Presented By:

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‘Metabolic Syndrome’ in the brain

Deficiency in omega-3 Fatty acid exacerbates dysfunctions In insulin receptor signaling and cognition.

Authors:
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Overview and Objectives

- Introduction
- Methods
- Results
- Discussion
- Conclusion

- Metabolic dysfunction affects brain function.
- This is shown in this paper using the effects of **metabolic syndrome** in rats induced by a high **fructose** diet.
- Unhealthy dietary habits (such as high fructose intake) can be partially counteracted by **omega 3 fatty acid** dietary supplementation.

- High sugar consumptions impair cognitive functions (memory) and disrupts insulin signaling by engaging molecules associated with energy metabolism and synaptic plasticity.
- **Omega 3 Fatty Acid** returns body to metabolic homeostasis.

Metabolic Syndrome (MetS)

- **Metabolic syndrome** is a disorder of energy utilization and storage.
- It increases morbidity (disease) and decreases life expectancy.
- Characterized by increased insulin resistance, hyperinsulinemia, hypertension and hypertriglyceridemia.
- Caused by high **fructose** intake
- It is diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal obesity, elevated blood pressure, elevated fasting plasma glucose, high triglycerides, and low high-density cholesterol (HDL) levels.

Hyperinsulinemia: high insulin levels
Hypertension: High blood pressure
Hypertriglyceridemia: high triglyceride levels in blood

Fructose

- **Fructose** is a simple sugar, or monosaccharide
- Fructose + Glucose = Sucrose
- High dietary fructose consumption contributes to an **increase in insulin resistance index**, **insulin** and **triglyceride levels**.
- High fructose diet leads to hepatic oxidative damage, altered lipid metabolism in rats.

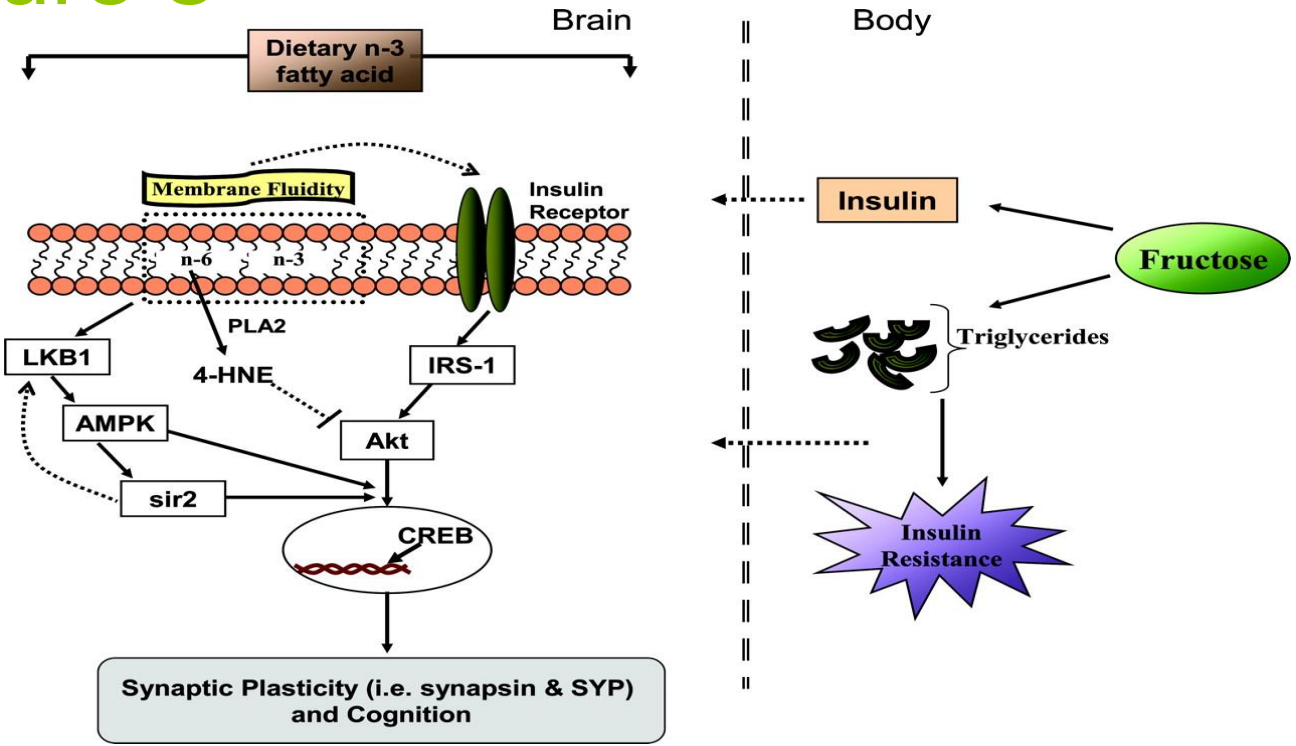
Omega 3 Fatty Acids

Docosahexaenoic acid (**DHA**) - This paper studies the ability of DHA to counteract MetS.

- A primary structural component of the human brain, cerebral cortex, skin, and retina.
- Supports learning and memory in Alzheimer's disease and brain injury.
- Important for brain development and plasticity.
- It can be synthesized from alpha-linolenic acid or obtained directly from maternal milk or fish oil.

α-Linolenic acid (ALA)- found in seeds (chia, flaxseed), nuts (walnuts), and many common vegetable oils.

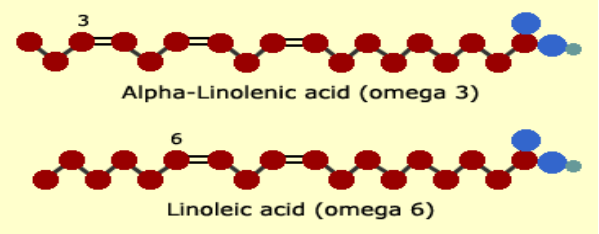
Figure 8


Brain
Body
Dietary n-3 fatty acid
Membrane Fluidity
Insulin Receptor
n-6
n-3
PLA2
LKB1
4-HNE
IRS-1
AMPK
Akt
sir2
CREB
Synaptic Plasticity (i.e. synapsin & SYP) and Cognition
Insulin
Fructose
Triglycerides
Insulin Resistance

Terminology

Omega 3 vs Omega 6 FA

- ***n*-6 fatty acids** have a double bond at the sixth carbon from the end of the carbon chain.
  - Arachidonic Acid (AA)
- ***n*-3 fatty acids** have a double bond at the third carbon atom from the end of the carbon chain.
  - Eicosapentaenoic acid (EPA)
  - Docosahexaenoic acid (DHA)
  - Alpha-linolenic acid (LNA)
- Peroxidation of membrane bound n-6 AA generates 4-HNE
- Fructose intake disrupts the plasma membrane by increasing 4-HNE
  - 4-HNE is 4-hydroxynonenol that is produced by lipid peroxidation in cells



Peroxidation: oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.

Experimental Design

- Used adult male Sprague- Dawley rats

- Kept in a polyacrylic cage with standard room temp (22-24C) and 12 hr light and dark cycle.
- Acclimatized on standard rat chow for 1 week.

- Trained on the Barnes Maze Test for 5 days, two trials per day to learn the task.
- Randomly assigned to diet groups of 6 rats each.
- To test memory retention, 2 trials were given after 6 weeks of diet experimentation.

n-3 diet	n-3 def
n-3 diet/Fru	n-3 def/Fru

Diet Composition

- Two custom diets: one n-3 and one n-3 def.
- Both had same basal macronutrients, vitamins, minerals, and basal fats (hydrogenated coconut and safflower oils).
- n-3 added through flaxseed oil (0.5%) LNA and docosahexaenoic acid capsule oil (1.2%) DHA.
- The fructose solution (15%) was substituted as drinking water for n-3 diet/Fru and n-3 def/Fru

Barnes Maze Task

- A circular surface with 18 circular holes around its circumference.
- Visual cues (colored shapes or patterns), are placed around the table in plain sight of the animal.
- The rats were trained to locate a dark escape chamber hidden under one of the holes.
- Start: place rat under cylinder cover at middle of maze for 10 sec.
- End: after rat enters escape chamber or 5 minutes passed.

