KDIGO CKD-MBD Implementation Summit
Keio Plaza Hotel
Tokyo, Japan

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

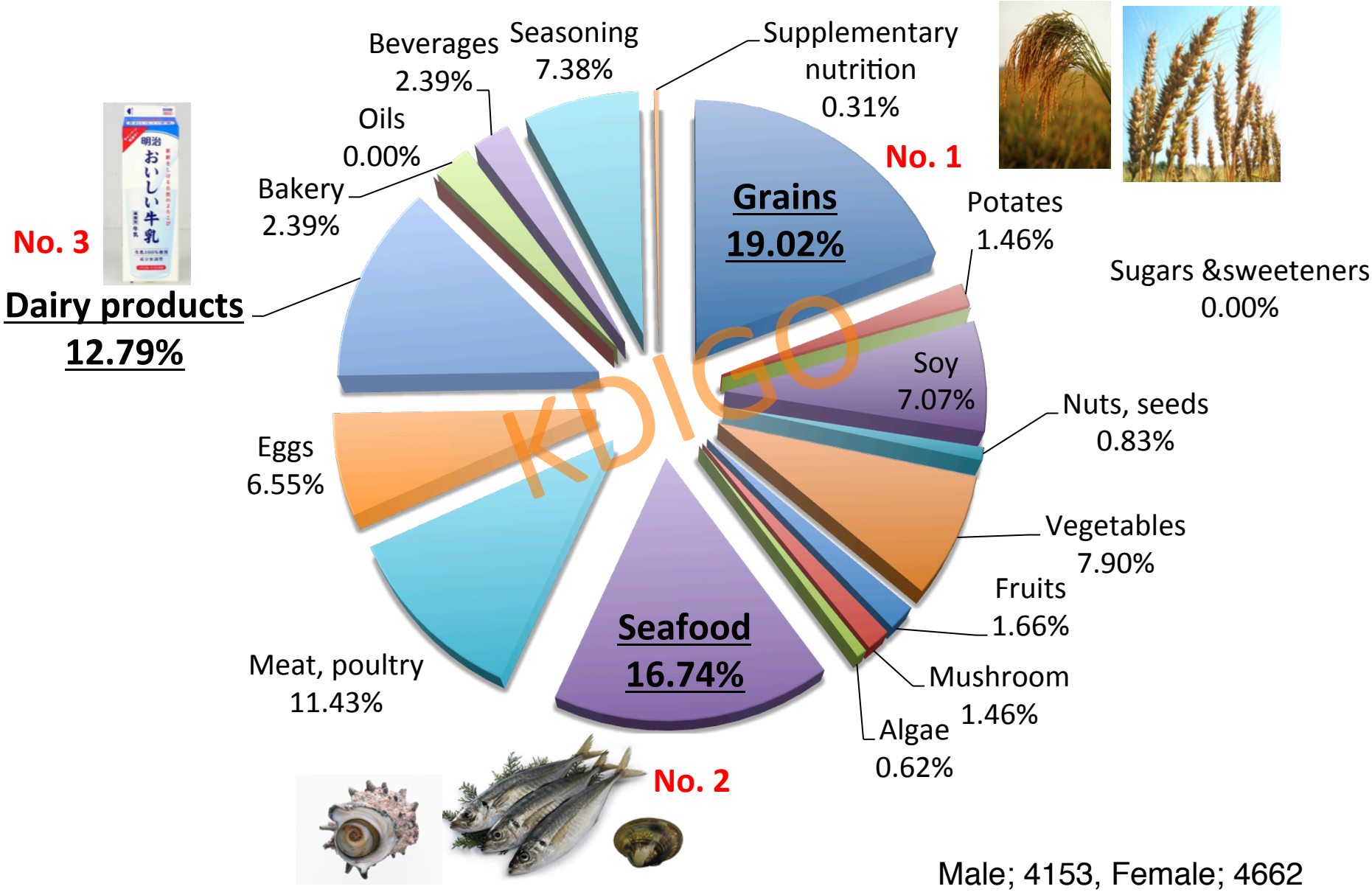
Hiroko Segawa

Department of Molecular Nutrition, Institute of Biomedical Sciences, Tokushima University Graduate School

- 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.

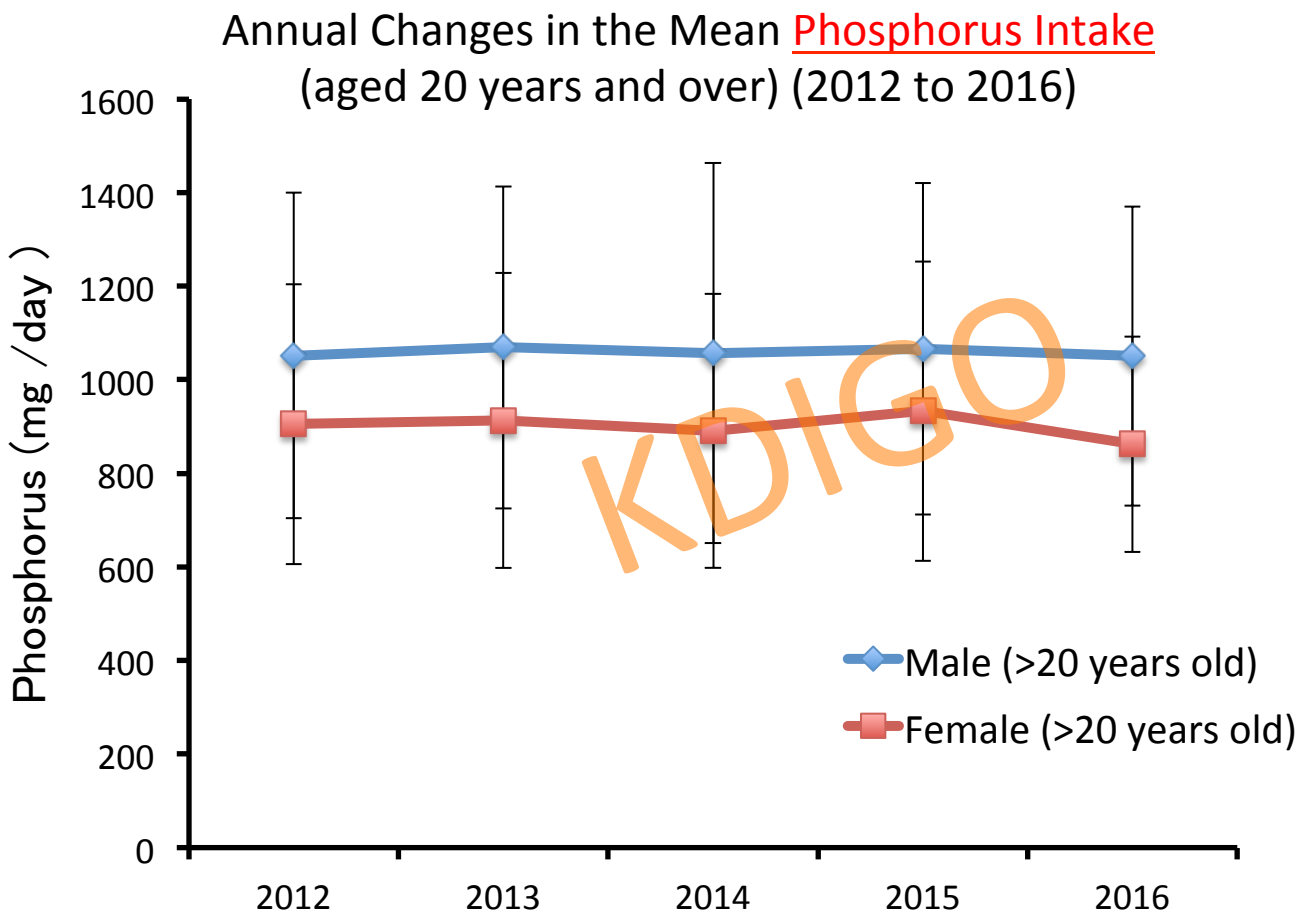
Sources of phosphorus in Japanese food supply, 2010



Male; 4153, Female; 4662

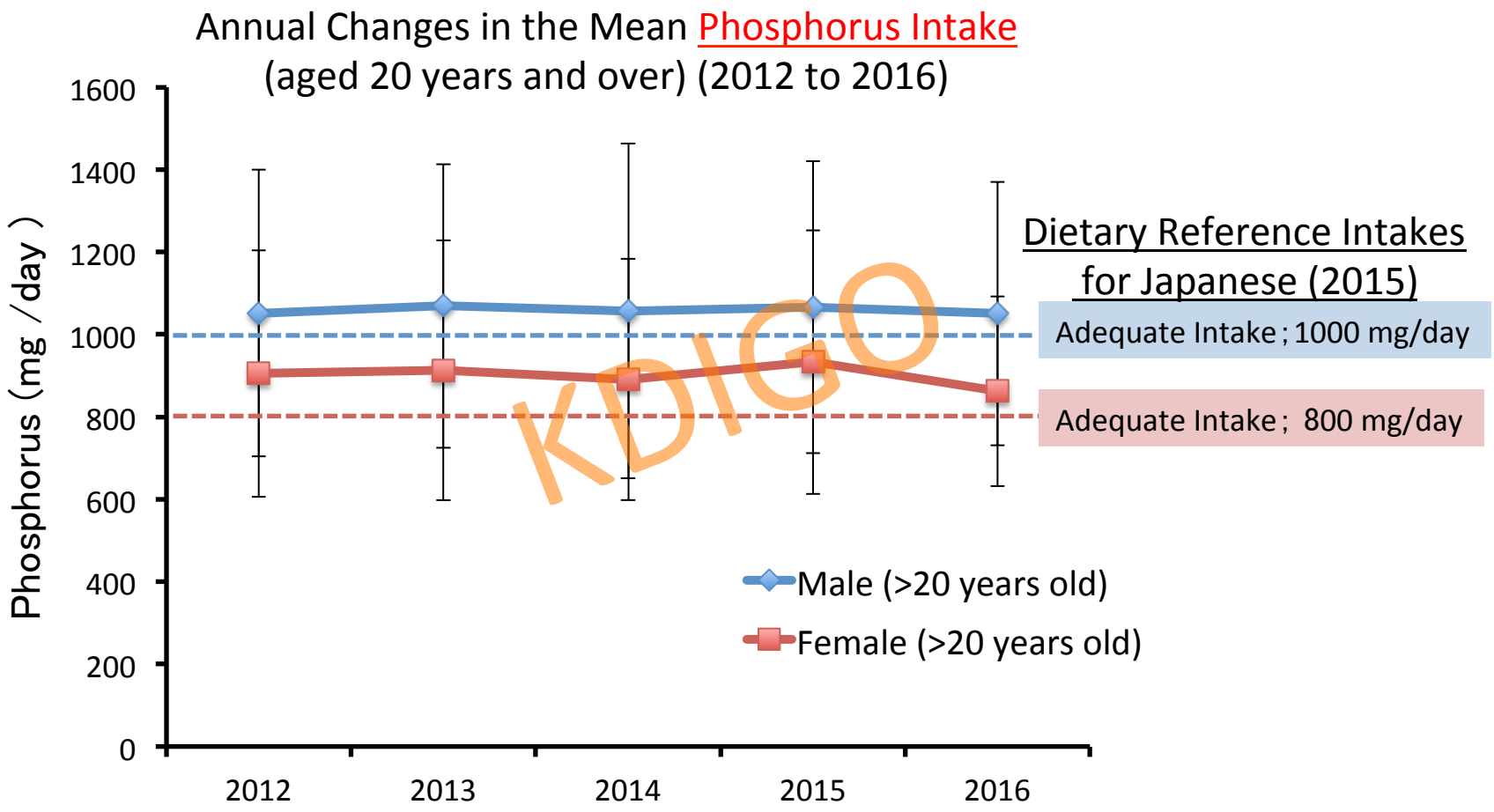
Cited; 2010 National Health and Nutrition Survey, Table 9

