

Extraction and administration of Spermidine using NaDES for treating neurodegenerative diseases



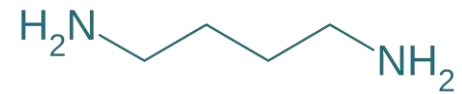
PhD Project
Lavinia Attili
27th March 2024
Tutor Manuela Cervelli



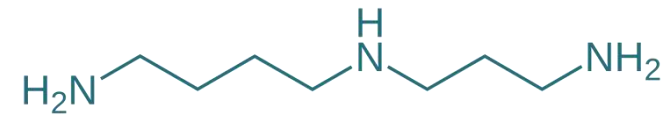
Polyamine



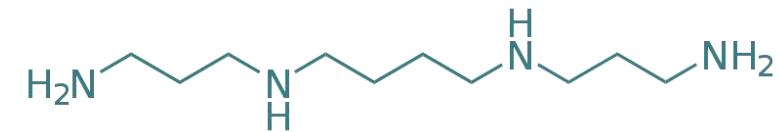
Putrescine (PUT)



Spermidine (SPD)

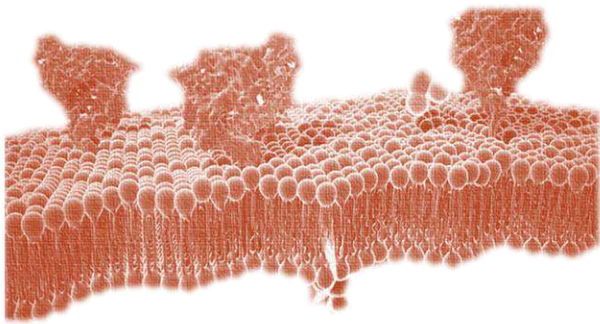


Spermine (SPM)



Polyamine's Cellular Functions

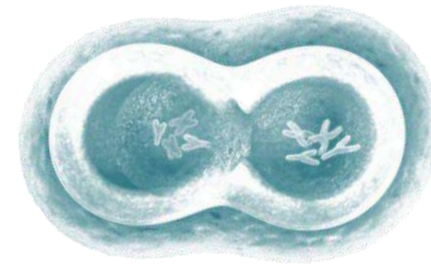
- Membrane stability
- Ionic channels specific modulation (iGluR, Kir)

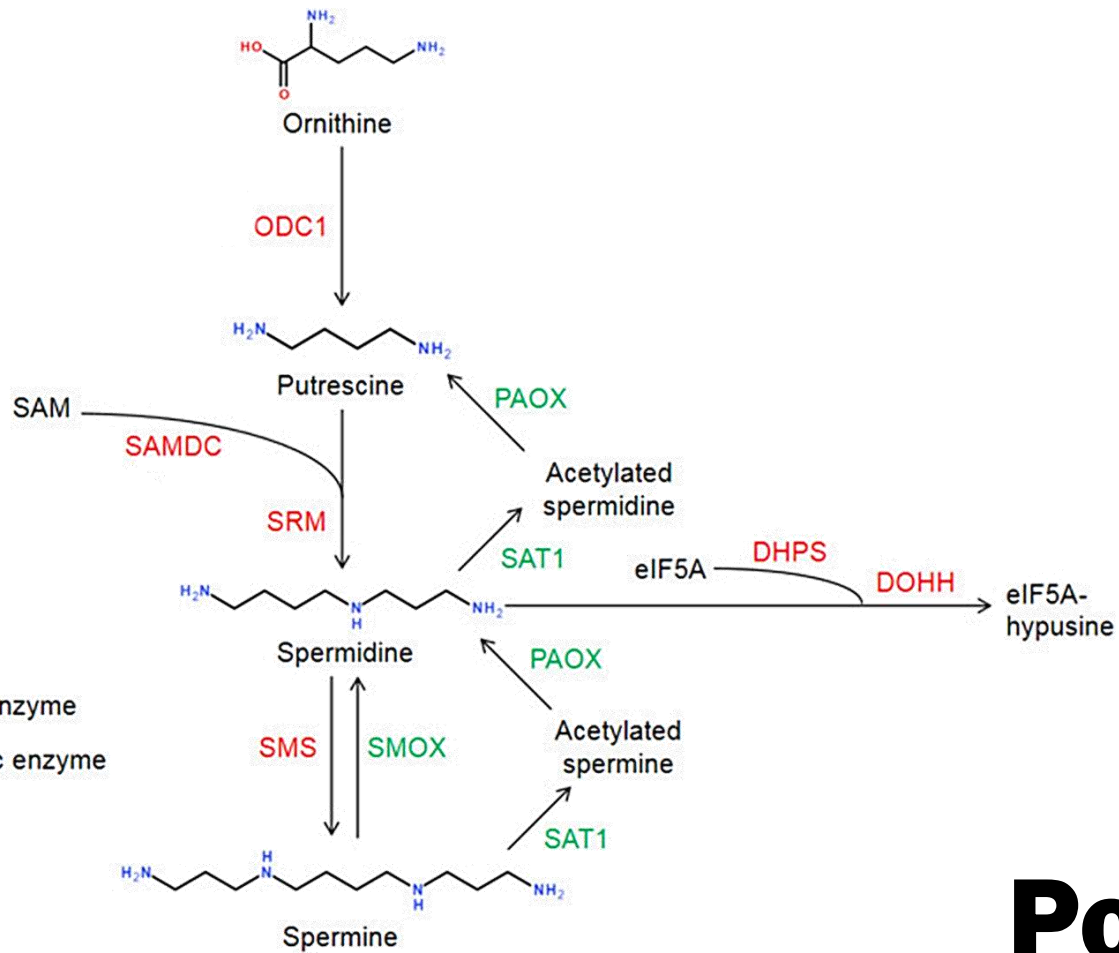


- Nucleic acid binding
- DNA stability
- Chromatin structure maintenance
- Regulation of gene expression



- Cell cycle regulation
- Proliferation and differentiation
- Apoptosis regulation





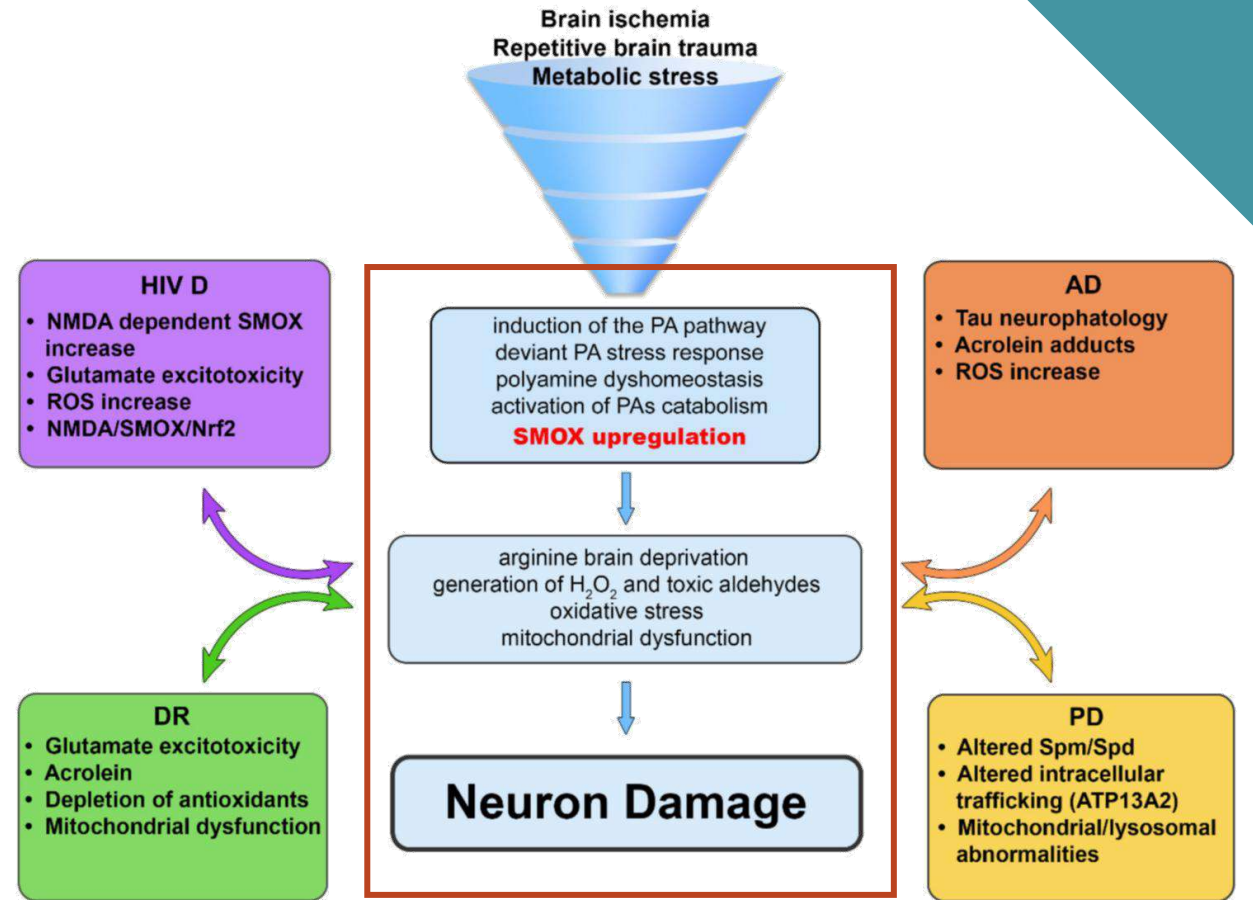
Adapted from Huang et al., 2020

Polyamine Metabolism

Polyamine in SNC

A polyamine deregulation has been found in the following neurologic pathologies:

