Lactulose as a Prebiotics, and Enhancement of Calcium and Magnesium Absorption

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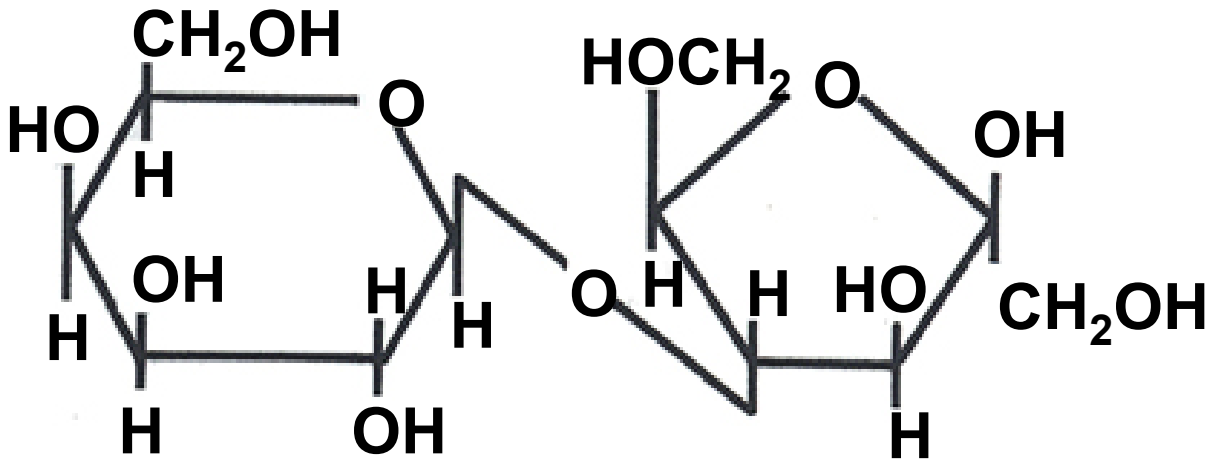
- **Introduction to lactulose**
- **Physiological function of lactulose as prebiotics**
- **Enhancements of calcium and magnesium absorptions by lactulose**

Introduction of Lactulose

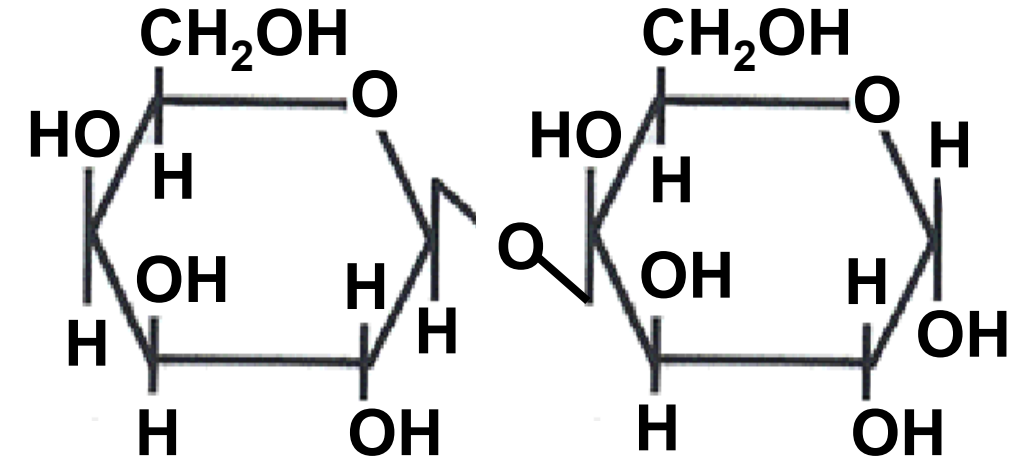
Molecular formula of **lactulose**



4-*O*-β-D-galactopyranosyl-D-fructofuranose

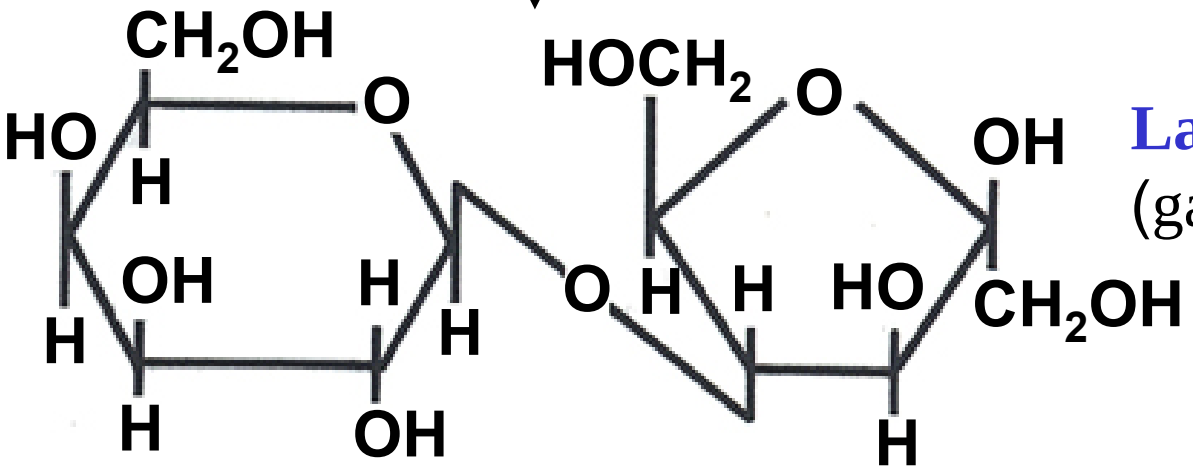
$C_{12}H_{22}O_{11}$: Mr=342.3 g/mol

Formation of lactulose



Lactose
(galactose - **glucose**)

Isomerization



Lactulose
(galactose - **fructose**)

Physicochemical characteristics

	Anhydride form	Trihydrate form
Appearance	White crystalline powder	White crystalline powder
Molecular formula	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}3H_2O$
Water content	0%	13.6%
Melting point	169°C	68°C
Heat of solution	- 4 kJ/mol	34 kJ/mol
Degree of sweetness	0.48 - 0.62	no reports

