CHAPTER 2

STUDY 1

Development and Evaluation of the Short-term Effect of Cognitive Training Program for Individuals with Mild Cognitive Impairment: A Preliminary Study

2.1 Introduction

The prevalence of dementia worldwide is expected to increase progressively in future years as the ageing population and the life expectancy continues to increase. The incidence of dementia increases with advancing age and it presents a major public health problem that impacts elder's ability to maintain physical, occupational and social function (23). Patients can live up to 20 years with progressive dementia, with Alzheimer's disease (AD) being the most prevalence and devastating form (1). Due to the irreversible and progressive AD symptoms, an early intervention in individuals at high risk of progression to AD is a critical process for prevention or delay cognitive and functional decline. Mild Cognitive Impairment (MCI) is proposed to be a transitional stage before converting from normal ageing to dementia (2); therefore people with MCI represent an ideal target for early intervention approach.

Recent evidence suggests that a cognitive training program can improve cognitive functions of individuals with MCI (8-13). Previous studies examined the effectiveness of various cognitive training programs such as multifaceted rehabilitation, Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE), and computer-assisted training (8, 10, 13). The beneficial effects of a cognitive training program in delaying cognitive function declines have been reported (10-12). However, a major constraint about these techniques is the feasibility in clinical set-up and they only give a general scope of training making it difficult to replicate. To overcome this limitation, it is of interest to develop a specific cognitive training program for individuals with MCI by targeting solely on the cognitive components affected early in the course of

the disease. These cognitive components were episodic memory, attention, and executive functions (24, 25). We speculated that this specialized cognitive training would empower these vulnerable impairment aspects of individuals with MCI.

The evidence of brain-behavior relationship is increasingly available in recent years due to the advanced knowledge in the neuroimaging field. Another aim of this study was to examine whether the present cognitive training program could induce changes at the cortical level. We were interested in monitoring the alteration in cortical molecular and cellular mechanisms by using Magnetic Resonance Spectroscopy (MRS). MRS is a standard technique used in clinical practice to provide an insight into the cortical neurochemistry condition by way of measuring the neurometabolites related to cortical cellular energy. Previous MRS studies revealed that individuals with MCI showed the hippocampal and posterior cingulate cortex neurochemistry abnormalities including reduction in N-acetylaspartate (NAA)/Creatine (Cr), NAA/Choline (Cho), NAA/Myoinositol (mI) ratios as well as elevation in mI/Cr ratio (26-30). Therefore, MRS measurement could be used to monitor cortical neurochemistry changes in response to cognitive training in individuals with MCI. Studies that examined the effects of cognitive training program on the neurochemistry changes are scarce. Valenzuela et al (31) have demonstrated that memory training using the Method of loci (MOL) technique could enhance the hippocampal neurochemistry in healthy older adults. However, it is still unknown whether cognitive training can induce neurochemistry changes in individuals with MCI. In the present study, we investigated the neurochemistry biomarkers in three brain regions including the hippocampus, the prefrontal cortex (PFC) and the anterior

cingulate cortex (ACC) that are responsible for episodic memory, attention, and executive functions (32-34).

Collectively, the aim of this study was to investigate the short-term effect of the specific cognitive training program on cognitive functions and cortical neurochemistry in individuals with MCI. To the best of our knowledge, this is the first study that determined the effect of a cognitive training program on both the behavioral and cellular levels in individuals with MCI. These findings would advance our understanding about the underlying mechanism responsible for cognitive training in individuals with MCI. Consequently, an effective interventional approach for individuals with MCI may be implemented at an early stage.

2.1.1 Research questions, purposes and hypotheses of the study

Research question

Can a cognitive training program focused on episodic memory, attention and executive function improve cognitive functions and induce changes of the cortical neurochemistry in individuals with MCI?

Purposes

1) To develop a cognitive training program for individuals with MCI that focuses on the core impairment aspects including episodic memory, attention and executive functions.
2) To evaluate the short-term effect of the specific cognitive training on cognitive functions in individuals with MCI.

3) To evaluate the short-term effect of the specific cognitive training on cortical neurochemistry in individuals with MCI.

Hypotheses

1) After completing the cognitive training course, participants with MCI in the experimental group would demonstrate significant improvement in cognitive functions (i.e. episodic memory, attention and executive functions) determined by the improvement of the score on cognitive tests (i.e. increasing Logical Memory score, Digit Span score, Block Design score and reducing time to complete Trail Making Test).

2) After completing the cognitive training course, MCI participants in the experimental group would demonstrate significant improvement in cortical neurochemistry biomarkers on both left and right sides of three brain regions (i.e. the hippocampus, PFC and ACC) determined by the changes of ratio value (i.e. increasing of NAA/Cr, NAA/Cho, Cho/Cr ratios and decreasing of mI/Cr ratio).

2.2 Literature Reviews

2.2.1 Mild Cognitive Impairment

Identifying the state that will predict the subsequent development of dementia could be useful for initiating early interventions. At present, many researchers in the field of ageing and dementia focus their studies on people who are at the transitional state between normal ageing and early dementia known as MCI (2, 35, 36). A longitudinal study has reported that the prevalence rate of MCI ranges from 3-53% among community-dwelling elders (37). The annual incidence rate is estimated at 1-1.5% with depressive symptoms, increasing age and low education reported as risk factors (38). The rate of conversion of MCI to dementia ranges from 10-15% per year (39, 40). The prevalence and incidence rate of MCI is varied due to the differences of MCI diagnostic criteria as well as the dissimilarity of assessment procedures.