- Alzheimer Disease
- Parkinson Disease
- Dementia
- Epilepsy



nature
cell biology

ARTICLES

Induction of autophagy by spermidine promotes longevity

Tobias Eisenberg¹, Heide Knauer¹, Alexandra Schauer¹, Sabrina Büttner¹, Christoph Ruckenstuhl¹, Didac Carmona-Gutierrez¹, Julia Ring¹, Sabrina Schroeder¹, Christoph Magnes², Lucia Antonacci¹, Heike Fussl¹, Luiza Deszcz^{3,4}, Regina Hartl^{3,4}, Elisabeth Schraml⁵, Alfredo Criollo^{6,7,8}, Evgenia Megalou⁹, Daniela Weiskopf¹⁰, Peter Laun¹¹, Gino Heeren¹¹, Michael Breitenbach¹¹, Beatrix Grubeck-Loebenstein¹⁰, Eva Herker¹², Birthe Fahrenkrog¹³, Kai-Uwe Fröhlich¹, Frank Sinner², Nektarios Tavernarakis⁹, Nadege Minois^{3,4,14}, Guido Kroemer^{6,7,8,15} and Frank Madeo^{1,15}

nature
neuroscience

ARTICLES

Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner

Varun K Gupta^{1,2}, Lisa Scheunemann¹, Tobias Eisenberg³, Sara Mertel¹, Anuradha Bhukel^{1,2}, Tom S Koemans⁴, Jamie M Kramer⁴, Karen S Y Liu^{1,2}, Sabrina Schroeder³, Hendrik G Stunnenberg⁵, Frank Sinner^{6,7}, Christoph Magnes⁶, Thomas R Pieber^{6,7}, Shubham Dipt⁸, André Fiala⁸, Annette Schenck⁴, Martin Schwaerzel¹, Frank Madeo³ & Stephan J Sigrist^{1,2}

Science

Spermidine in health and disease

Frank Madeo, Tobias Eisenberg, Federico Pietrocola and Guido Kroemer

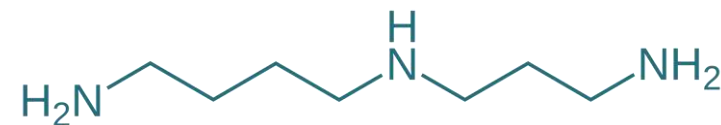
Science **369** (6374), eaan2788
DOI: 10.1126/science.aaan2788

Having your longevity and eating too

Although caloric restriction has clear benefits for maximizing health span and life span, it is sufficiently unpleasant that few humans stick to it. Madeo *et al.* review evidence that increased intake of the polyamine spermidine appears to reproduce many of the healthful effects of caloric restriction, and they explain its cellular actions, which include enhancement of autophagy and protein deacetylation. Spermidine is found in foods such as wheat germ, soybeans, nuts, and some fruits and vegetables and produced by the microbiota. Increased uptake of spermidine has protective effects against cancer, metabolic disease, heart disease, and neurodegeneration.

Science, this issue p. eaan2788

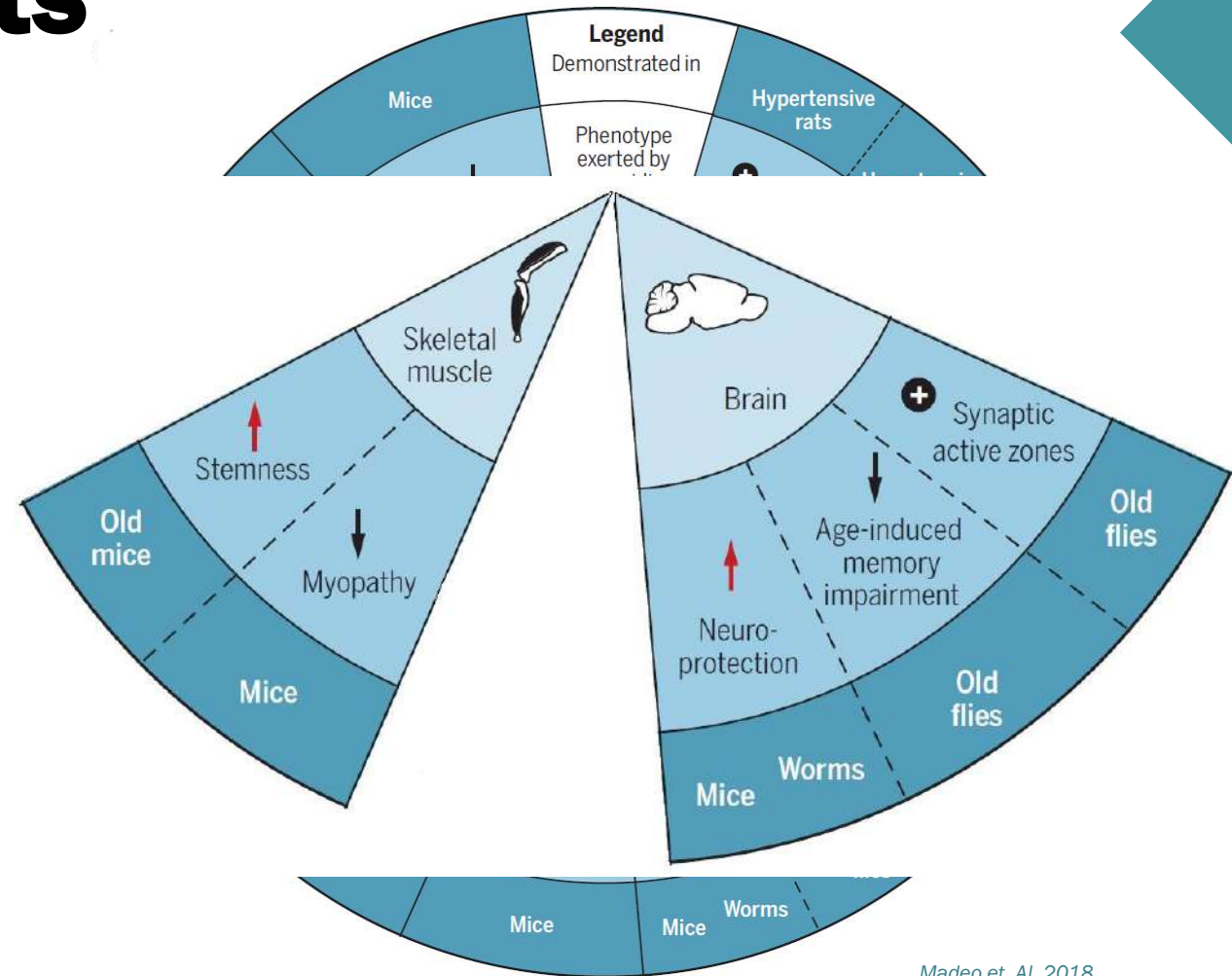
Spermidine



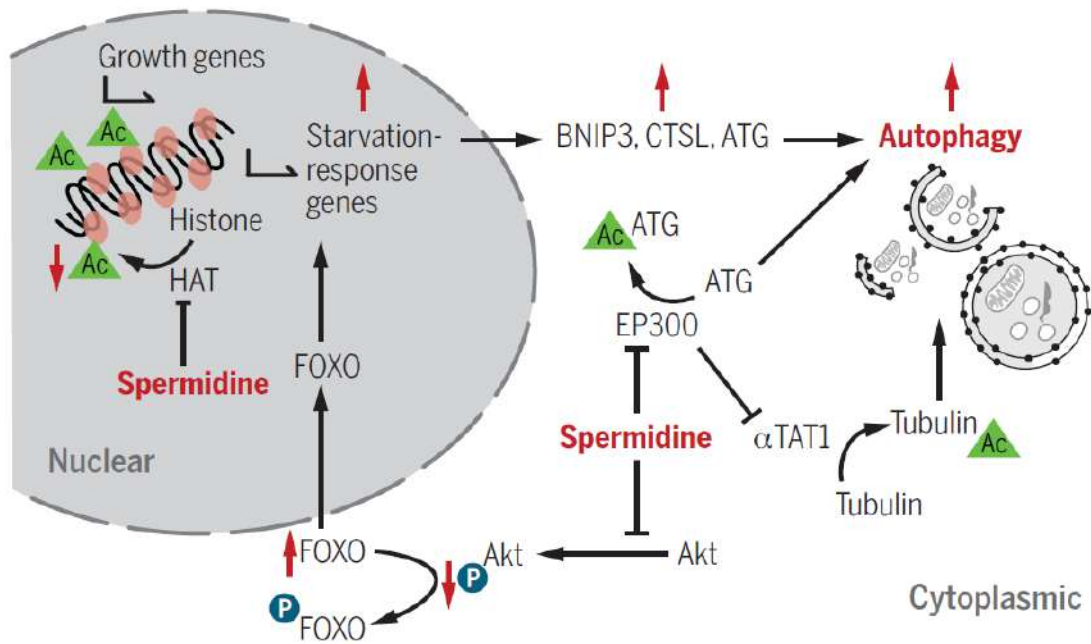
Spermidine Effects

These effects depends on the SPD involvement in the following processes:

