

Lipidomics of Alzheimer's disease: a liver peroxisomal dysfunction in the metabolism of omega-3 fatty acids

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Abstract : *Reduced brain levels of docosahexaenoic acid (DHA), a neurotrophic and neuroprotective fatty acid, might contribute to cognitive and visual decline. A multi-organ lipidomic approach revealed that the levels of free DHA, DHA-containing phospholipids precursors and DHA-metabolites were decreased in various brain regions of subjects with Alzheimer's disease (AD), compared to control subjects. Furthermore, liver DHA content was lower in AD patients than control subjects. Shorter chain omega-3 fatty acids precursors for DHA, were elevated in liver of AD patients, whereas expression of peroxisomal D-bifunctional protein, which catalyzes the conversion of tetracosahexaenoic acid into DHA, was selectively reduced. Lower DHA levels in the liver were associated with cognitive impairment and with higher prevalence of eye-diseases. The evidence presented herein indicate that a deficit in liver biosynthesis of DHA possibly lessens the flux of this neuroprotective fatty acid to brain and eyes, contributing to cognitive and visual impairments.*

Key words : *DHA, omega-3 fatty acids, liver, Alzheimer's disease, cognition, eyes*

Emerging epidemiological and molecular evidence indicates that a variety of lipid abnormalities are associated with Alzheimer's disease (AD) and, possibly, involved in its pathogenesis (Cunnane *et al.*, 2009). Risk factors for AD – such as aging, genetic vulnerability and environmental factors – alter specific lipid pathways in brain and peripheral tissues, and these alterations may influence in turn AD progression (*figure 1*). For example, the inheritance of certain forms of the lipid-carrier protein apolipoprotein E (APOE) increases the risk of AD and brain tissue from AD patients shows significant changes in lipid composition. Among the alterations most consistently observed in AD are decreases in levels of docosahexaenoic acid (C22:6, DHA), an omega-3 fatty acid derived from diet or synthesized in the liver. Although a large number of pre-clinical and clinical evidence suggests that DHA decreases the risk of developing AD, it is still unclear

how AD affects the metabolism of this essential omega-3 fatty acid.

Further understanding of the DHA metabolism in a complex disorder such as AD requires a more integrated lipidomics strategy, which can be regarded at three different levels (*figure 2*). First, from the molecular point of view, putting together lipidomics information with genomic, transcriptomic, proteomics and metabolomics data would give access to a global view of molecular changes underlying age-related cognitive decline and AD (*figure 2*). The second level of integration is from a more functional perspective, taking into account the demographic characteristics and clinical information of the subjects under study (*figure 2*). This level of information would allow relating the lipid changes with the initiation and progression of cognitive decline and AD. The third possible level of integration is from a

more holistic prospect, taking under consideration the interaction between peripheral organs and brain (*figure 2*). Indeed, most of the brain DHA derive from peripheral tissues through either biosynthetic processes (e.g., in liver) or dietary absorption. Consequently, systemic changes in lipid metabolism occurring in the peripheral tissues might strongly affect the structure and functioning of the brain. Only few studies have dealt with multi-organ interaction in AD and, at the moment, the role of peripheral lipid metabolism during cognitive decline and AD development is still unclear.

Together, this integrative holistic approach, which could be called "Systems Lipidomics" (*figure 2*), could open a new dimension for both differential and functional analyses of multifactorial disorders. In the present review, we analyze the application of a multi-organ

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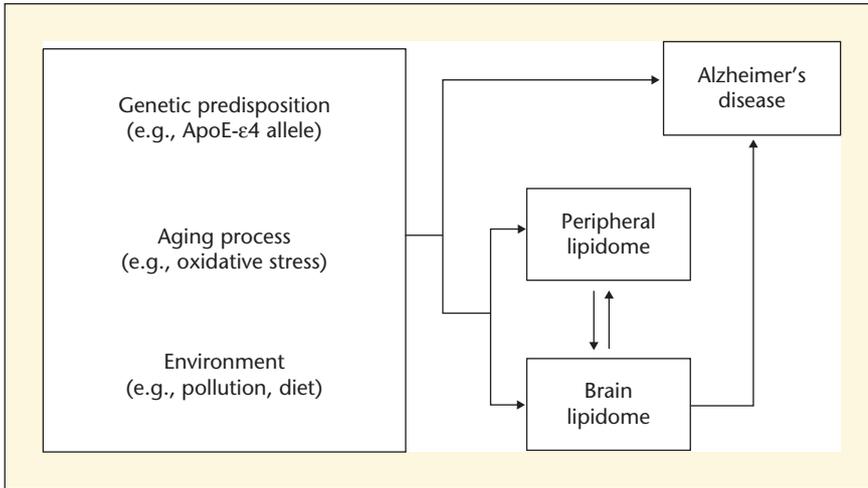


