



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

**Nobuo Seki**

Food Research & Development Laboratory,  
Morinaga Milk Industry Co., Ltd.,



# Contents

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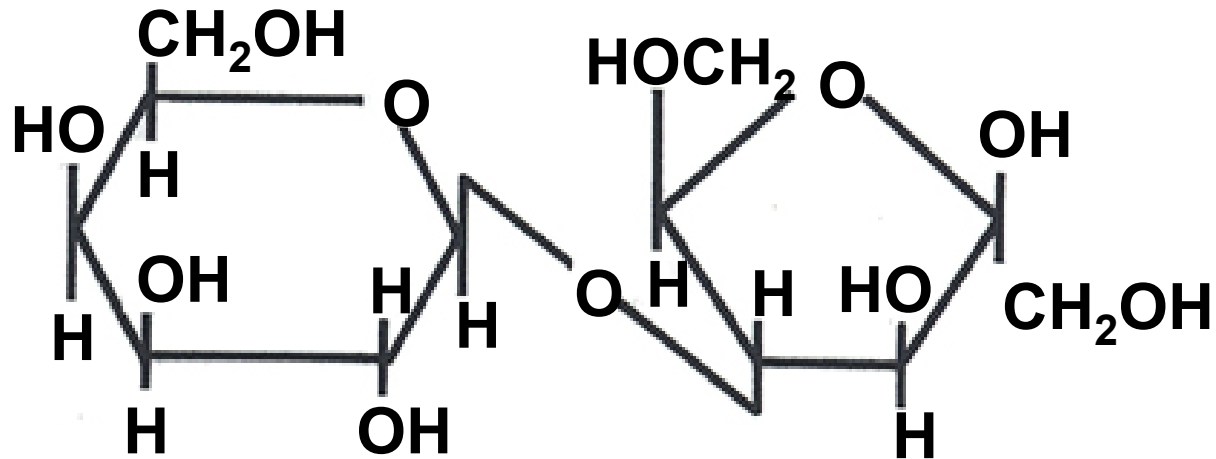
- **Introduction to lactulose**
- **Physiological function of lactulose as prebiotics**
- **Enhancements of calcium and magnesium absorptions by lactulose**



# **Introduction of Lactulose**



# Molecular formula of lactulose

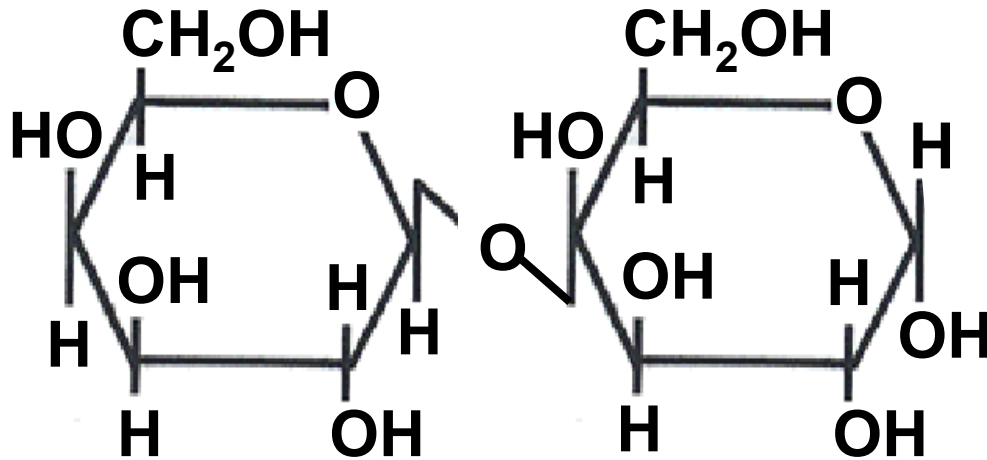


**4-O-β-D-galactopyranosyl-D-fructofuranose**

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

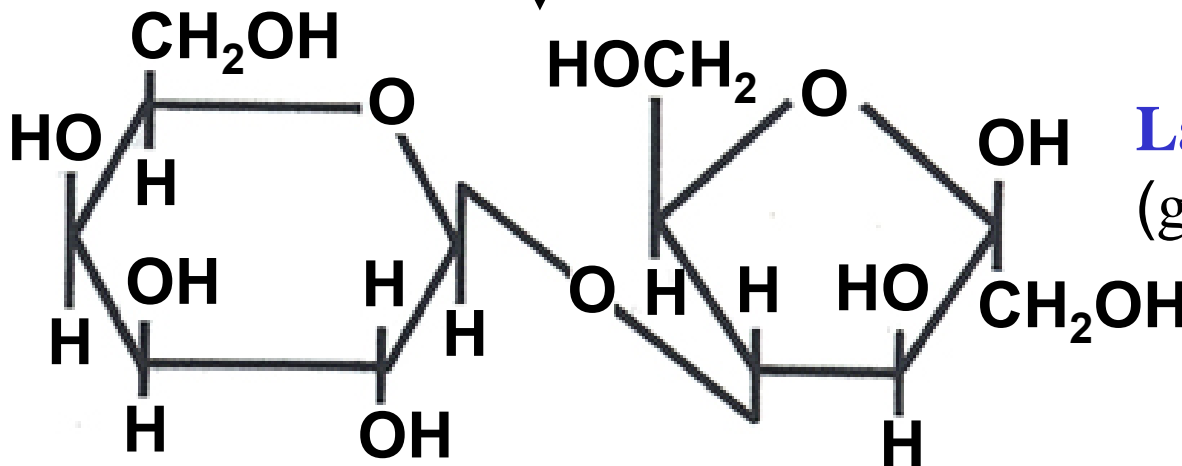


# Formation of lactulose



**Lactose**  
(galactose - **glucose**)

Isomerization



**Lactulose**  
(galactose - **fructose**)



# Physicochemical characteristics

	Anhydride form	Trihydrate form
Appearance	White crystalline powder	White crystalline powder
Molecular formula	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11} \cdot 3H_2O$
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

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**Lactulose syrup & powder**