- Autophagy activation
- Hypusination
- Free Radicals Scavenger
- Inibition of microglia and astrocytes activation
- Reduction of pro-inflammatory cytokines' levels



Autophagy



Madeo et. Al, 2018

Spd administration in various models

↑ translation of autophagy related genes

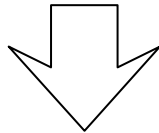
→ Autophagy induction

→ Life span extension

Hypusination

Spd as substrate of hypusination of eIF5A leads to:

- Autophagy improvement
- Mitochondrial activity improvement

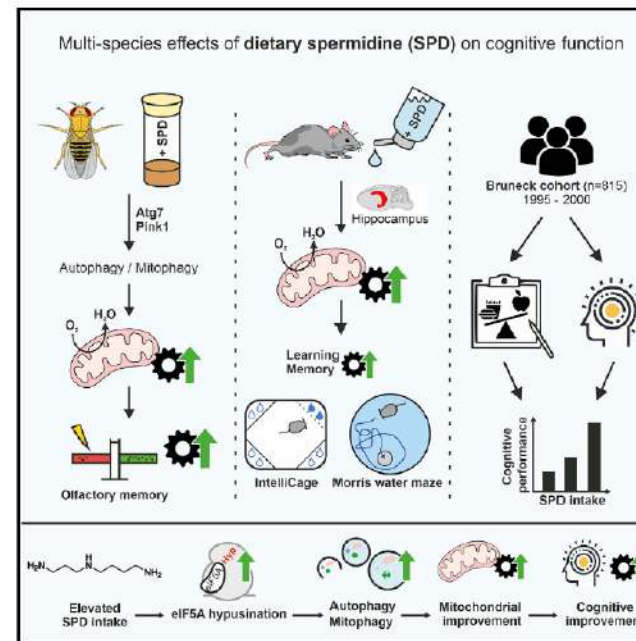


Cognitive improvement

Cell Reports

Dietary spermidine improves cognitive function

Graphical abstract



Article

Authors

Sabrina Schroeder, Sebastian J. Hofer, Andreas Zimmermann, ..., Stefan Kiechl, Tobias Eisenberg, Frank Madeo

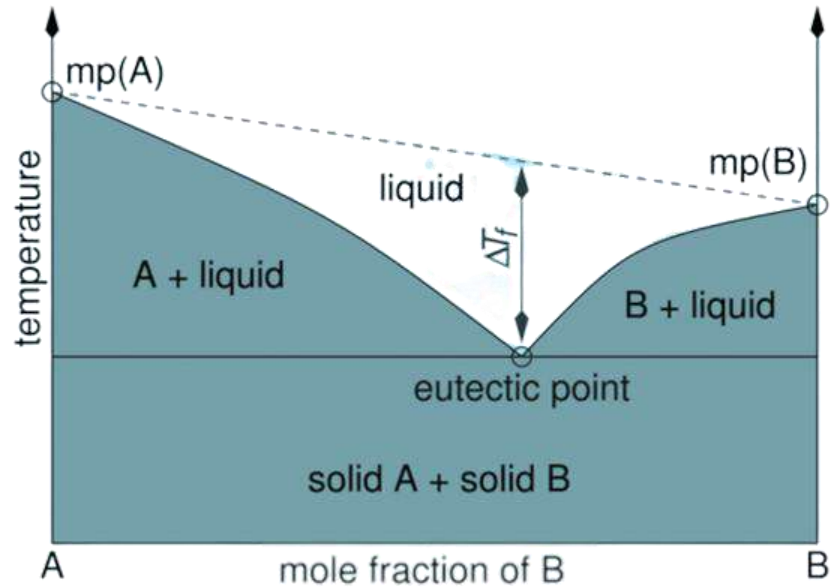
Correspondence

stefan.kiechl@i-med.ac.at (S.K.), tobias.eisenberg@uni-graz.at (T.E.), frank.madeo@uni-graz.at (F.M.)

In brief

Schroeder et al. report on the beneficial effects of dietary spermidine intake on cognitive performance in flies, mice, and humans. They link the polyamine's effects to elevated mitochondrial function in neuronal tissue, which depends on functional autophagy (Atg7) and mitophagy (Pink1).

- Deep Eutectic Solvent, characterized by a melting point lower than each solvent's component's melting point.
- Liquid at room temperature or lower.



DES



Carbohydrates,
aminoacids,
polialchols and
amines

DES

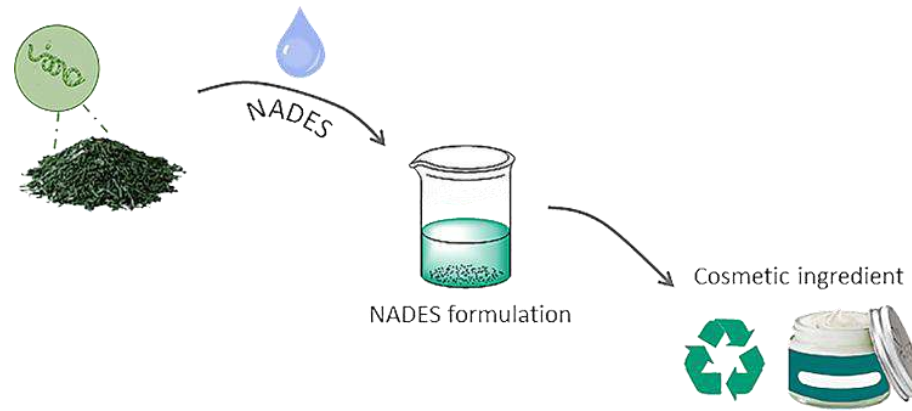


NaDES



NaDES

- Insignificantly volatile at room temperature
- Miscible in water
- Non-flammable
- Highly viscous
- Efficient solvents for complete dissolution and extraction of various compounds



NaDES can be used to extract bioactive substances from plants, food products or agroindustrial waste, and it can represent a

FULL NATURAL COMPLEMENTARY PRODUCT READY TO USE

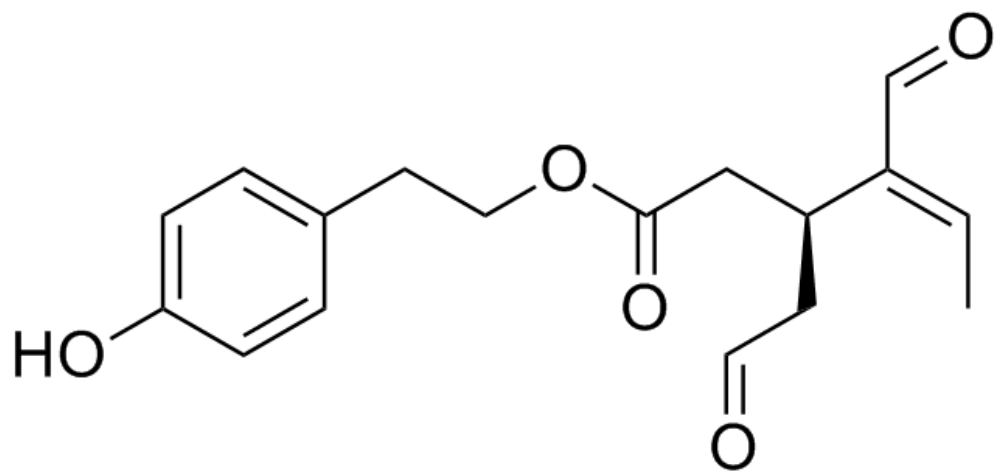
Extraction Sources

Polyamine/food item	Portions in grams	Polyamines mg/portion
Spermidine		
Soybean, cooked	190	9.7
Peas, green	140	9.1
Pear	125	6.6
Lentil soup	250	5.5
Mushroom	50	4.4
Red beans	190	3.7
Broccoli	100	3.6
Cauliflower	100	3.0
Chicken, steak	125	2.2
Popcorn	50	2.1
Cheese	20	2.0
Potato, cooked	150	1.8

Table 1

Food product	n	Spermidine				Country and reference
		Mean	SD	Min	Max	
Cereals						
Wheat flour	2	9.6	—	—	—	Japan (4) ^b
Bread, white	3	5.1	—	5.0	5.2	UK (22) ^a
	10	7.8	0.06	—	—	France (38) ^e
Whole grain	3	24.4	—	21.3	27.4	UK (22) ^a
	5	13.1	1.5	10.2	14.8	Norway (16) ^a
	10	15.8	0.1	—	—	France (38) ^e
Oat bread	10	9.4	0.06	—	—	France (38) ^e
Pasta, cooked	3	7.2	—	7.0	7.3	UK (22) ^a
Corn	2	43.2	—	—	—	Japan (15) ^b
Breakfast cereals, mixed	10	24.2	—	24.1	24.4	UK (2) ^a
Rice	6	0.4	—	0.3	0.6	Japan (6) ^c
Rice, polished	2	3.9	—	—	—	Japan (4) ^b

