Old dog, new tricks – roles of H$_2$S and NAD$^+$ in angiogenesis and health

Abhirup Das
The evolution of frailty and aging

- Decreased ability to exercise
- Decreased response to exercise

Vascular dysfunction

Health and fitness

Diet, Small molecules (AMPK, resveratrol, PPARδ)

Cardiovascular Diseases
Arterial Diseases
Sarcopenia
Dementia
Osteoporosis
Calorie restriction/Exercise/Bio-Stress

- Insulin/IGF-1
- amino acids
- Glucose
- ATP
- NAD$^+$

- mTOR
- AMPK
- Sirtuins

Longevity
Methionine Restricted Diet

Perrone et al. Experimental Gerontology 2013

CGL ↑

H₂S ↑

Hine et al. Cell 2015
MR diet induces angiogenesis *in vivo*
CGL is necessary for MR mediated angiogenesis
H₂S production by CGL is necessary for angiogenesis.
Endothelial SIRT1 modulates capillary density

SIRT1 knock-out mouse (ESKO)

Quadriceps

WT        ESKO

6 mo old

SIRT1 overexpression mouse (ESTO)

Quadriceps

SIRT1$^{STOP}$        ESTO

6 mo old
Endothelial SIRT1 affects exercise capacity

SIRT1 knock-out mouse (ESKO)  SIRT1 overexpression mouse (ESTO)

WT  ESKO  SIRT1STOP  ESTO

Distance run to exhaustion (m)

6 mo old
Endothelial SIRT1 doesn’t affect muscle parameters

Skeletal muscle ➔
- Mass
- Fiber types
- Oxidative capacity ➔ No changes
SIRT1

NAD$^+$

Aging

Muscle microvasculature

Exercise capacity

NMN $\rightarrow$ NAD$^+$ $\rightarrow$ SIRT1 $\rightarrow$ ?

Gomes et al. Cell 2013
NMN increases capillary density and blood flow

Contrast ultrasound
Relative blood volume

Photoacoustic tomography

Peak enhancement
Lower limb

Peak enhancement (AU)

Soluble O₂ %

# of capillaries/
# of myofibers

<table>
<thead>
<tr>
<th>NMN</th>
<th>Vehicle</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
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<tbody>
<tr>
<td>iT1KO</td>
<td>WT</td>
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**Note:** Significance levels indicated by * and **.
NMN treatment improves exercise capacity

- Vehicle
- NMN

Low intensity test
- Distance run to exhaustion (m)

High intensity test
- Distance run to exhaustion (m)

Skeletal muscle
- Mass
- Fiber types
- Oxidative capacity

No significant changes
NMN promotes angiogenesis under ischemic conditions

Hind-limb surgery model of ischemia
Wildtype vs SIRT1-KO

Gastrocnemius muscle
20 days post-surgery

<table>
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<th>WT</th>
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<th>KO</th>
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<tr>
<td>NMN</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Peak enhancement (dB)</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
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<th>KO</th>
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<td>NMN</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td># of capillaries/# of myofibers (ischemic muscle)</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
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NMN promotes exercise induced angiogenesis

Vehicle          NMN
10 mo old

** NMN     -         -        -        +       +
Axitinib     -         -        +       -        +

Exercise training
Sed

# of capillaries/
# of myofibers

0 1 2 3 4 5
Quadriiceps
capillary density

Sedentary     Exercised

10 mo old
Spheroid Assay

**Sprouting Spheroid**

Cell spheroid  Sprouting

<table>
<thead>
<tr>
<th>NMN</th>
<th>VEGF</th>
<th>Number of sprouts per spheroids</th>
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<tr>
<td></td>
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<tr>
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<tr>
<td>+</td>
<td>+</td>
<td>20</td>
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*** *** #

VEGF  NMN

- -
- +
+ +

[Bar graph showing the number of sprouts per spheroids with error bars and statistical symbols.]

**Notes:**
- NMN: Nicotinamide mononucleotide
- VEGF: Vasculogenic mimetic factor
- Sprouting: The formation of new blood vessels from pre-existing vessels
H$_2$S augments the effects of NMN on angiogenesis

NMN + NaHS treatment for 1 month, 32-month old

- **Quadriceps**
  - Capillary density
  - NMN + NaHS treatment increases capillary density significantly.

- **Treadmill test**
  - Distance run to exhaustion (m)
  - NMN + NaHS treatment increases distance significantly.

- **TUNEL assay**
  - % apoptotic capillaries
  - NMN + NaHS treatment decreases % apoptotic capillaries significantly.

* indicates significant difference from Vehicle.
** indicates significant difference from NaHS.
# indicates significant difference from NMN.

[Graphs showing data for each treatment group.]
Skeletal muscle

Ischemia
Exercise

VEGF

Endothelial cell

Aging

SIRT1
NAD^+

H2S
NMN

Notch1

Neovascularization in muscle

“Synergistic Hormetics”
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Brenna Osborne
Sanket Joshi

BRIL UNSW
Tzong-tyng Hung
Brendan Lee
Circadian clock

Small Molecule

Diet

Exercise

Mitochondrial activity

SIRT1-7

FOXO NF-κB p65

LXR PGC-1α PPARα

PPARγ

PGC-1α FOXO

FOXO UCP2

Skeletal muscle

Insulin sensitivity

Fatty acid oxidation

Brain/CNS

Neurodegeneration

Heart

Inflammation

Cardioprotection

Liver

Fatty acid oxidation

Gluconeogenesis

WAT

Lipogenesis

Pancreas

Insulin secretion

Haigis and Sinclair, Annual Reviews, 2010