

Study Protocol and Statistical Analysis Plan

Official Title: 18-Month Double-Blind, Placebo-Controlled Study of Curcumin

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Specific Aims

Several lines of evidence suggest that the neuropathological and clinical decline leading to Alzheimer disease (AD) begins years before patients develop the full AD clinical syndrome (NINCDS-ADRDA diagnostic criteria; McKhann et al, 1984). Mild memory complaints build gradually years before patients develop dementia. The neuropathological hallmarks of AD, "preclinical" neuritic plaques (Braak & Braak, 1991) and neurofibrillary tangles (Price & Morris, 1999), are also present years prior to clinical diagnosis. These abnormal protein deposits correlate strongly with cognitive decline.

Preclinical amyloid deposits may begin decades prior to dementia onset. In fact, diffuse plaques in non-demented elderly persons are associated with an accelerated age-related cortical cholinergic deficit, consistent with preclinical AD (Beach et al, 1997; Arai et al, 1999). Also consistent with a prolonged preclinical disease stage is our own work showing that position emission tomography (PET) measures of cerebral glucose metabolism vary according to AD genetic risk (apolipoprotein E-4 [APOE-4]) and predict cerebral metabolic and cognitive decline in people with mild cognitive complaints (age-associated memory impairment [AAMI]; Small et al, 2000). Such observations have stimulated interest in preclinical AD markers or biomarkers of brain aging that may assist in tracking treatments of AAMI and related conditions. New PET imaging methods now make it possible to provide in vivo measures of cerebral amyloid neuritic plaques (e.g., florbetapir-PET; Clarke et al, 2011) and tau neurofibrillary tangles (e.g. FDDNP-PET; Small et al, 2006, 2009).

Despite these previous research findings, clinical trials (including those using biomarkers as response measures) and subsequent treatment recommendations have been limited to patients with the full clinical dementia syndrome or mild cognitive impairment (MCI), a condition that increases the risk for developing dementia (Petersen et al, 2001). Cholinesterase inhibitors are currently the only drugs that have FDA clearance for treatment of AD, but previous studies (e.g., Ringman et al, 2005) suggest that other interventions, such as dietary or herbal supplements, may benefit cognition, and possibly interrupt the accumulation of abnormal amyloid protein deposits in the brain. For example, curcumin (diferulomethane), a low molecular weight molecule with antioxidant and anti-inflammatory activities that is derived from dietary spice, may have both cognitive-enhancing and anti-amyloid properties (Ringman et al, 2005; Yang et al, 2005).

To address such issues, we propose to build upon our group's previous longitudinal brain imaging and genetic risk studies in people with age-related memory decline. Because previous studies suggest that curcumin may improve cognitive ability and prevent the build-up of age-associated plaques and tangles in the brain, we will perform a double-blind, placebo-controlled trial of curcumin to test the following hypotheses:

1. People with age-related cognitive decline (i.e., MCI, AAMI or normal aging),, who receive curcumin 90 mg twice each day will show less evidence of cognitive decline (as measured with neuropsychological assessments) than those receiving placebo after 18 months.
2. People with age-related cognitive decline who receive an oral dose of curcumin 90 mg twice each day will show less build-up of plaques and tangles (as measured with FDDNP-PET imaging) than those receiving placebo after 18 months.
3. People with age-related cognitive decline, who receive curcumin 90 mg twice each day, will show decreased measures of inflammation in the blood compared with those receiving placebo after 18 months.
4. Cognitive change, FDDNP-PET measures, and treatment response will vary according to genotypes found to influence age at dementia onset (e.g., apolipoprotein E [APOE] TOMM40).

Because curcumin may alter inflammatory markers in the blood, we will draw blood samples at baseline and at 18 months and freeze them for later analyses.

To test these hypotheses, up to 132 subjects, with age-related cognitive decline will be enrolled (Crook et al, 1986; Petersen et al, 2001). Subjects will be randomized, using a double-blind design, to one of two treatment groups: curcumin (three 30 mg capsules twice each day) or placebo, and followed for 18 months. FDDNP-PET scanning will be performed at baseline and at 18 months. Magnetic resonance imaging (MRI) scans also will be performed for co-registration of PET and assistance in identifying regions of interest. Neuropsychological assessments will be performed at baseline, after 9 months of treatment and at the conclusion of the clinical trial (18 months). Blood will be drawn at baseline to perform genotyping.

Background and Significance

Prevalence and Impact of Age-Related Memory Loss

Forgetfulness and memory loss are common experiences of aging. The mildest form of age-related memory decline is known as age-associated memory impairment or AAMI (Crook et al, 1986). Only about 1% of such cases develop dementia each year. A more severe form of memory loss is mild cognitive impairment or MCI, often defined by significant declines in delayed recall but without other functional impairments. Approximately 10% of people 65 years or older suffer from MCI, and approximately 10% develop AD each year (Petersen et al, 2001). More severe forms of cognitive impairment, such as AD, afflict between 5% and 10% in this same age group and account for the most striking rise in dementia incidence in the very old (Small et al, 1997). The disorder progresses gradually, altering memory, higher intellectual function, language, praxis, and visual-spatial and other cognitive abilities. Patients eventually become bedridden and require total care. Neuropathological characteristics include neuritic plaques, neurofibrillary beta-amyloid peptide tangles, neuronal and neuritic damage and excessive deposition.