National Health and Nutrition Survey Japan



Cited; 2012 - 2016 National Health and Nutrition Survey,

National Health and Nutrition Survey Japan

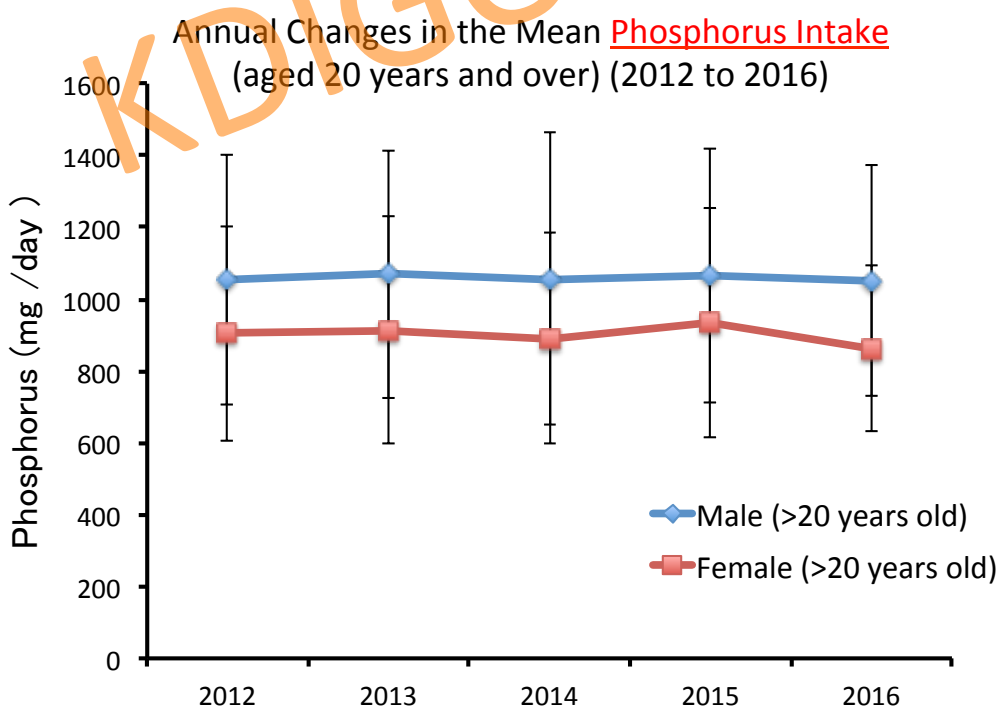


Cited; 2012 - 2016 National Health and Nutrition Survey,

- 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.



Dietary phosphorus intake by dietary records and 24-hour urine collection in Japanese adults.

Original Article

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 Takeda[3] and Hidekazu Arai[1]

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

ARTICLE

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 Shinozaki[1] · Kentaro Murakami[2] · Keiko Asakura[3] · Ken Uechi[1] · Satomi Kobayashi[4] · Shizuko Masayasu[5] · Satoshi Sasaki[1,4]

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- 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.

Shinozaki N. et al. European Journal of Clinical Nutrition (2018) 72:517–525

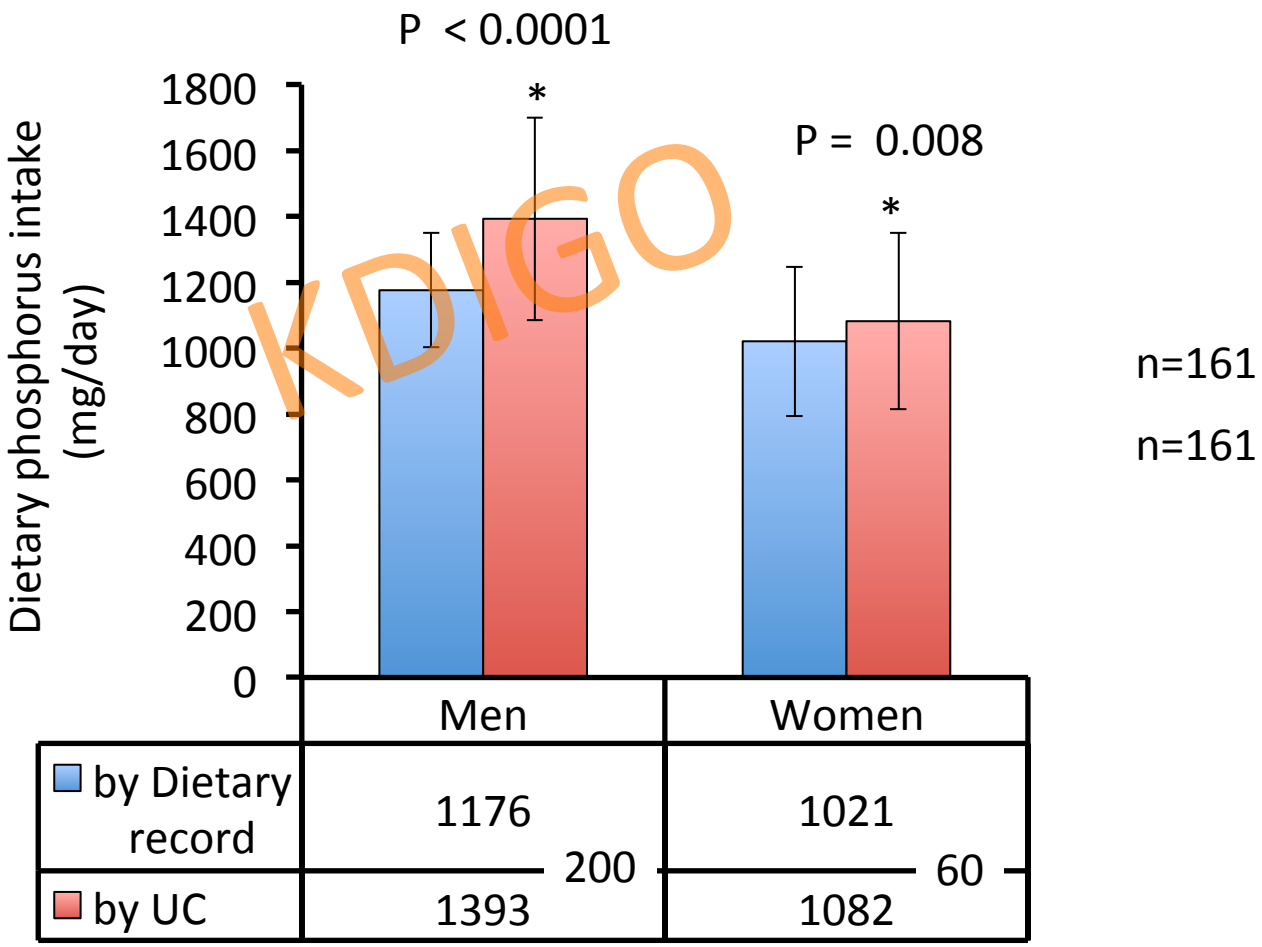
- Body weight and were measured
- BMI was calculated.
- Information on age, education, medical history, current medication use, physical activity, and smoking
- Estimates of physical activity were calculated.
- Dietary intake was assessed by 4-day semi-weighted dietary records.
- Urine collections were 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

Variable	Men (n = 161) Mean ± SD or n (%)	Women (n = 161) Mean ± SD or n (%)	Total (n = 322) Mean ± SD or n (%)
Age (years)	45.2±13.1	44.3±13.0	44.7±13.0
Body height (cm)	170.3±5.5	157.5±5.7	163.9±8.5
Body weight (kg)	69.2±10.7	55.3±8.6	62.2±11.9
BMI (kg/m^2)	23.8±3.2	22.3±3.3	23.1±3.3
Physical activity (MET × h)	37.3±5.8	37.8±5.6	37.5±5.7

