between acquisitions. Ultimately, the goal of an MRS acquisition is to maximize the signal-to-noise ratio (SNR: defined here by the maximum signal of the $NAA_{CH3}$ peak divided by the standard deviation of noise in the spectrum) and minimize the linewidth of peaks. Another important factor that impacts the SNR of a spectrum, in addition to TE and TR, is the number of averages, or the number of acquisitions. Signal from the metabolites of interest will remain relatively constant, whereas the noise in a scan is random between

each acquisition, thus increasing the number of acquired spectra and averaging them helps better distinguish the signal from the noise. The linewidth of peaks in a spectrum is largely determined by the magnetic field homogeneity within the voxel, where greater homogeneity produces more narrow linewidths. To maximize field homogeneity, the current within specially designed coils is adjusted to apply magnetic fields that sum together to create a more uniform field within the voxel of interest (e.g. shimming).

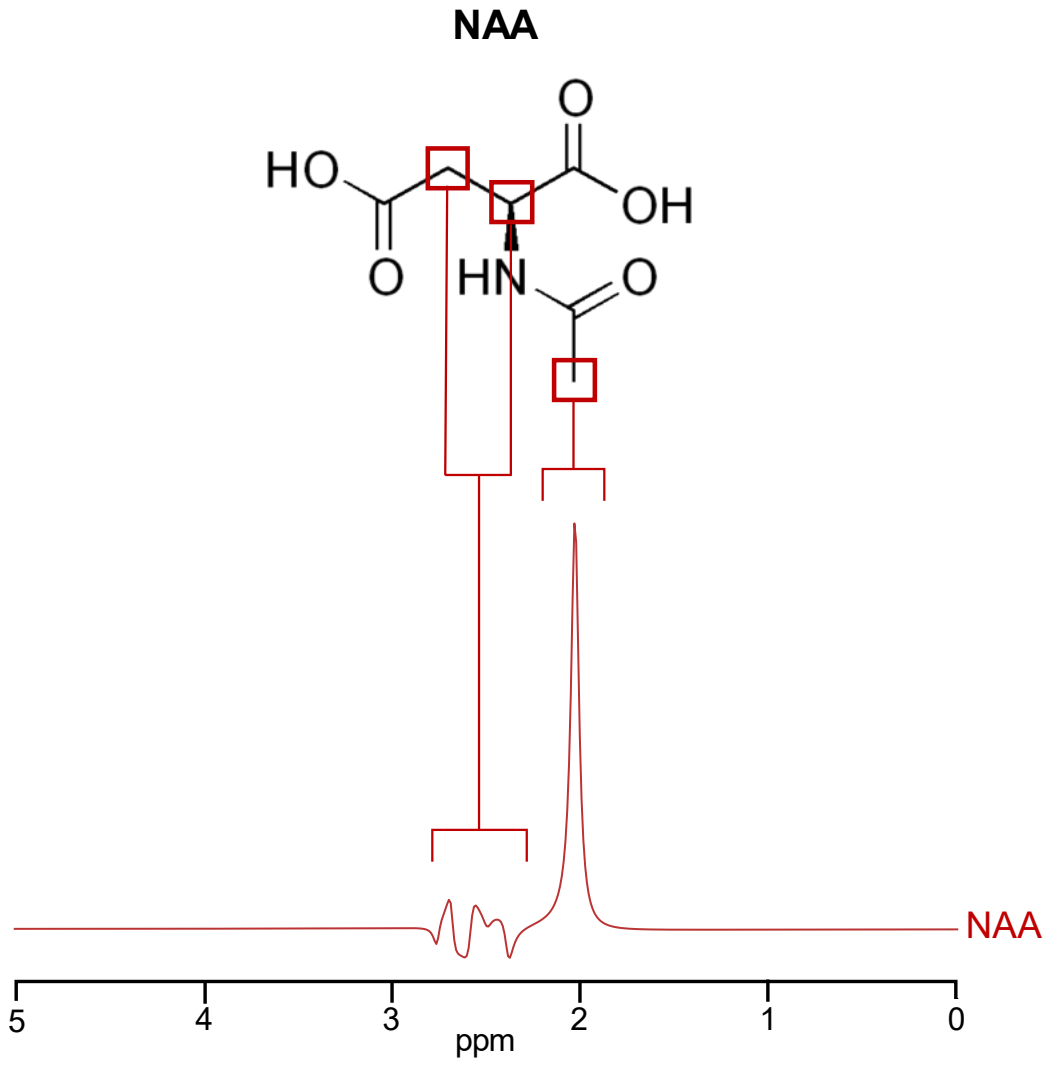


**Figure 1.7** N-acetyl aspartate (NAA) $^{1}$H-MR spectra and associated active nuclei in molecular structure. Spectra was collected at 3.0 T (TE/TR = 135ms/2000ms). The $NAA_{CH3}$ peak used for SNR measurements is found at 2 ppm. The peaks in the 2.3-2.8 ppm range represent J-coupled peaks from the CH and $CH_2$ groups.

### 1.5.3 Post-processing

#### 1.5.3.1 QUALITY & ECC

Before quantification of metabolite levels from the spectrum, the raw data from the MRI scanner is first converted into a format that can be visualized using fitMAN, a custom built software application that has been previously validated. Within the fitMAN package it is possible to perform QUALITY (quantification improvement by converting lineshapes to the Lorentzian type) deconvolution, which refines the data by converting metabolite lineshapes into a Lorentzian shape, or eddy current correction (ECC), which helps remove lineshape artifacts that may occur during acquisition. fitMAN can combine these tools together into QUECC (QUALITY deconvolution + ECC), which utilizes the best features of each correction; QUALITY deconvolution is applied to the initial portion of the time domain signal and ECC correction to the remaining points.

#### 1.5.3.2 Fitting

After lineshape correction, fitMAN performs an iterative non-linear least square fitting process in the time domain to determine the optimal contribution of each metabolite lineshape to the in vivo spectrum [69]. The goal of a fit is to optimize the linear addition of line shapes to replicate the in-vivo spectrum (**Figure 1.8**). Once this is known, the area underneath each peak can be used to determine the metabolite concentration in the tissue.

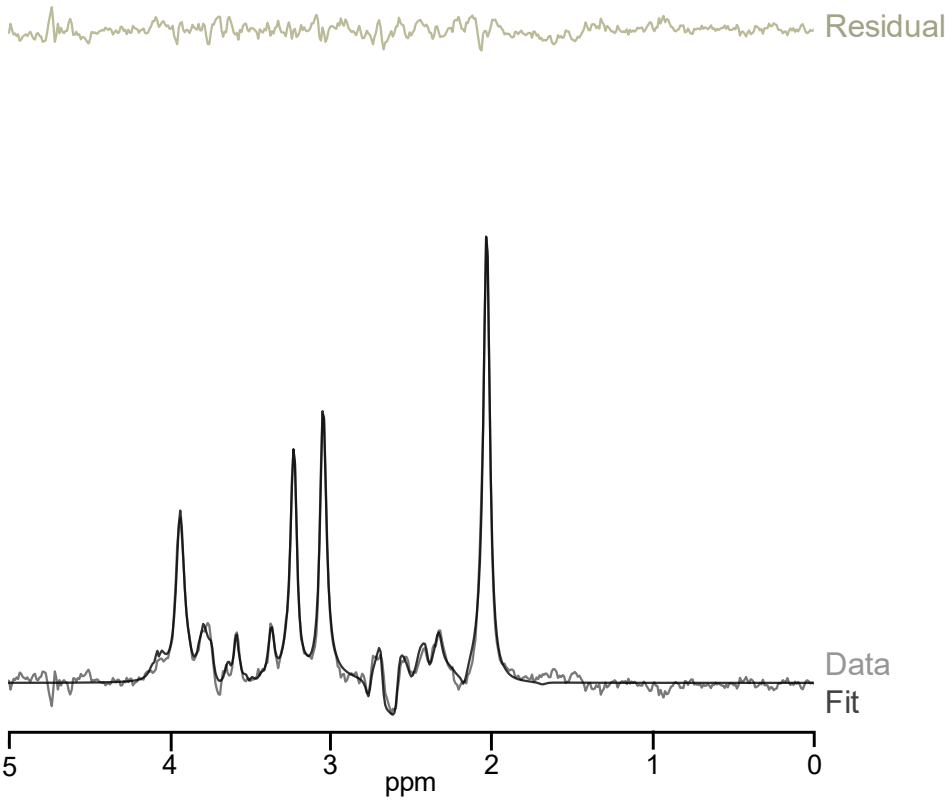


**Figure 1.8** $^{1}$H-MR spectra collected at 3.0 T (TE/TR = 135ms/2000ms) fit using fitMAN. Grey: raw data acquired; black: fit generated by fitMAN; beige: residual signal between raw data and fit

### 1.5.3.3 Spectral Quantification

To quantify the absolute concentration of metabolites present in the spectrum, two acquisitions are needed: one with water supressed, and the other with water unsuppressed. The concentration of water is 10,000 times greater than most metabolites of interest. Thus, before PRESS is initiated, a water suppression method such as chemical shift saturation selection (CHESS) is applied (**Figure 1.4**). CHESS consists of a train of RF pulses emitted with a carrier frequency and bandwidth set to the resonant frequency of water protons. After each RF pulse, a strong spoiler gradient is applied, which dephases the signal from water such that it removes the bulk of the signal contributed by water during PRESS. After post-processing and removal of any residual water signal, the

final water suppressed spectrum only contains peaks of nuclei from the metabolites of interest. Importantly, however, an MRS spectrum has arbitrary units of intensity and does not directly reflect a concentration. Empirically, the concentration of water within the brain can be approximated knowing the concentration of water within different brain tissue types. Therefore, by referencing the signal in a suppressed acquisition to an unsuppressed scan (no CHESS scan before PRESS) and multiplying by the known water concentration, the concentration of known metabolites can be calculated. This calculation is completed using BARSTOOL, another custom lab-made software previously validated. To increase the accuracy of the measurement, BARSTOOL (v4.1) also incorporates the fractions of grey matter, white matter, and cerebral spinal fluid within the voxel determined by segmenting $T_1$-weighted images acquired during the same scan. This information, along with corrections for metabolite $T_1$ and $T_2$ relaxation time constants is used to determine metabolite tissue concentration [70].

### 1.5.4 Metabolites

The spectroscopy acquisition protocol used in this thesis (PRESS) incorporated a long echo-time, which limited the detectable metabolites in the spectrum to N-acetyl aspartate (NAA), choline, (Cho), creatine (Cr), and myo-inositol (Myo). These metabolites are more easily detected and quantified due to a combination of factors including concentration and lineshape.

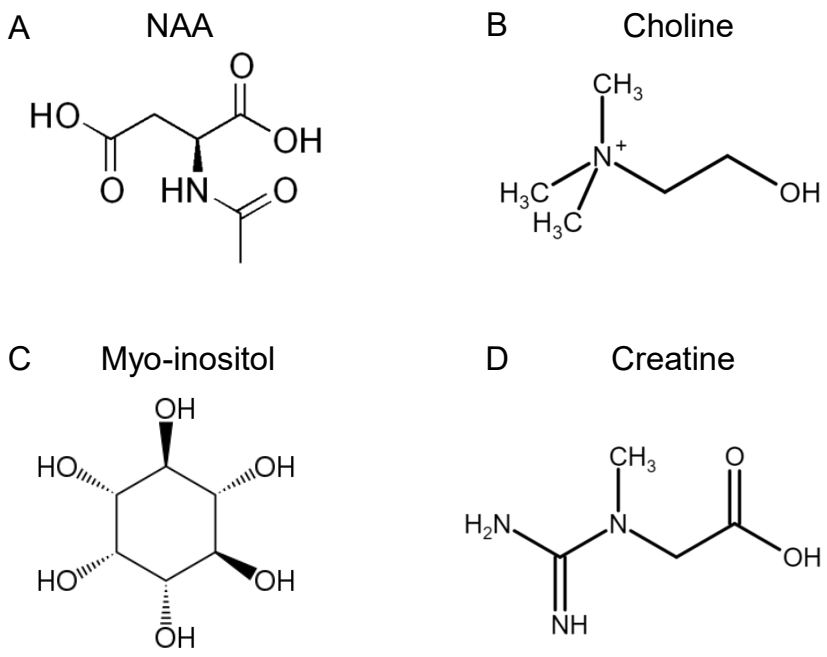


**Figure 1.9** $^{1}$H-MRS Metabolite Structures **A)** N-acetyl aspartate (NAA); **B)** Choline; **C)** Myo-inositol; **D)** Creatine

### 1.5.4.1 NAA

NAA is an amino acid found in neurons in the brain that is associated with mitochondrial metabolism [71]. The main peak of NAA resonates at 2.01 ppm [68]. NAA is synthesized by neurons using acetyl coenzyme A and aspartate. Reduced NAA levels may indicate neuronal loss, mitochondrial dysfunction, and decreased neuronal energy reserves, which are commonly observed in dementia [71], [72]. NAA is also involved in myelination, with decreased NAA levels potentially affecting the myelination process [73]. In individuals with MCI, NAA concentrations are typically lower compared to cognitively normal individuals, and these levels tend to decline further as someone progresses to dementia [74]–[76].

### 1.5.4.2 Choline

Cho is composed of signal from glycerophosphorylcholine and phosphorylcholine and resonates at 3.2 ppm in the spectrum [73], [77]. These compounds are involved in the metabolism of phosphatidylcholine, the most prevalent phospholipid in neuron

membranes. Changes in the Cho resonance detected in $^{1}$H-MRS reflect changes in membrane phospholipid turnover, either as increased membrane synthesis or degradation [78], [79]. Studies have reported increased Cho levels in MCI and AD, likely associated with membrane breakdown in chronic neurodegeneration and inflammatory processes [80], [81].

#### 1.5.4.3 Myo-inositol

Myo, a cyclic sugar alcohol, is present in the brain and can be observed as multiplets around 3.5 ppm on a $^{1}$H-MRS spectrum. Myo plays a role in intracellular secondary messenger signaling and is a precursor to phosphatidylinositol, a membrane phospholipid [82]. Myo is found in higher concentrations within glial cells than in neurons, where increased Myo may be indicative of glial cell activation and inflammation [83], [84]. Several $^{1}$H-MRS studies demonstrate elevated Myo levels in individuals with MCI and AD, which is associated with gliosis and neuroinflammatory processes [74], [76], [85], [86].

#### 1.5.4.4 Creatine

Cr is marker of energy metabolism and is found at 3.0 ppm in the spectrum [87]. With the signal composed of both creatine and phosphocreatine, together they reflect the energy demands of cells and are often reported as total Cr because they cannot be resolved [87]. In the MRS literature, Cr is often used as a reference molecule as Cr levels are relatively consistent between sex and throughout age. However, some studies have shown that Cr concentrations tend to vary within pathology [88]. Studies have found that absolute Cr levels sometimes decline in AD, either as a cause or a consequence of reduced neuron functioning and degenerative processes [88]. Alternatively, increases in Cr and decreases in NAA concentration (low NAA/Cr ratio) may be indicative of heightened inflammation because of the greater metabolic demand of astrocytes and reduced neuron functionality [89], [90]. Hence, the use of metabolite ratios with respect to Cr can be valuable and may provide more context to the metabolic processes occurring.

## 1.6 Thesis Overview

The first chapter of this thesis serves to introduce important concepts such as MCI, intervention options for people with MCI, gait impairment, dual task cost, and MRS metabolites that can be measured in M1. The overall objective of this thesis was to determine whether five months of combined PE, CT, and VitD supplementation could improve M1 metabolite profile and DTC on gait speed in people with MCI. We hypothesized that combining all three interventions would result in greater NAA, and lower Cho and Myo than all other treatment arms. A second objective of this thesis was to relate temporal changes in DTC on gait speed with changes in M1 neurochemistry. It was hypothesized that people who showed greater DTC gait speed over the trial would exhibit decreased NAA, and increased Cho and Myo. Briefly, this study found that combining PE and CT showed restorative effects on neuron function by increasing NAA/Cr levels compared to participants who only completed PE. Further, there was an inverse relationship between changes in DTC gait speed and changes in NAA, as well as an inverse relationship changes in DTC gait speed and changes in Cho, thus only partly supporting our hypothesis.