Figure 1. Scheme illustrating the multifactorial etiology of AD. Risk factors for AD (which include genetic-predisposition, aging and environmental factors such as a nutritional deficit in omega-3 fatty acids) influence interacting lipidomes throughout the body. Over time, accumulating lipid compounds compound with those factors to increase the risk for AD.

Systems Lipidomics approach to study the metabolism of omega-3 fatty acids in AD.

Free DHA levels in AD brain

With some inconsistency, deficits in DHA-containing phospholipids have been

reported in AD brains (Cunnane *et al.*, 2009). Only one previous study reported the levels of free DHA to be lower in hippocampus of AD patients than control subjects (Lukiw *et al.*, 2005). We measured the brain levels of free DHA by liquid chromatography/mass spectrometry (LC/MS) from mid-frontal cortex samples of 17 control subjects and 37 AD patients, which were matched for age

and post-mortem interval. DHA levels were significantly lower ($P < 0.05$) in mid-frontal cortex of AD patients compared to those of control subjects (figure 3 A) (Astarita *et al.*, 2010).

DHA-containing Phospholipids in AD brains

Free DHA is in equilibrium with DHA-containing glycerophospholipids (figure 4), which could be hydrolyzed into free DHA by phospholipases A₂. The levels of the 1-stearoyl, 2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine were significantly lower in mid-frontal cortex of AD patients than control subjects (figure 3B).

DHA metabolites in AD brains

Free DHA may be converted by a lipoxygenase-mediated pathway into neuroprotectin D1 (NPD1) (figure 4), a lipid signal with marked anti-inflammatory and neuroprotective properties (Hong *et al.*, 2003). Previous work by Lukiw *et al.* (2005) reported NPD1 levels are decreased in temporal lobes of subjects with AD. In our study, we observed that NPD1 levels were strikingly decreased in mid-frontal cortex of subjects with AD compared to control subjects ($P = 0.0018$). Because NPD1 has been implicated in the survival and repair of neuronal cells, such deficit could contribute to the brain damage and neural death observed in AD brains.

DHA levels in AD livers

Liver's capacity to generate DHA is critical to keep the bioavailability of this fatty acid within a normal range, especially when food does not provide a sufficient supply of this nutrient (Rapoport *et al.*, 2007; Rapoport *et al.*, 2007). A properly functioning liver synthesizes DHA from shorter-chain omega-3 precursors, such as α -linolenic acid (ALA, C18:3, omega-3) and eicosapentaenoic acid (C20:5, omega-3) (Rapoport *et al.*, 2007; Scott and Bazan, 1989; Burdge and Calder, 2005) (figure 5). A cascade of elongase and desaturase enzymes localized in the endoplasmic reticulum of the hepatocyte progressively add carbon units and