Biochemical Analysis

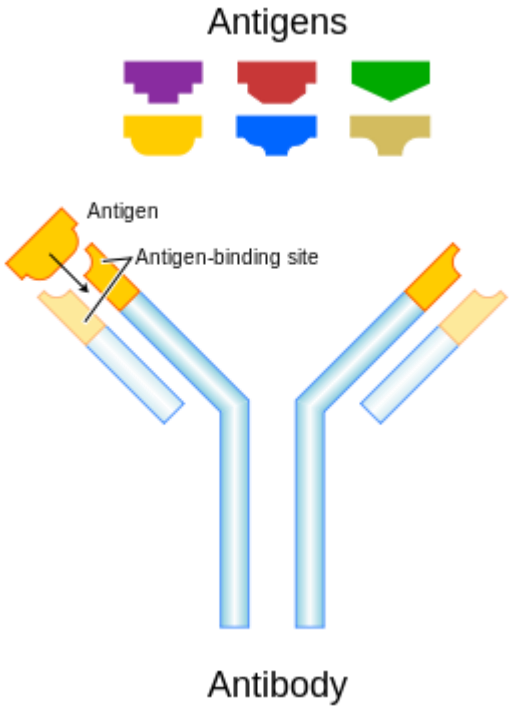
- Blood collected from rat tail after overnight fasting for serum samples
- Measured glucose, insulin, and triglyceride levels.
- Homeostasis model assessment ratio (HOMA-R) was then calculated. This is an index of insulin resistance
    - HOMA-R = fasting glucose x fasting insulin / 22.5

Fatty Acid Analysis

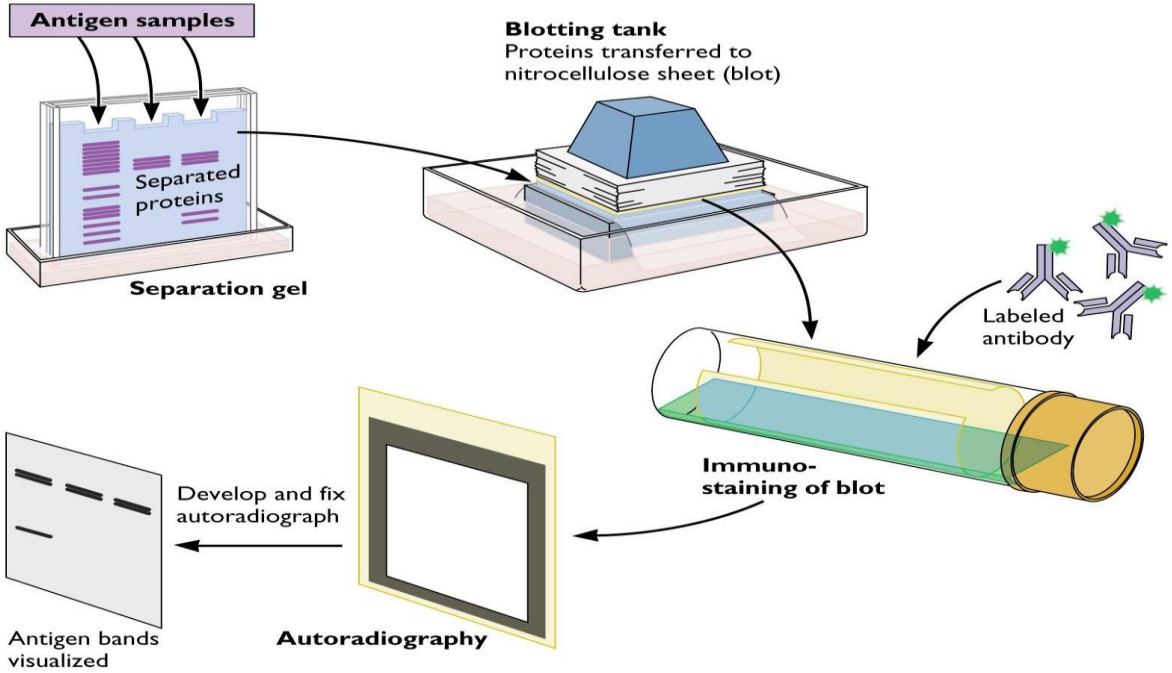
- Total Lipids were then extracted from the brain tissues of the rats.
- The lipids were analyzed on a chromatograph
- A chromatograph separates liquids, in this case the lipids, out of the ground tissue.

Immunoblotting

- Immunoblotting uses antibodies to identify target proteins in a protein mixture.
- They involve identification of protein target via antigen-antibody specific reactions.
- Proteins are first separated by gel-electrophoresis by charge and transferred onto membranes (blotting).
- The membrane is overlaid with a primary antibody for a specific target.



Immunoblotting

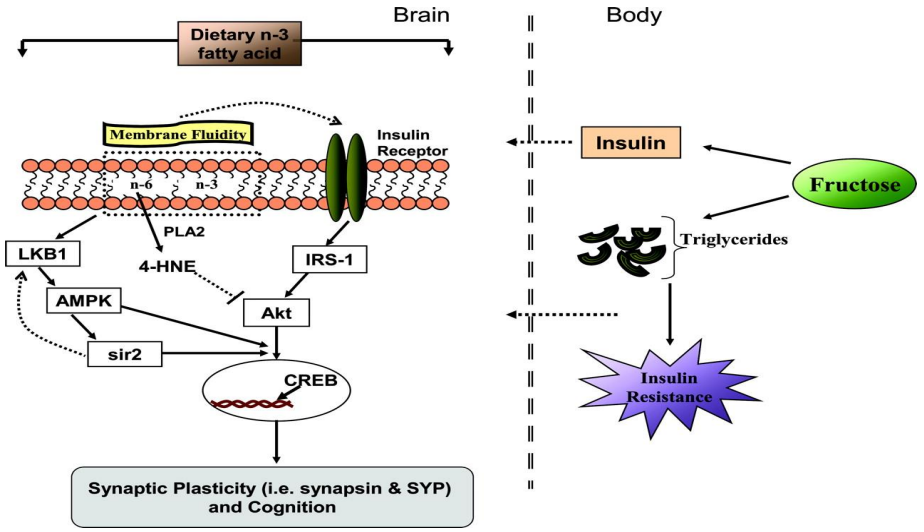


Immunoblotting

- Used for hippocampal tissue
  - **1-** the tissue was dissolved in lysis buffer
  - **2-** The liquid was centrifuged and the supernatant was collected
  - **3-** protein concentration of the supernatant was checked
  - **4-** Protein samples were run through a polyacrylamide gel where they were separated by charge through gel electrophoresis.
  - **5-** Then the gel was electrotransferred to a PVDF- polyvinylidene difluoride- membrane (non-reactive).
  - **6-** non-fat milk blocks non specific binding sites
  - **7-** Then membranes incubated with primary antibodies

Immunoblotting

- Antibodies
  - **- Anti- Actin**
  - -Anti- LKB1
  - - Anti pAMPK
  - -Anti-p-synapsin
  - -Anti- synpasin
  - -Anti- 4HNE
  - -Anti- IR
  - -Anti- CREB
  - -anti Sir2
  - -Anti- Synaptophysin
  - - Anti AMPK
  - - Anti- pAkt
  - -Anti-Akt



Immunoprecipitation

- Used to determine the expression of Insulin Receptor
- Precipitates a protein antigen out of solution.
- This process can be used to detect a particular protein from a sample of many proteins.