## 1.7 References

[1] "NAVIGATING THE PATH FORWARD FOR DEMENTIA IN CANADA REPORT"., (2022).

[2] R. C. Petersen. "Clinical Practice: Mild Cognitive Impairment". In: *The New England Journal of Medicine* 364.23 (2011), pp. 2227–2261.

[3] Y.-H. Liu *et al.* "Immunotherapy for Alzheimer disease—the challenge of adverse effects". In: *Nature Reviews Neurology* 8.8 (2012), pp. 465–469.

[4] J. R. Sims *et al.* "Donanemab in Early Symptomatic Alzheimer Disease: The TRAILBLAZER-ALZ 2 Randomized Clinical Trial". In: *JAMA* 330.6 (2023), pp. 512–527.

[5] H. W. Querfurth and F. M. LaFerla. "Alzheimer's Disease". In: *The New England Journal of Medicine* 362.4 (2010), pp. 329–344.

[6] G. Verdile *et al.* "Inflammation and Oxidative Stress: The Molecular Connectivity between Insulin Resistance, Obesity, and Alzheimer's Disease". In: *Hindawi* 2015. (2015), pp. 1–17.

[7] A. L. Palmer and S. S. Ousman. "Astrocytes and Aging". In: *Frontiers in aging neuroscience* 10. (2018), pp. 1–14.

[8] M. Rusek *et al.* "Ketogenic Diet in Alzheimer's Disease". In: *International Journal of Molecular Sciences* 20.16 (2019), pp. 3892.

[9] K. M. Langa and D. A. Levine. "The diagnosis and management of mild cognitive impairment: A clinical review". In: *JAMA* 312.23 (2014), pp. 2551–2561.

[10] "How are MCI and Dementia Treated". In: *Journal of the American Academy of Physician Assistants* 23.1 (2010), pp. 18–21.

[11] J. R. Thyrian *et al.* "Systematic, early identification of dementia and dementia care management are highly appreciated by general physicians in primary care - Results within a cluster-randomized-controlled trial (DelpHi)". In: *Journal of Multidisciplinary Healthcare* 9. (2016), pp. 183–190.

[12] "World Alzheimer's Report 2011: The benefits of early diagnosis and intervention"., (2011).

[13] K. B. Rajan *et al.* "Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020-2060)". In: *Alzheimer's & Dementia* 17.1 (2021), pp. 1966–1975.

[14] F. Chang, T. Patel, and M. E. Schulz. "The "Rising Tide" of dementia in Canada: What does it mean for pharmacists and the people they care for?". In: *Canadian Pharmacists Journal* 148.4 (2015), pp. 193–199.

[15] R. C. Petersen *et al.* "Mild Cognitive Impairment". In: *Archives of Neurology* 56.3 (1999), pp. 303–308.

[16] K. Yaffe *et al.* "Subtype of mild cognitive impairment and progression to dementia and death". In: *Dementia and Geriatric Cognitive Disorders* 22.4 (2006), pp. 312–319.

[17] A. J. Mitchell and M. Shiri-Feshki. "Temporal trends in the long term risk of progression of mild cognitive impairment: A pooled analysis". In: *Journal of Neurology, Neurosurgery and Psychiatry* 79.12 (2008), pp. 1386–1391.

[18] R. C. Petersen *et al.* "Mild Cognitive Impairment Ten Years Later". In: *Archives of Neurology* 66.12 (2009), pp. 1447–1455.

[19] P. Heyn, B. C. Abreu, and K. J. Ottenbacher. "The effects of exercise training on elderly persons with cognitive impairment and dementia: A meta-analysis". In: *Archives of Physical Medicine and Rehabilitation* 85.10 (2004), pp. 1694–1704.

[20] J. C. Davis *et al.* "An Economic Evaluation of Resistance Training and Aerobic Training versus Balance and Toning Exercises in Older Adults with Mild Cognitive Impairment". In: *PLoS ONE* 8.5 (2013), pp. 1–9.

[21] B. K. Pedersen. "Physical activity and muscle–brain crosstalk". In: *Nature Reviews Endocrinology* 15.7 (2019), pp. 383–392.

[22] A. M. W. Petersen and B. K. Pedersen. "The anti-inflammatory effect of exercise". In: *Journal of Applied Physiology* 98.4 (2005), pp. 1154–1162.

[23] W. Japa *et al.* "Effect of Resistance-Exercise Training on Cognitive Function in Healthy Older Adults: A Review". In: *Journal of Aging and Physical Activity* 20. (2012), pp. 497–498.

[24] J. Galloza, B. Castillo, and W. Micheo. "Benefits of Exercise in the Older Population". In: *Physical Medicine and Rehabilitation Clinics of North America* 28.4 (2017), pp. 659–669.

[25] C. W. Cotman, N. C. Berchtold, and L.-A. Christie. "Exercise builds brain health: key roles of growth factor cascades and inflammation". In: *Trends in Neurosciences* 30.9 (2007), pp. 464–472.

[26] D. Weisman, E. Hakimian, and G. J. Ho. "Interleukins, Inflammation, and Mechanisms of Alzheimer's Disease"., in *Interleukins*, pp. 505–530.

[27] J. Reijnders, C. van Heugten, and M. van Boxtel. "Cognitive interventions in healthy older adults and people with mild cognitive impairment: A systematic review"., *Ageing Research Reviews*, 12.1. pp. 263–275, Jan. 2013.

[28] S. B. Chapman *et al.* "Neural mechanisms of brain plasticity with complex cognitive training in healthy seniors". In: *Cerebral Cortex* 25.2 (2015), pp. 396–405.

[29] G. M. McPhee *et al.* "Cognitive training and Bacopa monnieri: Evidence for a combined intervention to alleviate age associated cognitive decline". In: *Medical Hypotheses* 95. (2016), pp. 71–76.

[30] C. Annweiler *et al.* "Vitamin D and Ageing: Neurological Issues ". In: *Neuropsychobiology* 62. (2010), pp. 139–150.

[31] L. Tripkovic, L. R. Wilson, and S. A. Lanham-New. "Vitamin D2 vs vitamin D3: They are not one and the same". In: *Nutrition Bulletin* 42.4 (2017), pp. 331–337.

[32] R. P. Heaney *et al.* "Vitamin D3 Is More Potent Than Vitamin D2 in Humans". In: *The Journal of Clinical Endocrinology & Metabolism* 96.3 (2011), pp. 447–452.

[33] C. Annweiler *et al.* "Vitamin D and Caudal Primary Motor Cortex: A Magnetic Resonance Spectroscopy Study". In: *PLOS One* 9.1 (2014), pp. 1–5.

[34] C. Annweiler *et al.* "Dietary intake of vitamin D and cognition in older women: A large population-based study". In: *Neurology* 75.20 (2010), pp. 1810–1816.

[35] R. O. da Costa *et al.* "Vitamin D (VD3) Intensifies the Effects of Exercise and Prevents Alterations of Behavior, Brain Oxidative Stress, and Neuroinflammation, in Hemiparkinsonian Rats". In: *Neurochemical Research* 48.1 (2023), pp. 142–160.

[36] E. Medhat *et al.* "Exercise enhances the effectiveness of vitamin D therapy in rats with Alzheimer's disease: emphasis on oxidative stress and inflammation". In: *Metabolic Brain Disease* 35.1 (2020), pp. 111–120.

[37] J. H. Kang *et al.* "Effect of vitamin D on cognitive decline: results from two ancillary studies of the VITAL randomized trial". In: *Scientific Reports* 11.1 (2021), pp. 1–14.

[38] T. Keisala *et al.* "Premature aging in vitamin D receptor mutant mice". In: *Journal of Steroid Biochemistry and Molecular Biology* 115.3–5 (2009), pp. 91–97.

[39] M. E. Moore *et al.* "Evidence that vitamin D 3 reverses age-related inflammatory changes in the rat hippocampus". In: *Biochemical Society Treansactions* 33.4 (2005), pp. 573–577.

[40] M. J. Berridge. "Vitamin D cell signalling in health and disease". In: *Biochemical and Biophysical Research Communications* 460.1 (2015), pp. 53–71.

[41] D. Wong *et al.* "Vitamin D Supplementation May Protect Against Oxidative Stress in a Vitamin D Deficient Double-Transgenic Mouse Model of Alzheimer's Disease". In: *Alzheimer's & Dementia* 13. (2017), pp. 746–747.

[42] D. Eyles *et al.* "Vitamin D3 and brain development". In: *Neuroscience* 118.3 (2003), pp. 641–653.

[43] C. S. Latimer *et al.* "Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats". In: *Proceedings of the National Academy of Sciences of the United States of America* 111.41 (2014), pp. 4359–4366.

[44] L. Bennett *et al.* "Vitamin D2-Enriched Button Mushroom (Agaricus bisporus) Improves Memory in Both Wild Type and APPswe/PS1dE9 Transgenic Mice". In: *PLoS ONE* 8.10 (2013), pp. 1–17.

[45] E. G. A. (Esther) Karssemeijer *et al.* "Positive effects of combined cognitive and physical exercise training on cognitive function in older adults with mild cognitive impairment or dementia: A meta-analysis". In: *Ageing Research Reviews* 40. (2017), pp. 75–83.

[46] M. Lipowski *et al.* "Improvement of Attention, Executive Functions, and Processing Speed in Elderly Women as a Result of Involvement in the Nordic Walking Training Program and Vitamin D Supplementation". In: *Nutrients* 11.6 (2019), pp. 1–17.

[47] N. W. Bray *et al.* "Combining exercise with cognitive training and vitamin D3 to improve functional brain connectivity (FBC) in older adults with mild cognitive impairment (MCI) Results from the SYNERGIC trial". In: *GeroScience* (2023),.

[48] H. B. Menz *et al.* "Reliability of the GAITRite® walkway system for the quantification of temporo-spatial parameters of gait in young and older people". In: *Gait and Posture* 20.1 (2004), pp. 20–25.

[49] M. Montero-Odasso *et al.* "Quantitative gait analysis under dual-task in older people with mild cognitive impairment: A reliability study". In: *Journal of NeuroEngineering and Rehabilitation* 6.35 (2009), pp. 1–6.

[50] J. Verghese *et al.* "Gait dysfunction in mild cognitive impairment syndromes". In: *Journal of the American Geriatrics Society* 56.7 (2008), pp. 1244–1251.

[51] J. Verghese *et al.* "Abnormality Of Gait as a Predictor Of Non-Alzheimer's Dementia". In: *New England Journal of Medicine* 347.22 (2002), pp. 1761–1768.

[52] R. Camicioli *et al.* "Motor slowing precedes cognitive impairment in the oldest old". In: *Neurology* 50.5 (1998), pp. 1496–1498.

[53] T. Buracchio *et al.* "*The Trajectory of Gait Speed Preceding Mild Cognitive Impairment". In: *Archives of Neurology* 67.8 (2010), pp. 980–986.

[54] M. Montero-Odasso *et al.* "Motor and Cognitive Trajectories Before Dementia: Results from Gait and Brain Study". In: *Journal of the American Geriatrics Society* 66.9 (2018), pp. 1676–1683.

[55] H. H. Dodge *et al.* "In-home walking speeds and variability trajectories associated with mild cognitive impairment". In: *Neurology* 78.24 (2012), pp. 1946–1952.

[56] M. M. Montero-Odasso *et al.* "Association of dual-task gait with incident dementia in mild cognitive impairment: Results from the gait and brain study". In: *JAMA Neurology* 74.7 (2017), pp. 857–865.

[57] P. Ali *et al.* "Associations between gait speed and brain structure in amnestic mild cognitive impairment: a quantitative neuroimaging study". In: *Brain Imaging and Behavior* 16.1 (2022), pp. 228–238.

[58] J. M. Hausdorff and A. S. Buchman. "What links gait speed and MCI with dementia? A fresh look at the association between motor and cognitive function". In: *Journals of Gerontology* 68.4 (2013), pp. 409–411.

[59] S. W. Hunter *et al.* "A framework for secondary cognitive and motor tasks in dual-task gait testing in people with mild cognitive impairment". In: *BMC Geriatrics* 18.202 (2018), pp. 1–7.

[60] M. Montero-Odasso *et al.* "Gait and cognition: A complementary approach to understanding brain function and the risk of falling". In: *Journal of the American Geriatrics Society* 60.11 (2012), pp. 2127–2136.

[61] M. Montero-Odasso *et al.* "The Motor Signature of Mild Cognitive Impairment: Results From the Gait and Brain Study". In: *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 69.11 (2014), pp. 1415–1421.

[62] M. Montero-Odasso, S. W. Muir, and M. Speechley. "Dual-task complexity affects gait in people with mild cognitive impairment: The interplay between gait variability, dual tasking, and risk of falls". In: *Archives of Physical Medicine and Rehabilitation* 93.2 (2012), pp. 293–299.

[63] E. Kandel and J. Schwartz. "*Principles in Neural Science,*"

[64] C. Annweiler *et al.* "Motor cortex and gait in mild cognitive impairment: A magnetic resonance spectroscopy and volumetric imaging study". In: *Brain* 136.3 (2013), pp. 859–871.

[65] N. T. Petersen *et al.* "Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking". In: *Journal of Physiology* 537.2 (2001), pp. 651–656.

[66] B. Godde. "More automation and less cognitive control of imagined walking movements in high versus low fit older adults". In: *Frontiers in Aging Neuroscience* 2.139 (2010), pp. 1–13.

[67] M. G. Lacourse *et al.* "Brain activation during execution and motor imagery of novel and skilled sequential hand movements". In: *NeuroImage* 27.3 (2005), pp. 505–519.

[68] R. A. de Graaf. "*In Vivo NMR Spectroscopy,*"

[69] R. Bartha, D. J. Drost, and P. C. Williamson. "Factors affecting the quantification of short echo in-vivo 1 H MR spectra: prior knowledge, peak elimination, and filtering". In: *NMR in Biomedicine* 12. (1999), pp. 205–216.

[70] "BARSTOOL v41".

[71] M. Bittšanský, D. Výbohová, and D. Dobrota. "Proton magnetic resonance spectroscopy and its diagnostically important metabolites in the brain". In: *General Physiology and Biophysics* 31.1 (2012), pp. 101–112.

[72] L. Calderón-Garcidueñas *et al.* "A Critical Proton MR Spectroscopy Marker of Alzheimer's Disease Early Neurodegenerative Change: Low Hippocampal NAA/Cr Ratio Impacts APOE 4 Mexico City Children and Their Parents". In: *Journal of Alzheimer's Disease* 48. (2015), pp. 1065–1075.

[73] H. Stringaris *et al.* "Inflammatory CNS Demyelination: Histopathologic Correlation with In Vivo Quantitative Proton MR Spectroscopy". In: *American Journal of Neuroradiology* 20.9 (2023), pp. 1619–1627.

[74] K. Kantarci. "Proton MRS in mild cognitive impairment". In: *Journal of Magnetic Resonance Imaging* 37.4 (2013), pp. 770–777.

[75] M. G. Targosz-Gajniak *et al.* "Magnetic resonance spectroscopy as a predictor of conversion of mild cognitive impairment to dementia". In: *Journal of the Neurological Sciences* 335.(1-2) (2013), pp. 58–63.

[76] K. Kantarci *et al.* "MRI and MRS predictors of mild cognitive impairment in a population-based sample". In: *Mayo Clinic* 81.2 (2013), pp. 126–133.

[77] A. J. Ross and P. S. Sachdev. "Magnetic resonance spectroscopy in cognitive research". In: *Brain Research Reviews* 44. (2004), pp. 83–102.

[78] J. Klein. "Membrane breakdown in acute and chronic neurodegeneration: focus on choline-containing phospholipids Review Article". In: *Journal of Neural Transmission* 107. (2000), pp. 1027–1063.

[79] M. M. Gonzales *et al.* "Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults". In: *Brain Topography* 26.1 (2013), pp. 126–134.