# Historical development of lactulose

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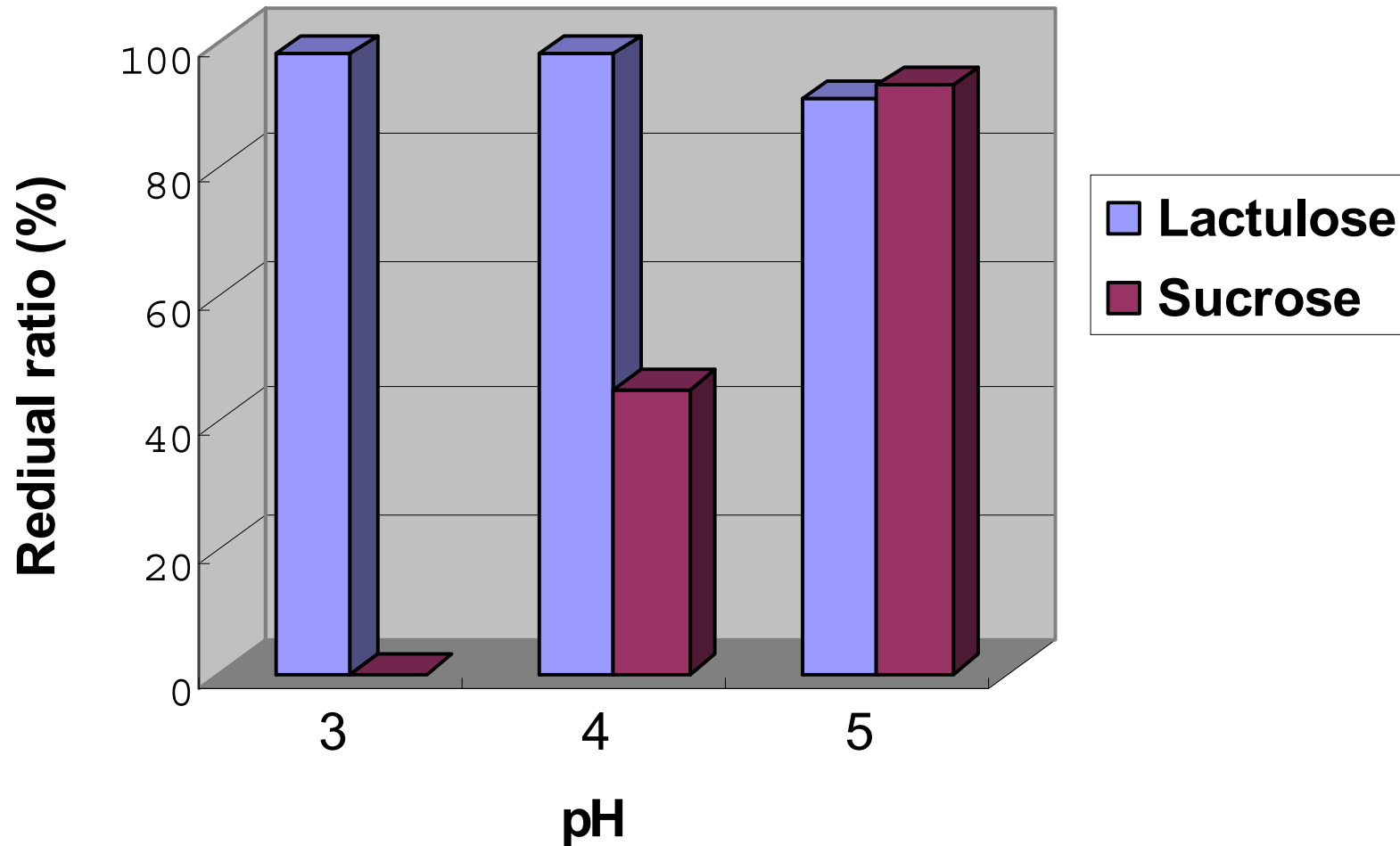
- 1930 Preparation by Montgomery and Hudson
- 1957 Discovery as a growth factor for bifidobacteria by Petuely
- 1959 Application to chronic constipation by Petuely
- 1966 Application to chronic portal systemic encephalopathy  
by Bircher