Mild cognitive impairment can be divided into four broad subtypes depending on whether a single or multiple domain are impaired, and whether only memory or other cognitive domains are impaired (41). Single-domain amnestic MCI (aMCI) is characterized by subjective or/and objective memory impairment while multiple-domain aMCI is defined by memory deficit and additional complaints of difficulties in non-memory cognitive domains such as executive function and visuospatial function. Single-domain non-amnestic MCI (naMCI) is characterized by single non-memory cognitive domain deficit while multiple-domain naMCI is characterized by multiple cognitive domain impairments without a memory deficit. The most common form of MCI is the amnestic type which is estimated to have 3-5% prevalence among

persons aged 65 years or older (40, 42). Amnesic MCI seems to represent an early sign of AD whereas the outcomes of the naMCI subtype appear more heterogeneous including vascular dementia, frontotemporal dementia or dementia with Lewy bodies.

To date, the definition of MCI has been formulated by several research groups, however, core criteria that are widely used in both clinical and research practice are Petersen's criteria (2) including 1) presence of a subjective memory compliant from the patients and family, 2) presence of an objective memory impairment, 3) preserved general (global) cognitive function, as determined by a clinician's judgment based on performance on a standard cognitive test (i.e. Mini-Mental Status Examination MMSE)), structured interview with the patient, and an informant report, 4) absence of significant functional impairment, and 5) absence of clinical dementia, as determined by National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's disease and Related Association (NINCDS-ADRDA) criteria for AD, as judged by an experienced AD clinician. For individuals who have a normal MMSE score but their cognitive status is questionable, Montreal Cognitive Assessment (MoCA) is often used to assist in describing and quantifying the cognitive deficit. MoCA was originally designed to assist in the diagnosis of MCI by helping to demonstrate objective cognitive loss (43). Individuals with MCI often demonstrate low MoCA scores (< 26 points, from a total of 30 points) with normal MMSE score (≥ 24 points, from a total of 30 points).

Oxidative stress plays an important role in the pathogenesis of MCI. Recent studies revealed that individuals with MCI have increased brain oxidative damage to vulnerable cerebral tissues before the onset of symptomatic dementia (44-46).

Normally, the brain tissue is particularly susceptible to free radical attack because 1) it contains large amounts of polyunsaturated fatty acid and transitions metal, 2) it has a high metabolic rate and 3) it has poor antioxidant defenses (47-50). Under normal circumstance, the brain is protected from such damage by a careful balance between free radicals and antioxidant mechanisms which include antioxidant enzyme and free radical scavenging chemicals. However, in individuals with cognitive impairment, this balance appears to be disturbed by decreasing in the activity of antioxidant enzyme (e.g. Superoxide dismutase, Glutathione peroxidase and Catalase) together with an increasing of free radicals caused oxidative damage to all the major macro-molecules in cells including lipids, essential protein and Deoxyribonucleic acid (DNA) (47, 51). Furthermore, the presence of an apolipoprotein E 4 allele and a small hippocampus volume is proposed to be the potential factors for accelerated the rate of conversion from MCI to AD (52, 53).

2.2.2 Cognitive training in individuals with MCI

Over the past ten years, only few studies investigated the non-pharmacological treatment effects of cognitive training technique among individuals with MCI. One such technique is the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) that focused on memory, reasoning, and speed of processing trainings (8, 54). This technique comprises 2 stages including primary training stage (10 sessions over 5-6 weeks) and booster training stage (4 sessions over 3 weeks). The booster training was performed 11 months after the end of the primary training. The results demonstrated that older adults with objectively-defined memory impairment showed

improvement on reasoning and speed of information processing performance after completing the ACTIVE program. However, a major constraint of ACTIVE technique is the feasibility in clinical set-up due to its long term training over 2 years. Another technique, computer assisted cognitive training program, was aimed at stimulating specific cognitive functions (e.g. attention, memory and speed of processing) (55, 56). Ott-Chervet et al (56) demonstrated that older adults with memory complaint improved their performance in attention, vigilance, and memory after completing 3-week computer-assisted cognitive training program. In addition, Gunther et al (55) examined the short-term and long-term effect of the computer-assisted cognitive training program in older adults with age-associated memory impairment. The results showed that after completing 14-week training course, older people showed improvement on working memory and speed of processing. Moreover, the improvement on these cognitive functions was maintained over 5-month follow-up. However, the major limitation of this technique is that it requires user's computer skill and high cost technical equipment.

Recently, a number of studies have emphasized the multifaceted rehabilitation program on promoting cognitive function in individuals with cognitive impairment (10-12). Results showed the beneficial effects of multifaceted intervention on cognitive and functional performance as well as transfer effects to some practical situations. To date, this technique was proposed to be a competent method due to its efficient in ameliorate the severity of MCI symptoms. However, a major concern about this technique is that a multifaceted intervention contains of several components as cognitive, motor and psychosocial aspects which resulted in certain limitations. Firstly, in

order to cover all training aspects, it takes quite a long duration for training. Secondly, since the program only gives a general scope of training, the training protocols and their emphasis were different across studies, making it difficult to replicate. Thirdly, the program does not focus on the patients' specific cognitive impairment. Lastly and importantly, it is not known how each training component contributes to the patients' cognitive improvement. To overcome these limitations, we were interested to develop a specific cognitive training program for individuals with MCI by focusing solely on cognitive component. In this study, cognitive component was highlighted on the crucial cognitive functioning which includes episodic memory, attention and executive function. Based on clinical evidences, these cognitive aspects are part of the core cognitive impairments in those individuals with MCI converting to AD and most likely to deteriorate early in the course of MCI (24, 25).

2.2.3 Evidence from Magnetic Resonance Spectroscopy in individuals with MCI

Normal ageing process has been associated with some degree of neuron and volume loss, accompanied by increasing adjacent glial cells (57). Recent studies have suggested that these changes are quantitatively less than previously thought and that they seem to be attributable to reductions of neuronal cell size rather than neuronal cell number (58). Furthermore, it is thought that the cognitive impairment associated with normal ageing is due to neuronal dysfunction rather than to loss of neurons or synapses. The decline in levels of synaptic proteins involved in structural plasticity of axons and dendrites has suggested that disturbed mechanisms of plasticity may contribute to

cognitive dysfunction during ageing (59). Although the histological evidence cannot be observed directly by non-invasive technique, neuroimaging technique including Magnetic Resonance Imaging (MRI) can be used as a quantitative analysis of cerebral structures in pathologic cognitive ageing. Evidences from MRI studies have indicated a progressive reduction in cerebral hemispheres and an increase in ventricular volumes as well as presence of characteristic white matter hyperintensities in the older adults who proposed to be at greater risk for developing cognitive impairment (60, 61). Specifically, a significant changes is found in the hippocampal and entorhinal regions that are affected early in the course of MCI disease (62, 63). Therefore, the changes in brain structures may lead to cognitive decline in individuals with MCI.

