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The efficiency of intestinal calcium absorption is greater under inulin intake in aged rats than in young or adult rats: a stable isotope approach.

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Short title: effects of aging and inulin on Ca and Mg absorption

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ABSTRACT

Previous studies have shown that non-digestible inulin-type fructan intake can increase intestinal mineral absorption in both humans and animals. However, this stimulatory effect on intestinal absorption may depend on experimental conditions such as duration of fermentable fiber intake, mineral diet levels and animals' physiological status, in particular their age. The aim of this study was to determine the effect of inulin intake on Ca and Mg absorption in rats at different age stages. Eighty male Wistar rats of four different ages (2, 5, 10 and 20 months) were randomized into either a control group or a group receiving 3.75% inulin in their diet for 4 days and then 7.5% inulin for three weeks. The animals were fed fresh food and water *ad libitum* for the duration of the experiment. Intestinal absorption of Ca and Mg was determined by fecal monitoring using stable isotopic tracers. Ca and Mg status was also assessed. Absorption of Ca and Mg was significantly lower in the aged rats (10 and 20 mo) than in the young and adult rat groups. As expected, inulin intake increased Ca and Mg absorption in all four rat groups. The extent of the stimulatory effect of inulin on absorption of Ca and Mg differed between the minerals studied. Inulin had a greater effect on Ca absorption in aged rats whereas its effect on Mg absorption remained similar across all four rat age groups. These results show that the beneficial effect of inulin on the intestinal absorption of Ca and Mg may vary between young adult, adult and aged rats. Further studies are required to explore this effect over longer inulin intake periods, and to confirm these results in humans.

Key words: Inulin, intestinal absorption, status, calcium, magnesium, fermentation, stable isotope, age, rat

Abbreviations: Ca: calcium; Mg: Magnesium; ICP/MS: Inductively coupled plasma/mass spectrometry, OS: oligosaccharides; SCFA: Short-chain fatty acids;

INTRODUCTION

When non-digestible inulin-type fructans reach the large intestine, they are fermented by the local microflora and stimulate the growth of bifidobacteria and lactobacilli, which may have health-promoting functions [1-3]. Several studies have demonstrated that rats fed with prebiotic fructans absorbed more Ca and Mg than control rats, despite an increase in total fecal mass [4-6]. Indeed, the products of fructan fermentation can influence the intestinal absorption of Ca and Mg in many ways. Short-chain fatty acids (SCFA) are fermentation products that are responsible for lowering the pH of cecal content, which in turn increases mineral solubility, leading to improved mineral absorption [7]. SCFA can directly influence mineral absorption by forming complexes with the minerals, thereby increase their uptake by the intestinal cells [8, 9]. It is thought that the bacterial metabolites (e.g. butyrate) can stimulate the intestinal epithelium and increase its absorptive capacity [10]. These various factors are closely linked to the nature of the prebiotic carbohydrates and to experimental conditions [7, 11, 12]. Inulin has been shown to have generally high and consistent effects on intestinal Mg absorption in both animals and humans [13], but the effects of inulin on calcium (Ca) absorption seem to be dependent on experimental conditions (dose of inulin, dietary Ca content, experiment duration, animal age and mineral requirements). In this study, we investigated the relationship between animal age and the stimulatory effect of inulin on intestinal absorption and retention of Ca and Mg using a stable isotope approach following short-term administration of inulin in rats aged from 2 to 20 months.

MATERIALS AND METHODS

Materials and reagents

The enriched Ca isotope (^{44}Ca) as CaCO_3 and the enriched Mg isotope (^{25}Mg) as MgO were obtained from Chemgas, (Boulogne, France). The atomic abundances of these enriched isotopes were as follows: $^{40}\text{Ca}=3.41\%$, $^{42}\text{Ca}=0.09\%$, $^{43}\text{Ca}=0.03\%$, $^{44}\text{Ca}=96.45\%$ $^{46}\text{Ca} < 0.01\%$ $^{48}\text{Ca}=0.02\%$ and $^{24}\text{Mg}=1.6\%$, $^{25}\text{Mg}=97.8\%$, $^{26}\text{Mg}=0.6\%$. HNO_3 (ultrapure), Mg and beryllium standard solutions (1 g/L) were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available. Distilled water was used throughout. A Perkin-Elmer 6100DRC system (Perkin-Elmer Instruments, Courteboeuf, France) equipped with a Meinhard nebulizer was used for isotopic measurement, and a Perkin Elmer AA800 (Perkin Elmer Instruments, Courteboeuf, France) was used for total Mg measurement.

