Workshop on Biomarkers of Inflammation  
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Nutrition & Inflammation

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I will not talk about **Anti-inflammatory foods**
The two hits:
Non-resolving metabolic & pro-inflammatory stress

The liver & the two hits

Obesity & NASH - Interaction of the white adipose tissue & the liver

Too much saturated fat & macrophages

Human Nutrigenomics examples

Conclusions
The two hits
Non-resolving metabolic & proinflammatory stress

a

Nutrients (dietary signals)

Signalling through sensor mechanisms

Genes (normal genotype)

Normal phenotype

Homeostasis

b

Nutrients (dietary signals)

‘Hit 1’
Metabolic stress

‘Hit 2’
Proinflammatory stress

Signalling through sensor mechanisms

Genes (sensitive genotype)

Sensitive phenotype

Onset of disease

‘Healthy’ signatures

‘Stress’ signatures

2003
“2 hits” in Metabolic Syndrome
Too much metabolic & inflammatory stress

Definition (DPS):
- Central obesity (usually BMI > 30 kg/m²)
- plus 2 of the following:
  - TGs > 150 mg/dL
  - HDL < 40-50 mg/dL
  - Blood pressure
  - Hyperglycemia

Complications of Chronic Diabetes:
- chronic kidney disease
- cardiovascular disease
- peripheral nerve damage
- eye disease & blindness
- non-healing skin ulcers, usually leading to amputations
- non-alcoholic fatty liver disease, which can lead to cirrhosis

2012
THE LIVER & THE 2 HITS

First hits
- ↑ LCFA delivery to the liver
  - High fat diet
  - High carbohydrate diet
  - CD36−/− mouse
- ↑ de novo LCFA and TG synthesis
  - Ob/Ob mouse
  - Db/Db mouse
  - Yellow-obese agouti mouse
  - PEPCK-NSREBP-1a mouse
  - aP2-NSREBP-1c mouse
- ↓ VLDL synthesis and export
  - MTT−/− mouse
- ↓ LCFA oxidation/metabolism
  - PPARα−/− mouse
  - MTP−/− mouse
  - JVS mouse
  - AOX−/− mouse
  - MCD Diet
  - Drugs (Oestrogen, etc)

Second hits
- ↑ Oxidative stress & lipid peroxidation
  - MCD Diet
  - MTP−/− mouse
- Pro-inflammatory cytokine release
  - TNFα
  - TNFβ
- Lipopolysaccharide
- Ischaemia-reperfusion injury

Obesity and insulin resistance

Inflammation

Steatosis

1st Hit

2nd Hit

Steatohepatitis

PPARα

Normal
More steatosis in mouse livers from PPARα -/- mice on a high fat diet
PPARα controls acute phase response induced by HF diets

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### Graphs

**Lipocalin 2**

- **Wildtype**
  - LFD: Empty bar
  - HFD: Empty bar

- **PPARα-/-**
  - LFD: Top bar with a significant increase
  - HFD: Top bar with a significant increase

**SAA**

- **Wildtype**
  - LFD: Empty bar
  - HFD: Empty bar

- **PPARα-/-**
  - LFD: Top bar with a significant increase
  - HFD: Top bar with a significant increase

**CXCL10/IP-10**

- **Wildtype**
  - LFD: Empty bar
  - HFD: Empty bar

- **PPARα-/-**
  - LFD: Top bar with a significant increase
  - HFD: Top bar with a significant increase
Increased plasma levels of chemokines in HF-diet fed PPARα-/- mice

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Nutrition and NAFLD

FA oxidation ↔ De novo lipogenesis
via: - PPAR activation
via: - gene expression regulation (SREBP-1, FXR, AMPK, PGC-1α)
- bypassing PFK (provides lipid precursor carbon)

Bile acid metabolism
via: - FXR activation
- taurine

Lipoprotein secretion

LIVER

?→ NAFLD progression

NAFLD progression → LIVER

NAFLD progression

Glutathione

Oxidative stress/damage

ER stress

Higher endotoxin levels

GIP response

Bacterial overgrowth

INTESTINE

Adipogenesis

FA oxidation

MUFA/PUFA

Protein

Isoflavones

SFA

Fructose

Choline

Antioxidants

Higher endotoxin levels

NAFLD progression

Steatosis

NASH

Lipoprotein secretion

Steatosis

NASH

FA oxidation

De novo lipogenesis

Bile acid metabolism

Lipoprotein secretion

ER stress

Higher endotoxin levels

FA oxidation

Adipogenesis
OBESITY & NASH
INTERACTION OF WAT & LIVER
Interaction between WAT and liver tissue essential for NASH/NAFLD in C57Bl/6 mice

• stratification on body weight

run-in diet

20 weeks diet intervention

• plasma collection

• multiple protein assays

• liver

• frozen sections: histological feat.

• lipid content

• RNA extraction: Affx microarrays

Mouse genome 430 2.0

quality control & data analysis pipeline

• ep. white adipose tissue

• paraffin sections: histological feat.

