Comparative analysis of fatty acids in mice brain: effect of inflammation

Charlotte Madore, Stéphane S. Grégoire, Veronique de Smedt-Peyrusse, Nathalie Castanon, Niyazi Acar, Lionel Brétillon, Sophie Layé, Corinne Joffre

BRAIN LIPIDS 2011

Journées CHEVREUL 2011

LIPIDS & BRAIN 2

Paris (FIAP Jean Monnet)
Monday 28 to Wednesday 30 March 2011

SPEAKERS ABSTRACTS
POSTERS ABSTRACTS

Scientific Committee

Bernadette Delplanque, Philippe Guesnet, Havard Bentsen, Jean Marc Alessandri, Nicole Pages, Pierre Astorg.

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The French Society for the Study of Lipids (SFEL, ex AFECG) is pleased to invite you to participate to the Journées Chevreul 2011 Lipids & Brain which will be held in Paris. This meeting is a follow-up to previous successful conferences held in Paris in 2007 (Journées Chevreul Lipids & Brain) and in Oslo in 2008 (the Brain Lipids Conference). It will be devoted to both fundamental and applied research on the metabolism and the biological effects of polyunsaturated fatty acids (PUFA) within the central nervous system and will address their bearing on brain health and disease.

There is a growing interest in omega-3 PUFA, the precursor (ALA: AlphaLinolenic Acid) or the Long-chains (LCn-3), since we know that docosahexaenoic acid (DHA, 22:6n3), the longest and most unsaturated fatty acid found in nervous membranes, is involved in the maintenance of normal neurophysiological function. Challenges in this field of research are depicting the different cellular processes modulated by DHA during growth and ageing (synaptogenesis, neurogenesis, neuronal apoptosis and inflammation) and to investigate the involved mechanisms. They are also understanding the mechanisms of control of PUFA metabolism in the brain and at the blood-brain barrier level. Many clinical and epidemiological studies have been conducted to assess the implications of the intake of omega-3 PUFA, (precursor or LCn-3), during pregnancy and lactation (maternal nutrition) on brain infant development and its impact on the metabolism during childhood. Moreover the potential roles of DHA and eicosapentaenoic acid (EPA, 20:5n3) in the management of depression and age related neuronal diseases including pathologies of the retina have been the topic of several studies.

The next Journées Chevreul 2011 Lipids & Brain in Paris will provide an opportunity to extend our understanding on these fields of research and on the relationship between nutrition, PUFA and central nervous system functions. They will honor Professor Nicolas Bazan from the LSU Neuroscience Center of Excellence (New Orleans) for his research conducted on DHA and the Neuroprotectin D1 and their roles in many neurological diseases, such as Alzheimer disease and Stroke. We look forward to welcoming you to this exciting meeting.

MEDAILLE CHEVREUL : Dr Nicolas BAZAN

DHA in stroke, Alzheimer's and blinding retinal degenerations: coping with neuroinflammation and cell survival

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Monday, March 28

9h30 –> Registration and Coffee

INTRODUCTION

10h15 –> Cunnane Stephen, CA

SIGNALLING MECHANISMS AND METABOLISM OF N-3 PUFA IN THE BRAIN

Chair: Alessandri Jean-Marc, Cunnane Stephen

10h45 –> Docosahexaenoic acid: metabolism and neurotrophic signaling mechanisms
Kim Hee-Yong, USA

11h15 –> The role of omega-3 fatty acids in hippocampal neurogenesis
Dyall Simon, UK

11h45 –> Valorisation and biodisponibility of polyunsaturated fatty acids
Linder Michel, FR

12h15 –> Lunch / Poster Session

14h00 –> Maintaining brain PUFA concentrations: uptake and rapid metabolism
Bazinet Richard, CA

14h30 –> Graded dietary n-3 PUFA deprivation identifies how brain PUFA metabolism is regulated
Rapport Stanley, USA

15h00 –> Reversion of kainate-induced seizures in magnesium-deficient mice is improved by omega-3 diet (rapeseed oil)
Pages Nicole, FR

15h15 –> Regulation of endocannabinoid signalling by dietary fatty acids: implications for brain function, obesity and the metabolic syndrome
Di Marzo Vincenzo, IT

15h45 –> Coffee break / Poster Session

PUFA AND NEURODEVELOPMENT - FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

Chair: Guesnet Philippe, Delplanque Bernadette

16h15 –> Post birth n-3 LCPUFA supplementation in women and children – Who benefits?
Gibson Robert, AU

16h45 –> Early behaviour and development influnced by omega-6 and omega-3 status
Strandvik Birgitta, SE

17h15 –> The role of omega-3 fatty acids in child development
Osendarp Saskia, NL

17h45 –> Synergistic effect of omega-3 PUFA deprivation and early life maternal separation in rats placed in an uncontrollable adverse situation
Mathieu Géraldine, FR

18h00 –> Poster Session

Tuesday, March 29

PUFA AND NEURODEVELOPMENT - FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

Chair: Guesnet Philippe, Delplanque Bernadette

9h00 -> The role of n-3 LCPUFA in pregnancy.
Makrides Maria, AU

9h30 -> Brain DHA levels of young rat are related to mother omega-3 status, and to ALA levels and fat matrix of the diet: impact of dairy products.
Delplanque Bernadette, FR

10h00 -> Different omega-3 sources for maternal diet during pregnancy and DHA in the developing rat brain
Childs Caroline, UK

10h30 -> Coffee break / Posters

11H00 CHEVREUL MEDAL : PR BAZAN NICOLAS, USA
DHA IN STROKE, ALZHEIMER'S AND BLINDING RETINAL DEGENERATIONS: COPING WITH NEUROINFLAMMATION AND CELL SURVIVAL

12h15 -> Lunch / Poster Session

13h45 -> AG SFEL

PUFA AND NEURODEVELOPMENT - FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

14h30 -> Alteration of brain insulin and leptin signaling promotes energy homeostasis impairment and neurodegenerative diseases
Taouis Mohammed, FR

15h00 -> Brain ciliary neurotrophic factor (CNTF) and hypothalamic control of energy balance.
Vacher Claire-Marie, FR

PUFA and Depression

Chair: Astorg Pierre, Bentsen Havard

15h15 -> Relationship of n-3 and n-6 in risk of clinical depression: results from the Nurses' Health Study
Lucas Michel, USA

15h45 -> The efficacy of omega-3 supplementation for major depression: a randomized controlled trial
Lesperance François, CA

16h15 -> Coffee break / Poster Session

16h45 -> EPA but not DHA appears to be responsible for the efficacy of omega-3 LC-PUFA supplementation in depression: evidence from a meta-analysis of randomized controlled trials
Martins Julian, UK

17h15 -> Effects of PUFA supplementation evidenced by brain imaging
Puri Basant, UK

17h45 -> Spadin, a Sortilin-derived peptide, targeting rodent TREK-1 channels: a new concept in the antidepressant drug design
Heurteaux Catherine, FR

18h00 -> Poster Session

20h00 -> **GALA DINER at Bel Canto Restaurant**

Wednesday, March 30

PUFA AND OCULAR PATHOLOGIES

Chair: Alessandri Jean-Marc, Pages Nicole

8h45 –> Needs in omega-3 and ocular pathologies
Bretillon Lionel, FR

9h15 –> Very Long Chains fatty acids (C35) in normal eye and ocular pathologies
Berdeaux Olivier, FR

PUFA AND NEUROPROTECTION

Chair: Alessandri Jean-Marc, Bentsen Havard

9h30 –> Multi nutrient concept for enhancing synapse formation and function: science behind a medical food for Alzheimer's disease
Sijben John, NL

10h00 –> Alpha-linolenic acid for stroke protection: from experimental studies on brain preconditioning to nutrition
Blondeau Nicolas, FR

10h30 –> N-3 Polyunsaturated Fatty Acids (PUFA) deficiency aggravates the loss of the protective function of astrocytes in rat brain during aging.
Denis Isabelle, FR

10h45 –> Coffee break / Poster Session

11h00 –> Omega-3 fatty acids and acute neurological trauma
Michael-Titus Adina, UK

11h30 –> Neuroinflammation, well-being and dietary Omega-3 fatty acids
LAYE Sophie, FR

12h00 –> Schizophrenia, PUFA and glutathion
Solberg Dag and Bentsen Havard, NO

12h30 –> Lunch / Poster Session

PUFA, Cholesterol and Alzheimer Disease

Chair: Astorg Pierre, Delplanque Bernadette

14h00 –> Brain cholesterol in normal and pathological aging
Mulder Monique, NL

14h30 –> Defective liver biosynthesis of DHA correlates with cognitive impairment in Alzheimer's disease
Astarita Giuseppe, USA

15h00 –> Mediterranean diet and cognitive decline: what role for N-3 PUFA
Barberger-Gateau Pascale, FR

15h30 Thesis Award

15h45 Conclusions

Guesnet Philippe, FR

SPEAKERS ABSTRACTS

INTRODUCTION

Does dietary docosahexaenoic acid protect the aging brain? A major challenge

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Amongst lipid nutrients, few if any sustain greater interest than the omega-3 polyunsaturated fatty acid - docosahexaenoic acid (DHA). With few exceptions, prospective epidemiological studies suggest that fish and DHA help preserve cognition in the elderly. From a public health perspective, it therefore seems prudent to recommend moderate fish consumption throughout adulthood to help protect cognition during aging. Nevertheless, currently, the epidemiological studies supporting long term fish consumption as a factor reducing the risk of Alzheimer's disease (AD) are not well supported by either DHA analyses of plasma or brain, nor by clinical trials with DHA supplements. Why not? A few studies show that plasma and cortical brain DHA is lower in AD but, overall, this literature shows AD brain and plasma contain the same amount of DHA as in age-matched controls. Hippocampal DHA may be lower in AD but this remains to be confirmed and does not explain why most studies show DHA in frontal and temporal cortex is not different, despite these regions being significantly affected in AD. Our recent data suggest plasma DHA in AD is lower in some plasma lipids but higher in others, an effect that is not specific for DHA. We believe there are several reasons why lower dietary DHA intake increases the risk of AD on a long term population basis yet is not reflected by lower plasma or brain DHA levels or by clinical trials with DHA supplements in AD:

(i) In general, neither AD nor elderly control groups are sufficiently well defined.
(ii) Risk of AD probably involves insufficient intake of a cluster of nutrients not just DHA.
(iii) Normal aging is associated with changes in DHA metabolism, irrespective of DHA intake.
(iv) AD further perturbs metabolism of fatty acids in general not just DHA.
(v) Degenerative processes in the AD brain may well increase peroxidation of DHA.

The confusing and disparate results of plasma and brain DHA analyses in AD probably reflect a combination of these biological as well as methodological variables; without addressing these issues, the likelihood of success in clinical trials using DHA in AD would seem to be low. DHA is not the only nutrient in fish nor is it the only nutrient that would potentially reduce the risk of AD; perhaps clinical trials using a combination of nutrients would have greater chance of success. New tools are available that may shed light on the metabolism of DHA in humans and its possible role in brain aging. They include ^{13}C-DHA, GC-MS analysis of DHA species from AD brain, ^{11}C-DHA for PET studies, and various forms of MRI. The aging brain has broadly the same vulnerabilities as in the infant brain. Although DHA is now well-accepted in infant nutrition, this was not always the case; it was a hard fought battle lasting several decades. AD is a heterogeneous disease for which no recent pharmaceutical trial has been successful. The development of DHA as a nutraceutical for the prevention and/or treatment of AD will therefore require the same lengthy investment of energy and resources as it did in infant nutrition, including acceptance of the same uncertainties about eventual success.

NSERC, CIHR, CFI, CRC, FRSQ, FQRNT and the Université de Sherbrooke provided financial support of our research on this topic.

SIGNALLING MECHANISMS AND METABOLISM OF N-3 PUFA IN THE BRAIN

Docosahexaenoic Acid: metabolism and neurotrophic mechanisms

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Docosahexaenoic acid (DHA, 22:6n-3) is particularly enriched in neuronal tissues mainly as membrane phospholipids (Kim HY, 2007). Maintenance of a high DHA concentration in brain is essential for proper neuronal function, suggesting a unique role played by this fatty acid for an optimal membrane structure and function in the nervous system. In search for mechanisms supporting essential function of DHA, we have investigated the role of DHA in membrane modification and cell signaling leading to neuronal survival and development. We found that DHA promotes neuronal survival primarily by increasing PS, the major acidic phospholipid in cell membranes. DHA uniquely increases PS, specifically in neuronal cells by serving as the best substrates for PS biosynthesis (Kim HY, 2007, Kim HY et al., 2004). The plasmenyl and recycling endosomal membranes are the major sites of PS localization in the cytoplasmic face, indicating that the plasma membrane offers a specific target surface for interaction of cytosolic proteins with PS (Calderon F and Kim HY, 2008). N-3 fatty acid deficiency decreases the PS content selectively from neuronal membranes through the inhibition of PS biosynthetic activities. Reduction of PS from neuronal membranes affected membrane translocation and activation of Raf (Kim HY et al., 2000), PKC (Kim HY et al., 2010) and Akt (Akbar M et al., 2005), which was found to be also PS-dependent (Huang BX et al., 2011), consequentially compromising neuronal survival. DHA also promotes neurite growth, synaptogenesis and glutamatergic synaptic function in developing hippocampal neurons (Cao D et al., 2009), supporting observed positive effects of DHA in hippocampus-related functional outcome. Unique metabolism of DHA in neural cells as well as its role in promoting neuronal survival and development along with associated molecular mechanisms will be presented.

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Akbar M, Calderon F, Wen Z and Kim HY. Docosahexaenoic Acid: A positive modulator of Akt signaling in neuronal survival. *Proc. Natl. Acad. Sci.* U.S.A. **102**, 10858-10863, 2005.

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The role of omega-3 fatty acids in hippocampal neurogenesis

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Neurogenesis occurs in limited areas of the adult mammalian brain, and has been reported in the hippocampus of rodents and man. Neurogenesis is enhanced in conditions associated with enhanced synaptic plasticity and following neuronal injury, suggesting a role for neurogenesis in cognition and brain repair.

Omega-3 polyunsaturated fatty acids (PUFAs) have been shown to promote hippocampal neurogenesis in a variety of models. Importantly, recent work has shown that the fat-1 transgenic mouse, an animal model of endogenous omega-3 PUFA enrichment, exhibits enhanced neurogenesis, with concomitant improvements in spatial memory compared to wild type mice (HE et *al*. 2009). During ageing, the rate of neurogenesis declines significantly and there is a strong correlation between memory impairment in hippocampal-dependent tasks and this decline. Interestingly, there is a strong correlation between omega-3 PUFA and hippocampal-dependent memory tasks, and we have recently shown that supplementation of aged rats with omega-3 PUFA partially reverses the age-related decline in neurogenesis (DYALL *et al*. 2010). Thus omega-3 PUFA positively influence neurogenesis, and these effects may contribute to improved cognitive performance. However, the mechanisms by which omega-3 PUFA regulate neurogenesis remain unclear, although a number or putative targets have been suggested.

The aims of this paper are to review the role of omega-3 PUFA in hippocampal neurogenesis, and explore some of the potential mechanisms of action which may underlie the observed effects.

