Why You Should Eat (More) Dark Chocolate and Oysters:
and other tails from the NAFLD mystery series

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University of Louisville

February 24th, 2022
My Research Challenge: Chronic Liver Disease

Kills over 2 million people in the U.S. each year!

What do you think it is the most common cause of Chronic Liver Disease?

Top 10 Causes of Death Worldwide

The Answer

Nonalcoholic fatty liver disease (NAFLD)
Nonalcoholic Fatty Liver Disease

- **Normal**
- **Obesity, Insulin Resistance**
- **Genetics, Sex Exposures**
- **NAFL**
- **NAFLD**
- **NASH**
- **Fibrosis/Cirrhosis**

- **Total fat > 5%**
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- **Total fat > 5%**
- **Inflammation**
- **Inflammation**
- **Cellular Damage**
- **Scar Tissue**

Not reversible
Nonalcoholic Fatty Liver Disease

Avg. Global Prevalence → 25%

North America: 24.13%
Europe: 23.71%
Middle East: 31.79%
Africa: 13.48%
South America: 30.45%
Asia: 27.37%
The Prevalence of NAFLD Is on the Rise!

Pooled Average
Major Risk Factors for NAFLD in the United States

9 out of 10 (90%) severely obese individuals have NAFLD

6 out of 10 (60%) diabetics have NAFLD

1 out of 4 people have NAFLD, independent of weight

Other Risk Factors

Environmental Toxicants
Cadmium: “a major health concern” - WHO

Naturally occurring, non-essential metal

Found in soils
zinc, lead, and copper ores

Occupational & Environmental risk factor

Anthropogenic Sources
phosphate fertilizers, fossil fuel combustion, waste incineration

2015 - The Animas River, Colorado

Environmental exposures to cadmium are linked to the development of several diseases
Cadmium Exposure is a Risk Factor for NAFLD

The liver is a major target organ of cadmium toxicity and accumulation

Exposures

Epidemiologically

Positive Correlation
✓ NAFLD
✓ NASH

Experimentally
✓ Increased hepatic lipids

(Hyder et al 2013)
(Ba et al 2017, Go et al 2015)

How cadmium influences the initiation and progression of diet-induced NAFLD is not well understood.

Studies do not consider:
1. Environmental exposures can be life-long
2. The risk of developing adulthood disease is correlated with adverse stimuli in utero.
What is the impact of whole-life exposure to low dose cadmium on the development of NAFLD?
Exposure Paradigm

F₀  14 weeks Breeding Groups

F₁  Second Litter Male Offspring

Control

5 ppm Cadmium

ND

HFD

ND

F₁  Diet-Induced NAFLD

Conception (3 wks old)

Weaning

Birth

24 weeks of Diet

0 or 5 ppm Cd (drinking water)

IPGTT, DS (26 wks old)

Sacrifice (27 wks old)

Diet (ND or HFD)
Cadmium Exacerbates Diet-Induced NAFLD!

**Liver Injury**

<table>
<thead>
<tr>
<th>Whole Animal Characterization</th>
<th>Control</th>
<th>5 ppm Cd</th>
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</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>↑↑↑</td>
<td>↑↑↑↑</td>
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<tr>
<td>% Fat</td>
<td>↑↑↑</td>
<td>↑↑</td>
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<tr>
<td>IPGTT AUC</td>
<td>↑↑↑</td>
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<tr>
<td>Plasma Insulin</td>
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**NAFLD Characterization**

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<tr>
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<tbody>
<tr>
<td>Liver:Tibia Ratio</td>
<td>↑</td>
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<tr>
<td>ALT</td>
<td>↑</td>
<td>↑↑↑↑</td>
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<tr>
<td>Steatosis</td>
<td>↑↑↑</td>
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<tr>
<td>Oil Red O</td>
<td>↑</td>
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↑ (trend); ↑↑ (Statistically Significant Increase)

