‘Metabolic Syndrome’ in the brain

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

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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.
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

Membrane Fluidity

n-6

n-3

Insulin Receptor

PLA2

4-HNE

LKB1

AMPK

sir2

Akt

CREB

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

Insulin

Fructose

Triglycerides

Insulin Resistance
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

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**Terminology**

**Introduction**

**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.
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.
Methods

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
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
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-synapsin
  - Anti-4HNE
  - Anti-IR
  - Anti-CREB
  - Anti-Sir2
  - Anti-Synaptophysin
  - Anti-AMPK
  - Anti-pAkt
  - Anti-Akt
**Methods**

**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.
Results

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

<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
Cognitive functions

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

Results
Metabolic Markers

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

<table>
<thead>
<tr>
<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>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>n-3 diet</td>
</tr>
<tr>
<td>n-3 def</td>
</tr>
<tr>
<td>n-3 def/Fru</td>
</tr>
<tr>
<td>n-3 diet/Fru</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
- 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 respond to normal actions of insulin hormone
Results

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
Results

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 $\rightarrow$ ↓ pTyrIR
- N-3 FA + fructose diet $\rightarrow$ ↑ pTyrIR

pTyrIR: tyrosine phosphorylation of insulin receptor

A

$\text{pTyrIR levels}$

$\text{n-3 diet}$ $\text{n-3 def}$ $\text{n-3 def/Fru}$ $\text{n-3 diet/Fru}$
Insulin receptor signaling

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

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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 correlation between Insulin resistance index and pTyrIR levels](image_url)
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

- $\uparrow$ ADP $\rightarrow$ $\uparrow$AMP $\rightarrow$ AMPK activation
- LKB1 activation $\rightarrow$ AMPK activation

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

- N-3 FA deficient diet $\rightarrow$ ↓ pLKB1

pLKB1: phosphorylated LKB1
Energy metabolism

- Positive correlation between pLKB1 and DHA
Energy metabolism

- Negative correlation between pLKB1 and arachidonic acid (AA)
Results

Energy metabolism

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

- N-3 FA deficiency + fructose diet $\rightarrow$ ↓ Sir2
- N-3 FA diet $\rightarrow$ ↑ 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
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.
Synaptic 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
Synaptic plasticity

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

FA composition in the brain

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

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
## Results

### FA composition in the brain

<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-6 (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.
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, ↑ 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 → ↓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 maintenance 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.
Discussion

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, synapsin 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.
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