References

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Valorisation and biodisponibility of polyunsaturated fatty acids

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The beneficial health effects of long chain polyunsaturated fatty acids (LC-PUFAs) are widely described in the literature, including benefits to coronary heart disease, cancer, inflammatory, and neurodegenerative diseases. An overview of the chemical structure, the different marine sources and conversion pathway from the essential dietary precursors is presented. Different lipid forms emerged on the market (triacylgglycerols, fatty acids, ethyl esters, phospholipids) to respond to the PUFA dietary guideline recommendations. However, the efficiency of these molecules depends on their bio-availability and upon the regiodistribution of the fatty acids esterified on the glycerol backbone. Several studies showed that the best carrier form of LC-PUFA seems to be an acylphosphoglycerol. Phospholipids contain 2-5 fold more DHA compared to marine triacylglycerols. The orally ingested type of DHA-ester determines whether absorbed DHA is packaged into forms that are accessible by specific tissues. Several animal studies reported an accumulation of 2-3 fold more radiolabeled DHA-phospholipids in the brain compared with DHA-TAG. Recent data suggest that brain preferentially absorbs DHA as *sn*-2 Lyso-PC compared with unesterified DHA. Different approaches are used to increase drugs and PUFA bioavailability. Mixtures of lipid classes, nano-emulsion or nanoliposome forms are used as LC-PUFA delivery systems, even in the drastic conditions found in the gastrointestinal tract.

Maintaining brain PUFA concentrations: uptake and rapid metabolism

Richard P Bazinet, PhD

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The brain is particularly enriched in glycerophospholipids with either arachidonic or docosahexaenoic acid esterified in the stereospecifically numbered-2 position. In this talk, I will review how polyunsaturated fatty acids (PUFA) enter the brain, and the mechanisms that regulate their concentrations within brain phospholipids. Whereas little evidence exists to support the incorporation of PUFA from lipoproteins into the brain, the incorporation rates of arachidonic and docosahexaenoic acid from the plasma unesterified pool into brain phospholipids closely approximate independent measures of their consumption rates by the brain. Upon entry into the brain, certain PUFA are highly conserved with extensive recycling within phospholipids, whereas others, such as eicosapentaenoic acid, are rapidly and extensively removed from the brain, in part, due to b-oxidation. Altered PUFA metabolism has been implicated in several neurological disorders, including bipolar disorder and Alzheimer's disease. Identifying the mechanisms by which PUFA enter and are handled within the brain could lead to new therapeutic approaches for these disorders and novel imaging methods.

Imaging plasma docosahexaenoic acid (DHA) incorporation into the brain in vivo, as a biomarker of brain DHA metabolism and neurotransmission

Stanley I. Rapoport, Mireille Basselin and Epolia Ramadan

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It is possible to image regional brain docosahexaenoic acid (DHA, 22:6n-3) incorporation into the brain *in vivo*, as a biomarker of brain DHA metabolism and neurotransmission in health and disease. This can be accomplished in rats with quantitative autoradiography and in humans with positron emission tomography (PET), by injecting radiolabeled DHA intravenously, determining regional brain radioactivity after a period of brain uptake, and normalizing brain radioactivity to the plasma input (integrated plasma radioactivity). The rate of brain DHA incorporation calculated in this way in rats was shown to correspond to the rate of brain DHA consumption by metabolism, determined following intravenous injection of radiolabeled DHA. This equivalence agrees with evidence that DHA cannot be synthesized *de novo* in vertebrates and that its nutritionally essential diet-derived circulating precursor, α-linolenic acid (α-LNA, 18:3n-3), is largely lost by β-oxidation after entering brain from plasma. This equivalence also justifies experiments to image how diet, drugs, disease and experimental manipulation alter regional brain DHA consumption, and how *in vivo* consumption depends on different phospholipase A_2 (PLA_2) enzymes. In one experiment, chronic visual deprivation reduced brain DHA incorporation on neuroimaging in regions (including superior colliculus) of the brain visual system, confirming a role for DHA in membrane remodeling. With regard to PLA_2 specificity, we confirmed a key role for Ca^{2+}-independent $iPLA_2$ in brain DHA metabolism and neurotransmission. Acute injection in unanesthetized rats of N-methyl-D-aspartate (NMDA), which increases entry of extracellular Ca^{2+} into the cell through the NMDA receptor, produced a robust arachidonic acid (AA, 20:4n-6) signal mediated by Ca^{2+}-dependent cytosolic $cPLA_2$, but no DHA signal. Additionally, mice in which $iPLA_2\beta$ (IVA) was knocked out showed markedly reduced brain DHA signaling at baseline and in response to the cholinergic agonist arecoline, supporting a major role for $iPLA_2\beta$ in DHA neurotransmission. Mutations in the gene for $iPLA_2\beta$ are associated with dystonia-Parkinsonism and other human motor disorders, so that DHA incorporation might be used as a biomarker for genetic $iPLA_2\beta$ and related abnormalities.

Lowering of threshold to kainate-induced seizures occurs in magnesium–deficient mice and may be partly reversed by chronic rapeseed oil diet and by acute magnesium chloride hexahydrate administration

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Magnesium deprivation increases brain vulnerability to inflammatory/oxidative and convulsant stresses. In mice, this nutritional model is suitable for *in vivo* evaluation of anticonvulsant, neuroprotective, and antiinflammatory/antioxidant compounds. Chronic magnesium deprivation based on a vegetable oils diet without ω-3 in mice is a nutritional *in vivo* model for a decreased threshold to NMDA receptor activation. This nutritional model responds remarkably to acute magnesium supply, further supporting that magnesium administration is a promising approach of glutamate-mediated brain disorders. On the other hand, recent data indicate that diet enriched with 5% rapeseed oil (rich in ω-3 alpha-linolenate [ALA]) improves protection against experimental seizures to a higher extent than diet containing 5% corn/sunflower oil (rich in PUFA).

In the wake of previous studies, the present work originally highlights a lowering of threshold to kainate seizures in OF1 mice induced by chronic exposition (27 days) to nutritional deprivation in magnesium (35 vs 930 ppm in normal diet). The shift observed in this threshold was operated from 45 mg/kg (normal magnesium fed animals) to 14.5 and to 17.5 mg/kg in mice given a magnesium-deficient diet based on corn: sunflower or rapeseed oils respectively Partial reversions (218 and 154%) in magnesium deficiency-driven drop of kainate seizure threshold were provided by acute intraperitoneal administration of magnesium chloride hexahydrate (at 28 mg magnesium/kg, threshold was re-heightened to 31 and to 27 mg kainate/kg, under corn:sunflower and rapeseed oils, respectively). In the present series of experiments, previously reported abilities of acute magnesium administration and of rapeseed oil-based magnesium-deficient diet to protect fully and partly, respectively, mice against audiogenic seizures were again observed. As a preliminary conclusion, ω3PUFA versus PUFA without ω3 enrichments of magnesium deficient diets reduce both the drop in kainate seizure threshold (would be less brain aggressive) and the capacity to reverse this drop through magnesium administration.

Key words

Rapeseed Oil, corn: sunflower oil, omega-3, alphalinolenic acid, audiogenic seizure test, magnesium deficiency Nutritional magnesium deprivation, Kainate receptor, ebselen, magnesium chloride hexahydrate, Kainate-induced seizure, seizure threshold

Regulation of endocannabinoid signalling by dietary fatty acids: implications for brain function, obesity and the metabolic syndrome

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Omega-3 polyunsaturated fatty acids (ω-3-PUFA) are known to affect several brain functions, to ameliorate several metabolic risk factors for cardiovascular disease and, at the same time, to alter the tissue levels of arachidonic acid (AA) esterified to phospholipids. During the last decade, we investigated, in collaboration with several research groups, the effects of dietary ω-3-PUFA supplementation on the tissue levels of the endocannabinoids, anandamide and 2-arachidonoylglycerol, two AA-containing, phospholipid-derived mediators that act mostly by stimulating the activity of two G-protein-coupled receptors, the cannabinoid receptors of type 1 and 2 (CB1 and CB2). Since cannabinoid receptors and endocannabinoids are emerging as key players in the control of synaptic function, on the one hand, and metabolism, on the other hand, we have also investigated if prolonged supplementation of food with dietary ω-3-PUFA affects these functions via alterations of this endocannabinoid system.

Studies on the stress response and on metabolic parameters were carried out, following dietary manipulations in rat dams, by means of behavioral observations in pups, or following milk supplementation with ω-3-PUFA in post-natal mice and piglets. Dietary administration of ω-3-PUFA was also carried out in two animal models of obesity (obese Zucker rats and mice with diet-induced obesity) and in a human clinical study. Finally, experiments were also performed in isolated adipocytes, following addition of AA or long chain ω-3-PUFA to the culture medium for 48 hours. In all cases, the tissue levels of endocannabinoids and their AA-containing biosynthetic precursors were measured by different lipid profiling LC-MS techniques.

In general, brain endocannabinoid levels were much less sensitive to dietary ω-3-PUFA supplementation in adult obese rats than in new-born pups of dams treated during gestation and lactation, or than the levels in peripheral tissues and adipocytes. Pups of dams treated with a high fat diet together with ω-3-PUFA as fish oil (FO) exhibited an enhancement of the stress response that was partly associated with changes in hippocampal and hypothalamic endocannabinoid levels. In rodent models of obesity, a tissue-specific reduction of peripheral endocannabinoid levels was observed, especially if ω-3-PUFA were administered as krill oil *vs*. FO. This reduction in endocannabinoid tone was associated with beneficial effects on several parameters of the metabolic syndrome. Levels of AA-containing endocannabinoid biosynthetic precursors were generally down-regulated following ω-3-PUFA supplementation, suggesting that this type of dietary regimen affects the levels of endocannabinoids in part by reducing their biosynthesis.

Overall, our data suggest that prolonged administration of ω-3-PUFA may both contribute to an enhanced stress response in newborns from treated dams, and promote metabolic benefits in treated obese adults, in part by interfering with endocannabinoid biosynthetic precursor availability. The obese animal metabolic data were supported by findings in a recent human clinical trial in overweight participants.

PUFA AND NEURODEVELOPMENT – FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

Post birth n-3 LCPUFA supplementation in women and children – Who benefits?

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Over the past 2 decades there has been a marked shift in the fatty acid composition of the typical Western diet towards increased intake of the n-6 fatty acid linoleic acid (LA, 18:2n-6), largely as a result of the replacement of saturated fats with plant-based PUFA. Whilst health agencies internationally continue to advocate for high n-6 PUFA intake combined with increased intakes of preformed n-3 long chain polyunsaturated fatty acids (LCPUFA) docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) for pregnant women and infants and to reduce the incidence of CVD, there are questions as to whether this is the best approach. LA competes with alpha linolenic acid (ALA, 18:3n-3) for endogenous conversion to long chain derivatives and LA also inhibits incorporation of DHA and EPA into tissues. Thus, high LA levels in the diet generally result in low n-3 LCPUFA status. Pregnancy and infancy are developmental periods during which the fatty acid supply is particularly critical. The importance of an adequate supply of n-3 LCPUFA for ensuring optimal development of infant brain and visual systems is well-established in preterm infants, and there is now evidence that the supply of n-3 LCPUFA also influences a range of growth, metabolic and immune outcomes in childhood. This review will question of whether the there are ways of improving the n-3 LCPUFA status of women and children other than supplementing the diet with marine oils rich in n-3 LCPUFA. Development of prudent public health strategies for improving the health of women and children must take into account economic and marine sustainability.

Early behaviour and development are influenced by omega-6 and omega-3 status

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It is now shown that the famines in the Second World War 1945 and in China 1959 have given long-term effects, probably by epigenetic mechanisms. Essential fatty acids (EFA) are potent modulators of gene expression (1). In animal experiments modulation of the essential fatty acid content of the diet to dams give long-term effects on the offspring into adult life (2-8).
We have followed a cohort of late premature infants from birth to 5 years of age. The diet reported by the mothers contained low EFA, especially omega-3 (9). Fatty acid analyses were performed in early breast milk and in mothers´ and infants´ plasma phospholipids early after birth and at gestational age 40 and 44 weeks and related to mothers´ dietary intake (9). The development of the infants were assessed with Brazelton Neonatal Behavioral Assessment Scale (BNBAS) at term (40 weeks) and 44 weeks (10) and with Bayley´s Scales of Infant Development (Second Edition (BSID-II) at 3, 6, 10 and 18 months corrected age. At 40 weeks and 3 months videotapes were made of the infants' spontaneous motor behavior to assess the quality of their general movements (GMs). The results of the tests were compared to the early fatty acid pattern and in multiple linear regression adjusted for confounding background factors.
At 40 and 44 weeks of age the neurological development was negatively associated to omega-6 fatty acids and positively to eicosapentaenoic acid (20:5ω3, EPA), but not to docosahexaenoic acid (22:6ω3, DHA). The motor quality was negatively correlated to the omega-6/omega-3 ratio and to mead acid, considered an indicator of EFA deficiency. Arachidonic acid (20:4ω6, AA) in breast milk was negatively correlated to Orientation (BNBAS) (β -.491, R^2 = 0.55, p=0.011) and Range of States (β -.374, R^2 = 0.24, p=0.032). At 3 months was the mental development negatively associated to the ratio in early breast milk between linoleic acid (18:2ω6, LA) and alpha-linolenic acid (18:3ω3, ALA) (β -0.36, p=0.006), and at 6 months the LA concentration in early breast milk was negatively associated both to mental (β -0.40, p=0.002) and emotional (β -0.41, p=0.001) regulation. Positive correlations to the infants´ plasma phospholipid concentrations of DHA at 44 weeks were found at 10 and 18 months for emotional regulation and orientation, and at the same time negative correlations between the ratio of AA/DHA to mental development (β -0.32, p=0.021 and β -0.37, p=0.008) and at 18 months also to Orientation (BSID-II) (β -0.46, p=0.002). Mothers´ education had the highest impact of the background factors.
The results consistently showed a negative influence of omega 6 on the early development in premature infants and positive correlations to DHA were first seen after 10 months of corrected age. It would be important and necessary to confirm our results in a larger study, especially in the context that the modern Western diet contains high amounts of omega-6 fatty acids and low amounts of omega-3 fatty acids, thus given also to the pregnant mothers.

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The role of omega-3 fatty acids in child development

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Omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) are important constituents of the maturing brain and therefore considered crucial for brain development in utero and in early infancy.

However, it is uncertain whether n-3 LCPUFA supplementation during pregnancy and lactation can have beneficial, sustainable effects on visual or cognitive development. Beneficial effects on child cognitive function after supplementation with EPA and DHA during pregnancy and lactation were observed at 4 years of age, but not at 3, 6 months or 7 years.

In term infants LCPUFA when given in relative high dosages, seems to improve visual acuity, but not cognitive function. Evidence for an effect of LCPUFA supplementation of preterm infants remains inconclusive.

In children older than 2 years of age, epidemiological evidence suggests an association between psychiatric or neurodevelopmental disorders and omega-3 fatty acid deficiencies. However, the evidence from randomized controlled trials exploring the impact of omega-3 fatty acids on cognitive performance or brain function in school-aged children is not conclusive.

In conclusion, n-3 LCPUFA are highly present in the maturing brain and are important for normal brain functioning and development. When provided in relative high dosages, n-3 LCPUFA may improve visual acuity in term infants. However, it remains unclear whether supplementation with n-3 LCPUFA during pregnancy, early infancy, and childhood can improve cognitive function.