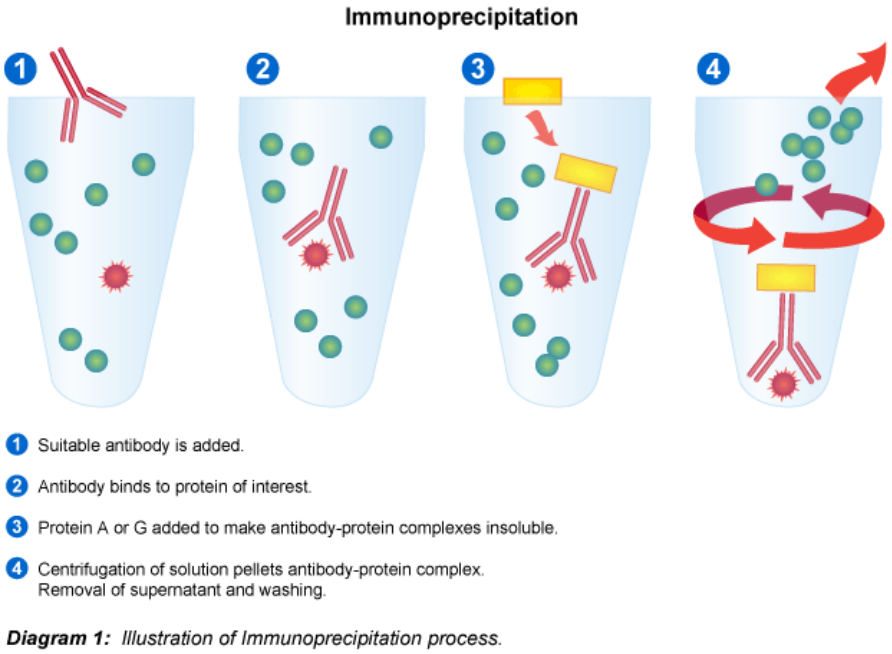


Diagram 1: *Illustration of Immunoprecipitation process.*

Statistical Analysis

- Analyzed by ANOVA- analysis by the difference in group means
- Analyzed by Newman-Keuls to determine statistical difference among group means.
- The **Newman–Keuls** method is a stepwise multiple comparisons procedure used to identify sample means that are significantly different from each other
- P value <0.05 is statistically significant

Overview

- Fructose and n-3 fatty acid dietary experiments
  - body weight, caloric intake, food, and water consumption
  - cognitive function
  - metabolic markers
  - insulin resistance
  - insulin receptor signaling
  - energy metabolism
  - synaptic plasticity
  - lipid peroxidation

Body weight, caloric intake, food and water consumption

- No significant differences observed in body weight, food intake, and water intake among any of the control and variable groups
- Slight preference towards fructose drinking in comparison to food intake

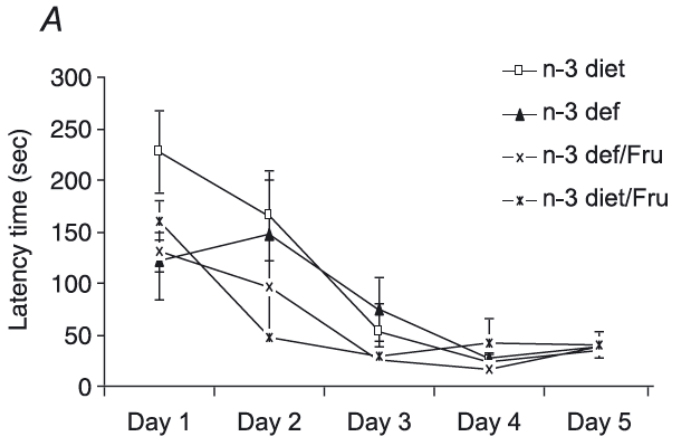
Table 1. Body weight, caloric intake, food and water consumption in groups subjected to *n*-3 and *n*-3 deficient diets with or without fructose water

	Body weight (g)	Food intake (g day^{-1})	Water intake (ml day^{-1})	Caloric intake (kcal day^{-1})
n-3 diet	508.2 ± 13.51	26.18 ± 1.28	30.77 ± 1.49	109.6 ± 4.14
n-3 def	492.5 ± 5.43	25.72 ± 0.605	33.18 ± 2.07	102.8 ± 2.17
n-3 def/Fru	512.8 ± 9.72	22.0 ± 1.52	45.72 ± 8.21	110.2 ± 7.14
n-3 diet/Fru	522.5 ± 24.44	22.58 ± 0.993	41.91 ± 4.90	117.4 ± 2.17

Values are expressed as mean ± SEM.

Cognitive functions

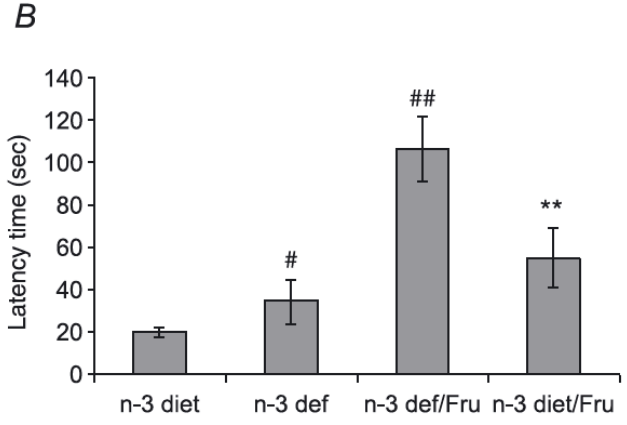
- Spatial learning in the Barnes Maze test
- Prior to experimental diet exposure, all groups observed
  - Decrease in latency time to find the escape hole
  - Similar latency time
  - Thus, all rats were in the same cognitive condition prior to experimental diets



Latency time: time interval between stimulation and response; can be thought of as time delay between cause and effect

Cognitive functions

- Memory retention in the Barnes Maze test
- After experimental diet exposure, all groups observed
  - N-3 FA deficient diet → ↑ latency times → memory impairment
  - N-3 FA deficient + fructose diet → ↑ latency times → memory impairment
  - N-3 FA + fructose diet → improved memory impairment
  - Thus, dietary n-3 deficiency influences vulnerability for fructose induced changes



Metabolic Markers

- Metabolic markers for metabolic dysfunction:
  fasting blood glucose, insulin, & triglyceride levels

Table 2. Blood glucose, insulin and triglyceride levels in groups subjected to *n*-3 and *n*-3 deficient diets with or without fructose water

	Glucose level (mg dl^{-1})	Insulin level (ng ml^{-1})	Triglyceride level (mg dl^{-1})
n-3 diet	81.17 ± 3.02	1.46 ± 0.24	91.17 ± 10.69
n-3 def	77.17 ± 4.26	1.56 ± 0.30	142.0 ± 10.60#
n-3 def/Fru	106.0 ± 6.55##	3.28 ± 0.21##	218.8 ± 23.04##
n-3 diet/Fru	99.83 ± 3.13	2.54 ± 0.16*	166.2 ± 17.65*

Values are expressed as mean ± SEM. #$P < 0.05$, ##$P < 0.01$: significant difference from *n*-3 diet; *$P < 0.05$: significant difference from *n*-3 def/Fru; ANOVA (one-way) followed by Newman–Keuls test.

