Metabolic Syndrome in the Brain:
Deficiency in Omega-3 Fatty Acids exacerbate dysfunctions in insulin receptor signalling and cognition

Group TBD:
Madeline Haff, Bikem Sonmezler, Ben Brooks, Rosie Chu, and Nicholas Anguetta
So what? Why should I care about MetS & n-3 fatty acid?
OUR CULTURE IS CHANGING

Fat-Shaming

fit is not a destination it is a way of life

SUPER SIZE ME
How is it all connected?

Buttock augmentation with fat grafting: 11,505 procedures, up 15 percent from 2013 to 2014

Buttock implants: 1,863 procedures up 98 percent from 2013 to 2014

Buttock lift: 3,505 procedures up 44 percent from 2013 to 2014

Pectoral implants: 1,054 procedures - increased 208 percent from 2013 to 2014

Male breast reductions: 26,175 procedures in 2014 - increased 29 percent since 2000
HEALTH EQUITY
There is no greater wealth than the health of our collective societies

HEALTH EQUITY IS...
The attainment of the highest level of health for all people. Achieving health equity requires valuing everyone equally with focused and ongoing societal efforts to address avoidable inequalities, historical and contemporary injustices, and the elimination of health and health care disparities.

HEALTH EQUITY MATTERS...
Health is a basic human right. It is a key determinant of economic and social development and has a positive impact on people’s life chances and opportunities.

A Framework for Health Equity

SOCIAL INEQUALITIES
Race, Class, Gender, etc.

INSTITUTIONAL POWER
Corporations & other Businesses
Schools
Healthcare Access

NEIGHBORHOOD CONDITIONS
Social, Physical, Environmental, Residential Segregation

RISK BEHAVIORS
Smoking
Nutrition
Physical Activity
Violence
Sex

DISEASE & INJURY
Infectious & Chronic Disease, Injury (intentional & unintentional)

DEATHS
Infant Mortality, Life Expectancy

Health Inequities
(Social Factors)

Health Disparities
(Health Status)

"Health Inequities are differences in health status & mortality rates across population groups that are systemic, avoidable, unfair and unjust."

-Margaret Whitehead

Source: Adapted from ACPHD from the Bay Area Regional Health Inequities Initiative, Summer 2008
We want the ratio of n-6:n-3 to be about 1:1, but it is typically as high as 16:1 because the Western diet is high in n-6 and low in n-3.
Animals and Experimental Design

- Adult male Sprague-Dawley rats were used in an experiment to test “Metabolic Syndrome” in the brain (simulated through high fructose diet).
- Six rats per group were housed individually in poly-acrylic cages with 12 hour light/dark cycle.
- Two custom diets were randomly used, n-3 deficiency diet and n-3 diet; differentiated in the amount of n-3 fatty acids.
- Both diets had the same amount of basal macronutrients, vitamins, minerals, and basal fats.
Barnes Maze Test

- Rats trained to find a hidden dark escape chamber
- Rats were trained through two trials per day for five days
- To test learning and memory functions the rats were tested on the maze before and after experimental diets
- Then randomly assigned omega-3 fatty acid diet (n-3 diet) or omega-3 fatty acid deficient diet (n-3 def) diet, with or without fructose solution
Trials recorded by an overhead camera in the center of the maze
Trials started by placing the rat in a covered chamber in the center of the maze
After 10 second delay the chamber was raised
In order to assess memory retention, two trials were given after 6 weeks of experimental diets
Six weeks after experimental diets, a Barnes maze test was performed for 1 day to evaluate memory retention
Metabolic Syndrome by High Fructose Intake

- High fructose diet mimics MetS in rats
- The deficiency of n-3 fatty acid caused increase in latency time, indicating memory impairment, further worsened by fructose intake
- The effect of fructose on memory in n-3 deficiency diet was enhanced by the n-3 diet
- Furthermore, fructose induced changes are influenced by dietary n-3 deficiency
Metabolic Syndrome by High Fructose Intake (Cont.)