Neuropathological Changes in Non-Demented Persons

Neuropathological, neuroimaging, and clinical research support the idea that the dementing process leading to AD begins years before a clinical diagnosis of probable AD can be confirmed (McKhann et al, 1984). Post-mortem studies of non-demented older people (Price & Morris, 1999) indicate that tangle density in healthy aging correlates with age, but that some cases demonstrate widely distributed neuritic and diffuse plaques throughout neocortex and limbic structures. This preclinical AD group also shows increased tangles. Braak and Braak (1991) have shown that neurofibrillary tangle density increases in some individuals, presumably those who will eventually develop AD, very early in adult life, perhaps even by the fourth decade. The diffuse amyloid deposits in middle-aged non-demented subjects are consistent with an early stage of AD pathology and suggest that the pathological process progresses gradually, taking 20 to 30 years for the clinical manifestation of dementia (Arai et al, 1999). Beach and co-workers (1997) found high diffuse plaque density in non-demented older persons in the entorhinal cortex and inferior temporal gyrus, in association with acetylcholinesterase fiber density. Our studies (Small et al, 1995, 2000), confirmed by others (Reiman et al, 1996), indicate lower regional brain metabolism in middle-aged and older persons with a genetic risk (APOE-4), lending further support for a prolonged presymptomatic AD stage.

Early Detection Measures

Numerous approaches to early detection of AD have been studied, including antemortem measures from various human tissues (skin, blood, and cerebrospinal fluid), brain imaging, and neuropsychological performance (Reagan Institute, 1998). For this project, FDDNP-PET will be used to measure binding of amyloid neuritic plaques (NPs) and neurofibrillary tangles (NFTs).

FDDNP-PET Scan

We enrolled 83 volunteers with self-reported memory problems who had undergone neurologic and psychiatric evaluation and PET. On the basis of cognitive testing, 25 volunteers were classified as having AD, 28 as having MCI, and 30 as having no cognitive impairment (healthy controls). PET was performed after injection FDDNP. All subjects also underwent 2-deoxy-2-[F-18]fluoro-D-glucose (FDG) PET, and 72 underwent MRI. Global values for FDDNP-PET binding (average of the values for the temporal, parietal, posterior cingulate, and frontal regions) were lower in the control group than in the group with mild cognitive impairment

($P < 0.001$), and the values for binding in the group with mild cognitive impairment were lower than in the group with Alzheimer's disease ($P < 0.001$). FDDNP-PET binding differentiated among the diagnostic groups better than did metabolism on FDG-PET or volume on MRI. We concluded that FDDNP-PET scanning can differentiate persons with MCI from those with AD and those with no cognitive impairment. This technique is potentially useful as a noninvasive method to determine regional cerebral patterns of amyloid plaques and tau neurofibrillary tangles.

In another study (Small et al, 2009) we assessed a volunteer sample of 76 middle-aged and older persons without dementia (mean age, 67 years) including 36 with mild cognitive impairment. Of the 72 subjects with genetic data, 34 were APOE-4 carriers. For all regions studied, cognitive status was associated with increased FDDNP binding ($P < .02$ to $.005$). Older age was associated with increased lateral temporal FDDNP binding. Carriers of APOE-4 demonstrated higher frontal FDDNP binding than noncarriers. In the mild cognitive impairment group, age was associated with increased medial and lateral temporal FDDNP binding, and APOE-4 carriers had higher medial temporal binding than noncarriers. We concluded that impaired cognitive status, older age, and APOE-4 carrier status are associated with increased brain FDDNP-PET binding in persons without dementia, consistent with previous clinical and postmortem studies associating these risk factors with amyloid plaque and tau tangle accumulation. Stratifying subject groups according to APOE-4 carrier status, age, and cognitive status may therefore be an informative strategy in future clinical trials using FDDNP-PET.

Curcumin

Curcumin (diferuloylmethane), a yellow pigment in the spice turmeric (also called curry powder), has been used for centuries as a treatment for inflammatory diseases. Extensive research within the past two decades has shown that curcumin mediates its anti-inflammatory effects through the down-regulation of inflammatory transcription factors, enzymes and cytokines (Aggarwal & Sung, 2009).

Research also has focused on the potential cognitive and anti-amyloid benefits of the curry spice curcumin (Ringman et al, 2005). Prepared from the turmeric plant, curcumin has been taken orally as a remedy for a variety of ailments, ranging from dyspepsia to liver disease. Recent studies have found it to be well tolerated. Yang and associates (2005) have found that curcumin inhibits the formation of amyloid beta oligomers and fibrils and that it binds to plaques and reduces amyloid in vivo. Curcumin also has potential cholesterol-lowering and anti-inflammatory properties, which could further benefit brain health (Ringman et al, 2005). Curcumin has the capacity to bind amyloid plaques and possibly prevent their aggregation and thus the potential to alter FDDNP binding values in normal aging and patients with MCI.