## 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

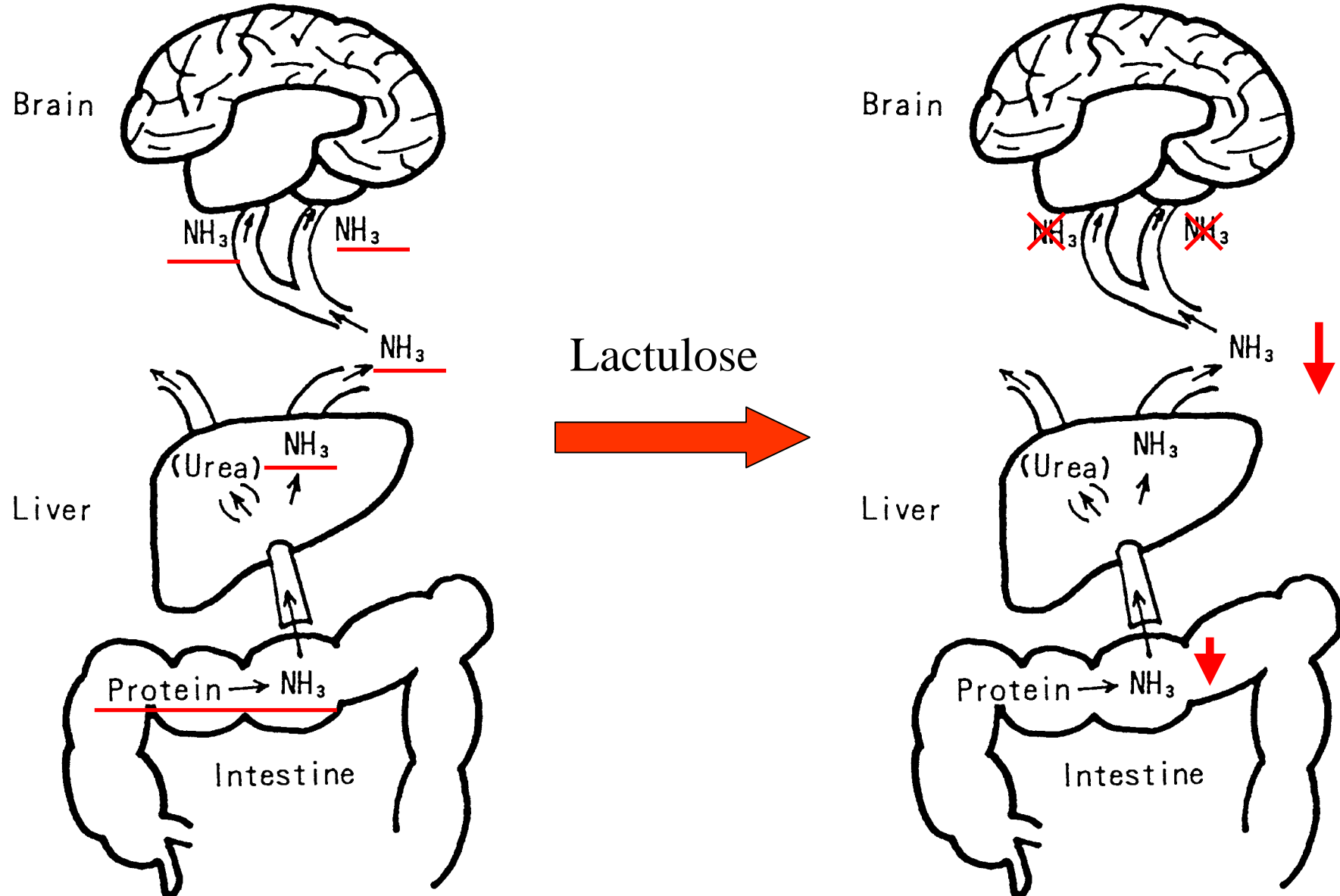


Syrup form



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



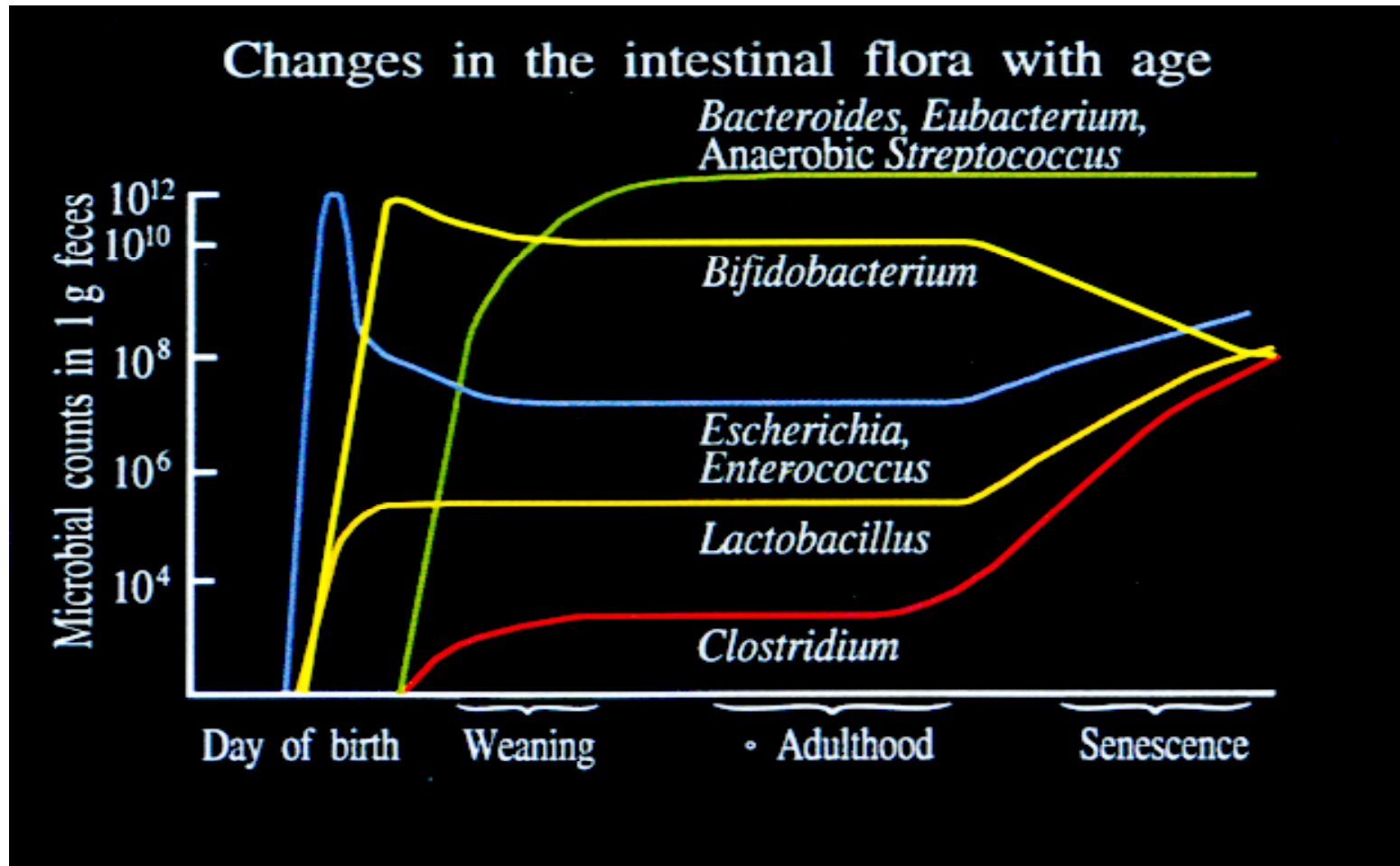
Cat milk



# **Physiological function of Lactulose as a prebiotics**



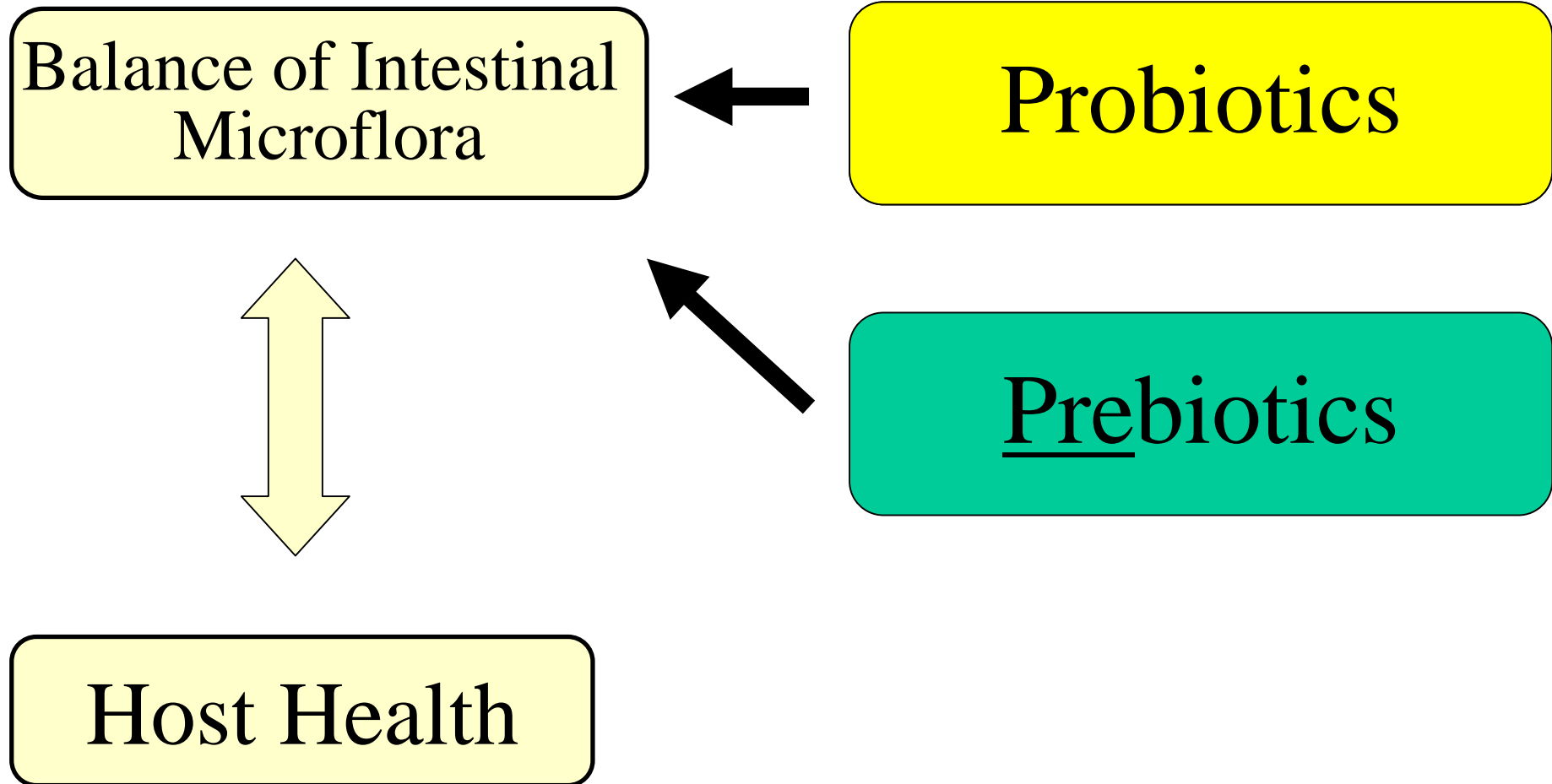
# Changes in Intestinal Flora with Age





# Probioteics

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# Prebiotics

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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.

