“Resolving Inflammation: Novel Control Pathways for Chronic Health Conditions”

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• Unresolved inflammation in chronic health conditions (i.e. obese subjects and associated disorders)

• Specialized pro-resolving lipid mediators (SPM).

• Deficit of SPM in chronic metabolic diseases.

• Biological properties of SPM on insulin sensitive tissues.

• In vitro actions of SPM in adipocytes, macrophages and liver cells.

• Translational studies in patients with chronic metabolic disorders.
Prevalent obesity comorbidities

**INCREASED RISK:**

- Type 2 diabetes (60-90%)
- Dyslipidemia (High LDL, TAG: 25-40%)
- Hypertension (>140/90: 70-80%)
- Vascular disease (CHD, stroke: 50-75%)
- Liver disease (20-40%)

Non-Alcoholic Fatty Liver Disease (NAFLD)

Association Between Visceral Fat and Insulin Resistance

Adapted from Carey et al. Diabetes 1996

"A Family", 1989 by Fernando Botero
Non-Alcoholic Fatty Liver Disease (NAFLD)

Prevalence in Obese Individuals:

- >40%
- >20%
- 5-10%

Steatosis
Fat accumulates in the liver

Steatohepatitis
Fat plus inflammation

Fibrosis/Cirrhosis
Scar tissue replaces liver cells

Non-Alcoholic (NAFLD)

Alcoholic (ALD)
Up-regulation of genes involved in the desaturation of fatty acids in patients with NASH

Biosynthesis of unsaturated fatty acids

FADS1 (D5 desaturase)
SCD-1 (D9 desaturase)
FADS2 (D6 desaturase)
ELOVL6
ELOVL5
SC5DL

López-Vicario and Clària. Gut 2014
Table. Hepatic fatty acid composition in Control and NAFLD patients

<table>
<thead>
<tr>
<th>PUFA</th>
<th>Control</th>
<th>NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0, Palmitic acid</td>
<td>22.38 ± 0.12</td>
<td>29.77 ± 0.63***</td>
</tr>
<tr>
<td>C16:1, Palmitoleic acid</td>
<td>0.57 ± 0.02</td>
<td>0.67 ± 0.01*</td>
</tr>
<tr>
<td>C18:0, Stearic acid</td>
<td>12.18 ± 0.39</td>
<td>6.44 ± 0.17***</td>
</tr>
<tr>
<td>C18:1, Oleic acid</td>
<td>22.99 ± 1.25</td>
<td>41.15 ± 0.80***</td>
</tr>
<tr>
<td>C18:2n-6, Linoleic acid</td>
<td>17.24 ± 0.48</td>
<td>12.69 ± 0.18**</td>
</tr>
<tr>
<td>C18:3n-6, γ-linolenic acid</td>
<td>2.64 ± 0.14</td>
<td>0.66 ± 0.02***</td>
</tr>
<tr>
<td>C20:3n-6, DGLA</td>
<td>1.25 ± 0.05</td>
<td>0.44 ± 0.01***</td>
</tr>
<tr>
<td>C20:4n-6, AA</td>
<td>7.31 ± 0.33</td>
<td>1.33 ± 0.03***</td>
</tr>
<tr>
<td>C22:4n-6, Adrenic acid</td>
<td>0.45 ± 0.13</td>
<td>0.07 ± 0.002</td>
</tr>
<tr>
<td>C18:3n-3, Linoleic acid</td>
<td>0.34 ± 0.01</td>
<td>0.29 ± 0.01*</td>
</tr>
<tr>
<td>C20:5n-3, EPA</td>
<td>1.92 ± 0.31</td>
<td>0.11 ± 0.004*</td>
</tr>
<tr>
<td>C22:5n-3, DPA</td>
<td>0.38 ± 0.03</td>
<td>0.14 ± 0.003**</td>
</tr>
<tr>
<td>C22:6n-3, DHA</td>
<td>3.69 ± 0.24</td>
<td>0.50 ± 0.03***</td>
</tr>
</tbody>
</table>

All values are expressed as % area/gram liver tissue.  
*P<0.05, ** P<0.01, *** P<0.001 vs. Control.
Adipokines in Obesity

**Complement Factors:**
Adipsin, complement factor B

**Cytokines:**
Leptin, adiponectin, resistin

**Hormones:**
TNFα, IL-6, IL-8, IL-10, MCP-1

**Enzymes:**
PAI-1, ACE, LPL

**Growth Factors:**
VEGF, HGF

**Insulin Resistance**
NAFLD

**Obese**

- **Anti-inflammatory Insulin Sensitizing**
  - Adiponectin

- **Pro-inflammatory Insulin Resistant**
  - Leptin, TNFα, IL-6, MCP-1

**Unbalanced Adipokine Release**
Obesity Triggers Adipose Tissue Inflammation

Adapted from Wellen and Hotamisligil. J Clin Invest. 2003
Unresolved inflamed Adipose Tissue = Target