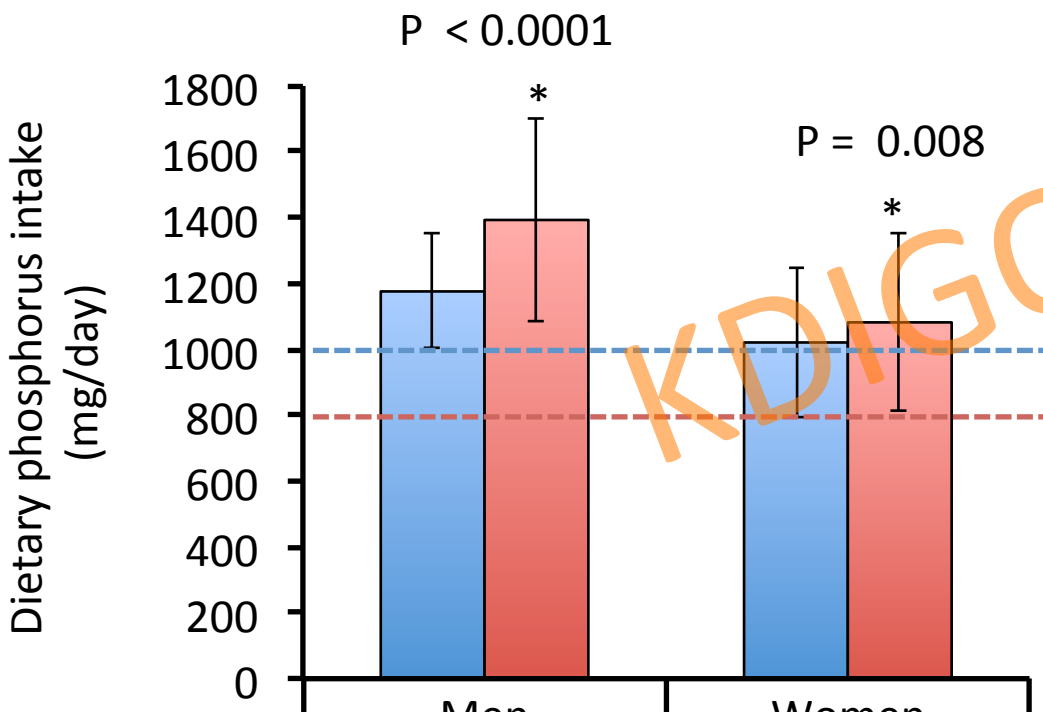
Shinozaki N. et al. European Journal of Clinical Nutrition (2018) 72:517–525

Phosphorus intake estimated by Urine Collection was higher than that estimated by Dietary record



Shinozaki N. et al. European Journal of Clinical Nutrition (2018) 72:517–525

Phosphorus intake was sufficiently higher than Adequate intake in DRI.



	Men	Women
by Dietary record	1176	1021
by UC	1393	1082
	200	60

Dietary Reference Intakes for Japanese (2015)

Adequate Intake (AI) ; 1000 mg/day

Adequate Intake(AI) ; 800 mg/day

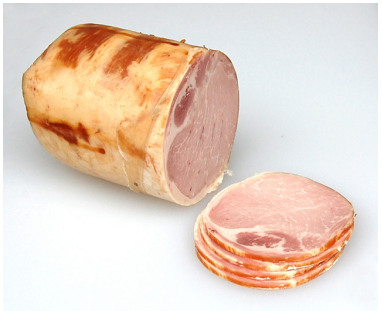
Dietary phosphorus

Organic phosphorus

Inorganic phosphorus

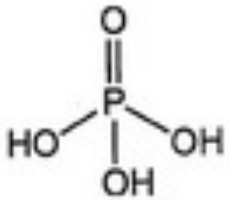
Animal protein
Vegetable protein

Food additives



<u>Mono-phosphate salts</u>

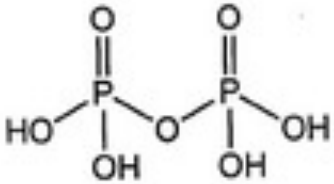
ex) Disodium hydrogen phosphate
Trisodium hydrogen phosphate