PMID: 34990729
Zinc (Zn) is an essential trace element

Cadmium competes with Zinc

Molecular Mimicry
- Active Zn
- Inactive Cd

Cadmium disrupts Zinc homeostasis

Zinc dyshomeostasis

Type 2 Diabetes
NAFLD

Zn levels are associated with acute and chronic liver disease

Zn protects against diabetes-induced damage
- ↑ sensitivity to insulin
- ↓ inflammation and oxidative stress

(Zhang et al. 2012)

Cardiac hypertrophy associated with HFD-induced obesity is exacerbated by Zn deficiency and rescued by Zn supplementation

(Wang et al. 2016)

Zn supplementation can counteract Cd-induced inflammation and cell-cycle dysregulation

Can Zinc Rescue HFD-Induced, Cadmium-exacerbated NAFLD?

**Exposure Paradigm**

- **F₀** 14 weeks Breeding Groups
- **F₁** Second Litter Offspring

**Control**
- ND
- HFD

**5 ppm Cadmium**
- ND
- HFD

**Exposure Details**

- Conception
- Weaning (3 wks old)
- Birth
- 24 weeks of Diet
- 0.5 and 5 ppm Cd (drinking water)
- Diet (ND or HFD): Aim 2 (ZN, ZS (+))
- IPGTT, DS (26 wks old)
- Sacrifice (27 wks old)

30 or 90 mg zinc/4057 kcal representing normal and supplemented zinc diet, respectively
Zinc Supplementation Reduced Body Weight Gain and Percent Fat in HFD-Fed, Cadmium Exposed Mice
Zinc Supplementation Attenuates Cadmium-Exacerbated, HFD-Induced Liver Injury
Zinc Supplementation Recues Cadmium-exacerbated, HFD-induced NAFLD

Hematoxylin & Eosin (20X)

Normal Diet

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Zinc Supplementation Recues Cadmium-exacerbated, HFD-induced NAFLD

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Normal Zinc

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Steatosis Score

@ @

Control 5 ppm Cd Control 5 ppm Cd
Zinc Supplementation Recues Cadmium-exacerbated, HFD-induced NAFLD

Oil Red O (20X)

Normal Diet

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High Fat Diet

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Zinc Supplementation Rescues Cadmium-exacerbated, HFD-induced NAFLD

Oil Red O (20X)
Zinc Supplementation Increase Metallothionein in HFD-fed Mice

Metallothionein (MT)

Provide protection against metal toxicity

- Two separate globular domains
- 61–68 amino acids
- Up to 20 cysteine residues

Protein

MT -1 mRNA (Fold of Control)

- Control
- 5 ppm Cd

High Fat Diet

- Zinc Normal
- Zinc Supplement (+)

* p < 0.05 compared to group control; # p < 0.05 compared to corresponding normal zinc diet
Zinc Supplementation Inhibits Diet-Induced NAFLD

In a clinical setting, a NAFLD patient would already have a fatty liver and supplementation would occur after prognosis.
Implications for Zinc Treatment in Non-Alcoholic Fatty Liver Disease

Normal Diet (10%)

High Fat Diet (60%)

12 weeks

30 mg/kg

Normal Zinc

90 mg/kg

Added Zinc

8 weeks

EchoMRI GTT
Results After 12 Weeks of Diet

**Fat (% Body Weight)**

- **ND**
- **HFD**

**Lean Tissue (% Body Weight)**

- **ND**
- **HFD**

**Blood Glucose (mg/dL)**

- **Normal Diet**
- **High Fat Diet**

**IPGTT (AUC)**

- **Normal Diet**
- **High Fat Diet**

Data are represented as the mean ± SD, (****) p < 0.0001
Why Should You Eat (More) Dark Chocolate and Oysters?

Injured Liver → Happy Liver

Chocolate and Oysters as sources of zinc.
Thank you!

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John Wise Sr.

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