News in sodium balance

A. Tissue sodium in healthy and hypertensive patients
B. Sodium sensing in the interstitium and relationship to hypertension.

Jens Titze

Division of Clinical Pharmacology
Vanderbilt University

Interdisciplinary Center for Clinical Research
University of Erlangen
Sodium balance

Assumption 1: Steady state: what goes in, must come out.

Assumption 2: Body Na\(^+\) is accompanied by commensurate amounts of water.

Assumption 3: Body Na\(^+\) content is maintained within narrow limits.
Isotonicity of the internal environment: the two-compartment model
Na⁺ and maintenance of internal environment composition

Intake:
- 9 g NaCl

Excretion:
- Skin
- ADH
- RAAS
- Nerves

Thirst

ECV
- Na⁺
- Na⁺
- K⁺

ICV
- K⁺
- K⁺

≈ 140 mmol/L
(≈ 9 g NaCl/L)
What is the reverse experiment?
The metabolic ward.

- Temp. 18-25°C
- Humidity: 40-75%
- Air Pressure: 660 - 880 mmHg
- PCO$_2$ < 8 mmHg
- PO$_2$: 140-200 mmHg
A little help from our friends
Acknowledgements
Doing the reverse experiment: the Mars500 project

**A** Mars 105

<table>
<thead>
<tr>
<th>Salt intake (g)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
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<tr>
<td>9</td>
<td>150</td>
</tr>
<tr>
<td>12</td>
<td>200</td>
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</tbody>
</table>

**B** Mars 520

<table>
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**Urinary Na\(^+\) recovery:** 95% 95%

Source: esa.com
Doing the reverse experiment: the Mars500 project.

Great!
Let's go home!
The Mars500 Project: Value of an accurately UNaV sample

A

Single UNaV sample

Subject 16

All subjects

mmol

UNaV (mmol per day)

Na⁺ Intake (mmol per day)

Day

B

Seven consecutive UNaV samples

Subject 16

All subjects

mmol

UNaV (mmol per day)

Na⁺ Intake (mmol per day)

Day

Unpublished
The Mars500 Project: Does salt intake determine fluid intake?

A

\[ R^2 = 0.08 \]
\[ P < 0.001 \]

B

\[ R^2 = 0.37 \]
\[ P < 0.001 \]

C

\[ R^2 = 0.03 \]
\[ P < 0.001 \]

D

\[ R^2 = 0.37 \]
\[ P < 0.001 \]

E

\[ R^2 = 0.38 \]
\[ P < 0.001 \]

F

\[ R^2 = 0.14 \]
\[ P < 0.001 \]
The Mars500 Project: Does salt intake influence fluid intake?
The Mars500 Project: Dietary salt leads to urinary Na\(^+\) concentration.
Where is the salt?

Cell Metabolism
17: 125-131, 2013
1. Why do we want to see the sodium?

a) Because serum electrolytes do not tell us much about tissue electrolyte composition.

b) Because immune cells seem to regulate interstitial electrolyte homeostasis, and because interstitial electrolytes may boost immune responses.

c) Because tissue Na\(^+\) accumulation may be hidden disease entity.
$^{23}$Na MRI for detection of hidden Na$^+$ storage in humans

TE = 2…3 ms
Sodium storage and its coincidence with hypertension.
23Na MRI of tissue Na⁺ content

Man, 24 y, healthy

Man, 85 y, hypertension
Where is the salt?

A

Muscle Na⁺, women

Muscle Na⁺, men

Muscle Water

B

Skin Na⁺, women

Skin Na⁺, men

Skin Water

Hypertension, 2013
Sodium storage and its coincidence with hypertension.

MRI (TE 2.07 ms)

- Women
- Men

Na⁺ (mmol/L)

- P\(_{(\text{age})}\) < 0.01
- P\(_{(\text{gender})}\) < 0.01

VEGF-C (ng/ml)

- Controls
  - y = -0.037x + 7.49
  - r = -0.48
  - P < 0.05

DuoSet ELISA Development Kit
R&D Systems
Sandwich Elisa
Can we mobilize?

A  Women  
Skin Na⁺ content

MRI (TE 2.07 ms)  
Na⁺ (mmol/L)  

Control  HTN

B  Men  
Muscle Na⁺ content

MRI (TE 2.07 ms)  
Na⁺ (mmol/L)  

Control  HTN  HTN + Spiro

Systolic BP  

mmHg  

control  HTN

Systolic BP  

mmHg  

control  HTN  HTN + Spiro

Hypertension, 2013
Epidermis with blood capillary loop and an initial lymph vessel (Magnification)

BK = Blood capillary
IL = Initial lymph vessel
CP = Corium papilla

Initial lymph vessel
Phase of opening

E = Endothelial cells
AF = Anchor filaments
IF = Inflow of interstitial fluid
L = Outflow direction of lymph

Lymph capillary network

Lymph vessels (green)
1 Lymph capillary (initial lymph vessel)
2 Precollector
3 Lymph collector (lymphatic ducts)
4 Deep lymph vessels
5 Lymphangion
6 Lymphatic trunk
7 Confluence (junction) of the lymphatic trunks in the veins
8 Afferent lymph vessel
9 Efferent lymph vessel

Blood Vessels (veins = blue, arteries = red)
10 Superficial vein
11 Superficial artery
12 Deep veins
13 Deep artery
14 Blood vessels of the musculature
15 Blood vessels of the lymph node
Na⁺ storage in the interstitium: the three-compartment model

ECV

Serum

Interstitium

ICV


Is the skin interstitium hypertonic compared to plasma?