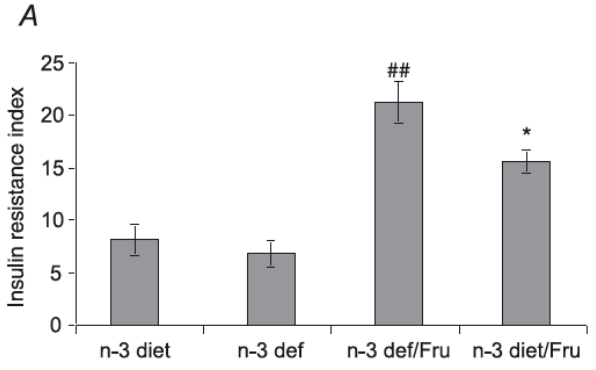
Metabolic marker: measurable metabolic change that characterizes a state of health or disease

Metabolic Markers

- Induced changes from dietary n-3 FA and fructose in metabolic markers:
  - N-3 FA deficiency diet → ↑ Triglyceride levels
  - N-3 FA deficiency + fructose diet → ↑ ↑ Triglyceride levels
  - N-3 FA deficiency + fructose diet → ↑ Glucose levels
  - N-3 FA deficiency + fructose diet → ↑ Insulin levels
  - N-3 FA + fructose diet → ↑ Insulin and triglyceride levels (alleviated fructose induced changes)

Insulin resistance

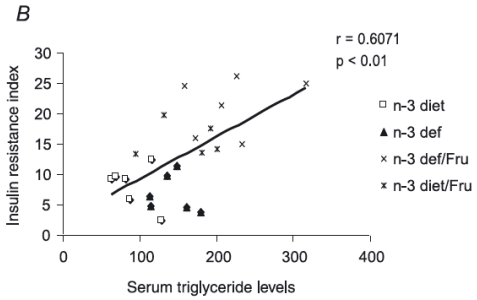
- N-3 FA deficiency diet → no change in insulin resistance index
- Effects of dietary n-3 FA on fructose induced insulin resistance
  - N-3 FA deficiency + fructose diet → ↑insulin resistance index
  - N-3 FA + fructose diet → ↑insulin resistance index (alleviated fructose induced changes)

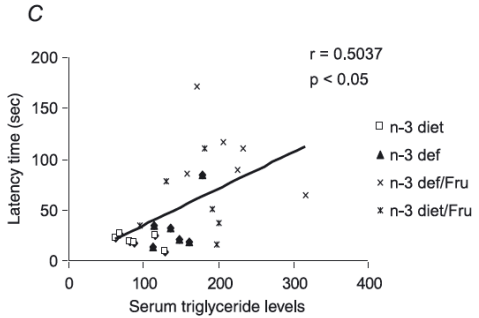


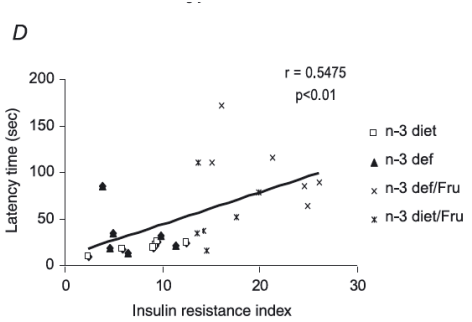
Insulin Resistance Index: the measure of the condition in which cells fail to response to normal actions of insulin hormone

Association between metabolic changes and cognitive behavior

- Correlated with triglyceride and insulin resistance levels with memory

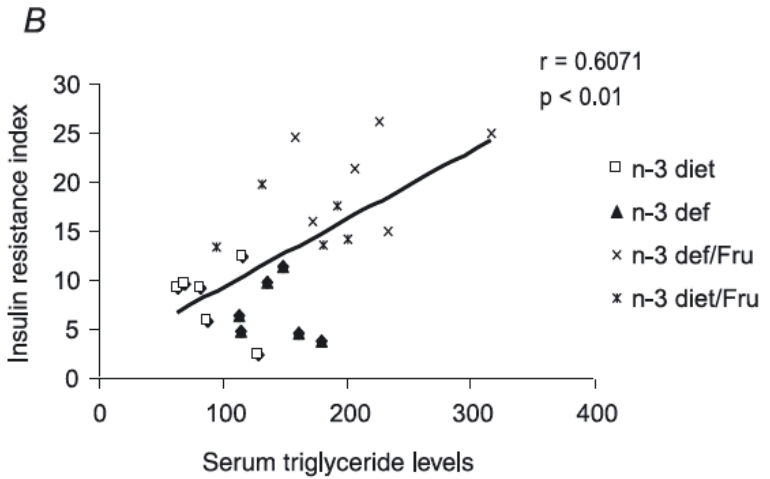






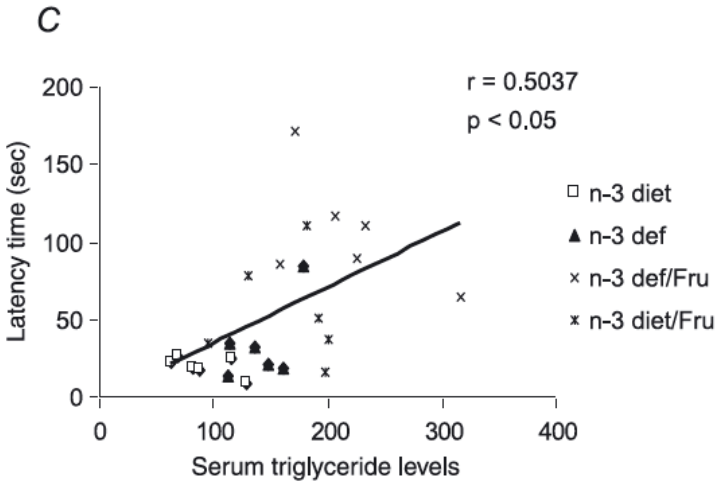
Association between metabolic changes and cognitive behavior

- Positive correlation between triglyceride levels and insulin resistance index



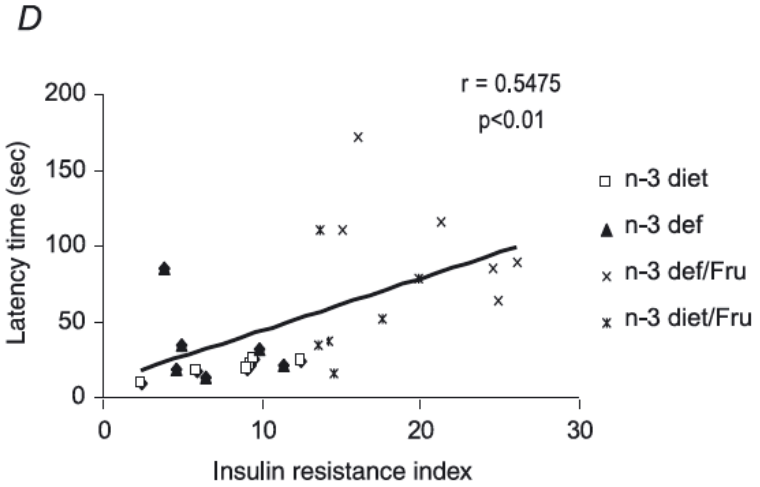
Association between metabolic changes and cognitive behavior

