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Abstract: Alzheimer's Disease (AD) and cardiovascular disease (CVD) are syndromes of aging that share analogous lesions and risk factors, involving lipoproteins, oxidative damage and inflammation. Unlike in CVD, in AD, sensitive biomarkers are unknown, and high risk groups are understudied. To identify potential prevention strategies in AD, we have focused on pre-clinical models (transgenic and amyloid infusion models), testing dietary/lifestyle factors strongly implicated to reduce risk in epidemiological studies. Initially, we reported the impact of non-steroidal anti-inflammatory drugs (NSAIDs), notably ibuprofen, which reduced amyloid accumulation, but suppressed few inflammatory markers and without reducing oxidative damage. Safety concerns with chronic NSAIDs led to a screen of alternative NSAIDs and identification of the phenolic anti-inflammatory/anti-oxidant compound curcumin, the yellow pigment in turmeric that we found targeted multiple AD pathogenic cascades. The dietary omega-3 fatty acid, docosahexaenoic acid (DHA), also

limited amyloid, oxidative damage and synaptic and cognitive deficits in a transgenic mouse model. Both DHA and curcumin have favorable safety profiles, epidemiology and efficacy, and may exert general anti-aging benefits (anti-cancer and cardioprotective.)

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August 18, 2005

Paul Coleman,
Editor in Chief
Neurobiology of Aging

Dear Paul

Thank you for the opportunity to submit this paper for the special Sparks Workshop issue of Neurobiology of Aging on 'Obesity, Diabetes, Mood, and Cognition'. My manuscript entitled "*Prevention of Alzheimer's Disease: Omega-3 Fatty acid and Phenolic Antioxidant Intervention*" s by myself, Giselle P. Lim, Fusheng Yang, Bruce Teter, Aynun Begum, Qiulan Ma, Marni E. Harris-White and Sally A Frautschy **has not been and will not be submitted elsewhere.**

Sincerely, Greg Cole

A handwritten signature in black ink that reads "Greg M Cole".



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Paul Coleman,
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Dear Paul

Disclosure of funding. There is no **financial association between** any authors and a commercial company that makes a product that figures prominently in the article entitled "*Prevention of Alzheimer's Disease: Omega-3 Fatty acid and Phenolic Antioxidant Intervention's*" by myself, Giselle P. Lim, Fusheng Yang, Bruce Teter, Aynun Begum, Qiulan Ma, Marni E. Harris-White and Sally A Frautschy.

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Sincerely, Greg Cole

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NBA, 'Obesity, Diabetes, Mood, and Cognition'

Special Issue Sparks Workshop

Prevention of Alzheimer's Disease:
Omega-3 Fatty acid and Phenolic Antioxidant Interventions

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Summary

Alzheimer's Disease (AD) and cardiovascular disease (CVD) are syndromes of aging that share analogous lesions and risk factors, involving lipoproteins, oxidative damage and inflammation. Unlike in CVD, in AD, sensitive biomarkers are unknown, and high risk groups are understudied. To identify potential prevention strategies in AD, we have focused on pre-clinical models (transgenic and amyloid infusion models), testing dietary/lifestyle factors strongly implicated to reduce risk in epidemiological studies. Initially, we reported the impact of non-steroidal anti-inflammatory drugs (NSAIDs), notably ibuprofen, which reduced amyloid accumulation, but suppressed few inflammatory markers and without reducing oxidative damage. Safety concerns with chronic NSAIDs led to a screen of alternative NSAIDs and identification of the phenolic anti-inflammatory/anti-oxidant compound curcumin, the yellow pigment in turmeric that we found targeted multiple AD pathogenic cascades. The dietary omega-3 fatty acid, docosahexaenoic acid (DHA), also limited amyloid, oxidative damage and synaptic and cognitive deficits in a transgenic mouse model. Both DHA and curcumin have favorable safety profiles, epidemiology and efficacy, and may exert general anti-aging benefits (anti-cancer and cardioprotective.)

Running Title

DHA, Curcumin and Prevention of Alzheimer's

The authors declare that they have no competing financial interest

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Introduction.

Alzheimer's (AD) resembles cardiovascular disease (CVD) since both show age-dependent accumulation of lipophilic material in multicellular plaque lesions, which involves a macrophage lineage (microglial) inflammatory response, oxidative damage and injury to surrounding cells. The genetic (ApoE alleles) and environmental (high homocysteine, statin use/ cholesterol/ inflammatory markers/ dietary fats/ fish/wine / type II diabetes, low exercise, etc) risk factors for AD and CVD show strong overlap, suggesting that prevention approaches for reducing CVD may be relevant to AD. Studies with CVD in both animal models and the clinic have identified relevant epidemiological risk factors that can be applied to development of successful prevention of CVD. Two major advantages for CVD prevention have been adequate biomarkers (blood lipid profiles) and the opportunity to conduct prevention trials in at-risk patients with secondary cardiovascular "events" as endpoints. Unfortunately, the lack of suitable biomarkers, "second events" and the cost and time involved in conducting prevention trials for AD have prohibited testing prevention in clinical trials; most testing has been in AD patients. Clinical trials in AD patients with vitamin E, and several NSAIDs (naproxen, cyclooxygenase-2 (COX-2) inhibitors) have shown mostly minimal or no effects on cognitive decline and AD progression. These failures emphasized the need for better screening in preclinical animal models (expressing familial AD genes or after amyloid peptide infusion into the CNS) using drugs with good epidemiology and proven safety. Amyloid models have permitted testing interventions limiting β -amyloid and associated oxidative damage and inflammation. Similar studies in tau transgenics to limit tau/tangle pathology are underway.

