May 5th, 12:00 AM

Plasmalogen Deficiency: A Risk Factor for Dementias and Potential Treatment Target

Mitchel A. Kling
Rowan University

Mallika Mendu
Rowan University

Rima F. Kaddurah-Daouk
Duke University School of Medicine

Sheldon Jordan
Pacific Neuroscience Group

Dayan B. Goodenowe
Prodrome Sciences, Inc.

Follow this and additional works at: https://rdw.rowan.edu/stratford_research_day

Part of the Geriatrics Commons, Medical Neurobiology Commons, Nervous System Diseases Commons, Neurology Commons, Neurosciences Commons, and the Pathological Conditions, Signs and Symptoms Commons
Let us know how access to this document benefits you - share your thoughts on our feedback form.

Kling, Mitchel A.; Mendu, Mallika; Kaddurah-Daouk, Rima F.; Jordan, Sheldon; and Goodenowe, Dayan B., "Plasmalogen Deficiency: A Risk Factor for Dementias and Potential Treatment Target" (2022). *Stratford Campus Research Day*. 138.
https://rdw.rowan.edu/stratford_research_day/2022/May5/138

Plasmalogen deficiency: a risk factor for dementias and potential treatment target

Mitchel A. Kling[1,2], Mallika Mendu[1], Rima F. Kaddurah-Daouk[3], Sheldon Jordan[4], Dayan B. Goodenowe[5]

[1]Rowan School of Osteopathic Medicine; [2]Perelman School of Medicine, University of Pennsylvania; [3]Duke University School of Medicine; [4]Pacific Neuroscience Group; [5]Prodrome Sciences, Inc.
Department of Geriatrics and Gerontology, RowanSOM

ABSTRACT

Altered lipid metabolism is implicated in the risk of sporadic Alzheimer's disease (AD) and related dementias (ADRD); however, the precise mechanisms accounting for findings from observational studies remains to be fully elucidated.

Plasmalogens are a subclass of integral membrane phospholipids with unique properties that appear to play important roles relevant to the pathophysiology of AD and ADRD, including vesicle fusion necessary for synaptic neurotransmitter release, modulation of membrane fluidity and microdomain dynamics, membrane antioxidant functions, and neuroprotection. Like the more familiar phosphatides, plasmalogens are synthesized on a 3-carbon glycerol backbone; however, they differ from phosphatides by the presence of a vinyl ether linkage at the 1st (sn1) glycerol carbon atom in place of the acyl ester linkage present at sn1 in phosphatides, and at sn2 in both lipid subclasses. Plasmalogens bearing the omega-3 fatty acid docosahexaenoic acid (DHA), a key component of fish oils, are the most abundant plasmalogen species in cerebral cortex membranes.

Circulating plasmalogen levels are decreased in older individuals, and are further decreased in AD and Mild Cognitive Impairment (MCI). In addition, reduced indices of plasmalogen biosynthesis and/or remodeling are significantly correlated with elevated cerebrospinal fluid (CSF) concentrations of total tau, which is a biomarker of AD and certain other neurodegenerative diseases. This correlation suggests a functional relationship between reduced plasmalogen availability and neurodegeneration.

Endogenous plasmalogen synthesis requires the integrity of peroxisomes for the attachment of an alkyl side chain to the sn1 glycerol carbon. Decreased peroxisome function may be a key factor underlying the decrease in circulating plasmalogens with aging and with neurodegenerative diseases such as AD and ADRD.

Preclinical data indicate that oral administration of a precursor phospholipid compound, DHA-containing alkyl-diacylglycerol, or DHA-AAG, can increase circulating DHA-containing plasmalogens in a peroxisome-independent manner, as conversion to plasmalogens from this precursor requires only the endoplasmic reticulum.