In a study in patients with high-risk or pre-malignant lesions (Cheng et al, 2001), curcumin was taken orally for 3 months with a starting dose of 500 mg/day. If no toxicity was observed, then the dose was escalated to another level in the order of 1,000, 2,000, 4,000, 8,000, and 12,000 mg/day. The concentration of curcumin in serum and urine was determined by high pressure liquid chromatography (HPLC). A total of 25 patients were enrolled in this study. There was no treatment-related toxicity up to 8,000 mg/day. Beyond 8,000 mg/day, the bulky volume of the drug was unacceptable to the patients. The serum concentration of curcumin usually peaked at 1 to 2 hours after oral intake of curcumin and gradually declined within 12 hours. The average peak serum concentrations after taking 4,000 mg, 6,000 mg and 8,000 mg of curcumin were 0.51 ± 0.11 microM, 0.63 ± 0.06 microM and 1.77 ± 1.87 microM, respectively. Urinary excretion of curcumin was undetectable. The investigators concluded that curcumin was not toxic to humans up to 8,000 mg/day when taken by mouth for 3 months (Cheng et al, 2001).

In a phase I clinical trial of oral curcumin (Sharma et al, 2004), 15 patients with advanced colorectal cancer consumed 0.45 to 3.6 grams daily for up to 4 months. Patients tolerated daily oral doses of 3.6 grams, which the authors advocated for evaluation of cancer prevention treatments.

In order to improve absorption and bioavailability of curcumin, curcumins dispersed with colloidal nanoparticles have been developed. The form of curcumin to be used in the proposed study (Theracurmin) is such a nanoparticle form. Dose-escalation, safety, and pharmacokinetic studies of Theracurmin have been performed at Kyoto University Hospital in Japan (Kanai et al, 2011). Theracurmin was studied in 6 healthy human volunteers at a single oral dose of 150 mg. After an interval of 2 weeks, the same subjects then received Theracurmin at a single dose of 210 mg. Plasma curcumin levels were measured at 0, 1, 2, 4, 6, and 24 hours after Theracurmin intake using HPLC. One subject reported grade 1 diarrhea after intake of 150 mg Theracurmin. No other toxicities were observed. C (max) for Theracurmin at 150 and 210 mg was 189 ± 48 and 275 ± 67 ng/ml (mean \pm SEM), respectively, and the area under the curve for 24 h was estimated to be $2,649 \pm 350$ and $3,649 \pm 430$ ng/ml \times h (mean \pm SEM), respectively. The $t(1/2)$ was estimated to be 9.7 ± 2.1 h for 150 mg and 13.0 ± 3.3 h for 210 mg. The investigators concluded that Theracurmin can safely increase plasma curcumin levels in a dose-dependent manner at least up to 210 mg without saturating the absorption system.

The absorption efficacy of this form of curcumin has been compared with that of curcumin powder (Sasaki et al, 2011). Healthy human volunteers were administered orally 30 mg Theracurmin or curcumin powder. The area under the curve of Theracurmin was 27-fold higher than that of curcumin powder. Because of this higher potential absorption, we will be using much lower doses of Theracurmin (180 mg daily) compared with that found to be tolerable using standard curcumin oral preparations (3,600 to 8,000 mg daily). Although systemic human safety studies provide data for only 4 months of treatment and the proposed study is for 18 months, we will be monitoring subjects every 3 months for safety, tolerability and emergence of adverse events.

Other Safety and Dosage Considerations

Theracurmin exhibits pharmacological safety based on trials in humans demonstrating the bioavailability of single doses and clinical trials using curcumin in both animals and humans. The absorption of curcumin into the blood has been documented previously (Anand et al, 2007). Curcumin has low bioavailability due to its poor water solubility and absorption, rapid metabolism, and rapid systemic elimination. A study by Yang and associates (2007) showed that 10 mg/kg of curcumin given intravenously to rats yielded a maximum serum curcumin level of 0.36 ± 0.05 μ g/mL, whereas 500 mg/kg of curcumin administered orally only yielded a 0.06 ± 0.01 - μ g/mL maximum serum level in rats. The absorption of curcumin in this study was only about 1%. A number of delivery strategies, including adjuvants, nanoparticles, liposomes, micelles, and phospholipid complexes, have been explored to enhance the intestinal absorption of curcumin (3-7). Sharma and colleagues (2001) showed that there was no detectable curcumin or its metabolites in the blood or urine after the administration of 440–2,200 mg of curcuma extract per day (containing 36–180 mg of curcumin) for up to 29 days to patients with advanced colorectal cancer. Cheng and co-workers (2001) demonstrated that the peak concentrations of curcumin in the serum after administration of 4, 6, and 8 g of curcumin (given in the form of tablets obtained from a commercial source, with each tablet containing 500 mg curcumin) were 0.51, 0.64, and 1.77 mM, respectively. Moreover, these investigators found that doses below 4 g were barely detectable. Lao et al. (2006) could not detect curcumin in the serum of volunteers given 0.5, 1.0, 2.0, 4.0, 6.0, or 8.0 g of curcumin. This was provided in a capsule form as a standardized powder extract, obtained commercially, containing a minimum 95% concentration of the three curcuminoids of curcumin, bisdemethoxycurcumin, and demethoxycurcumin. However, these authors found that curcumin levels reached 50.5 and 51.2 ng/ml sera by 4 hours in 2 subjects administered 10 and 12 g of curcumin, respectively.