- Positive correlation between fructose induced memory deficits and triglyceride



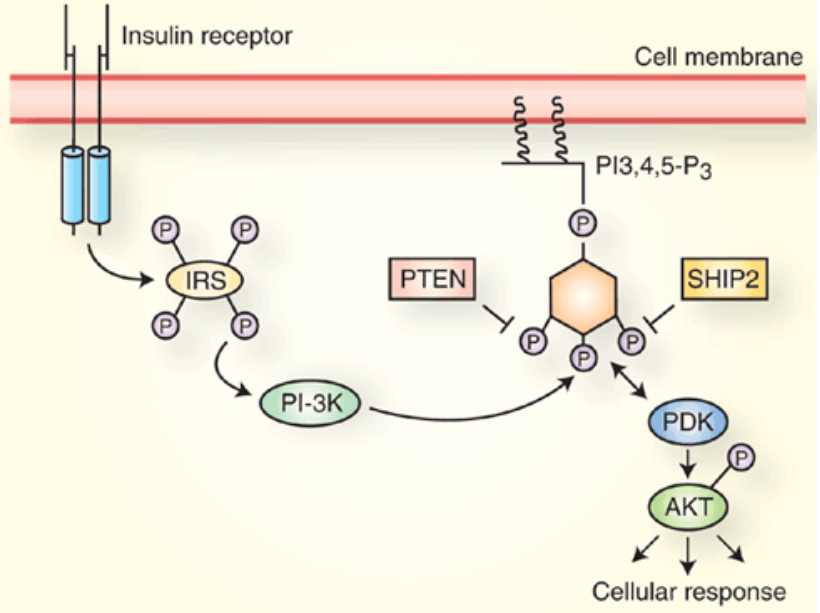
Association between metabolic changes and cognitive behavior

- Latency time varied in proportion to insulin resistance → memory relies on levels of insulin resistance index



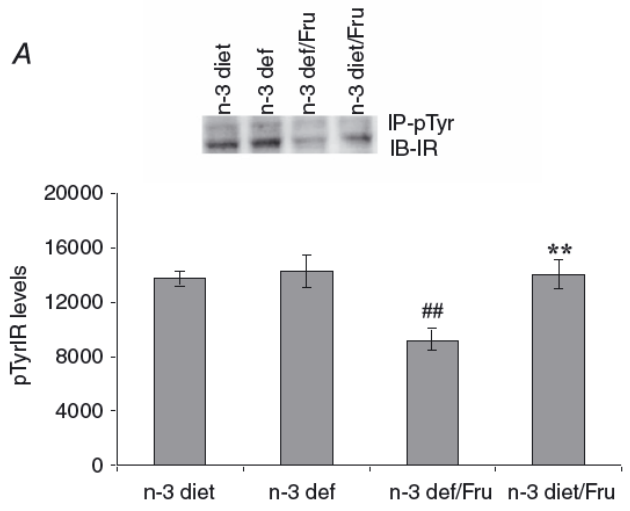
Insulin receptor signaling

- Assess levels of insulin receptor tyrosine phosphorylation and Akt phosphorylation according to the experimental diets



Insulin receptor signaling

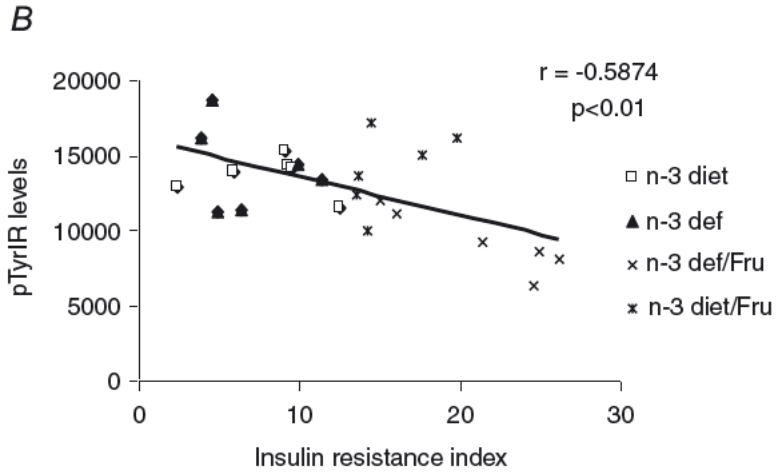
- N-3 FA deficiency + fructose diet → ↓ pTyrIR
- N-3 FA + fructose diet → ↑ pTyrIR



pTyrIR: tyrosine phosphorylation of insulin receptor

Insulin receptor signaling

- Negative correlation between insulin resistance index and pTyrIR levels
  → increased insulin resistance disrupts insulin receptor signaling

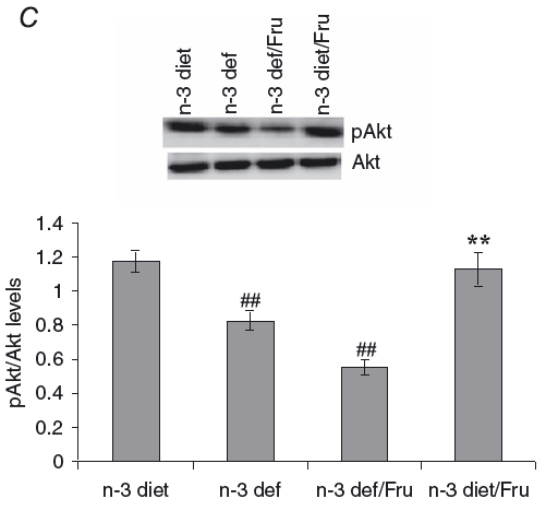


Insulin Resistance Index: the measure of the condition in which cells fail to response to normal actions of insulin hormone

pTyrIR: tyrosine phosphorylation of insulin receptor

Insulin receptor signaling

- N-3 FA deficiency diet → ↓ pAkt
- N-3 FA deficiency + fructose diet → ↓↓ pAkt
- N-3 FA + fructose diet → ↑ pAkt
  (alleviates fructose induced change)