CNS inflammation in AD is characterized by reactive microglia and elevated IL-1 and complement factors [3] . A β aggregates can stimulate oxidation in multiple ways: including neuronal H₂O₂ production [4], radical production via iron or copper binding [22] and pro-inflammatory stimulation of peroxynitrite or superoxide. Both oxidative damage [4, 24, 28] and

inflammation [3] begin early in AD accompanying amyloid accumulation and neurodegeneration. AD risk is reduced by antioxidant [20, 34] and non-steroidal anti-inflammatory drug (NSAID) [11] intake suggesting these might be useful prevention methods.

We initially tested the NSAID (ibuprofen) that had the strongest epidemiological rationale. With low dosing designed to model apparently protective chronic NSAID consumption in populations with reduced AD risk, ibuprofen suppressed amyloid accumulation in APPsw transgenic mice [16] but reduced a surprisingly limited subset of inflammatory markers, notably IL-1 β and downstream murine ACT mRNA, but not iNOS, macrosialin or CD11c mRNA [19]. Further, some NSAIDs including ibuprofen (but not naproxen and COX-2 inhibitors) selectively lowered A β 1-42 production without inhibiting A β 1-40 or NOTCH in vitro or in vivo [8, 32]. Although ibuprofen has the strongest rationale for use in AD prevention, it has not been tested. (Side-effect concerns halted COX-2 inhibitor and naproxen prevention trials in 2004.) Efficacy (and possibly even safety) issues with vitamin E leaves us with few viable approaches to chronic control of oxidative damage and inflammation in AD and other diseases of aging.

Phenolic Antioxidants. Many potent dietary antioxidants occur in plant fruiting bodies, seeds or roots and contain one or more phenol groups that contribute to potent antioxidant activities presumably selected to protect the plant germ line. Grapes, apples, many types of berries, pomegranates, green tea and many other plant sources are rich sources of these antioxidant compounds that often have potent antioxidant and anti-inflammatory properties and related health benefits. Notable among these, resveratrol from red wine, the green tea catechins and the turmeric extract curcumin have received intensive study for potential disease prevention or treatment and are worthy of consideration for AD.

Curcumin is a combined polyphenolic antioxidant/ NSAID that targets AD pathogenesis at multiple sites. Chronic oxidative damage and inflammation occur in AD and likely contribute

to neurodegeneration. Although other groups have some evidence for protection, in our hands, neither ibuprofen nor vitamin E supplements reduced protein carbonyls, one marker for oxidative damage. In contrast, a polyphenolic tumeric component, diferulomethane (curcumin) lowered F2 isoprostanes and carbonyls as measures of oxidative damage, reduced IL-1 β , iNOS and CD11b as markers of activated microglia and limited cognitive deficits, postsynaptic marker loss and amyloid accumulation [9, 15].

Curcumin has the potential to suppress the AD pathogenic cascade at multiple sites. Curcumin's structure is two methoxyphenol groups separated by a β -diketone bridge that confers iron and other metal chelating activity. Metals have been implicated in A β aggregation and toxicity. Thus, while curcumin is a direct radical scavenger, it is a more potent antioxidant and inhibitor of lipid peroxidation, particularly metal catalyzed peroxidation [29]. Curcumin is also a good inhibitor of expression of inflammatory cytokines, COX-2, and iNOS by virtue of inhibition of JNK/ AP-1 and NF κ B-mediated gene transcription [1]. All of these factors (IL-1, TNF α , COX-2, iNOS, JNK, NF κ B) have been implicated in A β toxicity or A β -induced AD pathogenesis in different AD models, indicating potential as a multi-target intervention. Beyond this, curcumin has other anti-amyloid activities. Because curcumin is a good inhibitor of cytokines, it may also limit inflammatory and oxidative damage induction of BACE1, the β -secretase enzyme that makes the initial step in amyloid production [12]. Our data suggest that, under conditions of elevated oxidative damage and inflammation, curcumin can reduce BACE1 expression in vivo. Curcumin is not simply immunosuppressive, but immunomodulatory, simultaneously inhibiting cytokine and microglial activation indices related to neurotoxicity, but increasing mRNA and immunostaining for both CD11c and macrosialin, markers for microglial phagocytosis that may clear amyloid [Cole & Morihara, unpublished data]. As previously reviewed, curcumin's hydroxyls provide polar groups 19 angstroms apart- resembling chrysin and the amyloid