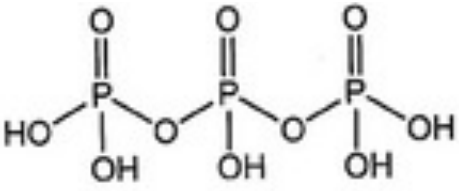
Orthophosphoric acid

<u>Poly-phosphate salts</u>

ex) Potassium polyphosphate
Calcium dihydrogen pyrophosphate



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.**

Moisture retention

Inflating

Binding

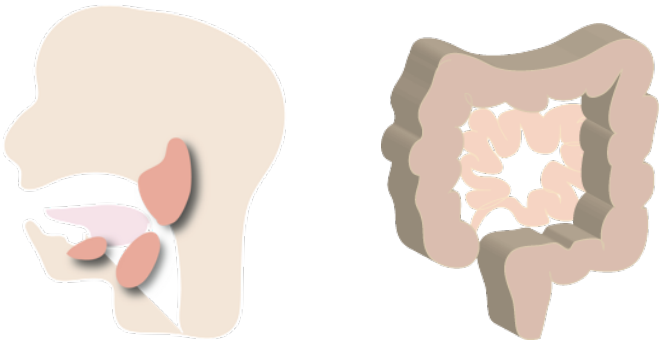
processed meat

Improve texture

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

Facilitate transport

Paracellular pathway

Transcellular pathway

$3Na^+$

Apical

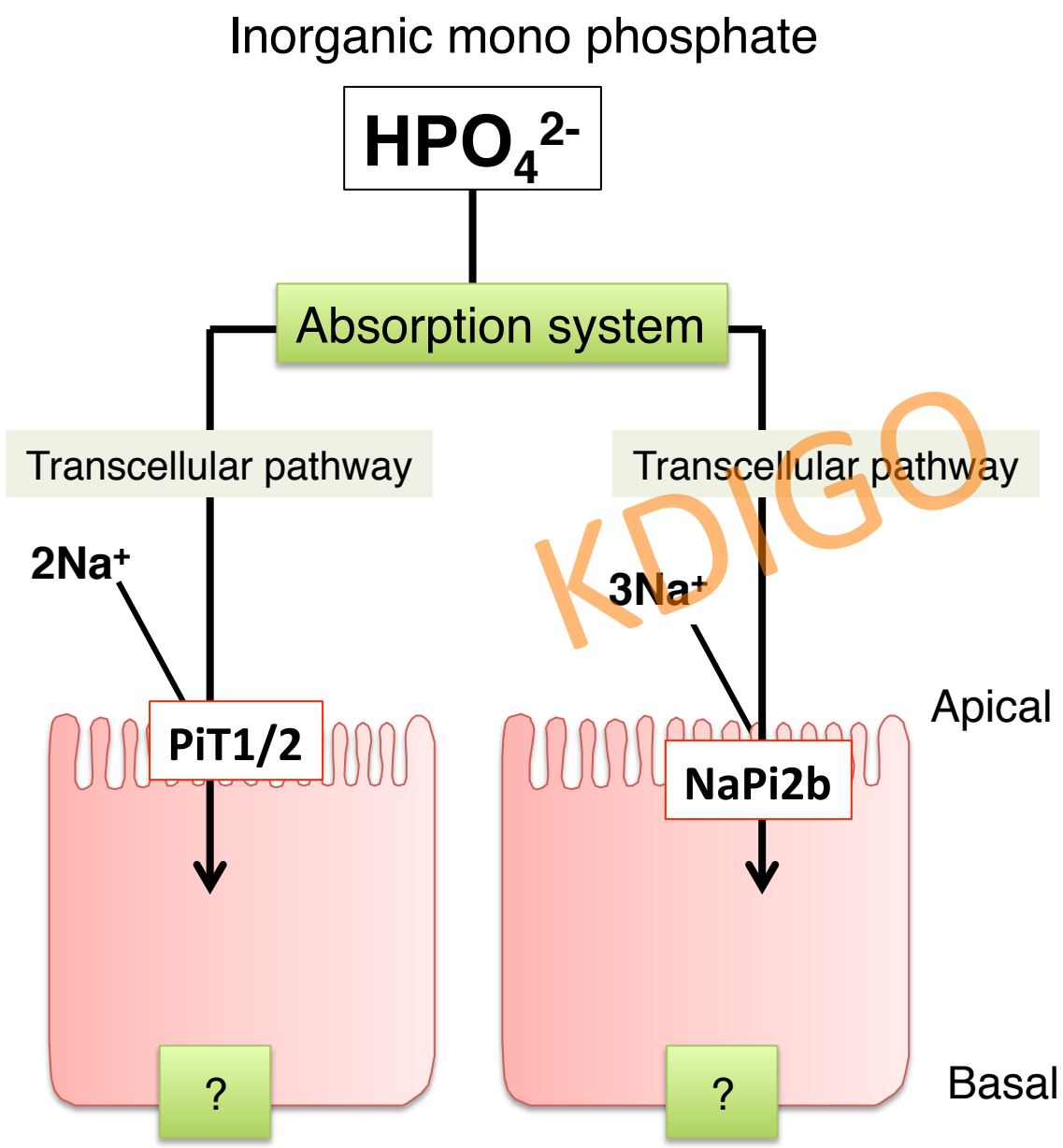
Transporter

Intestinal epithelial cell

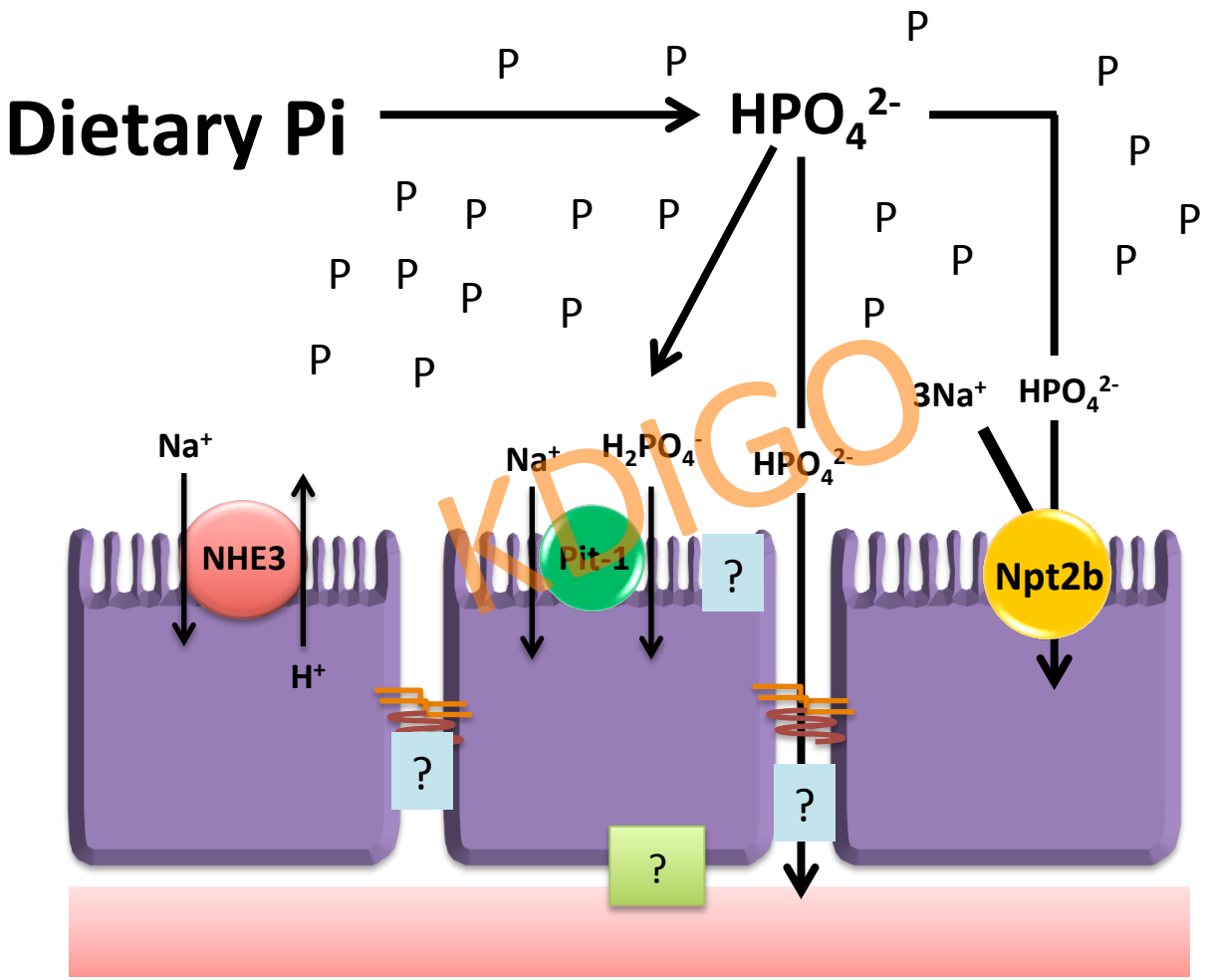
?

Basal

Transcellular pathway



Paracellular transport is a major system of intestinal Pi absorption



NHE3 inhibitor reduces phosphate absorption from the gut

J AmSoc Nephrol , 2015

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

J Am Soc Nephrol. 2017

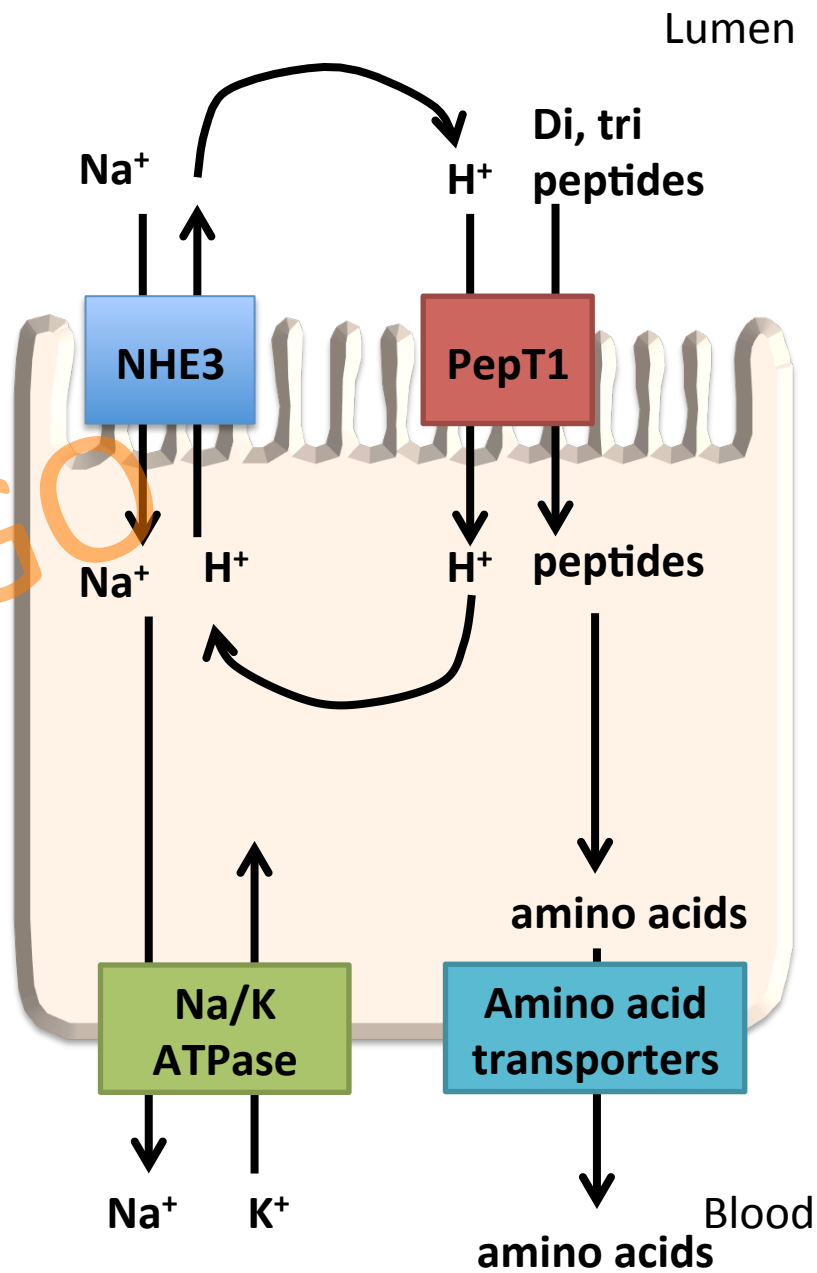
Effect of Tenapanor on Serum Phosphate in Patients Receiving Hemodialysis

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

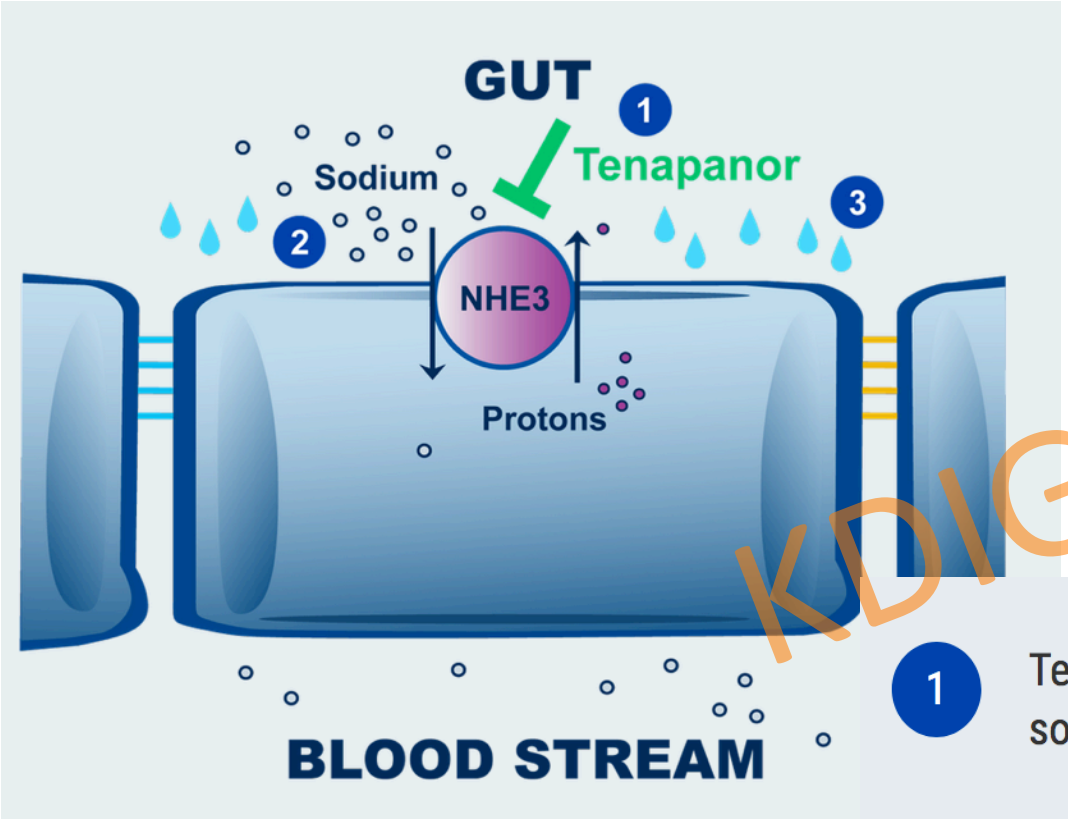
NHE3/SLC9A3 (Sodium+/hydrogen exchanger3)

- ✓ apical side iIntestine, 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.

