MONOAMINE OXIDASE CONTRIBUTION TO CARDIOVASCULAR DYSFUNCTION IN PATIENTS UNDERGOING SURGICAL REVASCULARIZATION

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An uninvited *eubacteria* was presumably incorporated into an *Archea-type host*

The initial antagonists began to rely on each other:

- The *eubacteria* evolved into the *mitochondria* and the responsibility for its replication was progressively shifted to the cell

- The host switched to the mitochondria the responsibility for energy production

Balaban et al, *Cell*, 2005
Mitochondrial ROS Generation

The Beauty...and the Belly of the Beast

Yet old habits are hard to break...
Mitochondrial ROS Generation

Respiratory Chain and Not Only...

Role: oxidative deamination of neurotransmitters & biogenic amines:

\[ R-\text{CH}_2-\text{NH}_2 + O_2 + H_2O \rightarrow R-\text{CHO} + \text{NH}_3 + H_2O_2 \]
## MAOs

### MAOs – 2 isoforms, MAO-A & MAO-B:

<table>
<thead>
<tr>
<th></th>
<th>MAO-A</th>
<th>MAO-B</th>
</tr>
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<tbody>
<tr>
<td><strong>Substrates</strong></td>
<td>Norepinephrine, Serotonin</td>
<td>Phenylethylamine, Benzylamine</td>
</tr>
<tr>
<td></td>
<td>Dopamine, Tyramine, Octopamine, Tryptamine</td>
<td></td>
</tr>
<tr>
<td><strong>Tissue distribution</strong></td>
<td>Heart, vessels, brain, intestine, liver, placenta, thyroid gland, skin</td>
<td>Brain, liver, kidney, platelets, heart, vessels, platelets, pancreatic islets</td>
</tr>
</tbody>
</table>

### MAO Inhibitors (MAOIs):

- **Irreversible & selective inhibitors:**
  - Clorgyline for MAO-A
  - Selegiline (Deprenyl) for MAO-B
  - Pargyline for MAO-A & B

- **Reversible & selective inhibitors:**
  - Moclobemide for MAO-A
  - Lazabemide for MAO-B
- Implicated in neurodegenerative & psychiatric disorders
- Reported to increase with ageing
- Involved as contributors in the cardiovascular system to the:
  - Myocardial injury caused by post-ischemic reperfusion (Parini’s group)
  - Maladaptative myocardial hypertrophy & heart failure (Di Lisa’s group)

Kaludercic et al, JMCC, 2014
MAOs Contributes to *In Vivo* Experimental Endothelial Dysfunction

- **Acute** endothelial dysfunction:  
  *LPS treatment*, 8 mg/kg, single injection, assessment after 12 h

- **Chronic** endothelial dysfunction:  
  *ANG II treatment*, 1.4 mg/kg/day via osmotic minipumps for 14 days

Sturza et al, *Hypertension*, 2013
In Vivo Exposure to Ang IIs / LPS Increased Endogenous MAO Expression in Murine Aortic Rings

**MAO A**

- CTL
- ANG II

**MAO B**

- CTL
- ANG II

**MAO A**

- CTL
- LPS

**MAO B**

- CTL
- LPS

mRNA Expression of Vascular MAOs (qPCR)

Sturza et al, Hypertension, 2013
Endogenous MAO Impaired Vascular Relaxation in the Ang II Model

The Effect Was Reversed by MAO Inhibition

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Aortic Rings
Endogenous MAO Impaired Relaxation in the LPS Model
The Effect Was Reversed by MAO Inhibition

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Aortic Rings

Sturza et al, Hypertension, 2013
MAO-A Inhibition Reduces H$_2$O$_2$ Production in Murine Aortas Harvested From *Ang II* and *LPS*-Treated Animals

Assessment of H$_2$O$_2$ Production in Diseased Vessels in the Presence vs. Absence of MAO Inhibitors (FOX Assay)

Which Is The Mechanism Underlying MAO Induction By LPS/ANG2?