Lactulose Syrup and Powder

Lactulose syrup & powder

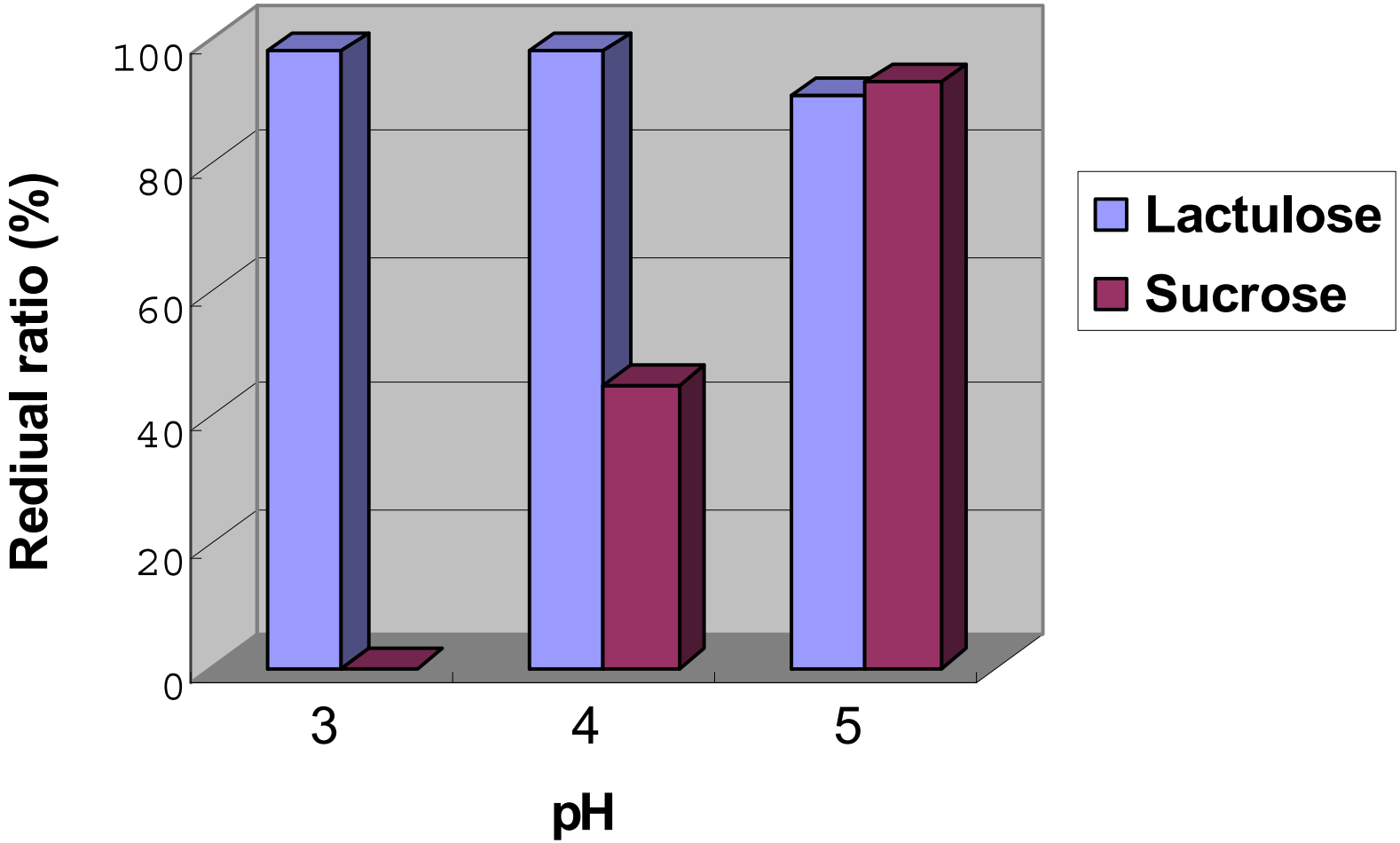
Historical development of lactulose

1930 Preparation by Montgomery and Hudson
1957 Discovery as a growth factor for bifidobacteria by Petuely
1959 Application to chronic constipation by Petuely
1966 Application to chronic portal systemic encephalopathy by Bircher

Applications at Morinaga Milk Industry

1960 Sale of first infant formula containing lactulose
1975 Pharmaceutical authorization of lactulose syrup
1992 Approval of lactulose for foods with specified health uses
1996 Sale of “MAIASA-SOHKAI” as a food with specified health uses
1997 Pharmaceutical authorization of lactulose crystalline powder

Thermostability of lactulose



Heating：130 degree, 10 minutes

Concentration of solution: 10%：(w/w)

Production site of lactulose

Fuji Plant (Japan)

Milei Plant (Germany)