Animals and diets

Eighty male Wistar rats aged 2, 5, 10 or 20 months were purchased from Janvier (Le Genest Saint Ile, France). They were fed a commercial pellet diet (Ssniff R/S-breeding - until 3 mo, then Ssniff R/S maintenance from 3 to 24 mo age). Two groups were formed for each age bracket to receive either a control diet or a semi-purified diet containing inulin until the end of the experiment. The composition of these two diets is given in Table 1. Tested inulin was purchased from Orafti, Tienen, Belgium (Raftaline®). The target Ca and Mg levels in these diets were 5000 mg Ca/kg and 500 mg Mg/Kg diet. Powder diet (100 g) was made up with 100 ml of distilled water to form a kind of semi-liquid food prepared on-site each day. Chemical analysis of the diets offered confirmed the expected Ca and Mg contents in the experimental diets: 5107 mg Ca/kg and 5050 mg Ca/kg, and 495 mg Mg/kg and 514 mg Mg/kg in the control and inulin diets, respectively. Chemical analysis showed that the inulin contained approximately 40 mg Ca/kg and less than 1 mg Mg/kg. Dietary inulin level was

1 maintained at 3.75% during the first 4 days and then 7.5% from day 5 until the end of the
2 experiment. The 8 rat groups were given fresh food and water daily, made available *ad*
3 *libitum*. Food consumption and body weight were recorded weekly. Throughout the
4 experiment, the rats were housed two per cage (wire-bottomed to limit coprophagy) in a
5 temperature-controlled room (22°C) with dark period from 08:00 pm to 08:00 am. Total
6 experiment duration was 30 days. All procedures complied with the Institute's ethical
7 guidelines on the care and use of laboratory animals.

8

9 **Preparation of stable isotope solution**

10 215 mg of the ^{44}Ca (in carbonate form = 508 mg) and 255 mg of the ^{25}Mg (in oxide form =
11 412 mg) were first individually moistened with 2 ml of distilled water. One ml of HCl 12N
12 (ultrapure) was added to the ^{44}Ca suspension and two ml of HCl 12N was added to the ^{25}Mg
13 suspension to transform the carbonate and the oxide into soluble chlorides of Ca and Mg,
14 respectively. Each solution was then diluted with 50 ml of distilled water, both solutions were
15 then mixed, and pH was adjusted to between 3 and 6 with 1 N sodium hydroxide solution.
16 The resulting study solution was then completed to 150 ml with distilled water and
17 maintained for several days at +4°C until utilization. Total and isotopic Ca and Mg contents
18 were checked before use.

19 The rats were transferred to metabolic cages and housed individually three days before the
20 beginning of the isotopic balance study to allow them to adapt to their new environment.
21 Animals received by gavage about 1.7 ml of isotopic solution. The urine and faeces of each
22 rat were quantitatively collected for four consecutive days, and excreted isotopes in these two
23 media and in the gavage solution were quantitatively determined by ICP/MS, as described
24 below.

25

1 **Sampling procedures**

2 The rats were sacrificed just after the dark period (between 08:00 am and 10:00 am), i.e. at
3 a time when cecal fermentation was still very active. After anesthesia (40 mg sodium
4 pentobarbital/kg body weight), blood was withdrawn from the abdominal aorta, placed into
5 tubes containing sodium heparin and centrifuged at 1,000 g for 10 minutes. Plasma samples
6 were stored at 4°C for mineral analysis. The cecum, complete with contents, was removed
7 and weighed (total cecal weight). The cecal wall was flushed clean with ice-cold saline,
8 blotted on filter paper, and weighed (cecal wall weight). For each rat, duplicate samples of
9 cecal contents were collected into 2 ml microfuge tubes and immediately frozen at -20°C until
10 analysis. The pH of cecal content was determined on site using a Sentron pH-system 1001
11 portable pH-meter (Sentron Europe B.V. Ac Roden, The Netherlands). Supernatants of the
12 digestive contents were obtained by centrifuging one of the two microfuge tubes at 20,000 g
13 for 10 minutes at 4°C, and then frozen until analysis. One tibia was also sampled for Ca and
14 Mg analysis.

15

16 **Analytical procedures**

17 Ca and Mg concentrations were determined in the plasma and urine after adequate dilution
18 into 0.1% (w/v) lanthanum chloride. Diet aliquots, fecal materials and tibia were dry-ashed
19 (10 hours at 500°C) and dissolved with concentrated HNO₃ and H₂O₂ on a heating plate until
20 complete decoloration. The resulting mineral solutions were set at 10 ml with water and
21 adequately diluted in 0.1% lanthanum chloride. Mineral concentrations were measured by
22 atomic absorption spectrophotometry (on a Perkin-Elmer AA800) at wavelengths of 422 nm
23 for Ca and 285 nm for Mg.

24 For isotopic ⁴⁴Ca and ²⁵Mg determination, samples were appropriately diluted before
25 analysis using 1% HNO₃. Ca and Mg concentration and isotope ratios were determined by

1 ICP-MS using Ca and Mg as external standard and beryllium as internal standard. The
2 instrument operating conditions were set as follows after optimization with a solution of 1µg
3 indium/l: RF Power = 1050 W, Nebulizer Ar flow rate = 0.79 L/min, Auxiliary Ar flow rate =
4 1.2 L/min, Outer Ar flow rate = 15 L/min. Data acquisition parameters were set as follows:
5 Sweeps/reading = 50, Readings/replicate = 1, Number of replicates = 3, Dwell time = 50 ms
6 for ²⁴Mg, 75 ms for ⁹Be, ²⁵Mg, ²⁶Mg, and ⁴⁴Ca, 150 ms for ⁴²Ca and 300 ms for ⁴³Ca,
7 Scanning mode = peak hopping. DRC operating conditions (for ⁴²Ca, ⁴³Ca and ⁴⁴Ca) were as
8 follows: Cell Gas A Flow Rate = 0.5 L ammonia/min, RPa = 0, and RPq = 0.45.

