‘Metabolic Syndrome’ in the brain

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

Authors:
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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

Introduction

Dietary n-3 fatty acid

Brain

Membrane Fluidity

Insulin Receptor

n-6

n-3

PLA2

4-HNE

IRS-1

LKB1

AMPK

sir2

Akt

CREB

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

Body

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 (22-24°C) 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
- Separated proteins
- Blotting tank: Proteins transferred to nitrocellulose sheet (blot)
- Labeled antibody
- Develop and fix autoradiograph
- Antigen bands visualized
- Autoradiography
- Immuno-staining of blot
Methods

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

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.

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>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</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-3 diet</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>508.2 ± 13.51</td>
</tr>
<tr>
<td>492.5 ± 5.43</td>
</tr>
<tr>
<td>512.8 ± 9.72</td>
</tr>
<tr>
<td>522.5 ± 24.44</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

Cognitive functions

![Graph showing latency times across different dietary conditions](image)
Metabolic Markers

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

<table>
<thead>
<tr>
<th></th>
<th>Glucose level (mg dl⁻¹)</th>
<th>Insulin level (ng ml⁻¹)</th>
<th>Triglyceride level (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-3 diet</td>
<td>81.17 ± 3.02</td>
<td>1.46 ± 0.24</td>
<td>91.17 ± 10.69</td>
</tr>
<tr>
<td>n-3 def</td>
<td>77.17 ± 4.26</td>
<td>1.56 ± 0.30</td>
<td>142.0 ± 10.60#</td>
</tr>
<tr>
<td>n-3 def/Fru</td>
<td>106.0 ± 6.55##</td>
<td>3.28 ± 0.21##</td>
<td>218.8 ± 23.04##</td>
</tr>
<tr>
<td>n-3 diet/Fru</td>
<td>99.83 ± 3.13</td>
<td>2.54 ± 0.16*</td>
<td>166.2 ± 17.65*</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
Results

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

![Graph showing correlation between serum triglyceride levels and latency time](image)
Association between metabolic changes and cognitive behavior

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

Results
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 respond to normal actions of insulin hormone

**pTyrIR**: tyrosine phosphorylation of insulin receptor

*Results*

- Line of best fit: $B$
  - $r = -0.5874$, $p < 0.01$

Graph showing the relationship between Insulin resistance index and pTyrIR levels.
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

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

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

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)

\[ C \]

$pLKB1$: 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

Results
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

**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.
Synaptic 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
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 $\rightarrow \downarrow$ pSynapsin I
- N-3 FA deficiency + fructose diet $\rightarrow \downarrow$ pSynapsin I
Synaptic plasticity

- N-3 FA deficiency diet $\rightarrow \downarrow$ Synaptophysin
- N-3 FA deficiency + fructose diet $\rightarrow \downarrow$ 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 $\rightarrow$ $\uparrow\uparrow$ 4-HNE
- N-3 FA diet $\rightarrow$ $\uparrow$ 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
# 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-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 + ↑ 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.
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.
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