Although these cortical structural changes that obtain from MRI technique are often correlated with cognitive impairment, however, it is still unclear whether neuronal function declines with ageing. To answer this question Magnetic Resonance Spectroscopy (MRS) is used to assess molecular structure compounds or to detect the compounds' presence of the human brain (64). MRS allows in vivo, a non-invasive study to impinge a sample by emitting electromagnetic energy and that sample, either absorbs or emits energy, can be detected. Through the distribution and intensities of the measured energy, called a spectrum, one can obtain information about the physical and chemical properties of the sample. Clinical applications of MRS are presently evolving from pioneering experimental protocols for detecting and monitoring metabolic failures. In principal, MRS of the brain can be mainly determined from: 1) N-acetylaspartate (NAA), 2) Myoinositol (mI), 3) Choline (Cho), and 4) Creatine (Cr) (64-67). Analyses of these

cortical neurochemistry biomarkers can arise through absolute value (e.g. NAA concentration, Cr concentration and Cho concentration) and ratio value (e.g. NAA/Cr, NAA/Cho, mI/Cr, and Cho/Cr ratios). The ratio is a relationship between two absolute values of neurochemical compound. In adult normal brain, Cr concentration is considered to be relatively stable, thus Cr is usually used as the reference value to calculate other ratios (68). This present study used the ratio of NAA/Cr, Cho/Cr and mI/Cr for evaluation because it could be measured easily and quickly, and the Cr level is stable and shows constant signal intensity in the brain.

Commonly, NAA is a recognized marker for neuronal health and viability. The reduction of NAA has been observed in many white matter diseases, especially multiple sclerosis, temporal lobe epilepsy, and mitochondrial disease (69, 70). mI is a recognized precursor of demyelination and gliosis. To date, the exact pathophysiology resulting from significant alternation in mI is uncertain; however, evidence shows that the elevation of mI level can reflect not only an expansion in glial cell population (glial cell proliferation or enlargement) but also the occurrences of inflammatory process (70). mI can be elevated in some pathologic conditions such as gliosis, astrocytosis, AD and demyelinating disease (71). Cho is the compound containing in phosphorylcholine and glycerophosrylcholine. Cho is considered as a precursor and breakdown products of membrane phospholipids. The pathological process that leads to Cho elevation includes malignant transformation of tumors and active demyelination (72, 73). Finally, Cr is an indicative of oxidative metabolism that can reflect energetic metabolism of neuron and glia cells. Systemic disease (e.g. liver disease and renal disease) can lead to Cr

concentration reduction (74). In addition, physical fitness, medications and obesity were found to be associated with neurochemical level. Erickson et al (75) showed that high fitness older people (determined by VO_2 peak) had greater NAA concentration compared to older people with low fitness. As for medications, previous studies demonstrated that antipsychotic drugs appeared to promote NAA concentration as well as increase NAA/Cr and Cho/Cr ratios in the dorsolateral prefrontal cortex (DLPFC) in individuals with schizophrenia (76, 77). Several studies were interested to investigate the effects of neuroprotective drugs on neurochemistry in people with bipolar disorders. The results demonstrated that treated patients had significantly greater NAA concentration in the PFC than untreated patients (78-80). Furthermore, study by Gonzales et al (81) revealed that high Body Mass Index (BMI) was associated with elevations in mI/Cr ratio in occipitoparietal gray matter that reflected poor osmotic regulation and microglia inflammation.

Recent MRS studies in individuals with MCI have found the cortical metabolite abnormalities in some brain areas including medial temporal lobe, hippocampus (which is located in the medial temporal lobe) and posterior cingulate gyri. Kantarci et al (28) revealed that at posterior cingulate gyri Cho/Cr ratio was higher and the NAA concentration was lower for MCI persons than those ratios in cognitively normal subjects. Furthermore, Schuff et al (30) showed that MCI patients had less NAA concentration in the medial temporal lobe compared to intact cognitively elders and NAA losses in this region were larger in MCI subjects who converted to dementia than in MCI subjects who remained stable. Interestingly, decreased NAA in medial temporal lobe

correlated stronger with increasing memory deficits on the delayed recall test than hippocampal volume. In contrast to NAA concentration, differences of hippocampal volume (determined by MRI) between MCI and control subjects were not significant. Researchers concluded that NAA measurement was a better predictor for memory deficits than volume measurement (30).

Beside the medial temporal lobe, hippocampus and posterior cingulate cortex, a recent study using Positron Emission Tomography (PET) revealed that individuals with MCI also showed a progressive metabolic decreased in the PFC and ACC (82). In this present study, we chose to examine the changes of neurochemistry in three brain areas (i.e. hippocampus, PFC and ACC) because: 1) evidence of impairment in these areas in individuals with MCI and 2) these areas are responsible for the cognitive function (i.e. episodic memory, attention, and execution) that were trained. Therefore, these areas could be considered as a target area in monitoring the neurochemistry changes associated with training.

At present, only few studies examined the effect of cognitive training on neurochemistry metabolism. Lovden et al (83) demonstrated that performing a spatial navigation task in a virtual environment could enhance hippocampal NAA concentration in young adults. Moreover, according to a study carried out by Valenzuela et al (31), Method of loci (MOL) memory program appeared to enhance hippocampal neurometabolites in healthy elders (determined by increasing Cho and Cr concentration).