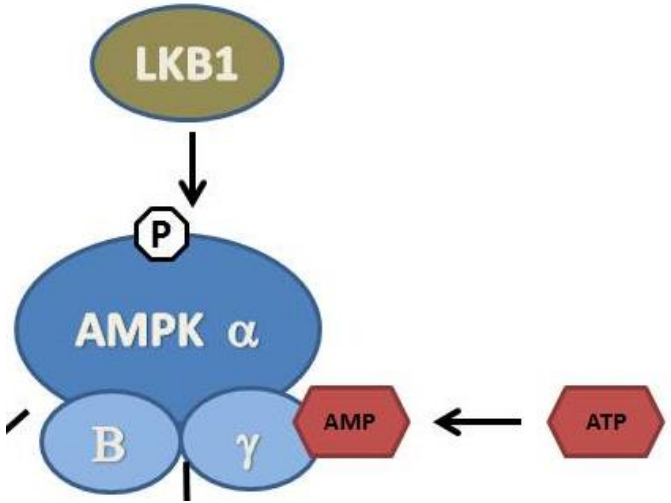


pAkt: phosphorylation of protein Akt

Energy metabolism

- ↑ ADP → ↑AMP → AMPK activation
- LKB1 activation → AMPK activation

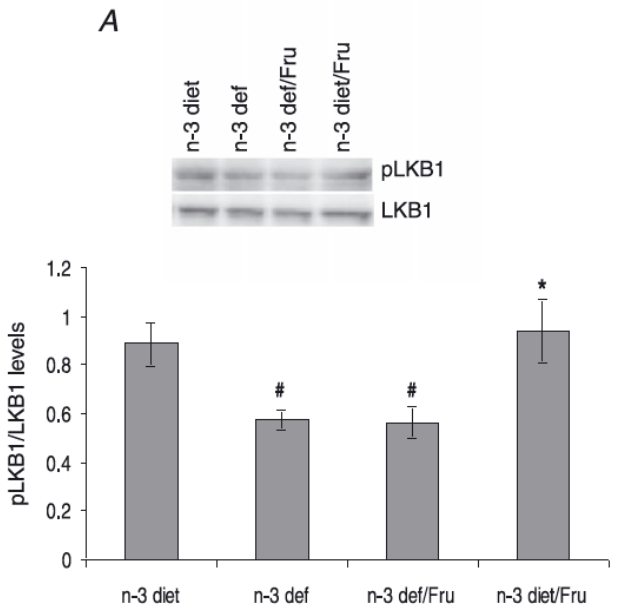
AMPK ← AMP ← (*Adenylate kinase*) ADP



AMPK: AMP-activated protein kinase
LKB1: kinase upstream of AMPK

Energy metabolism

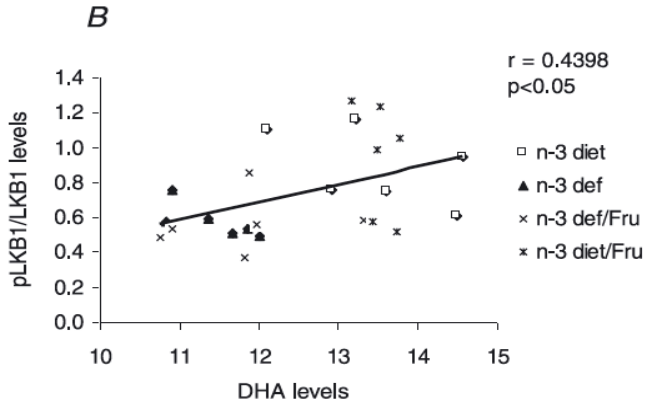
- N-3 FA deficient diet → ↓ pLKB1



pLKB1: phosphorylated LKB1

Energy metabolism

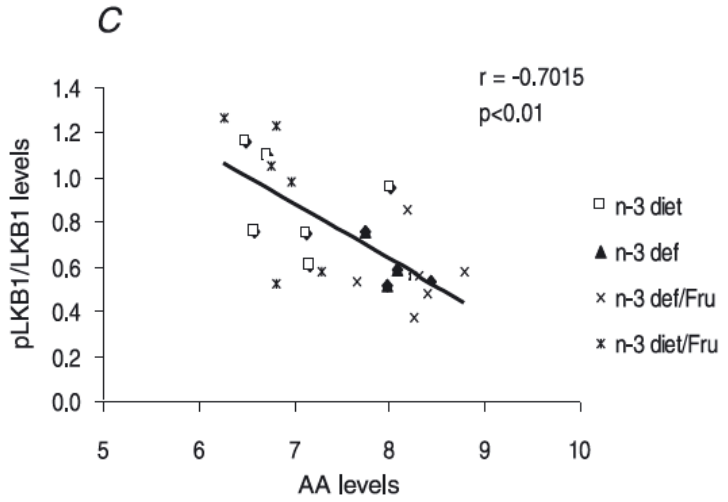
- Positive correlation between pLKB1 and DHA



pLKB1: phosphorylated LKB1
DHA: docosahexaenoic acid, n-3 fatty acid

Energy metabolism

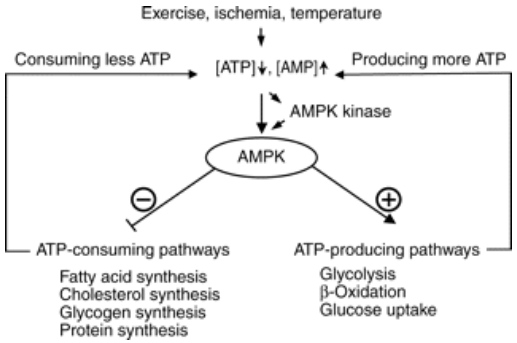
- Negative correlation between pLKB1 and arachidonic acid (AA)

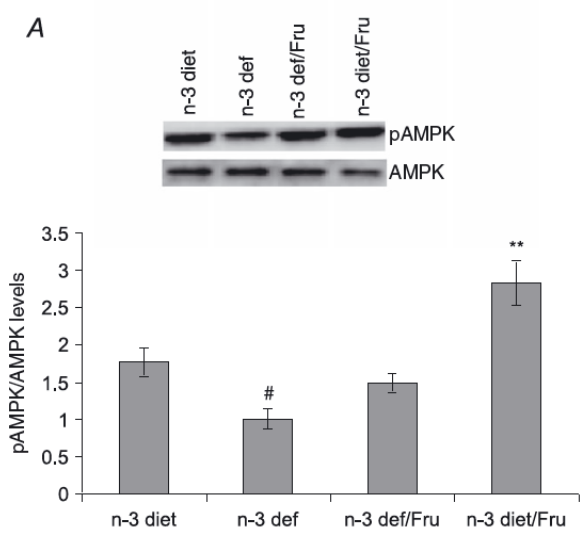


pLKB1: phosphorylated LKB1
AA: arachidonic acid, n-6 fatty acid

Energy metabolism

- N-3 FA deficiency diet → ↓ pAMPK → ↓ energy metabolism
- N-3 FA + fructose diet → ↑ pAMPK
- N-3 FA diet → ↑ pAMPK

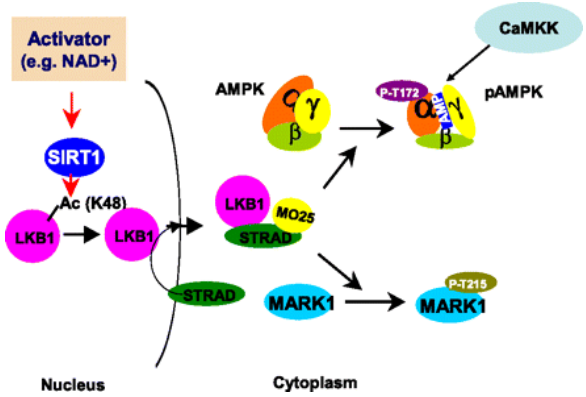


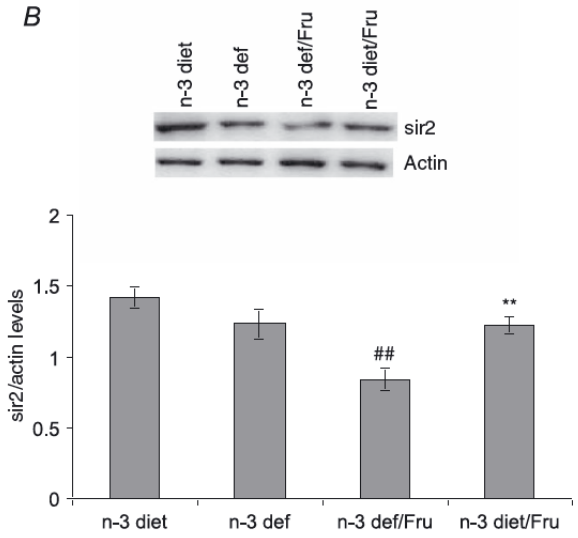


pAMPK: phosphorylated AMPK

Energy metabolism

- N-3 FA deficiency + fructose diet → ↓ Sir2
- N-3 FA diet→ ↑ Sir2



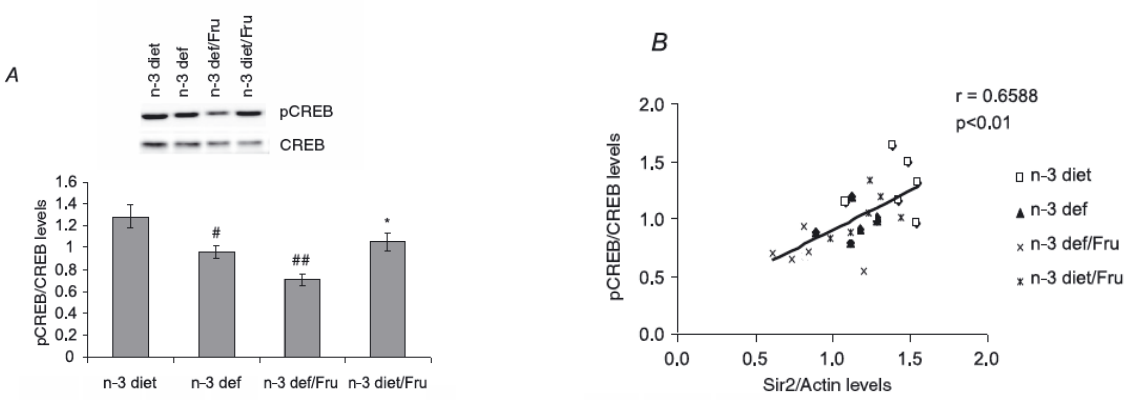


Sir2: yeast protein that plays a role in stress and is responsible for lifespan–extending effects of calorie restriction