We present here data showing that: 1) oral administration of a single dose of DHA-AAG at 100mg/kg to 6 (4M/2F) healthy subjects aged 23-56 increased circulating plasmalogen levels by 80% within 24 hours; and 2) daily oral administration of DHA-AAG to 22 persons (11M/11F), aged 37-84 (mean= 69) yr, with mild to moderate cognitive impairment [CDR: 0.5 (N=14); 1 (N=4); 2 (N = 4)] on an ascending-dose schedule of 1.0 ml/day for 30 days, followed by 2.0 ml/day for 60 days, followed by 4.0 ml for 30 days, increased serum DHA plasmalogens by > 2-fold by the end of the treatment period. DHA-AAG was well-tolerated by both groups of individuals in these 2 studies.

These findings suggest that DHA-AAG may be a useful agent for correcting plasmalogen deficiency associated with aging and aging-associated cognitive disorders.

Future studies will examine the effect of plasmalogen repletion with DHA-AAG on cerebrospinal fluid plasmalogen concentrations, and effects on cognitive function and other clinical outcomes.

INTRODUCTION

Altered lipid metabolism is implicated in the pathophysiology of Alzheimer's disease (AD) and related dementias (ADRD).
- The underlying mechanism(s) remain to be fully elucidated.
- One possible mechanism: altered balance between cholesterol and phospholipids such as plasmalogens containing very long chain fatty acids such as the omega-3 fatty acid docosahexaenoic acid (DHA).

Plasmalogens are **ether lipids** that are **integral membrane components**; those containing DHA
- are enriched in neuronal membranes
- play critical roles in **neuronal synaptic function**, as **membrane antioxidants**, and in other AD-relevant processes.

The liver is a key site for synthesis of plasmalogens incorporated into circulating lipoproteins and delivered to peripheral sites, including the CNS.

Activity of liver peroxisomes is crucial for plasmalogen synthesis; deficient synthesis can reduce plasmalogens' availability to the brain and contribute to impaired synaptic function and cognition.

Circulating plasmalogens are known to be progressively lower in older adult age cohorts, particularly in those 60 years and older.

Previous studies have found reductions in circulating and postmortem brain plasmalogen levels in Alzheimer-type dementias.
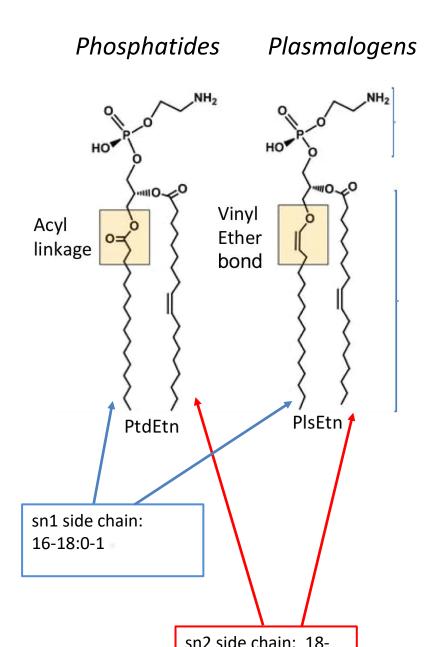
AIMS

1. Examine predetermined ratios and indices of circulating ethanolamine plasmalogen synthesis and/or remodeling in patients with dementia or mild cognitive impairment (MCI) due to Alzheimer's disease (AD)
2. Examine the relationship between indices of circulating ethanolamine plasmalogen synthesis and/or remodeling on cerebrospinal fluid (CSF) biomarkers of AD pathology (amyloid-beta, tau)
3. Examine the effect of an orally-absorbed plasmalogen precursor lipid (DHA-AAG) on circulating plasmalogen levels and relevant ratios.