2.3 Methods

The study consists of two parts which included development of specific cognitive training program in individuals with MCI and evaluation of the short-term effects of the developed cognitive training program.

2.3.1 Part I. Development of the cognitive training program

The cognitive training program was developed by integrating the knowledge on MCI and the literature review of previous cognitive training program in individuals with MCI. The developed cognitive training program underwent a critical appraisal from experts in the view of clinical as well as theoretical standpoints. Lastly, the cognitive training program was tried out on two MCI volunteers. Comments from the users were then used to refine the final cognitive training program.

2.3.2 Part II. Evaluation of the short-term effect of the cognitive training program

2.3.2.1 Participants

Ten individuals with MCI aged 50 years or older participated in the study. All of them were recruited from the Outpatients Department at Suanprung and Maharaj Nakorn Chiang Mai Hospitals, Chiang Mai, Thailand. The diagnosis of MCI was performed by an experienced neurologist using Petersen's criteria as follows (2):

1) A self-reported memory complaint, corroborated by an informant interview
2) A score on a standardized memory test rated as 0.5 on Clinical Dementia Rating (CDR)

3) Normal general cognitive function, as determined by a clinician's judgment based on a structured interview with the patient and an informant report and adjusted Mini-Mental State Examination (MMSE) score greater than 23. The MMSE score is adjusted based on age (+1 for age ≥ 80 years) and years education (+1 for years education < 9) (84, 85).
4) No or minimal impairment in activities of daily living (ADLs) or instrumental Activity of Daily Living (IADL), as determined by clinical review with the patients and informant interview
5) Not sufficiently impaired, cognitively and functionally, to meet National Institute of Neurological and Communicative Disorders and Stroke/ Alzheimer's disease and Related Association (NINCDS-ADRDA) criteria for AD, as judged by an experienced AD clinician

Inclusion criteria

The participant was included in this study if he/she:

1) Presence of cognitive impairment determined by the score on the Montreal Cognitive Assessment (MoCA) lesser than 26 (43)
2) Stable general health with no significant cerebral vascular disease (86), no infection, infarction or focal lesions as revealed by a computed tomographic (CT) or MRI brain scan within 12 months before screening

3) Able to comprehend instructions and willing to participate

Exclusion criteria

1) Presence of neurological conditions (e.g. Parkinson's disease, Stroke) or/and acute or/and chronic disease that could not be controlled (e.g. Asthma, Hypertension, Diabetes mellitus, Coronary artery disease)
2) Presence of depressive symptoms defined as a score > 12 on the Geriatric Depression Scale (GDS) (87)
3) Presence of surgery history which using metal implantation (e.g. cardiac pacemaker implantation)
4) Uncorrected visual and hearing impairment
5) Presence of color blindness
6) Unwillingness to undergo MRS
7) Participation in other cognitive training programs

2.3.2.2 Materials

1) Personal data collection form (Appendix A)
2) Standard neuropsychological tests (i.e. Logical Memory-Delayed Recall, Logical Memory-Recognition, Digit Span forward/backward, Trail Making Test A and B and Block Design) (Appendix B)
3) Cognitive training kits (Appendix C)
   - Episodic memory training:

-Task 1: a scheme of participant's house and its associated with word lists

-Task 2: an audio recorder of a short story

- Attention:

-Task 1: pictures and stop watch

-Task 2: an audio recorder of songs

-Task 3: pictures and stop watch

- Executive function:

-Task 1: a list of simulated executive function tasks and its related equipment

-Task 2: cards and stop watch

4) MRI/MRS Equipment (Philips Achieva 1.5 Tesla) with Proton Spectroscopy Head coil; Quadrature transmit/receive coil for comprehensive neurologic examination

2.3.2.3 Independent and dependent variables

Independent variables were:

1) Group: Control group and Experimental group

2) Testing Condition: Pre-training and Post-training

Dependent variables were:

1) Cognitive functions:

- Episodic memory (Logical Memory-Delayed Recall and Logical Memory-Recognition)

- Attention (Digit Span forward/backward)
- Executive function (Block Design and Trail Making Part B-A)

2) Neurochemistry biomarkers:

- Metabolite ratios

  -NAA/Cr ratio

  -NAA/Cho ratio

  -Cho/Cr ratio

  -mI/Cr ratio

2.3.2.4 Procedures

The study protocol was submitted for approval by the Human Ethical Review Board of the Faculty of Associated Medical Sciences, Chiang Mai University (Appendix D). Participants who were eligible to participate in the study based on the inclusion and exclusion criteria were informed about the purposes and procedure of the study (Appendix E) before signing an informed consent (Appendix F). Demographic data of the participants which consisted of medical conditions and medication usage were recorded. Participants matched by age, gender, and education were randomized within pairs to either the control or the experimental group. Randomization was performed by opening an opaque sealed envelope that contained a card indicating code for the control ("C") or the experimental ("E") group. Participants in the experimental group (n=5) received cognitive training while those in the control group (n=5) did not receive the cognitive training. Participants in the experimental group took part in an 18-session cognitive training program for about 45-60 minute per session, 3

days per week for 6 consecutive weeks. The order of three cognitive trainings (i.e. memory, attention, and executive functions) was randomized across sessions. The time for each cognitive training task was about 15 minutes with 5 minutes rest. The researcher gave training to one participant at a time. The training schedule for each participant was present; however, the participants were allowed to re-schedule if they were unavailable on that day. After each training session, the researcher recorded participant's performance in the log book. The difficulty of the training was progressed once a week based on individuals performance. The more complex cognitive tasks were assigned when the participants could perform prior tasks correctly 100%.