SIRT1: mammalian homolog of yeast Sir2

Synpatic plasticity

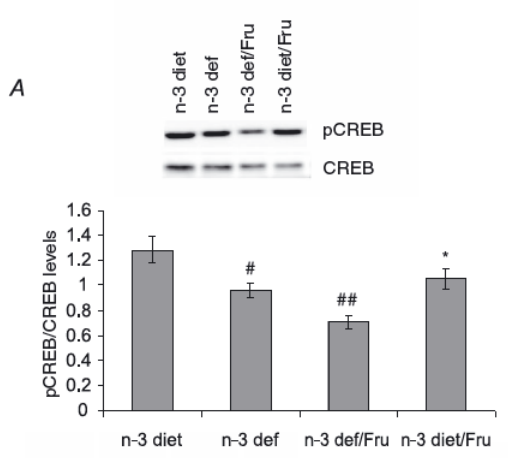
- cAMP-response element binding (CREB) protein plays a role in synaptic plasticity and cognitive functions



Synaptic plasticity: ability of synapses to strengthen or weaken over time, in response to increases or decreases in their activity
CREB: cellular transcription factor that binds to cAMP response elements

Synpatic plasticity

- N-3 FA deficiency diet→ ↑ pCREB
- N-3 FA deficiency + fructose diet→ ↑↑ pCREB
- N-3 FA + fructose diet→ ↑ pCREB
  - Thus, n-3 FA can counter-regulate fructose induced alterations in synaptic plasticity via CREB

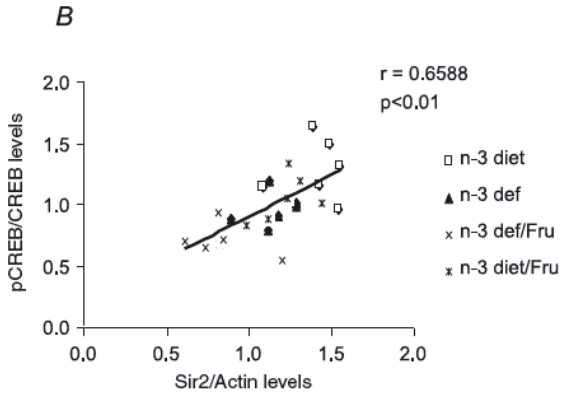


Synaptic plasticity: ability of synapses to strengthen or weaken over time, in response to increases or decreases in their activity

CREB: cellular transcription factor that binds to cAMP response elements

Synpatic plasticity

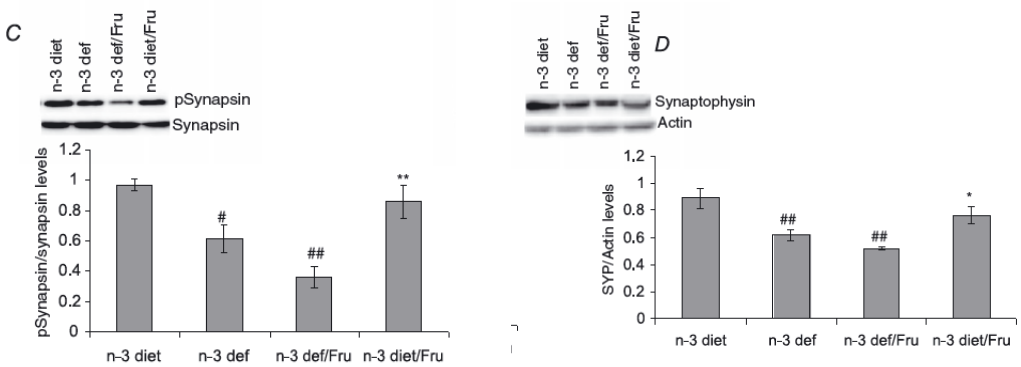
- Positive correlation between Sir2 and CREB
  - Thus, Sir2 involved in hippocampal plasticity and cognitive function



Sir2: yeast protein that plays a role in stress and is responsible for lifespan–extending effects of calorie restriction
CREB: cellular transcription factor that binds to cAMP response elements

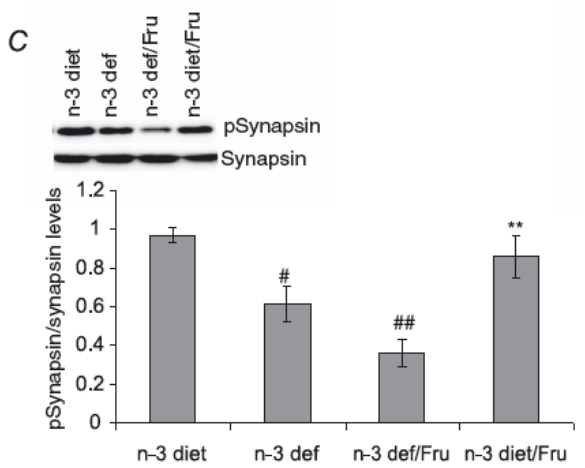
Synpatic plasticity

- Synapsin I: synaptic marker that regulates neurotransmitter release at the synapse
- Synaptophysin: marker for synaptic growth



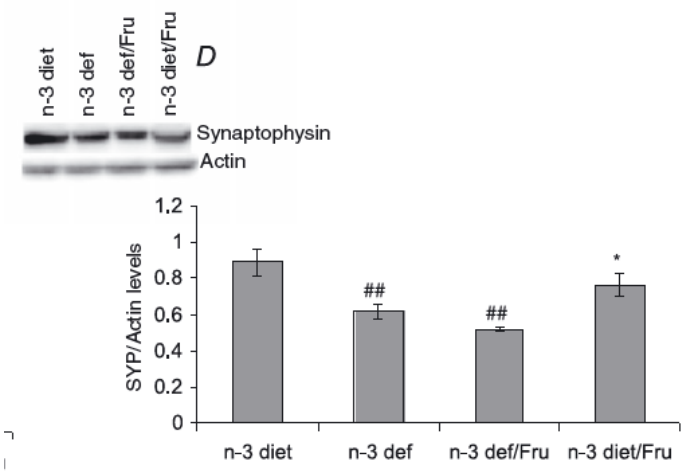
Synpatic plasticity

- N-3 FA deficiency diet → ↓ pSynapsin I
- N-3 FA deficiency + fructose diet → ↓ pSynapsin I



Synpatic plasticity

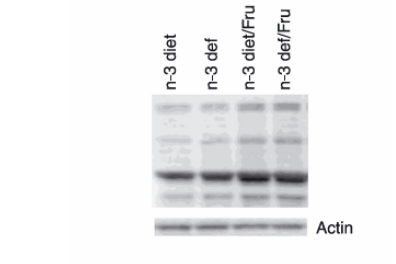
- N-3 FA deficiency diet → ↓ Synaptophysin
- N-3 FA deficiency + fructose diet → ↓ Synaptophysin

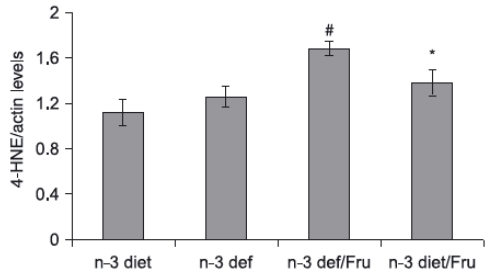


Lipid peroxidation

- Lipid peroxidation: oxidative degradation of lipids in which free radicals attacks, or stealing of, electrons from lipids resulting in cell damage
- N-3 FA deficient + fructose diet → ↑↑ 4-HNE
- N-3 FA diet → ↑ 4-HNE