binding dye Congo Red. Thus, curcumin binds and labels plaques in vitro and in vivo and inhibits amyloid aggregation in vitro and amyloid accumulation in vivo [33]. Curcumin also inhibits neurotoxic A β oligomer formation and oligomer-dependent A β toxicity in vitro [33]. Curcumin's multiple anti-amyloid activities may contribute to the suppression of amyloid in vivo and its anti-amyloid activity remains effective in aged mice- even after amyloid deposits are well-established. Since this compound has multiple direct and indirect anti-amyloid actions including metal chelating, phagocytosis enhancing, antioxidant and cholesterol lowering activity (reviewed in [15, 33] and possible suppression of BACE1 induction, it is likely that no one mechanism is involved. Curcumin also inhibits known amyloid response pathways including JNK kinase and iNOS expression in vitro [5, 12, 23] and in vivo [10, 13] and in CNS (our data). Thus curcumin should be able to suppress not only A β but key aspects of the response to A β that are JNK and iNOS dependent including LTP inhibition [30], reported to depend on JNK, iNOS, and microglial radicals [31]. With safety and so many potentially relevant therapeutic activities, curcumin has to be a strong candidate for AD prevention or treatment. While we initially screened many antioxidants, including several polyphenolics in an A β infusion model and selected curcumin as the most promising candidate, one might expect utility from other dietary polyphenolics.

Omega-3 fatty acids. Neurons and synapses are highly enriched in long chain unsaturated fatty acids, the most vulnerable of these being the omega-3 fatty acid, docosahexaenoic acid (DHA, C22:6 (n-3)) with 6 double bonds. Synapse loss is a close correlate of cognitive decline in AD, and synapses are sites of A β generation and accumulation. DHA is oxidized in AD to produce elevated F4-isoprostanes or neuroprostanes [18]. Because DHA is heavily concentrated in neurons, its oxidation products are a good index of neuronal oxidative damage.

Fatty fish are the major dietary source of DHA, and reduced fish or DHA intake increases risk for AD [17]. Aged Tg2576 APP^{sw} transgene-positive mice placed from 17-22

months on a safflower oil rich, DHA-depleting diet exhibited increased oxidative damage and transgene-dependent loss of CNS DHA and massive (70-95%) loss of postsynaptic proteins like the actin-regulatory drebrin, a dendritic spine protein known to be 70-90% lost in AD [7, 27] and NR2b [6]. Greater depletion of DHA in Tg2576 may be driven by A β aggregates causing DHA oxidation. DHA depletion also caused transgene-dependent caspase activation and deficits in the neuroprotective “insulin signaling pathway” PI3-kinase>Akt) similar to AD. Large deficits observed in the PI3-K p85 α subunit observed with high safflower oil were only partially explained by mRNA loss and may be secondary to caspase activation. While DHA only partially suppressed p85 α subunit loss, it still improved neuroprotective AKT, BAD and GSK3 β phosphorylation (unpublished results), likely through facilitation of AKT translocation and activation [2]. Although we did not detect reduced inflammatory cytokines in DHA fed AD model mice, the arachidonic acid levels were significantly reduced, suggesting potential NSAID-like activity of dietary omega-3 to reduce pro-inflammatory prostaglandin intermediates. Other AD-relevant DHA actions may include effects on metabolism to form neuroprotectin D1 or other anti-apoptotic or neuroprotective metabolites [21].

DHA depletion aggravated cognitive deficits in the Morris Water Maze in the Tg2576 mouse, while DHA supplementation was protective [7] and reduced amyloid, even late in life [14]. Anti-amyloid activities include modulation of secretase activities via membrane “fluidity”/ protein mobility changes or Akt>GSK regulation of γ -secretase [25] and induction of anti-amyloidogenic transthyretin [26]. Like fish oil, DHA from algal sources can be safely taken as a supplement at high doses. All of DHA’s neuroprotective effects may also be relevant to cognitive decline in normal aging. Both DHA and EPA are also likely protective against vascular disease, one of the other major causes of age-related dementia. Since both the cost and toxicity issues are minimal, marine omega-3 lipids are suitable for primary prevention.

Conclusions. Age-related diseases frequently cause oxidative damage and inflammation that should be preventable, but plausible antioxidants and anti-inflammatory approaches have not proven useful. In particular, recent data with antioxidants like vitamin E and NSAIDs, including the COX-2 inhibitors and naproxen, have raised concerns about both their chronic safety and efficacy. Because of its potent anti-carcinogenic activity, curcumin has already been through extensive pre-clinical toxicology and clinical testing and has a very favorable safety profile [1, 13]. Because of its availability and low cost, coupled with preclinical data showing its potential for intervention at multiple sites in AD pathogenesis, curcumin is now in clinical trials in mild to moderate AD patients under an FDA approved IND by the UCLA Alzheimer Center. Omega-3 fatty acids have an even stronger safety profile, a long history of use and proven health benefits for CVD, yet typical Western diets have less than 30% of the roughly 200-300 mg of DHA recommended by expert panels. Epidemiology shows risk reduction of 60% associated with modest increases in DHA intake or plasma levels. DHA works well in slowing AD pathogenesis in mice with a human AD gene and is safe enough to include in infant formula. It should be a strong candidate for use in primary prevention.

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Competing interests statement

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