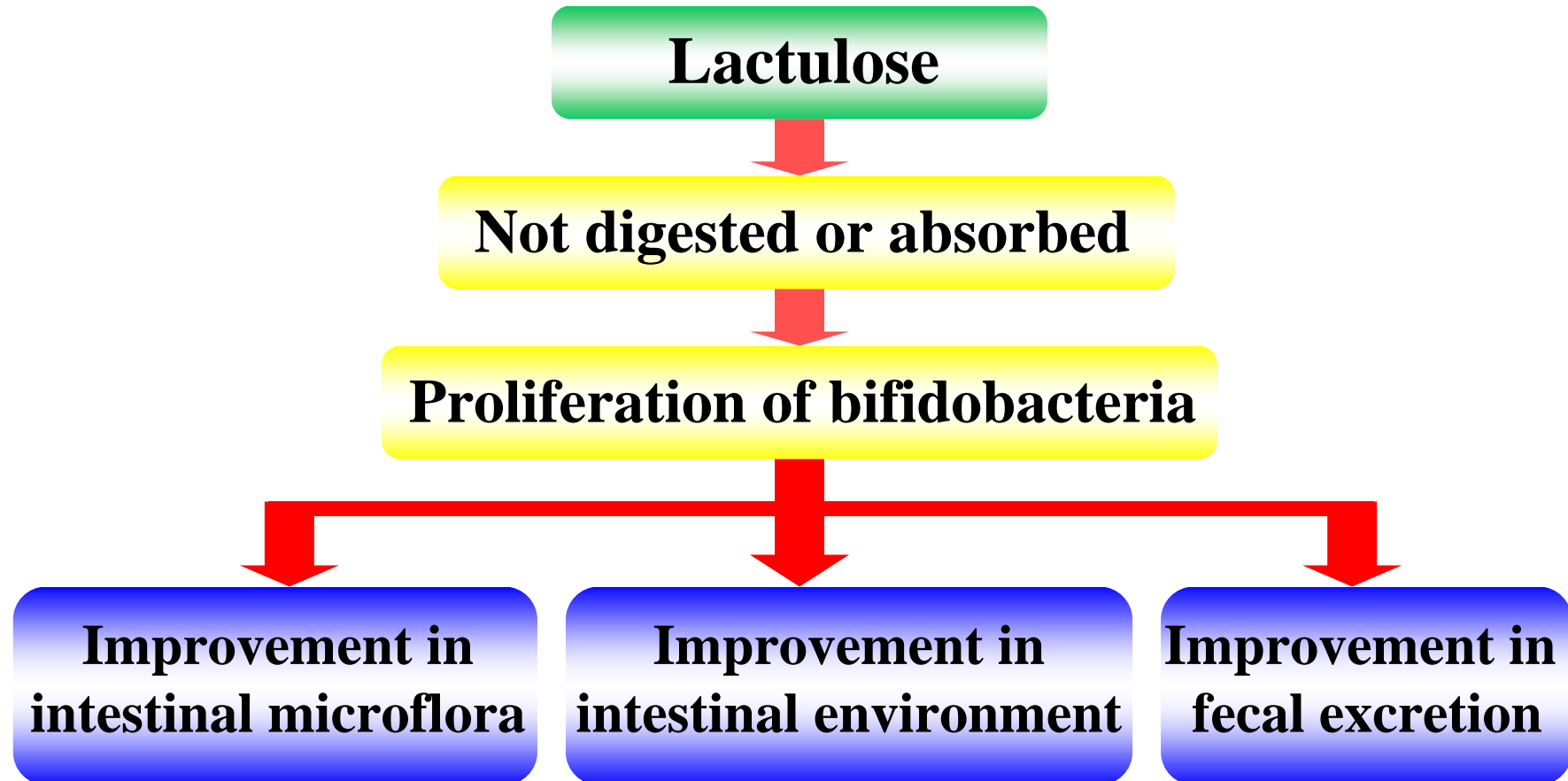
Gibson et.al. (1995)

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



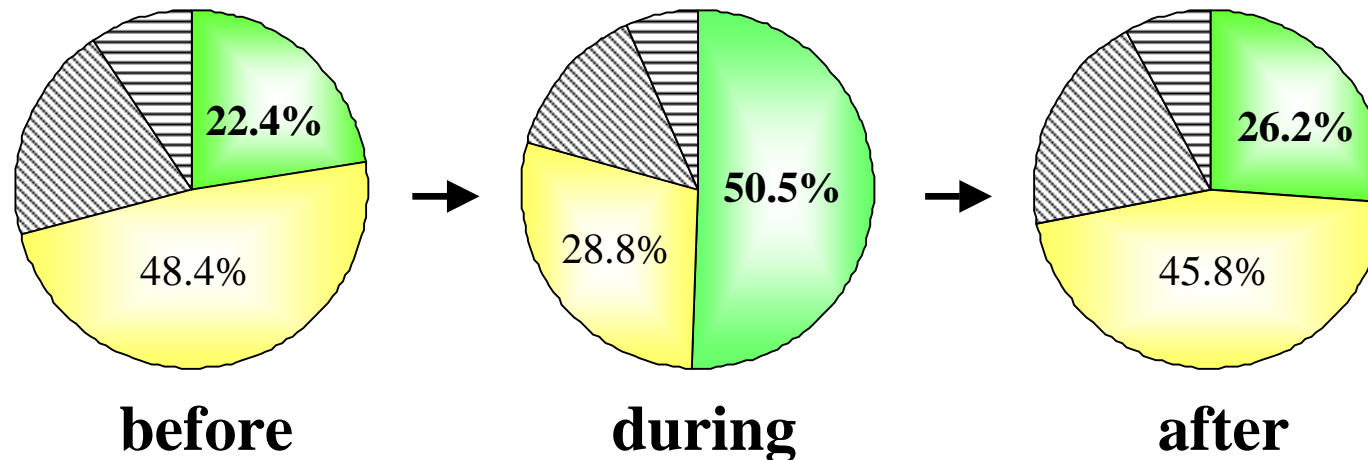
# Lactulose as probiotic

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# Improvement in the fecal microflora