Adapted from Ali et al., 2011



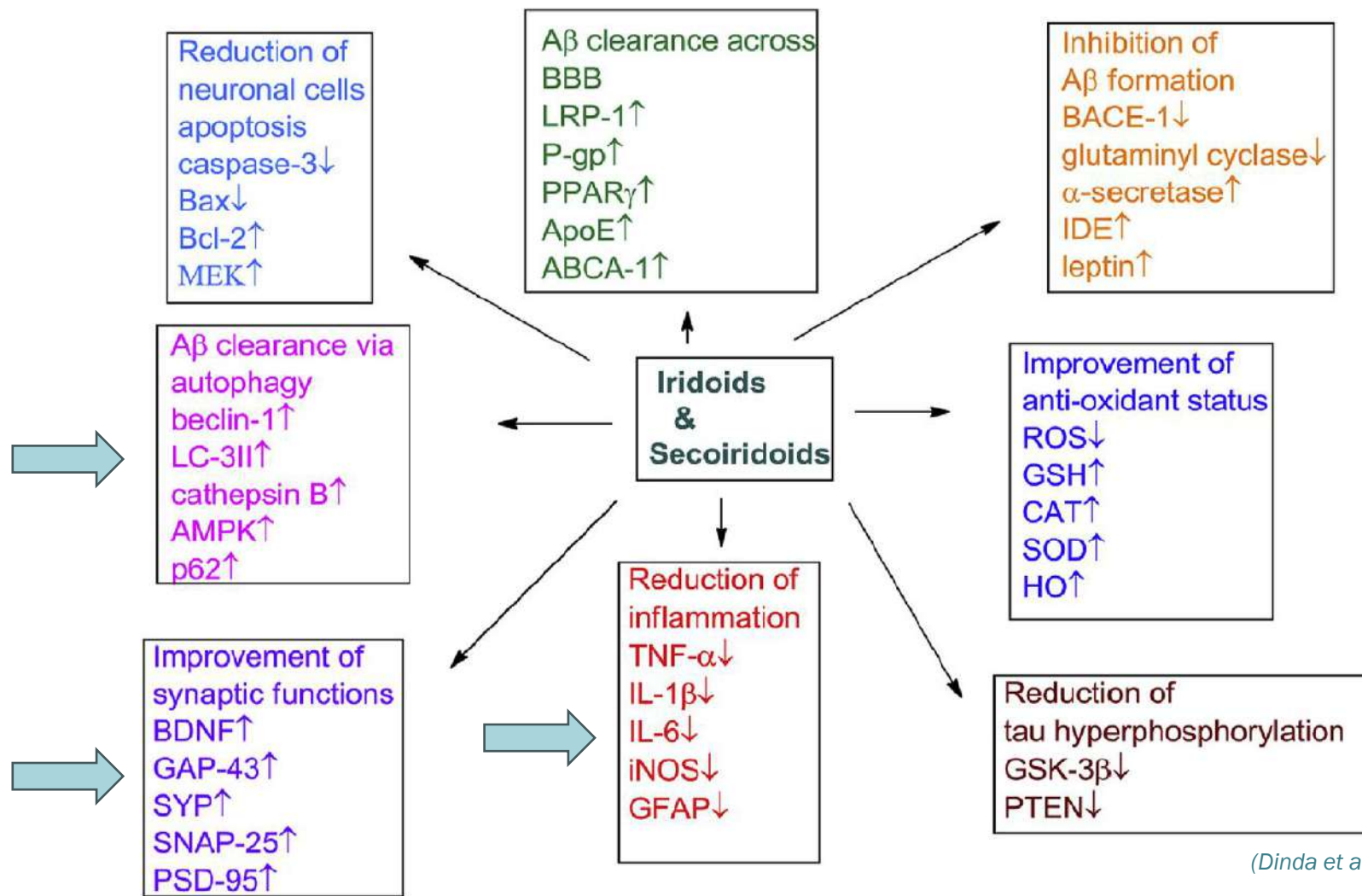
$C_{17}H_{20}O_5$

Monophenol only found in extra-virgin olive oil (EVOO), tagged as secoiridoids.

Extracted from EVOO using NaDES by ACTIVE ITALIA.



Oleocanthal (OC)



OC Mechanisms and Effects

Oleocanthal in SNC

- Antimicrobial
- Anticancer
- Anti-oxidant
- Anti-inflammatory
- Neuroprotective effects



antioxidants



Article

Dietary Oleocanthal Supplementation Prevents Inflammation and Oxidative Stress in Collagen-Induced Arthritis in Mice

Tatiana Montoya ¹, Marina Sánchez-Hidalgo ¹, María Luisa Castejón ¹, María Ángeles Rosillo ¹, Alejandro González-Benjumea ² and Catalina Alarcón-de-la-Lastra ^{1,*}

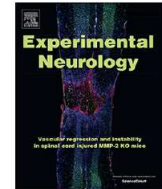


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Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr



Research Paper

Purified oleocanthal and ligstroside protect against mitochondrial dysfunction in models of early Alzheimer's disease and brain ageing

Rekha Grewal^a, Martina Reutzel^a, Benjamin Dilberger^a, Hannah Hein^a, Jens Zotzel^b, Stefan Marx^b, Joachim Tretzel^b, Alla Sarafeddinov^b, Christopher Fuchs^b, Gunter P. Eckert^{a,*}



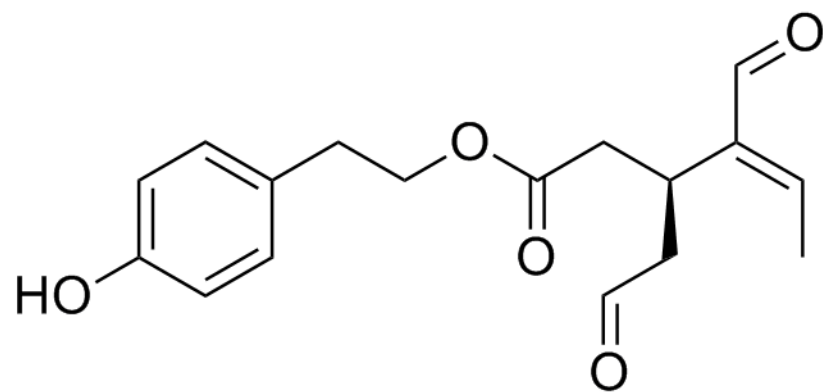
International Journal of
Molecular Sciences



Review

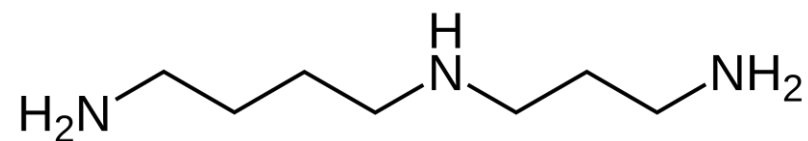
An Appraisal of the Oleocanthal-Rich Extra Virgin Olive Oil (EVOO) and Its Potential Anticancer and Neuroprotective Properties

Raffaele Infante ^{1,†}, Marco Infante ^{2,*}, Donatella Pastore ¹, Francesca Pacifici ³, Francesca Chiereghin ¹, Gina Malatesta ³, Giulia Donadel ⁴, Manfredi Tesaro ³ and David Della-Morte ^{1,3,5}



Oleocanthal

+



Spermidine

using NaDES

partnership with ACTIVE ITALIA



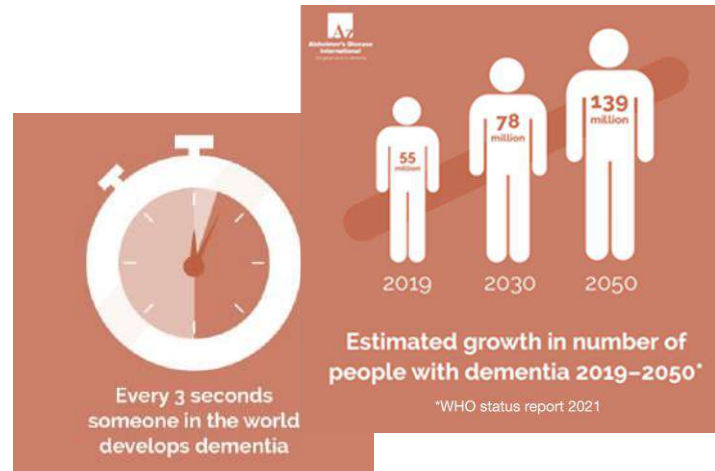
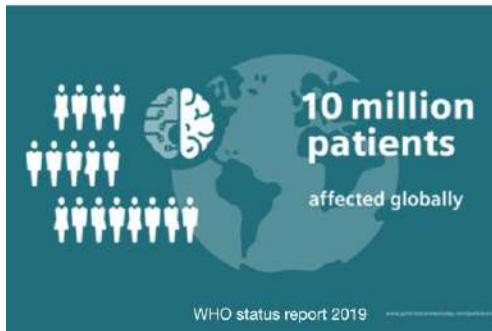
Synergical activity

?