- In vitro incubation (12 h) of aortic segments with Ang II / LPS ± pharmacological inhibitors

- mRNA isolation -> cDNA synthesis
  -> qPCR for MAO A & B

Sturza A, et al., Hypertension, 2013
Pharmacological inhibitors

- Irbesartan (100 µmol/L, AT1 receptor antagonist)
- Wortmannin (20 nM, PI3-kinase inhibitor)
- NωNitro-L-arginine-methylester (L-NAME 100 µmol/L, NOS inhibitor)
- AG490 (10 µmol/L, Janus kinase 2 inhibitor)
- Pyrolidin dithiocarbamate (PDTC, 50 µmol/L, NFκB inhibitor)
- RO-31-8220 (1 µmol/L - PKC inhibitor)
- U0126 (10 µmol/L, ERK1/2 kinase inhibitor)
- SB203580 (10 µmol/L, p38 MAPK inhibitor)

Pharmacological inhibitors$^2$

- **Apocynin** (10,100 µmol/L, Nox inhibitor)
- **Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)** 10 µmol/L, respiratory chain uncoupler
- **Dinitrophenol** (DNP, 10 µmol/L, respiratory chain uncoupler)
- **Oligomycin** (Oligo, 10 µM, complex V inhibitor)
- **Myxothiazol** (Myxo, 10 µmol/L, complex III inhibitor)

Ang 2-Induction Of Vascular MAO Is Mediated By AT-1 Receptors, PI3 Kinase And NF-Kappa B But Not By NADPH Oxidase

Sturza A, et al., Hypertension, 2013
LPS-Induction Of Vascular MAO Is Mediated By NF-Kappa B And PI3 Kinase But Again Not By NADPH Oxidase

Sturza A, et al., Hypertension, 2013
Diabetes Mellitus & Mitochondrial Dysfunction

- **Diabetes mellitus** is associated with increased prevalence of cardiovascular morbidity
- **Mitochondrial impairments** contribute substantially to the development of cardiac dysfunction in diabetes and evolution towards HF

![Diagram showing the relationship between diabetes mellitus and mitochondrial dysfunction, highlighting increased fatty acids, glucose, and acyl-CoA, leading to oxidative damage and contractile dysfunction.](image)
MAOs Impaired Vascular Relaxation In Type 2 Diabetes

The Effect Was Reversed By MAO A & B Inhibition

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Rat Aortic Rings

MAOs-Related $H_2O_2$ Production Was Increased In T2 Diabetes

The Effect Was Partially Reversed By MAO A & B Inhibition

MAOs Impaired Vascular Relaxation In T1 Diabetes

The Effect Was Reversed By MAO A & B Inhibition

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Rat Aortic Rings

MAO A & B Inhibition Reduced H$_2$O$_2$ Production In Aortic Rings From Rats With T1 Diabetes

**Graph:**

- **Y-axis:** nM H$_2$O$_2$ / mg tissue / h
- **X-axis:** CLORG SELEG
- **Legend:**
  - CTL
  - DIAB
  - *
  - #1
  - #2
  - #3

**FOX Assay - H$_2$O$_2$ production**

MURINE VASCULAR AND CARDIAC RINGS EXPRESS MORE MAO-B vs MAO-A & MAO-B ISOFORM IS UPREGULATED IN T1DM

Assessment of Vascular Expression of MAOs (qPCR, IH, confocal microscopy)

MAOs-related oxidative stress contribute to early cardiovascular dysfunction in patients with coronary heart disease (CHD) and preserved ejection fraction with/without diabetes mellitus (DM)
### MATERIAL and METHODS