Synergistic effect of N-3 polyunsaturated fatty acids deprivation and early life maternal separation in rats placed in an uncontrollable adverse situation

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Background: Low concentrations of n-3 polyunsaturated fatty acid (PUFA) and chronic stress are implicated in susceptibility to mood disorders.
Methods: One group of rats was fed an n-3 PUFA-deficient and the other a balanced diet from conception and throughout their lives. Half of them suffered early chronic stress by separation from their mothers (MS) from day 6 to 21 (6 hrs/day) and the behavior of all rats was tested at three months. Anxiety was assessed with the cross-maze task, before and after behavioral tests involving two aversive tasks differing in the control that the rat could exert: (1) conditioned fear involving 5 paired tone-shock associations and (2) an avoidance brightness discrimination task in a Y maze.
Results: MS significantly increased basal anxiety. All rats were more anxious after the behavior tests, but the n-3 PUFA-deficient rats that had experienced MS were most anxious. An n-3 PUFA-deficit also increased their expression of fear in inescapable situation while the MS rats were the more fearful.
Conclusion: A lack of n-3 PUFA and MS both modulated the emotional status and acted in synergy when rats were placed in an uncontrollable stressful situation. The n-3 PUFA-deficiency exacerbated the effects of early stress in the adult rats, supporting the notion that PUFA-unbalanced diet is a risk factor in emotional disorders.

PUFA AND NEURODEVELOPMENT – FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

The role of n-3 long chain polyunsaturated fatty acids (LCPUFA) in pregnancy

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The last few years have witnessed the emergence of a multitude of supplements for pregnant and lactating women with n-3 (or omega-3) fatty acids, predominantly as marine or fish oil. These marine oils have been added to prenatal vitamins and minerals as well as to food products specifically designed for women in the perinatal period. The main motivation behind these supplements appears to be the suggestion that an increased supply of docosahexaenoic acid (DHA, 22:6n-3), a key n-3 long chain polyunsaturated fatty acid (LCPUFA), during the last trimester of pregnancy and early postnatal life may enhance the development of the fetal and infant brain, clearly building on the extensive body of work relating to LCPUFA supplementation of infant formulas for term and preterm infants.

The metabolic demand for DHA is increased during pregnancy and lactation because of the extra needs of the fetus, expanded maternal cell mass and placenta as well as to deliver DHA to breast milk. However, maternal dietary DHA intake in the perinatal period is low and it is not clear whether adaptive metabolic mechanisms, such as amenorrhea or increased DHA synthesis from precursor fatty acids or increased mobilization of fat stores, are capable of meeting the increased DHA requirements. Consequently randomized controlled trials are important to determine whether additional dietary DHA in pregnancy modifies maternal or infant health outcomes. The available randomized comparisons of n-3 LCPUFA supplements with regard to pregnancy duration and pregnancy outcome consistently demonstrate a modest increase in the duration of gestation that also results in modest increases in birth size. On the other had the randomized comparisons of DHA supplements vs placebo with outcomes relating to maternal depression, infant visual acuity and development have not consistently demonstrated benefit to either the mother or child.

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Brain docosahexaenoic acid (DHA) levels of young rat are related to mother omega-3 status, and to α-linolenic acid (ALA) levels and fat matrix of the diet: impact of dairy products.

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DHA is the major brain FA and its deficit during gestation and/or lactation could have dramatic impacts on brain functions and mental health. Whether the alterations, could be corrected or not by omega 3 dietary intake after weaning was poorly investigated.

Our objectives were: F*irst (Study I)*, to evaluate the impact of dietary ALA-deficient or -rich diets during gestation and lactation on the brain DHA levels of young male rats (newborns, weaning) and the possibility to modulate DHA incorporation after weaning by submitting the rats during 6 weeks to 3 diets : deficient, low or rich ALA diets*; S*e*cond (Study II)* to evaluate the efficacity of dairy fat to reverse brain DHA in meanling deficient rats comparatively to usual vegetable blends used in infant formula.

Procedure: Two groups of dams were fed during gestation and lactation with: 1/ a low ALA-diet (0.4%) based on Palm/Soya (97/3, 5% Fat) and 2/ a rich ALA-diet (8%) containing only Rapeseed oil (10% fat).

Study I: After weaning, young males (n=10) born from deficient and ALA-rich dams received 10% fat diets for 6 weeks: either an ALA-deficient Palm (ALA 0.4%) or an ALA-rich Rapeseed diet (ALA 8%).

Study II: After weaning, young ALA-deficient males (n=10) received 10% fat diets for 6 weeks: Butter and Palm diet blended or not with Rapeseed/Sunflower oils to mimic infant formula; the ALA-deficient Palm/Soya diet (0.4%); ALA intermediary diets (1.5%) blended either with Butter or Palm (similar to infant formulas); pure Butter and Rapeseed fat diets (ALA 0.8 and 8% respectively).

Results / Conclusions:

StudyI - As expected, newborn and weanling pups from deficient dams had brain DHA levels 2 times lower than those from ALA-rich dams. The brain DHA levels of the post weaning rats receiving a deficient diet for 6 weeks were more dependent of the dams status than of their own diet: 30% less for those born from ALA-deficient dams, indicating that an ALA-rich diet during gestation and lactation is protecting against ALA deficiency during post weaning growth, at least for 6weeks. - The brain DHA levels of young rats receiving post weaning the ALA-rich diet for 6 weeks allowed a recovery for those born from deficient dams, but they did not reach the values of those born from ALA-rich dams (10% less).

Study II Six-week post-weaning restoration of brain DHA levels in young omega3-deficient rats is better with Butter compared to Palm/Rapeseed blends. The 1.5%ALA blends from Butter and Palm increase the DHA levels respectively by 80% and 60%. The brain DHA-increase with pure Butter diet (0.8%ALA) is more than 3 times the increase obtained with the 0.4%ALA Palm-blend. The pure Butter diet is as efficient as the pure Rapeseed diet to restore completely the normal levels of brain DHA, despite 10 times less ALA levels in the fat matrix.

Conclusion ALA-rich diet during gestation and lactation is protecting against ALA deficiency and preserve brain DHA levels during post weaning growth, at least for 6 weeks. Butter fat diets seem to be the best to restore post-weaning the levels of DHA in the brain of deficient-omega3 rats. The use of Dairy fat should be reconsidered for infant formula.

Different dietary omega-3 sources during pregnancy and DHA in the developing rat brain

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Background: DHA accumulates in the brain in early life, and infant formulas containing DHA have demonstrated benefits upon cognitive function.

Objective: To investigate the effect of pregnancy and diet on the fatty acid composition of maternal rat tissues, and the effect of maternal diet during pregnancy upon the DHA content of fetal rat tissues.

Procedure: Experimental diets (n = 6) were provided for 20 days to virgin female rats, or from day 1 to 20 of gestation to pregnant rats. The diets were low fat (LF, 3% w/w) soya bean oil, high fat (HF, 13% w/w) soya bean oil, HF sunflower oil; HF linseed oil or HF salmon oil. Maternal and fetal tissues were collected for FA analysis by gas chromatography. Differences between dietary groups were assessed using one-way ANOVA with Bonferroni correction for multiple comparisons.

Results: Significant diet x pregnancy interactions were observed, with the DHA content of the maternal liver significantly higher in pregnant rats provided with a HF linseed oil diet compared to those on a soya bean oil based diet (Liver PC and PE $p<0.001$), while no such effect was observed in virgin females. Maternal diet during pregnancy was found to have a significant effect upon the fatty acid composition of fetal brain phospholipids, including DHA in brain PE ($p <0.001$). The ALNA rich linseed oil diet was as effective at inducing high levels of brain PE DHA (mean % total FA 17.1) similar to those seen with the salmon oil diet (mean % total FA = 18.0), with no significant difference between these two diets (Bonferroni $p = 1.000$). In all other maternal and fetal tissues assessed, the salmon oil group had significantly higher DHA than the linseed oil group (Bonferroni post hoc comparisons: fetal plasma PC $p < 0.001$; fetal liver PC $p < 0.001$; fetal liver PE $p < 0.001$).

Conclusion: The ability of ALNA-rich linseed oil diet to raise DHA levels in the fetal brain to match those achieved by salmon oil feeding indicates that DHA is preferentially incorporated into the fetal brain and that maternal and fetal plasma phospholipid DHA content is not a suitable marker of brain DHA content. Higher ALNA intake increases the DHA content of maternal liver phospholipids in pregnant, but not virgin, females. ALNA supplementation may therefore be a useful tool in promoting DHA supply to the fetal brain among pregnant women who are unable to or do not wish to consume oily fish.

CHEVREUL MEDAL: Pr Nicolas BAZAN

After obtaining his MD in Tucuman (Argentina), Nicolas Bazan was a postdoc at P&S, Columbia University, and Harvard Medical School. In his first lab at the Clarke Institute of Psychiatry (Toronto) his contributions to neuroscience and medicine began with the discovery that brain ischemia or seizures releases unesterified arachidonic and docosahexaenoic acids (DHA), which became known as the "Bazan effect" (citation classic - "Neural Stimulation or Onset of Cerebral Ischemia Activates Phospholipase A2", Current Contents, 30:10, 1991). Then, he found that platelet activating factor (PAF) is released by injury or stimulation in the brain and eyes; uncovered PAF binding in synaptic and intracellular membranes (1990); defined PAF-mediated regulation of early gene expression (1989); and found a role for PAF in long-term potentiation and memory (1994-95). He then uncovered that DHA supply to the nervous system is liver regulated (1989). He discovered that in photoreceptor renewal, retinal pigment epithelium recycling retains DHA within photoreceptors by a "short loop" (RPE-to-photoreceptors) and a "long loop" (liver-to-retina) (1985). He found that Usher's Syndrome patients have DHA shortage in the blood, implicating the long loop in retinal degenerations (1986). He then discovered enzyme-mediated formation of DHA derivatives in the retina and coined the term docosanoids (1984). He and his colleagues discovered the synthesis and bioactivity of the first docosanoid, neuroprotectin D1 (2003-4), which arrests apoptosis in retinal pigment epithelial cells at the pre-mitochondrial level, and is neuroprotective in brain ischemia-reperfusion and in cellular models of Alzheimer's disease. Then he found a decrease in NPD1 in the CA1 area of Alzheimer's patients, and that NPD1 promotes down-regulation of pro-inflammatory genes and of pro-apoptotic Bcl-2 proteins, and neuronal and glial cell survival from Aβ toxicity. As part of his translational research and as an inventor he hold more than 20 patents that includes a family of new analgesics, analogs of paracetamol devoid of liver toxicity; novel PAF antagonists that protect the brain from inflammation and damage; and applications for neuroprotectin D1 in brain and retina injury, aging, and neurodegenerative diseases, such as Alzheimer's and macular degeneration. The recognitions he received include the Javits Neuroscience Award, NINDS, NIH; elected to Royal Academy of Medicine, Spain; elected fellow, Royal College of Physicians of Ireland, Dublin; and Proctor Medal, the highest honor of The Association for Research in Vision and Ophthalmology. He is the founding director of the LSU Neuroscience Center of Excellence, New Orleans. He is on the editorial board of several journals including Editor-in-Chief of the 25 year old Molecular Neurobiology. He is a member of the BDPE NIH Study Section (2010-2014) and is member of the Senate of the DZNE, Germany. Most recently he has written the novel "Una Vida: A Fable of Music and the Mind", a compassionate, heartfelt story that takes readers on a tantalizing journey into the secret recesses of the brain of a hauntingly gifted jazz musician whose memory is slipping into the abyss of Alzheimer's.

Docosahexaenoic Acid (DHA) in Stroke, Alzheimer's Disease, and Blinding Retinal Degenerations: Coping with Neuroinflammation and Sustaining Cell Survival

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The significance of the selective enrichment in omega-3 essential fatty acids (docosahexaenoyl – DHA- chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (e.g. photoreceptors, synaptic membranes) has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neurodegenerations, we contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1,10*R*,17*S*-dihydroxy-docosa-4*Z*,7*Z*,11*E*,13*E*,15*E*,19*Z* hexaenoic acid). This mediator is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20 C arachidonic acid family member of essential fatty acids not enriched in the nervous system. We found that NPD1 is promptly made in response to oxidative stress and brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-β peptide. Thus we envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations. We provide here recent experimental examples that highlight the specificity and potency of NPD1 spanning beneficial bioactivity during initiation and early progression of neurodegenerations: 1) Photoreceptors renew membrane disks containing the phototransduction apparatus and DHA intermittently via shedding of their tips and phagocytosis by retinal pigment epithelial (RPE) cells. At the same time, new membrane disks are made at the base of the outer segments; their length remains constant and cell integrity is maintained remarkably unchanged throughout many decades. This outcome occurs in spite of the fact that the photoreceptors are in an oxidative stress-prone environment (light, high O_2 consumption, high polyunsaturated fatty acid fluxes, etc). We show that phagocytosis of photoreceptor disks promotes via NPD1 synthesis specific refractoriness to oxidative stress-induced apoptosis in RPE cells, which in turn fosters homeostatic photoreceptor cell integrity. Disruptions of the sentinel role of NPD1 in photoreceptor renewal may participate in macular degeneration and other retinal degenerations leading to blindness. 2) In brain ischemia-reperfusion, DHA (i.v.) one hour after two hours of middle cerebral artery occlusion (MCAO) leads to penumbra protection with an entended time window of protection (up to five hours) and with concomitant NPD1 synthesis. Anti-apoptotic BCL-2 family of proteins availability is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is the arrival of leukocytes due to neurovascular unit breakdown. 3) NPD1 is drastically reduced in CA1 areas from Alzheimer's patients. Thus we have explored the significance of NPD1 in cellular models that recapitulate part of the Alzheimer's pathology. Human neurons and astrocytes challenged by amyloid-β or by overexpressing APPsw (double Swedish mutation) show that NPD1 downregulates amyloidogenic processing of amyloid-β precursor protein, switches off pro-inflammatory gene

PUFA AND NEURODEVELOPMENT – FROM MATERNAL PREGNANCY AND LACTATION TO INFANT DEVELOPMENT

Alteration of brain insulin and leptin signaling promotes energy homeostasis impairment and neurodegenerative diseases

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The central nervous system (CNS) controls vital functions, by coordinating peripheral and central signaling pathways and networks in a coordinated manner. Historically, the brain was considered to be an insulin-insensitive tissue. But, new findings demonstrating that insulin is present in different regions of the mammalian brain, in particular the hypothalamus and the hippocampus. Insulin acts through specific receptors and dialogues with numerous peptides, neurotransmitters and adipokines such as leptin. The cross-talk between leptin and insulin signaling pathways at the hypothalamic level is clearly involved in the control of energy homeostasis (Niswender and Schwartz, 2003; Benomar et al., 2009). Both hormones are anorexigenic through their action on hypothalamic arcuate nucleus by inducing the expression of anorexigenic neuropetides such as POMC (pro-opiomelanocortin, the precursor of αMSH) and reducing the expression of orexigenic neuropeptide such as NPY (Neuropeptide Y). Central defect of insulin and leptin signaling predispose to obesity (leptin –resistant state) and type-2 diabetes (insulin resistant state). Obesity and type-2 diabetes are associated to deep alterations in energy homeostasis control but also to other alterations of CNS functions as the predisposition to neurodegenerative diseases such as Alzheimer's disease (AD). AD is a neurodegenerative disorder characterized by distinct hallmarks within the brain. Postmortem observation of AD brains showed the presence of parenchymal plaques due to the accumulation of the amyloid beta (AB) peptide and neurofibrillary tangles. These accumulations result from the hyperphosphorylation of tau (a mictrotubule-interacting protein). Both insulin and leptin have been described to modulate tau phosphorylation and therefore in leptin and insulin resistant states may contribute to AD. The concentrations of leptin and insulin cerebrospinal fluid are decreased type2 diabetes and obese patients. In addition, the concentration of insulin concentration in the cerebrospinal fluid of AD patients is diminished.