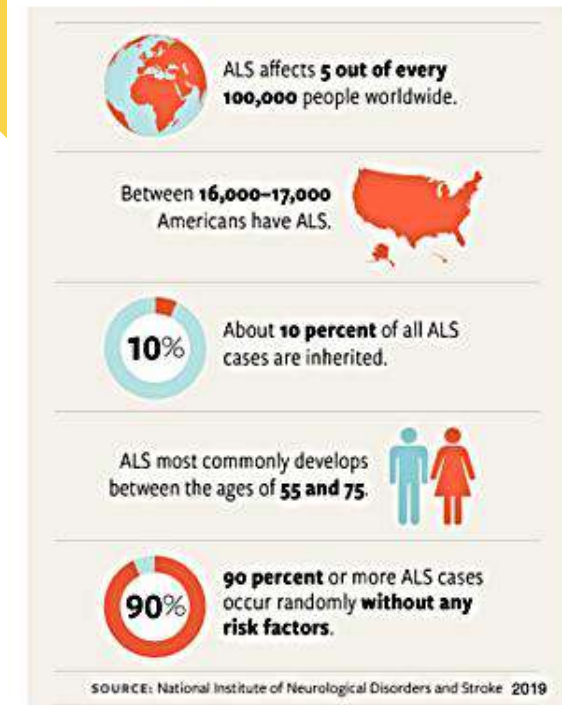
Neurodegenerative Diseases

Common pathogenic mechanisms, unfaithful therapeutic outcome

Parkinson's disease



Amyotrophic lateral sclerosis



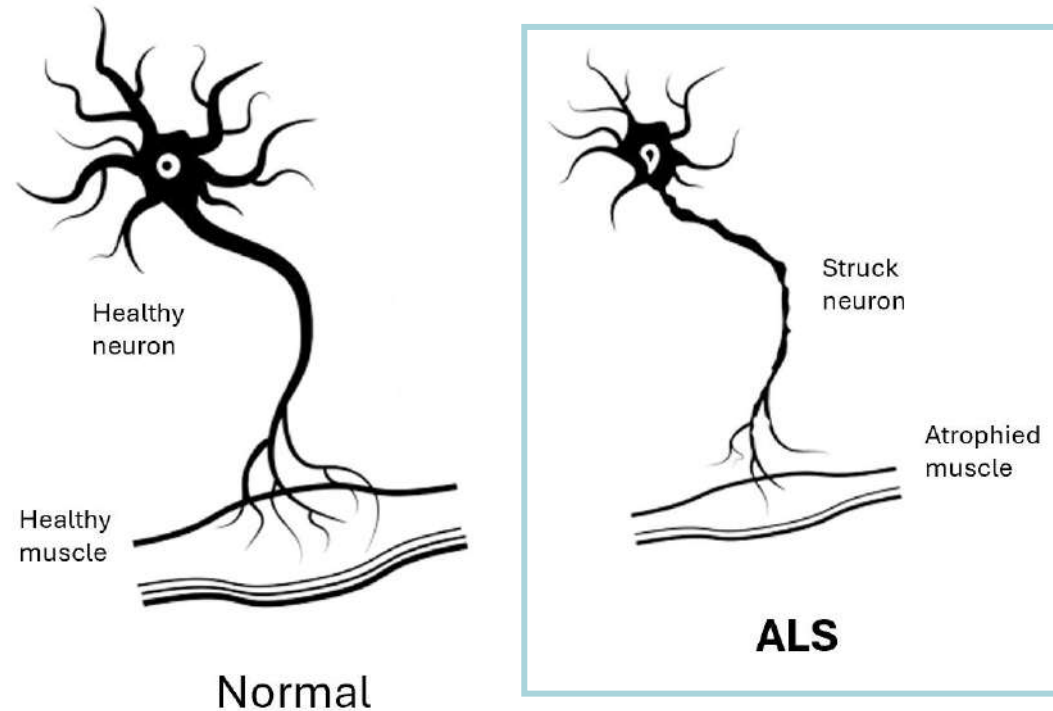
Urgence to find new and efficient therapies,
but also to understand the underlying mechanisms in the pathogenesis

Amyotrophic Lateral Sclerosis (ALS)

A = "a" is for absent, "myo" for muscles and "trophic" as a reference of nutrition

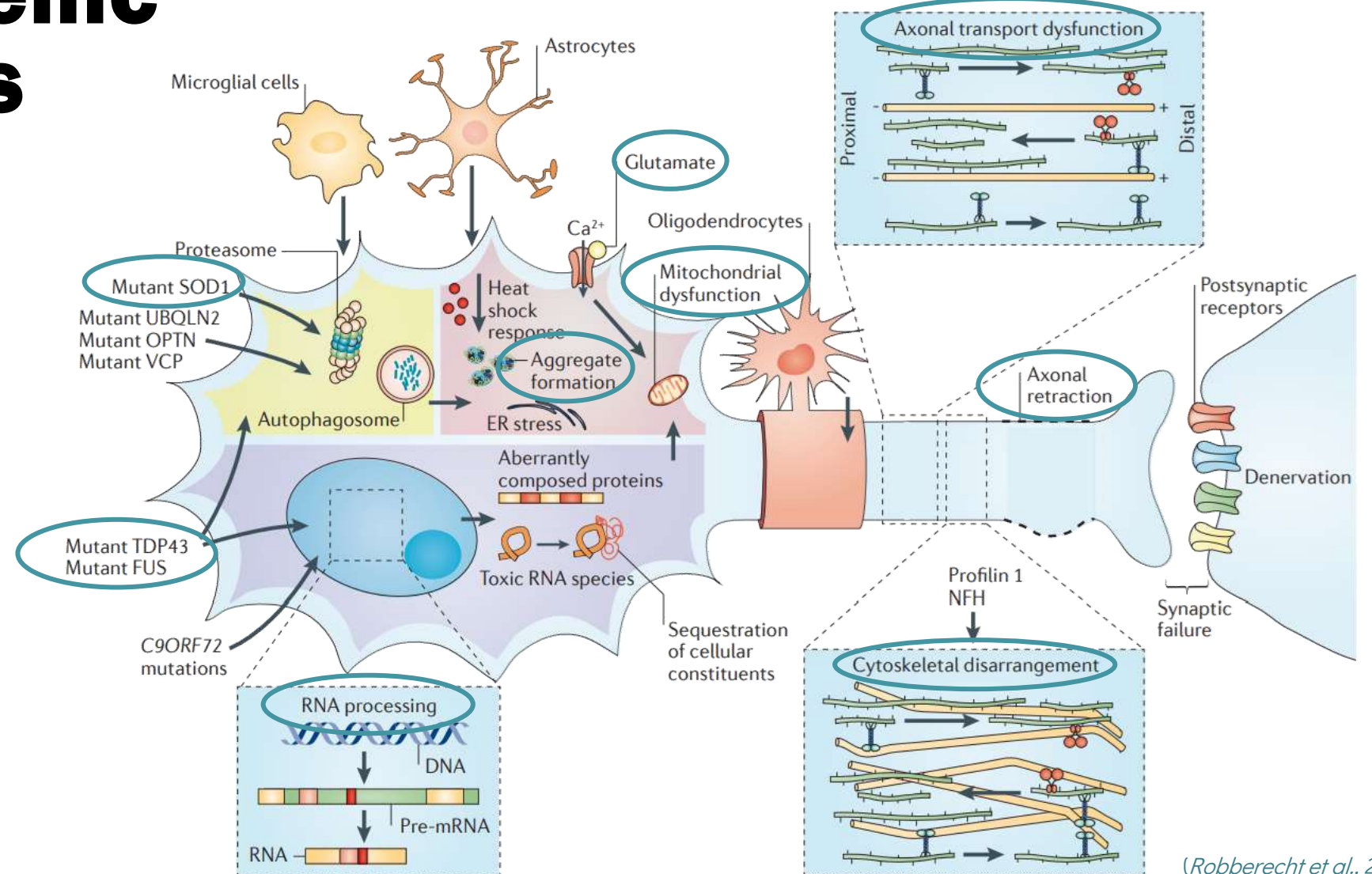
L = reference to the spinal cord area where the damaged neuron are located

S = sclerosis, neuronal degeneration that leads to stiffening



Incidence: 3 cases each 100.000 inhabitants/year in Italy, and a prevalence of 10 each 100.000 inhabitants in Europe (source: <https://www.aisla.it/>).