The safety and pharmacokinetics of the preparation to be used in the proposed studies (Theracurmin) was tested in six healthy human volunteers. Following single oral doses of 150 mg and 210 mg respectively separated by a two week washout period, plasma curcumin levels were measured at 0, 1, 2, 4, 6, and 24 h after Theracurmin intake using high performance liquid chromatography (HPLC) (see figure below) . One subject reported grade 1 diarrhea after intake of 150 mg of Theracurmin. No other toxicities were observed in this study. Cmax for Theracurmin at 150 mg and 210 mg was 189 ± 48 and 275 ± 67 ng/ml (mean \pm S.E.M.),

respectively and the area under the curve for 24 h was estimated to be 2649 ± 350 and 3649 ± 430 ng/ml \times h (mean \pm S.E.M.).

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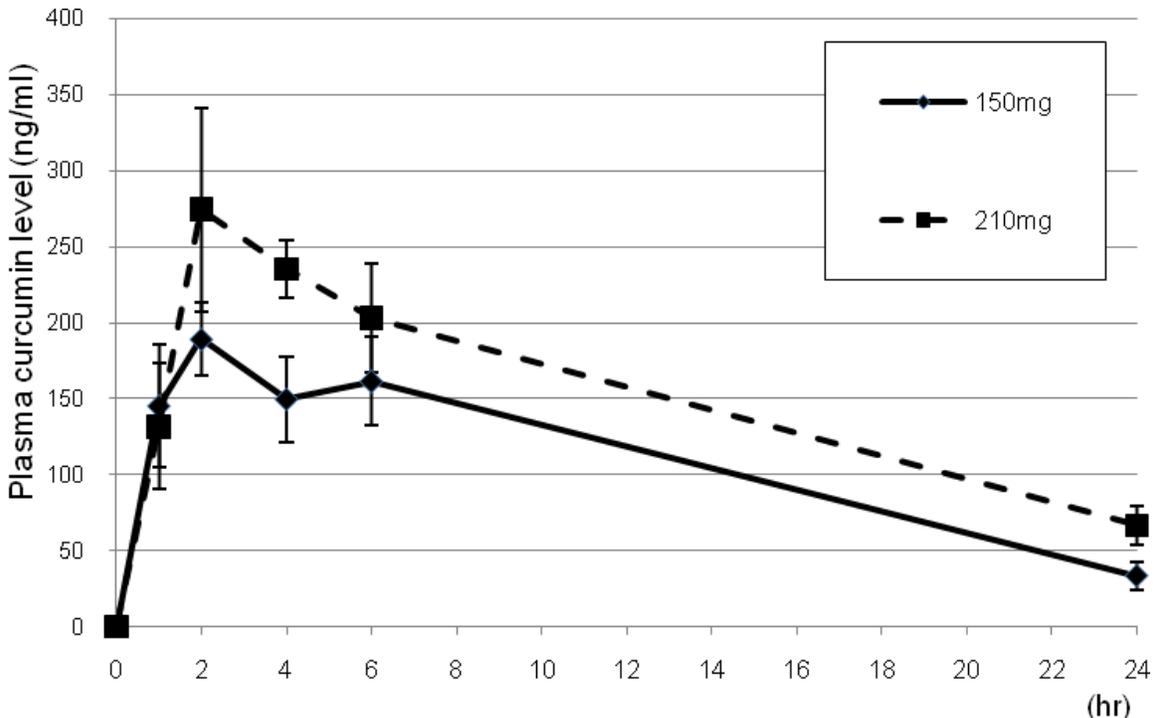


Figure 1: Plasma curcumin level in ng/ml following single doses of 150 or 210 mg of Theracurmin in healthy volunteers.

Research Design and Methods

The study is a double-blinded, randomized clinical trial. Subjects, investigators, psychometrists and research coordinators will be blinded to the type of supplement (curcumin versus placebo).

Theravalues Corporation will compound the study drug under the name of "Theracurmin," packaged in 465 milligram capsules, 30 of which are curcumin. The capsules will be provided to the UCLA Investigational Drug Pharmacy for dispensation. The company will also provide placebo capsules. The capsules will be labeled as A or B by the manufacturer.

Subjects in all portions of the study will have the procedures outlined below. The following procedures are performed solely for research purposes.

Visit 1 (Intake): 2 hours total

- Informed Consent and Clinical Assessment (1 hour)

- Routine Laboratory Blood Draw, DNA Blood Draw and Electrocardiogram (EKG) (1 hour)

Visit 2 (Baseline): 4.5 hours total

- Neuropsychological Evaluation (2 hrs)
- [F-18]FDDNP-PET/CT scan (1 hr)*
- MRI Scan (30 min)*
- Initial supply of supplement or placebo: done once all procedures above are complete.