Applications of lactulose

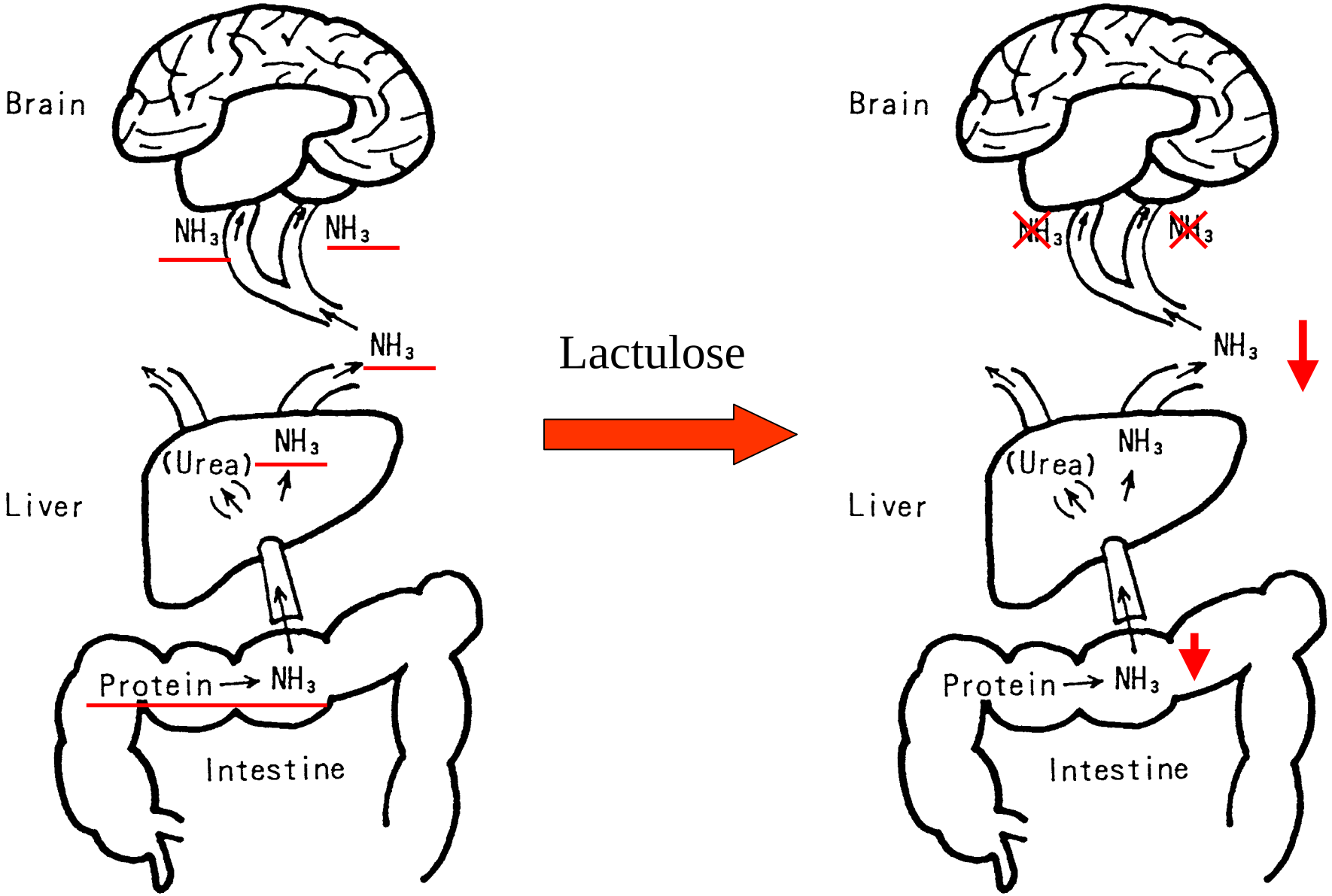
Pharmaceuticals

- for hepatic encephalopathy
- for chronic constipation

Syrup form

Powder form

Mechanism of hepatic encephalopathy



Applications of lactulose

Food and nutrition

- infant formula
- health foods

Infant formula

Soft drink

Yogurt

Applications of lactulose

Animal feed

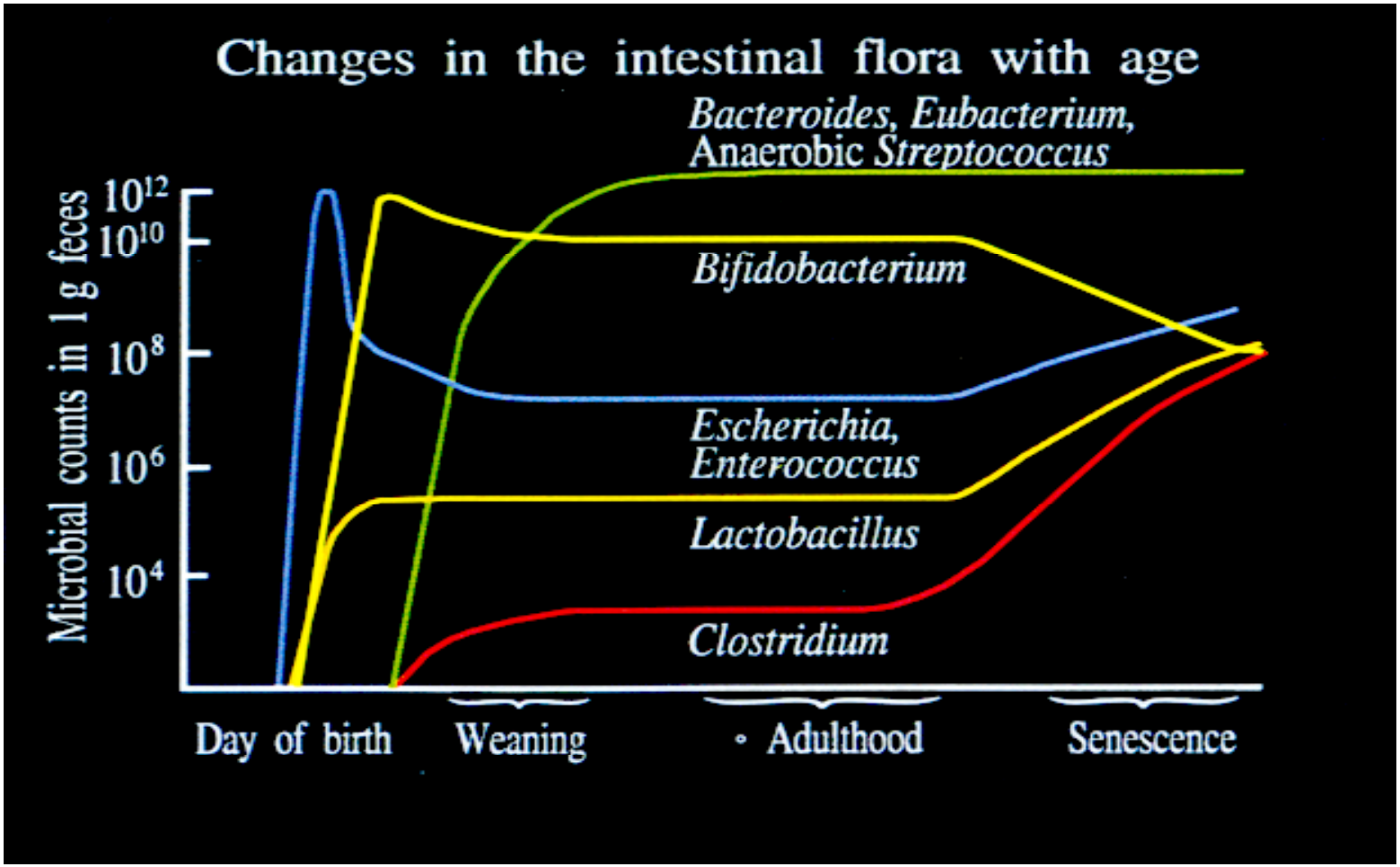
- piglets
- cattle
- dogs and cats

Dog food

Cat milk

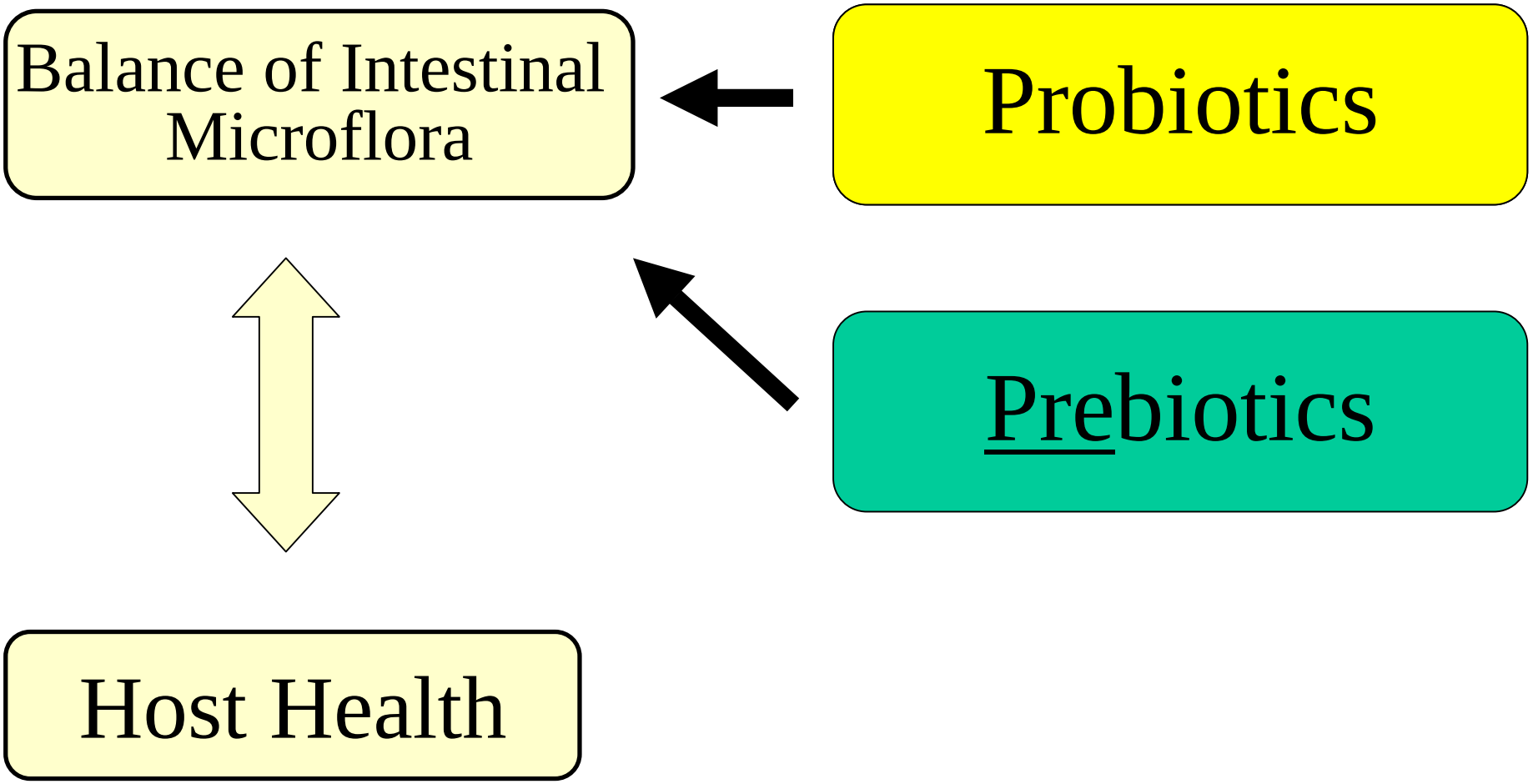
Physiological function of Lactulose as a prebiotics

Changes in Intestinal Flora with Age



Bifidobacteria Microflora 1: 3 (1982)