ALS Pathogenic Mechanisms



(Robberecht et al., 2013)

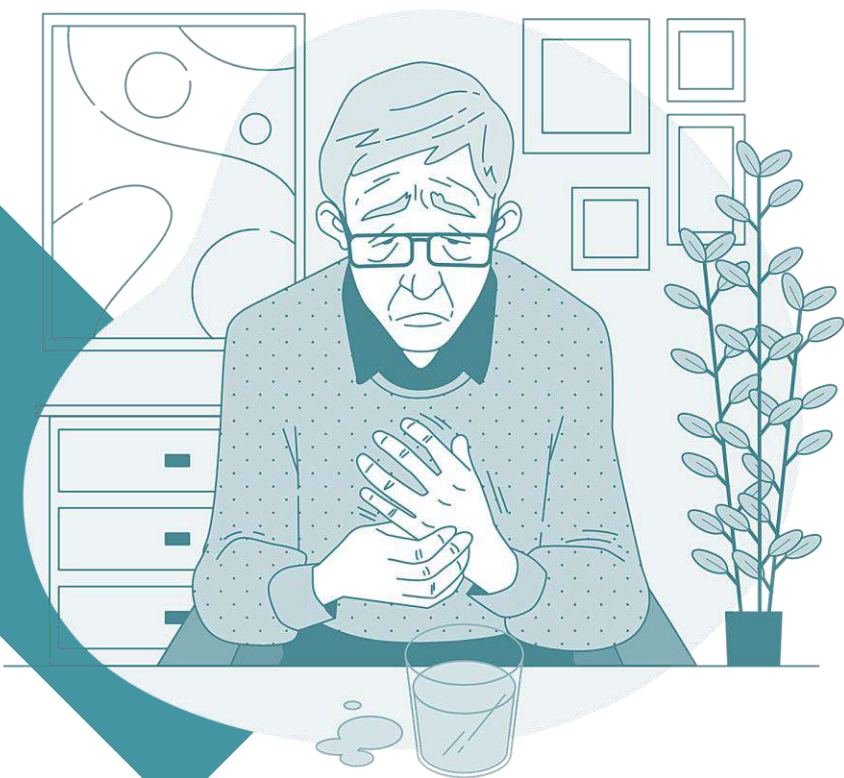
ALS Therapeutic Options



Edaravone –intravenous therapy, it slows the decline of daily functions, but has several collateral effects.

Riluzole – oral medication, elongates the patient's life span, with heavy collateral effects.

The absence of efficient therapies →search new and innovative drugs



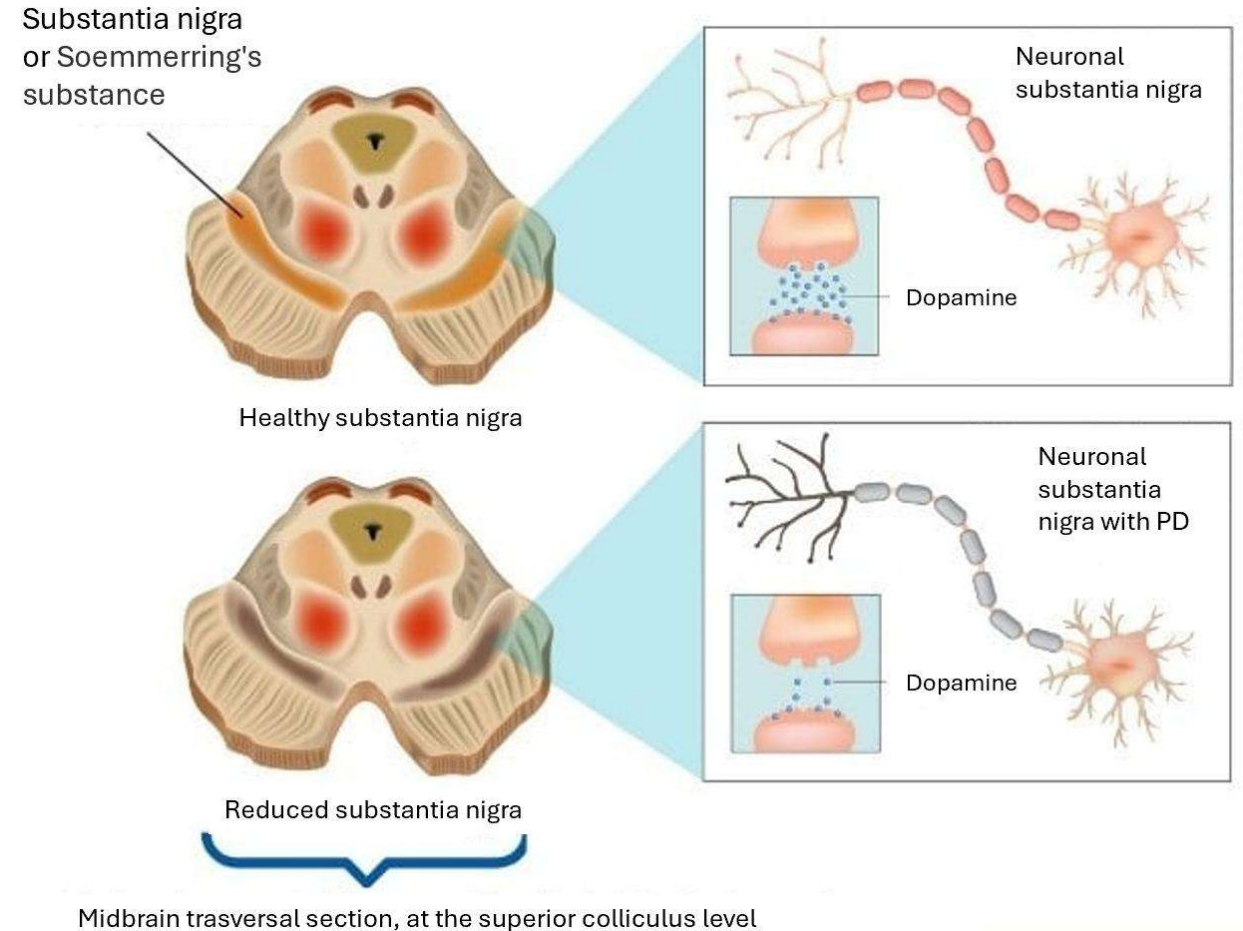
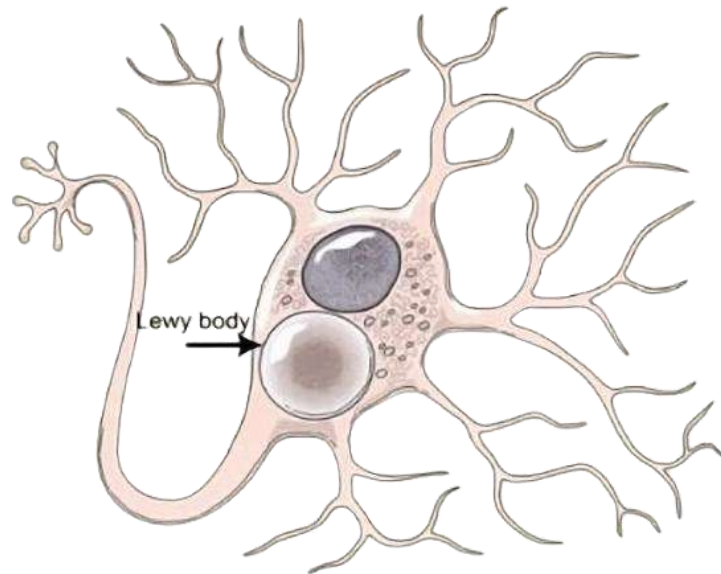
2nd most common progressive neurodegenerative disorder

Annual incidence per 100,000 people ranges from less than 10 to more than 20

Risk factors: aging, family history, pesticide exposure and environmental chemicals

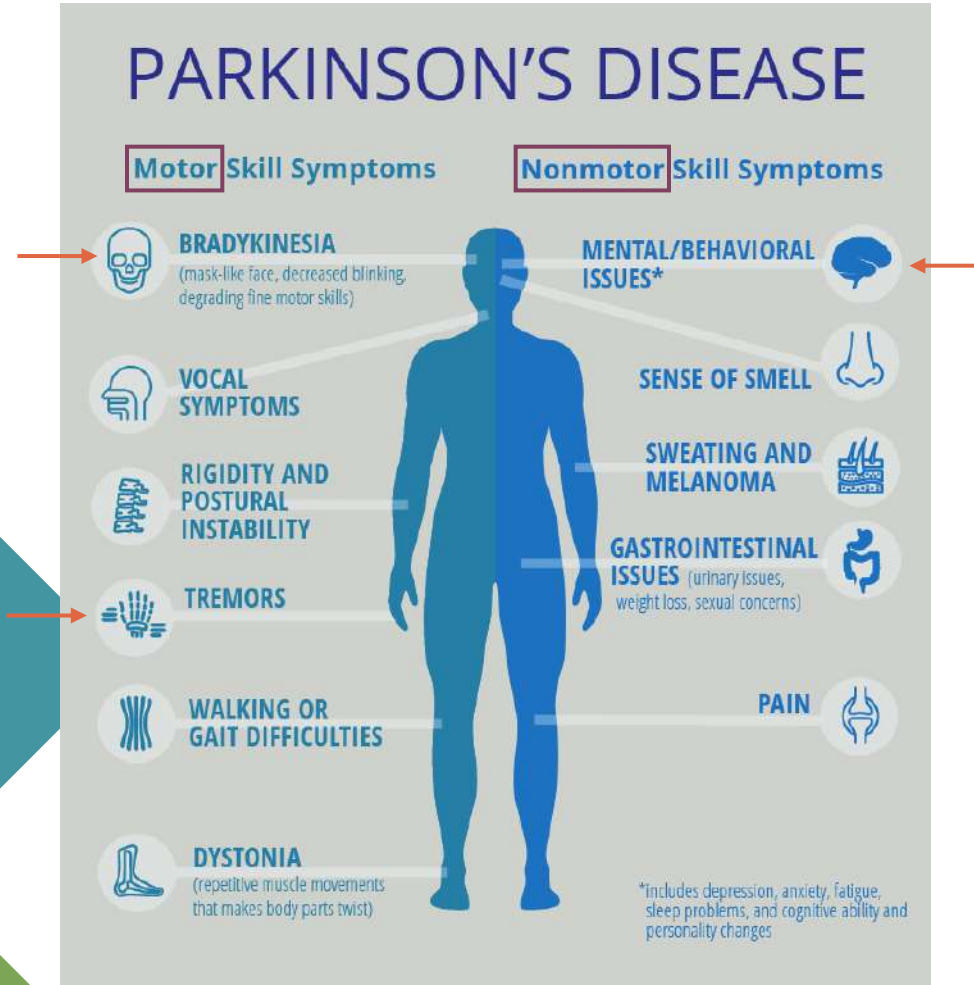
Parkinson Disease

- Loss or degeneration of dopaminergic neurons in the *substantia nigra* of the midbrain
- Development of neuronal Lewy Bodies (aggregates of α -synuclein and ubiquitin)