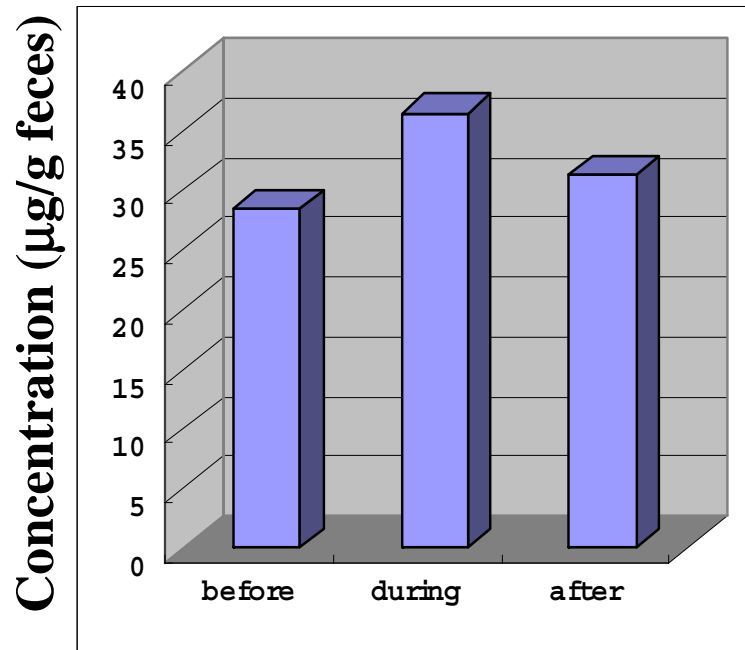


**Changes in the fecal microflora induced by  
ingestion of 4 g of lactulose**

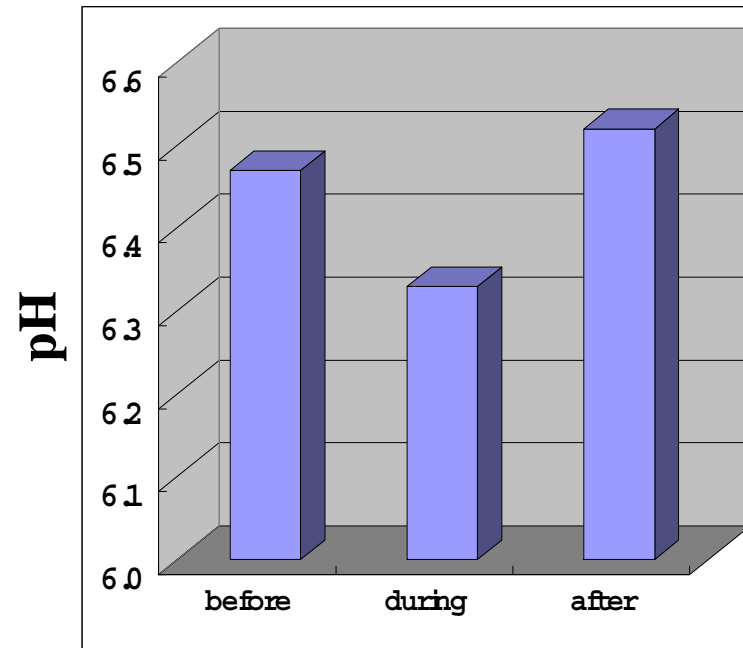




# Improvement in intestinal environment: fecal acetic acid and fecal pH



Fecal acetic acid



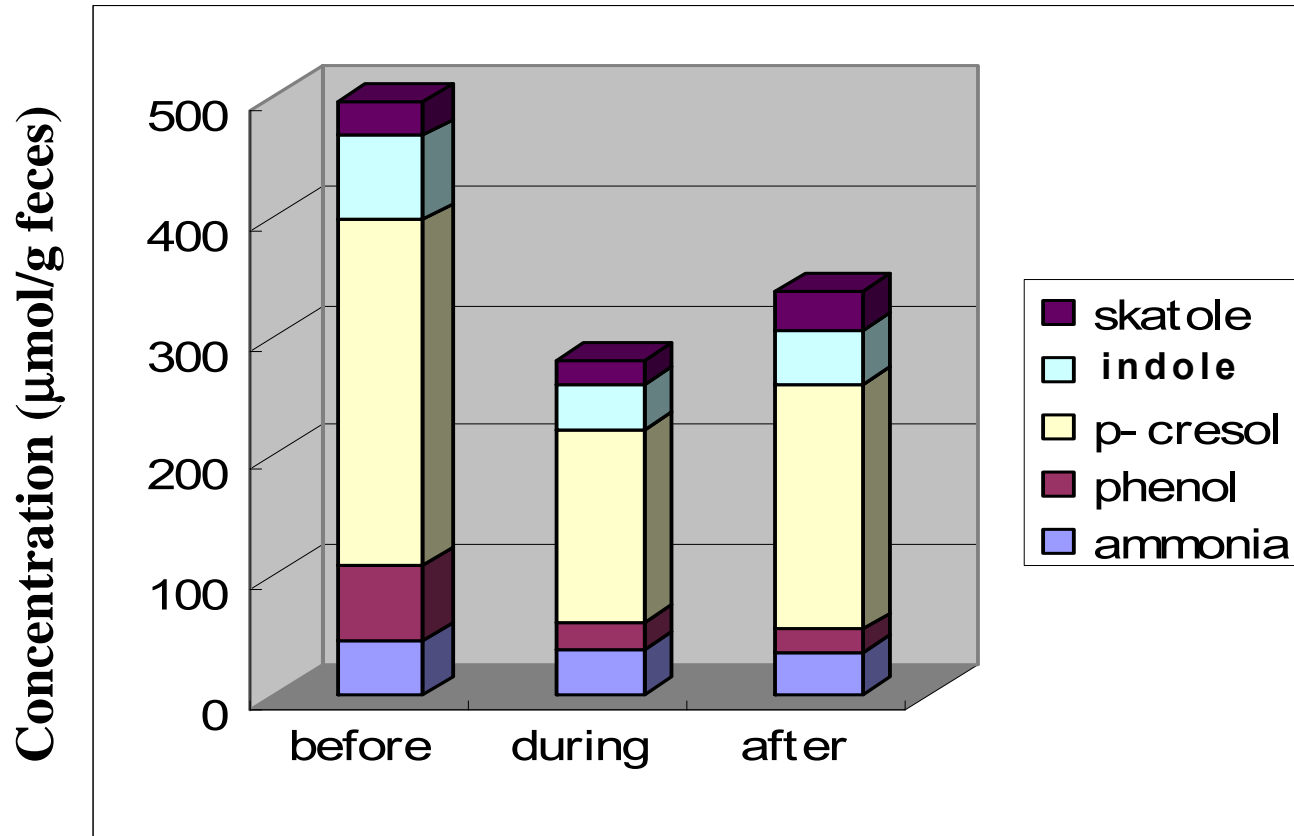
Fecal pH

**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

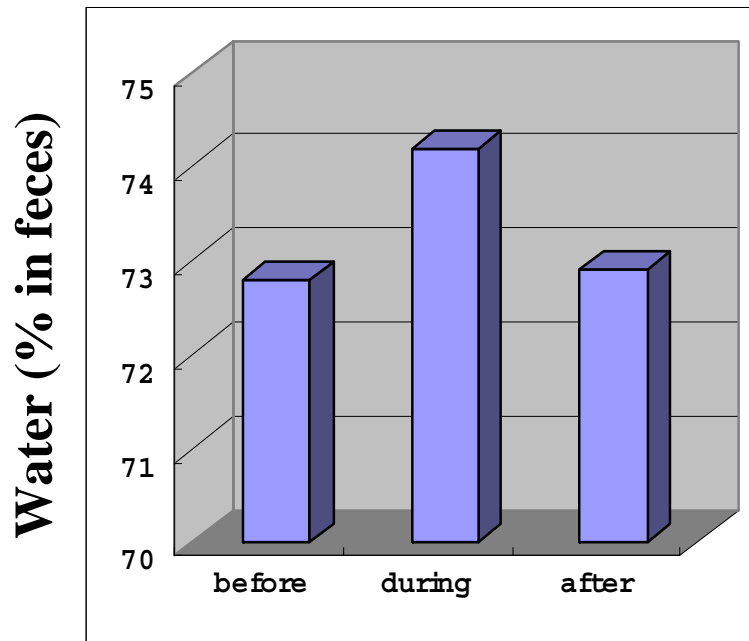


**Decrease in intestinal putrefactive substances  
induced by ingestion of 4 g of lactulose**

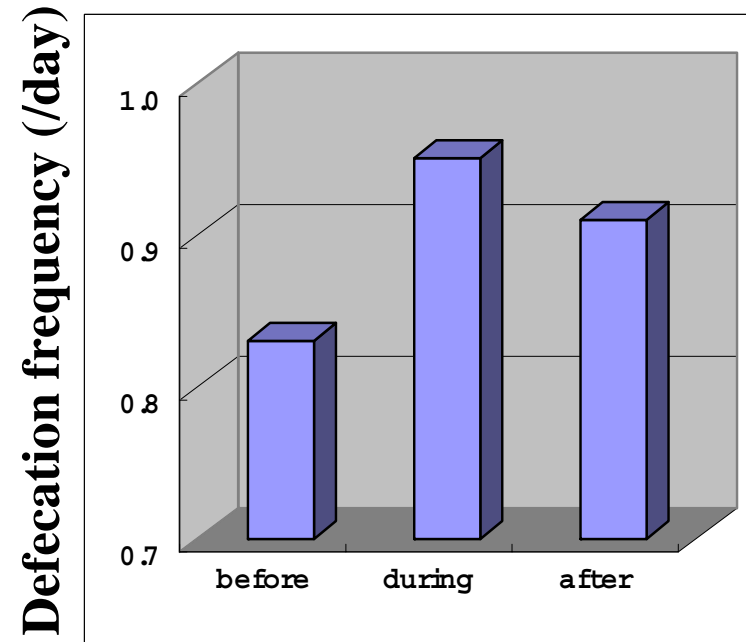
Mizota et al., *Milchwissenschaft* 57: 312 (2002)



# 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

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- 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**

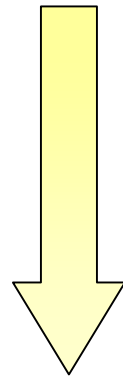
**An adequate calcium intake  
prevents osteoporosis,  
hypertension, cancer  
and kidney stones etc.  
Body fat reduce (?)**



# Absorption of calcium in milk

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**Calcium content of milk is high**