Probioteics



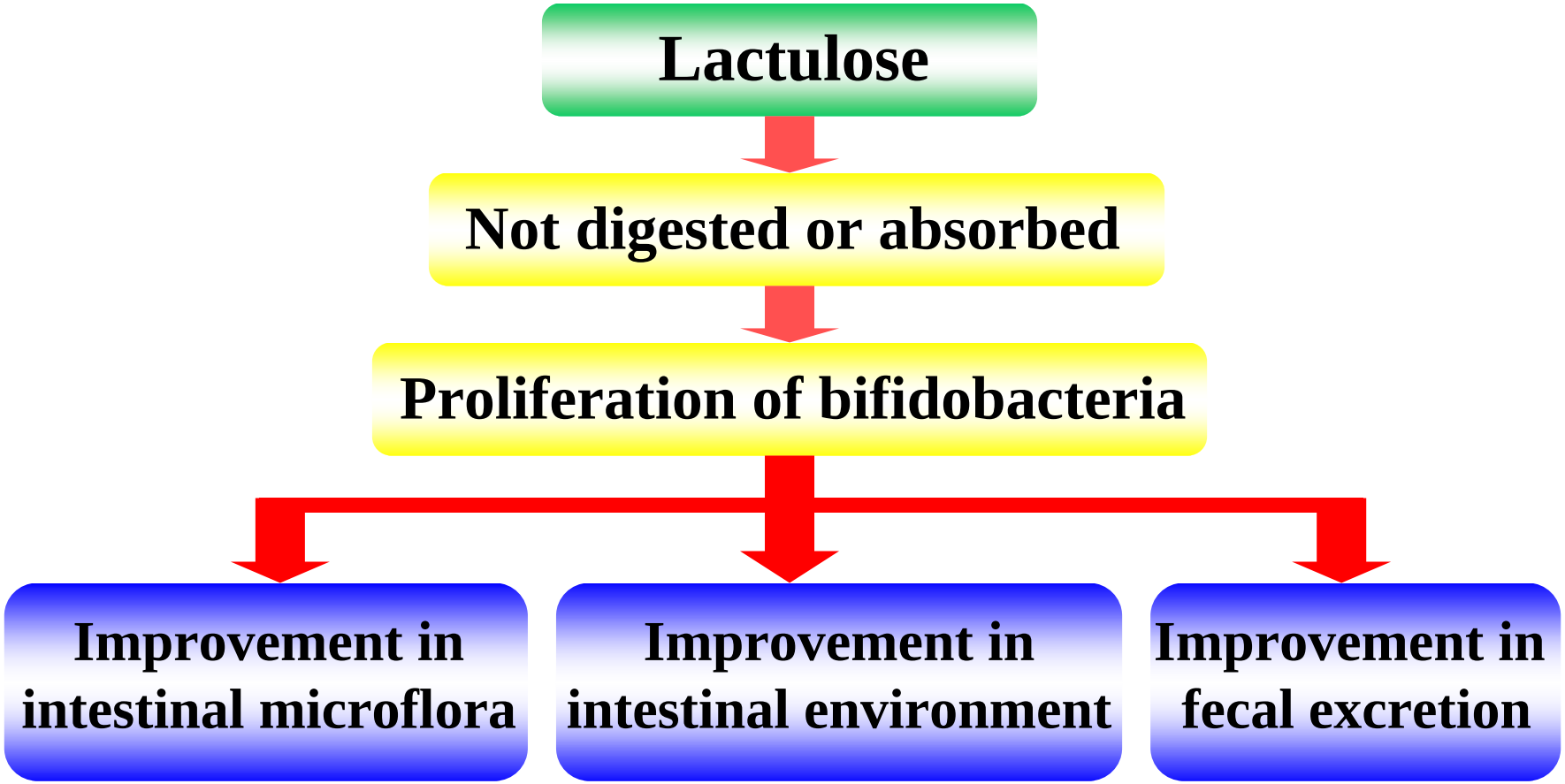
Prebiotics

Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health.

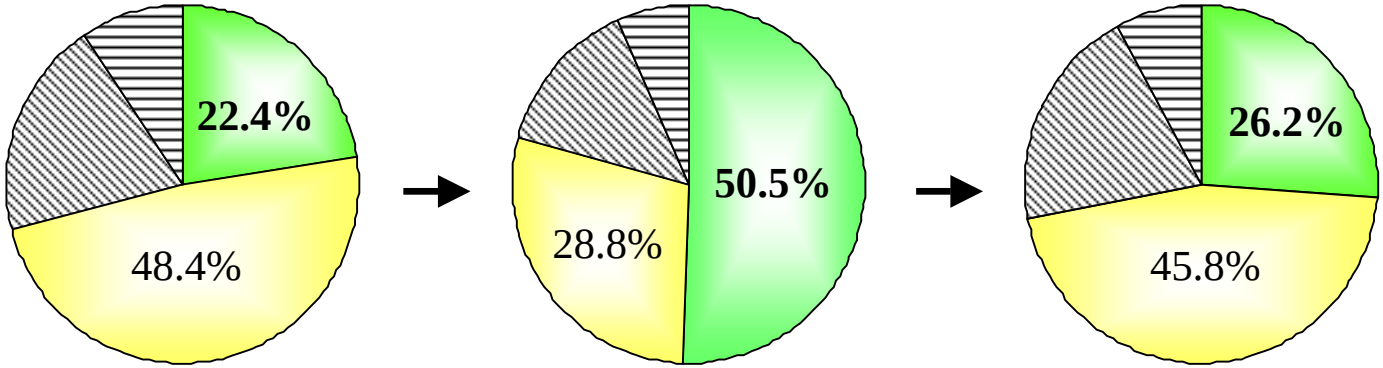
Gibson et.al. (1995)

Gibson et. al., J. Nutr. 125: 1401 (1995)

Lactulose as probiotic



Improvement in the fecal microflora

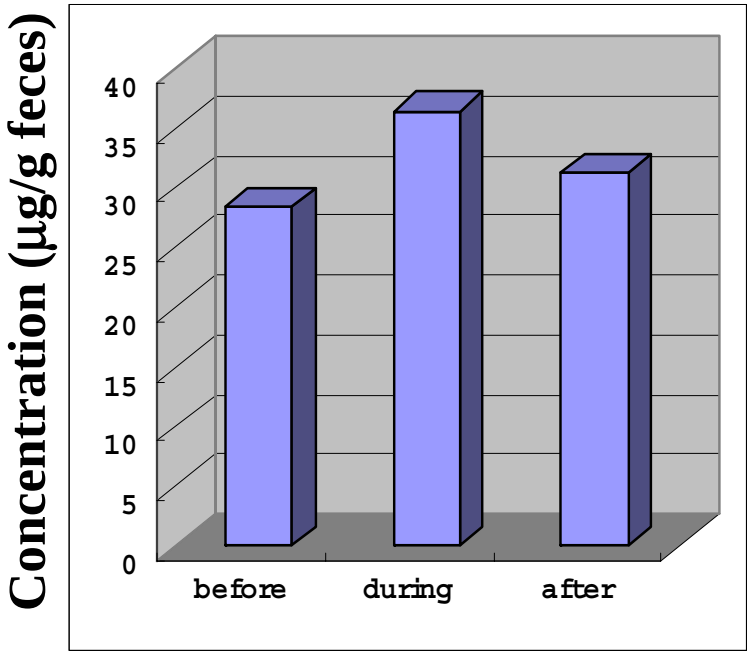


Changes in the fecal microflora induced by ingestion of 4 g of lactulose

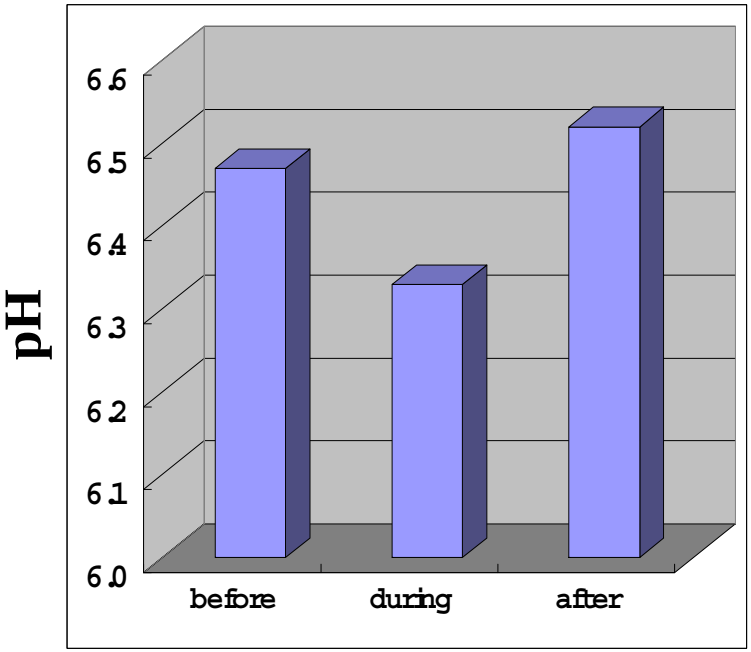
Bifidobacteria **Bacteroides**
Eubacteria **Others**

Mizota et al., Milchwissenschaft 57: 312 (2002)