Taken together, these data clearly links deficiency of leptin and insulin signaling to both alterations of energy homeostasis control and predisposition to AD. Furthermore, environment changes leading to insulin and leptin-resistance may promote these defects, such as high fat diet.

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Brain Ciliary Neurotrophic Factor (CNTF) and hypothalamic control of energy homeostasis

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The neural cytokine CNTF (Ciliary Neurotrophic Factor) was originally characterized as a neurotrophic factor that promotes the survival of a broad spectrum of neuronal cell types and that enhances neurogenesis in adult rodents.
We have recently shown that CNTF participates in the control of energy balance. Indeed, CNTF and its receptor are expressed in the anorexigenic neurons of the arcuate nucleus (ARC), a key hypothalamic region controlling food intake, and CNTF levels are inversely correlated to body weight in rats fed a hypercaloric diet. Thus hypothalamic CNTF may act, in some individuals, as a protective factor against weight gain during hypercaloric diet and could account for individual differences in the susceptibility to obesity.
Moreover, CNTF is involved in the differentiation of astrocytes during the post-natal period, as well as in the regulation of anorexigenic neuronal activity in adulthood, in the ARC. Interestingly, we have demonstrated in adult rats that CNTF expression is stimulated by fatty acids and that CNTF receptor expression is influenced by the maternal diet.
In conclusion, these data indicate that brain CNTF, which depends upon diet conditions, represents a putative therapeutic target in the treatment of numerous major pathologies, including neurodegenerative diseases and obesity.

PUFA AND DEPRESSION

Relationship of n-3 and n-6 with clinical depression risk: results from the Nurses' Health Study

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Background: A relative decrease intake of n-3 to n-6 fatty acids has been implicated in the pathogenesis of depression. However, the associations between different sources of dietary n-3, n-6, and their ratio, and the risk of depression have not been prospectively studied.
Methods: We prospectively studied 54,632 women who were 50 to 77 years of age and free from depressive symptoms at baseline. Information on diet was obtained from validated food frequency questionnaires completed four times before baseline (1984, 1986, 1990, 1994). Clinical depression was defined as reporting both physician-diagnosed depression and regular antidepressant medication use. Relative risks (RR) of clinical depression, adjusted for age and other possible risk factors, were estimate using Cox-proportional hazard models.
Results: During 10 years of follow-up (1996-2006), 2,823 incident cases of clinical depression were documented. Long-chain n-3 intake from fish was not associated with depression risk (multivariate RR for 0.3 g/day increment=0.99 [95% CI: 0.88, 1.10]). Intake of alpha-linolenic acid (ALA) was not associated with depression risk, except in multivariate model adjusted for n-6 linoleic acid (LA). For each 0.5 g/day increment of ALA, multivariate RR was 0.82 (95% CI, 0.71 to 0.94). Interaction between ALA and LA was found to be significant for depression risk (P=0.02). Within quintiles of LA, a 0.5 g/day increment in ALA was inversely associated with depression in the first, second and third LA quintiles (RR=0.57 (95% CI, 0.37 to 0.87); 0.62 (95% CI, 0.41 to 0.93); 0.68 (95% CI, 0.47 to 0.96) respectively) but not in the fourth and fifth quintiles.
Conclusions: The results of this large longitudinal study do not support a protective effect of long-chain n-3 from fish or fish intake on depression risk. However, this study provides support for the hypothesis that higher ALA and lower LA intakes might reduce depression risk, but this relation warrants further investigation.

The Efficacy of Omega-3 Supplementation for Major Depression: A Randomized Controlled Trial

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Purpose: Epidemiological studies suggest that lower levels of omega-3 fatty acids are associated with higher rates of depression. In addition, although several small randomized clinical trials have suggested that eicosapentaenoic acid supplementation may be beneficial as an add-on treatment for major depression, the data is far from being conclusive. Finally, the efficacy of eicosapentaenoic acid supplementation has not been evaluated as a stand-alone treatment.

Methods: We conducted a multi-site, double-blind, randomized clinical trial to evaluate the efficacy of 8 weeks of eicosapentaenoic acid treatment in comparison to matched-placebo in 432 individuals with an episode of major depression in eight outpatients clinics in Canada. Patients were randomized to either 1050 mg of eicosapentaenoic acid and 150 mg of docosahexaenoic acid (DHA) divided into 3 capsules, or matched-placebo (sunflower oil with fish flavor). Eligible participants included those not responding to antidepressants (supplement could be administered as an add-on treatment in those not responding to antidepressants), and those unable to tolerate antidepressants or who refused antidepressants despite physician recommendation (as a stand-alone treatment). The primary outcome measure was the 30-item Inventory of Depressive Symptoms, Self-Rated (IDS-SR). The secondary efficacy outcome was the Montgomery-Asberg Depression Rating Scale (MADRS).

Results: Recruitment began in October 2005 and the final sample of 432 was completed in December 2008 with follow-up continuing until February 2009. Some 68.5% of participants were women, 40.3% of the patients were taking at least one antidepressant at baseline, 72.5% had a recurrent depressive episode and 52.8% had a co-morbid anxiety disorder. The mean age was 46 years. The adjusted mean difference between EPA and placebo was 1.32 points (95% C.I.: -.20 to 2.84; p=0.088) on the IDS-SR30 and 0.97 (95% C.I.: -.012 to 1.95; p=0.053) on the MADRS. Subgroup analyses revealed a significant interaction of comorbid anxiety disorders and study group (p=.035). For patients without comorbid anxiety disorders (n=204), EPA was superior to placebo, with an adjusted mean difference of 3.17 points on the primary outcome, the IDS-SR30 (95% C.I.: 0.89 to 5.45; p=.007) and 1.93 points on the secondary outcome (95% C.I.: 0.50 to 3.36; p=.008) on the MADRS. However, there was no evidence of efficacy for patients with comorbid anxiety disorder. The treatment was well tolerated with 83.6% of the subjects completing the planned 8-week study with the recommended dosage.

Conclusions: We designed and conducted what is as yet the largest randomized controlled trial of omega-3 supplementation for the treatment of major depression. There was only a trend towards superiority of EPA supplementation over placebo in reducing depressive symptoms for the full sample of subjects that included patients with comorbid anxiety disorders and those with chronic and resistant depressive episodes, patients usually excluded from antidepressant trials conducted by the pharmaceutical industy. However, there was a clear benefit of EPA among MDE patients without comorbid anxiety disorders. This may suggest that comorbid anxiety may not be as responsive to EPA supplementation as MDE either because of different pathophysiology or because higher dosages may be needed.

EPA but not DHA appears to be responsible for the efficacy of omega-3 LC-PUFA supplementation in depression: evidence from an updated meta-analysis of randomized controlled trials

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Background: Epidemiological and case-control data suggest that increased dietary intake of omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA) may be of benefit in depression. However, the results of randomized controlled trials are mixed and controversy exists as to whether either eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) or both are responsible for the reported benefits.
Objective: To update a recently published meta-analysis (Martins JG, 2009) of double-blind, placebo-controlled, randomized controlled trials examining the effect of ω3 LC-PUFA supplementation where depressive symptoms were a reported outcome. The differential effectiveness of EPA versus DHA has been reassessed through meta-regression and subgroup analyses.
Design: Studies were selected using the PubMed database on the basis of the following criteria: i) randomized design; ii) placebo controlled; iii) use of an ω3 LC-PUFA preparation containing DHA, EPA or both where the relative amounts of each fatty acid could be quantified; and iv) reporting sufficient statistics on scores of a recognizable measure of depressive symptoms.
Results: 370 studies were identified (22/01/2011) of which 34 met the above inclusion criteria (6 additional to Martins JG, 2009) and were therefore included for analysis. Using a random effects model, overall standardized mean depression scores were reduced in response to ω3 LC-PUFA supplementation as compared with placebo (standardized mean difference = –0.245, 95% CI = –0.385 to –0.105, z = –3.437, $p < 0.001$). However, significant heterogeneity and evidence of publication bias was present. Meta-regression studies showed a significant effect of higher levels of baseline depression and EPA dose on therapeutic efficacy. Subgroup analyses showed significant effects for: i) diagnostic category (bipolar disorder and major depression showing significant improvement with ω3 LC-PUFA supplementation versus mild to moderate depression, perinatal depression, chronic fatigue and non-clinical populations not); ii) therapeutic as opposed to preventative intervention; ii) adjunctive treatment and to a lesser extent monotherapy; and iv) supplement type. Symptoms of depression were not significantly reduced in 3 studies using pure DHA ethyl ester (standardized mean difference = 0.001, 95% CI = –0.330 to 0.332, z = 0.004, $p = 0.997$), in 2 studies using a mixture of DHA and EPA ethyl esters (standardized mean difference = 0.067, 95% CI = –0.226 to 0.361, z = 0.451, $p = 0.652$), or in 6 studies using fish oil supplements containing greater than 50% DHA (standardized mean difference = 0.023, 95% CI = –0.175 to 0.222, z = 0.229, $p = 0.819$). In contrast, symptoms of depression were significantly reduced in 13 studies using fish oil supplements containing greater than 50% EPA (standardized mean difference = –0.513, 95% CI = –0.840 to –0.185, z = –3.065, $p = 0.002$) and in 10 studies using pure EPA ethyl ester (standardized mean difference = –0.360, 95% CI = –0.597 to –0.123, z = –2.976, $p = 0.003$). However, further meta-regression studies showed significant inverse associations between efficacy and study sample size and duration, thus limiting the confidence of these findings.
Conclusions: This updated meta-analysis provides evidence that EPA may be more efficacious than DHA in treating depression. However, owing to the identified limitations of the included studies, larger, well-designed randomized controlled trials of sufficient duration are needed to confirm these findings.

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Effects of PUFA supplementation evidenced by brain imaging

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This lecture will examine how *in vivo* changes in structural neuroimaging studies, using the tools of sinc interpolation-based non-rigid body registration and affine registration-based voxel-based morphometry, and in proton neurospectroscopy studies, to study the effects of PUFA supplementation in neuropsychiatric disorders. In respect of 31-phosphorus neurospectroscopy scans, until recently the primary way non-invasively to study brain cell membrane phospholipids in humans *in vivo* has been through the quantification of membrane phospholipid metabolism by means of ascertaining the levels of phosphomonoesters and phosphodiesters. However, this only gives an indirect measure of the cell membrane phospholipid molecules. Ideally, one would want to study the brain cell membrane motion-restricted phospholipids directly, but it has previously proved almost impossible to obtain high-resolution magnetic resonance spectra of membrane phospholipids in living tissues owing to the large chemical-shift anisotropy of the ^{31}P confined to what is, in terms of nuclear magnetic resonance, the relatively rigid structure of the cell membrane, whether this be the external cell membrane or the membrane of an intracellular organelle. In this lecture, a description will also be given of an approach to measure directly the level of brain cell membrane phospholipid molecules.

Spadin, a Sortilin-derived peptide, targeting rodentTREK-1 channels : a new concept in the antidepressant drug design

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Depression has a life time prevalence of up to 20% but the molecular components leading to the disease are still poorly understood. By using knock-out (KO) mice we have previously identified the TREK-1 potassium channel as an important target in the regulation of depression. Then, we hypothesize that a blocker of TREK-1 channel should be an efficient antidepressant (AD). Here, we showed that Spadin, a peptide derived from Sortilin (also called NTSR3), able to inhibit TREK-1 channel activity, behaves as an AD molecule. Mouse behaviors were studied through five different animal models, namely the Porsolt Forced Swim, the Tail Suspension, the Conditioned Suppression of Motility, the Learned Helplessness and the Novelty-Suppressed Feeding tests. Spadin treated mice displayed a behavior similar to that of naive fluoxetine treated or TREK-1 KO mice. Spadin effects on 5-HT neurotransmission were also studied by *in vivo* electrophysiological measurements. An intraperitoneal spadin injection increased the efficacy of the 5-HT neurotransmission particularly in neurons from the Dorsal Raphe Nucleus, these particular neurons discharged within an amplified frequency, up to 7 Hz instead of 1.5-2 Hz for the untreated mice. More importantly and contrary to conventional antidepressants, an intravenous 4- day treatment with spadin, induced a strong antidepressant effect and enhanced hippocampal phosphorylation of CREB protein and neurogenesis. Our data point out spadin as a putative AD of new generation with a rapid onset of action. Spadin can be regarded as the first AD natural peptide identified. It opens a new way to address the treatment of depression.

PUFA AND OCULAR PATHOLOGIES

Needs in omega 3 and ocular pathologies

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Life expectancy at birth has regularly increased decade after decade, especially since the beginning of the 20th century: 15 years have been gained over the past 50 years. Changes in living and dietary habits during this time period have been associated with the development of various pathologies which represent a growing socio-economic burden. Among age-related disorders, ocular diseases are the second most prevalent ones after 65 years. Age-related Macular Degeneration (AMD) is the leading cause of visual impairment after the age of 50 years. Age is the prominent risk factor for AMD and is accompanied with both endogenous (including genetics) and environmental factors, such as smoking habits and dietary factors (diet rich in cholesterol and saturated fatty acids) (KLEIN R *et al*. 1999). AMD is characterized by the loss of cells at the most central area of the retina, called macula. The term "retina" encompasses both the neural retina and the retinal pigment epithelium (RPE). The neural retina is a highly structured neurosensory tissue that is responsible for the transduction pathway. The transduction pathway is initiated in photoreceptors where the light stimulus is coded into an electrical signal. This signal is transmitted to neighboured neurons and transferred to the brain via the optic nerve. The RPE is the cellular and metabolic interface between the neural retina and choriocapillaris through Bruch's membrane. The close association between RPE and photoreceptors is one of the factors that promote the efficacy of RPE to, in the one hand, provide nutrients and oxygen to photoreceptors and, in the other hand, eliminate the metabolic debris originating from shedding of the outer segments. Epidemiological data suggest that dietary habits privileging the consumption of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs: EPA and DHA) participate to prevent from the development of AMD (SANGIOVANNI JP *et al*. 2009). The mechanisms underlying the effects of omega-3 LC-PUFAs remain unclear until now. The role of DHA in promoting the efficacy of the retina to respond to light stimulus has been established for a long time. Recent experimental data suggest that EPA and DHA may be converted into active metabolites which would protect against neurodegeneration of the retina (BAZAN NG 2009). The purpose of this presentation is to review data highlighting or suggesting the role of omega 3 LC-PUFAs in the retina and their associations with ocular diseases.

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Very Long Chains FA in normal and ocular pathologies

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The retina is one of the vertebrate tissues with the highest content of polyunsaturated fatty acids (PUFA). A large proportion of the retinal glycerophospholipids, especially those of photoreceptor membranes, consist of dipolyunsaturated molecular species. Studies have reported that dipolyunsaturated phosphatidylcholine (PC) molecular species that are present in both rod- and cone-dominant retinas, contain 22:6n-3 as one of the acyl chains, the other one being very-long-chain (C24–C36) polyunsaturated fatty acids (VLC-PUFA; four, five or six double bonds). The majority of these PC species containing VLC-PUFA (VLC-PC) are localized in photoreceptor outer segments where the phototransduction reactions take place.
Recently, at least three different mutations in a gene called *elongation of very long chain fatty acids 4* (*ELOVL4*) have been identified in patients with Stargardt-like macular dystrophy type 3 (STD3), a dominantly inherited form of juvenile macular degeneration leading to vision loss. Based on sequence homology with ELOVL1, 2, 3, and 5 proteins, which are implicated in the elongation of saturated, monounsaturated, or polyunsaturated fatty acids (PUFA) from 18 to 26 carbons, the ELOVL4 protein was predicted to have similar function. These findings suggest that retinal health depends on the presence of C28-C36 PUFA in addition to that of docosahexaenoic acid (DHA, 22:6n-3).

