Mecanismo de acción de Souvenaid: nuevos datos preclínicos

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Synapse loss is structural basis of deficits in people with AD; our lead for intervention target

Physical Basis of Cognitive Alterations in Alzheimer’s Disease: Synapse Loss Is the Major Correlate of Cognitive Impairment

Reduced number of synapses

Synapse loss AD is confirmed in >30 publications
Meta-analysis of synapse loss and synaptic pathology in AD

Meta-analysis highlights the presence of synapse loss and synaptic pathology in AD

• Synapse numbers are lower in AD
• A variety of synaptic protein markers are lower in AD
Insight behind our approach: Dietary precursor control of neural membrane synthesis

The Kennedy pathway for biosynthesis of neuronal membrane

\[ \text{EPA} \xrightarrow{\text{PL Choline}} \text{PHOSPHOCHOLINE} \xrightarrow{\text{Uridine}} \text{CTP} \xrightarrow{\text{CDP-CHOLINE}} \text{DAG} \xrightarrow{\text{brain PHOSPHATIDYLCHOLINE}} \text{increased membrane formation} \]

Synapses consist principally of neuronal membranes

Axon terminal

Dendritic spine

Neurite


Prof Richard Wurtman at MIT, Boston, USA
Insight behind our approach: Dietary precursor control of neural membrane synthesis

The Kennedy pathway for biosynthesis of neuronal membrane

- EPA
- DHA
- PL Choline
- Uridine
- PHOSPHOCHOLINE
- CTP
- CDP-CHOLINE
- DAG
- brain PHOSPHATIDYLCHOLINE

Synapses consist principally of neuronal membranes

Cholesterol
Proteins
Intracellular side
Phospholipids


Prof Richard Wurtman at MIT, Boston, USA
Dietary precursors can be rate-limiting: synergy between dietary precursors

**Brain phospholipids (PE)**

![Graph showing brain phospholipids (PE) levels for different treatments with control (choline), UMP, DHA, and UMP + DHA.](image)

B-vitamins enhance the bioavailability of DHA and choline via the PEMT pathway

Dietary B-vitamin enrichment leads to elevated plasma choline levels

Dietary B-vitamin enrichment leads to elevated plasma DHA levels
Dietary phospholipids increase precursor availability

**Phospholipids as cofactor**

Dietary phospholipids enhance the absorption of DHA and EPA from the gut into the enterocytes and the lymph → increased availability of DHA and EPA


Means without a common letter differ, p < 0.05
Dietary phospholipids increase precursor availability

**Phospholipids as a precursor**

Dietary phospholipids are digested into several phospholipid precursors:

- Fatty acids
- Lysophospholipids, phosphatidic acid
- Glycerol, monoglycerides
- Choline and ethanolamine

**KENNEDY PATHWAY**

- EPA
- DHA
- Phosphocholine
- Choline
- Uridine
- CTP
- CDP-Choline
- DAG
- Phosphatidylcholine
- Increased membrane formation

**Dietary phospholipids increase rat plasma and brain choline**

![Graph showing increased plasma choline](image)

- Control
- Fish oil
- Fish oil + PLs

Synaptic membrane synthesis induced by DHA+UMP depends on antioxidant adequacy.

**Kennedy Pathway**

- EPA → PLA₂ → PL, CHOLINE → CTP
- Uridine → CTP
- DHA → CDP-Choline → DAG → SEL

Increased membrane formation.

**Phospholipids**

- **Total**
  - AOX low
  - AOX high
  - Fish oil & UMP

- **PC**
  - AOX low
  - AOX high
  - Fish oil & UMP

*P < 0.05 vs. AOX low

Cansev, et al. (2016) Alz&Demen
The Kennedy pathway for biosynthesis of neuronal membrane

- B6
- FO
- EPA
- DHA
- PL Choline
- Uridine
- CTP
- CDP-Choline
- DAG
- SEL
- brain PHOSPHATIDYLCHOLINE
- increased membrane formation

Synapses consist principally of neuronal membranes

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Insight behind our approach: Dietary precursor control of neural membrane synthesis

The Kennedy pathway for biosynthesis of neuronal membrane

- **B6 FO**
- **B12**
- **AO**

**B6** → **FO** → **B12** → **AO** → **EPA** → **DHA** → **PHOSPHOCHOLINE** → **CDP-CHOLINE** → **DAG** → **SEL** → **brain PHOSPHATIDYLCHOLINE** → **increased membrane formation**

**PL Choline** → **Uridine** → **CTP**

Synapses consist principally of neuronal membranes

Fortasyn increases membrane phospholipid levels in the hippocampus of aged rat

- Results confirm effects of DHA+UMP on synaptic membrane formation
- Time-dependent effects
Fortasyn nutrients increase neurite outgrowth

Neurite outgrowth in PC12 cells after 2 day supplementation with NGF + different nutrients

CTRL

DHA

DUC

FC

Savelkoul et al. (2016) Alz&Demen
Fortasyn nutrients increase neurite outgrowth

Neurite outgrowth (length) in PC12 cells after 2 day supplementation with NGF + different nutrients

- Complete Fortasyn mix induces the largest effects
- Similar results observed for # neurites / cell

Savelkoul et al. (2016) Alz&Demen
Fortasyn increases pre- and postsynaptic protein levels