[80] D. J. Meyrhoff *et al.* "Axonal injury and membrane alterations in Alzheimer's disease suggested by in vivo proton magnetic resonance spectroscopic imaging". In: *Annals of Neurology* 36.1 (1994), pp. 40–47.

[81] S. Maul, I. Giegling, and D. Rujescu. "Proton Magnetic Resonance Spectroscopy in Common Dementias". In: *Frontiers in Psychiatry* 11.769 (2020),.

[82] G. Nick. "Inositol as a treatment for psychiatric disorders: a scientific evaluation of its clinical effectiveness". In: *Townsend Letter for Doctors and Patients* 255 (2004), pp. 51–53.

[83] C. Richter-Landsberg, D. Leibfritz, and K. W. Ords. "Multinuclear NMR Studies on the Energy Metabolism of Glial and Neuronal Cells Hypotaurine Multinuclear NMR spectroscopy Myo-inositol Neurons and glia cells Phosphocreatine TCA cycle". In: *Dev Neurosci* 15. (1993), pp. 289–298.

[84] N. Glanville *et al.* "Differences in the metabolism of inositol and phosphoinosifides by cultured cells of neuronal and glial origin". In: *Biochimica et Biophysica Acta* 1004.2 (1989), pp. 169–179.

[85] W. Huang *et al.* "High Brain myo-Inositol Levels in the Predementia Phase of Alzheimer's Disease in Adults With Down's Syndrome: A 1 H MRS Study". In: *American Journal of Psychiatry* 156.12 (1999), pp. 1879–1886.

[86] S. E. Rose *et al.* "A 1H MRS study of probable Alzheimer's disease and normal aging: Implications for longitudinal monitoring of dementia progression". In: *Magnetic Resonance Imaging* 17.2 (1999), pp. 291–299.

[87] E. Béard and O. Braissant. "Synthesis and transport of creatine in the CNS: importance for cerebral functions". In: *Journal of Neurochemistry* 115.2 (2010), pp. 297–313.

[88] V. Rackayova *et al.* "Creatine in the central nervous system: From magnetic resonance spectroscopy to creatine deficiencies". In: *Analytical Biochemistry* 529. (2017), pp. 144–157.

[89] E.-M. Ratai *et al.* "Brain creatine elevation and *N* -acetylaspartate reduction indicates neuronal dysfunction in the setting of enhanced glial energy metabolism

in a macaque model of NeuroAIDS". In: *Magnetic Resonance in Medicine* 66.3 (2011), pp. 625–634.

[90] J. Urenjak *et al.* "Proton Nuclear Magnetic Resonance Spectroscopy Unambiguously Identifies Different Neural Cell Types". In: *The Journal of Neuroscience* 13.3 (1993), pp. 981–989.

# Chapter 2

# 2 The Effects of Physical Activity, Cognitive Stimulation, and Vitamin D on Gait Speed and Primary Motor Cortex Metabolism in Individuals with Mild Cognitive Impairment: A $^{1}$H-MRS Analysis from the SYNERGIC Trial


## Abstract

Mild cognitive impairment (MCI) serves as a transitional phase preceding dementia. In individuals with MCI, changes in walking patterns have been linked to the progression towards dementia. Research utilizing magnetic resonance spectroscopy has identified a correlation between neurochemistry in the primary motor cortex (M1) and the speed of walking while performing dual tasks in MCI. Although studies suggest that interventions such as exercise, cognitive training, and vitamin D supplementation may be beneficial for MCI, the collective impact of these treatments on both walking speed and M1 metabolism remains uncertain. In this study, a group of participants diagnosed with MCI (N=75) were divided into five different intervention groups, and the cost of performing dual tasks on walking speed and M1 metabolism was evaluated before and five months after the intervention. The findings revealed that cognitive training combined with exercise resulted in increased concentrations of N-acetylaspartate (NAA)/creatine in the M1 region, compared to exercise alone ($p<0.05$). Furthermore, individuals who exhibited an increased cost in dual task performance over time demonstrated greater declines in NAA and choline, further emphasizing the connection between walking speed and M1 functionality in MCI.


## 2.1 Introduction

Mild cognitive impairment (MCI) is an intermediary state between healthy cognitive aging and dementia [1], [2]. Unlike dementia, individuals with MCI are capable of performing daily living activities independently, despite significant cognitive impairment.

Moreover, having MCI increases the risk of progressing to dementia 10-fold [3], [4] although nearly one-third of patients remain stable as MCI, or revert back to healthy cognitive function [3], [5]. Therefore, interventions during the MCI phase could prolong individuals' independent living, consequently saving billions in financial resources yearly.

Gait impairment is common in the elderly, especially in those with MCI. Gait difficulties in MCI, expressed as slowness, tend to worsen over time increasing the risk of falls with injuries [6]. This decline can be potentially explained by cognitive impairment and damaged networks that facilitate automatic walking [7]–[11]. When gait becomes impaired, individuals will allocate more attentional resources to compensate for imbalance and instability. As cognitive impairment increases, it becomes challenging to walk and perform simultaneous cognitive tasks due to a reduced capacity of central resources to maintain safe and steady walking [12]. Hence, in addition to estimating cognitive impairment, dual task (DT) gait assessment paradigms have been used to reveal an individual's gait impairment free from cognitive compensation. DT paradigms utilize a secondary cognitive task while walking to gauge the extent of neurological impairment in individuals with MCI [12]. The additional task acts as a cognitive stressor as it creates a competition for limited executive resources, and ultimately exposes impairments in gait. It is understood that this cognitive-motor interference, specifically on reducing gait speed, is strongly associated with cognitive decline in the general population and can be detected years before the first clinical symptoms of MCI [8], [13]–[18]. The degree of change in gait speed between single task (ST: usual walking) and DT walking can be represented as the DT cost (DTC) [19]. This approach accounts for individual differences in gait characteristics, thereby reflecting the extent of cognitive interference on gait performance. Importantly, the DTC on gait speed has been shown to be a functional biomarker capable of predicting dementia progression in MCI [4], [20], [21], therefore, DTC is likely influenced by ongoing neurodegenerative processes in the brain.

In dementia, inflammatory and neurodegenerative processes are linked through a positive feedback loop. Degeneration triggers inflammation, which then recruits reactive oxygen species, leading to further degeneration and perpetuating the pathological cycle. Both

inflammation and degeneration are associated with impaired neuronal functioning [22], [23]. To non-invasively measure neuronal function and inflammation in MCI and dementia, a commonly used technique is $^{1}$H-Magnetic Resonance Spectroscopy (MRS), which detects cellular metabolic abnormalities in selected brain regions by measuring concentrations of molecules associated with metabolic mechanisms [24]. In MCI and dementia, most studies report reduced levels of N-acetyl aspartate (NAA), a molecule associated with neuronal functioning and neuroaxonal integrity, as well as increased choline (Cho) and myo-inositol (Myo), which are markers of cell-membrane synthesis and degradation, and glial cell activation, respectively [24]–[30]. Collectively, these three metabolites can be used to characterize aberrant neurological metabolism in MCI and dementia. Surprisingly, very few MRS studies have investigated the role of the primary motor cortex (M1) in gait speed, despite its role as the final integrator of motor control [31], [32]. A study by Annweiler et al., found that individuals with MCI who exhibited high levels of Cho relative to creatine (Cho/Cr) in the M1 region showed slower single task and DT gait performance [32]. They also observed that individuals with high Cho/Cr levels had significantly less M1 volume compared to those with low Cho/Cr levels, and that reduced M1 volume was also associated with slower single task and DT gait speed. It was suggested that poor neuronal functioning and inflammation explained these occurrences, however, the relationship between gait speed and M1 metabolism in MCI remains unclear [32].

The inconsistent response and potential adverse effects of current pharmacological treatments for dementia, have motivated clinicians to increasingly incorporate positive lifestyle changes early into MCI treatment plans [33], [34]. Several previous studies have investigated the effects of physical exercise (PE), cognitive training (CT), and vitamin D (VitD) supplementation on various aspects of cognitive and physical function in MCI and dementia [35]–[41]. These studies have shown that PE, CT and VitD separately can improve DT gait performance and decrease levels of MRS inflammatory markers in the brain. Expanding on prior research, the primary objective of the current study is to determine whether providing a five-month-long combined intervention of regular aerobic and resistance exercise (PE), multidomain dual task training with memory load (CT), and VitD supplementation to individuals with MCI can improve MRS metabolism within the

M1 region and DT gait performance, by decreasing DTC and increasing DT gait speed. We hypothesized that, compared to placebo, combined PE, CT, and VitD would show the greater increases in both absolute and relative levels of NAA, while reducing Cho and Myo concentrations, and that combined PE, CT, and VitD would show greater reductions on DTC gait speed. Further, although DTC gait speed has been used to predict decline in cognition in MCI [8], [18], it is unknown whether long-term changes in DTC gait speed are associated with changes in the M1 concentrations of MRS metabolites indicative of inflammation in MCI. Therefore, the second objective of this study was to determine whether changes in DTC gait speed correlate with changes in M1 metabolite levels during the intervention period. We hypothesized that improvements in DTC gait speed over time are associated with increased concentrations of NAA, and decreased Cho and Myo.

## 2.2 Methods

### 2.2.1 Participants

552 individuals were screened for eligibility for the Synchronized Exercises Remedies in Gait and Cognition (SYNERGIC) trial, a Canada-wide, longitudinal, multi-site randomized controlled clinical trial [42]. Inclusion and exclusion criteria were in accordance with the SYNERGIC protocol. 183 participants with MCI were eligible for the trial, recruited from London, Waterloo, Vancouver, or Montreal. Demographic information was collected during screening, and participants completed the Montreal Cognitive Assessment (MoCA) and Mini-Mental State Examination (MMSE) to establish baseline cognitive functioning.

### 2.2.2 MCI Definition

Classifying participants as having mild cognitive impairment (MCI) was deemed by scoring 0.5 on the global rating of the Clinical Dementia Rating scale and meeting four specific criteria [3]: (1) reporting subjective cognitive difficulties; (2) displaying measurable cognitive decline in areas such as memory, executive function, attention, or language [43], [44]; (3) maintaining independence in daily activities according to interviews with a healthcare professional [45]; and (4) not meeting the criteria for

dementia as outlined in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision) [46].

### 2.2.3 Study Arms

Subjects were randomly assigned to one of five exercise arms for a five-month term (20 weeks), varying in combinations of physical exercise (PE), sham PE (balance and toning (BAT)), cognitive training (CT), sham cognitive training (sCT), vitamin D supplementation (VitD), and placebo vitamin D supplementation (pVitD), as shown in **Figure 2.1**.

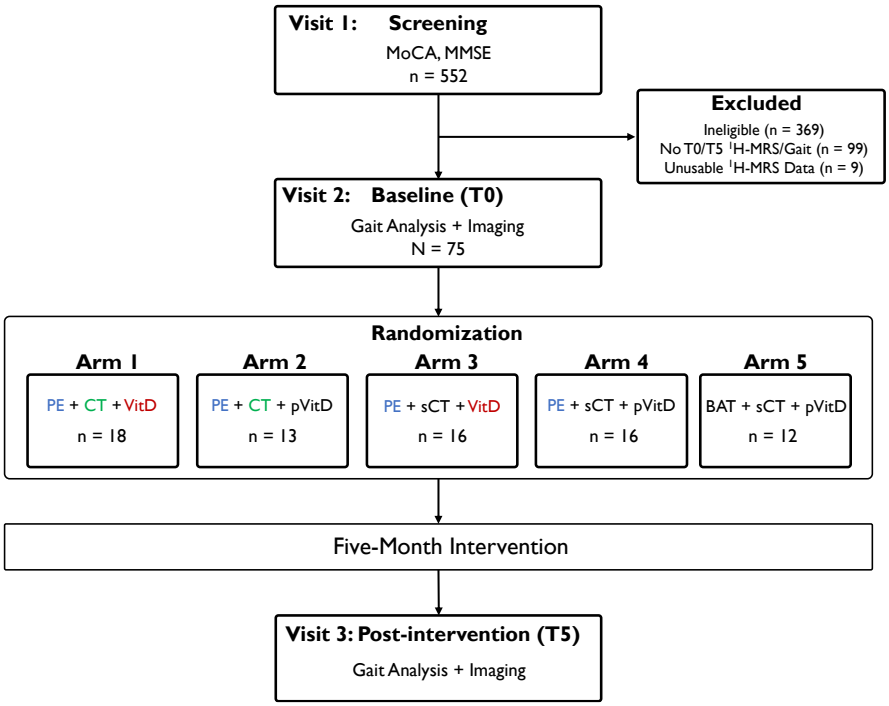


**Figure 2.1** Flow chart of the SYNERGIC study design, including data collection time points, and number of participants with MCI in each arm.

### 2.2.4 Interventions

A more detailed overview of the intervention regimens for PE, CT, VitD, and control can be found in the supplementary materials and in the published protocol [42]. Exercise instructors and physiologists who conducted the intervention were blinded to participant's arms.

#### 2.2.4.1 Physical Exercise & Balance and Toning

Each participant underwent five months of either PE or BAT led by a trained exercise instructor supervised by specialized an exercise physiologist for three 60-minute sessions per week in groups of up to eight participants for a total of 20 weeks. Briefly, PE consisted of a combination of aerobic exercise and progressive resistance training exercises, increasing in intensity and duration over the five months (**Table 4.1 and 4.2**), whereas BAT was provided to participants as a control exercise condition. These exercises focused on improving muscle tone and flexibility, without progressing in volume and intensity, consequently not increasing strength and cardiovascular capacity.

#### 2.2.4.2 Cognitive Training & Sham Cognitive Training

Each CT session involved tablet-based dual task training. The difficulty of each task was adjusted by an algorithm based on the individual's reaction time average after five trials to meet the functional level of each participant. To test working memory and multitasking abilities, participants were required to quickly provide responses for the given task, while also dividing their attention between two simultaneous tasks. The program used for the CT was originally intended for neurorehabilitation, previously validated in other clinical randomized trials [41], [47]–[49]. In sCT sessions, participants were asked to conduct an internet search for various local attractions within a city determined by the instructor at the start of each session. As a sCT task variation, participants were shown a 20-minute nature documentary film, followed by a brief questionnaire. CT and sCT sessions were held under the same conditions, using a tablet-based method within a quiet room removed from environment distractions.

#### 2.2.4.3 Vitamin D & Placebo Vitamin D

Subjects in the VitD arms were orally administered 30,000 IU of Vitamin D3 supplements each week for the duration of the trial (3 doses of 10,000 IU per week). 10,000 IU per day is an acceptable supplementation for older individuals, as determined by Health Canada, and thus was well within the safety margins for potential drug toxicity [50]. pVitD supplementation was matched to the schedule of VitD arms to control for any potential effects of social interactions during drug administration.

### 2.2.5 Neuroimaging Methods

All MRS data were acquired at a magnetic field strength of 3.0T using either a Siemens Magnetom Prisma Fit (N = 62) or Phillips Gyroscan (N = 13) MRI. Subjects were scanned at baseline (T0) and at the end of the trial five-months later (T5). Each imaging session began by acquiring a sagittal 3D T1-weighted anatomical image of the head with high grey matter/white matter contrast ([Siemens; Sequence: MP-RAGE TE/TR: 2.98ms/2300ms; inversion time: 900ms; flip angle: 9°; averages: 1; FOV (mm): 256 x 256 x 192; matrix: 256 x 256 x 256; channel: 32] [Phillips; Sequence: 3D TFE; TE/TR: 3.3ms/7.3ms; inversion time: 945ms; flip angle: 9°; averages: 1; FOV (mm): 256 x 247 x 192; matrix: 256 x 256 x 192; channel: 8]). Each brain was skull stripped (ROBEX) [51] and segmented (FSL, FAST) [52] for MRS metabolite quantification.