2.3.2.5 Assessment

All participants were assessed for their cognitive functions and neurochemistry biomarkers twice; before starting and after completing the training program. The pre-intervention assessment was conducted 1-3 days prior to training to use as a baseline data, while the post-intervention assessment was conducted 1-3 days after completion of the 6-week period. Cognitive functions were administered by a trained researcher. There were 5 standard neuropsychological tests (i.e. Logical Memory-Delayed Recall, Logical Memory-Recognition, Digit Span forward–backward, Trail Making Tests B-A and Block Design). The cognitive testing took about 1 hour. The neurochemistry biomarkers were assessed by an experienced radiologist. The duration of assessment was about 45-60 minutes. Both assessors were blinded to participant's group assignment.

Cognitive functions

The tests of cognitive functions included in the present study were the tests of memory, attention, and executive functions. With respect to the specific cognitive domains, episodic memory was examined using the Logical Memory-Delayed Recall and Logical Memory-Recognition subtests (88). During the test, the participants were instructed to listen carefully to two stories and remember the content of the stories. After a 30-min delay, they would be asked to repeat each story as close to the original story as possible (Delayed Recall) and provide "Yes" or "No" answer in response to a series of 30 questions (Recognition). Attention was assessed using Digit Span forward–backward test (89). In this test, a series of lists of numbers was presented verbally to the participants. The participants were asked to immediately repeat the numbers verbally either in the forward numerical order or the backward numerical order. Executive function was examined with Trail Making Tests B–A and Block Design (90, 91). To administer the Trail Making Test B, the participants were asked to draw a line to connect consecutive numbers in numerical order and letters in alphabetical order in an alternating sequence as quickly as possible. To administer Trail Making Test A, the participants were instructed to draw a line to connect consecutive numbers in numerical order as quickly as possible. Trail Making Tests B–A were measured as the difference between time taken to complete Parts B and A. To administer the Block Design test, participants were required to arrange blocks (on some sides they are all white; some are all red; and some are half red and half white) according to a presented model or picture as quickly and correctly as possible. The number of block increases from 4 to 9 blocks.

Neurochemistry biomarkers

The neurochemistry biomarkers were obtained by using 1HMRS in three different brain regions including the hippocampus, the PFC, and the ACC. These three regions on both left and right sides of the brain were investigated because previous studies revealed that they are responsible for episodic memory, attention, and executive functions (32). The 1H MRS was performed by using the automated single-voxel of 3.4 cm^3 (1.5 × 1.5 × 1.5 cm) with 1.5 T unit (Philips Achieva) equipped with head coils. T1-weighted images in axial, sagittal, and coronal planes were obtained for localizing the 1H MRS voxels. The point resolved spectroscopy (PRESS) pulse sequence with repetition time (TR) of 2,000 ms was used for the examination. An echo time (TE) of 31 ms was chosen to quantify metabolites with short transverse relaxation time.

2.3.2.6 Data Analyses

SPSS for Window version 11.5 was used for data analysis. Mann-Whitney U Test was used to compare the demographic data between the control and experimental groups. To identify the effects of the cognitive training program, the Wilcoxon Signed Ranks Test was conducted to compare the outcome measures between pre-training and post-training within each group. A significant level of 0.05 was set for all analyses.

2.3.2.7 Data collection location

The study was conducted at the Faculty of Associated Medical Sciences, Chiang Mai University.

2.4 Results

2.4.1 Development of the cognitive training program

The training program was developed by reviewing the literatures of previous cognitive training programs in people with MCI (8, 9, 12, 31). The cognitive training program emphasized on the core impairment aspects of individuals with MCI including episodic memory, attention and executive functions (24, 25). The developed cognitive training program underwent a critical appraisal from a cognitive neuroscientist, a geriatric psychiatrist, and a clinical psychologist. Lastly, the comments from two MCI volunteers were used to refine the final cognitive training program. The component and the content of each cognitive task are summarized in Table 1.

Table 1 Summary of the cognitive tasks included in the cognitive training program

Cognitive Task	Training Purpose	Testing Description
Episodic memory training:		
Task 1: MOL technique (12, 31)	To enhance the episodic memory ability by self-generated mental association technique	Practice to remember words by forming the association between the words and participant's familiar objects (in the room) via MOL technique
Task 2: Auditory memory training (9)	To facilitate the memory of event through auditory sense	Listen to a short story and remember the content of this story as much as possible. After a 30-minute delay, the story-related questions will be tested

Table 1 (continue)

Cognitive Task	Training Purpose	Testing Description
Attention:		
Task 1: Spotting the differences between two similar pictures (9)	To enhance visual attention performance by facilitating visual targeting perception	Identify the different points between two pictures as much as possible
Task 2: Detecting specific words in a song (9)	To enhance the ability of selective attention and speed of processing via auditory sense	Listen to the song and concurrently count the number of target words in the song
Task 3: Detecting the target letters (9)	To facilitate the ability of selective attention and speed of processing via visual sense by using perceptual task that involves an active scan of particular object (target) among other objects (distracter)	Search the target letters among distracter letters, for example search for a gray letter among black letter as quickly as possible

Table 1 (continue)

Cognitive Task	Training Purpose	Testing Description
Executive Function:		
Task 1: Simulated executive function tasks from activity of daily living (9)	To facilitate executive function skill including planning, organizing and problem solving which are closely related to activities of daily living	Practice complex tasks in activity of daily living such as simulating to make a food (create the menu, make a list of the ingredient, calculate the total cost of the purchase, check the change and tell the process to make a food)
Task 2: Categorization training (8, 9, 12)	To encourage abstract reasoning skills in categorizing the objects	Categorize the objects based on their function

2.4.2 Evaluation of the short-term effect of the cognitive training program

A total of ten individuals with MCI participated in this study. The demographic characteristics of the participants in the two groups are presented in Table 2. The Mann-Whitney U test was conducted to identify any demographic characteristic differences between the two groups. The result showed no significant differences between the two groups in all demographic variables.