9 Cecal SCFA concentrations, including acetic, propionic and butyric acid, were determined
10 by gas-liquid chromatography on portions of supernatant fractions of cecal contents as
11 previously described [14].

12

13 **Calculations**

14 Ca and Mg each have different stable isotopes with the following natural abundances:
15 ⁴⁰Ca=96.941%, ⁴²Ca=0.647%, ⁴³Ca=0.135%, ⁴⁴Ca=2.086% ⁴⁶Ca=0.004% ⁴⁸Ca=0.187% and
16 ²⁴Mg=78.99%, ²⁵Mg=10.00% and ²⁶Mg=11.01% [15]. ⁴⁴Ca and ²⁵Mg isotopic enrichments
17 were obtained, respectively, from the following equations: (⁴⁴Ca/⁴³Ca measured ratio -
18 ⁴⁴Ca/⁴³Ca baseline ratio)/(⁴⁴Ca/⁴³Ca baseline ratio) and (²⁵Mg/²⁶Mg measured ratio -
19 ²⁵Mg/²⁶Mg baseline ratio)/(²⁵Mg/²⁶Mg baseline ratio).

20 Non-absorbed ⁴⁴Ca and ²⁵Mg isotopes in the fecal or urine samples (coming only from the
21 ⁴⁴Ca or ²⁵Mg isotope labels) were calculated as follows:

22 for ⁴⁴Ca (mg) = (total fecal or urine Ca (mg) x (natural abundance ⁴⁴Ca x enriched ⁴⁴Ca))/(1
23 + (natural abundance ⁴⁴Ca x enriched ⁴⁴Ca));

24 for ²⁵Mg (mg) = (total fecal or urine Mg (mg) x (natural abundance ²⁵Mg x enriched
25 ²⁵Mg))/(1 + (natural abundance ²⁵Mg x enriched ²⁵Mg)).

1 Calculations were also made directly from ICP-MS data. The two modes of calculation
2 give the same results when the ICP-MS quantitative procedure is used [16].

3 Intestinal absorption of ^{44}Ca and ^{25}Mg was then calculated as administered ^{44}Ca or ^{25}Mg -
4 ^{44}Ca or ^{25}Mg excreted in the feces, and retention of ^{44}Ca and ^{25}Mg was calculated as
5 administered ^{44}Ca or ^{25}Mg - ^{44}Ca or ^{25}Mg excreted in the feces and in the urine.

6 Total cecal SCFA content ($\mu\text{mol}/\text{cecum}$) was calculated as the supernatant SCFA
7 concentration ($\mu\text{mol}/\text{ml}$) \times cecal water (ml/cecum).

8 Soluble Ca and Mg levels in the cecal contents were determined on the supernatant
9 concentration ($\mu\text{g}/\text{ml}$), and soluble Ca and Mg contents per cecum were calculated as (μg
10 Ca/ml or $\mu\text{g Mg}/\text{ml}$) \times cecal water (ml).

11

12 **Data analysis**

13 Values are given as means \pm SD, and data were tested by 2-way ANOVA using the
14 General Linear Models procedure of the Super ANOVA package (Abacus, Berkeley, CA).
15 Post-hoc comparisons were performed using Fisher's least significant difference procedures.
16 Differences of $p < 0.05$ were considered statistically significant. Simple linear correlation
17 analysis was used to assess the relationships between intestinal absorption of Ca and Mg and
18 other relevant parameters. Values of $p < 0.05$ were considered statistically significant.

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RESULTS

Food intake and growth rate

Inulin intake at the dose of 7.5% showed only a tendency to decrease animal food intake in this study. The slight decrease in food intake in inulin-fed rats led to a non-significantly lower growth rate ($p<0.10$) towards the end of the experiment in inulin-fed rats compared to controls. The lower calorific value of the inulin diets (-4%) compared to the control diets may also be responsible for this reduced weight gain. In addition, food intake decreased significantly with increasing age, as expected (data not shown).

Cecal fermentation parameters and total and cecal soluble Ca and Mg levels (table 2)

As expected, inulin intake significantly increased cecal wall weight and cecal content and significantly decreased the pH of cecal content. These variables did not change with rat age. In addition, inulin intake considerably increased the individual and total pools of SCFA in the cecal contents ($p<0.0001$). The effect of age on these SCFA pools was less clear. No significant age-related difference was observed amongst the control group rats, whereas in the inulin-fed group, the intestinal bacteria produced higher acetate, butyrate and total SCFA in the rats aged 10 mo than in the three other groups ($p<0.05$).