#### 2.2.5.1 $^{1}$H-Magnetic Resonance Spectroscopy

Anatomical images were used to localize a 20-mm isotropic voxel on the leg and foot region of the right primary motor cortex (**Figure 2.2A**). Spectra were acquired using point-resolved spectroscopy (PRESS) ([Siemens and Philips; TE/TR: 135ms/2000ms; voxel size: 8cm$^3$]. Both water-supressed (averages: 192) and water-unsuppressed (averages: 8) were collected to perform measure absolute metabolite levels using the unsuppressed water signal as an internal reference. Spectra were fitted using the fitMAN spectral analysis software [47], incorporating line shape corrections with QUECC (100 or 50 points for QUALITY deconvolution) or eddy current correction (ECC) [54]. The correction scheme used depended on the quality of the acquired data. Residual water signal in the water-suppressed spectrum around 4.7ppm was removed by subtracting resonance between 4.1 - 5.1 ppm identified by a Hankel singular value decomposition algorithm [55]. Suppressed spectra with signal-to-noise ratio (SNR: measured as the $NAA_{CH3}$ peak divided by the standard deviation of the noise) less than 30 were excluded from analysis. A suppressed spectrum was also excluded if the corresponding water unsuppressed spectrum had a linewidth greater than 12 Hz. The metabolite spectra were then fitted using a Levenberg-Marquardt minimization routine incorporating a template of prior knowledge of metabolite line shapes, as detailed in previous work [53], [55]. Absolute metabolite level measurements were calculated using BARSTOOL (v4.1) and

referenced to the voxel water signal following adjustment for the gray and white matter composition within the voxel [56]. Relative metabolite concentrations were calculated by dividing the metabolite of interest by the concentration of Creatine (Cr) ([metabolite]/Cr). The relative change in MRS metabolite levels was calculated and represented as the delta difference in concentration (Δ[metabolite] or Δ[metabolite]/Cr) between T0 and T5 (Δ[metabolite] = T5 [metabolite] – T0 [metabolite], Δ[metabolite]/Cr = T5 [metabolite]/Cr – T0 [metabolite]/Cr).

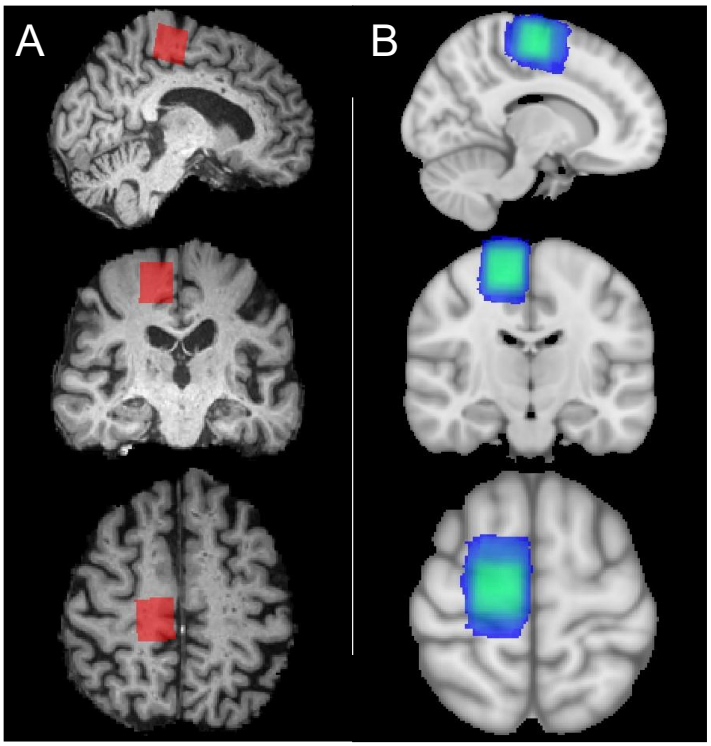


**Figure 2.2** $^{1}$H-MRS voxel placement. **A)** Voxel placement in the primary motor cortex of a single participant with mild cognitive impairment. Voxel is overlayed on a $T_1$ weighted 3D anatomical image **B)** Visual representation of the average voxel placement at both timepoints in the primary motor cortex registered to a Montreal Neurological Imaging (MNI) 152mm imaging standard template. Green indicates more overlap; blue indicates less overlap.

#### 2.2.5.2 MRS Voxel Overlap

Using the DICE coefficient, the within-subject overlap of voxel placement was measured between T0 and T5 [57]. Any participants with a DICE coefficient < 5% were excluded from the study. In addition, participants with voxels not placed in the motor cortex were removed from analysis. Voxel locations were registered to Montreal Neurological Imaging (MNI) space to calculate the DICE coefficient for each participant between data collection points.

### 2.2.6 Gait Analysis

Gait speed (cm/s) was recorded at T0 and T5 (**Table 2.3**). Gait measurements were collected on a computerized walkway embedded with pressure sensors. Each participant was asked to walk starting from one meter before the mat and ending one meter past the end of the mat to achieve steady-state walking and account for acceleration and deceleration within a trial. Gait speed was calculated by dividing the distance from the first to last footprint on the electronic mat by the time elapsed between them. Gait data for this analysis included a total of four different walking trials from each participant: three averaged normal walking trials at a self-selected pace (ST); and three different DT paradigms: a counting DT trial (cDT), a serial sevens trial (sDT), and an animal naming trial (nDT). This DT protocol has shown excellent test reliability in MCI [58]. During the ST, participants were asked to walk at their usual pace. The ST gait speed outcome for analysis was calculated by averaging the three ST trials. During the cDT and sDT trials, participants were instructed to walk at their usual pace while counting backwards from 100 by 1's, or by 7's aloud, respectively, without stopping their walking or counting. Participants with trouble performing the subtractions during the cDT or sDT were encouraged by a research assistant to continue walking on the electronic carpet while being assessed. In the nDT, participants were asked to name as many animals as possible aloud while walking without stopping their walking or talking. During a DT, refraining from instructing participants to prioritize either walking or the dual task is important as the data better captures natural multitasking conflict. Dual task cost (DTC) is a widely used metric in gait research to determine the level of gait impairment, calculated by taking the relative percentage difference in gait speed between each of the DTs and the

ST [4], [19]–[21]. Greater DTC is representative of greater cognitive interference on gait performance as it denotes the cognitive cost necessary to compensate for gait impairment (DTC = [SpeedST – SpeedDT]/SpeedST x 100) (Equation 1). The change in DTC for each participant was calculated and represented as the delta difference in DTC (ΔDTC) between T0 and T5 (ΔDTC = T5 DTC – T0 DTC).

### 2.2.7 Statistical Methods

To assess the effect of treatment intervention on our primary outcome measurements, separate univariate ANCOVAs were performed in SPSS (v29.0; SPSS) to compare the mean delta difference in metabolite concentration and DTC between the five treatment arms, controlling for age, sex, MoCA, years of education, and scanner type. Four of five sites (Western University, Wilfrid Laurier University, University of Waterloo, and Montreal University) collected MRS data using Siemens scanners, whereas data from the University of British Columbia was collected using a Philips scanner. Scanner type was also included as a covariate in the analyses. A post-hoc between-group comparison was conducted following the presence of significant main effects using Fisher's least significant difference test using an $\alpha = 0.05$. The five arms were then collapsed to investigate the relationship between ΔDTC and Δ[metabolite], and multiple linear regression was performed, adjusting for age, sex, MoCA, years of education, scanner type, and treatment arm. Parameter estimates (β) are provided with a 95% confidence interval using an $\alpha = 0.05$.

## 2.3 Results

### 2.3.1 Participant Characteristics

99 participants could not be included in the current analysis because of incomplete gait or imaging data collection at either timepoint. Additionally, two participants were removed due to incorrect voxel placements; three participants were removed due to artifacts in the MRS data that could not be fixed by post-processing; and four participants were removed following outlier analysis. A total of 75 participants with MCI were included in the analysis. No differences were found between arms for age, sex, MoCA, or years of education (**Table 2.1**). Spectra were successfully acquired from all included participants.

The average DICE coefficient for voxel overlap was 61% (**Figure 2.2B**). On average (±SD), the voxel contained 34.2 ± 7.1% grey matter, 50.5 ± 10.4% white matter, and 15.3 ± 5.8% cerebrospinal fluid. The average SNR for all MRS acquisitions was 77 ± 21, and the average linewidth was 4.2 ± 0.9 Hz. All data were fit successfully (**Figure 2.3**). T0 and T5 absolute and relative metabolite ratio (**Table 2.2**), and T0 and T5 DTC gait speed for the ST and DTs (**Table 2.3**), are reported for each treatment arm. No significant differences in T0 absolute or relative metabolite levels were found between arms, nor were there differences in T0 ST speed, DT speed, or baseline DTC for all DTs.

**Table 2.1** Demographic characteristics of each treatment arm.

| | **Total (N=75)** | **Arm 1 (PE + CT + VitD) (n = 18)** | **Arm 2 (PE + CT + pVitD) (n = 13)** | **Arm 3 (PE + sCT + VitD) (n = 16)** | **Arm 4 (PE + sCT + pViD) (n = 16)** | **Arm 5 (BAT + sCT + pVitD) (n = 12)** |
|---|---|---|---|---|---|---|
| **Clinical Measures** | | | | | | |
| Age, years | 73.8 (6.3) | 73.5 (6.4) | 73.3 (8.3) | 75.2 (7.6) | 72.5 (4.3) | 74.4 (4.9) |
| Sex, Females (%) | 37 (49) | 10 (56) | 3 (23) | 9 (56) | 9 (56) | 8 (67) |
| MoCA | 23.2 (2.6) | 24.2 (3.0) | 23.1 (2.3) | 22.6 (2.8) | 23.1 (2.1) | 22.7 (2.2) |
| MMSE | 27.4 (1.8) | 27.3 (2.1) | 27.7 (1.4) | 26.6 (2.3) | 27.6 (1.2) | 27.8 (1.6) |
| Education, years | 15.4 (3.9) | 14.8 (2.5) | 16.4 (4.1) | 14.3 (3.0) | 14.5 (3.3) | 17.8 (6.0) |

**Note:** Values are mean (±SD), n (%), or as indicated otherwise. Significant differences are denoted with matching letters ($p < 0.05$).

### 2.3.2 Effect of Treatment on $\Delta^1$H-MRS Metabolites & ΔDTC Speed

Changes in absolute metabolite levels and metabolite levels relative to Cr in all groups are summarized in **Figure 2.4A** and **2.4B**. There were no differences in ΔNAA ($p = 0.93$), ΔCho ($p = 0.20$), ΔMyo ($p = 0.36$), or ΔCr ($p = 0.42$) between treatment arms, nor were there any differences between arms for ΔCho/Cr ($p = 0.94$), or ΔMyo/Cr ($p = 0.33$). However, there was a significant main effect of treatment between arms for ΔNAA/Cr ($p = 0.04$). Post-hoc analysis revealed arms 1 and 2 had greater ΔNAA/Cr compared to arm

4, and arm 2 had greater ΔNAA/Cr compared to arm 5 (arms 1 and 4: p = 0.04; arms 2 and 4: p = 0.01; arms 2 and 5: p = 0.02).

Changes in DTC on gait speed for each treatment arm are shown in **Figure 2.5**. No differences in ΔDTC on gait speed were found between arms for any of the DTs (ΔcDTC: $p$ = 0.51; ΔsDTC: $p$ = 0.39; ΔnDTC: p = 0.78).

**Table 2.2** Absolute and relative concentrations of $^{1}$H-MRS metabolites in the primary motor cortex for each timepoint and treatment arm.

| | | **Total (N=75)** | **Arm 1 (PE + CT + VitD) (n = 18)** | **Arm 2 (PE + CT + pVitD) (n = 13)** | **Arm 3 (PE + sCT + VitD) (n = 16)** | **Arm 4 (PE + sCT + pViD) (n = 16)** | **Arm 5 (BAT + sCT + pVitD) (n = 12)** |
|---|---|---|---|---|---|---|---|
| **Neuroimaging Measures** | | | | | | | |
| **Metabolite** | **Time** | | | | | | |
| **Absolute, mM** | | | | | | | |
| NAA | T0 | 20.1 (2.4) | 20.3 (2.5) | 19.9 (1.7) | 21.0 (2.3) | 19.3 (2.8) | 19.9 (2.2) |
| | T5 | 20.1 (2.7) | 20.7 (2.9) | 19.4 (2.0) | 21.1 (2.7) | 19.3 (3.3) | 19.8 (2.1) |
| Cho | T0 | 3.2 (0.6) | 3.1 (0.6) | 3.3 (0.5) | 3.4 (0.5) | 2.9 (0.5) | 3.1 (0.6) |
| | T5 | 3.2 (0.6) | 3.2 (0.7) | 3.1 (0.5) | 3.4 (0.5) | 3.1 (0.6) | 3.2 (0.5) |
| Myo | T0 | 3.2 (0.7) | 3.3 (0.7) | 3.5 (0.8) | 3.3 (0.6) | 3.0 (0.7) | 3.1 (0.9) |
| | T5 | 3.2 (0.8) | 3.0 (1.0) | 3.2 (0.6) | 3.5 (0.6) | 3.0 (1.0) | 3.2 (0.4) |
| Cr | T0 | 10.7 (1.7) | 10.8 (1.6) | 10.8 (1.4) | 11.5 (1.8) | 10.0 (1.8) | 10.6 (2.0) |
| | T5 | 11.0 (2.1) | 11.1 (2.2) | 10.4 (1.7) | 11.8 (2.0) | 10.5 (2.4) | 11.0 (2.0) |
| **Relative, X/Cr** | | | | | | | |
| NAA/Cr | T0 | 1.89 (0.19) | 1.90 (0.20) | 1.86 (0.20) | 1.85 (0.14) | 1.95 (0.20) | 1.91 (0.22) |
| | T5 | 1.86 (0.22) | 1.91 (0.26)[a] | 1.88 (0.22)[b,c] | 1.81 (0.15) | 1.87 (0.24)[a, b] | 1.83 (0.23)[c] |
| Cho/Cr | T0 | 0.30 (0.03) | 0.29 (0.03) | 0.31 (0.03) | 0.30 (0.04) | 0.30 (0.03) | 0.30 (0.04) |
| | T5 | 0.29 (0.04) | 0.29 (0.04) | 0.30 (0.04) | 0.29 (0.03) | 0.30 (0.04) | 0.29 (0.03) |
| Myo/Cr | T0 | 0.30 (0.05) | 0.31 (0.07) | 0.32 (0.04) | 0.29 (0.04) | 0.30 (0.05) | 0.29 (0.06) |
| | T5 | 0.29 (0.05) | 0.27 (0.07) | 0.31 (0.04) | 0.30 (0.04) | 0.29 (0.07) | 0.29 (0.03) |

**Note:** Values are mean (±SD). Significant differences between arms are denoted with matching letters ($p < 0.05$).

**Table 2.3** Single and dual task gait speed and dual task cost for each timepoint and treatment arm.

| | | Total (N=75) | Arm 1 (PE + CT + VitD) (n = 18) | Arm 2 (PE + CT + pVitD) (n = 13) | Arm 3 (PE + sCT + VitD) (n = 16) | Arm 4 (PE + sCT + pVitD) (n = 16) | Arm 5 (BAT + sCT + pVitD) (n = 12) |
|---|---|---|---|---|---|---|---|
| **Gait Measurements** | | | | | | | |
| **Walking Task** | **Time** | | | | | | |
| **Speed, cm/s** | | | | | | | |
| Usual Gait | T0 | 121.9 (20.5) | 128.1 (23.3) | 125.2 (15.9) | 117.2 (18.0) | 122.3 (21.0) | 115.1 (23.1) |
| | T5 | 127.1 (20.4) | 136.7 (23.1) | 130.6 (22.5) | 117.7 (15.6) | 131.8 (15.3) | 116.0 (17.8) |
| Counting | T0 | 116.8 (23.8) | 126.8 (27.5) | 119.1 (24.0) | 113.3 (18.2) | 114.0 (20.7) | 107.9 (27.2) |
| | T5 | 122.5 (23.2) | 134.0 (23.1) | 128.2 (23.7) | 113.2 (16.3) | 125.0 (21.8) | 109.1 (24.9) |
| Serial 7s | T0 | 103.0 (28.3) | 115.5 (28.1) | 99.7 (38.8) | 98.1 (24.7) | 101.4 (21.2) | 96.6 (28.5) |
| | T5 | 108.0 (27.3) | 119.5 (27.6) | 110.4 (37.0) | 99.0 (21.8) | 111.5 (24.1) | 97.0 (21.9) |
| Naming Animals | T0 | 107.9 (26.6) | 116.2 (26.7) | 117.0 (24.1) | 99.4 (26.2) | 106.3 (23.5) | 99.6 (31.1) |
| | T5 | 114.8 (25.7) | 125.7 (25.4) | 119.6 (28.8) | 101.4 (22.9) | 118.8 (23.3) | 107.0 (22.6) |
| **Dual Task Cost, %** | | | | | | | |
| Counting | T0 | 4.6 (8.2) | 1.5 (6.1) | 5.8 (10.5) | 3.2 (8.5) | 6.5 (9.0) | 7.1 (6.0) |
| | T5 | 3.9 (7.8) | 1.9 (6.3) | 1.9 (5.3) | 3.7 (7.5) | 5.6 (8.2) | 6.9 (11.4) |
| Serial 7s | T0 | 15.9 (16.0) | 10.5 (11.3) | 21.5 (25.8) | 16.5 (15.9) | 16.1 (14.6) | 17.1 (10.7) |
| | T5 | 15.3 (14.8) | 12.6 (13.2) | 16.4 (22.3) | 15.9 (13.9) | 15.7 (14.6) | 16.8 (11.3) |
| Naming Animals | T0 | 11.8 (13.0) | 9.8 (8.2) | 7.2 (11.1) | 15.2 (16.8) | 12.1 (16.0) | 14.7 (10.4) |
| | T5 | 10.1 (11.7) | 8.2 (9.0) | 9.4 (14.0) | 13.9 (14.5) | 10.4 (10.8) | 8.1 (9.9) |

**Note:** Values are mean (±SD), or as indicated otherwise. Significant differences are denoted with matching letters ($p < 0.05$).