Table 1: Patients’ demographic, clinical data, and preoperative medication.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>CTRL (n = 25)</th>
<th>CHD (n = 30)</th>
<th>CHD-DM (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
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<tr>
<td>Age</td>
<td>63 ± 9.5</td>
<td>63 ± 8.5</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>15/10</td>
<td>26/4</td>
<td>16/4</td>
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<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.7 ± 5.7</td>
<td>27 ± 4</td>
<td>30 ± 3.2*†</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>184.8 ± 49.1</td>
<td>153.2 ± 33*</td>
<td>148 ± 23*</td>
</tr>
<tr>
<td>FPG</td>
<td>103.3 ± 20.2</td>
<td>105 ± 16</td>
<td>192 ± 64*†</td>
</tr>
<tr>
<td>LVEF</td>
<td><strong>55.7 ± 7.8</strong></td>
<td><strong>48.5 ± 8.4</strong></td>
<td><strong>48 ± 7.7</strong>*</td>
</tr>
<tr>
<td>HT</td>
<td>15 (60)</td>
<td>30 (100)*</td>
<td>20 (100)*</td>
</tr>
<tr>
<td>AF</td>
<td>3 (12)</td>
<td>2 (6.67)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Preoperative medication</strong></td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>5 (20)</td>
<td>30 (100)</td>
<td>20 (100)</td>
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<tr>
<td>β-blockers</td>
<td>20 (80)</td>
<td>30 (100)</td>
<td>20 (100)</td>
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<tr>
<td>Anticoagulants</td>
<td>25 (100)</td>
<td>30 (100)</td>
<td>20 (100)</td>
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<tr>
<td>Statins</td>
<td>18 (72)</td>
<td>30 (100)</td>
<td>20 (100)</td>
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<tr>
<td>Nitrates</td>
<td>8 (32)</td>
<td>21 (70)</td>
<td>15 (75)</td>
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<tr>
<td>Calcium channel blockers</td>
<td>5 (20)</td>
<td>8 (26.67)</td>
<td>7 (35)</td>
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<tr>
<td>ACE inhibitors</td>
<td>0</td>
<td>30 (100)</td>
<td>20 (100)</td>
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<td>Diuretics</td>
<td>25 (100)</td>
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<td>20 (100)</td>
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<tr>
<td>Insulin</td>
<td>0</td>
<td>0</td>
<td>5 (25)</td>
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<tr>
<td>Oral antidiabetics</td>
<td>0</td>
<td>0</td>
<td>20 (75)</td>
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<tr>
<td>Antibiotherapy</td>
<td>25 (100)</td>
<td>30 (100)</td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

MATERIAL and METHODS

Material: right atrial appendages

Methods:

- **Mitochondrial respiration:** *high-resolution respirometry*
  → permeabilized atrial fibers

- **MAO gene expression:** *qRT-PCR*

- **MAO protein expression:** *immunohistochemistry assay*
  → incubation with anti-MAO A and B antibodies

- **Oxidative stress evaluation - H₂O₂ release measurements:**
  - *confocal microscopy:* 2',7'-dichlorofluorescein diacetate (DCF) probe
  - *spectrofotometry:* Ferrous iron xylenol orange OXidation (FOX) assay

The Substrate-Uncoupler-Inhibitor Titration (SUIT) Protocol:

- **GMSt2 / (Rot)SSt2**: Glutamate + Malate / Succinate (+ rotenone) - *state 2*
- **DP**: ADP - OXPHOS capacity
- **AtrSt4**: atracyloside - *state 4*
- **FCCPE**: FCCP titrations - ETS capacity (uncoupled respiration)

Principles of Respirometry = OXPHOS
Complex I-Supported Respiration Is Depressed in CHD Patients With and Without Diabetes
Complex II-Supported Respiration Is Depressed Only in CHD Patients With Diabetes

- **Complex II-Supported Respiration**
  - **O2 flux per Volume (pmol.s⁻¹.ml⁻¹)**
    - **CTRL**
    - **CHD**
    - **CHD-DM**
    - **STATE 2**
    - **STATE 4**
    - **OXPHOS**
    - **ETS**

- Depression observed in **CHD-DM** patients compared to **CTRL** and **CHD**

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**Figure Legend**

- **State 2** and **State 4** reflect different metabolic states.
- Significance (**p < 0.01**) indicated where applicable.