Improvement in intestinal environment: fecal acetic acid and fecal pH



Fecal acetic acid

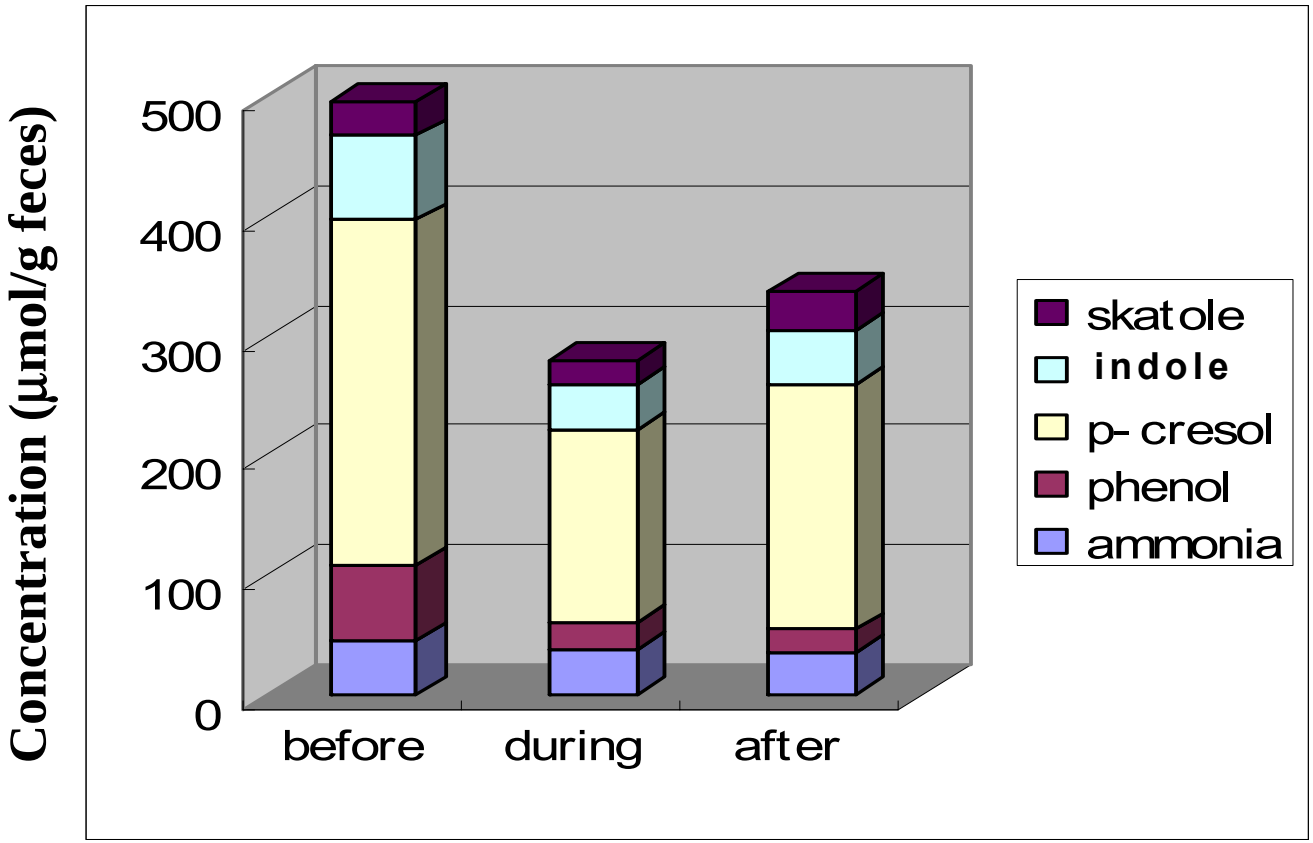


Fecal pH

Improvement in intestinal environment induced by ingestion of 4 g of lactulose

Mizota et al., Milchwissenschaft 57: 312 (2002)

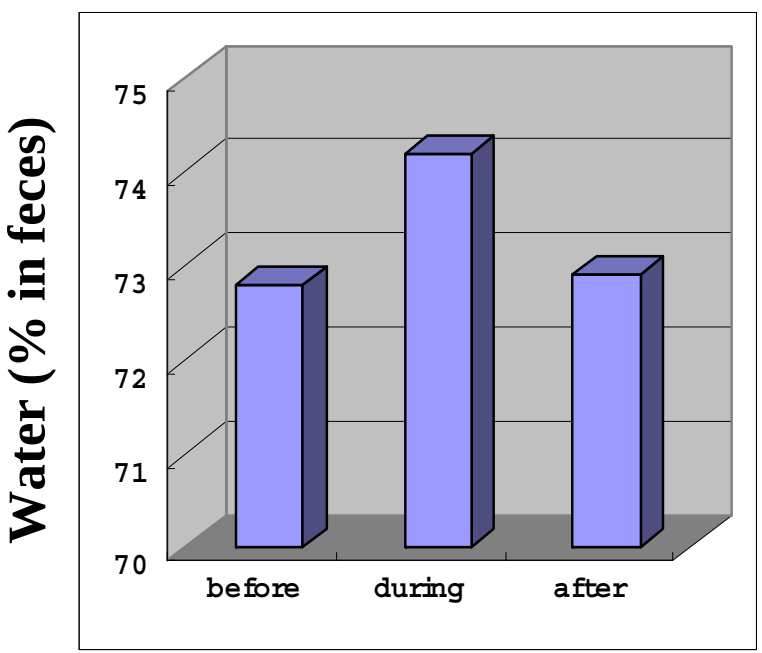
Improvement of intestinal environment: intestinal putrefactive substances



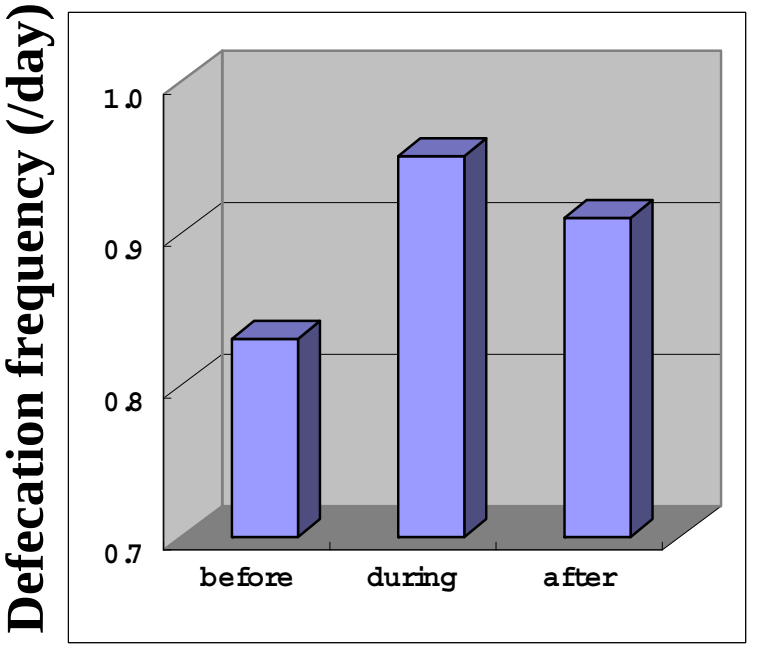
Decrease in intestinal putrefactive substances induced by ingestion of 4 g of lactulose

Mizota et al., Milchwissenschaft 57: 312 (2002)

Improvement in fecal excretion:
fecal water and defecation frequency



Fecal water



Defecation frequency

Improvement in fecal excretion induced by ingestion of 4 g of lactulose

Mizota et al., Milchwissenschaft 57: 312 (2002)

Soft drink: MAIASA-SOHKAI

A food for specific claims:
MAIASA-SOHKAI helps to increase intestinal bifidobacteria and maintain the gastrointestinal tract in good condition.

Lactulose
(bifidus factor)
4 g

Calcium
+
Multivitamin

Other physiological functions of lactulose

- Improvement of blood glucose responses
- Inhibition of secondary bile acid formation
- Activation of immune responses
- Treatment of salmonellosis
- Enhancement of mineral absorption

Enhancement of Calcium and Magnesium Absorption by Lactulose

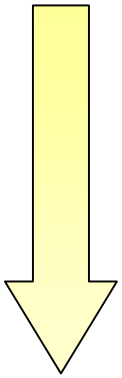
Calcium in milk

Calcium is one of the key nutrients in milk

An adequate calcium intake prevents osteoporosis, hypertension, cancer and kidney stones etc. Body fat reduce (?)