In this context, dipolyunsaturated PC molecular species containing VLC-PUFA in retina must be precisely characterized to improve our understanding of the pathogenesis of STD3. The different analytical approaches developed for the characterization and quantification of VLC-PUFA in biological samples will be reviewed in this talk. For 25 years, several current approaches using gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) have been used for the characterisation and quantification of VLC-PUFA. Theses methods allowed characterizing with precision the structures (carbon chain length and double bond positions) of the different VLC-PUFA in bovine retina. Thus, retinal C28-C36 fatty acids are polyunsaturated and belong to the n-3 and n-6 families. The n-6 family of C28-C36 fatty acids has four or five double bonds, while the n-3 family of VLC-PUFA contains five or six double bonds. Moreover, some studies allowed determining the position of VLC-PUFA in the VLC-PC species. All identified VLC-PC molecular species contained C22:6 in the *sn*-2 position and VLC-PUFA in the sn-1 position. But most of these conventional approaches are time-consuming, requiring successive extraction, chromatographic steps (HPLC, TLC) and often a derivatization step before. Recently, a normal HPLC-ESI-MS/MS method was proposed for the structural characterisation and the quantification of VLC-PC species in retinas from bovines and human donors. It directly analyses phospholipids as intact molecules and preserves the information based on the relative position of acyl radicals on the glycerol backbone. Since the results from molecular and biochemical studies led to the conclusion that VLC-PUFA are involved in the pathogenesis of STD3, this specific and accurate method may be useful to more precisely investigate the molecular mechanisms leading to photoreceptor dysfunction and death in STD3.

PUFA AND NEUROPROTECTION

Multi Nutrient Concept of Enhancing Synapse Formation and Function: Science behind a Medical Food for Alzheimer's Disease

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Alzheimer's Disease (AD) is the leading cause of dementia. Epidemiological studies suggest that AD is linked with poor nutritional status of nutrients including DHA, B-vitamins and vitamins E and C. Ongoing neurodegeneration, particularly synaptic loss, leads to the classical clinical features of AD, memory impairment, language deterioration, and executive and visuospatial dysfunction. The main constituents of neural and synaptic membranes are phospholipids. Animals given three circulating dietary precursors of phospholipids, DHA, UMP and choline, develop increased levels of brain phospholipids, synaptic proteins, neurite outgrowth, dendritic spines formation (i.e. the anatomical precursors of new synapses) and improve learning and memory[1]. Other nutrients act as co-factors in the synthesis pathway of neuronal membranes. For example B-vitamins are involved in methylation processes, thereby enhancing choline availability as synaptic membrane precursor. A multi-nutrient mixture including these nutrients may improve membrane integrity, thereby influencing membrane-dependent processes such as receptor function and APP (amyloid precursor protein) processing, as shown by reduced amyloid production and Abeta plaque burden, as well as Abeta toxicity[2]. Together, these insights provided the basis for the development of a medical food for patients with AD, Souvenaid®, containing a specific combination of nutrients (Fortasyn™ Connect) and designed to enhance synapse formation in AD. The effect of Souvenaid on memory and cognitive performance was recently assessed in a proof-of-concept study, Souvenir I, with 212 drug-naïve mild AD patients (MMSE 20-26). This proof-of-concept study showed that oral nutritional supplementation with Souvenaid given for 12 weeks improves memory in patients with mild AD[3]. To confirm and extend these findings, three additional studies were initiated. Two of these studies will be completed in 2011; Souvenir II (NTR1975), a 24-week study, enrolled 259 drug-naïve mild AD patients (MMSE≥20) in Europe and S-Connect (NTR1683), also a 24-week study, enrolled 527 mild-to-moderate AD patients (MMSE 14-24) using AD medication in the US. The EU-funded[4] LipiDiDiet study (NTR1705), a 24-month study, will enroll 300 prodromal AD patients to assess the effect on memory performance and will be completed in 2013.

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Alpha-linolenic omega-3 fatty acid for stroke protection: from experimental studies on brain preconditioning to nutrition.

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Stroke is the third leading cause of death. The only therapeutic is the restoration of cerebral blood flow by recombinant tissue plasminogen activator, but it is delivered only to around 5% of patients. In addition, therapeutics aimed at blocking the ischemic cascade, as identified in numerous preclinical studies, failed in clinical trials. This poor translation from experimental models to clinical trials led to a revisiting of the concept of what would be the "best-in class" drug against stroke. Indeed, neuroprotection being ineffective *per se*, the future (mono-, bi- or tri…) therapies should protect the whole neurovascular unit and targets time-dependent mechanisms.

Molecules that precondition the brain to ischemia, known as preconditioners offer a novel perspective in stroke protection. They elicit complex endogenous neuroprotective responses including pleiotropic mechanisms to block death pathways, to increase resistance and to promote survival. Besides chemical preconditioners, natural/endogenous compounds such as adenosine, glutamate, lysophospholipids, and omega-3 polyunsaturated fatty acids have been described as excellent preconditioners. This field opens new concepts in combating stroke, most notably drawing attention to the importance of prevention. Preconditioning could also explain and justify further interest in dietary functional foods/nutraceuticals as an important tool in preventing stroke damage.

While several epidemiologic studies suggested a beneficial effect of a seafood/omega-3-enriched diet in cerebral diseases, the omega-3-induced protective mechanisms are still poorly identified. This talk will show that alpha-linolenic acid (ALA), the omega-3 polyunsaturated fatty acids precursors protects from *in vivo* and *in vitro* models of stroke and fulfill the framework to identify the "best-in class" drug against stroke. ALA targets the whole neurovascular unit. ALA protects neurons from glutamate-mediated excitotoxicity *in vivo* and *in vitro*. ALA vasodilates brain collateral arteries *ex-vivo* that account for the improvement in cerebral blood flow observed after an ALA injection *in vivo*

ALA triggers time-dependent mechanisms promoting neuronal plasticity. *In vivo* and in *vitro,* ALA promotes neurogenesis, synaptogenesis, neurotrophic factors expression, that are known to promote stroke recovery.

Finally, increasing ALA intake via daily diet prevents the damage induced by middle carotid artery occlusion. Significant reduced mortality rate, infarct size and increased probability of spontaneous reperfusion in the post-ischemic period are also observed with diet enriched in ALA.

To conclude, the multi-potential effects of ALA may be used to create a global strategy for protecting and stimulating brain responses to achieve post-ischemic functional recovery. ALA supplementation through a modification of the daily diet could support stroke prevention.

n-3 Polyunsaturated Fatty Acids (PUFA) deficiency aggravates the loss of the protective function of astrocytes in rat brain during aging.

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Background

The unbalance between n-3 and n-6 PUFA in western diet may result in low brain DHA status that is supposed to participate to the decline of brain function with aging and to the occurrence of associated neuropathologies. However, the mechanisms linking DHA status in brain cells (neurons and astrocytes) and brain age-related disorders are still questioning.

Objective

We have shown that DHA regulates several functions of the astrocytes (Champeil-Potokar *et al* 2006, Grintal *et al* 2009). Because the neuroprotective role of astrocytes determines, in a large part, the brain resistance to age-related damages, we have investigated the possible consequences of an n-3PUFA deficiency on astroglial function in aging rats, focusing on glutamate scavenging and age-induced astrocytes hypertrophy (astrogliosis).

Procedure

Glutamate transport was investigated by measuring D-^{3}H-Aspartate uptake by freshly isolated brain homogenates and by quantifying astroglial glutamate transporters by western blotting. Astrogliosis was evaluated by immunohistological and western blotting determination of GFAP (Glial Fibrillary Acidic Protein). These parameters were compared in young (4 month-old) and old (22 month-old) rats receiving an n-3PUFA balanced or deficient diet. Their brain fatty acid status was evaluated in the main phospholipid classes.

Results

n-3 PUFA intakes had no influence on astroglial glutamate uptake or GFAP in young rats. Old rats exhibited a 30% reduced glutamate uptake, and a marked increase in GFAP, as compared to young ones. These age-related changes were exacerbated by n-3 PUFA deficiency: glutamate uptake was decreased by 20% and GFAP was increased by 40% in n-3 PUFA deficient old rats as compared to n-3 PUFA balanced old rats. VGLUT-1, a marker of glutamatergic transmission was also decreased in old n-3 PUFA deficient rats.

Conclusion

These results indicate that n-3PUFA deficiency aggravates the impairment of astroglial glutamate transport and the astrogliosis in aged rats. They suggest that a low brain DHA status contributes to the alteration of astrocyte function in the the glutamatergic circuits during aging.

Omega-3 fatty acids and acute neurological trauma

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Acute neurological trauma remains one of the clinical areas with the most significant unmet needs. In the central nervous system, acute trauma has two stages: the primary injury and the secondary injury. The former is irreversible, and is a direct consequence of the impact. The latter involves a complex series of processes that amplify tissue damage. Some of these processes are local, and involve for example degradation of phospholipids, which has deleterious consequences (Huang *et al* 2009), whereas other processes involve a systemic response. The aim of all treatments for neurological injury is : a) to confer neuroprotection and/or b) to promote neuroregeneration. The results reported so far with omega-3 fatty acids in animal models of neurotrauma suggest that these compounds have the potential to offer a novel therapeutic approach, and possibly even support prophylaxis. The long-chain omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, lead to increased neuronal and glial survival, they can limit the damaging neuroinflammation and they can also protect neurites after spinal cord injury (Lim *et al* 2010 ; Ward *et al* 2010). Neuroprotective effects reported initally in spinal cord injury are now supported by data in models of traumatic brain injury (Mills *et al* 2011 ; Bailes *et al* 2011). Eicosapentaenoic acid and docosahexanoic acid may activate a multitude of targets, including voltage and ligand-gated ion channels, transcription factors and G-protein coupled receptors. They can produce tissue-specific metabolites which have intrinsic activity, either on the same or on different targets compared to the parent compounds. The use of omega-3 fatty acids in spinal cord injury, brain injury and peripheral nerve injury will be discussed, focusing on new data and on issues which need to be addressed in order to translate successfully to the clinic the efficacy reported in the initial proof of concept animal studies.

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Neuroinflammation, well-being and dietary Omega-3 fatty acids

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Well-being is defined at the individual level as quality of life. The behavioral alterations that develop in sick individuals during the course of an episode of sickness due to infection and inflammation include depressed activity, depressed exploration, reduced social interaction, decreased food and water intake, fatigue, disappearance of body care activities and mood and cognitive disorder. These behavioral alterations are the signs of altered well-being. At the molecular level, these behavioral modifications are triggered by the action in the brain of proinflammatory cytokines released by brain macrophages (microglia) and astrocytes. Because polyunsaturated fatty acids (PUFA) have immunomodulatory properties and are highly incorporated in the brain, we examined whether dietary deficiency in n-3 PUFA modulates brain cytokine production and associated behavioral modifications. Our results demonstrate that the behavioral response linked to brain cytokines depends on the diet. Moreover, neuroinflammation, revealed by the brain cytokine production and glial cell morphology was differentially affected according to the level of docosahexaenoic acid (DHA). All together, these findings suggest that the n-3 PUFA provided by the diet play a key role in well-being by modulating neuroinflammation.

Validation of two subtypes of schizophrenia defined by polyunsaturated fatty acids in blood cells during an acute psychotic episode

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Background*:* We have completed two studies (I, II) exploring a bimodal distribution of polyunsaturated fatty acids (PUFA) in red blood cells (RBC) in schizophrenia and related psychoses. At the Lipids and Brain 2011 conference we report observational data from study I (1: Bentsen H *et al, in press; 2:* Bentsen H *et al, submitted*). It was part of a 16 weeks randomized controlled trial of ethyl-EPA and vitamins E+C. We also present the design of study II, focusing on the relation between glutathione and PUFA in a cohort from study I. **STUDY I. Material and Methods:** Patients aged 18-39 years with DSM-IV schizophrenia, schizoaffective or schizophreniform disorders were consecutively included at admission to hospital. RBC fatty acids were measured in 97 patients at baseline, as well as in 20 healthy controls. The primary outcome measures: (1) the bimodality test statistic T; (2) negative subscale of the Positive and Negative Syndrome Scale (PANSS). **Results:** (1) At baseline, RBC PUFA were highly significantly bimodally distributed among patients. One third of patients constituted a group ("low PUFA") who had PUFA levels at one fifth ($p<0.001$) of those in "high PUFA" patients and healthy controls, which did not differ. Bimodality was mainly accounted for by docosahexaenoic acid and arachidonic acid. Bimodality was confirmed after 16 weeks. Alpha-tocopherol was a robust predictor of PUFA at both occasions. Desaturase and elongase indices differed between PUFA groups. Smoking, gender, antipsychotic medication and dietary factors did not explain the bimodal distribution. (2) Low PUFA patients had more negative symptoms than high PUFA patients ($p=0.04$). This difference was enduring. Hypertriglyceridaemia (44 % of patients) was 3.4 times more likely in low than in high PUFA patients ($p=0.009$). Clozapine/olanzapine entailed a higher risk than other antipsychotics only in the low PUFA group (interaction $p=0.08$). Among low PUFA patients, serum glucose was higher ($p=0.03$), men were heavier ($p=0.05$), and mean corpuscular haemoglobin was higher than among high PUFA patients ($p=0.001$). **Conclusions STUDY I:** RBC PUFA were bimodally distributed among acutely ill patients with schizophrenia and schizoaffective disorder. Endogenous deficiencies of redox regulation or synthesis of long-chain PUFA in the low PUFA group may explain our findings. Compared to high PUFA patients, low PUFA patients had more negative symptoms and increased morbidity and mortality risk. **- STUDY II** was undertaken 4-6 years later, in collaboration with K Do and M Cuenod at the University of Lausanne. We examine mortality of patients from study I. We have recruited 55 patients from this study, as well as 51 healthy controls. We analyze PUFA and their links to glutathione genotypes, amino acids, redox regulation biomarkers, PANSS, diet assessed by a questionnaire, and Brain Derived Neurotrophic Factor. Subjects were assessed twice.

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PUFA, CHOLESTEROL AND ALZHEIMER DISEASE

Brain cholesterol in normal and pathological aging

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Aberrations in cerebral cholesterol homeostasis can lead to severe neurological diseases and have been linked to Alzheimer's Disease (AD). Many proteins involved in peripheral cholesterol metabolism are also present in the brain. Yet, brain cholesterol metabolism is very different from that in the remainder of the body. Insights into the regulation of cerebral cholesterol homeostasis will be presented, focussing on the cholesterol trafficking between astrocytes and neurons and on peripheral modulatory effects.

Astrocytes are a major site of cholesterol synthesis. They secrete cholesterol in the form of apolipoprotein E-containing HDL-like particles. After birth, neurons are thought to reduce their cholesterol synthesis and rely predominantly on astrocytes for their cholesterol supply. How exactly neurons regulate their cholesterol supply is largely unknown. A role for the brain-specific cholesterol metabolite, 24(S)-hydroxycholesterol, in this process was recently proposed. Recent findings strengthen the link between brain cholesterol metabolism and factors involved in synaptic plasticity, a process essential for learning and memory functions, as well as regeneration, which are affected in Alzheimer's Disease. Enhancement of the cholesterol-turnover in the brain was found to improve memory functions in an Alzheimer mouse-model. In contrast with what was initially assumed brain cholesterol homeostasis can be modulated by extra-cerebral factors.