*May be completed on separate visit

Visit 3 (3 months): 20 minutes in person or by mail/phone

- Review of possible adverse events
- Supplement compliance check and re-supply of supplement or placebo

Visit 4 (6 months): 2 hours total

- Neuropsychological Evaluation
- Review of possible adverse events
- Supplement compliance check and re-supply of supplement or placebo

Visit 5 (9 month): 20 minutes in person or by mail/phone

- Review of possible adverse events
- Supplement compliance check and re-supply of supplement or placebo

Visit 6 (12 months): 2 hours total

- Neuropsychological Evaluation
- Review of possible adverse events
- Supplement compliance check and re-supply of supplement or placebo

Visit 7 (15 months): 1 hour total

- Review of possible adverse events
- Supplement compliance check and re-supply of supplement or placebo

Visit 8 (18 month): 3.5 hours total

- Neuropsychological Evaluation (2 hrs)
- [F-18]FDDNP-PET/CT scan (1 hr)*
- Blood draw (15 minutes)
- Review of possible adverse events
- Supplement compliance check

*May be completed on separate visit

Informed consent, clinical assessment routine laboratory screening and EKG are done at the subject's first visit. These procedures are performed in a private office or at the Clinical Research Center (CRC) in a private room. The routine laboratory blood samples, DNA blood sample and EKG are performed at the CRC. The following includes additional details on the study visits.

Visit 1

A. Informed Consent:

If a potential subject calls the UCLA Longevity Center and indicates he/she is interested in the study, the following would happen:

1. The potential subject would be screened over the phone, by research personnel, to determine eligibility.
2. If eligible, the potential subject would come to the center and be evaluated by the clinician and screened for possible inclusion into the research study.
3. If the potential subject meets eligibility criteria, and once he or she agrees to participate, the subject would sign the consent form and be enrolled into the study.

4. With the subject's permission, the data collected by the clinician during the initial evaluation would be used in research study.
5. The data would then be reviewed by research personnel and if appropriate added to the research database.
6. If any of the procedures were performed within six months of enrollment, the subject would not have to repeat the PET or PET/CT scan or neuropsychological testing.

B. Clinical Assessment: This assessment includes a psychosocial history and mental status examination. The following standardized rating scales and questionnaires are used to verify the history, quantify degree of impairment, and assign subjects to diagnostic groups:

1. Beck Depression Inventory II (BDI-II)
2. Family History Questionnaire (completed by the Staff Research Associate)
3. Cognitive Screening: Montreal Cognitive Assessment Scale (MOCA)
4. Functional Activities Questionnaire (FAQ)
5. Profile of Mood States (POMS)
6. STOP-BANG Questionnaire for Sleep Apnea
7. Pittsburgh Sleep Quality Index (PSQ-I)
8. 7-day Sleep Diary

C. Routine Laboratory Blood Draw, DNA Blood Draw and EKG: After the informed consent and clinical assessment is completed, subjects will be taken to the Clinical Research Center (CRC) and enrolled as outpatients.

The following tests are performed:

- CBC, PLT, DIFF
- Free T4 Index
- TSH
- Vitamin B12
- Chem Panel
- Uric Acid
- RPR with FTA Confirmation
- Routine EKG
- Blood Draw for DNA Analysis. Additional blood will be drawn and frozen to assess inflammatory markers.

Visit 2

Once the results from Visit 1 have been reviewed by one of the investigators and deemed within normal limits, subjects will undergo the following procedures as baseline measures.

A. Neuropsychological Evaluation: The neuropsychological test battery includes:

- Tests Estimating Premorbid Intellectual Functioning (Baseline only)
 - Advanced Clinical Solutions Word Reading Test (formerly the Wechsler Test of Adult Reading).
- Core Battery
 - Brief Visuospatial Memory Test Revised (BVMT-R) [Visit 2 Only].
 - Buschke Selective Reminding Task (BSRT);
 - New York University Story Recall Test;
 - Conners' Continuous Performance Test;
 - CFL letter fluency test;
 - Animal Naming category fluency test
 - Trails A

Every Six Months: Completion of Sleep Diary and PSQ-I

Last Visit: Beck Depression Inventory II (BDI-II) and Profile of Mood States (POMS), Sleep Bang

Subjects are tested in a private office. Healthy and motivated subjects can generally complete the battery in a three-hour period, including time for periodic rest breaks.

B. [F-18]FDDNP-PET Scan or PET/CT Scan: Subjects undergo the [F-18]FDDNP-PET scan at the UCLA 200 Medical Plaza Nuclear Medicine Clinic. While in the scanner in a dimmed room, all subjects receive a 10 mCi intravenous injection of [F-18]FDDNP. During the scanning, each subject is kept quiet and exposed only to ambient room sound in a dimmed room, with eyes open and ears unplugged. The procedure will take 45 minutes of the subject's time.

Twenty-one blood samples (12 in the first two minutes and one each at 3, 4, 5, 7, 10, 20, 30, 45 and 60 minutes) are taken from a hand vein in a warm water bath (42 degrees centigrades) over 65 minutes for radiation counts that allow the calculation of the input function.

