In Vivo Responses of Phosphorus-based Food with Different Forms in Mice

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Dietary phosphorus management is important for not only renal patients but also healthy people with normal kidney function, because phosphorus intake is associated with osteoporotic fractures, left ventricular mass, and all cause mortality, even in healthy populations.
• The daily phosphate intake varies with age and gender.
• On a global scale, there are quite significant differences in diet compositions to be considered.
Sources of phosphorus in Japanese food supply, 2010

- **Grains**: 19.02%
- **Seafood**: 16.74%
- **Meat, poultry**: 11.43%
- **Eggs**: 6.55%
- **Bakery**: 2.39%
- **Beverages**: 2.39%
- **Soy**: 7.07%
- **Vegetables**: 7.90%
- **Fruits**: 7.90%
- **Mushroom**: 1.46%
- **Algae**: 0.62%
- **Sugars & sweeteners**: 0.00%
- **Nuts, seeds**: 0.83%
- **Supplementary nutrition**: 0.31%
- **Oils**: 0.00%

Cited: 2010 National Health and Nutrition Survey, Table 9

Male: 4153, Female: 4662
Annual Changes in the Mean **Phosphorus Intake** (aged 20 years and over) (2012 to 2016)

Cited; 2012 - 2016 National Health and Nutrition Survey,
Annual Changes in the Mean Phosphorus Intake (aged 20 years and over) (2012 to 2016)

Cited; 2012 - 2016 National Health and Nutrition Survey,
• Assessment of dietary phosphorus is complicated by inaccuracies in most nutrient composition databases due to the extensive and varied use of phosphorus-containing food additives.

• Intake from processed foods is particularly obscured by the fact that nutritional labels are not required to report the phosphorus content of food.
• Dietary phosphorus intake could be estimated from 24-h urine collection by urinary phosphorus excretion divided by phosphorus absorption rate (65%).

• Phosphorus absorption rates in previous studies vary widely possibly due to factors associated with its regulation, such as the type of phosphorus, other minerals and interindividual variation in prior nutrient status or transit time.
• Dietary records (DR) are a type of self-reported dietary assessment method.

• Although widely used, DR have inherent problems and requirement for participant literacy.

• These problems cannot be avoided in national representative surveys.

![Annual Changes in the Mean Phosphorus Intake](image)
Dietary phosphorus intake by dietary records and 24-hour urine collection in Japanese adults.

Estimate of dietary phosphorus intake using 24-h urine collection

Yuuka Morimoto,1 Masae Sakuma,1,* Hiroyuki Ohta,1 Akitsu Suzuki,1 Asami Matsushita,1 Minako Umeda,2 Makoto Ishikawa,2 Yutaka Taketani,3 Eiji Takeda3 and Hidekazu Arai1

1Laboratory of Clinical Nutrition and Management, Graduate School of Nutritional and Environmental Sciences and 2School of Nursing Sciences, The University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
3Department of Clinical Nutrition, Institute of Health Biosciences, University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan

Minerals, trace elements, Vit. D and bone health
Dietary phosphorus intake estimated by 4-day dietary records and two 24-hour urine collections and their associated factors in Japanese adults

Nana Shinozaki1 · Kentaro Murakami2 · Keiko Asakura3 · Ken Uechi1 · Satomi Kobayashi4 · Shizuko Masayasu5 · Satoshi Sasaki1,4
This observational study was based on data from a crosssectional multicenter survey conducted in February and March 2013.

The survey targeted healthy men and women aged 20–69 years living throughout Japan.

The total number of participants was 395 men and 396 women.
• Body weight and were measured

• BMI was calculated.

• Information on age, education, medical history, current medication use, physical activity, and smoking

• Estimates of physical activity were calculated.

• Dietary intake was assessed by 4-day semi-weighted dietary records.

• Urine collections were performed on two non-consecutive days.

• A total of 161 men and 161 women aged 20–69 years completed a 4-day dietary records and two 24-h urine collection.

• All analyses were performed using the statistical software package SAS version 9.4.

• Two-sided P-values <0.05 were considered statistically significant.
Table 1 Characteristics of 322 apparently healthy men and women participants aged 20–69 years, 2013

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 161)</th>
<th>Women (n = 161)</th>
<th>Total (n = 322)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.2±13.1</td>
<td>44.3±13.0</td>
<td>44.7±13.0</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>170.3±5.5</td>
<td>157.5±5.7</td>
<td>163.9±8.5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>69.2±10.7</td>
<td>55.3±8.6</td>
<td>62.2±11.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.8±3.2</td>
<td>22.3±3.3</td>
<td>23.1±3.3</td>
</tr>
<tr>
<td>Physical activity (MET × h)</td>
<td>37.3±5.8</td>
<td>37.8±5.6</td>
<td>37.5±5.7</td>
</tr>
</tbody>
</table>
Phosphorus intake estimated by Urine Collection was higher than that estimated by Dietary record

\[ P < 0.0001 \]

\[ P = 0.008 \]

<table>
<thead>
<tr>
<th>Dietary phosphorus intake (mg/day)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>by Dietary record</td>
<td>1176</td>
<td>1021</td>
</tr>
<tr>
<td>by UC</td>
<td>1393</td>
<td>1082</td>
</tr>
</tbody>
</table>

n=161
Phosphorus intake was sufficiently higher than Adequate intake in DRI.