Insight into the regulation of cerebral cholesterol homeostasis will provide possibilities to modulate the key steps involved and may lead to the development of therapies for the prevention as well as treatment of neurodegenerative diseases such as Alzheimer's Disease.

Defective liver biosynthesis of DHA correlates with cognitive impairment in Alzheimer's disease

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Reduced brain levels of docosahexaenoic acid (DHA), a neurotrophic and neuroprotective fatty acid, might contribute to cognitive and visual decline. Here, we investigated whether the liver enzyme system that provides DHA to the brain and the eyes is dysfunctional in Alzheimer's disease. DHA content was measured in post mortem liver samples from a cohort of 9 control subjects and 14 Alzheimer's disease patients by liquid chromatography/mass spectrometry (LC/MS). Expression of genes involved in the biosynthesis of DHA from dietary α-linolenic acid (ALA) was assessed by real-time polymerase chain reaction (RT-PCR). Liver DHA content was lower in Alzheimer's disease patients than control subjects. Liver DHA/ALA ratios correlated positively with MMSE scores, and negatively with global deterioration scale grades. Notably, the prevalence of eye-diseases was 78.5% in AD patients versus 33.5% in control subjects. Lower levels of liver DHA were associated with higher prevalence of eye-diseases. DHA precursors, including tetracosahexaenoic acid, were elevated in liver of Alzheimer's disease patients, whereas expression of peroxisomal D-bifunctional protein, which catalyzes the conversion of tetracosahexaenoic acid into DHA, was selectively reduced. DHA content was also measured in brain samples from an additional cohort of 17 control subjects and 37 Alzheimer's disease patients. Levels of free DHA, DHA-derived neuroprotectin-D1 and DHA-containing phospholipids such as 1-stearoyl,2-docosahexaenoyl-sn-3-glycero-phosphoethanolamine and 1-stearoyl,2-docosahexaenoyl-sn-3-glycero-phosphoethanolamine-*N*-arachidonoyl were decreased in temporal cortex, mid-frontal cortex and cerebellum of subjects with Alzheimer's disease, compared to control subjects. Mini Mental State Examination (MMSE) scores positively correlated with DHA/ALA ratios in temporal cortex and mid-frontal cortex, but not cerebellum. These results indicate that a deficit in liver biosynthesis of DHA possibly lessens the flux of this neuroprotective fatty acid to the eyes and brain, contributing to cognitive and visual impairments.

Mediterranean diet and cognitive decline: what role for N-3 PUFA?

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The Mediterranean diet (MeDi) is characterized by a high consumption of fruits, vegetables, legumes, cereals, and olive oil as the main source of added fat, a moderate consumption of fish and wine, and a low consumption of meat and dairy products as a source of saturated fat. Three longitudinal epidemiological studies, the WHICAP and the CHAP in the US, and the 3-City (3C) study in France, have shown that higher adherence to a MeDi was associated with slower cognitive decline in older persons. These protective effects might be mediated by omega 3 poly-unsaturated fatty acids (n-3 PUFA). We investigated this hypothesis in the 3C study.

The study population (mean age 75.9 y) consisted of 1050 subjects from Bordeaux (France) included in the 3C cohort. Adherence to the MeDi (scored as 0 to 9) was computed from a food frequency questionnaire and 24 hour recall. The proportion of each plasma fatty acid was determined. Cross-sectional analyses of the association between plasma fatty acids and MeDi adherence were performed by multi-linear regression. After adjustment for age, sex, energy intake, physical activity, smoking, body mass index, plasma triacylglycerol and apolipoprotein E genotype, plasma docosahexaenoic acid (DHA) and total n-3 PUFA were positively associated with MeDi adherence. Plasma eicosapentaenoic acid (EPA) was positively associated with MeDi adherence only in ApoE4 non-carriers. The n-6-to-n-3 PUFA, arachidonic acid (AA)-to-EPA, AA-to-DHA and AA-to-(EPA+DHA) ratios were significantly inversely associated with MeDi adherence. There was no association with n-6 PUFA.

These data suggest that the protective effect of the MeDi on cognitive functions might be partly mediated by higher plasma n-3 PUFA and lower n-6-to-n-3 PUFA ratios. However, we cannot exclude residual confounding by a general healthy lifestyle of "Mediterranean diet adherents". These results must be reproduced in other studies.

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THESIS AWARD

Nutrition and brain aging: role of fatty acids with an epidemiological perspective

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In the absence of identified etiologic treatment for dementia, the potential preventive role of nutrition may offer an interesting perspective. The objective of the thesis was to study the association between nutrition and brain aging in 1796 subjects, aged 65 years or older, from the Bordeaux sample of the three-City study, with a particular emphasis on fatty acids. Considering the multidimensional nature of nutritional data, several complementary strategies were used. At the global diet level, dietary patterns actually observed in the population were identified by exploratory methods (Samieri C, Jutand MA *et al*., 2008). Older subjects from the "healthy" pattern, who consumed more than 3.5 weekly servings of fish in men and more than 6 daily servings of fruits and vegetables in women, showed a better cognitive and psychological health. Adherence to the Mediterranean diet, measured according to a score-based confirmatory method, was associated with slower global cognitive decline after 5 years of follow-up (Féart C, Samieri C *et al*., 2009). At the nutrient biomarker level, higher plasma eicosapentaenoic acid (EPA), a long-chain omega-3 fatty acid, was associated with a decreased dementia risk, and the omega-6-to-omega-3 fatty acids ratio to an increased risk, particularly in depressed subjects (Samieri C, Féart C *et al*., 2008). EPA was also related to slower working memory decline in depressed subjects or in carriers of the ε4 allele of the ApoE gene. Docosahexaenoic acid was related to slower working memory decline only in ApoEε4 carriers (Samieri C, Féart C *et al*., in press).

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P1. The Essential Role of n-6 Long-Chain Polyunsaturated Fatty Acids in Animal Neurodevelopment: a Review of the Literature

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The most abundant long-chain polyunsaturated fatty acids (LCPUFA) in the brain are, respectively, arachidonic acid (ARA, 20:4n-6), docosahexaenoic acid (DHA, 22:6n-3), and adrenic acid (AdrA, 22:4n-6). These LCPUFA accumulate rapidly during the brain growth spurt from the 2nd semester of pregnancy through the first two years of postnatal life. Their ratio in the total lipid fraction does not change significantly. These n-3 and n-6 LCPUFA are major structural components of neural cell membranes and exert essential functional roles during late embryonic and postnatal development of the brain. DHA is highly enriched in the synaptic regions of gray matter, ARA is present throughout gray and white matter, and AdrA is particularly concentrated in myelin lipids within white matter (1-3). Little is known about the role of n-6 LCPUFA, particularly ARA, in neurodevelopment. The literature was searched for randomized controlled trials in animals reporting on the role of n-6 LCPUFA on various aspects of neurodevelopment.

Supplementation of excess doses of fish oil to rat and mouse dams was reported to decrease the offspring cerebral and deep brain tissue content of n-6 LCPUFA (particularly ARA) (4-10). These low brain ARA levels were associated with mixed outcomes in rat and mouse offspring; delayed auditory development was frequently reported in pups born to dams fed excess doses of fish oil throughout gestation and lactation (4, 6-9, 11-14). The observed reduced brain myelin deposition (6, 15) and prolonged neural transmission times (6, 7, 9, 16) were suggested to contribute to the delayed acoustic startle reflexes seen in these pups (6, 7, 9). Co-addition of ARA was able to restore brain ARA levels, epinephrine levels and acoustic response (12), indicating that neural tissue ARA is involved in this aspect of early neurodevelopment.

That ARA plays a role in early neurodevelopment is corroborated by the finding that supplementation of ARA (8, 17, 18) or a source of ARA (19) was able to overcome poor place-learning performance in pups born to diabetic dams with low ARA levels throughout pregnancy and nursing. In adult diabetic rats, ARA improved nerve conduction velocity (20). Dietary ARA given for 4 wk after birth to rat pups enhanced neurogenesis and acoustic prepulse inhibition, a measure of sensorimotor gating (21).

Essential fatty acid deficiency in rat pups produced reduced brain ARA levels and reduced proportions of myelin lipids (22, 23). ARA and LA fully and ALA partially restored the reduction in myelin lipids in these rats (23). A substantial increase in ARA was found in myelin of developing and regenerating nerves from rats fed a diet sufficient but not deficient in essential fatty acids (24, 25). Myelination represents a crucial event during the maturation of the visual, motor, and auditory system (26) prenatally and during the first years of life.

Animal trials suggest that n-6 LCPUFA (particularly ARA) play an essential role in neurodevelopmental processes such as neurogenesis and myelination.

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P3. Omega-3 fatty acids and brain glucose utilization: an ^{18}F-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) study in the rat

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Background – Several *in vivo* and *in vitro* studies suggest that docosahexaenoic acid (DHA, 22:6ω3), the main omega-3 fatty acids of brain membranes, may be a regulator of brain energy metabolism by affecting glucose metabolism and its transporter GLUT1.

Objective – The purpose of this study was to evaluate whether cerebral glucose metabolism measured by FDG-PET would reflect decreased GLUT1 expression in the brain of omega-3 deficient rats.

Methods – We measured the cerebral metabolic rate for glucose with FDG-PET for small animal (microPET) in adult rats (10 wk-old) receiving an omega-3 fatty acid-adequate (control, $n = 6$) or an omega-3 fatty acid-deficient (omega-3 def, $n = 6$) diet. Dynamic PET scans were performed during 45 min after injection of ^{18}F-FDG (60 MBq). Data were reconstructed and superposed with MRI slices of the rat brain to calculate radioactivity in regions of interest (ROIs). Fatty acid content in brain phospholipid classes was determined by gas chromatography and the mRNA and protein expression of GLUT1 using real-time PCR (TaqMan low-density array, TLDA) and western blotting, respectively.

Results – Omega-3 PUFA-deficient rats had 60-70% lower DHA in their brain membrane phospholipids. Significantly decreased ^{18}F-FDG uptake was observed in the brain of omega-3 deficient rats, corresponding to both a lower rate of FDG uptake during the early phase (0-15 min) and a lower plateau level of ^{18}F-FDG incorporation during the later plateau phase (15-45 min). The gene and protein expression of GLUT1 was also lower in the omega-3 def group.

Conclusion – In rats deficient in omega-3 PUFA, lower expression of GLUT1 in the brain is consistent with in vivo results using FDG-PET that show glucose hypometabolism in the brain. Dietary intake of omega-3 fatty acids therefore clearly appears to modulate brain glucose uptake, a mechanism by which omega-3 fatty acid status may influence cognitive function known to be at risk during aging.

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P5. Impact of omega-3 fatty acids from fish oil on cognitive functions in a non-human primate: *Microcebus murinus*

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Omega-3 (ω3) fatty acids are major components of brain cells membranes. They are essential nutrients that must be provided by food. The role of ω3 has been mainly investigated using chronically dietary deficient animal models. ω3-deficient rodents exhibit severe cognitive impairments (learning, memory) that have been linked to changes in neurotransmission processes. Since neurotransmission is highly dependent on energy supply, dietary ω3 fatty acids could play a role in brain glucose utilization. Thus, we suggested that impairments observed in ω3-deficient animals could be due to lower brain glucose utilization (Ximenes *et al*. 2002). Indeed we first demonstrated that animals fed a diet deficient in ω3 fatty acids had lower brain glucose utilization (Pifferi *et al*. 2005). We then demonstrated that ω3 supplementation increased expression of brain glucose transporters *in vivo* in rats (Pifferi *et al*. 2007) and uptake of glucose in rat brain endothelial cells and astrocytes cultured *in vitro* (Pifferi *et al*. 2010). Omega-3 fatty acids supplementation protocols have demonstrated an improvement in several cognitive parameters in rodents (reviewed in Fedorova *et al*, 2006). On the basis of these observations, we now propose that ω3 supplementation could improve cognitive performances in primates, and that this effect could be supported by a better brain glucose utilization.

The present study is based on the use of animals from the colony of Grey Mouse Lemurs (*Microcebus murinus*) of UMR CNRS-MNHN 7179 (Brunoy, France). Adult male mouse lemurs were fed either a control diet (CTL group, n=6) or a diet supplemented with a fish oil naturally rich in long-chain ω3 fatty acids (ω3 group, n=6). After 6 months of dietary treatment, cognitive and motor performances were tested using a circular platform test (to evaluate reference spatial memory) and an open field test (to evaluate anxiety) accompanied by a sensory-motor test and a jump test. Spontaneous locomotor activity was also monitored for one week in the home cage of the animals.

Animals fed the diet supplemented with ω3 fatty acids exhibited better performances in the reference spatial memory task compared to control animas ($p<0.05$), with more than 80% of success in this task for supplemented animals and less than 40% of success for control animals. Spontaneous locomotor activity in the home cage is also significantly reduced in ω3 supplemented animals ($p<0.01$) which exhibit a 50% decrease of spontaneous locomotor activity compared to controls. No significant difference between groups has been observed in anxiety assessed with the open field test.

These promising results suggest a positive impact of dietary ω3 fatty acids on cognitive performances in adult primates. These results are very encouraging in the context of aging during which cognitive decline is linked to lower brain glucose utilization.Omega-3 fatty acids represent a potential dietary strategy to maintain or to increase cognitive functions during aging. Further PET studies will be needed to assess the impact of ω3 on brain glucose utilization in this species.

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P7. Effect of dietary palmitic acid substitution either for myristic acid, lauric acid or short-chain saturated fatty acids on plasma lipid parameters, enzymatic activities and tissue fatty acid composition in the rat

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Dietary saturated fatty acids (SFA) are usually associated with negative consequences for human health. However, an increasing number of recent studies have shown that some SFAs have important and specific cellular roles, like N-myristoylation of proteins for myristic acid (C14:0) or regulation of gene expression for butyric acid (C4:0). Therefore, all dietary SFAs are not necessarily associated with a negative impact and their specific identity, individual intake level and dietary origin must be considered.

Further to the previous studies realized in the laboratory (Rioux *et al*., 2005; Rioux *et al*., 2008 and Legrand *et al*., 2010), the purpose of the present study was to distinguish the effect of each saturated fatty acid present in the butterfat. Therefore, to answer this question, we compared the effect of a substitution of dietary palmitic acid either for myristic acid, lauric acid or short-chain saturated fatty acids (C4:0-C10:0) on plasma lipid parameters, liver enzymatic activities and tissue fatty acid composition (adipose tissue, liver, plasma, red blood cells, brain and heart) in adult rats. The control diet (PALM) contained palmitic acid as the main source of saturated fat (25% of FA). In the 4 successive diets, 10% of palmitic acid initially present in the control diet was substituted for another saturated fatty acid group or single fatty acid: substitution for 10% synthetic short-chain SFA in the short chain SFA-enriched diet (SHORT), substitution for 10% synthetic lauric acid in the lauric acid-enriched diet (LAU), substitution for 10% synthetic myristic acid in the myristic acid-enriched diet (MYR) and substitution for 10% oleic acid (brought by olive oil) in the oleic acid-enriched diet (OL). Finally, the last saturated fat diet was supplemented with butterfat (BF) and contained therefore natural short- and medium-chain SFAs and myristic acid which is mainly in sn-2 position of the triglycerides. An important characteristic of the 6 diets was the presence of equal amounts of α-linolenic and linoleic acids (3% and 12% of fatty acids, respectively). Male rats were randomly assigned to 6 groups (n=6 per group) and fed *ad libitum* with the 6 isocaloric diets for 8 weeks.