Scanning is performed on a CTI/Siemens 831-08 tomograph (Siemens Corp, Hoffman Estates, 111) in three-dimensional acquisition mode (inter-plane septa removed), using double the previous standard axial sampling (Cherry et al, 1991, 1992). Imaging commences immediately after administration of the radiolabeled dye and lasts for 45 minutes.

Once subjects have completed the neuropsychological assessment and the PET scan in Visit 2, they will begin taking the supplement or placebo. Both the curcumin supplement and the placebo will be packaged in 465 milligram capsules to maintain blindness in both research personnel and subjects. Subjects will take three 465 milligram capsules twice a day (once in the morning and once at night). Unless otherwise notified by the investigator, subjects will be instructed to continue taking the supplement dose until the study's completion 18 month later.

C. MRI Scan: Subjects will undergo an MRI scan of the brain which will include fluid-attenuation inversion recovery (FLAIR) and 2D T2*-weighted gradient-recalled-echo (GRE) sequences. The MRI scan will also include an MPRage sequence for registration and cortical surface extraction with the PET scans. Scanning will be done using the 3-Tesla MRI scanner in the Staglin IMHRO Center for Cognitive Neuroscience at UCLA or the 1.5-Tesla MRI in the UCLA Department of Radiology. Sequences will have a slice thickness of less than 5mm and Echo Time (TE) of more than 20 ms. Subjects with more than 4 cerebral microhemorrhages (regardless of their anatomical location or diagnostic characterization as "possible" or "definite"), a single area of superficial siderosis, or evidence of a prior macrohemorrhage at screening or baseline will be excluded. MRI analysis will be performed by Neuroradiologist Noriko Salamon, MD, PhD or other UCLA radiologist.

*MRI may take place visit 1 or visit 2 depending on scheduling.

Visit 3

At 3 months, to ensure that subjects are taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed. Subjects will be provided a new supply of supplement or placebo.

Visit 4

At 6 months, to ensure that subjects are taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed. Subjects will be provided a new supply of supplement or placebo.

Visit 5

At the 9-month mark, subjects will be asked to return to complete another neuropsychological assessment. This evaluation contains the same tests as the aforementioned assessment. To ensure that subjects are taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed. Subjects will be provided a new supply of supplement or placebo.

Visit 6

At 12 months, to ensure that subjects are taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed. Subjects will be provided a new supply of supplement or placebo.

Visit 7

At 15 months, to ensure the subjects are taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed. Subjects will be provided a new supply of supplement or placebo.

Visit 8

At the conclusion of the study, the 18-month mark, subjects will be asked to return to complete a final neuropsychological assessment, [F-18]FDDNP-PET Scan and blood draw. Additional blood be drawn and frozen to assess inflammatory markers if curcumin outcomes are positive. To ensure that subjects were taking the supplement or placebo, staff will review for potential adverse events and safety of the supplement or placebo, and check to determine if subjects are taking them as prescribed.

Subjects who are unable to come to UCLA before running out of supplement may be mailed a refill via FedEx. Correct delivery will be ensured by mandatory signature upon receipt. Theracurmin pills are available on the market and can be ordered online and shipped home; no specific conditions are required. Although subjects will have to come to UCLA for the MRI, the review session may be conducted over the phone.

All study investigators will be notified of the possible occurrence of cerebral vasogenic edema, its imaging manifestations, and the clinical signs and symptoms that may accompany this phenomenon. They will also be instructed on the measures to be taken should cerebral vasogenic edema occur. Those measures include: discontinuation of study medication, more frequent serial MRI scans until the imaging abnormalities resolve, and consideration of treatment with high-dose dexamethasone should symptoms be severe.

Statistics and Data Analysis

Hypotheses:

1. People with age-related cognitive decline (e.g., MCI and/or AAMI) who receive a an oral dose of curcumin 90 mg twice each day, along with lifestyle counseling on healthy nutrition and exercise, will show less build-up of plaques and tangles (as measured with FDDNP-PET imaging) than those receiving placebo after 18 months.
2. People with age-related cognitive decline, who receive curcumin 90 mg twice each day along with healthy lifestyle counseling, will show less evidence of cognitive decline than those receiving placebo after 18 months.
3. Cognitive change, FDDNP-PET measures, and treatment response will vary according to genotypes found to influence age at dementia onset (e.g., apolipoprotein E [APOE] and TOMM40).

To test Hypotheses 1 and 2, the intervention and control groups will be compared on changes in performance on cognitive memory measures and FDDNP signals using a repeated measures analysis of variance. Based on our previous studies, we estimate an attrition rate of 10% in this age group of subjects. Hence we expect 60 subjects to complete the protocol in each group. Given an alpha level of 0.05 and power of 0.8, with 60 subjects per group, we can detect a moderate effect size of 0.51 of the change across testings between groups. Of course, the subject number for the intent-to-treat analysis will be higher since drop-outs will be included in this analysis.

To investigate the influence of genotype, we will determine, using a t-test, if the APOE-4 carriers in the intervention group show a significant improvement from baseline on the cognitive and amyloid measures compared to the non-APOE-4 carriers. We anticipate approximately 30 APOE-4 carriers out of the 60 subjects completing the intervention, so an

effect size of 0.72 would be detectable, at an alpha level of 0.05 and power of 80%. Assuming a common standard deviation of change for the two genetic groups, this effect size implies a difference between the genetic groups in the change across testings of 7.6 and 1.25 for the Buschke-Fuld Total and Benton Visual Retention Scores respectively.