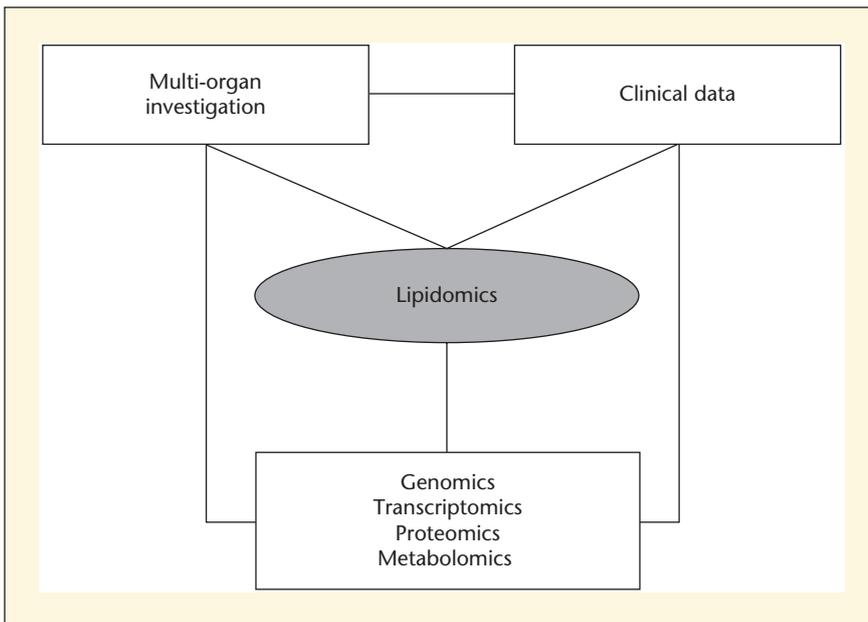


Figure 2. Systems Lipidomics for descriptive and functional analysis of complex disorders. Integration at both molecular and physiopathological levels is required for the understanding of complex diseases. It is to be noted that this schematic representation applies not only to AD, but to most human pathologies, and can be used for the identification and validation of novel biomarkers and therapeutic targets.

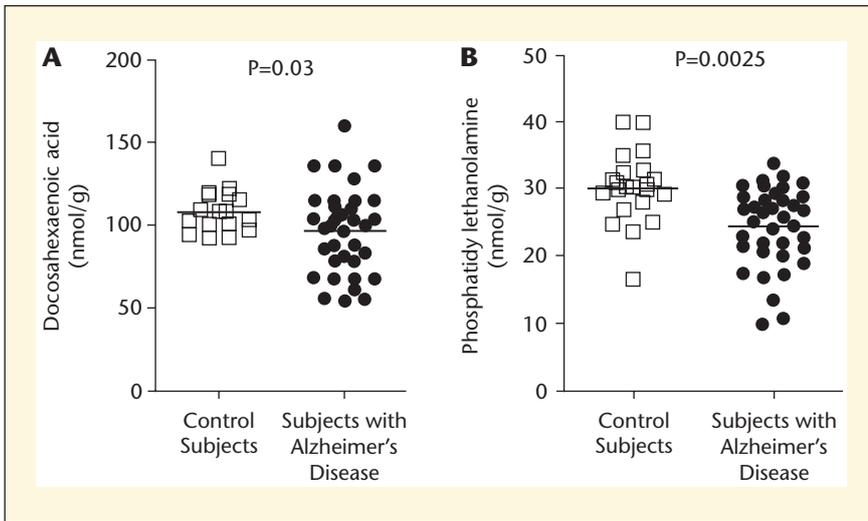


Figure 3. DHA content in AD brain. Levels of DHA (A) and DHA-containing phosphatidylethanolamine (1-stearoyl,2-arachidonoyl-3-glycerophosphoethanolamine, B) in mid-frontal cortex of control subjects (open squares) and subjects with AD (closed circles). Statistical analyses were conducted using a linear regression analysis adjusting for age, gender and post mortem interval.

double bonds to shorter-chain omega-3 fatty acids, producing the very-long-chain tetracosahexaenoic acid (C24:6, omega-3). This fatty acid is transported into peroxisomes and then converted to DHA by the sequential action of acyl-coenzyme A oxidases, D-bifunctional protein (DBP) and peroxisomal thiolases (Voss *et al.*, 1991, Sprecher *et al.*, 1995, Moore *et al.*, 1995, Su *et al.*, 2001) (figure 5). Liver-derived DHA reaches the

eyes and brain through the general circulation, probably bound to lipoprotein transporters, such as albumin (Scott and Bazan, 1989) (figure 4). Consequently, changes in DHA metabolism in the liver might strongly affect the structure and functioning of tissues that heavily rely on DHA bioavailability (*i.e.*, the eyes and the brain). Current evidence indicates that the liver-mediated DHA biosynthesis may be suffi-