Absorption of **calcium in milk**

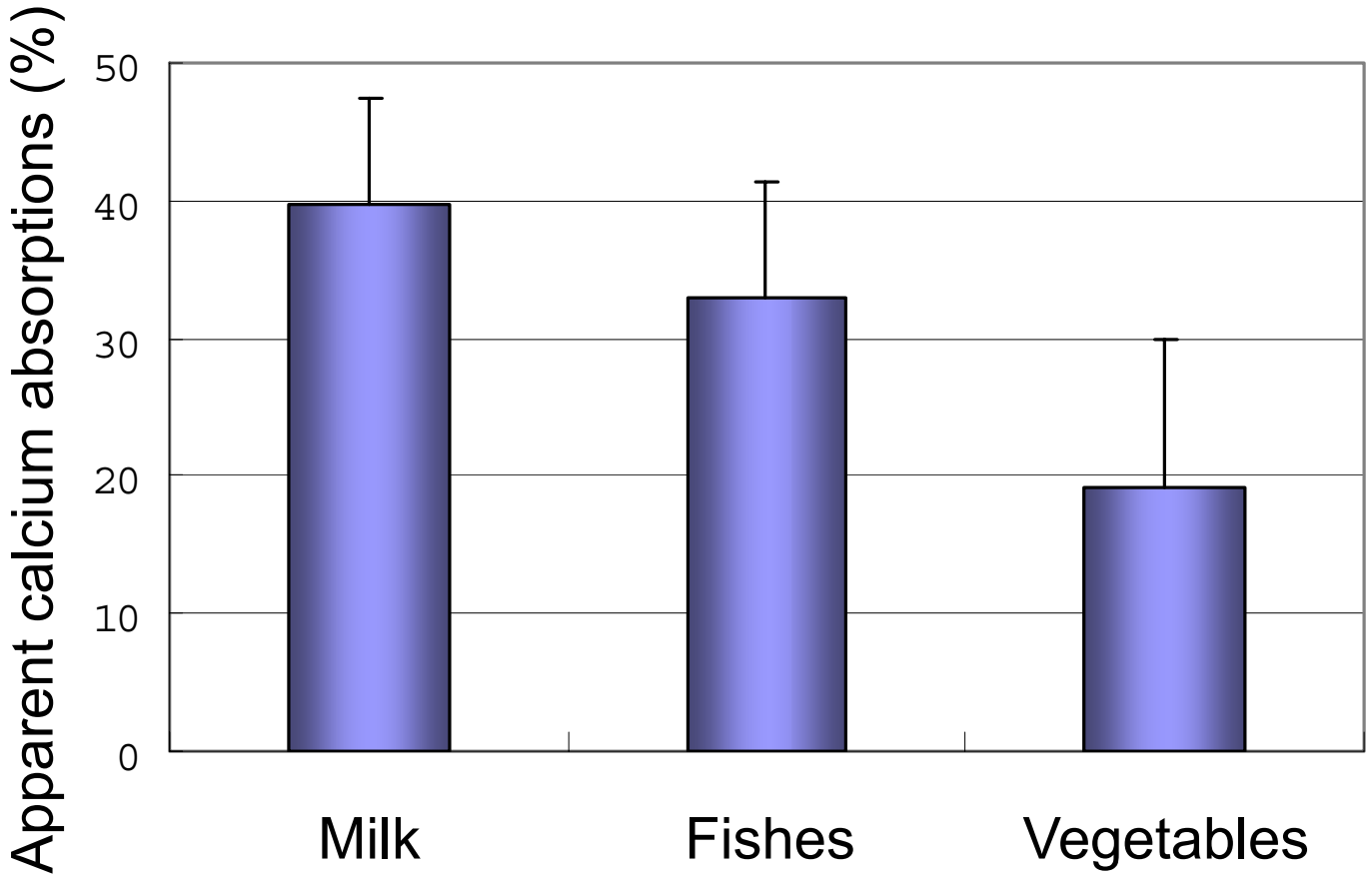
Calcium content of milk is high



Lactose
CPP (Casein phosphopeptide)
Vitamin D

Absorption is also high

Apparent calcium absorptions from foods



Uenishi et al., J Jpn Soc Nutr Food Sci 51: 259 (1998)

Enhancements of Ca and Mg absorptions

Calcium and **magnesium** in food

Does **lactulose** enhance
□□ the absorptions of both **Ca** and **Mg**
in humans?

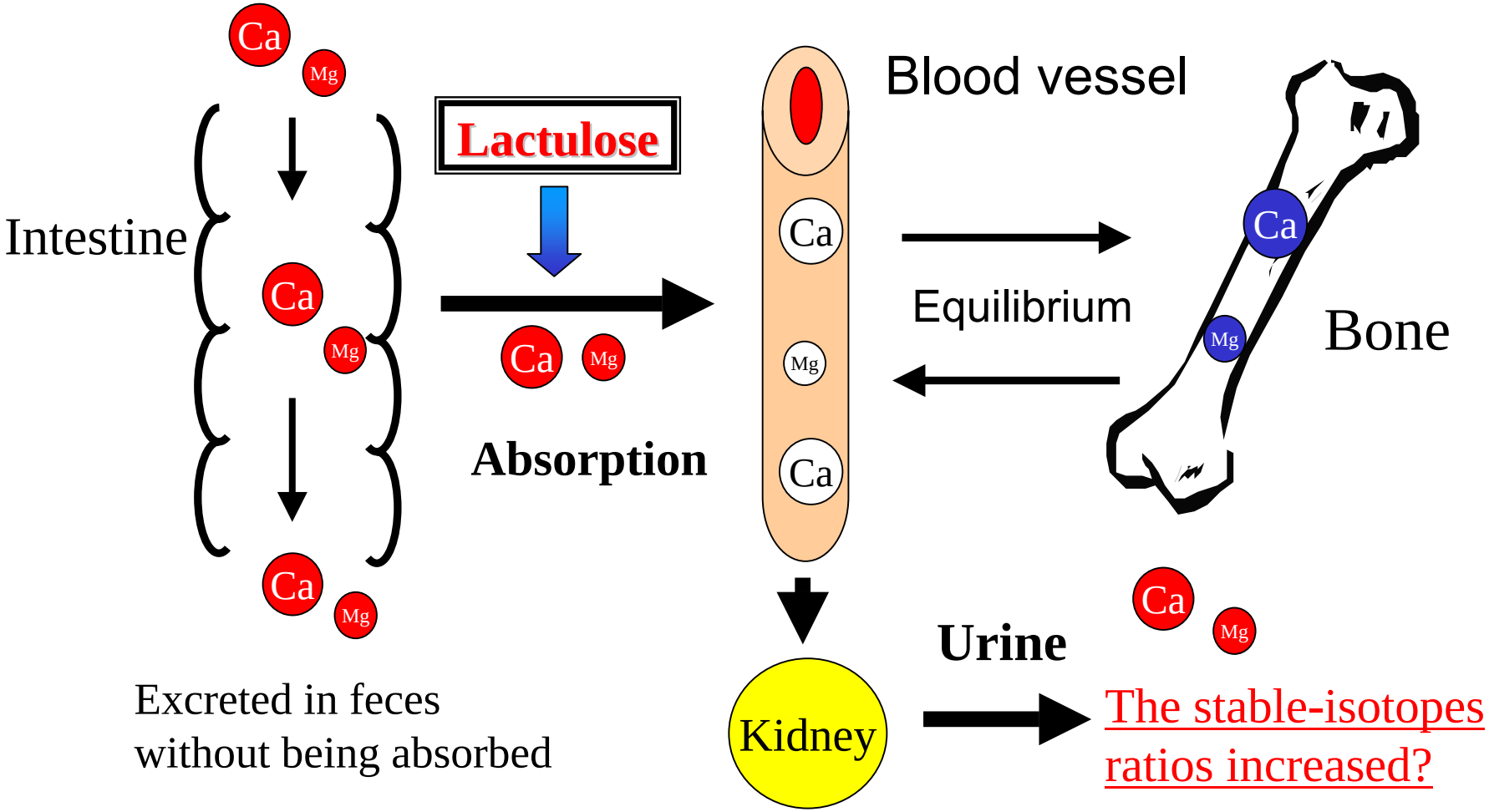
Absorption was evaluated using
a single-labeling isotope method

Evaluation method of Ca/Mg absorption

Stable isotopes

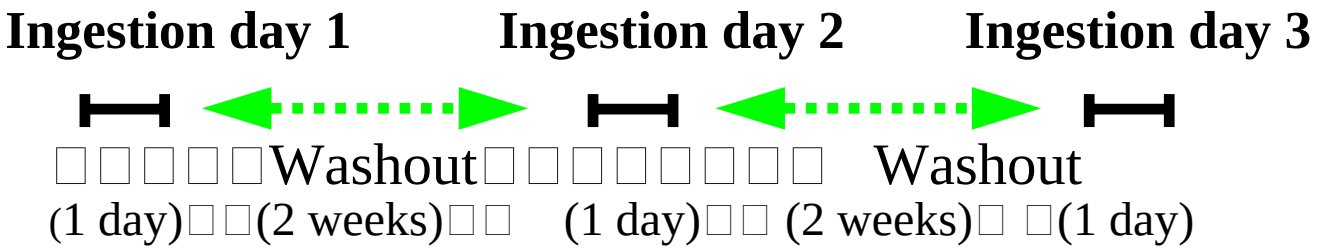
	NA*	ITF**
^{44}Ca	2.1% →	8.2%
^{25}Mg	10.1% →	27.3%