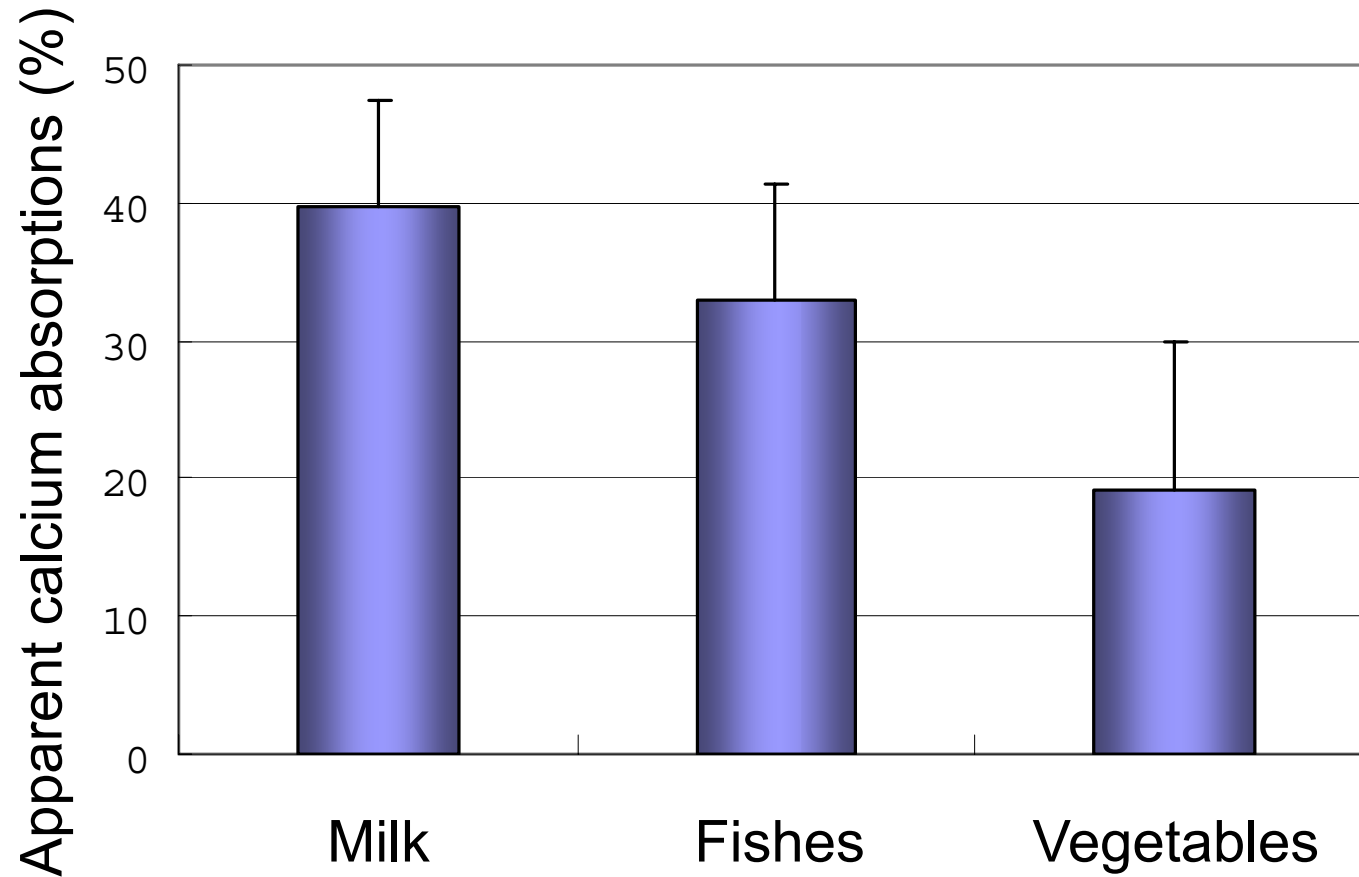


**Lactose**  
**CPP** (Casein phosphopeptide)  
**Vitamin D**

**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



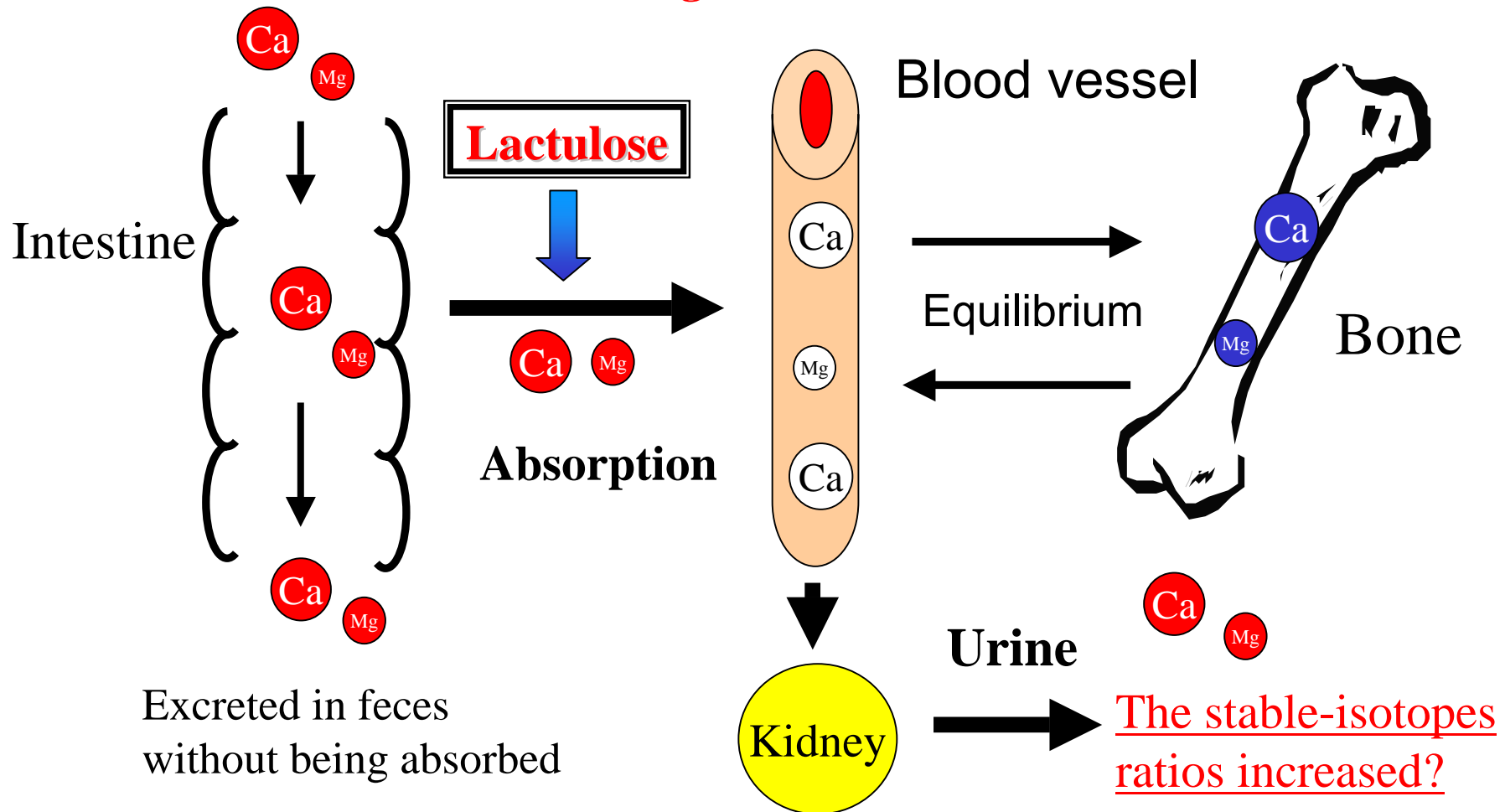
NA\*

ITF\*\*

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

$^{44}\text{Ca}$  2.1% → 8.2%

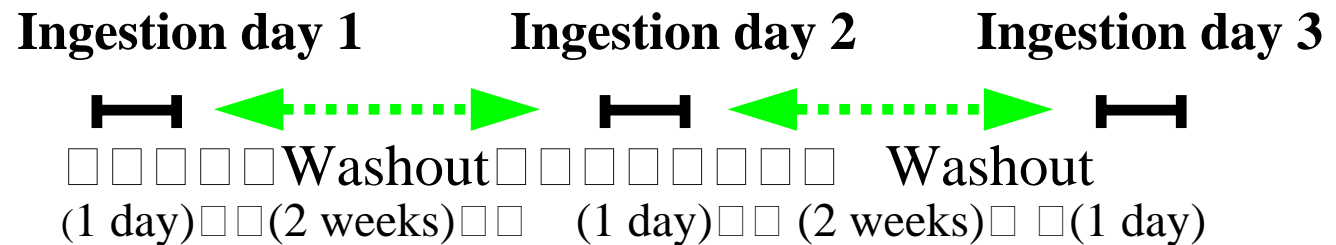
$^{25}\text{Mg}$  10.1% → 27.3%