Intestinal absorption and retention of calcium (table 3)

The amount of gavaged ^{44}Ca was about 1.60 mg/rat, which led to a fecal ^{44}Ca enrichment of 10% to 20% in the 4-day feces pool. Fecal ^{44}Ca excretion expressed as mg/g of feces or as mg/day increased significantly with age. Consequently, net (mg) and relative (%) ^{44}Ca absorption were significantly lower in the aged rats than in the young adult or adult rats. In addition, urinary ^{44}Ca excretion (mg) increased significantly with age. Consequently, net (mg) and relative (%) ^{44}Ca retention were considerably lower in the aged rats than in the young

1 adult or adult rats. Inulin intake significantly decreased fecal ^{44}Ca excretion, expressed as
2 $\mu\text{g/g}$ of feces or as μg , in all groups. Consequently, inulin intake significantly increased net
3 (mg) and relative (%) ^{44}Ca absorption. Moreover, inulin intake increased urinary ^{44}Ca
4 excretion (mg). Lastly, inulin intake significantly increased net (mg) and relative (%) ^{44}Ca
5 retention in the four age-related groups compared to the control diet groups.

6 The relative increase in ^{44}Ca absorption under inulin intake was 38%, 45%, 90% and 79%
7 in the four rat groups (2, 5, 10, 20 mo, respectively) compared to the same rat age groups
8 without inulin (Figure 1). This indicated that the stimulatory effect of inulin on ^{44}Ca
9 absorption was greater in the aged rats than in the young or adult rats.

10

11 **Intestinal absorption and retention of magnesium (table 4)**

12 The amount of gavaged ^{25}Mg was about 2.50 mg/rat, which led to a fecal ^{25}Mg enrichment
13 of 35% to 65% in the 4-day feces pool. Fecal ^{25}Mg excretion expressed as mg/g of feces or as
14 mg increased significantly with age. Consequently, net (mg) and relative (%) ^{25}Mg absorption
15 were significantly lower in the aged rats than in the young adult or adult rats. In addition,
16 urinary ^{25}Mg excretion (mg) increased significantly with age. Consequently, net (mg) and
17 relative (%) ^{25}Mg retention were significantly lower in the aged rats than in the young adult or
18 adult rats. As expected, inulin intake significantly decreased fecal ^{25}Mg excretion, expressed
19 as $\mu\text{g/g}$ of feces or as μg , in all groups. Consequently, inulin intake significantly increased net
20 (mg) and relative (%) ^{25}Mg absorption. Similarly, inulin intake increased urinary ^{25}Mg
21 excretion (mg). However, inulin intake led to significantly higher net (mg) and relative (%)
22 ^{25}Mg retention in all four groups compared to the control diet.

23 The relative increase in ^{25}Mg absorption under inulin intake was 54%, 53%, 54% and 55%
24 in the four rat groups (2, 5, 10, 20 mo, respectively) compared to the same rat age groups

1 without inulin (Figure 1). This indicated that stimulatory effect of inulin on ²⁵Mg absorption
2 was not age-dependent.

3

4 **Calcium and magnesium status (table 5)**

5 Mean plasma Ca varied from 95 to 102 mg/L, showing a tendency to increase with inulin
6 intake (+2%, $p = 0.0601$) and to decrease with increasing age (-1%, $p = 0.0619$). Mean bone
7 Ca varied from 202 to 228 mg/g dry weight, and was unaffected by inulin intake. However,
8 mean bone Ca increased significantly with increasing age. Mean plasma Mg varied from 16.9
9 to 18.2 mg/L, showing a tendency to increase with inulin intake (+3%, $p = 0.0570$). However,
10 mean plasma Mg was not modified by age. Plasma Mg increased in the inulin-fed aged rats
11 (+6.7%), whereas there was no increase in the young and adult rats (-0.3%). Mean red blood
12 cell Mg levels varied from 42.5 to 45.4 mg/L and remained unchanged when age increases or
13 under inulin intake. Mean bone Mg levels varied from 3.72 to 3.92 mg/g dry weight,
14 decreasing significantly with aging ($p < 0.0001$). However, mean bone Mg was unaffected by
15 inulin intake.

16

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DISCUSSION

1
2 Previous studies have repeatedly shown that intake of different inulin-type fructans can
3 variably increase mineral intestinal absorption in humans and animals [4, 5, 17-19]. Indeed,
4 inulin-type fructans strongly and consistently increase intestinal Mg absorption [12], whereas
5 their effect on Ca absorption seems to be dependent on experimental conditions such as inulin
6 type, dietary Ca levels, duration of fructan intake [20-22] and the animals' physiological state,
7 particularly age. It is well known that the absorption mechanisms of Ca and Mg differ
8 considerably [23, 24], which may explain the observed differences between these two
9 minerals in terms of inulin effect. In this study, we investigated the enhancing effect of
10 fructan intake on Ca and Mg intestinal absorption and balance in rats of different ages.