cient (and indeed essential) for human DHA requirements (Rapoport and Igarashi, 2009; Scott and Bazan, 1989). Indeed, human brain consumes DHA at the incredible slow rate of 3.8 mg/day (Umhau *et al.*, 2009). Considering that 0.5-10% of ingested ALA is converted in DHA, and an average ingestion of 1,400 mg/day of ALA (Albert *et al.*, 2005), the liver is able to synthesize DHA at rates of 7-140 mg/day, 1.8-36-fold, respectively, the human brain requirement (Umhau *et al.*, 2009). It appears, however, that an overall healthy liver is required for optimal DHA biosynthesis. Indeed, it has been reported that during conditions of hepatic stress such as in chronic alcohol intake (Pawlosky and Salem Jr, 1999, Frye and Salloum, 2006), and liver steatosis and injury (Araya *et al.*, 2004, Allard *et al.*, 2008), the levels of hepatic DHA are compromised.

To examine whether the metabolic conversion of ALA into DHA is altered in AD, we recently quantified the omega-3 fatty acids in liver samples from a cohort of 9 control subjects and 14 AD patients (Astarita *et al.*, 2010). DHA levels were lower in liver of AD patients compared with control subjects (table 1). The levels of the shorter chain omega-3 fatty acids that function as precursors for DHA were increased in liver of AD patients compared with control subjects (table 1). These results suggested that a dysfunction in the biosynthesis of DHA might be involved in the deficit of this essential fatty acid.

DHA biosynthesis in liver

To better understand the molecular step that may be dysregulated in the liver omega-3 metabolism, we performed mRNA analyses of targeted genes involved in the biosynthesis of DHA (table 2). The targeted analysis included both cytosolic enzymes, which are involved in the elongation and desaturation steps, and peroxisomal enzymes, which are involved in the peroxisomal biogenesis, fatty acid transport, and fatty acid beta-oxidation. The mRNA encoding the peroxisomal enzyme D-bifunctional protein (DBP, hydroxysteroid (17-beta) dehydrogenase 4, HSD17B4) was lower in the livers of AD patients than control subjects (table 2). No changes were observed for the other enzymes. Our results are in agreement with the reports showing a decline in the

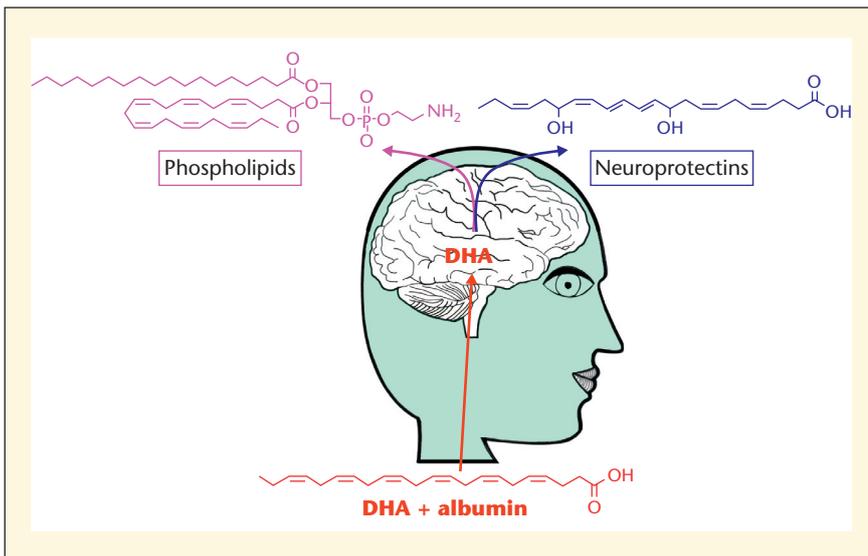


Figure 4. DHA transport through the blood to eye and brain tissues. Liver-derived DHA reaches the eyes and the brain (two tissues highly enriched in DHA) through the general circulation, most likely as a complex with lipid-binding proteins (*e.g.*, albumin) that are also synthesized in the hepatocytes. Once in the brain, free DHA is in equilibrium with complex lipids (*e.g.*, phosphatidylethanolamines) and metabolites (*e.g.*, neuroprotectins). The figure shows chemical structures of DHA-derived lipids quantified in our analyses.