Table 2 Demographic characteristics of participants in the control and experimental groups

Variables♦	**Control group (n = 5)**	**Experimental group (n = 5)**	***p*-value**
Age (yrs)	77.60 ± 6.07	78.40 ± 4.98	0.69
Male: Female	2 : 3	2 : 3	-
Educational level (yrs)	9.00 ± 5.20	12.40 ± 2.97	0.29
MMSE (score)	25.80 ± 2.18	25.50 ± 2.38	0.65
MoCA (score)	19.80 ± 3.70	19.80 ± 2.39	0.69
GDS (score)	3.40 ± 2.19	2.40 ± 1.34	0.42
Drugs (types)	4.00 ± 1.70	3.00 ± 1.30	0.24

♦Values are shown in mean ± standard deviation (SD); MMSE, total score = 30 points; MoCA, total score = 30 points; GDS, total score = 30 points

Evidence from cognitive function tests

Mann-Whitney U test revealed no significant differences between the two groups for all outcome measures at baseline ($p > 0.05$), indicating similar cognitive level between the two groups. The effect of the cognitive training program is demonstrated in Table 3. The Wilcoxon Signed Ranks Test revealed that after 6-week cognitive training, the participants in the experimental group demonstrated significant improvement in their performance on the Logical Memory-Recognition, Digit Span, and Trail Making Test ($p < 0.05$), while their performance on the Logical Memory-Delayed Recall improved to approach significance ($p = 0.06$). In contrast, the performance on all cognitive tests of the control group was similar between baseline and after 6 weeks ($p > 0.05$).

Paired-sample t-test showed significant change score from pre- to post-training for the Logical Memory-Recognition between the two groups. Specifically, the change score was significantly greater for the experimental group than those for the control group. Table 4 displays the changes of cognitive skills at post-training over the baseline between the two groups.

Table 3 Comparisons of cognitive skills outcome measures between pre-training and post-training for each group

Outcome measures♦	**Control group (n = 5)**		***p*-value**	**Experimental group (n = 5)**		***p*-value**
	Pre-training	**Post-training**		**Pre-training**	**Post-training**	
LM I (score)	10.40 ± 4.16	10.60 ± 4.87	1.00	8.08 ± 4.81	16.80 ± 6.82	0.06
LM II (score)	19.80 ± 0.37	20.80 ± 1.07	0.28	12.40 ± 3.67	22.80 ± 1.28	0.04*
DS (score)	13.80 ± 1.46	15.80 ± 1.91	0.22	12.00 ± 1.14	14.20 ± 1.16	0.03*
TMT (minute)	5.95 ± 1.65	4.69 ± 0.70	0.68	3.51 ± 0.94	2.12 ± 0.47	0.04*
BD (score)	9.60 ± 1.83	11.40 ± 1.81	0.35	13.60 ± 3.06	17.00 ± 0.89	0.33

♦ Values are shown in mean ± standard error of measurement (SEM). LM I, Logical Memory-Delayed Recall (total score = 75 points); LM II, Logical Memory-Recognition (total score = 30 points); DS, Digit Span (total score = 28 points); TMT, Trail Making Test, subtracting part B from part A (minute); BD, Block Design (total score = 51 points)

* Wilcoxon Signed Rank Test revealed significant difference at $p \leq 0.05$

Table 4 Comparison of changed scores (post-training – baseline) of cognitive skills outcome measures between the two groups

Outcome measures♦	**Change value†**		***p*-value**
	Control group (n = 5)	**Experimental group (n = 5)**	
LM I (score)	0.20 ± 4.49	8.0 ± 11.20	0.19
LM II (score)	1.00 ± 2.45	10.40 ± 6.43	0.02*
DS (score)	2.00 ± 3.32	2.20 ± 0.45	0.90
TMT (minute)	-1.26 ± 2.69	-1.39 ± 1.22	0.93
BD (score)	1.80 ± 4.15	3.40 ± 6.84	0.67

♦ Values are shown in mean ± standard error of measurement (SEM). LM I, Logical Memory-Delayed Recall (total score = 75 points); LM II, Logical Memory-Recognition (total score = 30 points); DS, Digit Span (total score = 28 points); TMT, Trail Making Test, subtracting part B from part A (minute); BD, Block Design (total score = 51 points)

* Paired-sample t-test revealed significant difference at $p \leq 0.05$

† Change of cognitive skills outcome measures calculated by subtracting post-training score from pre-training score

Evidence from neurochemistry biomarkers

Spectrum of single-voxel spectroscopy showed integral values of four major peaks including NAA at 2.02 parts per million (ppm), Creatine-phosphocreatine at 3.03 ppm, Choline-containing compounds at 3.22 ppm, and Myoinositol at 3.6 ppm. The ratios of NAA/Cr, NAA/Cho, Cho/Cr and mI/Cr were calculated for both sides of three brain regions (i.e. hippocampus, PFC and ACC). There was no significant difference between the two groups for all outcome measures at baseline ($p > 0.05$), except for the Cho/Cr in the left side ($p = 0.01$) and NAA/Cho in the right side ($p = 0.03$) of ACC as well as mI/Cho in left side of PFC ($p = 0.02$).

Comparisons of cortical neurochemistry biomarkers at pre-training (baseline) and post-training for each group are illustrated in Table 5 and 6. As for the control group, statistical analysis revealed no significant differences of all variables across the three brain areas between baseline and at 6-weeks after baseline ($p > 0.05$), except for the mI/Cr in the left PFC which decreased significantly at 6 weeks. For the experimental group, the ratio of mI/Cr in both sides of all three brain regions were statistically decreased after 6-week cognitive training ($p = 0.04$), while the ratio of other variables did not reach significant.

