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

Authors: Rahul Agrawal and Fernando Gomez-Pinilla
Overview and Objectives

- **Introduction**
  - 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.

- **Methods**
  - 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.

- **Results**
  - **Omega 3 Fatty Acid** returns body to metabolic homeostasis.

- **Discussion**

- **Conclusion**
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

Dietary n-3 fatty acid

Brain

Body

Insulin

Fructose

Triglycerides

Insulin Resistance

Membrane Fluidity

PLA2

IRS-1

Akt

AMPK

LKB1

4-HNE

CREB

Synaptic Plasticity (i.e. synapsin & SYP) and Cognition

Introduction
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.

<table>
<thead>
<tr>
<th>n-3 diet</th>
<th>n-3 def</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-3 diet/Fru</td>
<td>n-3 def/Fru</td>
</tr>
</tbody>
</table>
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.
**Methods**

**Immunoblotting**

- **Antigen samples**
- **Separation gel**
  - Separated proteins
- **Blotting tank**
  - Proteins transferred to nitrocellulose sheet (blot)
- **Labeled antibody**
- **Immuno-staining of blot**
- **Antigen bands visualized**
- **Autoradiography**
  - Develop and fix autoradiograph
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
Methods

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.

1. Suitable antibody is added.
2. Antibody binds to protein of interest.
3. Protein A or G added to make antibody-protein complexes insoluble.
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>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Food intake (g day(^{-1}))</th>
<th>Water intake (ml day(^{-1}))</th>
<th>Caloric intake (kcal day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-3 diet</td>
<td>508.2 ± 13.51</td>
<td>26.18 ± 1.28</td>
<td>30.77 ± 1.49</td>
<td>109.6 ± 4.14</td>
</tr>
<tr>
<td>n-3 def</td>
<td>492.5 ± 5.43</td>
<td>25.72 ± 0.605</td>
<td>33.18 ± 2.07</td>
<td>102.8 ± 2.17</td>
</tr>
<tr>
<td>n-3 def/Fru</td>
<td>512.8 ± 9.72</td>
<td>22.0 ± 1.52</td>
<td>45.72 ± 8.21</td>
<td>110.2 ± 7.14</td>
</tr>
<tr>
<td>n-3 diet/Fru</td>
<td>522.5 ± 24.44</td>
<td>22.58 ± 0.993</td>
<td>41.91 ± 4.90</td>
<td>117.4 ± 2.17</td>
</tr>
</tbody>
</table>

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

Results

![Graph showing latency times for different diet conditions]
Metabolic Markers

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

<table>
<thead>
<tr>
<th>Metabolic Markers</th>
<th>Table 2. Blood glucose, insulin and triglyceride levels in groups subjected to n-3 and n-3 deficient diets with or without fructose water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose level (mg dl⁻¹)</td>
<td>Insulin level (ng ml⁻¹)</td>
</tr>
<tr>
<td>n-3 diet</td>
<td>81.17 ± 3.02</td>
</tr>
<tr>
<td>n-3 def</td>
<td>77.17 ± 4.26</td>
</tr>
<tr>
<td>n-3 def/Fru</td>
<td>106.0 ± 6.55##</td>
</tr>
<tr>
<td>n-3 diet/Fru</td>
<td>99.83 ± 3.13</td>
</tr>
</tbody>
</table>

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 $\rightarrow$ ↑ Triglyceride levels
  - N-3 FA deficiency + fructose diet $\rightarrow$ ↑ ↑ Triglyceride levels
  - N-3 FA deficiency + fructose diet $\rightarrow$ ↑ Glucose levels
  - N-3 FA deficiency + fructose diet $\rightarrow$ ↑ Insulin levels
  - N-3 FA + fructose diet $\rightarrow$ ↑ 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)

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**Insulin Resistance Index**: the measure of the condition in which cells fail to respond 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

![Graph showing the relationship between serum triglyceride levels and insulin resistance index with correlation coefficient r = 0.6071, p < 0.01.](image)
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

![Graph showing association between latency time and insulin resistance index](graph.png)
Assess levels of insulin receptor tyrosine phosphorylation and Akt phosphorylation according to the experimental diets.
Insulin receptor signaling

- N-3 FA deficiency + fructose diet $\rightarrow$ $\downarrow$ pTyrIR
- N-3 FA + fructose diet $\rightarrow$ $\uparrow$ 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

**Results**

- **Insulin Resistance Index**: the measure of the condition in which cells fail to respond to normal actions of insulin hormone
- **pTyrIR**: tyrosine phosphorylation of insulin receptor

- Graph showing the relationship between insulin resistance index and pTyrIR levels with a correlation coefficient of $r = -0.5874$, $p < 0.01$
Insulin receptor signaling

- N-3 FA deficiency diet $\rightarrow \downarrow pAkt$
- N-3 FA deficiency + fructose diet $\rightarrow \downarrow\downarrow pAkt$
- N-3 FA + fructose diet $\rightarrow \uparrow pAkt$
  (alleviates fructose induced change)

$pAkt$: phosphorylation of protein Akt
Energy metabolism

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

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

Results
Synaptic plasticity

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

**A**

**B**

Results

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.
Synaptic plasticity

- N-3 FA deficiency diet $\rightarrow$ $\uparrow$ pCREB
- N-3 FA deficiency + fructose diet $\rightarrow$ $\uparrow\uparrow$ pCREB
- N-3 FA + fructose diet $\rightarrow$ $\uparrow$ 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.
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

Results

Synaptic plasticity

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

![Graph showing synaptic plasticity](image)
Synaptic plasticity

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

- N-3 FA deficiency diet $\rightarrow$ ↓ pSynapsin I
- N-3 FA deficiency + fructose diet $\rightarrow$ ↓ pSynapsin I
Synaptic plasticity

- N-3 FA deficiency diet $\rightarrow$ ↓ Synaptophysin
- N-3 FA deficiency + fructose diet $\rightarrow$ ↓ Synaptophysin

Results
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.
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
Results

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

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

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 $\rightarrow$ ↓ spatial memory
- N-3 FA deficient + fructose diet $\rightarrow$ ↓↓ spatial memory
Metabolic dysfunction and cognitive performance

- Obesity not a major contributor to altered memory function
- N-3 FA deficiency $\rightarrow$ fructose $\rightarrow$ hyperinsulinaemia, hyperglycaemia, triglyceride 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 $\rightarrow$ ↓pTyIR
- N-3 FA deficient $\rightarrow$ ↓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
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 maintenance of n-6 to n-3 FA ratio for synaptic plasticity, growth, and repair.

N-3 FA + fructose → maintained normal range

Discussion

Metabolic disturbances on neuronal signaling
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