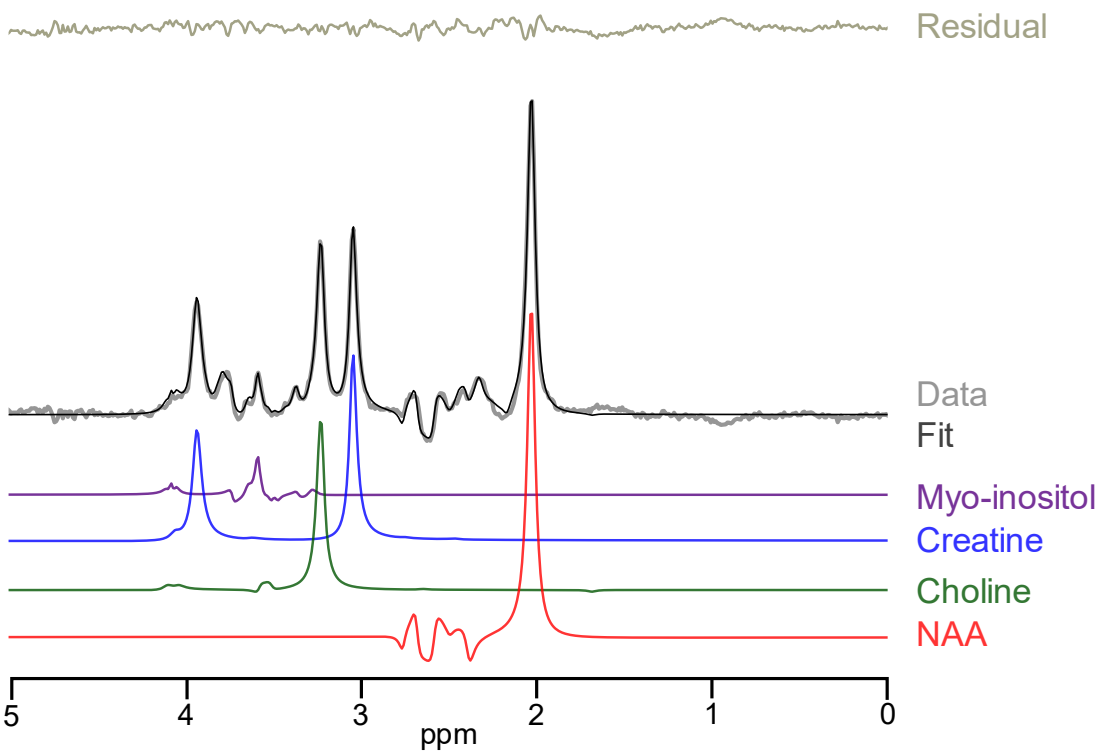


**Figure 2.3** 3.0T $^{1}$H-MRS spectrum from the primary motor cortex in one MCI participant (TE/TR = 135ms/2000ms). The spectrum following post-processing (light-grey), is superimposed on the fit generated by fitMAN (dark-grey) with the residual signal between the data and generated fit (beige) shown above. Individual metabolite components are shown below: N-Acetyl Aspartate (NAA) (red), Choline (green), Creatine (blue), and Myo-inositol (purple).

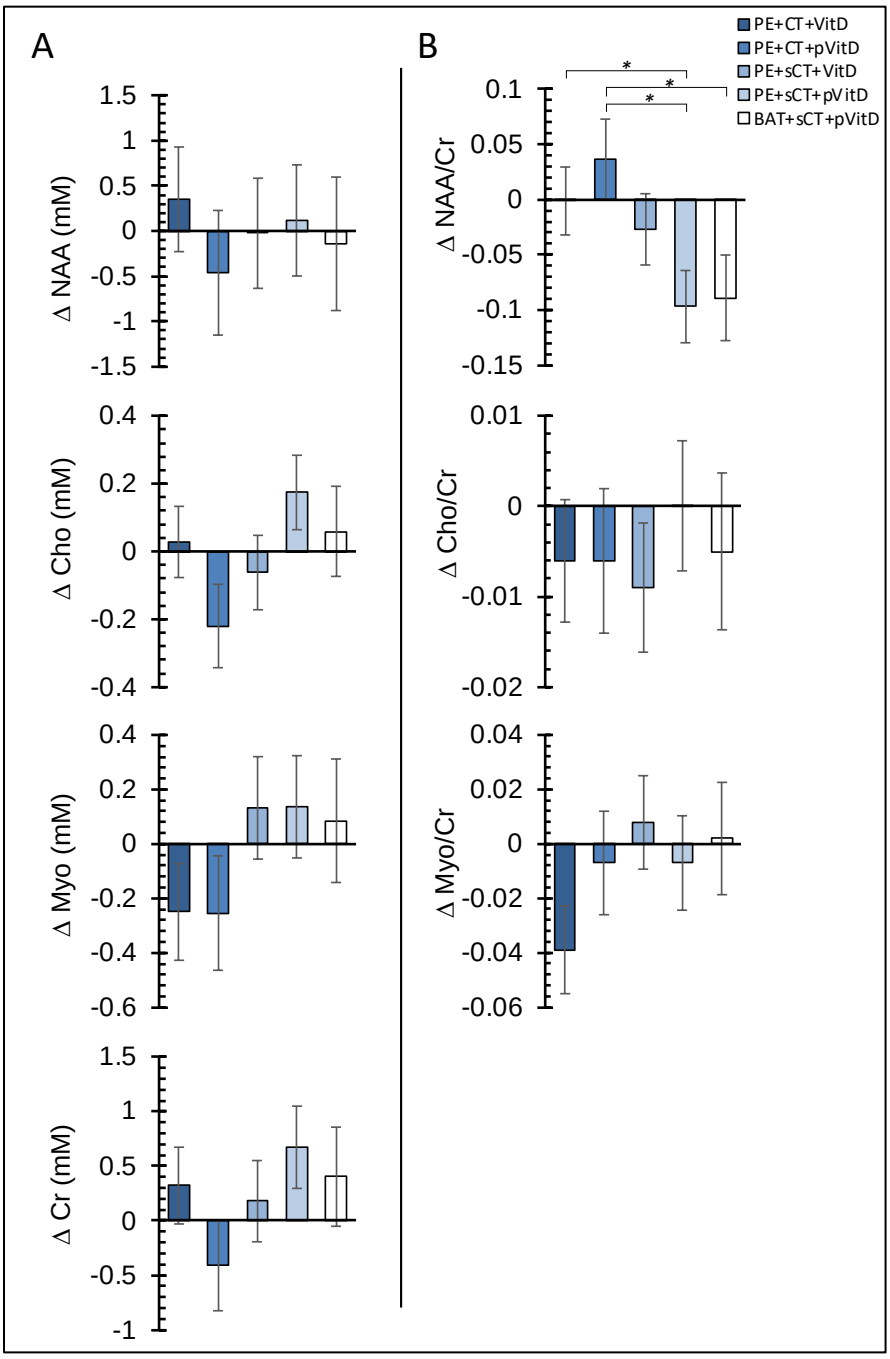


**Figure 2.4** Effect of treatment intervention on $^{1}$H-MRS mean delta change in metabolite levels between treatment arms after adjusting for age, sex, MoCA, years of education, and scanner type. A) Absolute metabolite concentrations (mM) B) Metabolite concentration relative to creatine concentration (unitless). Significant differences are denoted by asterisks ($p < 0.05$). Error bars = SEM.

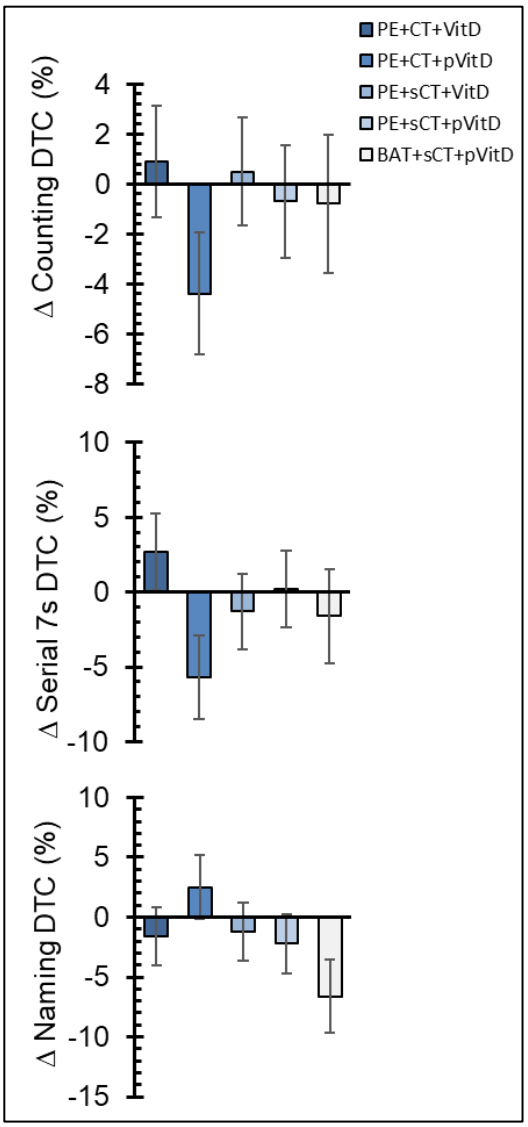


**Figure 2.5** Effect of treatment intervention on mean delta change in dual task cost on gait speed between treatment arms after adjusting for age, sex, MoCA, and years of education. Significant differences are denoted by asterisks ($p < 0.05$). Error bars = SEM.

### 2.3.3 Relationship Between Δ$^{1}$H-MRS Metabolism & ΔDTC Speed

The association between changes in dual task cost (ΔDTC) on gait speed, and the change in $^{1}$H-MRS metabolite concentration in the primary motor cortex is summarized in **Table 2.4A** and **2.4B**. Using multiple linear regression, ΔnDTC gait speed was inversely associated with ΔNAA concentrations (adjusted β = -9.240, $p$ = 0.01), and ΔCho concentrations (adjusted β = -1.471, $p$ = 0.02). ΔsDTC was negatively associated with

ΔNAA/Cr (adjusted β = -0.372, $p$ = 0.03). Additionally, ΔcDTC and ΔnDTC were not associated with any metabolite ratios. As a covariate, MoCA scores (adjusted β = -0.080, $p$ = 0.04, and adjusted β = -0.007, $p$ = 0.04, respectively) were both inversely related to Myo and Myo/Cr.

**Table 2.4** Association between delta changes in dual task cost (ΔDTC) on gait speed, and the delta change in $^{1}$H-MRS metabolite concentration in the primary motor cortex after adjusting for age, sex, MoCA, years of education, and scanner type. **A)** ΔDTC on gait speed for naming animals associated with Δ absolute metabolite concentration. **B)** ΔDTC on gait speed for serial sevens associated with Δ relative metabolite concentration.

**A.** Change in Naming Animals DTC Gait Speed on Change in Absolute Metabolite Concentration in the Primary Motor Cortex

| | **ΔNAA** | | **ΔCho** | | **ΔMyo** | |
|---|---|---|---|---|---|---|
| | ß (95% CI) | *p* | ß (95% CI) | *p* | ß (95% CI) | *p* |
| **ΔnDTC** | **-9.240 (-15.791 – -2.688)** | **0.006** | **-1.471 (-2.714 – -0.229)** | **0.021** | -1.049 (-3.025 – 0.927) | 0.292 |
| **Scanner** | -0.444 (-2.197 – 1.309) | 0.614 | 0.082 (-0.250 – 0.415) | 0.622 | **-0.604 (-1.132 – -0.075)** | **0.026** |
| Arm | -0.169 (-0.616 – 0.277) | 0.452 | 0.018 (-0.067 – 0.102) | 0.676 | 0.105 (-0.030 – 0.239) | 0.125 |
| Age | -0.011 (-0.106 – 0.084) | 0.815 | -0.005 (-0.023 – 0.013) | 0.569 | 0.006 (-0.023 – 0.034) | 0.697 |
| Sex | 0.617 (-0.538 – 1.772) | 0.290 | 0.038 (-0.181 – 0.257) | 0.730 | -0.052 (-0.400 – 0.296) | 0.767 |
| Education | -0.033 (-0.191 – 0.125) | 0.680 | -0.014 (-0.044 – 0.016) | 0.364 | -0.004 (-0.051 – 0.044) | 0.875 |
| **MoCA** | -0.042 (-0.299 – 0.215) | 0.746 | 0.006 (-0.043 – 0.055) | 0.802 | **-0.080 (-0.158 – -0.003)** | **0.042** |

**Table 2.4 Cont.**

**B.** Change in Serial Sevens DTC Gait Speed on Change in Relative Metabolite Concentration in the Primary Motor Cortex

| | **ΔNAA/Cr** | | **ΔCho/Cr** | | **ΔMyo/Cr** | |
|---|---|---|---|---|---|---|
| | ß (95% CI) | *p* | ß (95% CI) | *p* | ß (95% CI) | *p* |
| **ΔsDTC** | **-0.372 (-0.702 – -0.042)** | **0.028** | 0.025 (-0.051 – 0.101) | 0.517 | 0.044 (-0.125 – 0.212) | 0.606 |
| **Scanner** | **0.157 (0.063 – 0.251)** | **0.001** | **0.036 (0.015 – 0.058)** | **0.001** | **-0.050 (-0.097 – -0.002)** | **0.043** |
| Arm | **-0.025 (-0.048 – -0.002)** | **0.035** | 0.001 (-0.004 – 0.006) | 0.695 | 0.011 (-0.001 – 0.023) | 0.071 |
| Age | 0.001 (-0.004 – 0.006) | 0.737 | 0.000 (-0.002 – 0.001) | 0.434 | 0.000 (-0.002 – 0.003) | 0.780 |
| Sex | 0.047 (-0.016 – 0.110) | 0.142 | -0.002 (-0.017 – 0.013) | 0.786 | -0.005 (-0.037 – 0.027) | 0.769 |
| Education | -0.005 (-0.013 – 0.003) | 0.234 | -0.002 (-0.004 – 0.000) | 0.103 | -0.001 (-0.005 – 0.003) | 0.688 |
| **MoCA** | 0.004 (-0.009 – 0.018) | 0.547 | 0.001 (-0.002 – 0.005) | 0.361 | **-0.007 (-0.014 – 0.000)** | **0.039** |

Multiple linear regressions assessing the longitudinal associations between changes in dual task cost on gait speed (independent variables) and changes in metabolite concentration/ratio (dependent variables)[a] among participants with mild cognitive impairment, adjusted for potential confounders (n = 75). Δ = 5 months – 0 months (post trial – baseline). ß significant (i.e. $p < 0.05$) indicated in bold. ß = coefficient of regression corresponding to a change in metabolite concentration/ratio.

a Separated analyses were used for each model.