- Fructose rats showed increase in insulin resistance index
- N-3 deficient diet indicated insulin resistance developed in high fructose intake rats
- N-3 presence improved insulin resistance, indicating improved insulin sensitivity
Biochemical Analysis

- For biochemical analysis, blood was collected from rat tail vein after overnight fasting and then centrifuged to obtain serum samples.
- Glucose level was measured using a glucometer (Bayer’s Contour meter).
- Insulin levels were determined by an ELISA kit.
Fatty Acid Analysis

- Bligh & Dyer method was used to extract lipids from brain tissue
- Frozen brains homogenized in chloroform/methanol
- Liquid nitrogen tissues were ground to powder and subjected to extraction of total lipids
- Peaks of resolved fatty acid methyl esters were identified and quantified by comparison with standards
**Results**

(As differences from fructose-free, n-3 diet rats)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>0 + + Fructose intake increases blood glucose</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td>0 + ** MeTs ~ hyperinsulinemia; n3 reduces insulin levels in MeTs</td>
</tr>
<tr>
<td><strong>TGs</strong></td>
<td>+ ++ +* MeTs ~ high triglycerides; n3 reduces TG levels</td>
</tr>
<tr>
<td><strong>Latency</strong></td>
<td>+ ++ +* Both MeTs and lack of n3 impair cognition/memory</td>
</tr>
<tr>
<td><strong>IR</strong></td>
<td>0 + ** MeTs ~ Insulin resistance; n3 reduces MeTs-induced IR</td>
</tr>
<tr>
<td><strong>pTyrIR</strong></td>
<td>0 - 0** Both MeTs and n3 deficiency impair insulin-receptor signaling pathway in the hippocampus</td>
</tr>
<tr>
<td><strong>pAKT</strong></td>
<td>- -- 0*</td>
</tr>
<tr>
<td><strong>4-HNE</strong></td>
<td>0 + 0* n3 deficiency increases fruc-based lipid peroxidation risk</td>
</tr>
</tbody>
</table>

* implies both n3 deficiency and MeTs/fructose make this worse

** implies MeTs makes this worse, but dietary n3 reduces the severity

**Figure 3**

Tyrosine phosphorylation of insulin receptor (pTyrIR; A) and Akt phosphorylation (C) in groups subjected to n-3 and n-3 deficient diets with or without fructose water. B, negative correlation between insulin resistance index and pTyrIR levels. Values are expressed as mean ± SEM. #P < 0.05, ##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.
Results continued (As differences from fructose-free, n-3 diet rats)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLKB1</td>
<td>n-3 deficiency = low DHA ~ high AA, low LKB1</td>
</tr>
<tr>
<td></td>
<td>↓ n-3 deficiency → physiological stress, lower energy metabolism</td>
</tr>
<tr>
<td></td>
<td>↑ LKB1 → AMPK → Energy metabolism</td>
</tr>
<tr>
<td>AA</td>
<td>+ DPA (n6)</td>
</tr>
<tr>
<td>DHA</td>
<td>-</td>
</tr>
<tr>
<td>AMPK</td>
<td>-</td>
</tr>
<tr>
<td>Sir2</td>
<td>0</td>
</tr>
</tbody>
</table>

* implies both n3 deficiency and MeTs/fructose make this worse
** implies MeTs makes this worse, but dietary n3 reduces the severity

Dietary n-3 reduces effects of MeTs.

Figure 6
A, CREB phosphorylation. B, positive correlation between Sir2 levels and phosphorylated CREB. C, phosphorylation of synapsin. D, levels of synaptophysin (SYP) in groups subjected to n-3 and n-3 deficient diets with or without fructose water. Values are expressed as mean ± SEM. #P < 0.05, ##P < 0.01: significant difference from n-3 diet, *P < 0.05, **P < 0.01: significant difference from n-3 def/Fru; ANOVA (one-way) followed by Newman–Keuls test.
N-3 fatty acids balance the effects of Metabolic Syndrome

- Hyperglycemia
- Hyperinsulinemia
- Insulin Resistance
- Lipid peroxidation
- High Triglyceride level
- Inhibited Insulin receptor signaling
- Lower cognitive function/memory
- Physiological stress
- Inhibited hipp. plasticity/cognition
- Inhibited hipp. synaptic growth
- High Arachidonic Acid (AA) level
- Increased n-6/n-3 ratio

Dietary n3 at least partially alleviates all of these
Metabolic Dysfunction and Cognitive Performance

- Dietary n-3 fatty acid deficiency
- Decrease in maintenance of metabolic homeostasis
- Decrease in cognitive abilities
- Deficiency of n-3 → decline in spatial memory
  - in proportion to the intensity of index of insulin resistance
  - All parameters aggravated by increase in the fructose intake
- No difference observed in body weight and total caloric intake with either of the diets

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 function is affected by central and peripheral insulin resistance
  • Looked at several markers of metabolic function in serum
  • Increased consumption of fructose (particularly when combined with DHA deficiency) resulted in:
    - Hyperinsulinaemia
    - Hyperglycaemia
    - Increase in triglyceride levels
**Metabolic Dysfunction and Cognitive Performance**

Fructose may lead brain towards insulin resistance via its effects on TG.