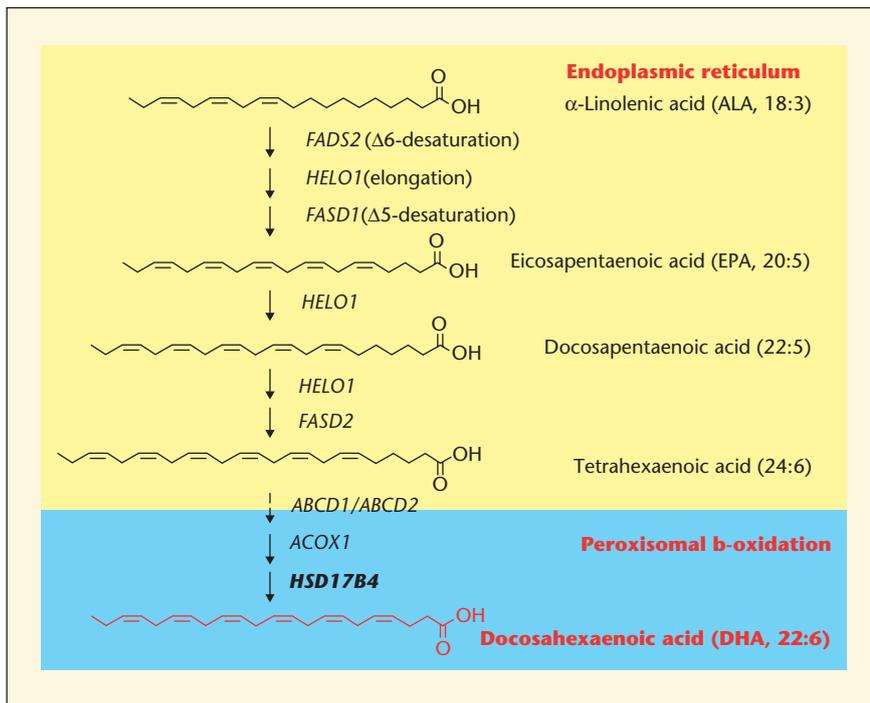


Figure 5. Overview of DHA biosynthesis in liver. Diet-derived ALA (C18:3 omega-3) is transformed into tetrahexaenoic acid (C24:6 omega-3) by the sequential action of $\Delta 6$ and $\Delta 5$ desaturases (encoded by the FADS2 and FADS1 genes, respectively) and elongases (such as that encoded by the HELO1 gene) present in the endoplasmatic reticulum. Tetrahexaenoic acid is transported into peroxisomes (shaded area), presumably by proteins encoded by the ABCD1 or ABCD2 genes, and then converted into docosahexaenoic acid (DHA, C22:6 omega-3) by sequential action of acyl coenzyme-A oxidase (encoded by the ACOX1 gene), D-bifunctional protein (encoded by the HSD17B4 gene), and various peroxisomal thiolases (not shown). The figure shows chemical structures of fatty acids quantified in our analyses.

peroxisomal function associated with aging (Youssef and Badr, 1999). A peroxisomal dysfunction could also explain the decrease in the synthesis of both plasmalogens (Zoeller and Raetz, 1986; Martinez, 1990; Favrelele *et al.*, 2000) and DHA (Burdge and Calder, 2005; Burdge *et al.*, 2003; Burdge *et al.*, 2002) observed in elderly people. Overall, this evidence supports the hypothesis that peroxisomal DHA biosynthesis may have a significant role in aging and AD.

Liver DHA is linked to cognitive impairment

Several, albeit not all, epidemiological and clinical studies suggest that higher intake of DHA decreases the risk of cognitive decline and dementia in elderly adults. Notably, our study revealed that liver DHA correlated with the most recent MMSE and global deterioration scale scores. The ratios were positively correlated with MMSE scores ($r=0.78$;

$P<0.0001$) and negatively correlated with global deterioration scale grades ($r=0.48$; $P=0.004$). Although the increased availability of DHA might directly affect the brain lipid composition and functioning, it is likely, however, that the positive effects excited by DHA are the result of multiple signaling events, many of which remain to be discovered. For example, DHA is known to play important roles in the cardiovascular system, which in turn may contribute to the clinical manifestation and the pathology of AD in the brain (Milionis *et al.*, 2008; Beach *et al.*, 2007). The roles of DHA on the vascular component and the cerebral parenchyma itself, however, have not been systematically explored (de la Torre, 2004).