...in the hippocampus of aged rats

![Graph showing presynaptic and postsynaptic levels in the hippocampus of aged rats.](image)


...in hippocampal and cortex areas of aged mice (wild-type and AD risk model, ApoE4)

![Graph showing postsynaptic levels in hippocampal and cortex areas of aged mice.](image)

Fortasyn nutrients improve membrane receptor function and density

Muscarinic G protein-coupled receptor activation *in vitro*

PC12 cells

APP/PS1 mice

Complete Fortasyn mix induces the largest effect
Fortasyn increases cholinergic transmission in the hippocampus of aged rats

- Microdialysis in freely moving rats
- CA1 region of hippocampus
- Fortasyn diet vs. Control diet
- 4 or 6 weeks dietary intervention

**Acetylcholine release**

- FC increases acetylcholine release
- Basal and after stimulation with atropine
- Time-dependent effects

**Acetylcholine tissue levels**

- Acetylcholine tissue levels also increased by FC

**ChAT levels**

- ChAT levels increased by FC
- ChAT is the enzyme that synthesizes acetylcholine from choline and acetyl-CoA

Fortasyn protects against abeta toxicity in a rat model of AD

- ICV Abeta1-42 infusion
- Resembling AD pathology

**Protection of cholinergic cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>ChAT Positive Cells NBM</th>
<th>VACHT Positive Cells NBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td>400</td>
<td>300</td>
</tr>
<tr>
<td>Abeta Control</td>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>Sham Fortasyn®</td>
<td>400</td>
<td>300</td>
</tr>
<tr>
<td>Abeta Connect*</td>
<td>400</td>
<td>300</td>
</tr>
</tbody>
</table>

* * indicates statistical significance compared to control.

de Wilde, et al. JAD 2011
Beta-amyloid production
Fortasyn reduces AD pathology

Transgenic mouse model of Alzheimer pathology
Fortasyn preserves integrity of white and gray matter in the brain of APP/PS1 mice.

**diffusion-tensor MRI**

- **White matter loss** (*reduced* FA) is observed in APP/PS1 mice on Control diet but not on Fortasyn diet; a diet with only fish oil and UMP (DEU) is less effective.

- **Gray matter loss** (*increased* MD) is observed in APP/PS1 mice on Control diet but not on Fortasyn or DEU diet.

**Fortasyn is most effective**
Fortasyn improves cerebral blood flow and cerebral blood volume

Reduced cerebral blood flow in APP/PS1 mice is restored by fish oil and UMP (DEU) diet and Fortasyn diet


Now replicated in ApoE-4 mice and normal mice (Wiesmann, Zerbi, et al. Neural Plast 2016)

In ApoE-ko mice, a vascular risk model for dementia, Fortasyn increased relative cerebral blood volume in midbrain and cortical areas

Spatial learning in the water maze is improved in APP/PS1 mice on a Fortasyn diet

Fish oil diet has no effect

Fortasyn is most effective

Precursors & cofactors enhance synapse formation & function

Summary basic science

Review

Targeting Synaptic Dysfunction in Alzheimer’s Disease by Administering a Specific Nutrient Combination

Nick van Wijk\textsuperscript{a,*}, Laus M. Broersen\textsuperscript{a}, Martijn C. de Wilde\textsuperscript{a}, Robert J.J. Hageman\textsuperscript{a}, Martine Groenendijk\textsuperscript{a}, John W.C. Sijben\textsuperscript{a} and Patrick J.G.H. Kamphuis\textsuperscript{a,b}

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12. Grimm (2011) JBC
15. de Wilde (2002) Brain Res
18. van Wijk (2014) JAD
19. de Wilde (2011) J Alz Dis
26. Savelkoul (2011) ADPD
27. Savelkoul (2016) in prep
31. Wiesmann (2013) JAD
32. Wiesmann (2016) Neur Plas
34. van Wijk (2016) Lipids
35. Van Wijk (2016) AAIC
Putative effects of Fortasyn nutrients on membrane-related pathology in AD

- **Nutritional precursors and cofactors**
  - Improved neuronal membrane formation and function
    - e.g. increased phospholipid synthesis via the Kennedy pathway
    - e.g. increased membrane fluidity
    - e.g. reduced γ-secretase activity and reduced binding of Aβ to membranes

- **Synapse formation**
  - Increased neurite outgrowth
  - Increased spine density
  - Increased synaptic proteins

- **Neurotransmission**
  - Increased neurotransmitter levels & release
  - Increased receptor signaling

- **Aβ pathology**
  - Reduced Aβ toxicity
  - Reduced Aβ production
  - Reduced plaque burden
  - Reduced neurodegeneration

- **Improved behavior, learning and memory**
Thank you
Additional slide
Synaptic membrane synthesis induced by DHA+UMP depends on antioxidant adequacy

**phospholipids**

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  - Fish oil & UMP
  - AOX high
  - Fish oil & UMP

- **PC**
  - AOX low
  - AOX high
  - AOX low
  - AOX high

**synaptic proteins**

- **PSD95**
  - AOX low
  - AOX high
  - Fish oil & UMP
  - AOX high
  - Fish oil & UMP

- **Synapsin-1**
  - AOX low
  - AOX high
  - Fish oil & UMP
  - AOX high
  - Fish oil & UMP

* P < 0.05 vs. AOX low

Cansev, et al. (2016) Alz&Demen