11

12 **1- Effect of animal age and inulin intake on Ca absorption:**

13 Our results clearly showed that aged rats exhibited less efficient intestinal absorption and
14 retention of Ca. ⁴⁴Ca absorption ranged from 48% without inulin to 66% under inulin intake
15 in the young and adult rats and from 15% without inulin to 27% under inulin intake in the old
16 and very old rats. ⁴⁴Ca absorption correlated negatively with rat age (Figure 2). This decline
17 in Ca absorption with age is not new, and has already been reported in animal and human
18 studies [25-27] and is largely confirmed in this study. This decline is primarily due to an
19 energy- and vitamin D-dependent Ca transport component in the elderly [28]. Our results
20 clearly showed that inulin intake increased the efficiency of Ca intestinal absorption and
21 retention. The mean ⁴⁴Ca absorption in the four rat control groups was 26.3% compared to
22 40.2% in the four inulin-fed groups, with an overall increase in ⁴⁴Ca absorption of 53%. These
23 results are in agreement with literature data showing that the effect of inulin on Ca absorption
24 seems to be optimal in the early weeks, then decreasing gradually with experiment duration
25 [20, 29, 30]. One possible explanation for this phenomenon is a down-regulation of the active

1 pathway of intestinal Ca absorption after several weeks of feeding inulin, as previously
2 reported [31, 32].

3

4 **2- Effect of animal age and inulin intake on Mg absorption:**

5 Our results showed that aged rats exhibited less efficient intestinal absorption and retention
6 of Mg. ^{25}Mg absorption ranged from 56% without inulin to 86% under inulin intake in the
7 young and adult rats and from 45% without inulin to 70% under inulin intake in the old and
8 very old rats. Indeed, ^{25}Mg absorption tended to correlate negatively with rat age (Figure 2).
9 This decline in Mg absorption with age is not well documented in the literature in either
10 animal or human studies. Few, if any, incomplete studies have reported an age effect on Mg
11 absorption [33-35], and the results are inconsistent. Hence, to our knowledge, this is the first
12 robust report to clearly show that Mg absorption decreases with age in the rat. Although Mg
13 absorption is generally described as a passive phenomenon, one component of this absorption
14 remains under hormonal control [36, 37], which may explain the observed results. Our results
15 clearly showed that inulin intake considerably increased Mg intestinal absorption and
16 retention efficiency. Mean ^{25}Mg absorption in the four rat control groups was 50.6%,
17 compared to 78.0% in the four corresponding inulin-fed rat groups, with an overall increase in
18 ^{25}Mg absorption of 54%. These results are in agreement with literature data showing that
19 inulin intake considerably increases Mg absorption in animals and humans (see recent review
20 [13]).

21

22 **3- Modulation of the stimulatory effect of inulin on Ca and Mg absorption by the rat** 23 **ages:**

24 Since Ca absorption is generally well controlled, the observed absorption increase under
25 inulin intake may be down-regulated (known as a feed-back phenomenon) in adult rats. Thus,

1 given that Ca absorption is low and the adaptative phenomenon less well controlled in aged
2 rats, we hypothesized that inulin intake would lead to a much greater increase in Ca
3 absorption in aged rats than in the young or adult rats. Conversely, since Mg absorption is
4 only weakly controlled with a generally consistent increase under inulin intake in adult rats,
5 we hypothesized that inulin intake would increase Mg absorption in both aged rats and young
6 or adult rats to the same extent.

7 The relative increase in ^{44}Ca absorption under inulin intake was 41.5% and 84.5% in the
8 adult and aged rats, respectively. This clearly indicated that the stimulatory effect of inulin on
9 ^{44}Ca absorption was greater in the aged rats than in the adult rats. Furthermore, the relative
10 increase in ^{25}Mg absorption under inulin intake was 53.5% and 54.5% in the adult and aged
11 rats, respectively. This indicated that the stimulatory effect of inulin on ^{25}Mg absorption was
12 not age-dependent. It is clear that inulin intake led to a much greater increase in ^{44}Ca
13 absorption in the aged rats than in the adult rats, whereas inulin intake led to a similar increase
14 in ^{25}Mg absorption in young, adult and aged rats, thus confirming the hypothesis we
15 formulated for this study.

16 In conclusion, as expected, our results clearly confirmed that short-term inulin intake
17 stimulates the absorption of both Ca and Mg. Furthermore, these results not only confirmed
18 that Ca absorption declines considerably with age but also showed for the first time that Mg
19 absorption also declines with age in the rat. Moreover, these results confirmed our hypothesis
20 of a greater stimulatory effect of inulin on Ca absorption in aged rats than in the young or
21 adult rats, and a similar stimulatory effect of inulin on Mg absorption in aged rats and young
22 and adult rats. Further studies are required to explore this effect on longer inulin intake
23 periods and to validate these results on the stimulatory effect of inulin on Ca and Mg
24 absorption in the elderly.

25

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Figure legends:

Figure 1:
Modulation of the stimulatory effect of inulin on the intestinal absorption of calcium and magnesium in adult and old rats.

Intestinal absorption in the control groups was normalized to 100% for each age group. The stimulatory effect of inulin (%) for a given age group was calculated as follows: $100 \times (\text{intestinal absorption in the inulin-fed age group} / \text{intestinal absorption in the same age group without inulin})$. The rats were given ^{44}Ca and ^{25}Mg after 14 days of inulin intake (7.5%), and fecal non-absorbed isotopes were determined in a 4d feces pool.