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**Data Insights**

- **OXPHOS** and **ETS** pathways are primarily affected in **CHD-DM** patients.
- Depression is most pronounced in **State 2**.
MAO mRNA Expression Was Comparable Among The Groups And MAO-B Was the Predominant Isoform

A-C. RT-PCR (mRNA expression: $2^{\Delta Ct}$) for MAO A and MAO B relative to the housekeeping gene EEF2α in atrial samples from CTRL group (A), CHD group (B), CHD-DM (C), n=10, *p < 0.05

D. RT-PCR (fold increase) for MAO A and MAO B relative to the housekeeping gene EEF2α in atrial samples (n=10/group).

Duicu et al, Oxidative Med Cell Longevity, 2016
MAO Protein Expression Was Comparable Among the Groups

MAO protein expression in human atrial samples. Immunohistochemistry for MAO A and MAO B in atrial samples (A) and the corresponding results expressed as a mean score of intensity (B) (n=10/group).

Duicu et al, *Oxidative Med, 2016*
H$_2$O$_2$ Generation Was Not Increased In Diabetic vs. Non-Diabetic Patients

Atrial H$_2$O$_2$ emission detected with DCF fluorescence (n=10/group)

H$_2$O$_2$ Emission Was Not Increased In Diabetic vs. Non-Diabetic Patients

Atrial H$_2$O$_2$ emission detected with FOX assay (n=5/group)

Duicu et al, Oxidative Med Cell Longevity, 2016
Ex Vivo Inhibition of MAOs Attenuated Atrial H$_2$O$_2$ Formation

Atrial H$_2$O$_2$ emission detected with FOX assay.

CONCLUSIONS

1. A substrate-independent impairment of respiratory function occurs in elderly patients with CHD and preserved EF in the presence of diabetes.

2. MAOs, in particular the MAO-B isoform, contribute to oxidative stress in elderly patients with CHD and preserved EF, regardless the presence of diabetes.
Cardiovascular pharmacology

Exposure of cardiomyocytes to angiotensin II induces over-activation of monoamine oxidase type A: Implications in heart failure

Maria Elena Manni, Marina Zazzeri, Claudia Musilli, Elisabetta Bigagli, Maura Lodovici, Laura Raimondi*

Department of Pharmacology, University of Florence, Italy
Working Hypothesis

MAO-derived ROS contribute to endothelial dysfunction in CHD patients with and without diabetes
MAO-A And MAO-B Expression In Human Mammary Arteries From CHD Patients **WITHOUT Diabetes**

Immune-histology for MAO-A (A) and MAO-B (B):
(a&d) entire arterial wall
(b&e) endothelial layer and muscular media
(c&f) adventitia with fibroblasts, nerves and vasa-vasorum.

*EC= endothelial cell, FB= fibroblast, IEL= internal elastic lamina*

Lighezan et al, *CJPP, 2016*
MAO-A And MAO-B Expression In Human Mammary Arteries From CHD Patients WITH Diabetes

Immune-histology for MAO-A (A) and MAO-B (B):
(a&d) entire arterial wall (b&e) endothelial layer and muscular media
(c&f) adventitia with fibroblasts, nerves and vasa-vasorum.
EC= endothelial cell, FB= fibroblast, IEL= internal elastic lamina
Ex Vivo MAO Inhibition Reverses Endothelial Dysfunction In Mammary Arteries From Coronary Diabetic & Non-Diabetic Patients

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Mammary Artery Rings

Lighezan et al, CJPP, 2016
The Presence Of An Intact Endothelium Is Crucial For The Vascular Effects Of MAOI

Endothelium-Dependent Relaxation in Response to Acetylcholine in Isolated Phenylephrine-Preconstricted Mammary Artery Rings

Lighezan et al, CJPP, 2016
Conclusions

1. MAO-related oxidative stress is a novel contributor to the endothelial dysfunction in CHD patients.

2. *Ex vivo* inhibition of MAOs restores the impaired endothelial function in human mammary arteries harvested from CHD patients with and without diabetes.
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We shall not cease from exploration.
The end of our exploring will be to arrive where we started from and know the place for the first time.

T.S. Eliot
MAO Limited Endothelial cGMP Accumulation in HUVEC

Sturza et al, *Hypertension*, 2013
Working Model For MAO-induced Endothelial Dysfunction

LPS & AngII

via NFkB, PI3-kinase

MAO Gene & Protein expression

H$_2$O$_2$ production

cGMP level

Endothelial dysfunction