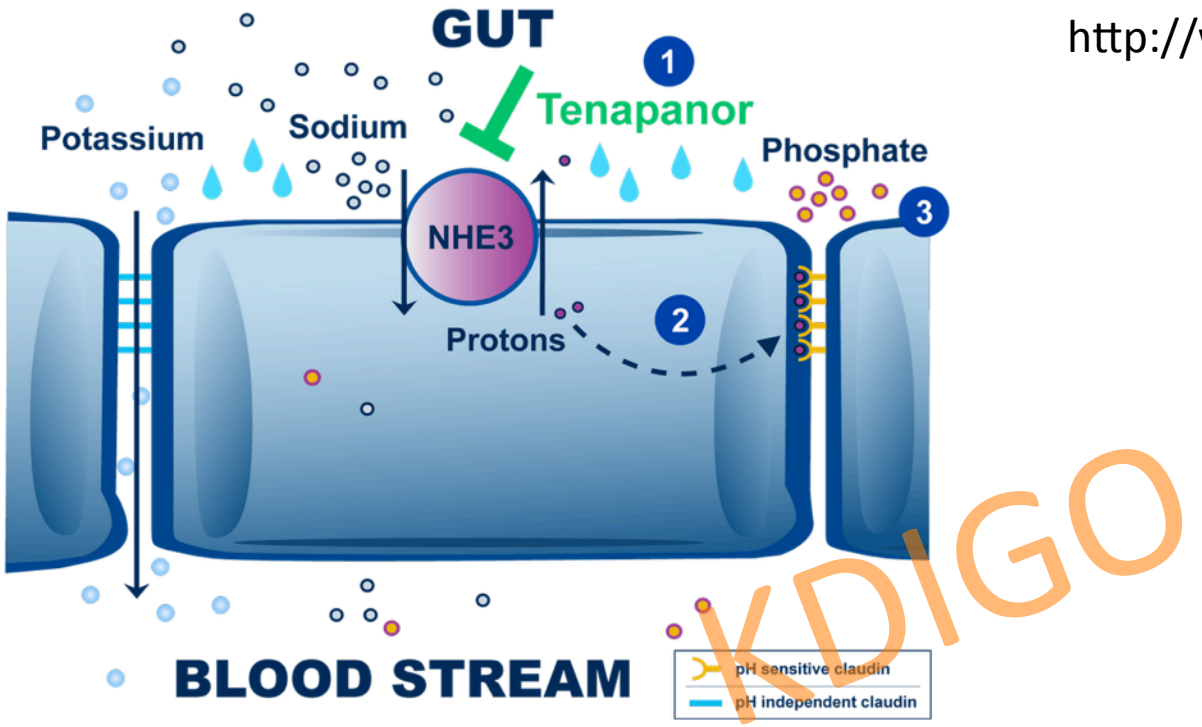
http://www.ardelyx.com/what-we-do/



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/

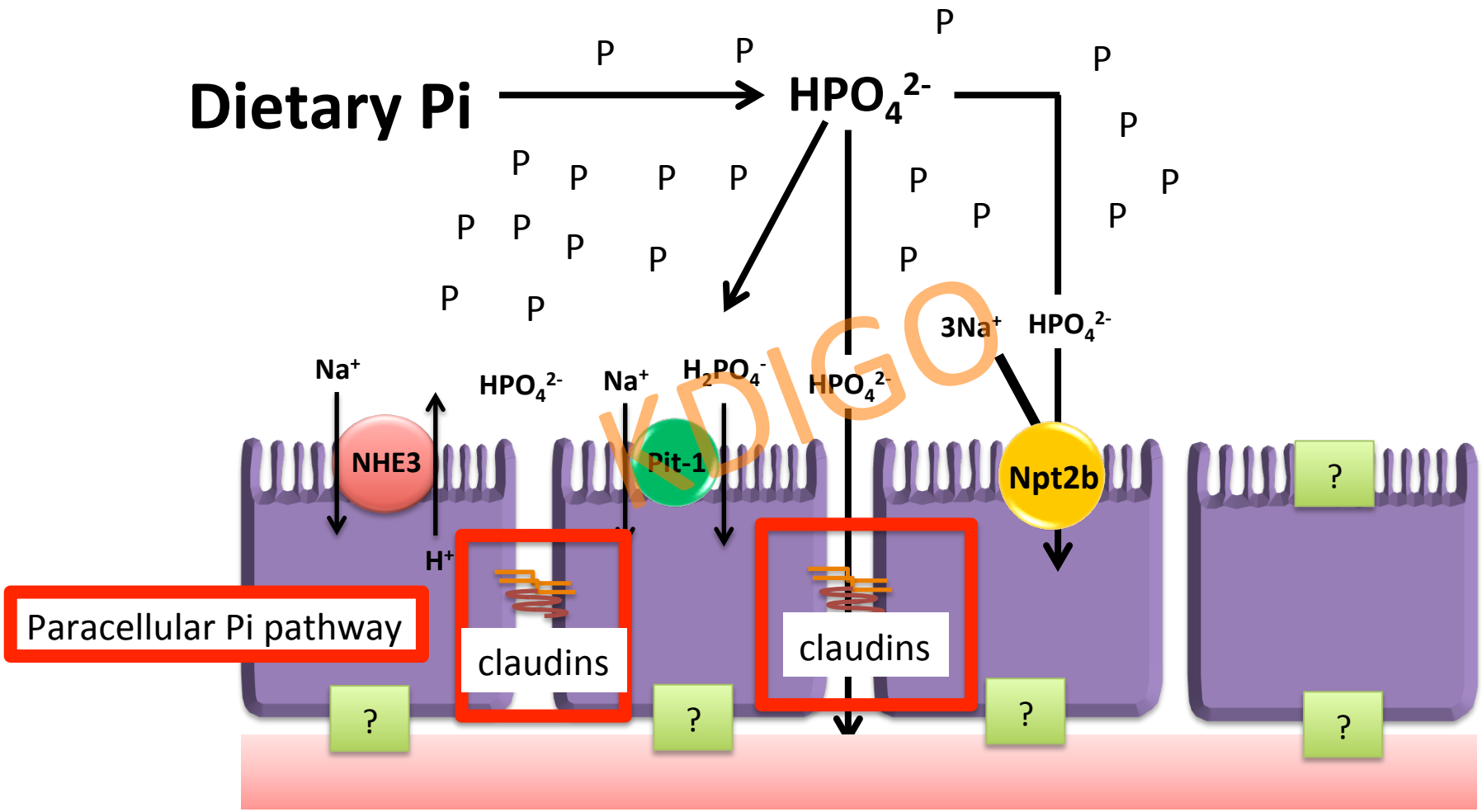


1. Tenapanor blocks NHE3, increasing sodium in the gut; NHE3 transports sodium in exchange for protons

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

3. Increased tight junction resistance reduces gut phosphate absorption

Transcellular and Paracellular Pi transport



Polyphosphate salt have more harmful effects than those of monophosphate salt on bone physiology and renal function.

✓ J Nutr Sci Vitaminol (Tokyo). 1999 Oct;45(5):595-608.

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

Matsuzaki H[1], Kikuchi T, Kajita Y, Masuyama R, Uehara M, Goto S, Suzuki K.

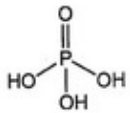
✓ Biosci Biotechnol Biochem. 2001 Apr;65(4):928-34.

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[1], Masuyama R, Uehara M, Nakamura K, Suzuki K.

Mono-phosphate

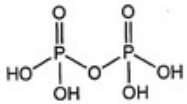
ex) Disodium hydrogen phosphate
Trisodium hydrogen phosphate