NAFLD
Resolution is Part of the Inflammatory Response

Modified from Meneghin and Hogaboam, J. Clin. Invest. 2007
Lipidomic Analysis of Self-Resolving Exudates

Adapted from Bannenberg and Serhan, Biochim. Biophys. Acta 2010

Zymosan A (1 mg intraperitoneal)

Collect peritoneal exudates

LC-MS/MS analysis

Various timepoints

(time) 0

PMN number

Exudate level

ResOLUTION INTERVAL

Resolution interval

Exudate level

Fatty acids (FA)

FA oxygenated products

Adapted from Bannenberg and Serhan, Biochim. Biophys. Acta 2010
Fatty Acids

- Saturated (No bond)
- Monounsaturated (1 bond)
- Polyunsaturated (>1 bond)

Examples:
- Palmitic acid
- Oleic acid
- Linoleic acid
Bioactive Lipid Mediators

Lipid Mediators Derived from Polyunsaturated Fatty Acids

**Omega-6**
- Arachidonic Acid 20:4n-6
- First double bond at 6 carbons from methyl end

**Omega-3**
- DHA 22:6n-3
- EPA 20:5n-3
- First double bond at 3 carbons from methyl end

**Inflammatory Mediators**
- Prostaglandins and Leukotrienes

**Anti-inflammatory Mediators**
- Resolvins, Protectins and Maresins

**SPM**
- Derived from EPA and DHA through 5-lipoxygenase and 15-lipoxygenase pathways

Flowchart showing the conversion of arachidonic acid, EPA, and DHA into various lipids and metabolites.
Bioactive DHA Metabolome

17S-HDHA

17S-HpDHA

[N]PD1

17S-HpDHA

17R-HpDHA

AT-Resolvins

5-LOX

15-LOX

DHA

12-LOX

Epoxidation Hydrolysis

Peroxidase

Hydrolase

14S-HpDHA

MaR1

MaR2

17S-HDHA

7-hydroperoxy-17S-HDHA

5-LOX

7(8)-epoxy-17S-HDHA

5-LOX

4-hydroperoxy-17S-HDHA

Peroxidase

4(5)-epoxy-17S-HDHA

Peroxidase

RvD5

RvD1

RvD2

RvD3

RvD4

RvD6

Serhan CN. Nature 2014
Bioactive Lipid Mediators in Adipose Tissue

**Pro-inflammatory Lipid Mediators**
- Prostaglandins
- Leukotrienes

**Specialized Pro-resolving Mediators (SPM)**
- Protectins, Resolvin,
- Maresins and Lipoxins

White Adipose Tissue

Adipocytes

Stromal Vascular Cells
(pre-adipocytes, macrophages)

Adipose Tissue Inflammation and
Endocrine Dysfunction in Obesity
Increased Omega-6-derived Eicosanoids in Obesity

Deficit of Pro-resolving Mediators in Obese Adipose Tissue

Impaired Formation of Pro-resolving Mediators in Patients with Peripheral Vascular Disease

**Figure:**

- **Left:** Graph showing relative intensity over time (0-16 minutes) for various metabolites, including 14-H(p)DHA, 17-H(p)DHA, 14-HDHA, 17-HDHA, and PD1.
- **Middle:** Graphs showing relative intensity over m/z (120-360 Da) for 17-HDHA, PD1, and 14-HDHA.
- **Right:** Graphs showing relative intensity over pM for MCP-1, Resistin, PAI, and IL-8 in control and PVD conditions.

**Table:**

<table>
<thead>
<tr>
<th>Source</th>
<th>Spearman’s correlation coeff.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous</td>
<td>18-HEPE (n=11), pg/g</td>
<td>0.76</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>14-HDHA (n=11), pg/g</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Clària and Serhan. Am. J. Physiol. (Cell. Physiol.) 2013*
Anatomical heterogeneity in human fat depots

Increased levels in self-resolving peri-wound human fat

Monohydroxy Biomarkers from DHA

Resolvins and Protectins from DHA

Clària and Serhan. Am. J. Physiol. (Cell. Physiol.) 2013
Pro-resolving Mediators Reduce Adipose Tissue Inflammation

**Adiponectin**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Leptin</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Lean</td>
<td>0.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Obese</td>
<td>0.5</td>
<td>&lt;0.001</td>
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</table>

**Leptin**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Leptin</th>
<th>P-value</th>
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<tbody>
<tr>
<td>V</td>
<td>0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LXA4</td>
<td>0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RvD1</td>
<td>0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RvD2</td>
<td>0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SPM</td>
<td>0.3</td>
<td>&lt;0.05</td>
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**IL-6**

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-6</th>
<th>P-value</th>
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<tbody>
<tr>
<td>V</td>
<td>8x10^6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RvD1</td>
<td>4x10^6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RvD2</td>
<td>3x10^6</td>
<td>&lt;0.05</td>
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**Transadipose Migration**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Transadipose Migration</th>
<th>P-value</th>
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<tbody>
<tr>
<td>V</td>
<td>140%</td>
<td>&lt;0.05</td>
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<tr>
<td>RvD1</td>
<td>100%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RvD2</td>
<td>80%</td>
<td>&lt;0.05</td>
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**LTB4**