<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 ± 10.50#</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 ± 17.55*</td>
</tr>
</tbody>
</table>

"Insulin resistance index → Triglyceride levels → Cognitive impairment"

Fructose may lead brain towards insulin resistance via its effects on TG.
Findings from previous experiments that support this theory

- Decreased ability of insulin to activate its signaling cascade
- TGs penetrate the BBB
- Impaired memory
- Hippocampal insulin signaling facilitated memory
- Insulin resistance accompanied with increased circulating TGs
Metabolic Dysfunction and Cognitive Performance

SUMMARY

Based on theirs and previous studies the hypothesis they came up with was:

Metabolic dysfunction leading to insulin resistance can affect memory performance through regulation of the insulin signalling system via its effects on TGs.

Fructose also contributes to the brain function. HOW??

More and more evidence shows that neuronal cells can metabolize fructose and fructose feeding increases the expression of fructose sensitive glucose transporters (GLUT5!!) in the hippocampus.

Possible that fructose or one of its metabolites directly induced the memory deficits that were observed in the experiment.
INFLUENCES OF METABOLIC DISTURBANCES ON NEURONAL SIGNALLING

Energy metabolism \(\rightarrow\) Membrane function \(\rightarrow\) metabolic dysfunction \(\rightarrow\) pathways that can lead to the disruption of the membrane homeostasis and detrimental consequences for neuronal function.

4-HNE \(\rightarrow\) (4-Hydroxynonenal), produces alterations in the function of key membrane proteins.

**How do we know this?**

Fructose intake \(\rightarrow\) Plasma membrane disruption

Peroxidation of membrane bound n-6 AA results in the generation of 4-HNE. (THIS IS BAD!)

Increase in the levels of 4-HNE (a marker of lipid peroxidation)

**What are Free radicals?**

- Free radicals are like robbers which are deficient in energy.
- Free radicals attack and snatch energy from the other cells to save for themselves.

Peroxidation \(\rightarrow\)
INFLUENCES OF METABOLIC DISTURBANCES ON NEURONAL SIGNALLING

Peroxidation of membrane bound n-6 AA results in the generation of 4-HNE.

Membrane peroxidation also produces alterations in insulin receptor signalling via Akt.
INFLUENCES OF METABOLIC DISTURBANCES ON NEURONAL SIGNALLING

Deficiency in DHA ➔ Deleterious effects of fructose on the stability of the plasma membrane
● Reflected by ↓DHA levels and ↑AA levels

AA ➔ (Arachidonic acid) Most abundant fatty acids in the brain.

Overexpression can lead to alterations in the conversion of AA into other bioactive molecules which can result in neurological disorders.

Relationship between n-3 and n-6 is important for the function of the plasma membrane which is also required for synaptic plasticity, growth and repair.
INFLUENCES OF METABOLIC DISTURBANCES ON NEURONAL SIGNALLING

The dietary n-6/n-3 ratio is of fundamental importance. Why?

N-3 and n-6 fatty acid families have PUFA precursors which are essential nutrients that cannot be synthesized in de novo in mammals.

They exist in plants as linoleic acid and alpha-linolenic acid and are metabolized by elongations and desaturations. (AA, EPA and DHA)
What happened to the n-6/n-3 ratio in the experiment?

**N-3 deficient diet** → increase in n-6/n-3 ratio (both alone and with presence of fructose)

Increase of ratio in the group fed on fructose: indicative of substitution of n-3 by n-6 in the membrane which may alter the membrane fluidity.

Why is this important?

(membrane fluidity) → disrupt membrane insulin receptor signalling + downstream cascades (Akt)

**ALTERATIONS IN SYNAPTIC PLASTICITY AND COGNITION**

**N-3 diet w/ fructose** → maintained the ratio (efficient in balancing harmful effects of high sugar diet)
Fructose intake disrupts plasma membranes as evidenced by an increase in the levels of 4-HNE (marker for lipid peroxidation)

Membrane peroxidation produces alterations in the function of key membrane proteins and insulin receptor signalling via Akt.

Influence of insulin resistance on lipid peroxidation and membrane homeostasis disruption is evident.

Increase of AA levels can lead to neurological disorders.

N6 to n3 ratio is important because the increase of this ratio results in the decrease in the membrane fluidity. This can lead to alterations in synaptic plasticity and cognition.
Looked at the effects of dietary manipulations on several markers of synaptic plasticity. (CREB, Sir-2, Synapsin 1 and Synaptophysin)

In order to assess synaptic plasticity:
Looked at Sir2-CREB relationship & Synapsin 1 and synaptophysin markers.
**IMPLICATIONS FOR SYNAPTIC PLASTICITY**

**CREB:** cAMP-response element binding protein

**Sir 2:** responsible for the lifespan-extending effects of calorie restriction

Both modulate synaptic plasticity and cognitive function.