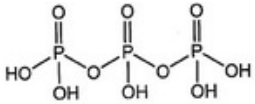
Orthophosphoric acid

Poly-phosphate

ex) Potassium polyphosphate
Calcium dihydrogen pyrophosphate

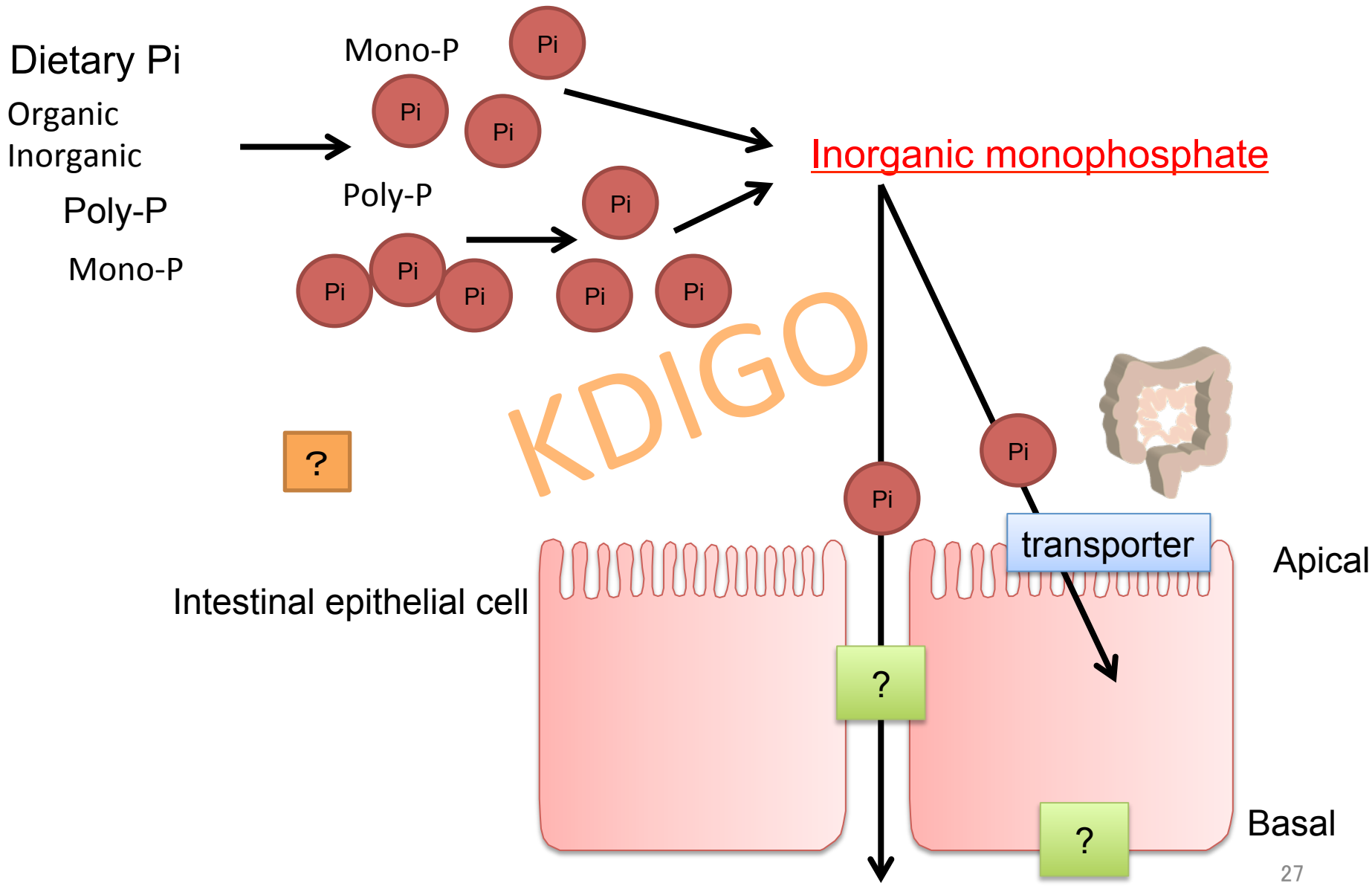


Pyrophosphoric acid



triphosphate

Gastrointestinal phosphate handling



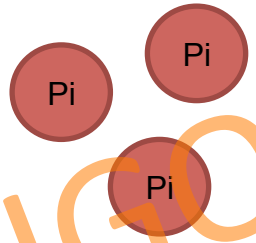
Animals and Diet

Animal
C57BL6J, 5 weeks of age ♂

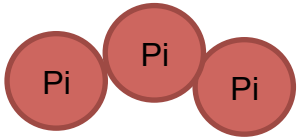
Test diet Group

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

Mono-P
High phosphate diet

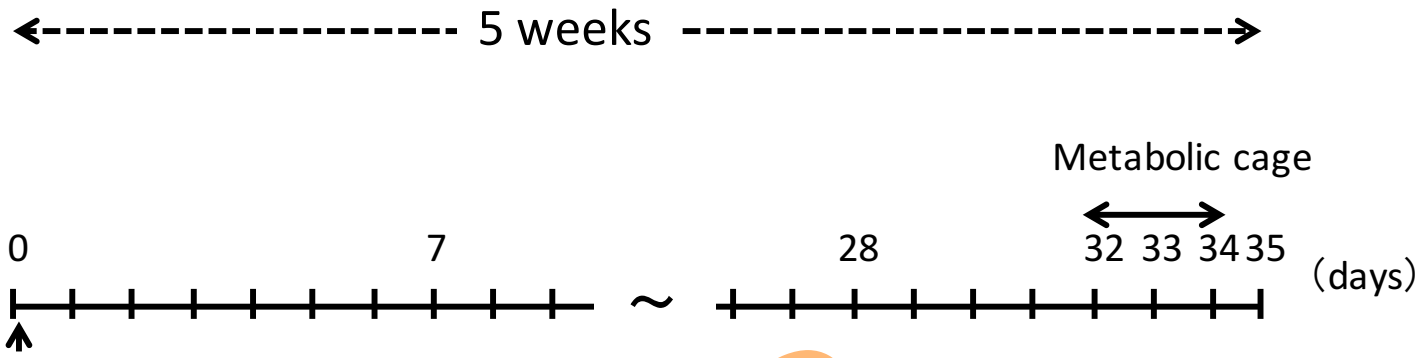


Poly-P
High phosphate diet



	Diet 100 g（g）			
	LP	CP	Mono HP	Poly HP
AIN93G modified Mix	59.5	59.5	59.5	59.5
Egg White	20	20	20	20
AIN93G modified Mineral Mix	1.5645	1.5645	1.5645	1.5645
Sucrose	10.4371	7.8871	5.2521	4.7421
$CaCO_3$	1.4984	1.4984	1.4984	1.4984
KH_2PO_4 or $K_5P_3O_{10}$	0	2.55	5.185	5.695
Soy Bean Oil	7	7	7	7
Total	100	100	100	100

Time schedule



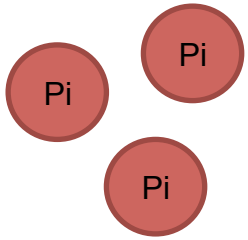
Start
5 weeks of age

Sacrificed
10 weeks of age

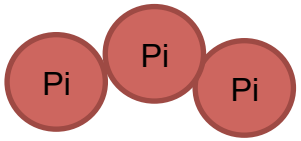
Test diet

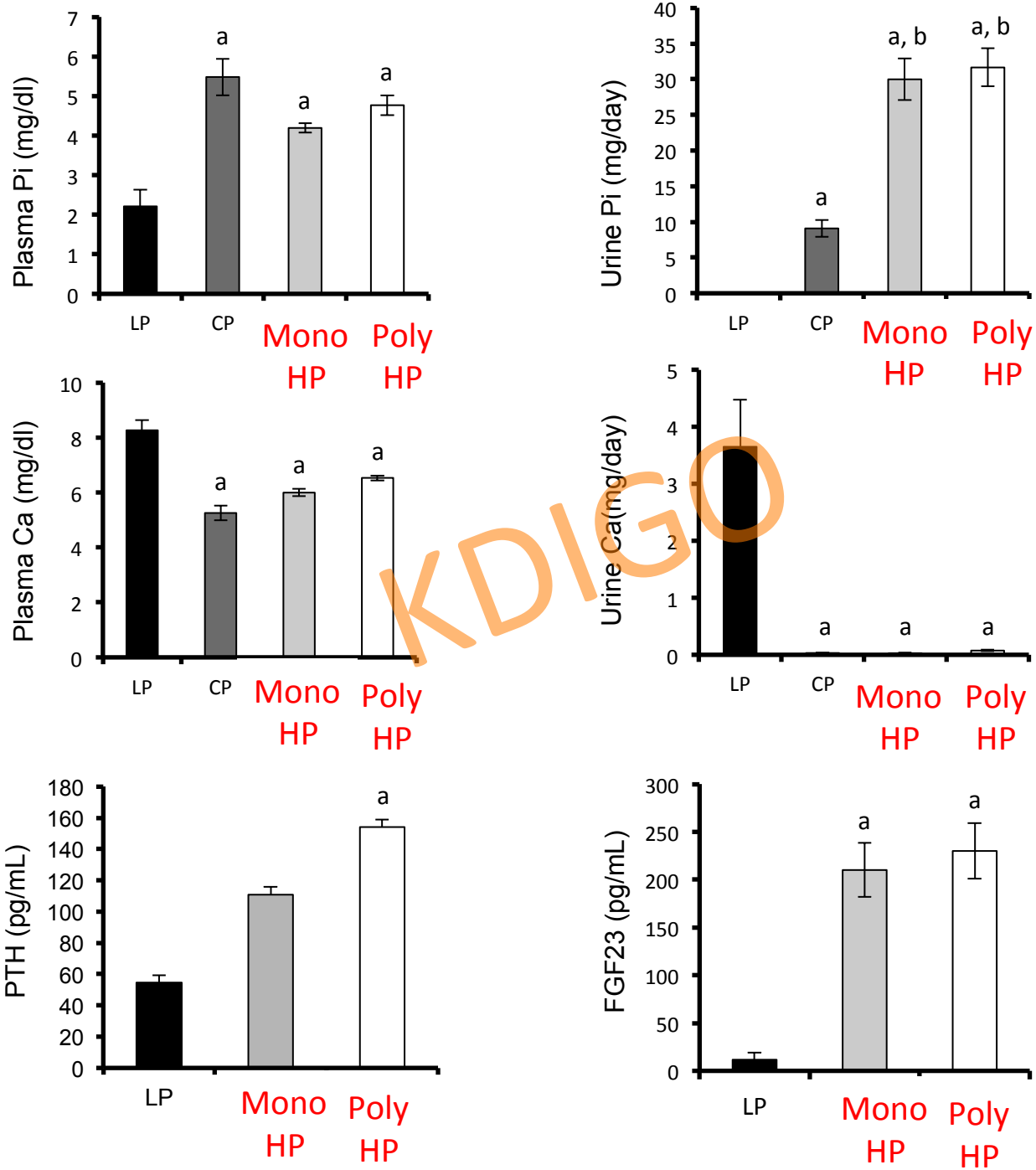
1. Low Pi diet
2. Control Pi diet
3. Monophosphate HP
4. Polyphosphate HP

Mono-P
High phosphate diet



Poly-P
High phosphate diet

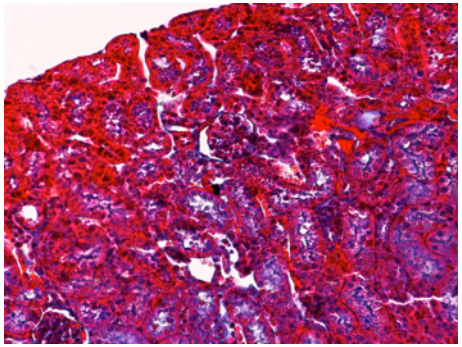



Plasma Pi (mg/dl)
0
1
2
3
4
5
6
7
a
a
a
LP
CP
Mono HP
Poly HP
Urine Pi (mg/day)
0
5
10
15
20
25
30
35
40
a
a, b
a, b
LP
CP
Mono HP
Poly HP
Plasma Ca (mg/dl)
0
2
4
6
8
10
a
a
a
LP
CP
Mono HP
Poly HP
Urine Ca(mg/day)
0
1
2
3
4
5
a
a
a
LP
CP
Mono HP
Poly HP
PTH (pg/mL)
0
20
40
60
80
100
120
140
160
180
a
LP
Mono HP
Poly HP
FGF23 (pg/mL)
0
50
100
150
200
250
300
a
a
LP
Mono HP
Poly HP

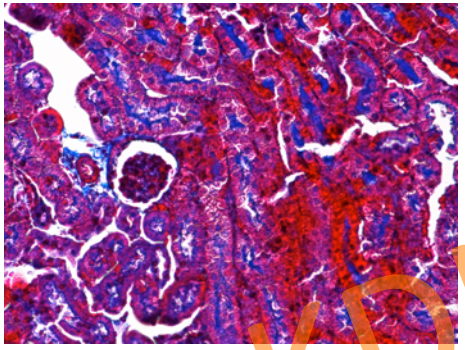
Polyphosphate diet induced renal fibrosis and calcification.