## 2.4 Discussion

The current study examined the effect of exercise and cognitive training in combination with VitD supplementation on changes in motor cortex metabolite levels and gait parameters in people with MCI. It was hypothesized that combined PE, CT, and VitD would yield increased M1 NAA, and reduced Cho and Myo over time compared to the control arm, as well as reduced DTC gait speed over time compared to control. In support of the first hypothesis, a significant effect of treatment was found for ΔNAA/Cr ratio, but not for other metabolite ratios or for absolute metabolite levels. Compared to those who

engaged in exercise and cognitive training with VitD supplementation (1 v 4), people who only did exercise showed greater impairment in M1 neuron function indexed by reduced NAA/Cr levels over time. Also, compared to controls and participants who only completed exercise, those who engaged in exercise and cognitive training showed increased neuronal functioning in M1. In contrary to our hypothesis, no differences in ΔDTC were found between the intervention arms. Consistent with our second hypothesis, when combining all arms of the study and performing multiple regression, greater ΔnDTC was associated with reduced absolute NAA, and greater ΔsDTC was associated with reduced NAA/Cr. However, opposite to our prediction, greater ΔnDTC was associated with reduced Cho.

Physical exercise, cognitive training, and vitamin D have each individually demonstrated benefits for brain health [35]–[41]. This study, therefore, is the first to examine the effects of various combinations of CT and VitD supplementation paired with PE on motor cortex brain metabolite levels in a well-defined MCI cohort. Contrary to our hypothesis, we did not identify a specific effect of PE on absolute metabolite levels (comparing arms 4 and 5). Although there is literature to support the benefits of PE on overall cognition in MCI [59]–[61], the literature regarding the effects of PE on MRS measured brain metabolite levels is inconsistent. One study found reduced Myo in female varsity athletes compared to a female sedentary control group, indicating reduced glial cell activity and inflammation [38]. Additionally, aerobic exercise increased NAA/Cr levels in the hippocampus and anterior cingulate cortex compared to inactive healthy controls [37], [62]. In contrast, a randomized trial found no changes in cerebral NAA/Cr in healthy middle-aged males following 12 weeks of aerobic exercise compared to control [63]. Another study found that healthy elderly participants who performed high intensity interval training for 3 years showed reduced hippocampal NAA/Cr compared to the healthy control group [64]. In the current study, PE alone did not increase NAA or NAA/Cr in the motor cortex. Instead, an increase in NAA/Cr level was observed only when PE was combined with CT but without VitD, suggesting intervention-specific effects of PE and CT on improving neuron metabolism and function.

Contrary to our hypothesis, VitD did not seem to influence NAA/Cr levels (comparing arms 1 and 2, or arms 3 and 4). Rather, the group receiving placebo VitD supplementation paired with PE+CT demonstrated the greatest gains in NAA/Cr over the five-month period compared to all other arms. There was a significant difference in ΔNAA/Cr between arm 1 and arm 4, however, we cannot attribute this difference to VitD along, as VitD and CT were provided in arm 1 while neither were provided in arm 4. VitD has been proposed to be linked to improved neuronal function due to its anti-inflammatory properties [65]. One study found that in people with MCI, those with lower serum VitD concentration were more likely to have lower NAA/Cr than those with higher serum VitD levels after controlling for other demographic factors [31]. Considering that all participants in our cohort were initially screened and excluded if they were VitD deficient, it is possible that additional VitD may be less effective at improving neuron functioning and reducing inflammation in individuals who are not VitD deficient. It is interesting however, that PE+VitD, regardless of CT, was able to maintain NAA/Cr levels, suggesting a protective effect of VitD on neuronal functional decline when combined with PE.

As expected, PE when paired with CT did affect motor cortex metabolite levels. Comparing the effect of CT in PE+VitD arms (arm 1 compared to 3), did not show any difference in ΔNAA/Cr. However, in PE+pVitD arms (arm 2 compared to 4), there was a significant difference in ΔNAA/Cr where people in arm 2 demonstrated a significantly greater increase in NAA/Cr after five months compared to arm 4, which exhibited the most substantial decline in NAA/Cr. Additionally, arm 2 had greater ΔNAA/Cr than the control arm (arm 5). These results suggest that multidomain PE, when complemented with dual task CT, may provide positive effects on neuronal function within M1. It is possible, however, that these benefits are not limited to M1. Research related to the effects of CT on MRS metabolite levels is scarce. Only one study has examined $^{1}$H-MRS metabolite levels following CT, reporting reduced Cho levels in the hippocampus of individuals with MCI who participated in memory training exercises [39]. It was suggested that reductions in Cho were associated with lower cell membrane degradation, and thus reduced inflammation. However, this study should be interpreted with caution as

it lacked a true MCI control arm, instead comparing hippocampal Cho levels to a yoga intervened group with MCI.

Notably, there were no statistically significant differences in NAA concentration changes between arms after the five-month treatment period. It is therefore possible that the differences in ΔNAA/Cr observed between arms is at least partially due to changes in Cr concentration. Cr is a marker of energy metabolism and is often used as a reference molecule in the MRS literature [66]. Within the context of disease, the simultaneous changes in Cr and NAA may suggest altered neuronal function and inflammation, while also accounting for participant-specific differences. A previous study in a NeuroAIDS macaque model showed that relatively high Cr and low NAA was associated with inflammation and a reduction in neuron count within the posterior cingulate [67]. Abnormally high levels of Cr can be associated with increased energy demand from glial cell activation and thus inflammation [68]. Myo is a marker of gliosis [24], and although the current study did not find statistically significant differences in Myo between arms, there was an inverse relationship between MoCA scores at baseline and ΔMyo, implying that those with more impaired cognition at baseline were more likely to show increased M1 inflammation. One study found that healthy older individuals who underwent five months of memory training exercises found reduced serum levels of cortisol and pro-inflammatory cytokines compared to controls [69]. A different study that examined strengthening attentional resources through mindfulness exercises found that individuals with MCI show reduced serum c-reactive protein, an inflammatory cytokine, providing evidence for neurological benefits of CT in MCI progression [39]. The current study supports the idea that providing CT in conjunction with PE may enhance neuron function while also reducing inflammation.

Contrary to our hypothesis, there were no differences in ΔDTC gait speed between arms for any of the dual tasks. PE and CT have many benefits on human health both physiologically and psychologically [35], [63]. The effect of combined PE and CT on gait speed in MCI is less well known. PE and CT have been shown to improve slow DT gait speed in the healthy elderly population as well as those with dementia [36], [70]. There is also evidence demonstrating null effects of resistance training or aerobic

exercise on gait [71], [72]. Additionally, some studies have shown benefits of tai chi, a balance-focused martial art, on DTC gait speed in elderly individuals with and without MCI [73]–[75], which may explain the trend of improvement in ΔnDTC gait speed over five months in the control arm. One limitation of studying DTC on gait speed alone is that it can be difficult to interpret due to the inability to quantify engagement in the dual task, as participants may have given priority to completing the walking test over the cognitive test, or vice versa [76]. For instance, if DTC on gait speed appears low in one participant, it is possible they may have done poorly on the cognitive task. Importantly, however, the current study cohort was restricted to those who completed both T0 and T5 MRS and consequently does not reflect the entire SYNERGIC cohort. Thus, the effect of PE, CT, and VitD on DTC gait speed cannot be ruled out and requires further investigation.

All arms were collapsed together to determine if there were any relationships between changes in DTC speed and changes in M1 metabolite levels, irrespective of treatment intervention. A significant inverse relationship between ΔnDTC, and ΔNAA concentrations was identified, as well as an inverse relationship between ΔNAA/Cr and ΔsDTC. It is unclear why each metabolite measurement is associated with a different DT. Still, these findings provide additional evidence to support DTC as a predictive measurement of neurological decline and strengthen our understanding of the physiological mechanisms that contribute to DT interference on gait speed in individuals with MCI [4], [77], [78]. NAA is a neuronal marker of neuroaxonal integrity and mitochondrial metabolism, where greater concentrations are indicative of enhanced neuron function. NAA is well documented as a metabolite that gradually declines throughout the progression of MCI to dementia, indicating a of loss of neuronal function and neurodegeneration [28], [37], [79]. Lower concentrations of NAA may be a sign of poor neurotransmission when attentional resources are consumed during the DT, leading to slower gait. In contrast to our results, Annweiler et al., did not find a relationship between NAA and DT gait speed in MCI, and rather found that NAA was specifically associated with stride time variability during the sDT [32]. However, they also found that slower ST and DT gait speed correlated with reduced M1 volume in MCI, further supporting M1 as a contributor to slow gait [32]. The current study suggests that the

relationship between NAA and DT speed in MCI cannot be detected through a single observation. Instead, changes in M1 neurochemistry and gait impairment may only be visible when observed longitudinally. Moreover, the nearly four times greater sample size in the current study, compared to the previous study, may have provided adequate statistical power to yield the observed relationship.

In the context of MRS, Cho is a marker of cellular membrane turnover, degradation, and synthesis. Cho has been inversely associated with M1 volume in MCI, which increased Cho has thought to be associated with increased inflammation and demyelination [32], [80]. Further, Cho was also reported to be inversely associated with ST and DT speed [32]. Contrary to our hypothesis and to the findings of Annweiler et al., we found that an improvement in nDTC gait speed was associated with greater levels of Cho. Although this finding seems counterintuitive, perhaps Cho may be increasing as a result of new dendritic and axonal connections forming within M1 in participants that improved in the nDT gait speed [37]. The Cho peak in 3.0 T $^{1}$H-MRS is mainly composed of both phosphocholine and glycerophosphocholine [81]. The former is a precursor molecule for phosphatidylcholine, the most prevalent phospholipid in neuron membranes, and is associated with cell membrane synthesis, while the latter is a residual product associated with the breakdown of cell membranes. Consequently, Cho changes in the MRS literature can be attributed to both constructive and destructive lipid metabolism [37], [82], [83]. Axonal growth is associated with heightened phosphatidylcholine synthesis after the application of nerve growth factors [84]. Gonzales et al., supports our finding as well as aerobic exercise increased NAA/Cr and Cho/Cr in healthy elderly participants compared to inactive controls, which they also attributed to increased Cho concentrations to increased synaptic connections [37]. Electroconvulsive therapy also increases hippocampal Cho in rats [85], a technique that is associated with neurogenesis [37], [86]. Moreover, following post-stroke administration of citicoline, an endogenous precursor to phosphatidylcholine, neurogenesis is observed through increased dendritic spine formation and greater expression of synaptic-related proteins [87]. Taking the above findings together with studies that show aerobic exercise increases dendritic formations in the hippocampus [88], [89], it is possible that the increase in M1 Cho and

improvement in nDTC on gait speed following five months of exercise is associated with neuroplasticity, however further studies are needed to confirm.

There are several limitations that should be considered for the current study. First, there was a substantial number of participants that dropped out of the trial due to COVID-19 restrictions. Second, MCI is a heterogenous syndrome with several subtypes that were not considered separately in this study [3], [28]. Thus, future work should compare the effects of PE, CT, and VitD on M1 metabolism in people with amnestic and non-amnestic MCI. Another limitation of this study was that the voxel placement in M1 was performed manually resulting in variation between timepoints and consequently leading to increased variance in metabolite level measurements. Many longitudinal MRS studies have the same issue and have used the DICE coefficient as was done in the current study to quantify the level of voxel overlap [38], [57]. Finally, the current study did not account for differences in diet regimens, sleep hygiene, and prescribed medications, which may also affect metabolite levels and gait speed measurements.

## 2.5 Conclusion

The current study demonstrates that providing five months of combined PE and CT to older adults with MCI can improve neuronal metabolism in M1, despite no improvements in DTC. Future studies should investigate how DTC is associated with neurometabolic changes in different brain regions as well as in different MCI sub-types to better characterize the effects of various treatment interventions.

## 2.6 References

[1] R. C. Petersen *et al.* "Mild Cognitive Impairment". In: *Archives of Neurology* 56.3 (1999), pp. 303–308.

[2] R. C. Petersen *et al.* "Mild Cognitive Impairment Ten Years Later". In: *Archives of Neurology* 66.12 (2009), pp. 1447–1455.

[3] R. C. Petersen. "Clinical Practice: Mild Cognitive Impairment". In: *The New England Journal of Medicine* 364.23 (2011), pp. 2227–2261.

[4] M. M. Montero-Odasso *et al.* "Association of dual-task gait with incident dementia in mild cognitive impairment: Results from the gait and brain study". In: *JAMA Neurology* 74.7 (2017), pp. 857–865.

[5] M. Storandt *et al.* "Rates of progression in mild cognitive impairment and early Alzheimer's disease". In: *Neurology* 59.7 (2002), pp. 1034–1041.

[6] F. Pieruccini-Faria *et al.* "Mapping Associations Between Gait Decline and Fall Risk in Mild Cognitive Impairment". In: *Journal of the American Geriatrics Society* 68.3 (2020), pp. 576–584.

[7] J. Verghese *et al.* "Gait dysfunction in mild cognitive impairment syndromes". In: *Journal of the American Geriatrics Society* 56.7 (2008), pp. 1244–1251.

[8] J. Dumurgier *et al.* "Gait Speed and Decline in Gait Speed as Predictors of Incident Dementia". In: *The Journals of Gerontology* 72.5 (2017), pp. 655–661.

[9] J. Verghese *et al.* "Quantitative gait dysfunction and risk of cognitive decline and dementia". In: *Journal of Neurology, Neurosurgery and Psychiatry* 78.9 (2007), pp. 929–935.

[10] M. Montero-Odasso *et al.* "The Motor Signature of Mild Cognitive Impairment: Results From the Gait and Brain Study". In: *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 69.11 (2014), pp. 1415–1421.

[11] M. Montero-Odasso *et al.* "Gait and cognition: A complementary approach to understanding brain function and the risk of falling". In: *Journal of the American Geriatrics Society* 60.11 (2012), pp. 2127–2136.

[12] D. J. Clark. "Automaticity of walking: Functional significance, mechanisms, measurement and rehabilitation strategies". In: *Frontiers in Human Neuroscience* 9.26 (2015), pp. 1–13.

[13] R. Camicioli *et al.* "Motor slowing precedes cognitive impairment in the oldest old". In: *Neurology* 50.5 (1998), pp. 1496–1498.

[14] M. Montero-Odasso *et al.* "Motor and Cognitive Trajectories Before Dementia: Results from Gait and Brain Study". In: *Journal of the American Geriatrics Society* 66.9 (2018), pp. 1676–1683.

[15] H. H. Dodge *et al.* "In-home walking speeds and variability trajectories associated with mild cognitive impairment". In: *Neurology* 78.24 (2012), pp. 1946–1952.

[16] M. M. Mielke *et al.* "Assessing the temporal relationship between cognition and gait: Slow gait predicts cognitive decline in the mayo clinic study of aging". In: *Journals of Gerontology* 68.8 (2013), pp. 929–937.

[17] R. K. MacAulay *et al.* "Improving Sensitivity to Detect Mild Cognitive Impairment: Cognitive Load Dual-Task Gait Speed Assessment". In: *Journal of the International Neuropsychological Society* 23.6 (2017), pp. 493–501.

[18] T. Buracchio *et al.* "The trajectory of gait speed preceding mild cognitive impairment". In: *Archives of neurology* 67.8 (2010), pp. 980–986.

