Renal dysfunction and metabolic syndrome: the chicken or the egg?

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Metabolic syndrome

- Hypertension
- Visceral Obesity
- Insulin Resistance
- High Triglycerides
- Low HDL-Cholesterol

Type 2 Diabetes
Hearth disease
Stroke
Fatty Liver Disease
Renal dysfunction
- Tubular atrophy
- Interstitial fibrosis
- Glomerulosclerosis
Metabolic syndrome & Renal dysfunction

- In 1974, first description of an association between Metabolic Syndrome & Nephrotic proteinuria.

- Serum lipid abnormalities (high TG, low HDL) are associated with significantly increased risk of CKD.

- Predict the development of renal dysfunction.

- Increasing BMI associates with increased risk of developing ESRD.

- Renal dysfunction appears long before hypertension/diabetes in Metabolic syndrome.

- Metabolic syndrome patients have 2.5-fold higher risk of developing CKD and 2-fold higher risk for microalbuminuria.

Prevalence of CKD

N° Metabolic Syndrome components

**Nlrp3 is a key modulator of diet-induced nephropathy and renal cholesterol accumulation**

*Pieter J. Bakker^1, Loes M. Butter^1, Lotte Kors^1, Gwendoline J.D. Teske^1, Jan Aten^1, Fayyaz S. Sutterwala^2, Sandrine Florquin^1,3 and Jaklien C. Leemans^1*

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**Western-diet**

- Weight gain
- Insulin resistance
- Dyslipidemia

**Renal pathology**

- Renal steatosis
- Cholesterol & phospholipid accumulation
- Renal inflammation & fibrosis
- Microalbuminuria
Kidneys in Murine model of Metabolic syndrome

Nile Red positive vacuoles
Phospholipid accumulation
Causes of renal dysfunction in Metabolic syndrome??

- Glomerular hyperfiltration
- Excess reabsorption
- RAAS activation
- Glomerular and tubulointerstitial remodeling/injury
- Physical compression by adipose tissue
  - Hyperlipidemia
  - Renal lipotoxicity

Effects of LDL on tubular epithelial cells?
• Lipoproteins from plasma of human healthy donors.

Experimental Approach
Harmful effects of LDL on TECs

Lactate Dehydrogenase (LDH) Cytotoxicity Assay

Apoptosis AnnexinV staining

HK2 cells
LDL-induced phospholipidosis formation in TECs

**HK2**

**ImmortoTEC**

**MDCK**

**Phospholipidosis:** lysosomal accumulation of phospholipids.

LipidTOX Phospholipidosis Red Detection Kit (Life Technologies).

**HK2** 3 days oxLDL

ctr nLDL

**MDCK**

**LDL**

100x
Enlargement of the lysosomal compartment upon LDL

HK2 cells – day 3

LysoTracker Red
labeling and tracking acidic organelles in live cells

3 days

LysoTracker Red  MFI

- Ctr LPDS
- nLDL
- oxLDL

* *
Enlargement of the lysosomal compartment upon LDL

HK2 cells – day 3/5

LysoTracker Red
labeling and tracking acidic organelles in live cells

5 days

LysoTracker Red  MFI

 ctr  nLDL  oxLDL

Ctr LPDS  nLDL  oxLDL

3 days  5 days

LAMP-2

β-actin
Impaired lysosomal acidification upon LDL

HK2 cells

LysoSensor Green: Low pH-dependent fluorescent dye
LysoTracker Red: Labeling acidic organelles

FC
Lysosensor MFI / Lysotracker MFI

![Graph showing the ratio of MFI for different treatments over 3 days. Ctr LPDS, nLDL, and oxLDL conditions are compared.](image-url)
Impaired lysosomal acidification upon LDL

**HK2 cells**

**LysoSensor Green**: Low pH-dependent fluorescent dye

**LysoTracker Red**: Labeling acidic organelles

**FC**

Lysosensor MFI / Lysotracker MFI

- Ctr LPDS
- nLDL
- oxLDL
LDL-loading induces autophagy

LDL - loading induces autophagy.