- Thus, n-3 FA deficient diets make the brain more vulnerable to fructose induced free radical attacks

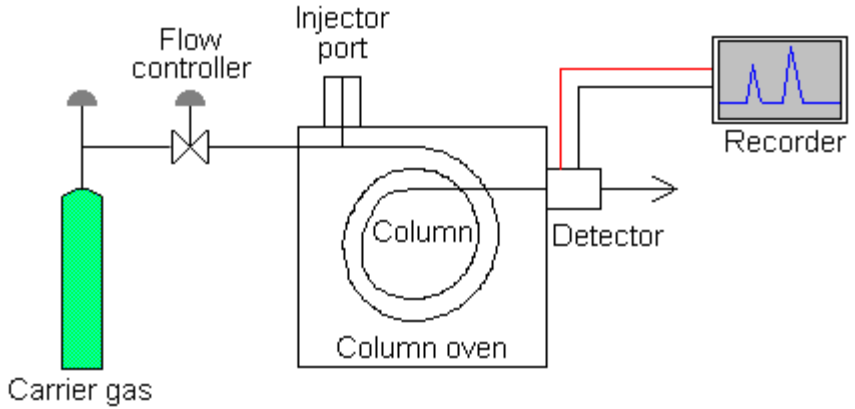




4-HNE: molecule that is produced by lipid oxidation

FA composition in the brain

- Gas chromatography: separating compounds by their vapor pressures without decomposition occurring



FA composition in the brain

- Profiled fatty acids in the brain observed during these experimental diets
- N-3 FA deficient (+ fructose) diet → no change in saturated or mono-unsaturated FA levels
  - Except in:
    - FA (22:6n-3) where DHA levels decreased
    - FA (22:5n-6) where DPA levels increased
    - FA (20:4n-6) where AA levels decreased
  - Exposure to n-3 FA diet reversed n-3 FA deficiency and fructose

- Increased ratio of n-6 FA to n-3 FA during n-3 deficiency and/or fructose
- Ratio of N-6 FA to n-3 FA can be counter-regulated by dietary n-3 FA

FA composition in the brain

Table 3. Fatty acid composition in groups subjected to *n*-3 and *n*-3 deficient diets with or without fructose water

Fatty acids	*n*-3 diet	*n*-3 def	*n*-3 def/Fru	*n*-3 diet/Fru
14:0	0.338 ± 0.019	0.299 ± 0.039	0.311 ± 0.004	0.391 ± 0.018
16:0	20.27 ± 0.274	20.57 ± 0.552	20.55 ± 0.377	21.00 ± 0.298
18:0	18.72 ± 0.150	19.32 ± 0.239	18.84 ± 0.199	18.81 ± 0.295
18:1	15.10 ± 0.214	14.34 ± 0.351	14.55 ± 0.208	14.84 ± 0.225
18:2*n*-6 (LA)	0.353 ± 0.020	0.310 ± 0.063	0.250 ± 0.012	0.340 ± 0.016
20:0	0.254 ± 0.012	0.246 ± 0.018	0.236 ± 0.017	0.238 ± 0.013
20:1	1.028 ± 0.020	0.963 ± 0.076	0.997 ± 0.045	0.953 ± 0.020
20:4*n*-6 (AA)	7.017 ± 0.228	8.149 ± 0.107##	8.265 ± 0.149##	6.821 ± 0.136**
22:0	0.290 ± 0.023	0.265 ± 0.017	0.285 ± 0.022	0.264 ± 0.010
22:5*n*-6 (DPA)	0.212 ± 0.010	0.968 ± 0.032##	0.912 ± 0.036##	0.222 ± 0.014**
22:6*n*-3 (DHA)	13.48 ± 0.388	11.44 ± 0.199##	11.77 ± 0.375##	13.52 ± 0.089**
24:0	0.652 ± 0.048	0.578 ± 0.035	0.679 ± 0.040	0.629 ± 0.023
24:1*n*-9	1.254 ± 0.078	1.167 ± 0.088	1.260 ± 0.064	1.212 ± 0.044
n-6/*n*-3	0.562 ± 0.010	0.824 ± 0.017##	0.803 ± 0.020##	0.546 ± 0.011**

Values are expressed as mean ± SEM. ##$P < 0.01$: significant difference from *n*-3 diet; **$P < 0.01$: significant difference from *n*-3 def/Fru; ANOVA (one-way) followed by Newman–Keuls test. LA, linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Metabolic dysfunction and cognitive performance

- N-3 FA deficiency compromises metabolic homeostasis and thus affects cognitive abilities
- N-3 FA deficient diet → ↓ spatial memory
- N-3 FA deficient + fructose diet → ↓↓ spatial memory

Metabolic dysfunction and cognitive performance

- Obesity not a major contributor to altered memory function
- N-3 FA deficiency + ↑ fructose → hyperinsulinaemia, hyperglycaemia, ↑ triglyercide levels

- Metabolic dysfunction leading to insulin resistance can affect memory performance through regulation of insulin signaling system

Hyperinsulinaemia: excess insulin in the blood
Hyperglycaemia: excess glucose in the blood

Insulin signaling in brain and metabolic dysfunction

- N-3 FA deficient → ↓pTyIR
- N-3 FA deficient → ↓pAkt

- N-3 FA maintains proper insulin signaling in the brain
- N-3 FA diets cope with challenges imposed by fructose

Metabolic disturbances on neuronal signaling

- Metabolic dysfunction potentiates pathways that can lead to disruption of membrane homeostasis which can ultimately negatively affect neuronal function
  - Alterations in insulin receptor signaling via Akt pathway
- Fructose intake disrupts plasma membrane with lipid peroxidation occurring
  - Dysfunction of membrane proteins

Metabolic disturbances on neuronal signaling

- N-6 and N-3 FA are essential nutrients that cannot be synthesized by the body
  - They exist in plants in forms like linoleic acid, which can be metabolized into arachidonic acid, eicosapentaenoic acid, and DHA
- Proper maintence of n-6 to n-3 FA ratio for synaptic plasticity, growth, and repair
- N-3 FA + fructose → maintained normal range

Dietary influences on energy homeostasis

- AMPK levels high in n-3 rats implies that n-3 conserves energy in ATP levels in hippocampus.
- NAD is activated by AMPK
- Fructose intake decreases Sir2 levels, but n-3 normalizes these levels
- n-3 deficiency with or without fructose decreased LKB1 Phosphorylation
- DHA increased Phosphorylation while AA decreased Phosphorylation.
- This implies that n-6 is harmful and n-3 is good.

Implications for synaptic plasticity

- AMPK regulates cAMP-response element binding (CREB) proteins
- CREB proteins play a major role in synaptic plasticity and cognitive functions
- CREB is correlated with Sir2, synapsin 1 and synaptophysin which are all related to synaptic plasticity.
- n-3 deficiency decreases Phosphorylation of CREB, synpasin 1 and synaptophysin.

Health Implications

- n-3 deficiency increases vulnerability to effects of fructose
- Causes disrupted IR signaling, cognitive functions like memory impairment, and homeostasis.
- n-3 improves neuronal function by supporting synaptic membrane fluidity, regulating gene expression and cell signalling.
- n-3 deficiency during brain maturation results in elevated anxiety behavior in adulthood.

Conclusion

EAT YOUR OMEGA 3 FATTY ACIDS!

Especially if your diet includes lots of sugar!

But also even if it does not!

*Disclaimer- do not eat OMEGA 6 FATTY ACIDS