Masson trichrome stain

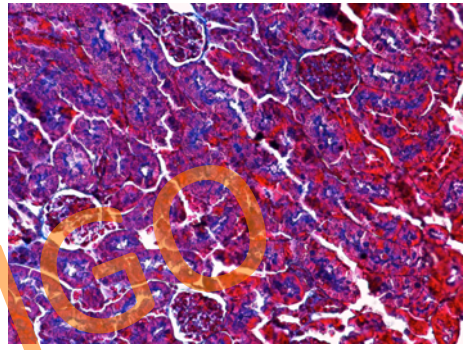
LP



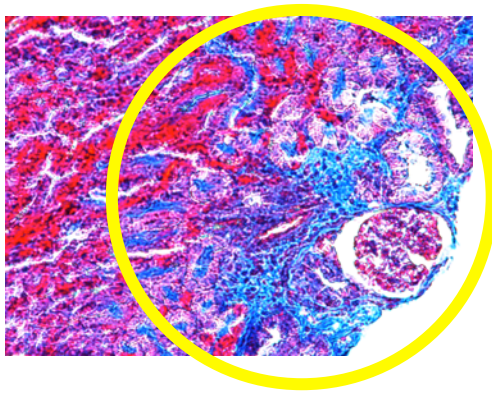
CP



Mono HP

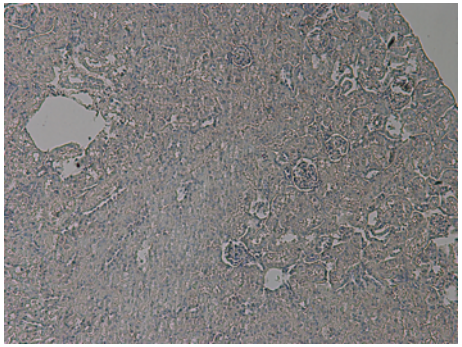


Poly HP

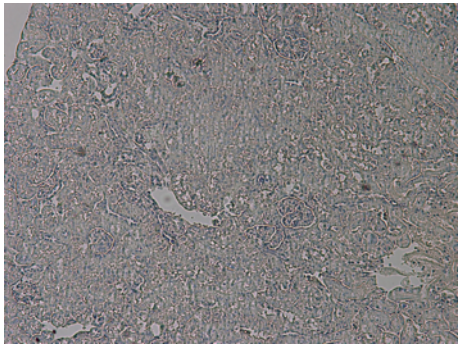


Von Kossa Calcium stain

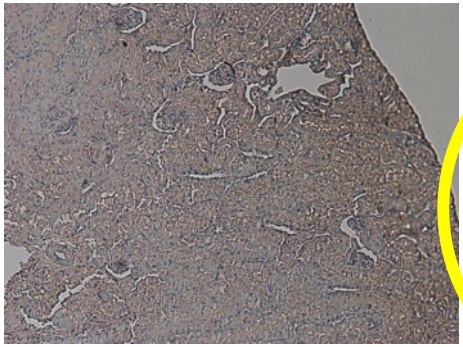
LP



CP

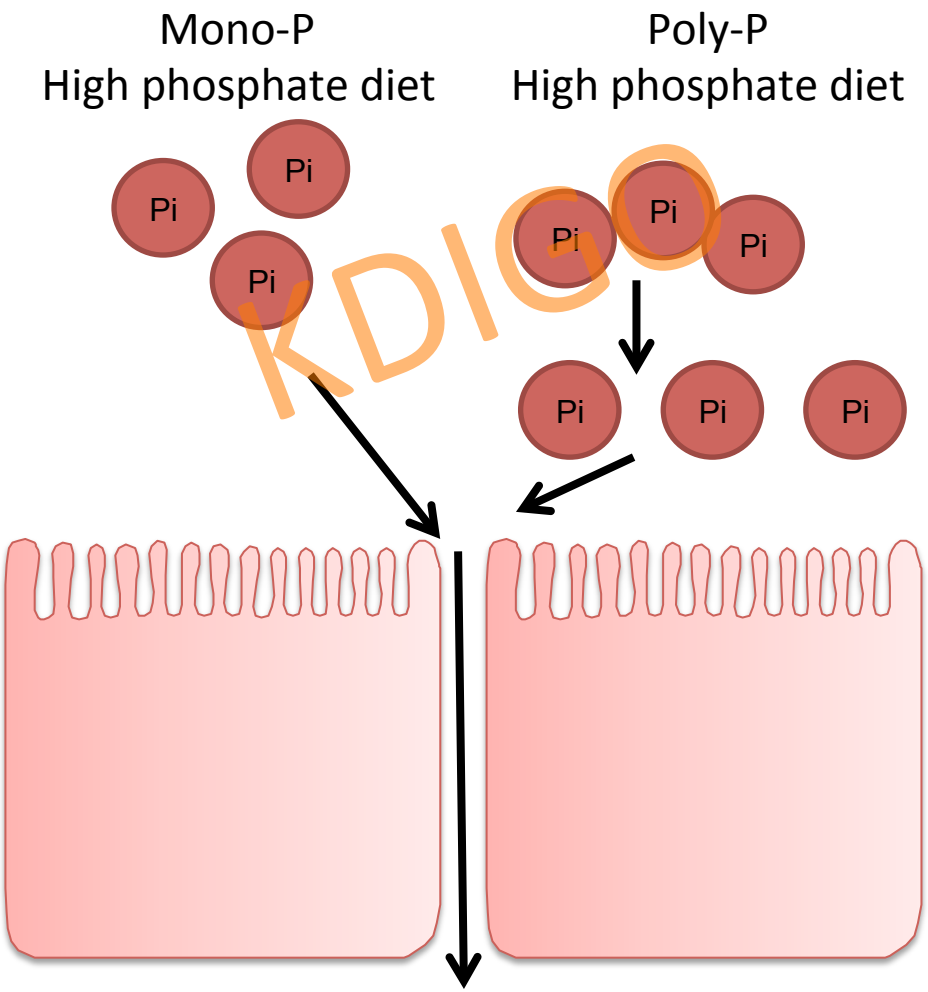


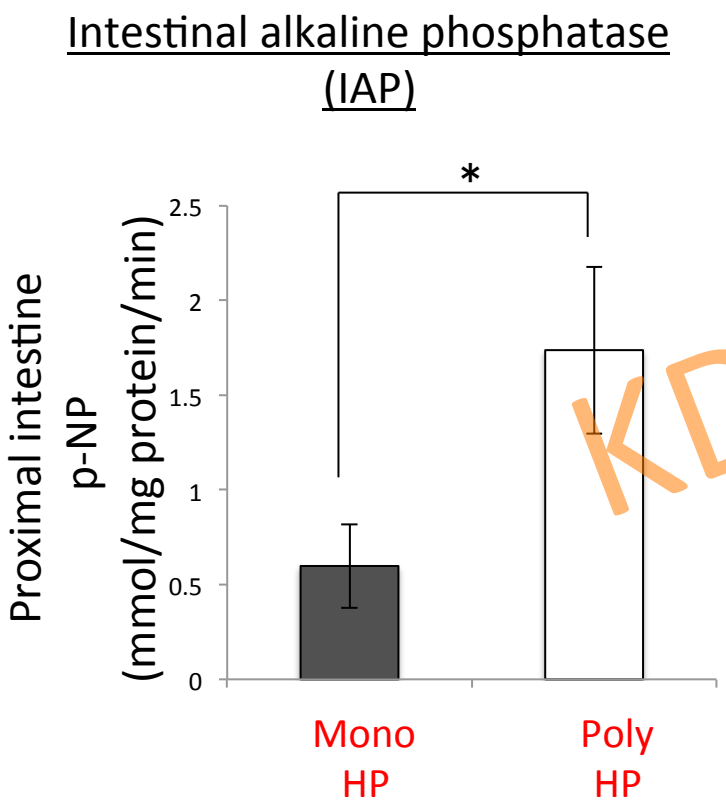
Mono HP

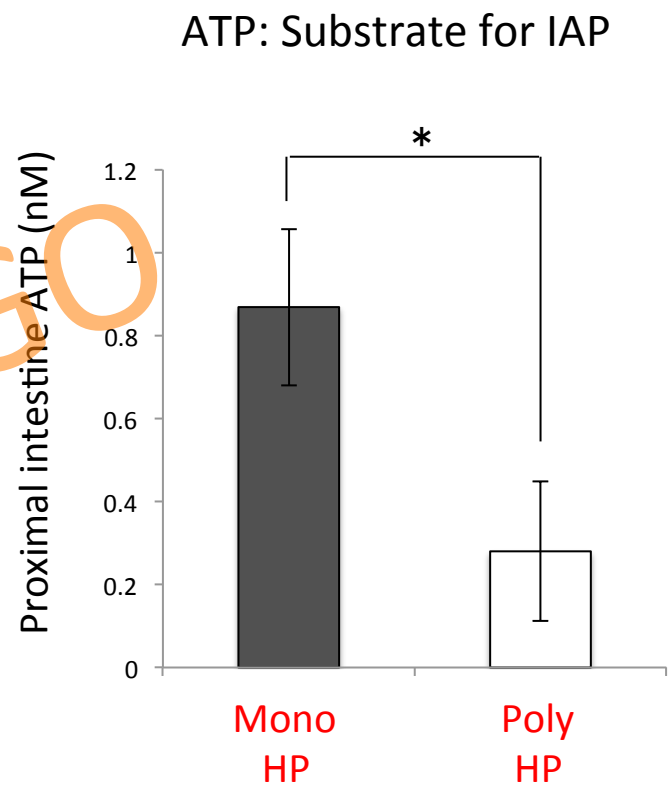


Poly HP

What is the difference between mono-P diet and poly-P diet?