PD Pathogenic Mechanisms

PD Symptoms and Therapeutic Option



Therapies:

- Carbidopa/Levodopa (Sinemet),
- Dopamine agonists
- Monoamine oxidase-B (MAO-B) inhibitors
- Injectable dopamine agonist
- Receptor inhibitors
- Anti-cholinergics
- Deep brain stimulation (DBS)

Collateral effects: behavioral problems, absence of neuroprotective effects

No therapy that stops the neurodegeneration

ATP13A2

- LOF mutations in juvenile PD
- Lysosomal transporter
- Protects mitochondrial functionality and lysosomal-mitochondrial axis
- Polyamine transporter

Article

ATP13A2 deficiency disrupts lysosomal polyamine export

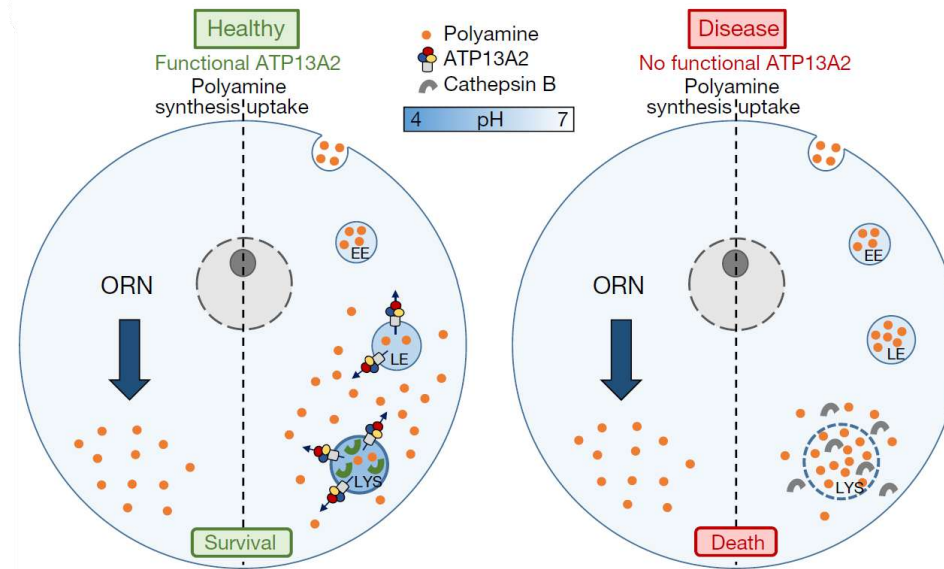
<https://doi.org/10.1038/s41586-020-1968-7>

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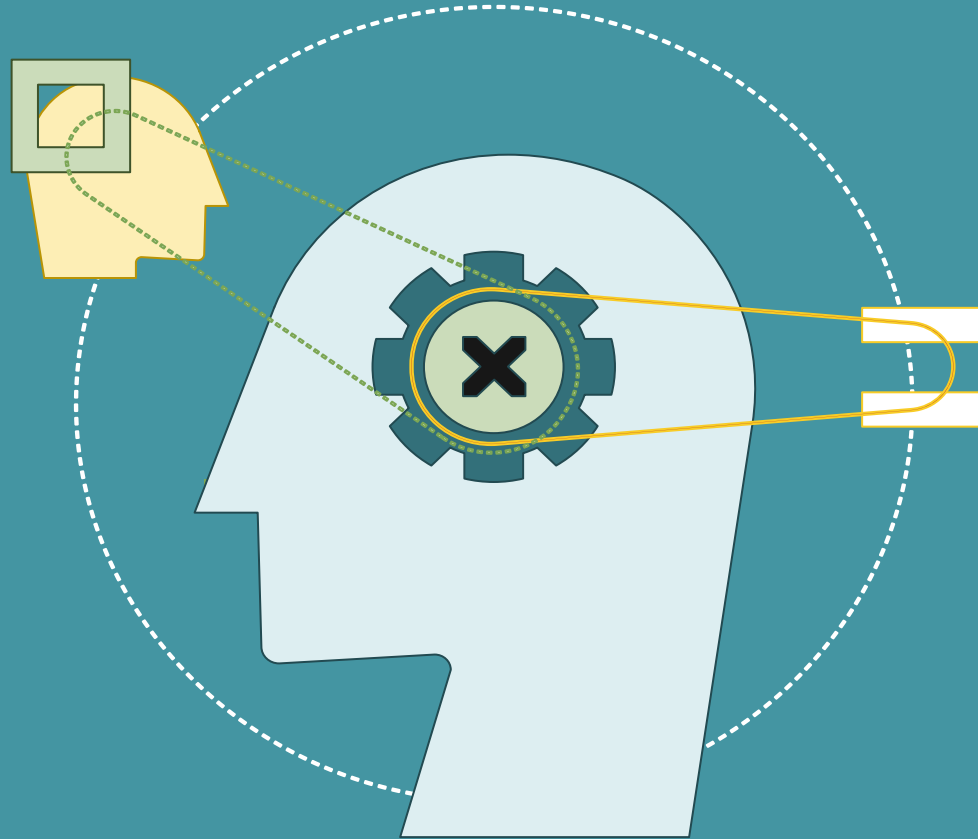
Sarah van Veen^{1,14}, Shaun Martin^{1,14}, Chris Van den Haute^{2,3}, Veronick Benoy¹, Joseph Lyons⁴, Roeland Vanhoutte⁵, Jan Pascal Kahler⁵, Jean-Paul Decuyper^{1,6,7,8}, Géraldine Gelders², Eric Lambie^{9,10}, Jeffrey Zielich⁹, Johannes V. Swinnen⁶, Wim Annaert⁷, Patrizia Agostinis^{9,11}, Bart Ghesquière¹², Steven Verhelst^{5,13}, Veerle Baekelandt², Jan Eggermont¹ & Peter Vangheluwe^{1*}



(van Veen et al., 2020)

Project Goals

- Use NaDES to extract Spd and OC from vegetal waste to obtain a supplement where Spd and OC are more concentrated or efficient.
- The supplement will be administrated to cellular lines and in vivo model of ALS and PD to test its efficiency, compared to commercial SPD alone.



PLANNING

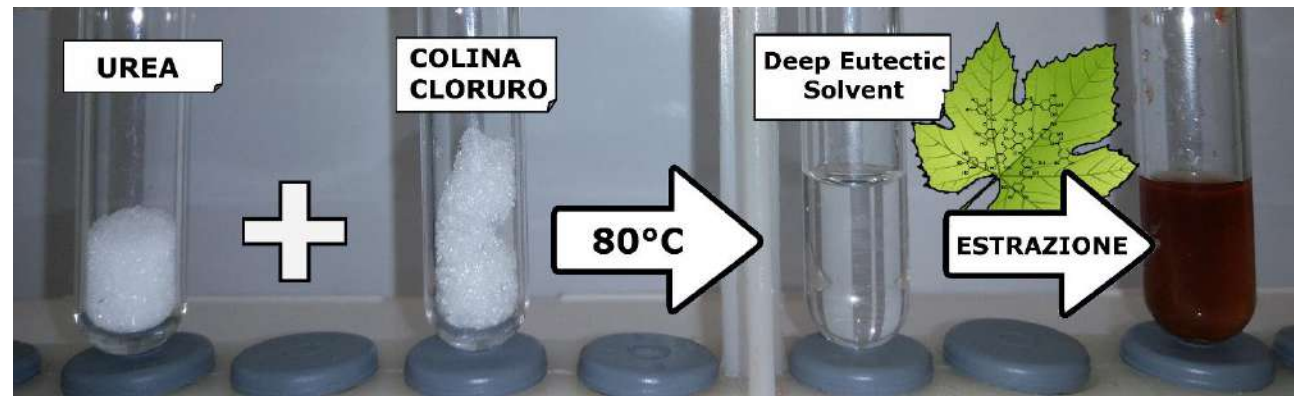


PHASE 1

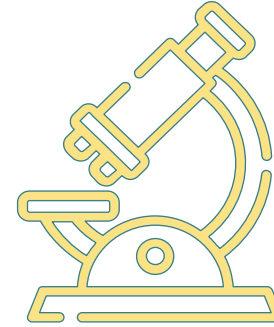
Developing methods to extract Spd from wheat germ and OC from olive oil using NaDES in collaboration with Active Italia



- Trying various combinations of coline chloride, betaine or proline with polioils, organic acids or carbohydrates.
- Evaluation of biocompatibility and toxicity on various cell lines.
- Isolation of Spd from wheat germs and OC from olive oil
- Union of Spd and OC extract in one solution



PLANNING



PHASE 2

Evaluation of the effect of Spd-OC-NaDES administration on cellular models of ALS and PD vs SPD and OC alone.