Chemical analysis

![Graph showing plasma [Na⁺], [Cl⁻], and Osmolality.]

Plasma and skin tissue osmolality in rats fed low- or high-salt diets.

JCI, 2013; 123: 2803-2815
Na⁺ storage in the interstitium: the three-compartment model


Ernest Henry Starling (1866 –1927)
How is microcirculation organized?

Traditional view  (Starling, J Physiol, 19, 312-326, 1896)

\[
\begin{align*}
P_{(i)} &= -2 \\
P(\pi) &= 0.1
\end{align*}
\]

\[
\begin{align*}
P_{(i)} &= -2 \\
P(\pi) &= 3
\end{align*}
\]

\[
\begin{align*}
P_{(c)} &= 35 \\
P(\pi) &= 28
\end{align*}
\]

\[
\begin{align*}
P_{(c)} &= 15 \\
P(\pi) &= 28
\end{align*}
\]

\[
P_{(eff)} = 9
\]

\[
P_{(eff)} = 8
\]
How is microcirculation organized - why not questioning Starling’s equation?

\[ \begin{align*}
\text{P}(\text{i}) &= -2 \\
\text{P}(\pi) &= 0.1 \\
\text{P}(\text{Na}) &\approx 5600 \\
\text{P}(\text{osmNa}) &= 5000 \text{ mmHg} \\
\text{Na}^+ + K^+ &\approx 160 - 240 \text{ mmol/L} \\
\text{Osm} &\approx 290 \text{ mOsm/kg} \\
\end{align*} \]
2. How can we see the sodium?

a) By chemical analysis (19th and 20th century).

b) Invasively by EDX-SEM microscopy.

c) Non-invasively by 23NaMRI (21th century).
Sodium sensing in interstitium and Relationship to hypertension
<table>
<thead>
<tr>
<th></th>
<th>LSD</th>
<th>HSD</th>
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<tbody>
<tr>
<td>Blood: [Na(^+) + K(^+)]</td>
<td>143 ± 2 mM</td>
<td>145 ± 2 mM</td>
</tr>
<tr>
<td>Skin: [Na(^+) + K(^+)]</td>
<td>177 ± 8 mM</td>
<td>191 ± 7 * mM</td>
</tr>
</tbody>
</table>

Agnes Schröder

Nature Medicine, 2009
VEGF-C 

TonEBP 

VEGFR-2 

eNOS 

Blood 

Skin Interstitium 

Kidney 

Urine 

Lymph Vessel 

Clearance 1 

Clearance 2 

Na⁺ 

Cl⁻ 

Part 1 

Part 2 

VEGFR-3
Questions:

1. MPS: regulators of electrolytes and blood pressure?

2. Lymphatic regulation of blood pressure?

3. Is blood pressure regulated in the skin?
Macrophages regulate skin electrolyte homeostasis and blood pressure

A

mRNA

Protein

Arbitrary Units

Ton EBP

VEGF-C

CD68

C

Skin chloride

SKCl

SKCl⁻ / SKW

mmol·DW⁻¹

mmol·ml⁻¹

n = 2

n = 3

n = 2

n = 3

B

FVB LSD

D

mmHg

AU

LCD

MAP

JCI, 2013; 123: 2803-2815
Questions:

1. Is the skin microenvironment hypertonic?

2. Are lymph capillaries important for blood pressure?

3. Is skin VEGF-C important for blood pressure control?
Lymphatic regulation of blood pressure?

Agnes Schröder
Bronek Pytowski
Lymphatic regulation of blood pressure?