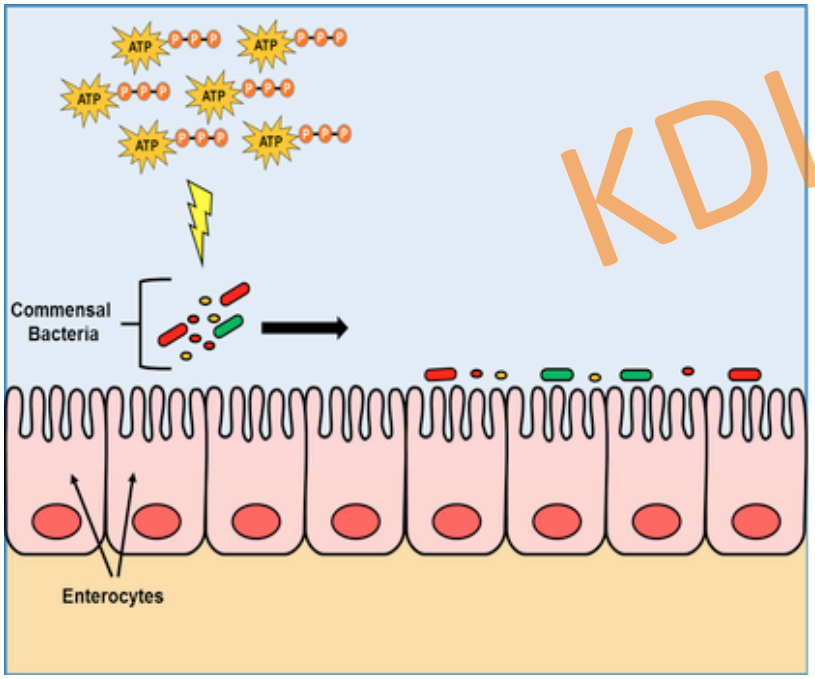


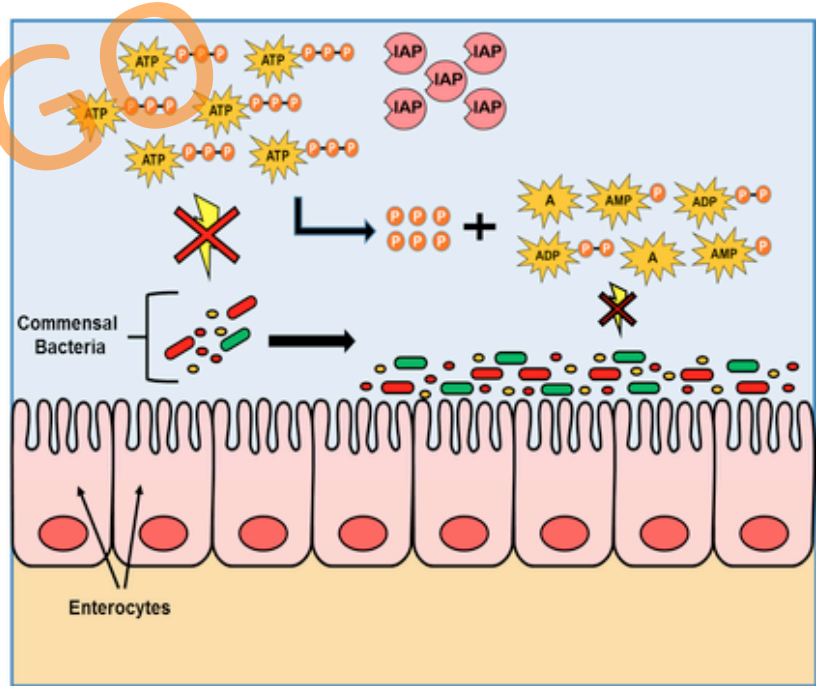

Intestinal alkaline phosphatase (IAP)
Proximal intestine p-NP (mmol/mg protein/min)
2.5
2
1.5
1
0.5
0
*
Mono HP
Poly HP


ATP: Substrate for IAP
Proximal intestine ATP (nM)
1.2
1
0.8
0.6
0.4
0.2
0
*
Mono HP
Poly HP

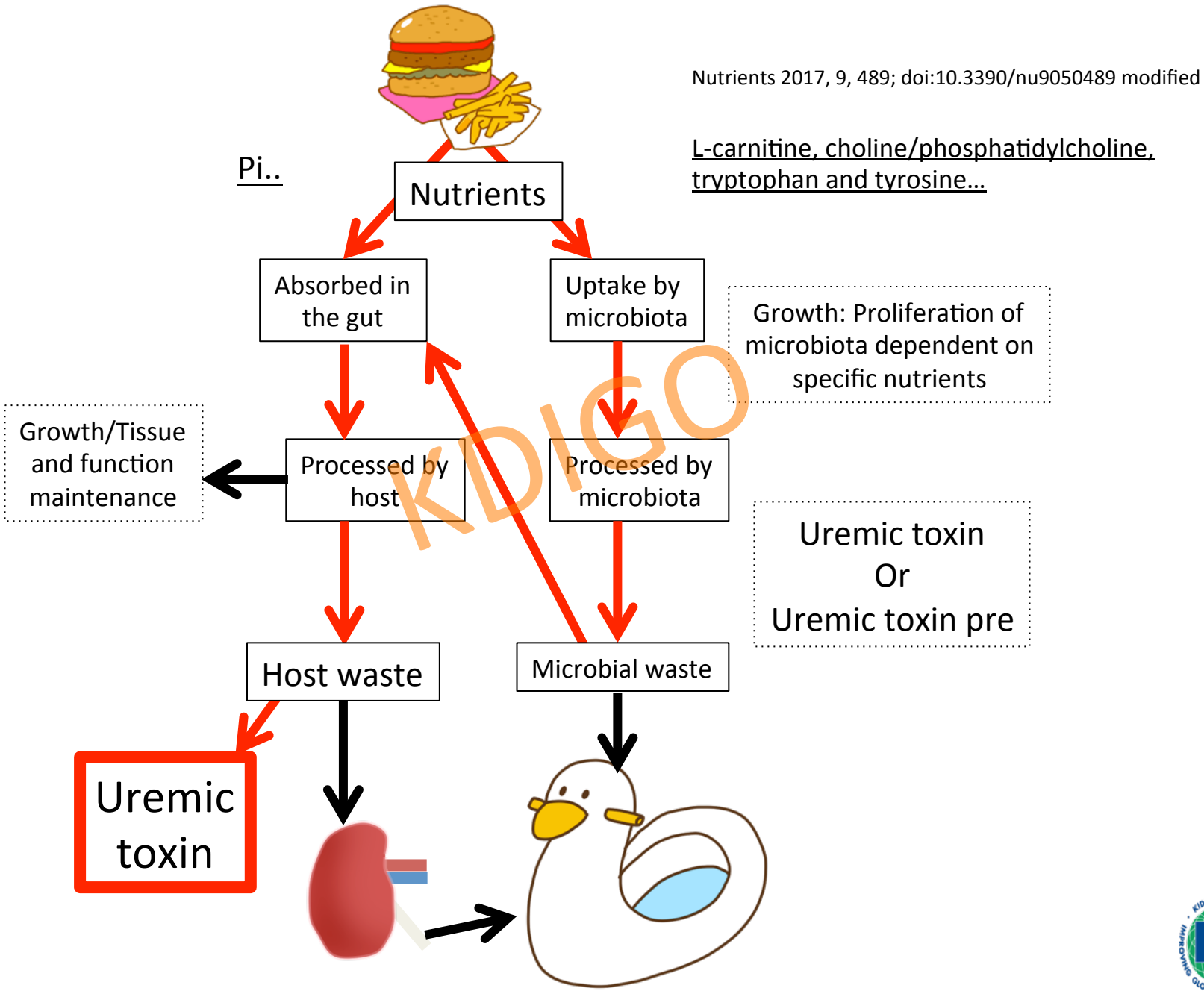
Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase

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. Hodin[1]

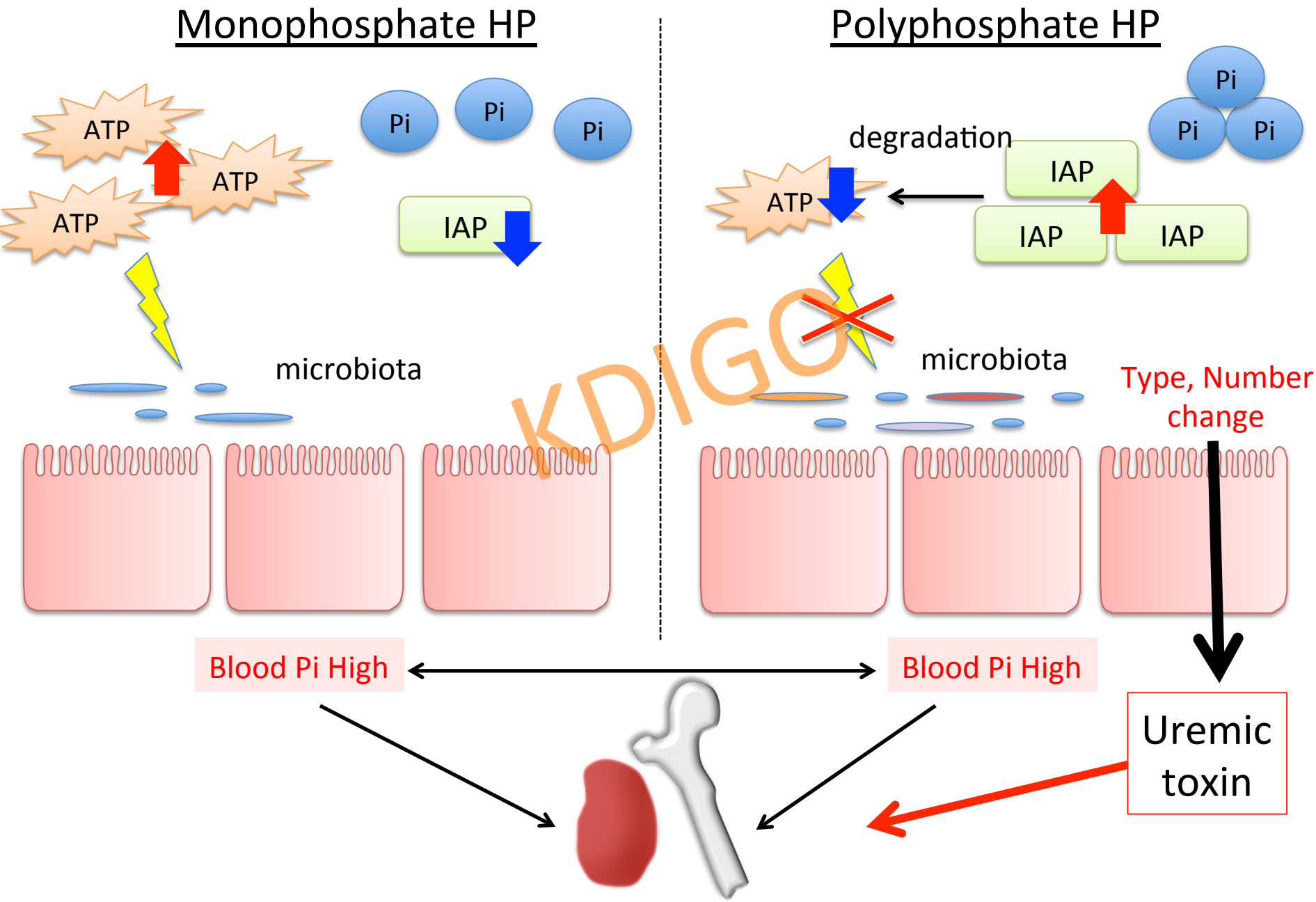




Microbiota–host interaction and modulation by dietary nutrients.



Hypothesis


Monophosphate HP
Polyphosphate HP
ATP
ATP
ATP
Pi
Pi
Pi
IAP
microbiota
Pi
Pi
Pi
degradation
IAP
IAP
IAP
ATP
microbiota
Type, Number change
KDIGO
Blood Pi High
Blood Pi High
Uremic toxin

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.