Figure 2 :
Correlation between intestinal absorption of Ca and Mg and rat ages.
The rats were given ^{44}Ca and ^{25}Mg after 14 days of inulin intake (7.5%), and fecal non-absorbed isotopes were determined in a 4d feces pool.

Table 1. Diet composition (g/kg) during the experiment

	Control diet	Inulin diets	
		3.75%	7.5%
Wheat starch	650	546.5	575
Casein	200	200	200
Corn oil	50	50	50
Cellulose	50	50	50
Mineral mix (AIN 1993)a	35	35	35
Vitamin mix (AIN 1993)b	10	10	10
DL-Methionine	3	3	3
Choline bi-tartrate	2	2	2
Inulin	0	37.5	75

a: Mineral mix AIN 1993 ensures the following mineral levels in the diets (mg/kg): Na, 1020; K, 3600; P, 4000; Ca, 5000; Mg, 500; Zn, 30; Fe, 35; Cu, 6; Mn, 54; Se, 0.1; I, 0.2; Cr, 2.

b: Vitamin mix AIN 1993 ensures the following mineral levels in the diets (mg/kg) : thiamine, 6; riboflavine, 6; pyridoxine, 7; nicotinic acid, 30; calcium pantothenate, 16; folic acid, 2; D-biotin: 0.2; and ($\mu\text{g/kg}$) cyanocobalamine (vitamin B12), 10; vitamin K, 50; and (IU/kg) vitamin A, 4000; vitamin E, 50; vitamin D, 1000.

1.1.

Table 2 : Effect of age and inulin intake and their interaction on cecum fermentation parameters and cecal Ca and Mg levels in rats

Cont 3Mo	Cont 6Mo	Cont 11Mo	Cont 21Mo	Inulin 3Mo	Inulin 6Mo	Inulin 11Mo	Inulin 21Mo	inulin	age interaction		
Cecal content pH	6.92±0.24	6.87±0.17	6.72±0.58	6.62±0.31	5.71±0.58	5.41±0.22	5.64±0.37	5.57±0.22	<0.0001	NS	NS
Cecal content, g	2.20±0.35	2.34±0.68	2.53±0.97	2.86±0.74	6.18±1.68	6.46±1.57	7.09±2.31	7.10±1.91	<0.0001	NS	NS
Cecal wall, g	0.87±0.07	1.11±0.22	1.32±0.29	1.25±0.15	1.80±0.37	2.32±0.29	2.51±0.39	2.46±0.30	<0.0001	NS	NS
Acetate, μmol/cecum	22.4±6.3	24.5±9.4	24.4±11.8	28.0±8.5	49.2±22.3	70.4±16.7	54.3±17.3	63.5±11.4	<0.0001	0.0334	NS
Propionate, μmol/cecum	5.49±1.48	6.31±2.20	5.11±2.10	6.11±1.72	15.56±12.29	12.84±5.04	9.96±4.86	11.26±3.92	<0.0001	NS	NS
Butyrate, μmol/cecum	9.90±2.70	8.63±3.10	6.37±2.78	8.01±3.38	48.13±25.12	69.51±32.50	39.98±18.42	43.15±8.63	<0.0001	0.0181	0.0368
Total SCFA, μmol/cecum	37.8±9.1	39.5±13.5	35.9±15.9	42.2±12.6	112.9±50.8	152.8±40.0	104.2±27.7	117.9±17.3	<0.0001	0.0238	0.0490

Table 3 : Effect of age and inulin intake and their interaction on intestinal absorption and retention of Ca in rats

	Cont 3Mo	Cont 6Mo	Cont 11Mo	Cont 21Mo	Inulin 3Mo	Inulin 6Mo	Inulin 11Mo	Inulin 21Mo	inulin	age	interaction
Administered ⁴⁴ Ca, µg	1637±46	1610±14	1602±17	1605±24	1593±19	1614±19	1621±25	1626±22	NS	NS	0.0003
Fecal ⁴⁴ Ca enrichment, %	12.5±3.3	17.3±2.4	20.2±4.3	18.7±2.8	10.9±4.2	17.8±6.2	17.4±2.1	21.2±3.1	NS	<0.0001	NS
Fecal ⁴⁴ Ca level, µg/g	112±30	182±35	218±45	204±38	76±30	152±49	163±26	188±26	<0.0001	<0.0001	NS
Fecal ⁴⁴ Ca excretion, µg	856±224	1139±153	1389±96	1366±115	541±223	926±142	1207±195	1192±142	<0.0001	<0.0001	NS
Intestinal ⁴⁴ Ca absorption, µg	781±206	471±153	213±90	239±117	1052±222	689±142	413±202	434±143	<0.0001	<0.0001	NS
Intestinal ⁴⁴ Ca absorption, %	47.8±12.9	29.3±9.4	13.3±5.6	14.9±7.3	66.1±13.9	42.7±8.8	25.4±12.4	26.7±8.7	<0.0001	<0.0001	NS
Urinary ⁴⁴ Ca enrichment, %	17.4±6.5	20.6±6.7	14.8±3.1	16.9±3.9	13.7±3.3	17.5±6.7	18.6±4.2	18.5±4.1	NS	NS	0.0569
Urinary ⁴⁴ Ca excretion, µg	15.3±5.9	14.8±6.0	25.8±10.7	28.1±7.9	23.5±7.2	21.8±9.9	49.0±11.8	40.3±12.5	<0.0001	<0.0001	0.0359
⁴⁴ Ca retention, µg	765±204	456±153	188±92	212±113	1029±219	667±135	364±203	394±145	<0.0001	<0.0001	NS
⁴⁴ Ca retention, %	46.9±12.8	28.3±9.5	11.7±5.7	13.2±7.1	64.6±13.8	41.3±8.3	22.4±12.4	24.2±8.9	<0.0001	<0.0001	NS