[19] S. W. Hunter *et al.* "A framework for secondary cognitive and motor tasks in dual-task gait testing in people with mild cognitive impairment". In: *BMC Geriatrics* 18.202 (2018), pp. 1–7.

[20] R. Beurskens and O. Bock. "Age-related Deficits of dual-task walking: A review". In: *Neural Plasticity* 2012. (2012), pp. 1–9.

[21] J. M. McDowd and F. I. M. Craik. "Effects of aging and task difficulty on divided attention performance". In: *Journal of Experimental Psychology* 14.2 (1988), pp. 267–280.

[22] G. Verdile *et al.* "Inflammation and Oxidative Stress: The Molecular Connectivity between Insulin Resistance, Obesity, and Alzheimer's Disease". In: *Hindawi* 2015. (2015), pp. 1–17.

[23] A. L. Palmer and S. S. Ousman. "Astrocytes and Aging". In: *Frontiers in aging neuroscience* 10. (2018), pp. 1–14.

[24] G. Öz *et al.* "Clinical proton MR spectroscopy in central nervous system disorders". In: *Radiology* 270.3 (2014), pp. 658–679.

[25] K. Kantarci *et al.* "Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease". In: *Neurology* 55.2 (2000), pp. 210–217.

[26] S. E. Rose *et al.* "A 1H MRS study of probable Alzheimer's disease and normal aging: Implications for longitudinal monitoring of dementia progression". In: *Magnetic Resonance Imaging* 17.2 (1999), pp. 291–299.

[27] W. Huang *et al.* "High Brain myo-Inositol Levels in the Predementia Phase of Alzheimer's Disease in Adults With Down's Syndrome: A 1 H MRS Study". In: *American Journal of Psychiatry* 156.12 (1999), pp. 1879–1886.

[28] K. Kantarci. "Proton MRS in mild cognitive impairment". In: *Journal of Magnetic Resonance Imaging* 37.4 (2013), pp. 770–777.

[29] K. Kantarci *et al.* "MRI and MRS predictors of mild cognitive impairment in a population-based sample". In: *Mayo Clinic* 81.2 (2013), pp. 126–133.

[30] G. Oeltzschner *et al.* "Neurometabolites and associations with cognitive deficits in mild cognitive impairment: a magnetic resonance spectroscopy study at 7 Tesla". In: *Neurobiology of Aging* 73. (2019), pp. 211–218.

[31] C. Annweiler *et al.* "Vitamin D and Caudal Primary Motor Cortex: A Magnetic Resonance Spectroscopy Study". In: *PLOS One* 9.1 (2014), pp. 1–5.

[32] C. Annweiler *et al.* "Motor cortex and gait in mild cognitive impairment: A magnetic resonance spectroscopy and volumetric imaging study". In: *Brain* 136.3 (2013), pp. 859–871.

[33] K. M. Langa and D. A. Levine. "The diagnosis and management of mild cognitive impairment: A clinical review". In: *JAMA* 312.23 (2014), pp. 2551–2561.

[34] "How are MCI and Dementia Treated". In: *Journal of the American Academy of Physician Assistants* 23.1 (2010), pp. 18–21.

[35] P. Plummer *et al.* "Effects of Physical Exercise Interventions on Gait-Related Dual-Task Interference in Older Adults: A Systematic Review and Meta-Analysis". In: *Gerontology* 62.1 (2015), pp. 94–117.

[36] L. Tay *et al.* "A Combined Cognitive Stimulation and Physical Exercise Programme (MINDVital) in Early Dementia: Differential Effects on Single- and Dual-Task Gait Performance". In: *Gerontology* 62.6 (2016), pp. 604–610.

[37] M. M. Gonzales *et al.* "Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults". In: *Brain Topography* 26.1 (2013), pp. 126–134.

[38] A. L. Schranz *et al.* "Brain Metabolite Levels in Sedentary Women and Non-contact Athletes Differ From Contact Athletes". In: *Frontiers in Human Neuroscience* 14. (2020), pp. 1–13.

[39] H. Yang *et al.* "Neurochemical and Neuroanatomical Plasticity Following Memory Training and Yoga Interventions in Older Adults with Mild Cognitive Impairment". In: *Frontiers in Aging Neuroscience* 8. (2016), pp. 1–9.

[40] D. Wong *et al.* "Vitamin D Supplementation May Protect Against Oxidative Stress in a Vitamin D Deficient Double-Transgenic Mouse Model of Alzheimer's Disease". In: *Alzheimer's & Dementia* 13. (2017), pp. 746–747.

[41] K. Z. H. Li *et al.* "Benefits of Cognitive Dual-Task Training on Balance Performance in Healthy Older Adults". In: *The Journals of Gerontology* 65.12 (2010), pp. 1344–1352.

[42] M. Montero-Odasso *et al.* "SYNERGIC TRIAL (SYNchronizing Exercises, Remedies in Gait and Cognition) a multi-Centre randomized controlled double blind trial to improve gait and cognition in mild cognitive impairment". In: *BMC Geriatrics* 18.93 (2018), pp. 1–15.

[43] B. Winblad *et al.* "Mild cognitive impairment - Beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment". In: *Journal of Internal Medicine* 256.3 (2004), pp. 240–246.

[44] R. C. Petersen *et al.* "Current Concepts in Mild Cognitive Impairment". In: *Archives of Neurology* 58.12 (2001), pp. 1985–1992.

[45] M. P. Lawton and E. M. Brody. "Assessment of Older People: Self-Maintaining and Instrumental Activities of Daily Living 1". In: *The Gerontologist* 3.1 (1969), pp. 179–186.

[46] S. B. GUZE. "Diagnostic and Statistical Manual of Mental Disorders, 4th ed (DSM-IV)". In: *American Journal of Psychiatry* 152.8 (1995), pp. 1228–1228.

[47] L. Bherer *et al.* "Transfer effects in task-set cost and dual-task cost after dual-task training in older and younger adults: further evidence for cognitive plasticity in attentional control in late adulthood". In: *Experimental aging research* 34.3 (2008), pp. 188–219.

[48] L. Bherer *et al.* "Testing the limits of cognitive plasticity in older adults: application to attentional control". In: *Acta psychologica* 123.3 (2006), pp. 261–278.

[49] L. Bherer *et al.* "Training effects on dual-task performance: are there age-related differences in plasticity of attentional control?". In: *Psychology and aging* 20.4 (2005), pp. 695–709.

[50] "Prescription Drug List (PDL): Vitamin D"., https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/prescription-drug-list/notices-changes/notice-amendment-vitamin-d.html.

[51] J. E. Iglesias *et al.* "Robust brain extraction across datasets and comparison with publicly available methods". In: *IEEE Transactions on Medical Imaging* 30.9 (2011), pp. 1617–1634.

[52] Y. Zhang *et al.* "Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm". In: *IEEE Transactions on Medical Imaging* 20.1 (2001), pp. 45–57.

[53] R. Bartha *et al.* "Factors affecting the quantification of short echo in-vivo 1 H MR spectra: prior knowledge, peak elimination, and filtering". In: *NMR in Biomedicine* 12. (1999), pp. 205–216.

[54] R. Bartha *et al.* "Spectroscopic Lineshape Correction by QUECC: Combined QUALITY Deconvolution and Eddy Current Correction". In: *Magnetic Resonance in Medicine* 44. (2000), pp. 641–645.

[55] M. N. E. Kassem and R. Bartha. "Quantitative Proton Short-Echo-Time LASER Spectroscopy of Normal Human White Matter and Hippocampus at 4 Tesla Incorporating Macromolecule Subtraction". In: *Magnetic Resonance in Medicine* 49. (2003), pp. 918–927.

[56] "BARSTOOL v41"., https://github.com/dwong263/MAGIQ/wiki/BARSTOOL-Overview.

[57] L. R. Dice. "Measures of the Amount of Ecologic Association Between Species". In: *Ecology* 26.3 (1945), pp. 297–302.

[58] M. Montero-Odasso *et al.* "Quantitative gait analysis under dual-task in older people with mild cognitive impairment: A reliability study". In: *Journal of NeuroEngineering and Rehabilitation* 6.35 (2009), pp. 1–6.

[59] C. Nascimento *et al.* "Physical Exercise in MCI Elderly Promotes Reduction of Pro-Inflammatory Cytokines and Improvements on Cognition and BDNF Peripheral Levels". In: *Current Alzheimer Research* 11.8 (2014), pp. 799–805.

[60] Cammisuli *et al.* "Aerobic exercise effects upon cognition in Mild Cognitive Impairment: A systematic review of randomized controlled trials". In: *Archives Italiennes de Biologie* 1 (2017), pp. 54–62.

[61] J. A. Blumenthal *et al.* "Lifestyle and neurocognition in older adults with cognitive impairments: A randomized trial". In: *Neurology* 92.3 (2019), pp. 212–223.

[62] L. Den Ouden *et al.* "The Influence of Aerobic Exercise on Hippocampal Integrity and Function: Preliminary Findings of a Multi-Modal Imaging Analysis". In: *Brain Plasticity* 4.2 (2018), pp. 211–216.

[63] S. Matura *et al.* "Effects of aerobic exercise on brain metabolism and grey matter volume in older adults: results of the randomised controlled SMART trial". In: *Translational Psychiatry* 7.7 (2017), pp. 1172–1172.

[64] L. S. Reitlo *et al.* "Hippocampal neurochemicals are associated with exercise group and intensity, psychological health, and general cognition in older adults". In: *GeroScience* (2023).

[65] C. Annweiler *et al.* "Vitamin D and Ageing: Neurological Issues ". In: *Neuropsychobiology* 62. (2010), pp. 139–150.

[66] J. F. A. Jansen *et al.* "$^{1}$H MR Spectroscopy of the Brain: Absolute Quantification of Metabolites". In: *Radiology* 240.2 (2006), pp. 318–332.

[67] E.-M. Ratai *et al.* "Brain creatine elevation and *N* -acetylaspartate reduction indicates neuronal dysfunction in the setting of enhanced glial energy metabolism in a macaque model of NeuroAIDS". In: *Magnetic Resonance in Medicine* 66.3 (2011), pp. 625–634.

[68] E.-M. Ratai *et al.* "Proton Magnetic Resonance Spectroscopy Reveals Neuroprotection by Oral Minocycline in a Nonhuman Primate Model of Accelerated NeuroAIDS". In: *PLoS ONE* 5.5 (2010), pp. 1–14.

[69] M. Pesce *et al.* "Memory training program decreases the circulating level of cortisol and pro-inflammatory cytokines in healthy older adults". In: *Frontiers in Molecular Neuroscience* 10. (2017), pp. 1–17.

[70] M. Schwenk *et al.* "Dual-task performances can be improved in patients with dementia: A randomized controlled trial". In: *Neurology* 74.24 (2010), pp. 1961–1968.

[71] U. Granacher *et al.* "Balance training and multi-task performance in seniors". In: *International journal of sports medicine* 31.5 (2010), pp. 353–358.

[72] A. Halvarsson *et al.* "Effects of new, individually adjusted, progressive balance group training for elderly people with fear of falling and tend to fall: A randomized controlled trial". In: *Clinical Rehabilitation* 25.11 (2011), pp. 1021–1031.

[73] B. Manor *et al.* "Functional benefits of tai chi training in senior housing facilities". In: *Journal of the American Geriatrics Society* 62.8 (2014), pp. 1484–1489.

[74] C.-L. Liu *et al.* "Effects of Exergaming-Based Tai Chi on Cognitive Function and Dual-Task Gait Performance in Older Adults With Mild Cognitive Impairment: A Randomized Control Trial". In: *Frontiers in Aging Neuroscience* 14. (2022), pp. 1–9.

[75] L. Hosseini *et al.* "Tai Chi Chuan can improve balance and reduce fear of falling in community dwelling older adults: a randomized control trial". In: *Journal of Exercise Rehabilitation* 14.6 (2018), pp. 1024–1031.

[76] G. Yogev-Seligmann *et al.* "How Does Explicit Prioritization Alter Walking During Dual-Task Performance? Effects of Age and Sex on Gait Speed and Variability". In: *Physical Therapy* 90.2 (2010), pp. 177–186.

[77] V. Koppelmans *et al.* "Systematic Review Neural Mechanisms of Motor Dysfunction in Mild Cognitive Impairment and Alzheimer's Disease: A Systematic Review". In: *Journal of Alzheimer's Disease Reports* 6. (2022), pp. 307–344.

[78] P. Ali *et al.* "Associations between gait speed and brain structure in amnestic mild cognitive impairment: a quantitative neuroimaging study". In: *Brain Imaging and Behavior* 16.1 (2022), pp. 228–238.

[79] A. Pfefferbaum *et al.* "In Vivo Brain Concentrations of N-Acetyl Compounds, Creatine, and Choline in Alzheimer Disease". In: *Archives of General Psychiatry* 56.2 (1999), pp. 185–192.

[80] J. R. L. Ferraz-Filho *et al.* "Application of magnetic resonance spectroscopy in the differentiation of high-grade brain neoplasm and inflammatory brain lesions". In: *Arquivos de Neuro-Psiquiatria* 67.2 (2009), pp. 250–253.

[81] A. J. Ross and P. S. Sachdev. "Magnetic resonance spectroscopy in cognitive research". In: *Brain Research Reviews* 44. (2004), pp. 83–102.

[82] S. Herminghaus *et al.* "Increased choline levels coincide with enhanced proliferative activity of human neuroepithelial brain tumors". In: *NMR in biomedicine* 15.6 (2002), pp. 385–392.

[83] J. Klein. "Membrane breakdown in acute and chronic neurodegeneration: focus on choline-containing phospholipids Review Article". In: *Journal of Neural Transmission* 107. (2000), pp. 1027–1063.

[84] W. Araki and R. J. Wurtman. "Control of membrane phosphatidylcholine biosynthesis by diacylglycerol levels in neuronal cells undergoing neurite outgrowth". In: *Cell Biology* 94.22 (1997), pp. 11946–11950.

[85] A. Sartorius *et al.* "Choline rise in the rat hippocampus induced by electroconvulsive shock treatment". In: *Biological Psychiatry* 53.7 (2003), pp. 620–623.

[86] T. M. Madsen *et al.* "Increased neurogenesis in a model of electroconvulsive therapy". In: *Biological Psychiatry* 47.12 (2000), pp. 1043–1049.

[87] R. Muralikrishna Adibhatla *et al.* "Effects of Citicoline on Phospholipid and Glutathione Levels in Transient Cerebral Ischemia". In: *Stroke* 32.10 (2001), pp. 2376–2381.

[88] V. A. Redila and B. R. Christie. "Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus". In: *Neuroscience* 137.4 (2006), pp. 1299–1307.

[89] A. M. Stranahan *et al.* "Running induces widespread structural alterations in the hippocampus and entorhinal cortex". In: *Hippocampus* 17.11 (2007), pp. 1017–1022.

# Chapter 3

# 3 Summary and Future Work

## 3.1 Summary

Mild cognitive impairment (MCI) is an intermediary state between healthy cognitive aging and dementia. The combined effect of physical exercises (PE), cognitive training (CT), and vitamin D (VitD) on primary motor cortex metabolism and gait impairment in people with MCI is not well understood. Further, the relationship between gait impairment in MCI, specifically, dual task cost (DTC) on gait speed, and M1 neurometabolic markers is scarcely studied. The primary aim of this thesis was to investigate whether five months of combined PE, CT, and VitD could alter M1 inflammation and DTC on gait speed in people with MCI. The second aim of our study was to understand whether there is a longitudinal relationship between changes in M1 metabolites and changes in DTC on gait speed in individuals with MCI, regardless of their treatment intervention.

This study measured M1 metabolism and DTC on gait speed before and after five months of a prescribed treatment intervention. For our first aim, we predicted that after five months, participants assigned all three treatment interventions would demonstrate higher M1 NAA levels, and lower Cho and Myo levels than each of the other treatment combinations. We also predicted that the triple intervention arm would show the greatest reduction in DTC on gait speed compared to all other arms. For our second aim, we predicted that individuals who showed a reduction in DTC on gait speed were more likely to demonstrate elevated NAA, and reduced Cho and Myo concentrations in M1.