• RNA extraction: real-time PCR
High fat diet-induced obesity

**Liver TG content**

- LFL: 40 mg TG/g liver
- LFH: 80 mg TG/g liver
- HFL: 120 mg TG/g liver
- HFH: 160 mg TG/g liver

**Hepatomegaly**

- Ratio LW/BW (%)
  - LFL: 4%
  - LFH: 6%
  - HFL: 8%
  - HFH: 10%

**ALT plasma activity**

- LFL: 20 UI
- LFH: 40 UI
- HFL: 60 UI
- HFH: 80 UI

Graphs showing trends over weeks under diet intervention with significance indicated by stars: *

**Graphs**

- BW gain (g) vs. weeks under diet intervention
- ALT activity (UI)
- Ratio LW/BW (%)

Table:

- Liver TG content (mg TG/g liver)
  - LFL
  - LFH
  - HFL
  - HFH

Significance markers: * (p < 0.05), ** (p < 0.01), *** (p < 0.001)
A subpopulation of mice fed HFD develops NASH
Immunohistochemical staining confirms enhanced liver inflammation and early fibrosis in HFH mice

HFL

HFH

Macrophage CD68

Collagen

Stellate cell GFAP
Upregulation of inflammatory and fibrotic gene expression in HFH responder mice
Adipose dysfunction in HFH mice
Change in adipose gene expression indicate adipose tissue dysfunction
Plasma proteins as early predictive biomarker for NASH in C57Bl/6 mice
Plasma proteins as early predictive biomarker for NASH in C57Bl/6 mice

Multivariate analysis of association of protein plasma concentrations with final liver triglyceride content
Conclusions

• The data support the existence of a tight relationship between adipose tissue dysfunction and NASH pathogenesis.

Duval et al. Diabetes 2010
How inflammation is initiated and developed in obesity

TOO MUCH SATURATED FAT & MACROPHAGES
Examination of the role of Angptl4 under conditions of lipid overload

Angptl4 +/-

Angptl4 +/-

Low fat

Low fat

High fat

High fat
Angptl4-/- mice on HFD become very ill
Systemic inflammation in Angptl4-/- mice fed HFD

[Bar chart showing fold change for various markers in Angptl4+/+ and Angptl4-/- mice fed different diets.]

- CD40 Ligand
- MCP-1
- MCP-3
- MCP-5
- MDC
- IL-5
- MIP-1gamma
- MIP-3beta
- MMP-9
- MPO
- TIMP-1
- vWF
- Factor VII
- CRP
- Haptoglobin
- IgA
- Fibrinogen

- SAA2
- Haptoglobin
- Lipocalin 2

Mice fed a high-fat diet (HFD) show increased expression of these markers compared to a low-fat diet (LFD).
Inflammatory response independent of microbiota
No effect of medium chain or PUFA TGs
Massive enlargement of mesenteric lymph nodes in Angptl4-/- mice fed HFD
Angptl4 protects against lipolysis and subsequent foam cell formation
Angptl4 protects against lipolysis and subsequent foam cell formation
Conclusion

- A high saturated fat diet causes massive inflammation in Angptl4-/- mice originating in mesenteric lymph nodes.
- In the absence of this protective mechanism, feeding a diet rich in saturated fat rapidly leads to enhanced lipid uptake into MLN-resident macrophages, triggering foam cell formation and a massive inflammatory response.

Lichtenstein et al. Cell Metab. 2010
HUMAN NUTRITION
Fish-oil supplementation induces anti-inflammatory gene expression profiles in human blood mononuclear cells

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Less inflammation & decreased pro-arteriosclerosis markers = Anti-immuno-senescence

“Obese-linked” pro-inflammatory gene expression profile by saturated fat

- The SFA-rich diet:
  - Induces a pro-inflammatory obese-linked gene expression profile
  - Decreases expression and plasma level of the anti-inflammatory cytokine adiponectin
- “Personal Transcriptomes”

Van Dijk et al. AJCN 2009
General conclusions

- (Over)nutrition and inflammation are intimately linked => non-resolving metabolic and pro-inflammatory stress (the two hits).
- It will be essential to get a better understanding of the very early events that lead to non-resolving organ inflammation and the precise role of nutrition (causal or preventive) in this pathophysiological development.
- We need biomarkers for organ function (“2 hit state”) to be able to specifically target and modulate.
- The challenge will be the translation of the findings from mice studies to the human situation (“individual” health).
Sander Kersten  
Rinke Stienstra  
Lydia Afman  
Guido Hooiveld  
Mark Boekschot  
Laetitia Lichtenstein  
Caroline Duval  
& NMG group

Christian Trautwein  
Folkert Kuipers  
Ben van Ommen  
& many more
Visceral adipose tissue of mildly obese individuals is characterized by enhanced caspase-1 activity levels and higher production of IL-1β as compared to subcutaneous adipose tissue.