Table 4 : Effect of age and inulin intake and their interaction on intestinal absorption and retention of Mg in rats

	Cont 3Mo	Cont 6Mo	Cont 11Mo	Cont 21Mo	Inulin 3Mo	Inulin 6Mo	Inulin 11Mo	Inulin 21Mo	inulin	age	interaction
Administered ²⁵ Mg, µg	2553±71	2511±22	2499±26	2504±38	2485±30	2518±30	2527±39	2536±35	NS	NS	0.0003
Fecal ²⁵ Mg enrichment, %	47.9±7.1	51.9±7.4	54.6±12.3	54.1±8.1	33.9±20.3	41.0±19.9	47.4±13.9	65.7±17.6	NS	0.0007	0.0273
Fecal ²⁵ Mg level, µg/g	149±26	184±34	205±44	204±38	51±33	70±35	91±31	120±35	<0.0001	<0.0001	NS
Fecal ²⁵ Mg excretion, µg	1138±201	1157±229	1311±200	1366±177	358±224	430±206	673±205	757±184	<0.0001	<0.0001	NS
Intestinal ²⁵ Mg absorption, µg	1415±187	1354±225	1188±199	1137±175	2127±224	2087±208	1855±232	1780±186	<0.0001	<0.0001	NS
Intestinal ²⁵ Mg absorption %	55.5±7.5	54.0±9.0	47.5±8.0	45.4±7.0	85.6±8.9	82.9±8.2	73.3±8.4	70.2±7.2	<0.0001	<0.0001	NS
Urinary ²⁵ Mg enrichment, %	29.3±2.7	29.1±3.5	28.0±3.7	28.2±2.32	34.2±3.8	35.5±2.4	33.5±3.7	36.7±6.5	<0.0001	NS	NS
Urinary ²⁵ Mg excretion, µg	398±64	323±36	298±80	292±88	699±91	792±167	633±146	551±164	<0.0001	0.0025	NS
²⁵ Mg retention, µg	1017±189	1031±225	890±179	845±142	1428±216	1295±192	1221±242	1228±165	<0.0001	0.0107	NS
²⁵ Mg retention, %	39.8±7.3	41.1±9.0	35.6±7.1	33.7±5.7	57.8±8.9	51.5±7.8	48.3±9.2	48.4±6.5	<0.0001	0.0081	NS

Table 5 : Effect of age and inulin intake and their interaction on status biomarkers of Ca and Mg in rats

	Cont 3M	Cont 6M	Cont 11M	Cont 21M	Inulin 3M	Inulin 6M	Inulin 11M	Inulin 21M	inulin	age	interaction
Plasma Ca, mg/L	98±4	98±5	95±6	100±5	102±5	99±4	98±3	100±4	<u>0.0601</u>	<u>0.0619</u>	NS
Tibia weight, mg dw	480±42	630±80	717±89	630±93	489±66	617±66	841±92	648±47	0.0424	<0.0001	0.0231
Bone Ca, mg/g dw	207±21	214±15	216±15	215±21	205±13	215±18	202±14	228±7	NS	0.0179	<u>0.0639</u>
Plasma Mg, mg/L	17.9±1.1	17.7±1.1	17.2±1.0	16.9±1.3	17.6±1.1	17.9±1.3	18.2±1.5	18.2±1.7	<u>0.0570</u>	NS	NS
Erythrocyte Mg, mg/L	45.4±3.8	44.2±4.7	42.5±3.4	43.4±3.0	44.9±4.9	43.8±2.5	44.3±3.6	43.8±3.9	NS	NS	NS
Bone Mg, mg/g dw	3.92±0.10	3.79±0.08	3.72±0.08	3.76±0.08	3.91±0.10	3.72±0.07	3.73±0.09	3.72±0.09	NS	<0.0001	NS

Figure 1: Efficiency of inulin intake on intestinal absorption of ^{44}Ca and ^{25}Mg in rats of different ages