<table>
<thead>
<tr>
<th>Condition</th>
<th>LTB4</th>
<th>P-value</th>
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<tbody>
<tr>
<td>V</td>
<td>25%</td>
<td>&lt;0.05</td>
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<tr>
<td>RvD1</td>
<td>20%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RvD2</td>
<td>15%</td>
<td>&lt;0.05</td>
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</table>

*Clària and Serhan. J. Immunol. 2012*
RvD1 Promotes M2 Polarization of Adipose Tissue Macrophages


Non-Alcoholic Fatty Liver Disease (NAFLD)

Pharmacological treatment

Life-style changes
Calorie Restriction + Physical Exercise

Lipid accumulation

Fat accumulates in the liver

Fat plus inflammation

Scar tissue replaces liver cells

RvD1

Adapted from Adams et al. CMAJ 2005
**RvD1 Actions on Hepatic Inflammation**

### Design of the Study

- **Chow** (Chow group, n=9)
- **HFD** (CT group, n=13)
- **CR + Plb** (CR group, n=8)
- **CR + RvD1** (300 ng/mouse·day, n=7)

**Switch from HFD to chow diet; calorie restriction (CR)**

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**Hematoxylin-eosin staining**

- **Chow**
- **CT**
- **CR**
- **CR+RvD1**

**F4/80 staining**

- **Chow**
- **CT**
- **CR**
- **CR+RvD1**

**Macrophage Infiltrate**

- **% Stained area**
  - **Chow**, **CT**, **CR**, **CR+RvD1**
  - **P<0.001**
  - **P<0.05**
  - **C**

**Protein expression**

- **CCR7 (M1 marker)**
  - **CT**, **CR**, **CR+RvD1**
  - **P<0.05**
- **Arg1 (M2 marker)**
  - **Arg1**, **b-actin**
  - **P<0.05**

---

**Rius and Clària. FASEB J. 2014**
RvD1 Anti-inflammatory Actions in Precision-Cut Liver Tissue Slices

Hypoxia

** mRNA expression (arbitrary units)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>COX-2</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>CCR7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RvD1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLL+Hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P<0.001

RvD1

Hypoxia + RvD1

Clodronate Liposomes (CLL)

Rius and Clària. FASEB J. 2014
RvD1 Modulates Hepatic MicroRNA levels

Placebo Group (n=6)
RvD1 Group (n=6)

% expression vs Placebo

Rius and Clària. FASEB J. 2014
Ingenuity Pathway Analysis (IPA)

RvD1

Rius and Clària. FASEB J. 2014
Fat-1 mice: transgenic expression of an omega-3 desaturase gene

C. elegans → Omega-3 desaturase → Microinjection into fertilized egg → Fat-1 mice

ω-3 index

WT

Fat-1

ω-6/ω-3

C_{13}H_{19}O_{2}=153
C_{17}H_{23}O_{2}=207.14

Relative concentration

López-Vicario and Clària. Gut 2014

White et al. Diabetes 2010
Hudert et al. PNAS 2006
Fat-1 mice are Protected from Hepatic Steatosis and Inflammation

**Adipose tissue**

WT

Fat-1

**Liver**

WT

Fat-1

WT Fat-1

Oil Red-O

Liver

H&E

F4/80+

Serum Insulin

GMG/dL

Serum Glucose

GMG/dL

JNK1

JNK2

P-JNK1

P-JNK2

P-JNK1/JNK1

P-JNK2/JNK2

López-Vicario and Clària. Gut 2014
Increased omega-3 epoxides in Fat-1 mice


Cell membrane

AA

COOH

DHA

COOH

EPA

COOH

CYP

PLA2

5,6-EET

8,9-EET

11,12-EET

14,15-EET

19,20-EDP

17,18-EEQ

5,6-DHET

8,9-DHET

11,12-DHET

14,15-DHET

19,20-DiHDPA

17,18-DiHETE

sEH

Inhibitor

Inactive metabolites

Omega-6 epoxides

Omega-3 epoxides

5,6-DHET

8,9-DHET

11,12-DHET

14,15-DHET

19,20-DiHDPA

17,18-DiHETE
Omega-3 epoxides regulate autophagy and ER stress in hepatocytes

Summary of Metabolic Actions of Omega-3-derived Lipid Mediators

Summary

- Un-resolved inflammation is a common finding in chronic health conditions.

- Chronic metabolic diseases have a remarkable deficit in SPM.

- SPM are endogenous immunoresolvents promoting the resolution of inflammation.

- SPM promote resolution by shifting macrophages toward a M2 phenotype and deactivating the inflammasome.

- SPM exert protective actions by blocking ER stress and improving survival in parenchymal cells.

- SPM are a promising discovery for chronic health conditions.
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