Results showed no significant effect of the diets on body weight and plasma total cholesterol. Concerning the FA composition of the tissues, the BF diet increased significantly the storage of the n-3 and n-6 PUFA in some tissues (adipose tissue, liver, plasma and heart) compared to the OL diet, but did not modify the FA composition in the brain and the red blood cells. We also showed that the SHORT, LAU, MYR and PALM diets did not modify the n-3 and n-6 PUFA profiles compared to the BF diet. Moreover, the diets had no effect on liver Δ6- and Δ5-desaturase activities, enzymes of PUFA biosynthesis.

In conclusion, in diets with identical well-balanced ALA/LA ratio, a 10% increase of individual SFA (short-chain fatty acids, lauric acid or myristic acid) with a concomitant decrease of palmitic acid has no effect on plasma lipid parameters and PUFA bioavailability. However, major differences in the n-3 and n-6 tissue storage appeared between the BF diet and OL diet. Therefore, it is difficult to distinguish the effect of each saturated fatty acid present in the butterfat because dairy product is a complex matrix.

P9. Beneficial effects of docosahexaenoic acid, on cognitive decline with aging and Alzheimer's disease

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Docosahexaenoic acid (DHA, C22:6n-3), one of the essential polyunsaturated fatty acids in the brain, exerts a markedly positive effect on neuronal cell properties. Many evidences have stressed the potential health benefits of DHA for cognitive decline with ageing and Alzheimer's disease (AD).

We previously found that dietary administration of DHA improves significantly cognitive learning ability in young and aged rats (GAMOH S *et al*. 1999, 2001) and also that the administration promotes the neurogenesis in the granule cell layer of dentate gyrus in adult rats (KAWAKITA E *et al*. 2006). Furthermore, we reported that DHA administration protects against (HASHIMOTO M *et al*. 2002) and ameliorates (HASHIMOTO M *et al*. 2005) the impairment of learning ability in amyloid β (Aβ)-infused AD model rats, with concurrent increases in DHA and decreases in the levels of lipid peroxide and reactive oxygen species in cortico-hippocampal tissues of the rats. Additionally, we reported that dietary DHA participates in eliminating the amyloid burden from the brains of AD model rats. Studies on whether DHA inhibits the *in vitro* polymerization of Aβ have revealed that DHA significantly inhibits the *in vitro* fibrillation of Aβ (HASHIMOTO M *et al*. 2008). DHA also significantly inhibits the *in vitro* fibrillation of Aβ and the amyloid fibrillation-induced apoptosis is reduced by DHA in cell culture (SHAHDAT H *et al*. 2009). These *in vitro* data support the *in vivo* suggestion that DHA ameliorates the cognitive deficits of AD by limiting Aβ polymerization in the brains of AD model rats

We investigated the relationships among cognitive function, dietary nutrition, and fatty acid components in plasma and erythrocytes of 53 65-year-old or older community dwellers in Shimane, Japan over a 4-year follow-up: DHA levels in erythrocytes were significantly higher in those who showed improvement or no change in their Hasegawa dementia rating scale (HDS-R) score than in those who showed a worse score. Recently, in this randomized, double-blind, placebo-controlled trail we observed beneficial effects of DHA+EPA supplement for 12 months on cognitive function in 65-year-old or older community dwellers in Shimane, Japan with very mild dementia .

These findings demonstrate that the consumption of DHA may improve cognitive function by preventing and/or delaying age-, or AD-related cognitive decline. DHA might be used as one of the therapeutic agents for the prevention or the slowing of cognitive impairment in very mild cases of AD.

P11. Effect of oils on neonatal brain development

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Brain is the most lipid-concentrated organ in the body, second only to the adipose tissue, the lipid constituting up to 50% of its total dry weight. These lipids play an important role in maintenance of membrane structure, function and fluidity. The study was undertaken to assess the incorporation of lipids into the membranes of brain and other organs in rat pups whose mothers. Adult female rats selected for the study were put on a diet containing different oils at 5% and 20% namely peanut oil, crude palm oil, fish oil and sunflower oil as the source of lipid. These were allowed to mate and conceive and the same diet was continued throughout the gestational period. The brain and other organs from the pups were collected at various stages pre-natal, pre-weaning and post natal. Lipid profile of the brain and other organs was analysed. Results indicate that docosahexaenoic acid and arachidonic acid are accumulated in the brain during the fetal stage, indicating that these fatty acids are essential and have to be provided in the diet through the maternal route.

P13. Synthesis of F_2-Dihomo-isoprostanes for the identification of early biomarkers of brain white matter oxidation in Rett syndrome

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Oxidative stress (O.S.) is involved in the pathogenesis of Rett syndrome (RTT),[1] a severe neurological disease affecting about 1 female newborn in 10,000 and caused in up to 95% by a mutation in the X-linked gene methyl-CpG binding protein 2 (*MeCP2*)[2] for which no effective therapy exists to date. Several biomarkers of O.S. are elevated in RTT, including isoprostanes (IsoPs), derived from free radical-catalyzed peroxidation of arachidonic acid (AA, 20:4 n-6).[3]

We have examined the potential value of F_2-dihomo-IsoPs, peroxidation products from adrenic acid (AdA, 22:4n-6) (a component of myelin),[4] as an early biomarker of brain white matter peroxidation in RTT. F_2-dihomo-IsoPs were determined by gas chromatography/negative ion chemical ionization tandem mass spectrometry (GC-NICI-MS/MS); the measured ions were the product ions at m/z 327 derived from the $[M-181]^-$ precursor ions (*m/z* 597) produced from both the derivatized ent-7(RS)-F_{2t}-dihomo-IsoPs and 17-F_{2t}-dihomo-IsoPs.

Plasma F_2-dihomo-IsoPs levels in age and gender matched healthy controls range between 0.8 and 1.2 pg/ml (mean ± SEM, 1.0±0.11 pg/ml). Plasma F_2-dihomo-IsoPs were increased by about 2 orders of magnitude (in RTT patients in stage I of the disease (185.9±68.9 pg/ml; stage I vs. controls, $p<0.0001$), while these values decreased by about 2 to 3 folds higher than in controls in later stages of the natural progression of the disease (stage II: 3.75 ± 0.43 pg/ml; stage III: 2.65± 1.15 pg/ml; stage IV: 2.53 ±0.54 pg/ml). The F_2-dihomo-IsoPs detected by GC-NICI-MS/MS correspond to the sum of the two synthesized isomers ent-7(RS)-F_{2t}-dihomo-IsoPs and 17-F_{2t}-dihomo-IsoPs. These data strongly emphasize the critical importance of IsoPs chemical synthesis for correctly identifying lipid peroxidation products in clinical practice.

In conclusion, our data indicate that plasma F_2-dihomo-IsoPs 1) represent a very early marker of the disease; 2) provide a better understanding of the pathogenic mechanisms in RTT; 3) are a possible therapeutic target for future attempts to arrest the progression of the disease using an early therapeutical intervention.

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P15. ELOVL2 controls the level of 24 - 30 PUFA; a prerequisite for male fertility and sperm maturation in mice.

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ELOVL2 is a member of the mammalian microsomal fatty acid elongation enzyme family (ELOVL) involved in the elongation of very long-chain fatty acids (VLCFAs) including polyunsaturated fatty acids (PUFAs) required for various cellular functions in mammals (JACOBSSON A *et al*. 2006, GUILLOU H *et al*. 2010).

Here, we used ELOVL2-ablated mice (*Elovl2*$^{-/-}$) and show that PUFAs with 24-30 carbon atoms of the ω-6 family in testis are indispensable for normal sperm formation and fertility in male mice (ZADRAVEC D *et al*. 2011). Furthermore, heterozygote *Elovl2*$^{+/-}$ male mice exhibit haploinsufficiency with reduced levels of C28:5 and C30:5n-6 PUFAs which gives rise to impaired formation and function of haploid spermatides. These new insights reveal a novel mechanism involving ELOVL2-derived PUFAs in mammals and a previously unrecognized role for C28 and C30 n-6 PUFAs in male fertility. In accordance with its suggested function *Elovl2*$^{-/-}$ mice have distorted levels of C20 and C22 PUFAs (including DPA and DHA) in serum within both the n-3 and n-6 series. However, dietary supplementation of C22:6n-3 could not restore male fertility of *Elovl2*$^{+/-}$ mice suggesting that the changes in n-6 fatty acid composition seen in the testis of the *Elovl2*$^{+/-}$ mice, cannot be compensated for by increased C22:6n-3 content.

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P17. Methylmercury alters the fatty acid composition in wt mice, but not in fat-1 mice.

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The western diet has a high content of n-6 poly unsaturated fatty acids (PUFA) at the expense of n-3 PUFA (SIMOPOULOS 2000). This creates an imbalance in the ratio of n-6/n-3 PUFA, which may make the body more susceptible to a variety of diseases including obesity, diabetes and mental disorders. Mammalian cells lack the gene (*fat-1*) encoding n-3 desaturase, an enzyme that converts n-6 PUFA to n-3 PUFA found in the nematode *Caenorhabditis elegans*. Transgenic mice which can produce n-3 PUFA from n-6 PUFA have been generated by insertion of the *fat-1* gene cloned from *C. elegans*. *Fat-1* mice are characterised by an n-6/n-3 ratio close to 1:1 in all cells (KANG JX 2007). In the present study, this mice model was used to study the effect of methylmercury (MeHg) exposure on fatty acid composition in several tissues. Seafood is a valuable dietary source of n-3 PUFA, but also contains contaminants such as MeHg (JAYASHANKAR *et al* 2010). The hypothesis of the present study was that less MeHg would be accumulated in the *fat-1* mice compared to the wild-type (wt) due to an "optimal" n-6/n-3 ratio in the *fat-1* mice. A total of 70 male C57BL/6 mice (7-14 weeks old) of two different phenotypes, *fat-1* and wt, were fed a control diet (AIN-76A, 5% sunflower oil) or a MeHg-spiked diet (3.4 mg Hg/kg feed, added as MeHg cysteine). Fatty acid profile and levels of MeHg were analysed in the brain, muscle, heart, kidney, blood and liver. The results showed no significant differences in accumulation of MeHg between wt and *fat-1* in any organs. The n-6/n-3 ratio differed among the tissues in both genotypes; in wt from 0.8 (brain) to 13.1 (liver), and in *fat-1* from 0.6 (brain) to 3.9 (blood). MeHg only affected the n-6/n-3 ratio in the organs of the wt mice, including the brain. In brain tissue, both the total amount of n-3 fatty acids and the amount of DHA were significantly lower in the wt mice exposed to MeHg. The total amount of n-6 fatty acids was higher while the content of arachidonic acid was unchanged in the brain of wt mice, whereas no such change was found in the *fat-1* mice. In conclusion, MeHg altered the fatty acid profile in all organs including brain of the wt mice but not in *fat-1* mice suggesting that the influence of MeHg on tissue fatty acid composition depends on the n-6/n-3 ratio.

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P19. Interactions of iron and Omega-3 fatty acid deficiencies in rats: effects on the metabolism, brain momoamine levels and spatial learning and memory fonctions

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Background Iron (Fe) and n-3 fatty acids (FAs) are essential for normal brain development. Combined deficiencies in Fe and n-3 FAs may have interactive effects on the structure and function of the central nervous system.

Objectives To investigate the effects of Fe and n-3 FA depletion and repletion, alone and in combination, on Fe and FA metabolism, brain monoamine levels and spatial learning and memory function in rats.

Procedure During a 5 week depletion period, male Wistar rats at 3 weeks of age were fed either a control, an iron deficient (ID), an n-3 fatty acid deficient (n-3 FAD) or an ID + n-3 FAD diet. After 5 weeks, part of the ID + n-3 FAD rats were allocated into 6 repletion groups receiving either an ID + α-linolenic acid/linoleic acid (ALA/LA) sufficient, an Fe + ALA/LA sufficient, an ID + DHA/EPA sufficient, an Fe + DHA/EPA sufficient, an ID + n-3 FAD or Fe sufficient + n-3 FAD diet for another 5 weeks. Spatial learning and memory was assessed at the end of both periods using the reference- and working-memory version of the Morris Water Maze (MWM) task. Monoamine levels as well as total phospholipid (TPL) FA composition and Fe concentration were examined in various brain regions at 5 and 10 weeks.

Results Significant Fe x n-3 FA interactions on working memory were found during the depletion and repletion period. Dopamine (DA) concentrations were increased by ID and n-3 FAD after the depletion period. Homovanillic acid (HVA) and norepinephrine (NE) concentrations were increased by n-3 FAD only, while ID decreased serotonin concentrations. Significant Fe x DHA interactions on several monoamine concentrations were found in brain regions after repletion. Long-chain omega-3 FAs were significantly decreased in plasma and red blood cells by ID alone or in combination with n-3 FAD after depletion and repletion. Decreased Fe concentrations were found in hippocampus of n-3 FAD rats. Additionally, Fe given in combination with DHA resulted in more efficient Fe repletion in olfactory bulb.

Conclusion ID and n-3 FAD have interactive effects on spatial memory function and monoamine metabolism in rats. TPL analysis suggests iron deficiency impairs n-3 FA status, most probably by affecting FA desaturation. This is the first study to show that n-3 FA status may modulate brain iron concentrations. Unilever Research Vlaardingen, NL is gratefully acknowledged for supporting this study.

P21. Comparative analysis of fatty acids in mice brain: effect of inflammation

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The contributions of long chain n-6 and n-3 polyunsaturated fatty acids (PUFA) to brain health occur in part via the regulation of inflammation by the synthesis of lipid mediators such as eicosanoids, resolvins and neuroprotectins, endocannabinoids. The ability of these PUFA to incorporate and modify the composition of cell membranes is one of the factors which determines the type of inflammatory mediators that will be produced during the inflammatory response. Arachidonic acid (AA) and docosahexaenoic acid (DHA) have opposite effects on the inflammatory signalling system. AA is the precursor of pro-inflammatory eicosanoids, whereas DHA is the precursor of anti-inflammatory molecules such as eicosanoids, resolvins and neuroprotectins. Moreover DHA displays anti-inflammatory effects and inhibits the production of pro-inflammatory cytokines. Hence, depending on the relative amounts of AA and DHA in the membranes, neuroinflammation will be either reduced or exacerbated. We first evaluated the topography of fatty acids in various brain structures of C57/Bl6 mice. Since the fatty acid composition of cell membranes can be changed by dietary means, we then assessed the fatty acid composition of the cortex after a nutritional intervention (n-3 PUFA deficiency) and in the genetic Fat-1 mouse model of endogenous conversion of n-6 into n-3 fatty acids. Then we evaluated the effect of inflammation on fatty acid composition. The consequences of low grade inflammation associated with aging or obesity on fatty acid composition of the brain cortex were studied in aged C57/Bl6 mice, in the senescent accelerated mouse (SAM) model, and in the obese db/db mouse. To compare, the effect of an acute inflammation was evaluated in C57/Bl6.

We found that the brain structures with the highest AA content were hippocampus and prefrontal cortex and those with the highest DHA were hippocampus, prefrontal cortex, cortex and cerebellum. All these structures and in particular the cortex were affected by an n-3 PUFA deficiency: we observed a decrease in DHA associated to an increase in AA. In Fat-1 mice, cortex was enriched in DHA and contained less AA than wild type mice. Inflammation was tested in different models. In the models of low grade inflammation in the cortex of aged mice, there was a decrease in DHA content that was reduced in Fat-1 mice as compared to their wild type counterparts. The same results were observed in C57/Bl6 mice, a decrease in DHA content in aged cortex mice that was reduced in DHA-supplemented mice. No changes in AA were detected. In SAM and obese mice, AA and DHA did not differ significantly from wild-type animals. Acute inflammation induced by lipopolysaccharide treatment did not affect both AA and DHA content in the cortex of C57/Bl6.