*NA: natural abundance
**ITF: isotope abundance in test food



Trial design

Three-group crossover design



Group A (*n*=8) **Placebo** **Low dose** **High dose**

Group B (*n*=8) **Low dose** **High dose** **Placebo**

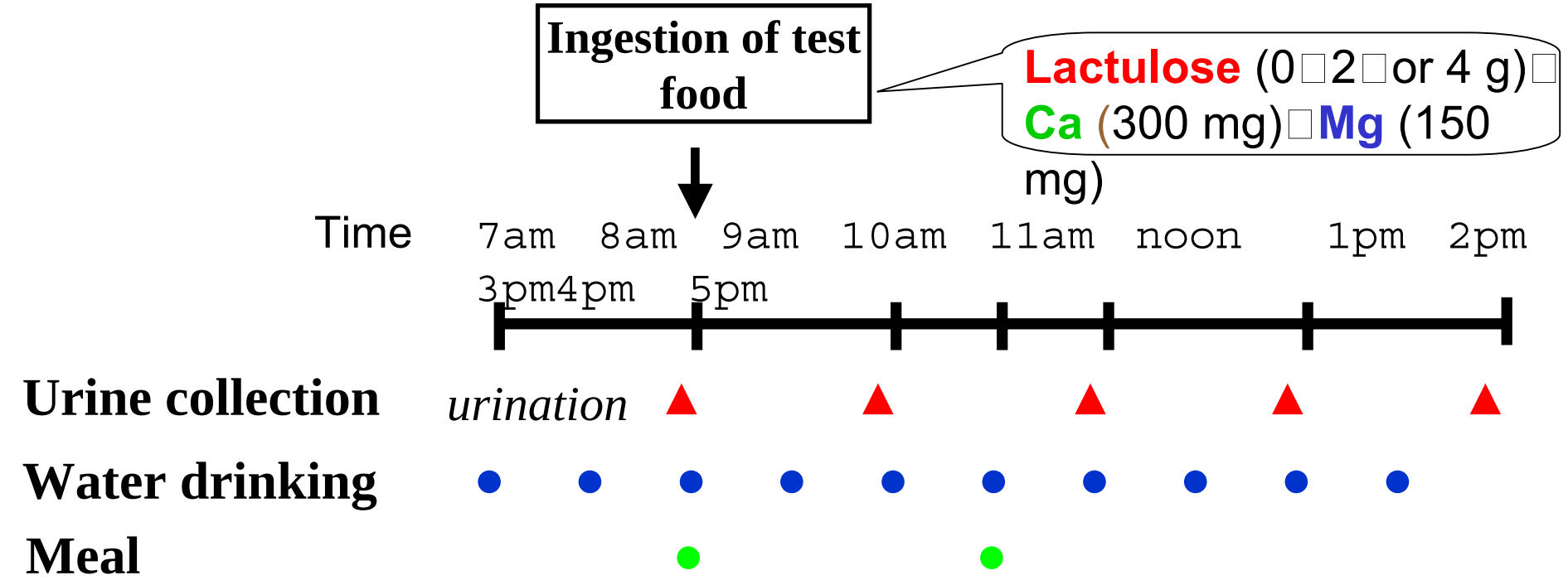
Group C (*n*=8) **High -dose** **Placebo** **Low dose**

(Healthy adult men)

Placebo:	Lactulose (**0 g**) + Ca (300 mg) + Mg (150 mg)
Low dose:	Lactulose (**2 g**) + Ca (300 mg) + Mg (150 mg)
High dose:	Lactulose (**4 g**) + Ca (300 mg) + Mg (150 mg)

Seki et al., J Nutr Sci Vitaminol 53: 5 (2007)

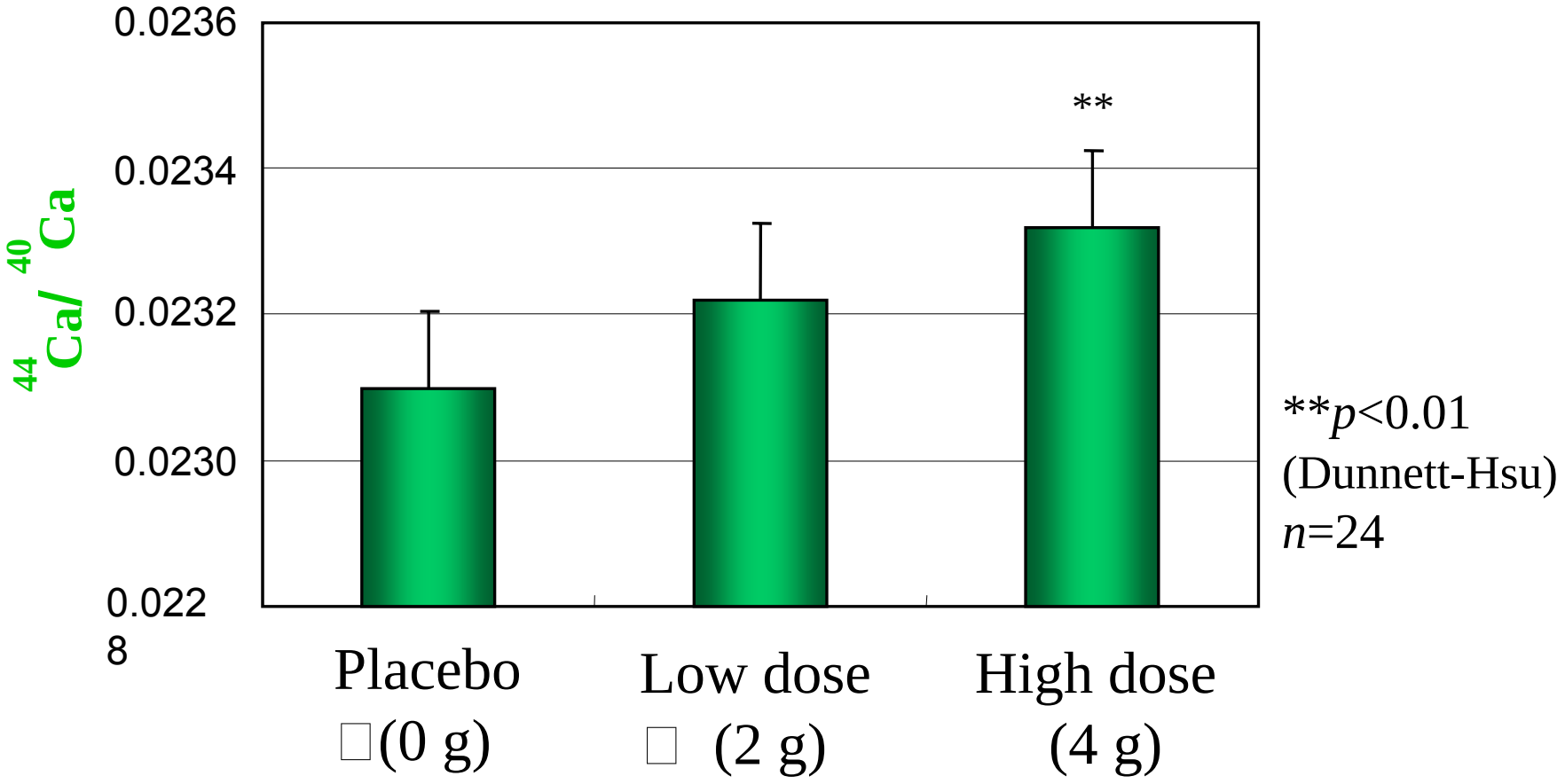
Schedule of the ingestion day



- □Test food was ingested at 0900 hours
- □All urine was collected every 2 h from 0900 to 1700 hours (▲)
- □Subjects drank 150 ml of water every hour (●)
- □Breakfast/lunch (●)

Seki et al., J Nutr Sci Vitaminol 53: 5 (2007)