Dietary phosphorus intake (mg/day)

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>by Dietary record</td>
<td>1176</td>
<td>1021</td>
</tr>
<tr>
<td>by UC</td>
<td>1393</td>
<td>1082</td>
</tr>
</tbody>
</table>

- Adequate Intake (AI); 1000 mg/day
- Adequate Intake (AI); 800 mg/day

*P < 0.0001

*P = 0.008
Dietary phosphorus

Organic phosphorus
- Animal protein
- Vegetable protein

Inorganic phosphorus
- Food additives
- Mono-phosphate salts
  - Disodium hydrogen phosphate
  - Trisodium hydrogen phosphate
- Poly-phosphate salts
  - Potassium polyphosphate
  - Calcium dihydrogen pyrophosphate

Phosphate-containing food

Organic phosphate

Orthophosphoric acid

Pyrophosphoric acid

triphosphate
Phosphorus additives are used for these functions......

- **Enhance Flavor**
- **Improve Color**
- **Extend the shelf life**
- **Moisture retention**
- **Improve texture**
- **Smoothness**
- **Binding**
- **etc.**

**Common Phosphorus Additives**

- Dicalcium Phosphate
- Hexameta phosphate
- Monocalcium Phosphate
- Phosphoric Acid
- Ca, Na, or K-polyphosphate
Pathways for phosphate absorption in the small intestine

- Dietary Pi
- Protein polyphosphate

Digestion system

Inorganic monophosphate

HPO$_4^{2-}$

Absorption system

Transcellular pathway

Paracellular pathway

Facilitate transport

Intestinal epithelial cell

Transporter

Apical

Basal

Dietary Pi

Protein polyphosphate

Dietary Pi

Protein polyphosphate
Transcellular pathway

Inorganic mono phosphate

$\text{HPO}_4^{2-}$

Absorption system

Transcellular pathway

$2\text{Na}^+$

$\text{PiT1/2}$

$?$

Transcellular pathway

$3\text{Na}^+$

$\text{NaPi2b}$

$?$

Apical

Basal
Paracellular transport is a major system of intestinal Pi absorption
NHE3 inhibitor reduces phosphate absorption from the gut

Gastrointestinal Inhibition of Sodium-Hydrogen Exchanger 3 Reduces Phosphorus Absorption and Protects against Vascular Calcification in CKD

Eric D. Labonté, Christopher W. Carreras, Michael R. Leadbetter, Kenji Kozuka, Jill Kohler, Samantha Koo-McCoy, Limin He, Edward Dy, Deborah Black, Ziyang Zhong, Ingrid Langsetmo, Andrew G. Spencer, Noah Bell, Desiree Deshpande, Marc Navre, Jason G. Lewis, Jeffrey W. Jacobs, and Dominique Charmot

Ardelyx, Inc., Fremont, California

Effect of Tenapanor on Serum Phosphate in Patients Receiving Hemodialysis

Geoffrey A. Block,* David P. Rosenbaum,† Maria Leonsson-Zachrisson,‡ Magnus Åstrand,‡ Susanne Johansson,‡ Mikael Knutsson,‡ Anna Maria Langkilde,‡ and Glenn M. Chertow§

*Denver Nephrology, Denver, Colorado; †Ardelyx Inc., Fremont, California; ‡AstraZeneca Gothenburg, Mölndal, Sweden; and §Division of Nephrology, Stanford University School of Medicine, Stanford, California
NHE3/SLC9A3
(Sodium+/hydrogen exchanger3)

- apical side intestine, colon, kidney....)
- Na+,H+ exchange
- plays a major role in transepithelial absorption of Na+ and water
- modulates the absorption of other nutrients
Tenapanor is discovered and developed for the patients with IBS-C.

1. Tenapanor blocks NHE3, which transports sodium in exchange for protons.
2. Blocking NHE3 increases sodium outside cells/in the gut.
3. Increased sodium increases water in the gut, which loosens stool, alleviating constipation.
In addition,

http://www.ardelyx.com/what-we-do/

Tenapanor blocks NHE3, increasing sodium in the gut; NHE3 transports sodium in exchange for protons

1. Elevated proton levels in cells selectively increase tight junction resistance to phosphate transport

2. Increased tight junction resistance reduces gut phosphate absorption
Transcellular and Paracellular Pi transport

Dietary Pi → HPO$_4^{2-}$

Paracellular Pi pathway

Claudins

NHE3

Pit-1

Npt2b

$3\text{Na}^+ \rightarrow \text{HPO}_4^{2-}$
Polypophosphate salt have more harmful effects than those of monophosphate salt on bone physiology and renal function.