Our study found that individuals who were subjected to five months of PE and CT with placebo VitD showed the greatest increase in mean M1 NAA/Cre levels in all arms and was significantly greater compared to the PE only and control arms ($p<0.05$). We also found that the triple intervention arm, on average, showed no changes in NAA/Cre levels over the five months, but was significantly different from the PE only arm who showed a

notable decrease in NAA/Cr over time ($p<0.05$). M1 Cho and Myo concentrations were not significantly different between any treatment arms, nor was DTC on gait speed different between arms.

In contrary to our hypothesis, the triple intervention arm did not show the greatest improvement in NAA levels. Considering the participants in this study were not VitD deficient, it is possible that additional VitD supplements were not able to improve neuron function. However, CT when paired with PE seemed to improve neuron function when VitD was not provided as NAA/Cr levels increased rather than remained stable, and removing CT induced a decline in NAA/Cr. The physiological mechanism explaining why neuron function improved in the motor cortex following combined PE and CT is unclear, though perhaps there was a global effect of improving neuron function.

Consistent with our hypothesis, we found an inverse relationship between changes in NAA/Cr and changes in serial sevens DTC on gait speed, as well as an inverse relationship between changes in absolute NAA and changes in naming animals DTC on gait speed. In contrast to our prediction, changes in naming animals DTC on gait speed were also inversely associated with absolute changes in Cho levels, while no relationship between DTC and Myo was found. The reason that Cho increased in individuals who showed reduced DTC could be explained by more synaptic formations and myelination occurring within M1, which is consistent with the increase in NAA levels.

This is the first study to examine how five months of combined PE, CT, and VitD influences M1 metabolism and gait impairment on a well-defined cohort of people with MCI. Additionally, this study is the first to examine the temporal relationship between M1 neurochemistry using magnetic resonance spectroscopy and gait impairment in MCI. These findings will help guide clinicians in constructing optimal treatment plans for their patients with MCI. Moreover, this study will serve to supplement gait research and motivate further exploration of M1 and its contribution to gait impairment in MCI.

## 3.2 Limitations

Several limitations should be considered when interpreting the results of this study. First and foremost, the current study included only a small subset of the participants from the SYNERGIC trial cohort because many participants had absent or incomplete MRS data. Therefore, the results presented regarding the effects of the interventions on DTC on gait speed are specific to this study and should not be extrapolated to the complete SYNERGIC cohort. Another limitation of this study is that the measure of DTC on gait speed fails to incorporate how well participants performed the cognitive task while walking. For example, it is possible that some participants who demonstrated faster DT gait speed may have made more errors, suggestive of poor task management and cognitive functioning. Lastly, the current study is limited by the inconsistent placement of the voxel in the M1 region between the baseline and post-trial acquisitions. It is possible that the voxel incorporated some tissue beyond M1 and therefore metabolite measures partially represent some regions surrounding M1. Consequently, the data reported may not entirely represent changes in M1 metabolism.

## 3.3 Future Work

This thesis was the first to identify MRS associated changes in the primary motor cortex of individuals with MCI who underwent an extensive five-month treatment regimen. Future work should follow a similar participant cohort and treatment protocol for a longer period of time to see whether the relationship between M1 metabolism and DTC on gait speed persists. This hypothetical study should also determine whether combined PE and CT continue to improve neuron function in M1. Additionally, future studies should acquire MRS data using a higher field MR scanner, that provides greater metabolite measurement precision, to better understand why increases in Cho are associated with reduced DTC on gait speed. Using an ultra-high magnetic field could allow quantification of phosphocholine and glycerophosphocholine concentration, which would help interpret whether the observed increases in Cho are associated with membrane degradation or synthesis. Finally, stride time variability and stride length variability have been associated with cognitive decline in MCI and have not been associated with changes in M1 neurochemistry. Thus, future research should also examine changes in other gait

parameters over time to determine whether they are associated with changes in M1 metabolite levels.

# Chapter 4

# 4 Appendices

## 4.1 Research Ethics

Western Research

**Date:** 1 August 2018

**To:** Dr. Manuel Montero Odasso

**Project ID:** 107670

**Study Title:** SYNchronizing Exercises, Remedies in GaIt and Cognition (SYNERGIC) Trial. A Randomized Controlled Double Blind Trial

**Application Type:** HSREB Amendment Form

**Review Type:** Delegated

**Meeting Date / Full Board Reporting Date:** 21/Aug/2018

**Date Approval Issued:** 01/Aug/2018

**REB Approval Expiry Date:** 19/Jul/2019

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Dear Dr. Manuel Montero Odasso ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

**Documents Approved:**

| Document Name | Document Type | Document Date |
|---|---|---|
| 107670_31July2018 | Protocol | 31/Jul/2018 |
| CCNA_LOICWestern_Waterloo_31July2018 | Consent Form | 31/Jul/2018 |
| SYNERGIC Protocol_v _11 June 2018 | Protocol | 11/Jun/2018 |

**Documents Acknowledged:**

| Document Name | Document Type | Document Date |
|---|---|---|
| NOL217410 _26July2018 | Sponsor Correspondence | 26/Jul/2018 |
| Summary of Changes HC _ 11 June 2018 | Summary of Changes | 11/Jun/2018 |

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Patricia Sargeant, Ethics Officer (ext. 85990) on behalf of Dr. Joseph Gilbert, HSREB Chair

***Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).***

**Figure 4.1** Research Ethics Form

## 4.2 Exercise Protocols

A detailed version of the published protocol for SYNERGIC can be found online.

### 4.2.1 Physical Exercise & Balance and Toning

Each subject underwent five months of either PE or BAT for three 60-minute sessions per week at a local fitness facility led by an exercise facilitator.

PE consisted of a combination of aerobic exercise and progressive resistance training exercises. The difficulty of the exercises was adjusted to each subject based on their individual functional ability. All subjects were given 10 minutes of warming up, including: bum kicks, dynamic hamstring stretching, quarter squats, ankle circles, dynamic calf stretching, split-step knee bends, side stepping with wrist circles, marching in place with arm swings, shoulder circles, arm reaches, hula hoop circles, and torso twists.

#### 4.2.1.1 Resistance Training

The resistance training portion of PE consisted of 5 exercises: lat pull, chest press, and seated row (upper body), leg flexion and leg press (lower body). With alternating between upper and lower body exercises, the number of reps, sets, and duration of the rest intervals intensified every 4 weeks to ensure subjects were increasing muscle strength. The load and volume of resistance training for the first 12 weeks was adjusted to target strength endurance, whereas the last 12 weeks were adjusted to target maximal strength. 30 – 60 second rests were permitted between sets. At the end of each PE session, subjects will be provided a 10-minute cool down period with ample stretching guided by the trainer.

**Table 4.1** Resistance training protocol across 20 weeks.

| Weeks | Sets | | | Repetitions | | | Rest between sets (sec) |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 | |
| 1-4 | 2 | 2 | 2 | 15-18 | 15-18 | 15-18 | 30 |
| 5-8 | 3 | 2 | 3 | 12-15 | 12-15 | 12-15 | 30 |
| 9-12 | 3 | 2 | 3 | 10 | 10 | 10 | 60 |
| 13-16 | 2 | 3 | 2 | 8 | 12 | 8 | 60 |
| 17-20 | 3 | 2 | 3 | 6 | 8 | 6 | 60 |

### 4.2.1.2 Aerobic Exercise

After completion of the resistance training session, participants were subjected to 10-20 minutes of aerobic exercises on various ergometers (ie: ellipticals, cycling, rowing, treadmill machines etc.), staircase climbs, or any freeform aerobic exercise that minimizes cognitive demand. Intensity was continuously measured using the Borg scale (0 – 10), with subjects targeting 5 – 6 in the first and second month, 6 – 7 in the third and fourth month, and 7 – 8 in the last month. Subjects were given 5-minute recovery periods between sets, consisting of various stretching, and breathing exercises.

**Table 4.2** Aerobic training protocol across 20 weeks

| Weeks | Sets | Duration (min) | Intensity (Borg 0-10) | Rest between sets (minutes) |
|---|---|---|---|---|
| 1-4 | 2 | 10 | 5-6 | 1 |
| 5-8 | 2 | 10 | 5-6 | 1 |
| 9-12 | 2 | 10 | 6-7 | 1 |
| 13-16 | 2 | 10 | 6-7 | 1 |
| 17-20 | 2 | 10 | 7-8 | 1 |

#### 4.2.1.3 Balance and Toning

BAT was provided to subjects as a control exercise condition, conducted in small groups of up to 8 subjects guided by a trainer. These exercises focused on improving muscle tone and flexibility, without increasing strength or cardiovascular ability. The resistance load and volume remained constant throughout the study, unless participants were unable to complete the necessary number of reps at the start of the exercise regimen. BAT subjects completed the 10-minute warm-up routine with subjects in PE arms before they began their exercises. Each session consisted of functional training (wall squats, standing calf raises, standing leg abduction/adduction, standing ball walk up & down, seated back row with resistance tube, shoulder retractions, wall push-ups, toe walking, heel walking, quarter squats, gluteus kickback holds, standing ball twists, shoulder circuits, yoga ball chest press, yoga ball shoulder abduction, yoga ball leg adduction squeeze), balance training (standing leg circumduction, tandem stance, single leg stance, tandem stance ball relay pass, partner ball pass in tandem stance, tandem forward & backward walking, toe taps on bench), agility training (4 step zig zag in place, 4 step zig zag walking, line and cone drills), and core training (core contractions seated on exercise ball, exercise ball seated ball pass, seated exercise ball marching, modified & full bug on floor). BAT subjects then regroup with the PE group to complete the same 10-minute cool down activity.

### 4.2.2 Cognitive Training & Sham Cognitive Training

Each CT or sCT session was about 30 minutes in length, involving tablet-based dual task training and took place before the PE session.

#### 4.2.2.1 Cognitive Training

To test working memory and multitasking abilities, subjects were required to maintain and prepare their responses for the given task, while also dividing their attention between two simultaneous tasks.

The difficulty of each task was adjusted to the functional level of each subject. All subjects were then sat at individual desks away from one another in a quiet room with

headphones. Participants performed a concurrent visuo-motor task (dual task combination) consisting of different sets of visual stimuli that had to be identified by tapping designated figures on the tablet. Discrimination tasks involved sets of items (i.e.: numbers, letters, celestial bodies, vehicles, animals, fruits). Each session involved two sets of items, one for each task. The combination of sets of items changed every four sessions.

Trained research assistants always gave instructions for each task combination in every training session. Participants completed a block of single-pure (SP) trials, followed by mixed-trial blocks with single-task trials (SM) and dual-mixed trials (DM: two tasks at once). Each training session included 80 SP trials, 128 SM trials, and 192 DM trials (with 40% of the DM blocks consisting of SM trials). During the first 30 training sessions of the DM trials, participants were asked to give equal priority to both hands. For the following 30 sessions, participants were instructed to vary their priorities between hands in different blocks. In total, participants completed 60 sessions.

Importantly, during the dual-mixed block of the training sessions, adaptive continuous feedback on performance was provided in the form of a speedometer. The speedometer's indicator moved and changed color (green, yellow, orange, or red) to indicate the response speed. Two speedometers (one for each hand) were displayed at the top of the screen, with each speedometer associated with one task. In equal priority blocks, participants were asked to keep both speedometers in at least the yellow zone and prevent them from turning red. In variable priority blocks, participants were asked to maintain the speedometer associated with the prioritized task in the green zone while the other speedometer could be at least in the orange zone. The color of the speedometers was determined by the average reaction time (RT) on the last three trials' RT for the DM block compared to the median RT for the SM trials multiplied by a factor of 1.5. In addition, feedback was provided at the end of each session, where participants were informed of their mean RT and accuracy achieved throughout the sessions presented in a histogram without explicit values.

#### 4.2.2.2 Sham Cognitive Training

Participants in the sCT arm were asked to conduct an internet search for 3 hotels, 3 touristic places, and 3 restaurants, in a city determined by the instructor at the start of each session. As a second task, subjects were provided a 20-minute National Geographic documentary film also chosen by the instructor at the beginning of the session. After completing the film, participants were to answer three questions about the film. The internet search and film tasks were alternated between sessions, each of which took roughly 20 minutes to complete. These control sessions were held under the same conditions as the CT group, using a tablet-based methods within a quiet room removed of distractions. Some studies have used ST as a control condition for DTs to account for exposing participants to the exact same type of task. However, subjects have also reported showing less interest in a single-task exercise due to a lack of stimulation and feeling of progression. Akin to Walton et al., the sham CT regiment of choice uses conditions more reliably matching effects of clinical interaction and patient expectation to the dual tasks.

### 4.2.3 Vitamin D & Placebo Vitamin D Supplementation

### 4.2.4 Vitamin D

Subjects in the VitD arms were administered 30,000 IU of oral Vitamin D supplements each week for the duration of the trial (3 doses of 10,000 IU per week). 10,000 IU per day is an acceptable supplementation for older individuals, as determined by Health Canada, and thus was well within the safety margins for potential drug toxicity. Additionally, prior studies have administered 30,000 IU per day to adult male subjects for five months, without any adverse side effects or changes in serum calcium concentrations. A review depicting the lowest level of risk for Vitamin D toxicity found that 40,000 IU per day was required to observe any adverse effects, further validating the safety of our VitD supplementation dosing and schedule.

### 4.2.5 Placebo Vitamin D

pVitD supplementation was matched to the schedule of VitD arms to control for any potential effects of social interactions during drug administration.

# Chapter 5

# Jack Elkas | Curriculum Vitae

## Education

**Western University** **London, ON**
*Master of Science (M.Sc.), Neuroscience* *2021-present*
Advisor: Dr. Robert Bartha

**University of Toronto** **Scarborough, ON**
*Bachelor of Science (B.Sc.), Neuroscience* *2016-2021*

## Publications

**2023 (In Preparation):** "*Exercise, Gait Speed, and Primary Motor Cortex Metabolism in Mild Cognitive Impairment: A $^{1}$H-MRS Analysis from the SYNERGIC Trial*", **Jack Elkas**, Frederico Pierucinni-Faria, Manuel Montero-Odasso, Robert Bartha

## Presentations and Abstracts

**2023 (Poster):** "'*Dual-Task Gait Predicts Changes in Choline in the Primary Motor Cortex of Older Individuals with Mild Cognitive Impairment*", **Jack Elkas**, Frederico Pierucinni-Faria, Manuel Montero-Odasso, Robert Bartha, International Society for Magnetic Resonance in Medicine Annual Meeting & Exhibition, Metro Toronto Convention Centre, Toronto, ON.

**2023 (Presentation):** "*Dual Task Gait Predicts Changes in Choline in the Primary Motor Cortex of Older Individuals with Mild Cognitive Impairment*", **Jack Elkas**, Frederico Pierucinni-Faria, Manuel Montero-Odasso, Robert Bartha, Imaging Network Ontario Symposium, March 29, 2023, DoubleTree, London, ON.

**2023 (Presentation):** "*Dual Task Gait Performance Predicts Choline Alterations in the Primary Motor Cortex of Mild Cognitive Impairment Patients*", **Jack Elkas**, Frederico Pierucinni-Faria, Manuel Montero-Odasso, Robert Bartha, Neuroscience Research Day, February 24, 2023, Western University, London, ON.

**2022 (E-Poster & Presentation):** "*Association of Gait and Inflammation in the Motor Cortex*", **Jack Elkas**, Frederico Pierucinni-Faria, Manuel Montero-Odasso, Robert Bartha, London Health and Research Day, April 29, 2022, Virtual, London, ON.

## Honours and Awards

**Educational Trainee Stipend ($600)** **Toronto, ON**
*International Society for Magnetic Resonance in Medicine Annual Meeting & Exhibition* *June 2023*

**Oral Presentation Award, 1st Place** **London, ON**
*Imaging Network Ontario Symposium* *March 2023*

**Oral Presentation Award, 1st Place** **London, ON**
*Neuroscience Research Day* *February 2023*

**Western Graduate Scholarship ($3000; Annually)** **London, ON**
*Western University* *2021-2023*