Table 5 Comparisons of cortical neurochemistry biomarkers at pre-training (baseline) and post-training for the control group

Outcome measures♦	Control group (n = 5)					
	Left		*p*-value	Right		*p*-value
	Pre-training	Post-training		Pre-training	Post-training	
H						
NAA/Cr	1.46 ± 0.30	1.51 ± 0.16	0.89	1.56 ± 0.28	1.50 ± 0.34	0.69
NAA/Cho	1.35 ± 0.41	1.68 ± 0.64	0.50	1.37 ± 0.24	1.40 ± 0.44	0.89
Cho/Cr	1.11 ± 0.11	0.95 ± 0.21	0.22	1.14 ± 0.17	1.10 ± 0.13	0.42
mI/Cr	0.89 ± 0.14	0.88 ± 0.12	0.89	0.67 ± 0.11	0.82 ± 0.11	0.08
PFC						
NAA/Cr	1.51 ± 0.03	1.50 ± 0.10	1.00	1.57 ± 0.18	1.62 ± 0.05	0.29
NAA/Cho	1.80 ± 0.37	1.74 ± 0.38	0.69	1.77 ± 0.40	1.68 ± 0.58	0.72
Cho/Cr	0.84 ± 0.20	0.88 ± 0.18	0.23	0.80 ± 0.11	0.84 ± 0.15	0.72
mI/Cr	0.84 ± 0.05	0.66 ± 0.22	0.04*	0.79 ± 0.14	0.72 ± 0.08	0.69
ACC						
NAA/Cr	1.56 ± 0.13	1.52 ± 0.16	0.50	1.47 ± 0.20	1.59 ± 0.04	0.11
NAA/Cho	1.48 ± 0.20	1.43 ± 0.25	0.35	1.44 ± 0.15	1.53 ± 0.52	0.69
Cho/Cr	1.07 ± 0.17	1.08 ± 0.19	0.36	1.12 ± 0.23	1.10 ± 0.10	0.89
mI/Cr	1.05 ± 0.09	0.97 ± 0.03	0.14	1.02 ± 0.13	0.95 ± 0.10	0.35

♦ Values are shown in mean ± SD. H, Hippocampus; PFC, Prefrontal Cortex; ACC, Anterior Cingulate Cortex; NAA, Nacetylaspartate; mI, Myoinositol; Cho, Choline: Cr, Creatine. * Wilcoxon Signed Rank Test revealed significant difference at $p \leq 0.05$

Table 6 Comparisons of cortical neurochemistry biomarkers at pre-training (baseline) and post-training for the experimental group

Outcome measures♦	Experimental group (n = 5)					
	Left		*p*-value	Right		*p*-value
	Pre-training	Post-training		Pre-training	Post-training	
H						
NAA/Cr	1.23 ± 0.21	1.45 ± 0.15	0.11	1.52 ± 0.11	1.51 ± 0.16	0.59
NAA/Cho	1.21 ± 0.17	1.52 ± 0.61	0.35	1.21 ± 0.43	1.68 ± 0.32	0.08
Cho/Cr	1.20 ± 0.48	1.10 ± 0.16	0.50	1.03 ± 0.17	0.92 ± 0.09	0.29
mI/Cr	0.86 ± 0.12	0.69 ± 0.06	0.04*	0.81 ± 0.12	0.67 ± 0.06	0.04*
PFC						
NAA/Cr	1.48 ± 0.24	1.58 ± 0.35	0.89	1.44 ± 0.20	1.44 ± 0.17	1.00
NAA/Cho	2.04 ± 0.13	1.90 ± 0.34	0.50	2.02 ± 0.14	1.80 ± 0.31	0.08
Cho/Cr	0.67 ± 0.12	0.79 ± 0.06	0.07	0.72 ± 0.06	0.80 ± 0.06	0.14
mI/Cr	0.98 ± 0.09	0.61 ± 0.11	0.04*	0.98 ± 0.18	0.66 ± 0.06	0.04*
ACC						
NAA/Cr	1.37 ± 0.19	1.50 ± 0.09	0.47	1.53 ± 0.14	1.55 ± 0.06	0.79
NAA/Cho	1.80 ± 0.37	1.85 ± 0.32	0.69	1.84 ± 0.29	1.82 ± 0.36	1.00
Cho/Cr	0.78 ± 0.11	0.81 ± 0.11	0.50	0.88 ± 0.10	0.85 ± 0.14	0.47
mI/Cr	1.12 ± 0.06	0.89 ± 0.08	0.04*	1.07 ± 0.08	0.72 ± 0.07	0.04*

♦ Values are shown in mean ± SD. H, Hippocampus; PFC, Prefrontal Cortex; ACC, Anterior Cingulate Cortex; NAA, Nacetylaspartate; mI, Myoinositol; Cho, Choline: Cr, Creatine. * Wilcoxon Signed Rank Test revealed significant difference at $p \leq 0.05$

2.5 Discussion

This present study aimed to develop and determine the effects of a cognitive training program that focused specifically on the core impairment aspects of individuals with MCI which are memory, attention and executive functions. The short-term effect of the developed program was determined both at the behavioral level (as evaluated by cognitive skills) and the cellular level (as evaluated by cortical neurochemistry biomarkers). Five participants with MCI in the experimental group took part in the 18-sessions cognitive training over a 6-week period while 5 age- and gender- matched individuals with MCI served as controls. After the 6-week program, significant improvement of cognition was demonstrated in the experimental but not the control group. Our findings are consistent with previous studies that demonstrated the effects of cognitive training in improving cognitive performance of individuals with MCI (8, 9, 12, 31).

With respect to the episodic memory, the experimental group demonstrated significant changes on the Logical Memory-Recognition part after completing the cognitive training program. In this study, episodic memory training was consisted of 2 subtasks including MOL technique and auditory memory training. The MOL technique is the strategy employed to memorize novel things by forming the linkage between familiar environment and the things requiring memorization via self-generated imaginary association (12, 31). The auditory memory training was focused to enhance participant's recall and recognition memory via auditory sense, the common way that people gain information in activities of daily living. In auditory memory task, self-generation of

imagery, one aspect of MOL technique was applied to facilitate the memory of the story. Therefore, the improvement of the Logical Memory-Recognition may be due to the effect of the episodic memory training that enhanced several cognitive functions including the generation of imaginary, linguistic association, working memory, and mental map retrieval as well as long-term memory. Moreover, previous study reported that repetitive practice is an effective strategy in assisting persons with cognitive impairment to gain new information (92). The improvement of the Logical Memory-Recognition may be due to the benefit of task-repetition effect which covers recognized the stories (auditory memory training). In this study, the performance on recall memory test although showed large change between pre-training and post-training but only reached a marginally significant level ($p = 0.06$). The result was not unexpected since a free recall task is typically harder than recognition task due to the low availability of cue information (93). These findings were consistent with previous studies that found the participants with MCI did not improve on the delayed recall text memory after completing an 8-week multifaceted rehabilitation program (94).