Positive correlation between Sir2 and CREB reflects interaction between Sir2 and regulation of plasticity and cognitive function in hippocampus.
To examine the modulatory role of dietary factors on synaptic functions they looked at Synapsin 1 and Synaptophysin.

**Synapsin 1**: a nerve terminal protein. Implicated in the regulation of neurotransmitter release during synaptic plasticity, synaptogenesis and neurite outgrowth.

Also regulated by CREB.

N-3 def decreases phosphorylation of CREB and synapsin 1; fructose potentiated this effect.
**Synaptophysin:** marker for synaptic growth, involved in calcium binding, channel formation, exocytosis.

Important player for regulating synaptic plasticity

Decreased levels with n-3 deficiency and fructose treatment.
IMPLICATIONS FOR SYNAPTIC PLASTICITY SUMMARY

Positive correlation between Sir2 and CREB reflects interaction between Sir2 and regulation of plasticity and cognitive function in hippocampus.

N-3 def decreases phosphorylation of CREB and synapsin 1; fructose potentiated this effect.

Synaptophysin shows decreased levels with n-3 deficiency and fructose treatment.

The n-3 supplementation: obligatory for normalizing this effect suggesting that n-3 fatty acids can restore the cognitive function by normalizing the action of insulin resistance on synaptic plasticity via CREB, synapsin I and synaptophysin.
Insulin Signalling in the Brain & Metabolic Dysfunction

- This study looked at IR phosphorylation by immunoprecipitation of phosphotyrosine, followed by immunoblotting of the IR (to show IR activation)
  - Found that phosphorylation of the IR and Akt were diminished after a DHA deficient diet, and were further aggravated by elevated fructose levels
  - Shows importance of DHA supplementation for proper insulin signalling in the brain
  - Necessity of DHA supplementation to cope with problems imposed by high fructose
**Insulin Signalling in the Brain & Metabolic Dysfunction**

- Correlation between ↑ insulin resistance and ↓ pTyrIR suggests association between peripheral insulin and disruption of insulin signalling in the brain
  - Memory deficits positively correlated with increased insulin resistance shows that insulin does cross the BBB and interact with neurons directly
- High levels of fructose paired with DHA deficiency leads to increased hippocampal insulin resistance (decreases in IR signalling)
**Dietary influences on energy homeostasis**

- ATP and NAD are molecules used in energy production of all cells; can be sensed by regulatory proteins like AMPK and Sirtuins
  
  - AMPK can sense low energy levels and activate or inhibit other molecules to restore energy balance
    
    - Increased AMPK phosphorylation
      - Activates mechanisms to conserve hippocampus ATP
    
    - Decreased AMPK phosphorylation
      - Disturbances seen in maintaining energy homeostasis
Dietary influences on energy homeostasis

LKB1 = upstream kinase that activates AMPK in response to AMP or ADP increases

NAD = crucial substrate for Sir2 function, activated by AMPK (dose-dependant)

- Decreased phosphorylation of LKB1
- Varied in direct proportion to DHA levels and inverse to AA levels
- Proper n-6/n-3 ratio needed to maintain proper energy homeostasis

- Reduced synaptic plasticity
- Reduced Sir2 in cortex and hippocampus
- Sir2 levels can be normalized with n-3
PUBLIC HEALTH IMPLICATIONS

- DHA is a crucial part of neuronal membranes, especially at synapse sites, and must be supplemented through diet
  - Helps in synaptic membrane fluidity, regulates gene expression and cell signalling
- Both diets high in fructose and diets with \( n-3 \) deficiencies can predispose you to MetS, further promote insulin resistance in the brain, and increase incidence of cognitive dysfunction related to these conditions
  - DHA supplementation even more important in cases of MetS and other metabolic dysfunctions!
PUBLIC HEALTH IMPLICATIONS

- Western diet very high in sugars (ex: HFCS), so DHA supplementation is crucial to help prevent the effects of MetS in the brain
- DHA deficiencies during early development and maturation can cause increased anxiety behaviors throughout adulthood
Conclusion

- Central IRs can dysfunction even while peripheral IRs still work
- Omega-3s and prevent the negative cognitive effects of a high fructose diet (like the US diet), especially in cases with metabolic problems
- Omega-3s are crucial for brain health, but also offer many benefits for the whole body