Biomarker levels will be analyzed between doses of curcumin using basic t-tests and/or ANOVAs. For the RCT, differences in cognitive and imaging markers will be analyzed between groups (active supplement vs. placebo) using the same basic statistics. For any more complex analyses in the dosing study or the RCT, we will consult our group statistician for the appropriate statistical procedures.

Inclusion Criteria

- a) Agreement to participate in the 18-month double-blind, placebo-controlled clinical trial of curcumin.
- b) Diagnostic criteria for mild cognitive impairment (MCI) or any age related memory decline according to standard criteria (Petersen et al, 2001; Crook et al, 1986).
- c) Age 50 to 90 years.
- d) No significant cerebrovascular disease: modified Ischemic Score of < 4 (Rosen et al, 1980).
- e) Adequate visual and auditory acuity to allow neuropsychological testing.
- f) Screening laboratory tests and EKG without significant abnormalities that might interfere with the study.

Exclusion Criteria

- a) Diagnosis of probable Alzheimer's disease (AD) or any other dementia (e.g., vascular, Lewy body, frontotemporal) (McKhann et al, 1984).
- b) Evidence of other neurological or physical illness that can produce cognitive deterioration. Volunteers with a history of stroke, TIA, carotid bruits, or lacunes on MRI scans will be excluded.
- c) Inability to undergo MRI.
- d) Evidence of Parkinson's disease as determined by the motor examination (items 18-31) of the Unified Parkinson's Disease Rating Scale (Fahn et al, 1987).
- e) History of myocardial infarction within the previous year, or unstable cardiac disease.
- f) Uncontrolled hypertension (systolic BP > 170 or diastolic BP > 100).
- g) History of significant liver disease, clinically-significant pulmonary disease, diabetes, or cancer.
- h) Current diagnosis of any major psychiatric disorder according to the DSM-IV TR criteria (APA, 2000).
- i) Current diagnosis or history of alcoholism or substance addiction.
- j) Regular use of any medication that may affect cognitive functioning including: centrally active beta-blockers, narcotics, Clonidine, anti-Parkinsonian medications, antipsychotics, benzodiazepines, systemic corticosteroids, medications with significant cholinergic or anticholinergic effects, anti-convulsants, or Warfarin. Occasional chloral hydrate use will be allowed, but discouraged, for insomnia.
- k) Use of more than one multivitamin per day. Vitamins other than the standard multivitamin supplement will not be allowed.
- l) Use of medications known to affect FDDNP-PET binding (e.g., ibuprofen, naproxen).
- m) Use of more than one daily baby aspirin (81mg) and/or use of any medication containing curcumin.
- n) Use of cognitive enhancing supplements (e.g. Ginkgo biloba).
- o) Use of any investigational drugs within the previous month or longer, depending on drug half-life.
- p) Pregnancy.
- q) HIV infection.
- r) Evidence of vasogenic edema; specifically, evidence of more than 4 cerebral microhemorrhages (regardless of their anatomical location or diagnostic

characterization as “possible” or “definite”) or a single area of superficial siderosis), or evidence of a prior macrohemorrhage at screening or baseline.

Potential Benefits

During the course of the screening and subject identification, untreated medical conditions may be recognized. However this study will not improve a subject's health or condition.

Although there is no known benefit to society at this time, it is hoped that society will benefit from the knowledge gained from this study. In particular, the results of the study may lead to the development of new treatments for memory decline and Alzheimer's disease.

Potential Risks/Discomforts and Management Strategies

1. SCREENING

Subjects may feel uncomfortable answering some of the screening questions. They do not have to answer any questions they do not wish to answer and they may stop at any time. Their participation in the screening is voluntary. A decision whether or not to participate in the screening will not affect their relationship with UCLA. Subjects will be told that they may not directly benefit from the screening.

Subjects' answers will be confidential. No one will know the answers except for the research team. If the subject decides to answer the questions of the screening interview, that will determine their eligibility for the research. If the subject qualifies for the study, the subject will be asked to read through and sign an informed consent. Their answers will be kept with their other research generated records. If they do not qualify for the study, their answers will be destroyed.

2. MAGNETIC RESONANCE IMAGING

Subjects may experience anxiety and/or claustrophobia from being in an enclosed space. They can ask to be taken out of the scanner at any time. Earplugs will be given during the MRI procedure.

3. CURCUMIN SUPPLEMENT

Nutritional supplements may cause some side effects or other reactions. Curcumin's widespread use in food without adverse effects supports its safety. Although rare, the most frequent side effects associated with curcumin are nausea and diarrhea. Any subjects who do not tolerate the curcumin will be discontinued from the study.

In small cancer prevention studies that assessed doses of up to 12,000 mg per day, curcumin was found to be safe in humans, although doses above 8,000 mg were found to be unpalatable. At doses higher than 12,000 mg, gastric irritation was reported.