As for the attention domain, the experimental group showed significant differences on the digit span forward-backward test between pre-training and post-training. Digit Span is widely used for measuring attention ability and immediate verbal recall (89). The improvement of attention performance may be explained by the training effect of attention training together with the effect of MOL training. The training of attention consisted of 3 subtasks including spotting the differences between two similar pictures, detecting the words in a song, and visual search task. The coexistence of

training purpose was to facilitate the ability to focus on a single stimulus while ignoring irrelevant stimuli as well as enhance the speed of processing ability via the visual and auditory senses. In addition, the MOL technique (subtask of memory training) may promote the working memory ability for temporarily storing and managing the information. Our findings not only showed the training effect but also provided some linkage between memory and attention ability in promoting information-processing functions. This notion is in line with the suggestion from previous studies that successful retrieving data required the attentional-demanding to encode and construct the information (95).

With regard to the executive function, the experimental group demonstrated significant improvement on the Trail Making Test (B-A) but not the Block Design test after completing the cognitive training program. Trail Making Test (B-A) performance is commonly used to assess executive function by accounting for visuospatial function ability (96, 97). Executive function refers to a diversity of higher cognitive processes that use and modify information from many cortical sensory systems to plan, monitor and execute a sequence of goal-directed complex actions (98). In the present study, simulated executive function tasks provided opportunities for individuals to actively participate in initiation, planning, and problem solving. Recent evidence has revealed that active involvement in tasks can promote the learning process by enhancing the ability to execute goal-directed complex actions (99). In this study, the results showed no significant changes on Block Design performance while there was significant improvement on the Trail Making Test (B-A). Although both tests have been widely used to assess executive

function skill (90, 91), these 2 tests are different in some aspects. Trail Making Test B-A mainly requires visual search and scanning, sequencing, attentional shifting and speed of processing (84, 100). Block Design test appears to require more complex skills than Trail Making Test due to it needs an adequate visual matching, 3-dimensional perception and construction abilities as well as requires mental flexibility in adjusting an ongoing action (101, 102).

Localized proton MRS was used to measure the biochemistry in both sides of the hippocampus, PFC, and ACC before and after the 6-week cognitive training. The Cr level is relatively constant in adult brain. Therefore, a decrease of mI/Cr ratio indicates that the mI value decreased. It is known that mI is a recognized marker for gliosis (103). Thus, a decrease of mI level after training in the experimental group may be due to the training effect in delaying the process of neuroglial cell damage in individuals with MCI. The NAA level, a marker for neuronal health and viability, however, did not significantly increase after training. One explanation is that NAA/Cr and NAA/Cho values had larger variation as compared to mI/Cr value so it was difficult to detect the changes of NAA level after training. Another explanation is that the changes of neuroglial cells may precede the neuronal damage during the course of AD pathology (104); therefore, the changes of mI level may be detected earlier than the changes of NAA level. Decreased mI level in the present study was accompanied by improvement in cognitive performance (i.e. memory, attention, and executive function). Thus, the mI level appears to be a promising candidate as one biomarker for monitoring of MCI improvement after cognitive training.

2.5.1 Clinical implications and limitations

To the best of our knowledge, this is the first study that determined the effect of a cognitive training program on both the behavioral and cellular changes in individuals with MCI. The cognitive training program that focuses on the core impairment aspects including episodic memory, attention and executive functions may help restore, or at least maintain the cognitive ability of individuals with MCI. Therefore, cognitive training program may be considered as an alternative option of non-pharmacological treatment to restore or slow down the cognitive decline in individuals with MCI.

While the present study demonstrates the beneficial effect of a cognitive training program to enhance cognitive performance of individuals with MCI, there are certain limitations. With respect to participant considerations, the small sample size in the present study may have weakened statistical power in detecting the changes of outcome measures. Future studies with larger sample sizes would enhance the power analysis and external validity of the study. In addition, homogeneity with respect to the participant education level as well as their cognitive level at baseline would ensure that the results were due to the cognitive training program. In this study, the magnitude of the standard deviations reveals that both the experimental and control groups have wide variability on the cognitive measures making detection of differences more difficult. Another limitation is concerning the lack of long term follow-up. Given that the post-training assessment was done immediately after training, it is unknown whether the improvement shown in

the experimental group can be sustained afterwards. Long term follow-up is needed to confirm our findings and to establish the long term effects of the cognitive training on both a behavioral- and a biochemical level. Training adherence is another important issue for training efficacy. In the present study, all participants were highly motivated to participate in the training program. Thus, the adherence rate was 100%. It is likely that the adherence to training rate will be lower when implementing the training program to people with MCI in community. It is also possible that cognitive improvement observed in the experimental group were influenced by the opportunity to engage in social interactions with the trainer. To ensure that the results were due to the cognitive training program, future study should provide the same kind of social interactions (without cognitive training) to the control group. Moreover, it is not known whether the improvement on cognitive outcome measures in this study could be generalized to daily life function. Therefore, it is important for future research to examine how improvement on theses psychological tests is related to functional changes.

2.5.2 Conclusion

This preliminary study demonstrated a beneficial effect of the cognitive training program in improving memory, attention, and executive function of individuals with MCI. The cognitive improvement was accompanied by the decrement of mI/Cr in both sides of the hippocampus, PFC and ACC. Thus, a cognitive training program may potentially be an effective interventional approach to delay the conversion from MCI to Alzheimer's disease. Further study is needed to confirm these preliminary findings.