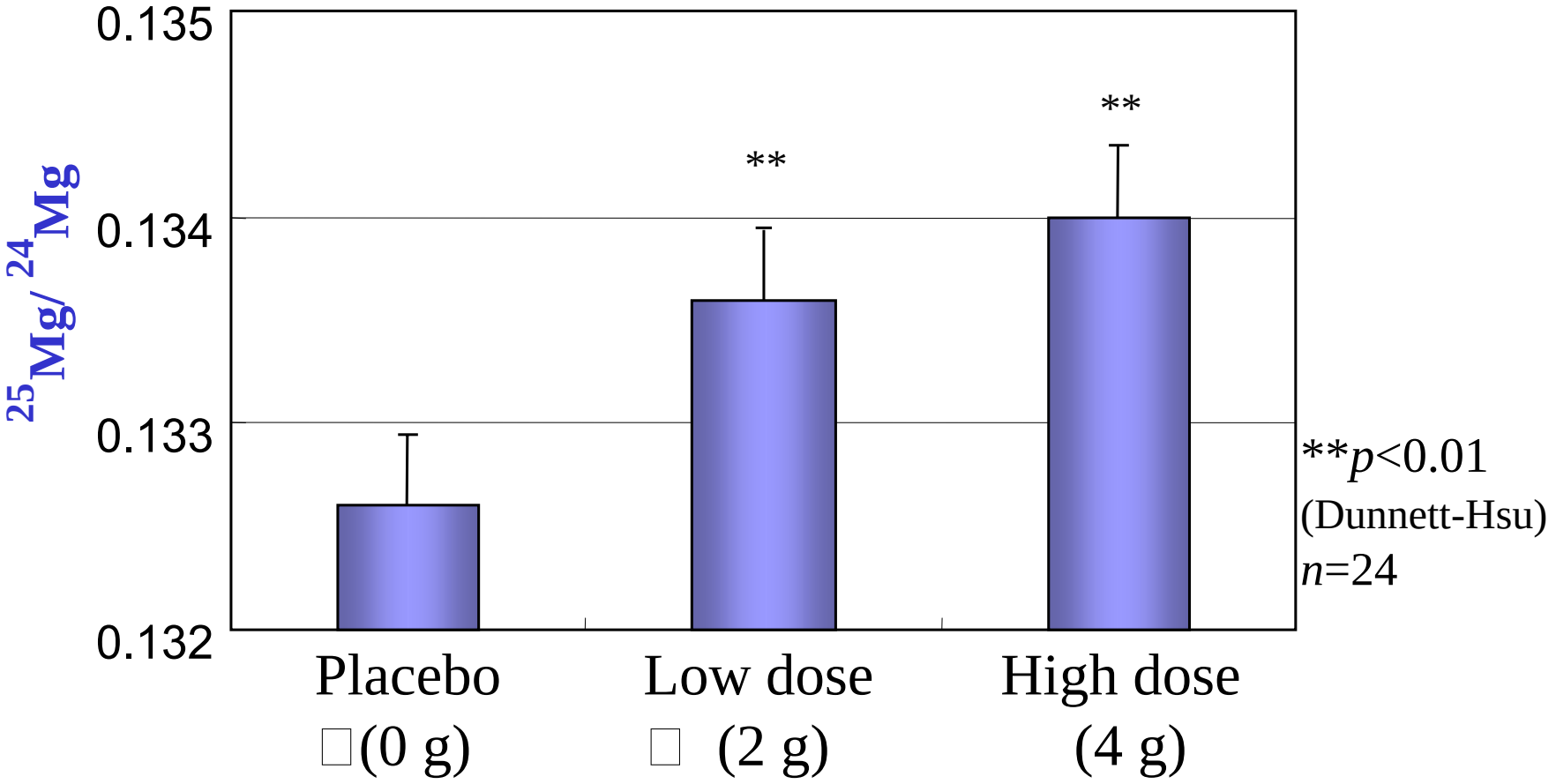
Effect of lactulose on ratio of urinary Ca



Ratio of Ca stable isotopes ($^{44}Ca/^{40}Ca$) in combined urine increased with lactulose dose

Combined urine: all of the urine collected for 8 h after test-food ingestion

Effect of lactulose on ratio of urinary Mg

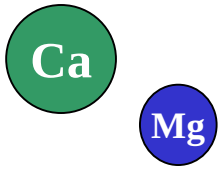


Ratio of Mg stable isotopes ($^{25}Mg/^{24}Mg$) in combined urine increased with lactulose dose

combined urine: all of the urine collected for 8 h after test-food ingestion

A single-labeling method for Urine

Oral intake of Stable Isotopes



Measure the ratios of stable isotopes in urine

Do minerals in urine come from test food or not?

Advantages

- No intravenous injection
- Direct check of the absorption for minerals

Disadvantages

- Needs cross over design
- Difficult to quantification

Hypothesized action mechanism

Arrival of lactulose at the large intestine

↓

Conversion of lactulose to organic acids

↓

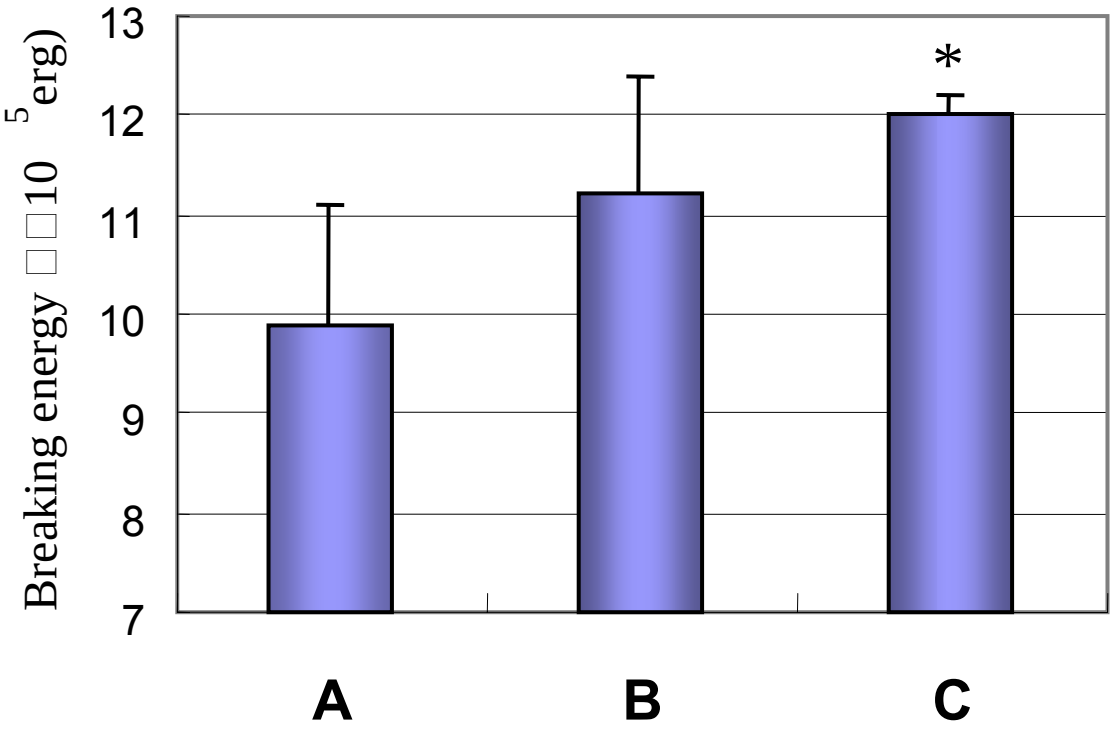
Decrease in intestinal pH

Direct effect of lactulose on the mineral absorption (?)

Direct effect of organic acids on intestinal permeability

Increases in solubilities of Ca and Mg salts

The effects of lactulose on rat bone



*p<0.05

A: Milk calcium　B: A+*B. longum*　C: A+*B. longum*+lactulose (3%)

The femur fracture properties of ovariectomized osteoporosis model rat

Igarashi et al., Bifidus 7: 139 (1994)

Example of product

4g Lactulose
350mg Calcium
175mg Magnesium
/2 sachets/day
For postmenopausal women

Summary

- **Lactulose has many physiological functions and can be applied in many areas as a probiotic**

- **Our clinical trials confirmed that low-dose lactulose has positive effects on calcium and magnesium absorptions in humans**

Thank you for your attention