FIGURES AND RESULTS

Serum/Plasma Plasmalogen Indices in AD, MCI, and Cognitively Normal (CN) Subjects: Participants Characteristics

ADNI-1, -GO, -2

Parameter	N	Total group (N=1545)	CN (N=364)	SMC (N=98)	EMCI (N=282)	LMCI (N=497)	AD (N=304)	Test Statistic
Age (yr) (SD)	1545	73.67 (7.23)	74.63 (5.79)	72.24 (5.63)	71.14 (7.52)	74.09 (7.58)	74.65(7.80)	F=14 d.f.=4,1540 P<0.001
% Male (#)	1545	54% (840)	48% (176)	42% (41)	55% (154)	61% (304)	54% (165)	Chi-square=21 d.f.=4 P<0.001
Education (yr) (SD)	1545	15.91 (2.85)	16.30 (2.77)	16.73 (2.56)	15.98 (2.66)	15.86 (2.90)	15.20 (3.01)	F=8.2 d.f.=4,1540 P<0.001
% APOE4 + (#)	1545	47% (726)	28% (103)	33% (32)	43% (120)	54% (270)	66% (201)	Chi-square=117 d.f.=4 P<0.001
ADAS/Cog13 score (SD)	1545	27.17 (2.66)	9.18 (4.39)	8.82 (4.08)	12.57 (5.39)	18.70 (6.64)	29.74 (8.11)	F=536 d.f.=4,1529 P<0.001
MMSE score (SD)	1545	27.17 (2.66)	29.08 (1.13)	29.00 (1.20)	28.33 (1.59)	27.16 (1.82)	23.23 (2.01)	F=449 d.f.=4,1540 P<0.001

U. Penn Alzheimer's Disease Center

Parameter	N (N = 112)	CN (N = 51)	MCI (N = 18)	AD (N = 43)	Test statistic and Group Effect
Age (mean ± std)	112	69.9 ± 9.2	68.1 ± 9.8	70.9 ± 5.9	71.5 ± 9.3	F=1.13 d.f.=2,109 P = 0.186
Gender, # F (% F)	112	59 (52.7%)	30 (59%)	4 (22%)	25 (58%)	Chi-square=8.0 d.f.=2 P = 0.018
Yrs. of Education (mean ± std)	112	16.0 ± 2.9	16.3 ± 2.8	15.7 ± 3.2	15.7 ± 2.9	F=0.70 d.f.=2,109 P > 0.2
# APOE4 + (%)	82	31 (37.8%)	13 (26.0%)	4 (36.4%)	14 (66.7%)	Chi-sq=10.3 d.f.=2 P=0.006
MMSE (mean ± std)	109	25.72 ± 5.34	29.16 ± 1.11	27.35 ± 1.46	20.98 ± 5.85	F=56.1 d.f.=2,106 P < 0.001

PE Platform Analyte Ratios and Indices Used in Statistical Analyses

Ratio/Index Name	Numerator	Denominator	PL-PX	PL/PE	PBV
PE 226_224	PE.P.16.0_22.6	PE.P.16.0_22.4	1		1
PE 205_224	PE.P.18.0_20.5	PE.P.16.0_22.4	1		1
PE 205_226	PE.P.18.0_20.5	PE.P.16.0_22.6	1		1
PE 226_PE226	PE.P.16.0_22.6	PE.16.0_22.6		1	1
PE 205_PE226	PE.P.18.0_20.5	PE.16.0_22.6		1	1

Plasmalogen levels impact APP processing resulting in increased amyloid production

Plasmalogen levels impact vesicular fusion rates which could lead to cognitive impairment

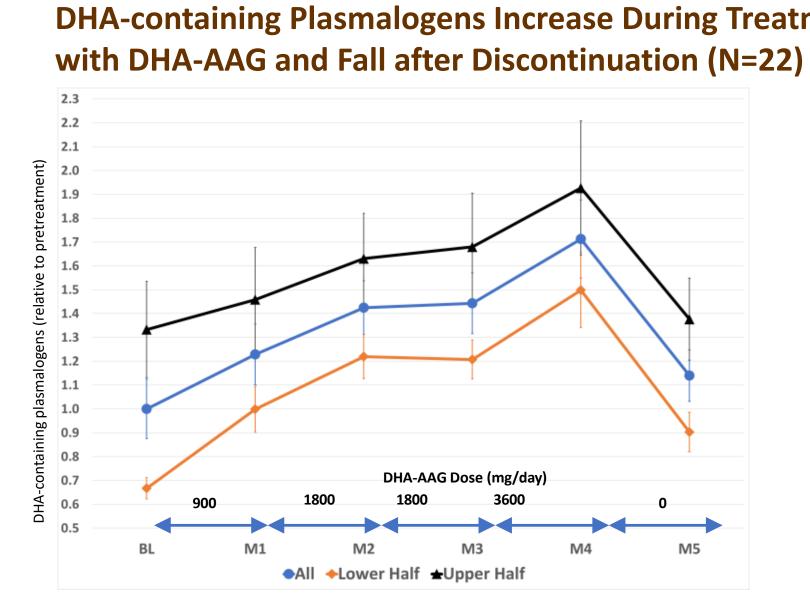Plasmalogen levels impact cholesterol esterification and efflux