WB

Ctr   nLDL   oxLDL

LC3B  I
LC3B  II
β-actin

ImmTEC

n/oxLDL

increase in LC3-II
decrease in LC3-I expression

HK2

LC3B  I
LC3B  II
β-actin
Autophagy as a protective mechanism

Without autophagy ⟷ More Apoptosis

ATG5 knockdown (Short hairpin RNA)
Impaired function after n/oxLDL treatment: Less absorption, ATP, integral mitochondria

**Glucose uptake 2-NBDG**

<table>
<thead>
<tr>
<th></th>
<th>MDCK</th>
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</thead>
<tbody>
<tr>
<td>Ctr LPDS</td>
<td>60 MFI FITC</td>
</tr>
<tr>
<td>nLDL</td>
<td>40 MFI FITC</td>
</tr>
<tr>
<td>oxLDL</td>
<td>50 MFI FITC</td>
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**Intracellular ATP**

<table>
<thead>
<tr>
<th></th>
<th>MDCK</th>
<th>HK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr LPDS</td>
<td>1.0 ATP/µg protein</td>
<td></td>
</tr>
<tr>
<td>nLDL</td>
<td>0.7 ATP/µg protein</td>
<td></td>
</tr>
<tr>
<td>oxLDL</td>
<td>0.5 ATP/µg protein</td>
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**Mitochondrial damage**

<table>
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<th>HK2</th>
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<tbody>
<tr>
<td>Ctr LPDS</td>
<td>1.5 % Q3/ %Q2</td>
</tr>
<tr>
<td>nLDL</td>
<td>2.0 % Q3/ %Q2</td>
</tr>
<tr>
<td>oxLDL</td>
<td>3.0 % Q3/ %Q2</td>
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**Oxidative stress - ROS**

<table>
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<tr>
<th></th>
<th>5 days</th>
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</thead>
<tbody>
<tr>
<td>Ctr LPDS</td>
<td>3000 MFI FITC</td>
</tr>
<tr>
<td>nLDL</td>
<td>4000 MFI FITC</td>
</tr>
<tr>
<td>oxLDL</td>
<td>5000 MFI FITC</td>
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</tbody>
</table>

* indicates statistical significance.
Impaired function after n/oxLDL treatment: Improper response to EGF

**Proliferation**

- % cells in S+G2/M

**Signaling activation**

- EGFR
- PI3K

**Cholesterol-rich microdomains**

- Cholera toxin subunit B- FITC

**Cholesterol-depletion**

- Methyl-β-cyclodextrin (MβC)

**Surface EGFR**

- MFI FITC

- % cells in S+G2/M

HK2

5 days
Low-grade inflammation in Metabolic syndrome

**Nlrp3** is a key modulator of diet-induced nephropathy and renal cholesterol accumulation

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**NLRP3 deficiency protects against**
- renal steatosis
- cholesterol & phospholipid accumulation
- fibrosis
- macrophage influx
- microalbuminuria

**NLRP3 mediates high fat diet-induced metabolic renal injury**
Low-grade grade metabolic inflammation: NLRP3 inflammasome

- Activated in CKD & diabetic nephropathy (DN)
- Triggers onset of DN
- Mediates diet-induced nephropathy & renal cholesterol accumulation

Mori et al., *Nat. Med.*, 2011
Low-grade grade metabolic inflammation: NLRP3 inflammasome

NLRP3 Inflammasome

- Cytoplasmic **innate immune** multiprotein complex
  - Nod-like receptor protein 3 (NLRP3),
  - adaptor ASC,
  - pro-caspase-1

- **IL-1β** and **IL-18** maturation

**dos Santos et al., Am. Journ. Physiol., 2012**
NLRP3 activation in metabolic overloading

Silencing inflammasome components

Phospholipidosis

HK2

shRNA  ASC

3 days

Ctr  nLDL  oxLDL

MIF PE

ASC  +  -  +  -  +  -
NLRP3 activation in metabolic overloading

Without NLRP3/ASC complex  →  Less Phospholipidosis

Phospholipidosis

**shRNA NLRP3**  
**sgRNA NLRP3**

3 days

- Ctr
- nLDL
- oxLDL

MFI PE

NLRP3

+  -  +  -  +  -  +  -
Absence of NLRP3 prevent excessive intracellular lipid deposition in proximal tubules

Nile Red staining
Absence of NLRP3 attenuates Western-diet-induced lipid accumulation in kidneys

WT  NLRP3 KO

Chol  *  *
PG  *  *
LPG  *
BMP  *
PC

PI  *
SPA  *
SPH  *
LacCer  *
Plasmalogens  *

p=0.057
PROPOSED MECHANISM

Plasma membrane

LDL

Lysosome

Mitochondria

Cholesterol

EGF

GFs

Signaling

V-ATPase

LC3 II

Autophagy

Phospholipids

Cholesterol

Triacylglycerols

Apolipoprotein
PROPOSED MECHANISM

- LDL in Plasma membrane
- Cholesterol
- Lysosome
- Mitochondria
- NLRP3
- Autophagy
- LC3 II
- IL-1β
- EGF
- GFs
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