4. PLACEBO

Subjects will be informed that there is a 50% chance of receiving placebo during the course of the study and the investigators do not anticipate any long-term benefits from use of placebo.

5. RADIATION EXPOSURE

We are exposed to radiation on a daily basis, both from natural (sun and earth) and man-made sources. In addition to the radiation that subjects may be exposed to as part of their clinical care, the subject may potentially receive one 10 mCi FDDNP- PET/CT scan in a year while participating in this research study. This amount is well under the amount considered safe.

If the FDDNP-PET/CT scan is performed, the total estimated radiation dose to the whole body would be 153 millirem, or 3% of the 5,000 millirem annual whole body limit allowed for adult radiation workers. The total estimated radiation dose to the primary critical organ (gallbladder wall) would be 544 millirem or 11% of the 5,000 millirem single dose limit allowed for radiation workers. The total estimated radiation dose to the secondary critical organ (liver)

would be 497 millirem, or 10% of the 5,000 millirem single dose limit allowed for radiation workers.

6. BLOOD DRAW FOR ROUTINE BLOOD ANALYSIS AND DNA SAMPLE

The risks include problems associated with blood drawing. This is a routine procedure performed under standard and sterile medical conditions. The potential side effects of removing blood may include momentary discomfort during the puncture, lightheadedness, faintness, and soreness and discoloration of the area for several days. In very rare instances, either bleeding or infection can develop at the venipuncture site. There is no more discomfort encountered than when blood samples are taken during periodic medical examinations or when blood is donated at a blood bank.

7. GENETIC ANALYSIS

The data collected for genetic analysis will not be kept in the subject's medical record and will remain strictly confidential. The potential side effects of genetic analysis of blood may, however, involve certain psychological and social risks in the advent of inadvertent disclosure. These risks include:

- a. Broad sharing of phenotype and genomic data (e.g. genotype, DNA sequence, expression profiles, etc.);
- b. Computer security breaches;
- c. Other unanticipated distributions arising from maintaining data in an electronic format;
- d. Privacy breaches (both those known and those unforeseen at this time);
- e. Uncertainty of findings related to genetic risk for a given disease or trait;
- f. Risks to relatives or identifiable populations or groups;
- g. Physical risks (such as those associated with collecting blood or other tissues samples).

Effective November 2009, federal legislation (The Genetic Information Nondiscrimination Act, or GINA) was passed to provide baseline protection against discrimination in employment and health insurances decisions across the nation.

8. NEUROPSYCHOLOGICAL EVALUATION

Subjects may experience feelings of failure, frustration, or anxiety induced by neuropsychological testing. The psychologist administering the tests is experienced in assessing persons and will convey a relaxed and confident attitude. Subjects have the right to refuse to answer any questions that they may not wish to answer. If a subject does have the above reaction to the evaluation, it should go away at the completion of the test. If the subject finishes the test and feels anxious, then the subject can talk to the psychologist who administered the test.

In the event that the subject tells the research staff that they are thinking about killing themselves or they answer yes to a question about having thoughts about suicide, the investigator will ask the subjects more questions about these thoughts. Depending on the answers to these questions, the research staff may:

- a. Provide the subject with referrals for treatment;
- b. Work with the subject to contact their personal physician, trusted family member, or therapist to discuss these thoughts of harming themselves;
- c. Work with the subject on a plan that may include getting them to a hospital for safety.

9. INJECTION DURING PET SCAN

The potential side effects of removing venous blood may include momentary discomfort during the puncture, lightheadedness, fainting, and soreness and discoloration for several days. In very rare circumstances, either bleeding or infection can develop at the venipuncture site.

10. UNFORESEEN RISKS

This study may include unforeseen risks. However, risks will be minimized because subjects will be monitored closely for the occurrence of any side effects, and the study treatments will be discontinued promptly if there appears to be a distressing reaction. Regular monitoring including physical examinations and vital signs (including weight) will be done during this

study to detect any possible adverse effects from treatment. Subjects will be advised to contact the study physician if they experience any unexpected reaction while participating. Also, subjects will be advised not to start any new medication, including over-the-counter medications, or have an elective surgery without approval of the study physician.

RISK/BENEFIT ANALYSIS

Both the risks and benefits are minimal for individual subjects. The results of the study could lead to more effective prevention and treatment of memory decline and Alzheimer's disease. The investigators believe the potential benefits from participation in this study outweigh the risks and, therefore, the risk benefit ratio is thought to be favorable.

DATA AND SAFETY MONITORING

An independent data and safety monitoring committee will be established and will review data on three occasions during the study. The committee will be informed of the occurrence of any serious adverse events if they arise.

SUBJECT COMPENSATION

Subjects will be paid as follows, up to a total of \$300.00. Parking will be covered by the study.

<u>Project Period</u>	<u>Amount to be paid</u>
Initial Study Visit	\$25.00 total
6 Week Mark	\$75.00 total
6 Month Mark	\$75.00 total
1 Year Mark	\$75.00 total
18 Month Mark/End of study	\$50.00 total

If the investigator terminates the study or the participant decides to withdraw, the participant will be paid based on the last project period completed.

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III. Research Design and Methods: Describe in detail the design and methodology of the study.

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