Low Levels of Plasmalogen Indices Are Associated with *Increased* Likelihood of AD and late MCI, and With *Greater* Cognitive Impairment

Plasmalogen/Phosphatide Indices and Component Ratios in ADNI and Penn Cohorts:

ADNI

Index/ratio	AD vs. CN		LMCI vs.CN		AD vs. LMCI		ADAS-Cog13		MMSE	
	coefficient	p value	q value	coefficient	p value	q value	coefficient	p value	q value	coefficient	p value	q value	coefficient	p value	q value
COMP_PBV	-0.2550	0.0050	0.0305	-0.2939	1.39E-04	7.97E-04	-0.0005	0.9944	0.9944	-0.1302	6.24E-06	2.77E-05	0.0379	6.50E-09	2.60E-08
COMP_PL_PX	-0.2436	0.0070	0.0305	-0.3300	2.89E-06	1.73E-04	-0.0005	0.9944	0.9944	-0.1349	3.24E-06	1.94E-05	0.0397	1.28E-09	1.25E-08
COMP_PL_PE	-0.1117	0.2277	0.3036	0.1323	0.0846	0.1450	-0.2786	6.15E-04	0.0025	0.0116	0.6961	0.7594	-0.0031	0.6464	0.8872
PL205_224	-0.2401	0.0076	0.0305	-0.1703	0.0228	0.0557	-0.1038	0.1764	0.2352	-0.0767	0.0075	0.0226	0.0323	5.99E-07	1.80E-06
PL205_226	-0.1034	0.2570	0.3084	0.1511	0.0508	0.1016	-0.3314	6.90E-05	4.14E-04	0.0592	0.0439	0.1055	0.0028	0.6765	0.8872
PL226_PE226	-0.2180	0.0154	0.0463	-0.3795	2.70E-06	3.24E-05	0.1247	0.1097	0.1646	-0.1484	2.95E-07	9.54E-06	0.0392	2.08E-09	1.25E-08
PL226_PE226	-0.0747	0.4181	0.4561	0.1102	0.1520	0.2028	-0.1963	0.0142	0.0244	-0.3676	1.17E-05	1.41E-04	0.0420	0.1501	0.2251
PL205_PE226	-0.1203	0.1901	0.2852	0.1746	0.0232	0.0557	-0.3676	1.17E-05	1.41E-04	0.0420	0.1501	0.2251	-0.0009	0.8910	0.9339

Penn

Index	AD | CN		MMSE	
	coefficient	p value	coefficient	p value
COMP_PBV	-0.7446	0.0296	0.1042	0.2084
COMP_PL_PX	-0.3480	0.1936	0.0443	0.5426
COMP_PL_PE	-0.6401	0.0191	0.1133	0.0949

Plasmalogen/Phosphatide Indices in ADNI and UPenn: *Low* Levels are Associated With *More* "AD-Like" Pattern of CSF Total Tau and Its Ratio to CSF Abeta[1-42]
No significant relationship of these indices with CSF Abeta[1-42]

ADNI:

Index	CSF Total tau (Ttau)			CSF Aβ[1-42]			Log[10](CSF Ttau/CSF Aβ[1-42])		
	Coefficient	P value	Q value	Coefficient	P value	Q value	Coefficient	P value	Q value
COMP_PBV	-6.994	7.77E-06	3.11E-05	1.970	0.173	0.694	-0.0408	4.39E-06	1.76E-05
COMP_PL_PX	-7.193	5.55E-06	3.11E-05	2.048	0.162	0.694	-0.0422	2.73E-06	1.64E-05
COMP_PL_PE	-0.038	0.981	0.981	0.518	0.727	0.988	-0.0004	0.9664	0.9664

UPenn:

Index	CSF TTau		CSF Abeta1-42		Ttau Abeta Ratio	
	coefficient	p value	coefficient	p value	coefficient	p value
COMP_PBV	-6.4904	0.3490	-4.1657	0.7276	-0.0316	0.5422
COMP_PL-PX	0.8036	0.8865	-9.1439	0.3451	0.0287	0.4938
COMP_PL/PE	-12.2309	0.0305	7.0682	0.4723	-0.0973	0.0206

Test Compound:
1-*O*-alkyl-2,3-*O*-diacyl-sn-glycerol (AAG)

Structure drawn using Lipidmaps Structure Drawing tool
www.lipidmaps.org

- Contains 16- or 18-carbon alkyl ether linkage to glycerol carbon sn1
- Contains DHA (22:6) or other acyl linkages at glycerol carbons sn2 and sn3
- Orally absorbed, converted to DHA-containing plasmalogens in liver
- Enzymatic conversion of sn1 alkyl to vinyl ether linkage occurs in ER, **not dependent on peroxisome function**
- Naturally produced in plasmalogen synthesis pathway; proposing natural product status to FDA
- Compound has been synthesized; letter to FDA being prepared including composition/purity and demonstrating production by cGMP methods

DHA-containing Plasmalogens Increase During Treatment with DHA-AAG and Fall after Discontinuation (N=22)

CONCLUSIONS

- These data provide further evidence that altered ethanolamine plasmalogen metabolism contributes to the risk of cognitive impairment in in AD and MCI.
- Failure of plasmalogen synthesis involving liver peroxisomes may adversely affect CNS synaptic and antioxidant functions and contribute to cognitive impairment in AD.
- An orally-absorbed DHA-containing alkyl-diacylglycerol (DHA-AAG) can increase circulating plasmalogens in healthy subjects and in patients with aging-associated cognitive decline.