# Trial design

## Three-group crossover design



Group A □  $n=8$  □ Placebo □      Low dose □□□□      High dose

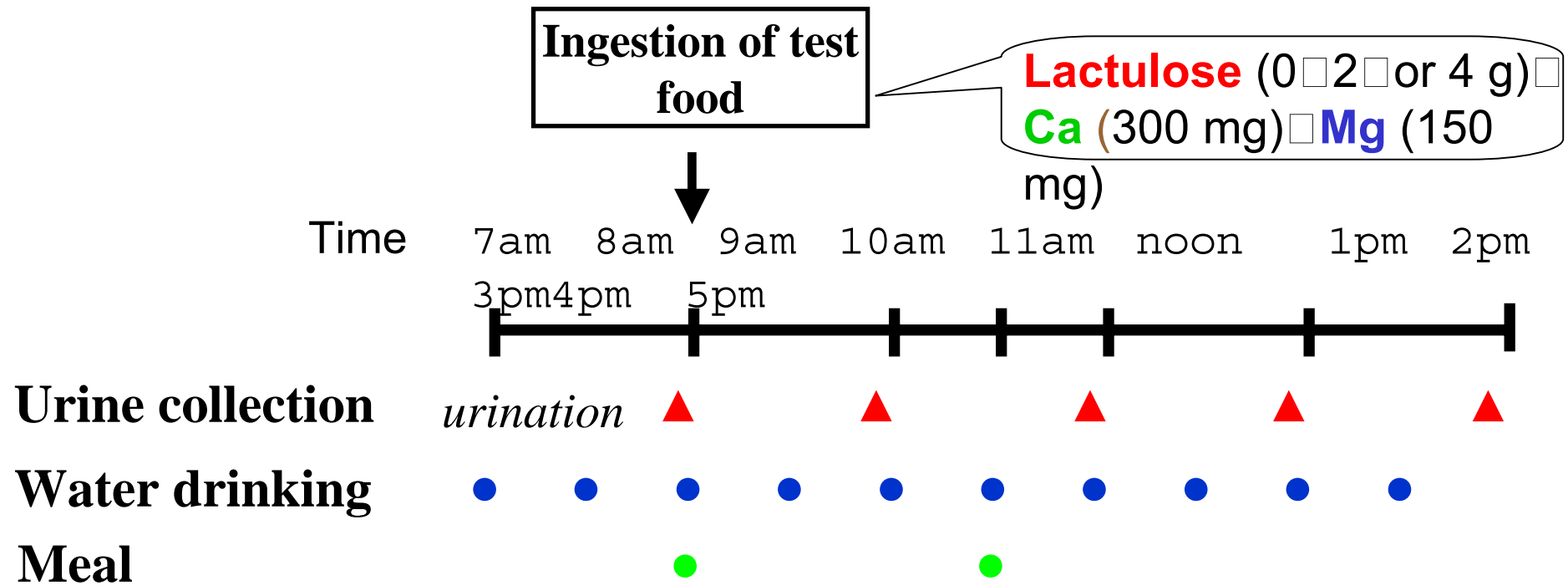
Group B □  $n=8$  □ Low dose □□□□      High dose □□□□□□ □ Placebo

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)

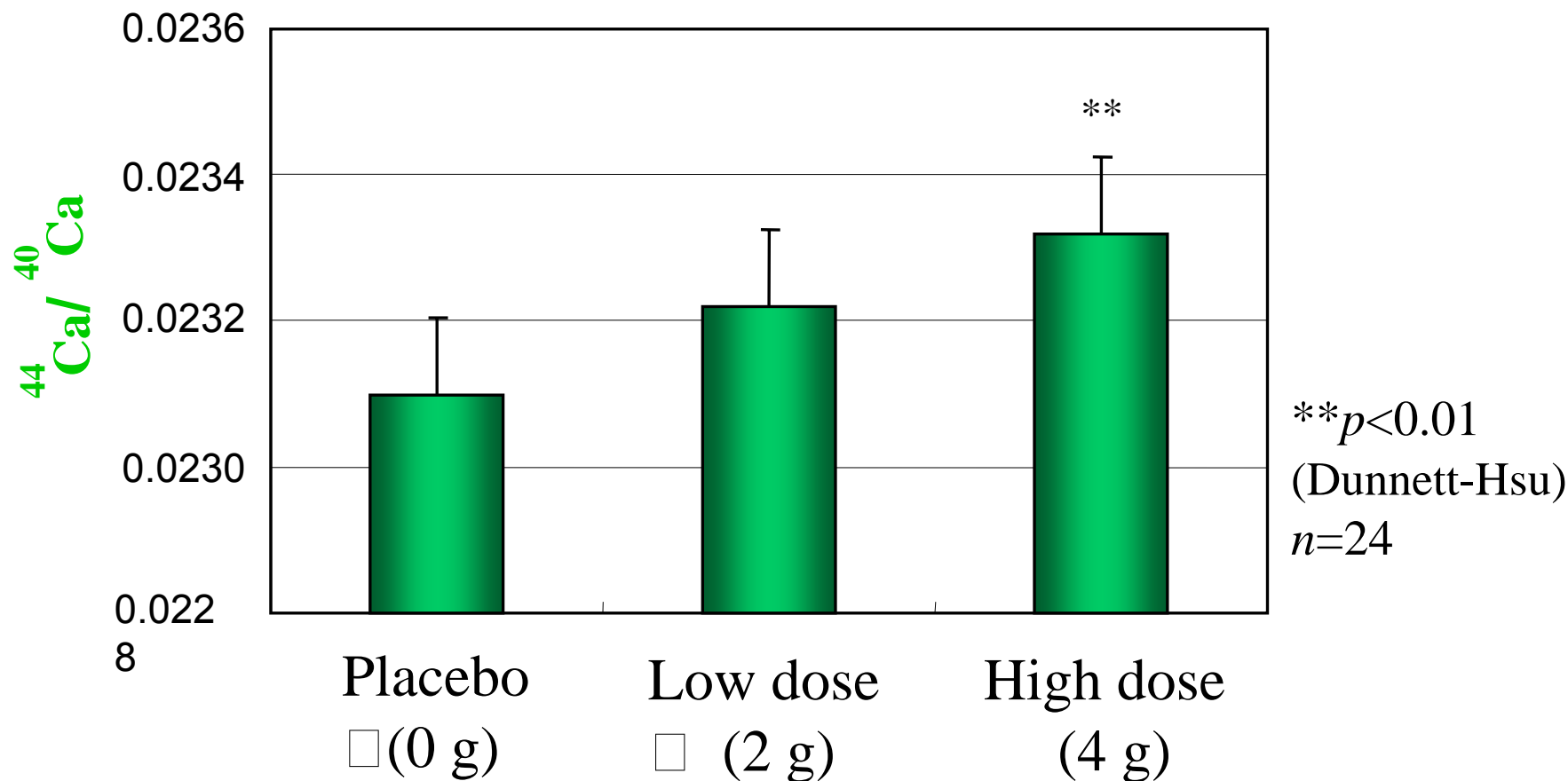
# Schedule of the ingestion day



- 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 (●)