In conclusion, our results demonstrated that AA and DHA contents in the brain were not evenly distributed in all regions and that they varied mainly according to the diet and with age. These changes may explain in part the differences observed in low grade inflammation model such as aging. In obesity model and in an acute inflammation, AA and DHA may have a role to play in inflammation via other pathways such as the regulation of gene expression.

Key-words: DHA, arachidonic acid, inflammation, brain regions, n-3 PUFA deficient diet.
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P23. Impact of a postnatal leptin antagonist on predisposition to obesity, on insulino-resistance and on hypothalamic microRNA patterns.

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The early post-natal period is crucial to establish accurate hypothalamic nuclei interconnections. In rodents, a leptin surge from d4 to d16 favors projections of arcuate nucleus (Arc) neurons to paraventricular nucleus (PVN) neurons (Ahima RS et al., 1998 ; Bouret SG et al., 2004). Once mature, the axis Arc-PVN participates in food intake and energy expenditure regulations. Axonal projections from the Arc are disturbed in *ob/ob* mice deficient in leptin (Bouret SG et al., 2004). Moreover, blocking postnatal leptin action in rats by a daily administration of a leptin antagonist leads to leptino-resistance in adulthood and predispose adults to obesity when fed high fat diet (Attig L et al., 2008). On the other hand, offspring of obese rat dams have an amplified and prolonged postnatal leptin surge which correlates with a durably energy homeostasis disturbance (Kirk SL et al., 2009). Mechanisms underlying predisposition to obesity by disturbing the level of postnatal leptin and by high-fat diets are still unknown.

In our experiment, rats received a daily i.p. injection of stabilized rat leptin antagonist (pegylated form : pRLA, 7.5μg/g/d ; n=35) or NaCl (n=36) from d2 to d13. At weaning, all animals were fed a chow diet until d125 and then switched to a high fat diet (HFD) until d153. We examined endocrine and metabolic parameters at different steps of the experiment : at weaning (d27), under chow diet (d90) or after one month challenge with HFD (d153). Insulinemia, adiponectinemia and glycemia were measured. Molecular and biochemical markers of metabolism and energy expenditure in hypothalamus, liver, muscle were analysed by qRT-PCR or western blot. In addition, microRNAs (miRs) of the hypothalamus were analyzed at d28 by a large-scale expression profiling approach (Taqman Low Density Array). MiRs with a two-fold change in expression were further looked for at d90 and d153.

Rats treated with pRLA exhibited a higher body weight at weaning (+12.5%), under chow diet (+10.3% at d125) and under HFD (+32.8% at d153). Plasma adiponectin levels were significantly lower in pRLA rats as compared to control rats, except after HFD challenge where no significant difference was detected. Plasma insulin levels were higher in the pRLA rats at d90 as compared to control rats, but after HFD (d153) this difference was lost. Furthermore, the HOMA index was significantly higher in pRLA group as compared to control group at d90 indicating an insulin-resistant state in the pRLA group. Molecular analyses of the hypothalami showed a transient two-fold enhancement in mRNAs encoding the leptin and insulin receptors as well as the energy expenditure AdipoR2 marker of d28-old pRLA rats. At d90, mRNAs coding negative regulators of leptin and insulin signaling pathways (PTP1B or SOCS3) were increased in pRLA rats compared to control. This increase was maintained after HFD challenge at d153. Expression of several miRs in pRLA hypothalamus was increased after HFD (d153). Molecular analyses of peripheral tissues showed (1) at d90 an increase in energy expenditure mRNAs coding for UCP2/3 and AdipoR1/R2 in pRLA muscle; (2) at d153 after HFD challenge, a decrease in pRLA muscle AdipoR1 mRNA, opposed to AdipoR1/R2 increase observed in pRLA liver.

In conclusion, these results demonstrate that blockade of early postnatal leptin action leads to a weight gain which is exacerbated under HFD. This blockade also contributes to the onset of insulin-resistance that is mirrored by a low level of circulating adiponectin, a lack of correlation between insulinemia and glycemia (measured also by the HOMA index) and up-regulations of PTP-1B and SOCS-3 at the hypothalamic level. Furthermore, the expression of energy expenditure markers is modified in the pRLA rats as compared to controls. Finally, the pRLA rats exhibit changes in microRNA patterns at the hypothalamic level which may reflect a deep and a long term alteration in gene expression patterns due to the blockade of early postnatal leptin action.

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P25. Acceptability of omega-3 enriched foods. An exploratory study of the senior consumer expectation.

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The design of nutritionally improved foods should envisage their acceptance by the targeted population. Accordingly, the acceptance of omega-3 enriched food by senior consumers can be a limiting factor for their efficiency in the prevention of age-mediated brain pathologies. The objective of this study was to evaluate senior attitude regarding omega-enriched foods

A qualitative study was led in March 2009 through 28 conversations among seniors living in Ecully, thus essentially targeting city-dwellers of superior and middle classes (Vercolier, 2009). For the retired people, the food plays an essential role for their health, they declare to prefer fresh products. Seniors enjoy consuming their "usual products": the choice for new products will be made on medical or family advice; they distrust assertions which are perceived as a marketing strategy to sell more. The knowledge of the subjects on lipids and omega-3 remain very vague. Seniors, however, are able to assert that omega-3 participate in the protection of face to face cardiovascular diseases. Margarines enriched in omega-3 are well accepted products and are rather sought.
A quantitative study was realized among a sample of 102 persons in France (Devillers, on 2009). The questionnaire consisted of three parts: consumer habits food, the knowledge on lipids and omega-3, and expectations for new enriched products. This study confirms that seniors look for a quality food supply, realize mostly themselves that their culinary preparations and are relatively little sensitive to assertions. They do not have enough knowledge on lipids or omega-3; however, 60 % of them are nevertheless ready to consume food rich in omega-3.

The results of these two studies converge and show that seniors are not frightened at the idea of consuming omega-3; on the contrary half of the seniors questioned in the quantitative study consume margarines enriched in omega-3. It would however be necessary to improve the information given to seniors about lipids and omega-3. To propose new food enriched in omega-3, it would be necessary to turn either to a "culinary assistant" used daily, or to a food which they already consume regularly, little transformed, in agreement with their search for quality products.

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P27. Reduced injury response to traumatic brain injury following diet supplementation with docosahexaenoic acid (DHA): Rat study

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Docosahexaenoic Acid (DHA), a long chain omega-3 fatty acid of marine origin, is a major constituent of neural membranes, representing ca. one fifth of their dry weight. (Yehuda et al, 1999). There is emerging evidence in several models of central nervous system pathology indicating that DHA may have therapeutic potential for ameliorating the outcome of traumatic brain injury (Mills et al, 2010 ; Bazan, 2005 & Hoyges et al, 2003). DHA appears to be retained by neuronal membrane phospholipids, as opposed to astroglial cells, which readily release it, probably reflecting a trophic or anti-apoptotic effect important for neuronal survival

Previous work by the authors (Mills & Bailes, 2011) has shown that post-injury administration of DHA and DHA/EPA (as fish oil) significantly reduces the number of injured axons following TBI. This poster looks at the prophylactic administration of DHA to Sprague-Dawley rats prior to injury, then measured anatomical /structural (axonal injury), cellular (markers of inflammation and cell death: CD68 and caspase-3) and behavioural (Morris Water Maze) outcomes. The results indicate that DHA, a proven safe and well-tolerated dietary supplement, could be advantageous for clinical use in populations at risk of traumatic brain injury, including participants of sports that involve significant head contact (e.g. rugby, soccer, boxing).

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P29. Fatty acid composition in the postmortem amygdala of patients with schizophrenia, bipolar disorder and major depression

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Background: We have previously investigated n-3 long-chain polyunsaturated fatty acids (LCPUFAs) in the postmortem hippocampus from subjects with schizophrenia and bipolar disorder, and controls; however, we found no significant differences except for small ones in n-6 LCPUFAs (Hamazaki et al J Psychiatr Res 2010). Other studies with postmortem orbitofrontal cortex showed abnormalities in n-3 LCPUFAs in individuals with schizophrenia and major depression. In the present study we investigated whether there were any abnormalities in LCPUFAs in the amygdala of schizophrenia, bipolar disorder and major depression compared to unaffected controls.

Methods: We obtained from the Stanley Medical Research Institute 15 amygdala samples each for schizophrenia, bipolar disorder, major depression and controls matched for age and sex. Amygdala tissues were scraped off from 3 consecutive frozen sections for microscopic slides (14 μm each) and homogenized. Total lipids were extracted and total phospholipid fractions were separated by thin-layer chromatography. The fatty acid composition was analyzed by gas chromatography.

Results: Unlike the previous studies with the orbitofrontal cortex and hippocampus, we found no significant differences in major LCPUFAs. The amounts of docosahexaenoic acid (%), the major n-3 LCPUFA, were 10.0 ± 0.3, 10.0 ± 0.3, 9.3 ± 0.3 and 9.7 ± 0.3 in schizophrenia, bipolar disorder, major depression and unaffected controls, respectively. The composition of arachidonic acid (%), the major n-6 LCPUFA, was 9.0 ± 0.2, 9.2 ± 0.1, 9.4 ± 0.2 and 9.4 ± 0.2 respectively.

Conclusions: Changes in LCPUFAs in these psychiatric disorders may be specific to certain brain regions. LCPUFAs in the amygdala may not be the etiology of these diseases.

P31. About the interest of HPTLC in the bio-clinical research: how does it work, and typical results in the lipid analysis

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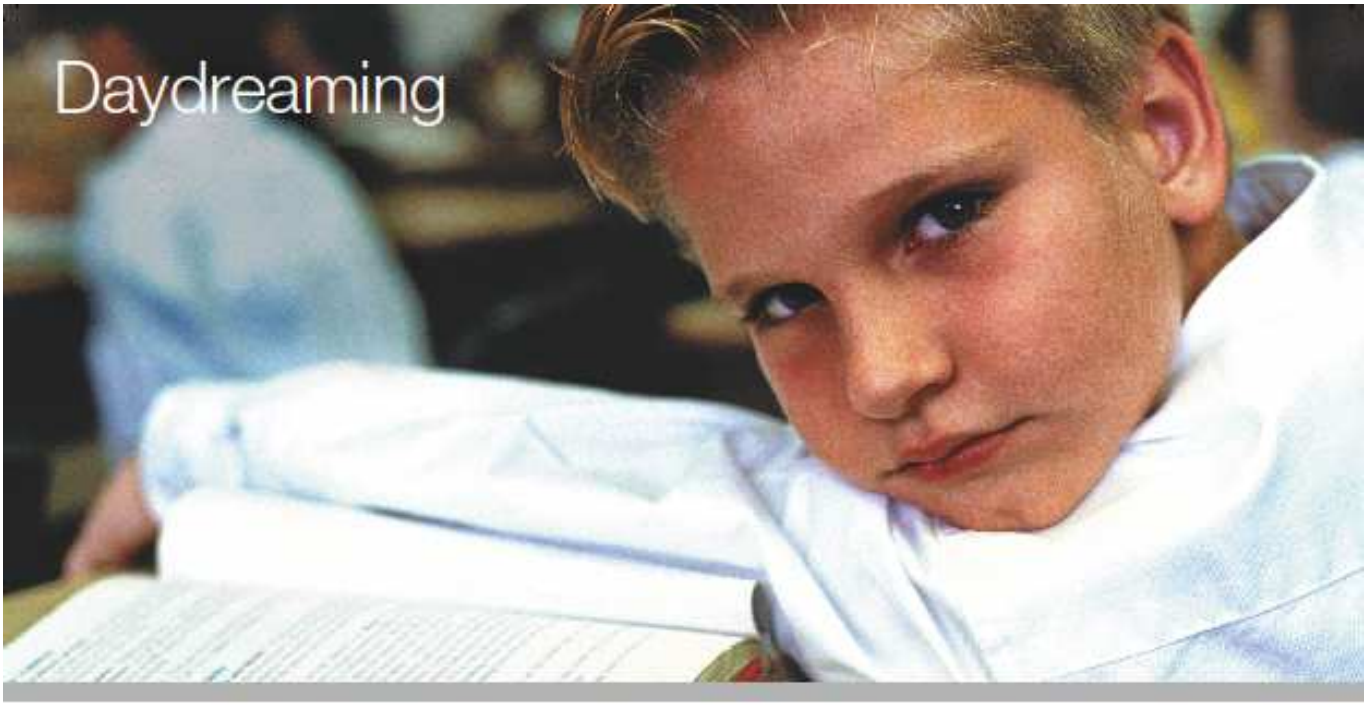
HPTLC (for high-performance thin-layer chromatography) is a rapid liquid chromatography, with a very wide application field due to its detection capabilities and low matrix sensitivity. It is a quantitative method which is hyphenated with MS. When many analysts are now confronted with situations where HPTLC is a suitable solution to their problems and might be favored over better known and more widely used analytical methods, they usually miss up-to-date and adequate information-training in HPTLC.
This poster will intend to show with some examples in the lipids analytical field, how does it work, and what are its possibilities and limits.
HPTLC is an off-line method where each step is fully independent and gives therefore an extreme flexibility. One key element is that all the sample content remains dried on the plate after separation, for any detection means. Therefore this method is favored in any case when the interesting compounds of the sample have no UV absorbance, which is the case of most of the lipids. The first step of the method is spray-on application, and enables HTS (high throughput screening). The automatic sampler (ATS) applies up to 30 samples on each side of a 20x10 plate, with any solvent, including polar ones as water. The second step, the migration of the plate, takes place in a horizontal anti-parallel chamber. This needs only a small volume of solvent, 3 mL for 30 samples, i. e. 100 μL / sample only, which is good for the economy and the ecology as well.
The speed of the analysis, which is proven to be much quicker than UPLC, is due to the parallel chromatography of many samples on the plate. The matrix tolerance of the chromatographic plate avoids heavy sample preparation steps, reduces time consumption and improves the results reliability.
Besides the various ways to develop an HPTLC plate, vertically or horizontally, manually or automatically, the ultimate method for high separation power is the AMD gradient method. Invented first for pesticides in water, it is widely used in lipids analysis. The potential of this method is both in high resolution and sensitivity because of the focusing effect of this method. This automated process is absolutely reproducible, and independent of matrix effect (i.e. the migration distances are the same with or without matrix for reference and sample).
The detection is a major strength of the HPTLC method, as the separated samples remain accessible on the plate. Many detection ways are compatible on the same plate, from densitometry, with or without staining, until anti-body antigenic reaction directly on the plate. HPTLC-MS interface, in elution mode enables today the extraction of the substances of the plate for on-line coupling with ESI-MS or for isolation and further analysis, through NMR for instance.
This poster would have reached its goal when it gives an overview on this HPTLC method and motivates to search for more information via the symposium for HPTLC (www.hptlc.com, 6-8th July 2011, Basel CH). Trials and analytical feasibility studies are also possible on request by the authors.

Keywords

HPTLC for high-performance thin-layer chromatography, HTS (high throughput screening) and quantitative chromatographic method, spray-on application, AMD gradient, immuno-staining, densitometry, HPTLC-MS interface.



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