SELECTED REFERENCES

. [1] Goodenowe DB, Cook LL, Liu J, Lu Y, Jayasinghe DA, Ahiahonu PW, et al. Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. Journal of lipid research. 2007;48:2485-98.
[2] Goodenowe DB, Haroon J, Kling MA, Zielinski M, Mahdavi K, Habelhah B, et al. The Effect of Oral Plasmalogen Precursor Treatment on Blood Plasmalogens, Oxidative Stress Biomarkers, Cognition, and Mobility in Cognitively Impaired Persons. Frontiers in Cell and Developmental Biology. 2022 (in revision).
[3] Goodenowe DB, Senanayake V. Relation of Serum Plasmalogens and APOE Genotype to Cognition and Dementia in Older Persons in a Cross-Sectional Study. Brain Sci. 2019;9.
[4] Gregoire L, Smith T, Senanayake V, Mochizuki A, Miville-Godbout E, Goodenowe D, et al. Plasmalogen precursor analog treatment reduces levodopa-induced dyskinesias in parkinsonian monkeys. Behav Brain Res. 2015;286:328-37.
[5] Han X. Lipid alterations in the earliest clinically recognizable stage of Alzheimer's disease: implication of the role of lipids in the pathogenesis of Alzheimer's disease. Current Alzheimer research. 2005;2:65-77.
[6] Han X, Holtzman DM, McKeel DW, Jr. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. Journal of neurochemistry. 2001;77:1168-80.
[7] Huynh K, Lim WLF, Giles C, Jayawardana KS, Salim A, Mellett NA, et al. Concordant peripheral lipidome signatures in two large clinical studies of Alzheimer's disease. Nat Commun. 2020;11:5698.
[8] Kling MA, Goodenowe DB, Senanayake V, MahmoudianDehkordi S, Arnold M, Massaro TJ, et al. Circulating ethanolamine plasmalogen indices in Alzheimer's disease: Relation to diagnosis, cognition, and CSF tau. Alzheimers Dement. 2020.
[9] Kling MA, Trojanowski JQ, Wolk DA, Lee VM, Arnold SE. Vascular disease and dementias: paradigm shifts to drive research in new directions. Alzheimers Dement. 2013;9:76-92.
[10] Maeba R, Maeda T, Kinoshita M, Takao K, Takenaka H, Kusano J, et al. Plasmalogens in human serum positively correlate with high- density lipoprotein and decrease with aging. Journal of atherosclerosis and thrombosis. 2007;14:12-8.
[11] Reitz C. Dyslipidemia and the risk of Alzheimer's disease. Current atherosclerosis reports. 2013;15:307.
[12] Wood PL, Khan A, Mankidy R, Smith T, Goodenowe DB. Plasmalogen deficit: a new and testable hypothesis for the etiology of Alzheimer's disease. In: De La Monte S, editor. Alzheimer's Disease Pathogenesis - Core Concepts, Shifting Paradigms, and Therapeutic Targets. Rijeka, Croatia: InTech; 2011. p. 561 - 88.
[13] Wood PL, Mankidy R, Ritchie S, Heath D, Wood JA, Flax J, et al. Circulating plasmalogen levels and Alzheimer Disease Assessment Scale-Cognitive scores in Alzheimer patients. Journal of psychiatry & neuroscience : JPN. 2010;35:59-62.
[14] Wood PL, Smith T, Lane N, Khan MA, Ehrmantraut G, Goodenowe DB. Oral bioavailability of the ether lipid plasmalogen precursor, PPI-1011, in the rabbit: a new therapeutic strategy for Alzheimer's disease. Lipids in health and disease. 2011;10:227.

METHODS

- We measured 8 phosphoethanolamines (PEs), including 4 ethanolamine plasmalogens and 4 closely-related phosphatidylethanolamines, in
  - >1500 baseline serum specimens from Alzheimer's Disease Neuroimaging Initiative (ADNI)-1,-GO, and -2 cohorts
  - 112 plasma samples from Penn ADC and CNDR Biobank (AD, MCI, and CN).
- Phosphatides: PtdEtn 16:0/18:3, **18:0/20:5, 18:0/22:4, 16:0/22:6**
- Plasmalogens: PlsEtn 16:0/18:2, **18:0/20:5, 16:0/22:4, 16:0/22:6**
- Stable isotope dilution, flow-injection analysis (FIA)-mass spectrographic (MS) method on an Ionics 3Q tandem mass spectrometer operating in the **negative ionization** atmospheric pressure chemical ionization (APCI) mode.
- Biochemical measurements carried out by Drs. Dayan Goodenowe and colleagues (now at Prodrome Sciences, Inc.)
- DHA-AAG was synthesized from ingredients generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA) under cGMP conditions by Dr. Goodenowe at Prodrome Sciences, Temecula, CA
- DHA-AAG was orally administered in a single dose of 100 mg/kg to 6 healthy subjects (4M/2F) aged 23-56. Blood was drawn for the target plasmalogen (PlsEtn 16:0/22:6) and related lipids at baseline and at 2, 6, 12, and 24 hours after administration.
- DHA-AAG (900 mg/mL) was given orally to 22 persons (11M/11F), ages 37-84 (mean = 69 yr) with mild to moderate cognitive impairment [CDR: 0.5 (N=14); 1 (N=4); 2 (N = 4)] on an ascending-dose schedule of 1.0 ml/day for 30 days (days 1-30), 2.0 ml/day for 60 days (days 31-90), and 4.0 ml for 30 days (days 91-120), followed by a 30-day wash-out period (days 121-150).

ACKNOWLEDGMENTS