The triage principle exemplified by vitamin K: how long-term micronutrient deficiencies lead to age-related diseases

Cees Vermeer

Classification of vitamin K-dependent proteins

<table>
<thead>
<tr>
<th>Hepatic:</th>
<th>Extra-hepatic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood clotting</td>
<td>Artery calcification inhibition</td>
</tr>
<tr>
<td>— Factors II, VII, IX, X</td>
<td>— Matrix Gla-Protein (MGP)</td>
</tr>
<tr>
<td>Clotting Inhibitors</td>
<td>Bone growth regulation</td>
</tr>
<tr>
<td>— Proteins C, S, Z</td>
<td>— Osteocalcin (OC)</td>
</tr>
<tr>
<td></td>
<td>Cell growth regulation</td>
</tr>
<tr>
<td></td>
<td>— Gas6</td>
</tr>
<tr>
<td></td>
<td>Unknown functions</td>
</tr>
<tr>
<td></td>
<td>— Gla-Rich Protein (GRP)</td>
</tr>
<tr>
<td></td>
<td>— 4 transmembrane Gla-proteins</td>
</tr>
<tr>
<td></td>
<td>— Periostin &amp; Periostin-Like Factor</td>
</tr>
</tbody>
</table>

The triage theory: during sub-optimal supply, vitamins are primarily utilized for functions important for immediate survival


- If blood clotting is disturbed there is an immediate risk for bleeding: life threatening
- If calcification inhibition is sub-optimal there is increased risk for vascular calcification: development of age-related disease in 10-20 years
- So: ‘evolution’ decided: blood clotting has 1st priority

Function of K vitamins

- uncarboxylated (uc) protein
  - ucOC, ucMGP
  - no functional activity
- carboxylated (c) protein
  - cOC, cMGP
  - biologically active form
  - proteins are called: Gla-proteins
The triage theory demonstrated

12 volunteers
Anticoagulated: INR = 2.0
Daily dose of K1
Weekly increment: 50 g/d
Measured: INR, ucOC

Different forms of vitamin K
K₁
K₂ (MK-4)
K₂ (MK-7)

Misconception about vitamin K: Healthy subjects are adequate in vitamin K

False: only the blood clotting factors (synthesized in the liver) are fully carboxylated in non-supplemented subjects; all extrahepatic Gla-proteins examined thus far were undercarboxylated for 20-30%. This is suggestive for extrahepatic vitamin K-insufficiency

Vitamin K₂ status and age-related disease: osteoporosis and osteoarthritis

P = 0.047
P = 0.005
Vitamin K\textsubscript{2} and prostate cancer: the EPIC study
- > 23,000 subjects
- Healthy, 35-65 yrs
- 14 years follow-up
  - Reduction of cancer incidence
  - Reduction of fatal cancers
  - Strong reduction of total cancer mortality
  - Also for most other forms of cancer

Only for K\textsubscript{2}

RR per 10 \( \mu \text{g} \) K\textsubscript{2}: 0.76

K\textsubscript{2} and cardiovascular health: the Rotterdam study
- Over 4,800 subjects
- Healthy elderly, 55 years and older
- 10 years follow-up
  - 50\% reduction of arterial calcification
  - 50\% reduction of cardiovascular death
  - 25\% reduction of all cause mortality

Only for K\textsubscript{2}

Hazard ratio for coronary heart disease risk per 10 \( \mu \text{g} \) increase of K intake
- PROSPECT Study cohort
- Included: 16,057 women 50-70 years old
- Longitudinal survey, follow-up period > 10 years

<table>
<thead>
<tr>
<th>Type of K</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_1 )</td>
<td>1.00</td>
<td>1.00-1.02</td>
<td>0.51</td>
</tr>
<tr>
<td>( K_2 )</td>
<td>0.91</td>
<td>0.85-0.99</td>
<td>0.04</td>
</tr>
</tbody>
</table>


Structure of Matrix Gla-Protein (MGP)
- Both domains strongly bind calcium
- Gla-domain is required for function
- No function known for Phosphoserine domain
In bone we see significant effects at 45 \( \mu \)g/day.

In arteries the first improvement was at 90 \( \mu \)g/day; this value may decrease at prolonged intake.

MK-7 helps decrease ucMGP in HD patients.

Vitamin K prevents vascular stiffening.

- **Vascular health study**
  - 120 postmenopausal women
  - 2 groups of 60
  - 3 years daily supplement:
    - vitamin D \( D_3 \) (8 \( \mu \)g) or
    - vitamin \( D_3 \) + vitamin K \( K_1 \) (1 mg)

- DC: vascular disensibility
- CC: vascular compliance
- E: Young's elasticity modulus
Conclusions for cardiovascular disease

- Vitamin K insufficiency of the vessel wall is widespread in the adult healthy population.
- This results in sub-optimal MGP carboxylation and thus in poor calcification-inhibitory activity.
- Poor MGP carboxylation is a strong and independent risk factor for vascular calcification and mortality.

General conclusions

- Besides clotting factors, osteocalcin and MGP there are 8 vitamin K dependent proteins the function of which is not or only partially known.
- Hence more physiological processes may turn out to be controlled by vitamin K.
- In this respect it is of great concern that vitamin K intake has been going down all the time during the last 2000 years.
- Recommendations for vitamin K intake should be shifted upwards and separate recommendations should be given for vitamins K1 and K2.

Business strategy for VitaK (1)

1. Find new diseases related with vitamin K insufficiency and file user-patent for the use of vitamin K supplements and fortified foods.

Business strategy for VitaK (2)

1. Find new diseases related with vitamin K insufficiency and file user-patent for the use of vitamin K supplements and fortified foods.
2. Design and develop simple tests to monitor vitamin K-insufficiency in specific tissues.
Business strategy for VitaK (3)

1. Find new diseases related with vitamin K insufficiency and file user-patent for the use of vitamin K supplements and fortified foods
2. Design and develop simple tests to monitor vitamin K insufficiency in specific tissues
3. License out or sell the patents in exchange for long-lasting R&D contracts

Approach to achieve growing business

1. Yearly “in house” R&D budget > € 300,000,–
2. 15-20 high impact scientific papers yearly
3. Long-lasting (3-10 years) R&D contracts with third parties
4. All K-related technology available
   • Analytical (sensitive detection of all forms of vitamin K)
   • In vitro (cell culture, ELISA-arrays, cell-free systems)
   • Animal models (K-deficient, transgenic, califying)
   • Clinical trials (up to 1,000 participants according to GCP guidelines)
   • Iso-9001 certification

Personnel employed at VitaK

President: Cees Vermeer, PhD
Vice-president: Leon Schurgers, PhD
Scientific staff: Elke Theuwissen, PhD
Lavenja Braam, PhD
AIOs: Ellen Craenenburg (MD)
Marij Kruijnen (Isc)
vacancy
Technicians: Elke Magodeyns, BSc
Kirsten Teunissen, BSc
Stefan Pantus, BSc
Nadia Drummen, BSc
Marjolein Harfis, BSc
Noura Ahmed, BSc
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vacancy