Experiments with mF4-31c1

E

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<tr>
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<tbody>
<tr>
<td>Anti-CD68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-β Actin</td>
<td></td>
<td></td>
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<tr>
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F

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<tbody>
<tr>
<td>Anti-eNOS</td>
<td></td>
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<tr>
<td>Anti-β Actin</td>
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<tr>
<td>Anti-p-eNOS</td>
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G

Lymph capillary density (AU)

MAP (mmHg)
Lymphatic regulation of blood pressure

a) Low salt diet

GV [AU]: 26.4
MAP [mmHg]: 113

40.2  25.2  31.4  24.3  34.3
115  123  109  119  117

b) High salt diet

GV [AU]: 45.3
MAP [mmHg]: 125

51.3  62.1  41.0  52.3  57.8  68.6  44.3
126  126  120  124  126  128  117

d) High salt diet + mF4-31c1

GV [AU]: 39.3
MAP [mmHg]: 129

9.9  33.4  17.4  21.8  9.1  15.2  25.2
142  130  152  146  151  142  134

JCI, 2013; 123:
Lymphatic regulation of blood pressure

Experiments with mF4-31c1

Lymph capillary density (AU)

MAP (mmHg)

SKCl - SKW (mmol·ml⁻¹)

JCI, 2013; 123: 2803-2815
Questions:

1. Is the skin microenvironment hypertonic?

2. Are lymph capillaries important for blood pressure?

3. Is skin VEGF-C important for blood pressure control?
Skin VEGF-C and blood pressure

Agnes Schröder
Kari Alitalo
Skin VEGF-C and blood pressure

JCI, 2013; 123: 2803-2815
Skin VEGF-C and blood pressure

Does not seem to be a kidney problem.
Skin VEGF-C and blood pressure

- **MAP (mmHg)**
  - wt, K14-FLT4
  - LSD, HSD
  - Anti-CD68, Anti-β Actin, Anti-VEGF-C, Anti-β Actin

- **SKCl / SKW (mmol·ml⁻¹)**
  - wt, K14-FLT4
  - LSD, HSD

- **Serum [Cl⁻] (mmol·L⁻¹)**
  - wt, K14-FLT4
  - LSD, HSD

- **Lymph Capillary Density**
  - wt, K14-FLT4
  - LSD, HSD

JCI, 2013; 123: 2803-2815
Lymphatic regulation of blood pressure

Asleep at the switch?

"Model" research

"Patient" research
ESRD patients
Sodium storage and its coincidence with hypertension.

MRI (TE 2.07 ms) Na⁺ (mmol/L)

Women
Men

P(age) < 0.01
P(gender) < 0.01

VEGF-C (ng/ml)

Controls

y = -0.037x + 7.49
r = -0.48
P < 0.05

95% Prediction Band

Age (years)

DuoSet ELISA Development Kit
R&D Systems
Sandwich Elisa
ESRD patients
Urine Cl Na
VEGF -C
Lymph Vessel Na+
Na+
EBP Cl-
Ton VEGFR-3
VEGFR-2
NO eNOS
Skin Interstitium
Blood
Kidney
Clearance 1
Clearance 2
Sodium removal in ESRD patients

A

Pre-HD

$\text{Na}^+ : 31.9 \text{ mmol/L}$

Post-HD

$\text{Na}^+ : 19.0 \text{ mmol/L}$

B

Skin $23\text{Na}$

P < 0.001

C

Muscle $23\text{Na}$

P < 0.001

mmol/L

Pre-HD Post-HD

Pre-HD Post-HD

mmol/L
ESRD patients

Man, 64 y, high Na\(^+\) removal

- Pre-HD sFLT4: 26 ng/ml
- Muscle Na\(^+\): 24.0 mmol/L
- Post-HD

Man, 77 y, low Na\(^+\) removal

- Pre-HD sFLT4: 38 ng/ml
- Muscle Na\(^+\): 22.1 mmol/L
- Post-HD

\[
y = -0.11x + 466.0 \\
R^2 = 0.251 \\
p < 0.05
\]
Summary

1. Isotonicity versus hypertonicity.

2. Clearance.

3. What is immune function?
Junior Research Group II

Agnes Schröder, PostDoc (biology)
Diana Friedrich, PhD student (biology)
Natalia Rakova, MD
Peter Linz, PhD (physics)
Anke Dahlmann, MD
Christoph Kopp, MD
Jennifer Goss (technician)
Ulrike Goller (technician)
Jonathan Jantsch

Karl Hilgers
Kai-Uwe Eckardt
Bernd Sterzel

Funded by:
IZKF (TP B13, Junior Research Group 2)
DFG (Ti-345/2, Lymphatics)
DLR/BMBF

Collaborators

Andreas Hess, Erlangen
Armin Kurtz, Regensburg
Bronek Ptykowski, ImClone, New York
Dominik Müller, Berlin
Dontscho Kerjaschki, Vienna
Franz-Xaver Beck, Munich
Friedrich C. Luft, Berlin
Hubertus Wagner, Kulmbach
Katharina Machura, Regensburg
Kari Alitalo, Helsinki
Mark Belakovsky, Moscow
Michael Deimling, Erlangen
Michael Uder, Erlangen
Moo Kwoon, Baltimore
Nicola Volpi, Modena
Peter Dietsch, Berlin
Tuomas Tammela, Helsinki
Wolfgang Neuhofer, Munich
Results:

Sodium during Therapy

Before Therapy (69.5 kg) (103 kg) (73.5 kg)

After Therapy (65.7 kg) (100 kg) (70.4 kg)