Liver DHA is linked to eye diseases

DHA plays an essential role in eye health. Very high levels of DHA are present in the retina, accounting for over half the total fatty acyl groups present in the phospholipids of rod outer segment membranes. DHA turnover in the retina is surprisingly slow and high levels of DHA appear to be maintained despite reductions in dietary intakes of omega-3 fatty acids. In this context, a blood stream "long loop" connect the supply of DHA to the biogenesis of excitable and photoreceptor membranes (Bazan, 1990). In retinal degenerative diseases, a shortage of blood DHA has been demonstrated, and a failure of the "long loop" from the liver is suggested to underlie these changes (Bazan *et al.*, 1986). Notably, the retina of patients with peroxisomal disorders has virtually no DHA (Martinez, 1992) and visual improvement has been obtained in these patients using DHA therapy (Noguer and Martinez, 2009).

Table 1. Levels of free omega-3 fatty acids (nmol/g) in liver of control subjects and subjects with AD.

Omega-3 Fatty acid	Control subjects	Subjects with AD	P-value
	Mean \pm SD; N=9	Mean \pm SD; N=14	
ALA (C18:3)	28.55 \pm 7.88	36.72 \pm 22.05	0.152
Eicosapentaenoic (C20:5)	44.57 \pm 14.74	67.4 \pm 29.46	0.012
Docosapentaenoic (C22:5)	21.51 \pm 8.97	32.39 \pm 13.82	0.019
Tetrahexaenoic (C24:6)	0.73 \pm 0.17	0.9 \pm 0.21	0.041
DHA (C22:6)	324.83 \pm 122.89	204.64 \pm 74.62	0.011

Statistical analyses were conducted using a linear regression analysis adjusting for age, gender and post mortem interval.

Table 2. Expression of genes involved in DHA biosynthesis and peroxisomal function in liver of control subjects and subjects with AD.

Gene	Control Subjects	Subjects with AD	P-value
Symbol	Mean±SD; N = 9	Mean±SD; N=14	
FADS2	0.02±0.029	0.016±0.0099	0.851
HELO1	0.011±0.012	0.006±0.0046	0.265
FADS1	0.037±0.061	0.024±0.026	0.693
ABCD1	0.027±0.034	0.02±0.024	0.500
ABCD2	8e-04±0.001	0.0011±0.0013	0.567
ACOX1	0.2±0.24	0.31±0.68	0.782
HSD17B4	0.18±0.045	0.14±0.046	0.048
PEX13	0.041±0.017	0.037±0.017	0.569
PEX14	0.0087±0.004	0.013±0.022	0.700
PEX19	0.018±0.018	0.012±0.0054	0.291

Statistical analyses were conducted using a linear regression analysis adjusting for age, gender and RNA integrity number.

Increasing evidence shows that, besides the brain, also the eyes, and particularly the retina, are affected in AD (Guo *et al.*, 2010; Hodge *et al.*, 2005). In our population, the prevalence of eye-diseases (i.e., a combination of cataract, glaucoma and macular degeneration) was 78.5% in AD patients versus 33.5% in control subjects. Lower DHA levels in the liver were associated with higher prevalence of eye-diseases (P=0.0016), indicating a previously unrecognized association between hepatic DHA availability and eye-health. These results emphasize the potential role of liver-derived DHA in visual health.

Conclusion

The use of a multi-organ systems lipidomics approach is contributing to our understanding of the regulation of DHA metabolism in AD. It appears that a reduced DHA bioavailability – due to a dysfunctional liver biosynthesis – leads to a series of molecular and functional changes that might contribute to the cognitive and visual impairments observed in AD patients. Further investigation on the role of the hepatic peroxisomal metabolism in brain and eye health is currently underway.

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