ALS cellular models

NSC-34 mouse cells:

- SOD1 (G93A)
- TDP43 (Q331K, M337V)
- FUS (R514G, R521G, P525L)
- C9ORF72 (2/10 e 31 expanded repeats)

Endpoint evaluation:

- Cellular viability
- ROS production
- Autophagy
- Mitochondrial metabolism
- Genetic expression analysis
- In-take and localisation of Spd-OC-NaDES

PD cellular models

SHSY-5Y human cells:

- Wild-type (WT-OE)
- Transport deficient ATP13A2

Endpoint evaluation:

- Cell death
- ATP cellular levels
- Mitochondrial membrane potential
- Mitochondrial generated ROS
- **BODIPY-spermidine up-take and redistribution**

At KU Leuven University

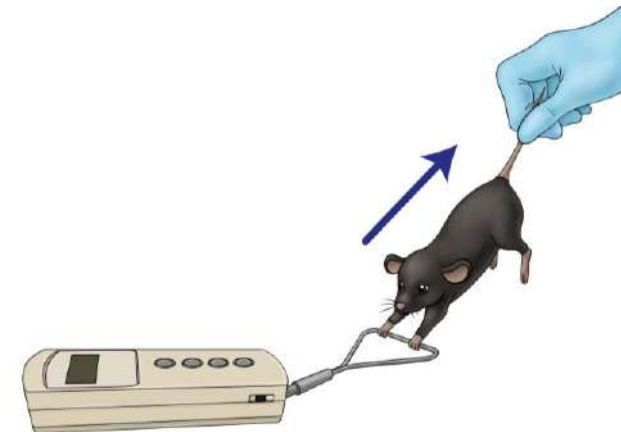
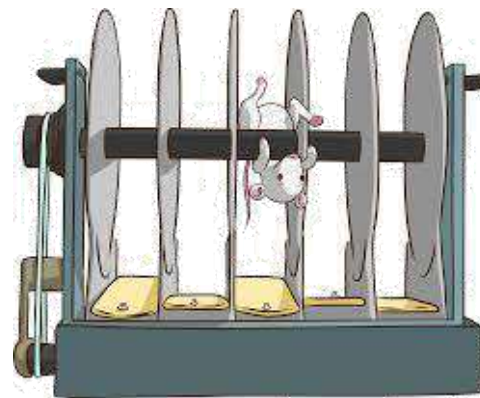
PLANNING

PHASE 3

Evaluation of the effect of Spd-OC-NaDES administration on in vivo murin models of ALS.

Administration of Spd-OC-NaDES to C57/black6 mice that overexpress the mutated protein SODG93A, from the first appearance of symptoms for two months or until the terminal phase of the pathology.

- Survival and phenotypic parameters analysis: muscular strength and functionality
- Transcriptional and proteomic analyses on muscular tissue and spinal cord

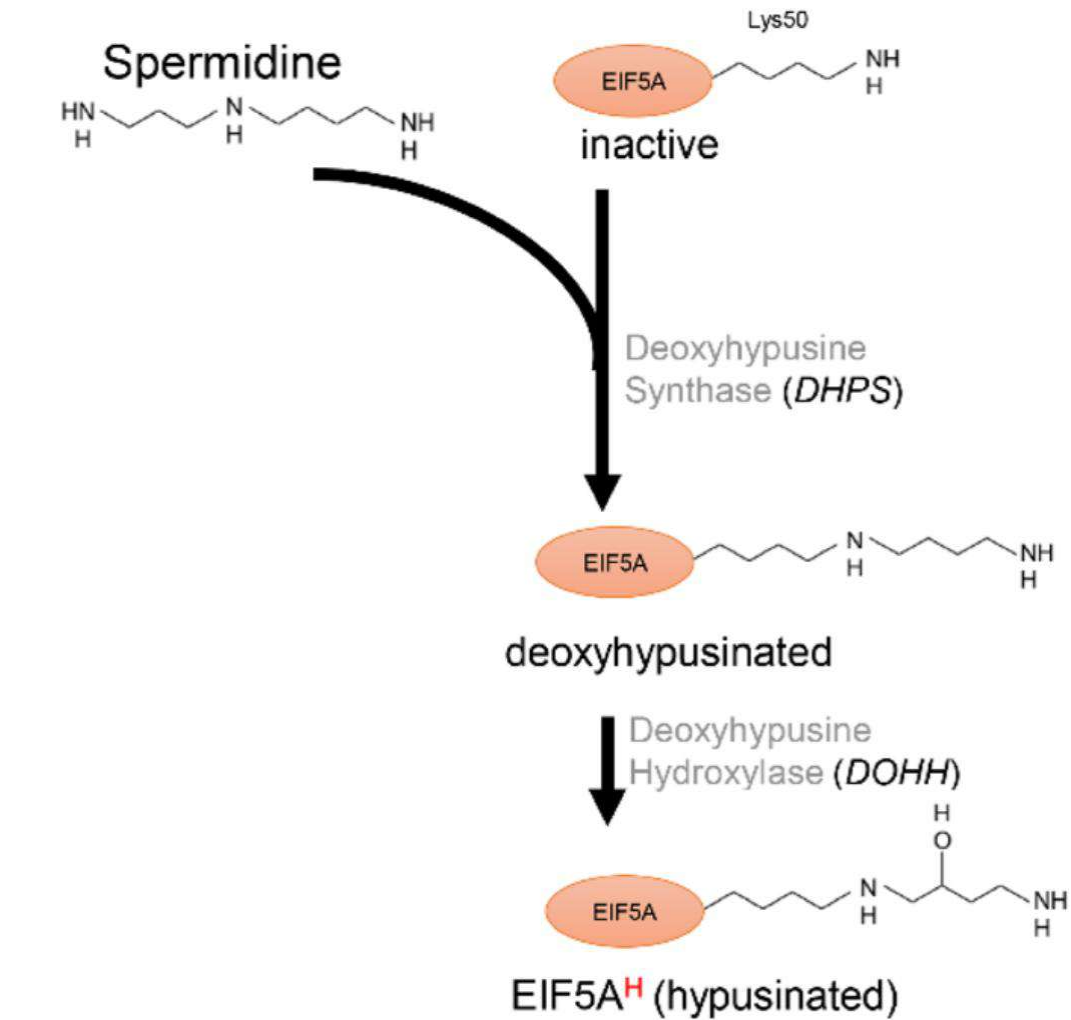




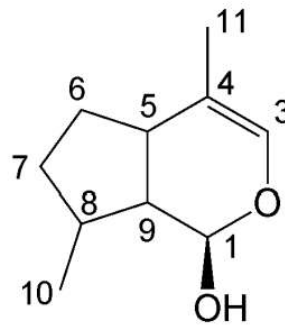
Thank you for your attention

All questions are welcome!

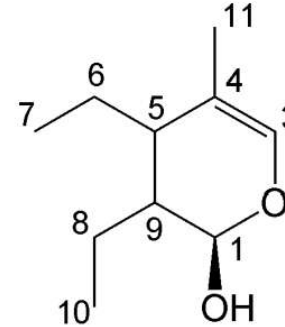
Hypusination



Secoiridoids



General iridoid skeleton



General secoiridoid skeleton

The secoiridoids are derived from iridoids by opening of the cyclopentane ring of the iridoids. Iridoids are abundant in Oleaceae.

Spd Extraction

This new process allows the extraction of polyamines from a complex natural matrix, such as wheat germ, thanks to various treatment, extraction and filtration steps.

The liquid obtained, rich in polyamines, is further processed in order to precipitate and crystallize the polyamines from the hydro-alcoholic solution. By the end, within **24-48 hours**, the polyamines can be collected by simple filtration. A **more concentrated product** is therefore obtained, compared to those currently available, and **suitable for food consumption**.

Spd Extraction

The powdered wheat germ matrix alone and that used for the supplement were, respectively, extracted for polyamine content using an acid water solution containing 0.4% (v/v) trifluoroacetic acid (TFA). The material containing the soluble polyamine content was sonicated for 15 min and then filtered at 0.22 μm . The polyamines (SPD, putrescine, and spermine) were separated and analyzed by HPLC-ESI-MS (Waters e2695 equipped with Acquity QDa detector) using a Luna Omega polar C18 column (Phenomenex, Torrance, CA, USA), 5 μm , 250 $\times$ 4.6 mm set at a temperature of 30 $^{\circ}\text{C}$. The mobile phase was composed of 15% methanol, 5% acetonitrile, and 80% TFA solution (0.4% in water), respectively. Detection parameters were as follows: positive ionization, 12 V cone voltage, and probe temperature of 600 $^{\circ}\text{C}$.