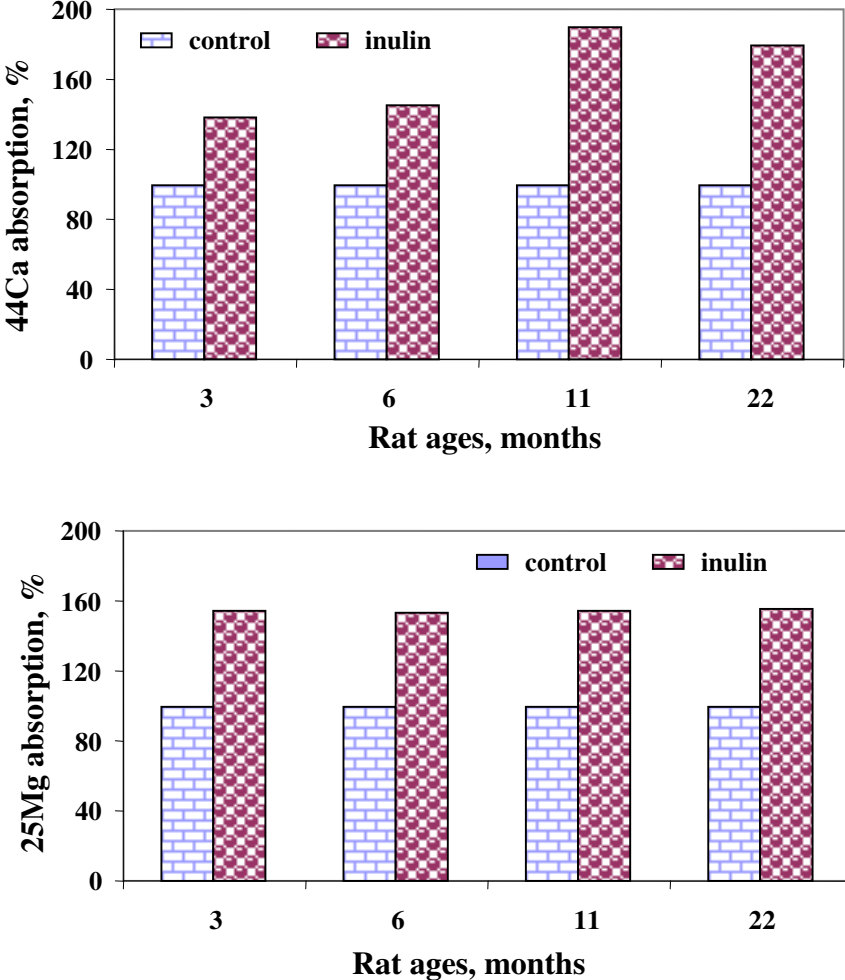


Figure 2: Relationship between aging and intestinal absorption of ^{44}Ca and ^{25}Mg in rats

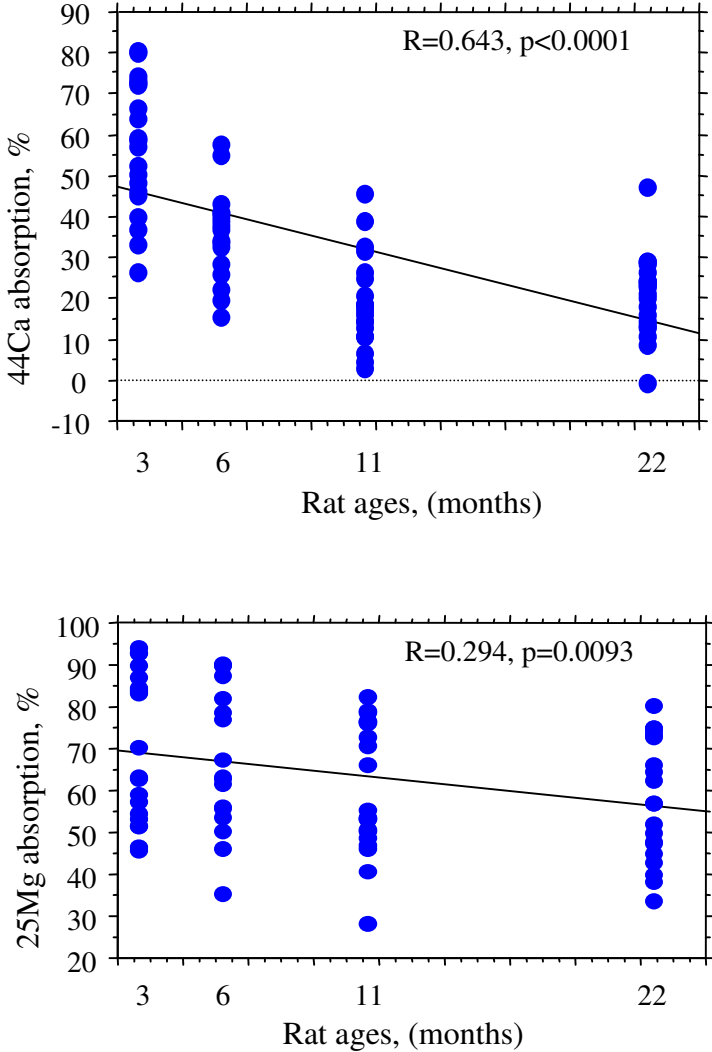


Figure 2