Comparison of various phosphate salts as the dietary phosphorus source on nephrocalcinosis and kidney function in rats.

- **Biosci Biotechnol Biochem.** 2001 Apr;65(4):928-34.
Greater effect of dietary potassium tripolyphosphate than of potassium dihydrogenphosphate on the nephrocalcinosis and proximal tubular function in female rats from the intake of a high-phosphorus diet.
Matsuzaki H, Masuyama R, Uehara M, Nakamura K, Suzuki K.

### Mono-phosphate vs. Poly-phosphate

<table>
<thead>
<tr>
<th>Mono-phosphate</th>
<th>Poly-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ex) Disodium hydrogen phosphate</td>
<td>ex) Potassium polyphosphate</td>
</tr>
<tr>
<td>Trisodium hydrogen phosphate</td>
<td>Calcium dihydrogen pyrophosphate</td>
</tr>
</tbody>
</table>

- Orthophosphoric acid
- Pyrophosphoric acid
- triphosphate
Gastrointestinal phosphate handling

Dietary Pi
- Organic
- Inorganic
- Poly-P
- Mono-P

Inorganic monophosphate

Intestinal epithelial cell

transporter

Apical

Basal
**Animals and Diet**

Animal  
C57BL6J, 5 weeks of age ♂

Test diet Group  
1. Low Pi diet  
2. Control Pi diet  
3. **Monophosphate HP**  
4. **Polyphosphate HP**

<table>
<thead>
<tr>
<th>Diet 100 g (g)</th>
<th>LP</th>
<th>CP</th>
<th>Mono HP</th>
<th>Poly HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN93G modified Mix</td>
<td>59.5</td>
<td>59.5</td>
<td>59.5</td>
<td>59.5</td>
</tr>
<tr>
<td>Egg White</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>AIN93G modified Mineral Mix</td>
<td>1.5645</td>
<td>1.5645</td>
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<tr>
<td>Sucrose</td>
<td>10.4371</td>
<td>7.8871</td>
<td>15.2521</td>
<td>14.7421</td>
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<tr>
<td>CaCO₃</td>
<td>1.4984</td>
<td>1.4984</td>
<td>1.4984</td>
<td>1.4984</td>
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<tr>
<td>KH₂PO₄ or K₅P₃O₁₀</td>
<td>0</td>
<td>2.55</td>
<td>5.185</td>
<td>5.695</td>
</tr>
<tr>
<td>Soy Bean Oil</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
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</table>
Time schedule

<table>
<thead>
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<th>Schedule</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>7</td>
<td>5 weeks</td>
</tr>
<tr>
<td>28</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

Metabolic cage

Sacrificed

Start
5 weeks of age

10 weeks of age

Test diet

1. Low Pi diet
2. Control Pi diet
3. Monophosphate HP
4. Polyphosphate HP
Polyphosphate diet induced renal fibrosis and calcification.

Masson trichrome stain

Von Kossa Calcium stain
What is the difference between mono-P diet and poly-P diet?

Mono-P High phosphate diet

Poly-P High phosphate diet
Proximal intestine ATP (nM)

Intestinal alkaline phosphatase (IAP)

ATP: Substrate for IAP

Proximal intestine p-NP (mmol/mg protein/min)
Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase

Kathryn T. Chen,¹,² Madhu S. Malo,¹ Angela K. Moss,¹ Skye Zeller,³ Paul Johnson,³ Farzad Ebrahimi,¹ Golam Mostafa,¹ Sayeda N. Alam,¹ Sundaram Ramasamy,¹ H. Shaw Warren,³ Elizabeth L. Hohmann,³ and Richard A. Hodin¹
Microbiota–host interaction and modulation by dietary nutrients.

**Nutrients**

- **Absorbed in the gut**
- **Uptake by microbiota**

**Growth**:
- Proliferation of microbiota dependent on specific nutrients

**Processed by host**
- Host waste

**Processed by microbiota**
- Microbial waste

**Uremic toxin**

**Uremic toxin pre**

**Pi..**

**L-carnitine, choline/phosphatidylcholine, tryptophan and tyrosine...**

*Nutrients 2017, 9, 489; doi:10.3390/nu9050489 modified*
Hypothesis

Monophosphate HP

Polyphosphate HP

Microbiota

Blood Pi High

Uremic toxin

Type, Number change
Summary

- Japanese people take higher dietary phosphorus than the adequate intake in DRI.
- Phosphorus intake estimated by Urine Collection was higher than that estimated by Dietary record.
- Hidden phosphorus
- Consider phosphorus content and the form.