## Effect of **lactulose** on ratio of urinary **Ca**

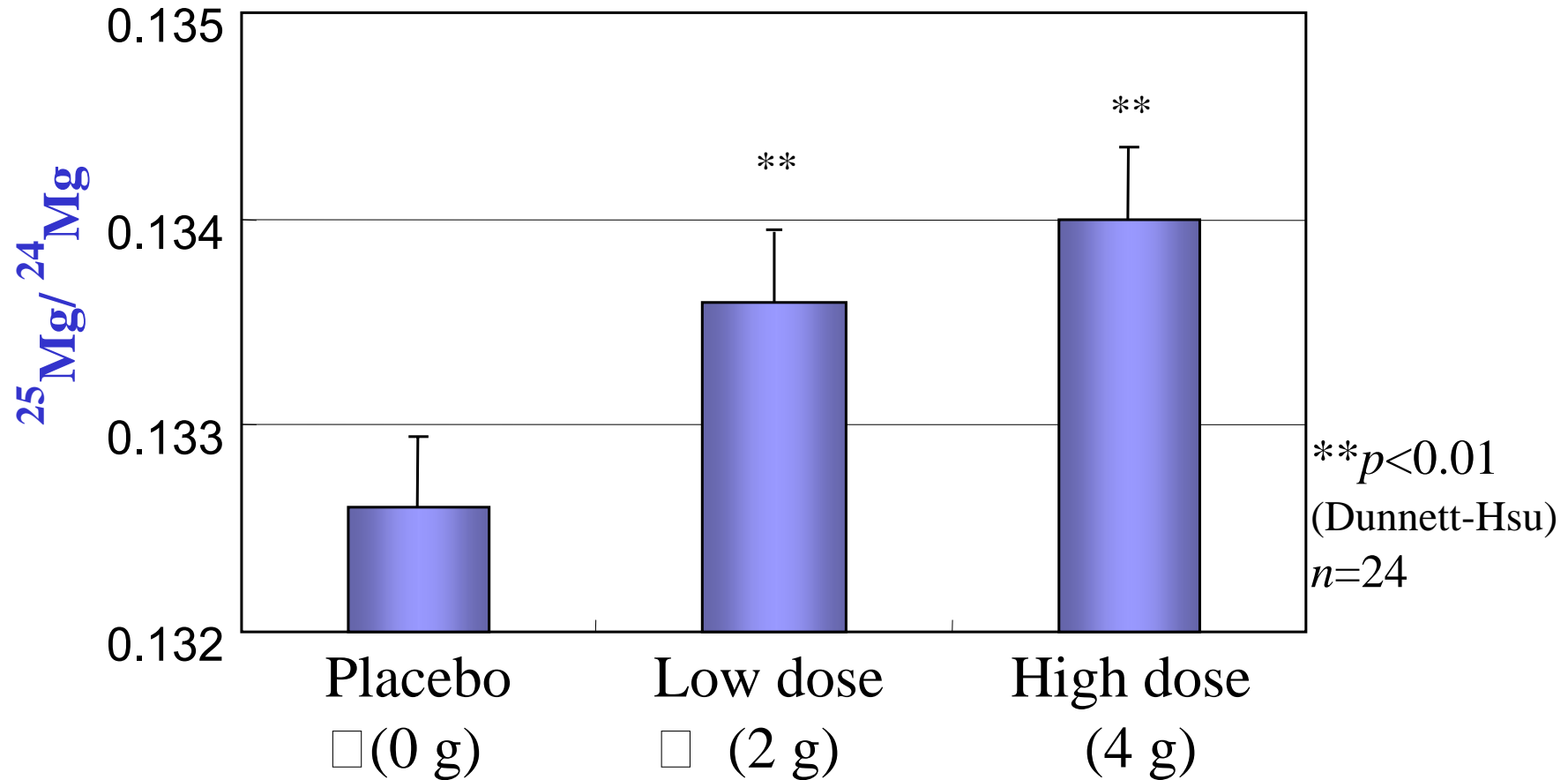


Ratio of Ca stable isotopes ( $^{44}\text{Ca}/^{40}\text{Ca}$ ) in combined urine increased with lactulose dose

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



## Effect of **lactulose** on ratio of urinary **Mg**



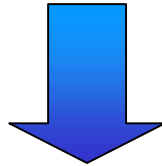
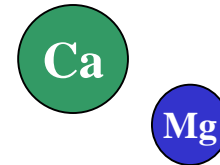
Ratio of Mg stable isotopes ( $^{25}\text{Mg}/^{24}\text{Mg}$ ) in combined urine increased with lactulose dose

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



# A single-labeling method for Urine

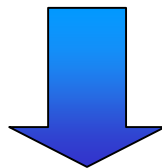
Oral intake of Stable Isotopes



Measure the ratios of stable isotopes in urine

## Advantages

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



Do minerals in urine come from test food or not?

## Disadvantages

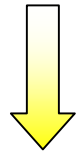
- Needs cross over design
- Difficult to quantification



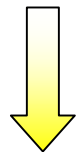
# Hypothesized action mechanism

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**Arrival of lactulose at  
the large intestine**



**Conversion of lactulose  
to organic acids**



**Decrease in intestinal pH**

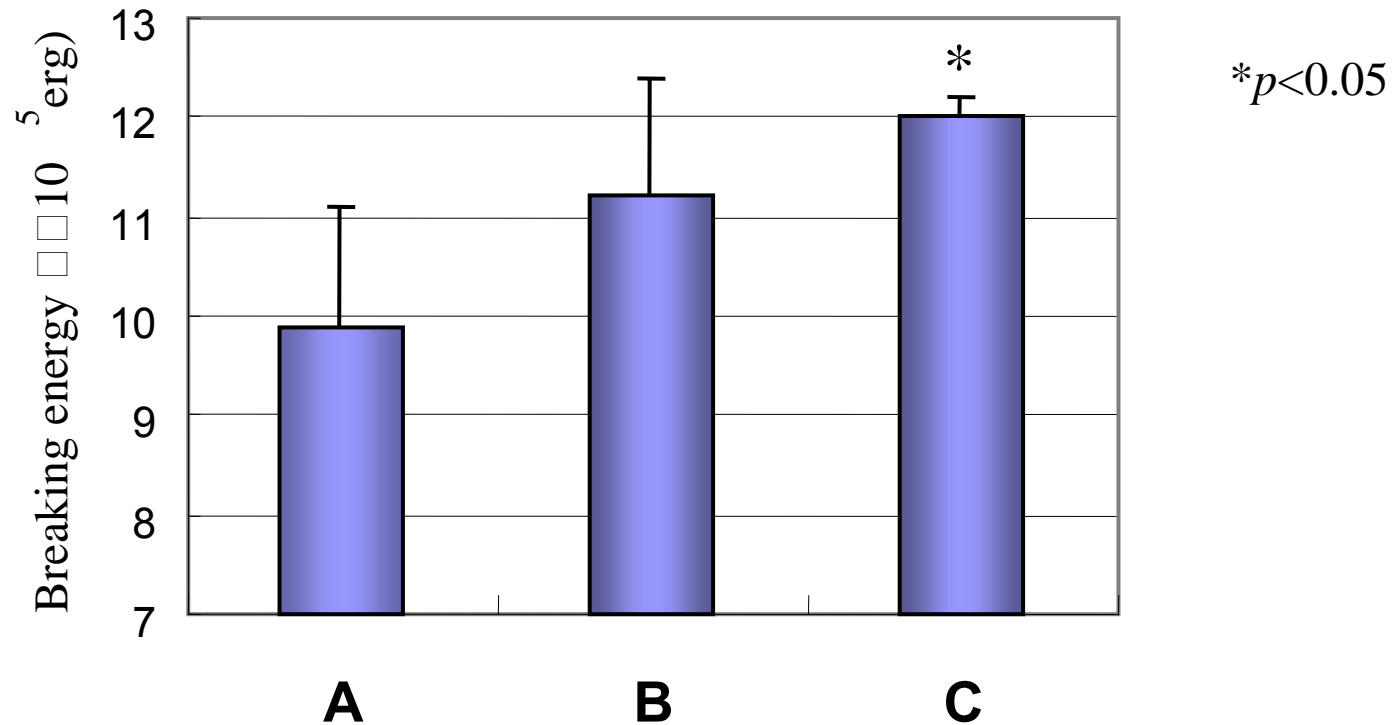
Direct effect of lactulose on  
the mineral absorption (?)

Direct effect of organic acids  
on intestinal permeability

Increases in solubilities of  
Ca and Mg salts



# The effects of lactulose on rat bone



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

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- **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