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Methodological study on endogenous calcium absorptivity using rats and ^{41}Ca tracing

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Abstract Elemental calcium plays an important role in human physiology. In order to study the relationship between Ca-intake, Ca-chemical formulation, and Ca-absorptivity, a balance experiment using a ^{41}Ca tracer technique in SD rats was conducted to measure the endogenous fecal calcium and true absorption of calcium. Apparent absorption of calcium was measured as a control to the endogenous calcium labeling experiment. These results show that by using ^{41}Ca labeled endogenous calcium in vivo, researchers could obtain the true calcium absorption data without extrinsic labeling. Therefore, the method was not affected by the chemical structure or type of calcium supplement and might be used in evaluating the absorptivity of marketed calcium supplements.

Keywords Endogenous calcium absorptivity · ^{41}Ca tracer · Accelerator mass spectrometry measurement

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1 Introduction

The important role played by calcium in mammalian organisms is now well recognized. Calcium is the major cation within bone mineral. Calcium also plays an important role as an intracellular messenger in many systems and cells [1, 2]. Although calcium is a microelement closely related to the health of the human body, the average actual calcium intake in China is, on average, 405 mg/d, which is only approximately 50% of the recommended dietary allowance [3, 4]. The calcium deficiency situation is especially serious in children, adolescents, and pregnant women, as reported by the Third National Nutritional Investigation in China (1992). However, recent studies have shown that some diseases may be caused by excess calcium supplementation (especially in patients with osteoporosis) [5–8]. Therefore, the accuracy of calcium absorptive measurements is very meaningful for reasonable calcium supplementation and prevention of diseases caused by calcium metabolism. Due to the poor distinction between calcium sources, lower measurement sensitivity, and radioactive damage that results from traditional calcium absorptivity measurement techniques, it is difficult to carry out accurate, systematic, and long-term research on this topic [9–11].

A common method of determining calcium absorptivity is using the relation $\zeta = (I - F)/I$, where ζ is the apparent calcium absorptivity, I is the calcium intake, and F is the calcium content in feces. However, F can be divided into two parts according to the relation $F = F_i + F_e$, where F_i is calcium of dietary origin and F_e is calcium of endogenous origin [12–14]. Thus, ζ cannot be used to replace calcium absorptivity accurately. We used a more reliable relation, $\eta = (I - F_i)/I = (I - F + F_e)/I$, to measure the calcium

absorptivity of rats, where η is the true calcium absorptivity. The primary difficulty in the determination is the measurement of F_e. A tracer should be used to label the calcium of endogenous origin and calculate the proportion of F_e in F. Because the radiation dose is small and it is not present in biological systems, ^{41}Ca can be used for biological tracer studies [15–17]. Currently, AMS (accelerator mass spectrometry) is the most effective method of measuring ^{41}Ca. Based on the high sensitivity of ^{41}Ca measurements made using CIAE-AMS and the innovative method of labeling endogenous calcium with ^{41}Ca, the calcium absorptivity of rats was studied in this work.

2 Experimental section

2.1 Animals treatment and ^{41}Ca tracing

^{41}Ca tracing and AMS were used to monitor the changes in the skeletal metabolism of rats by labeling endogenous calcium with ^{41}Ca. A total of 48 adult male SD rats (provided by Vital River Laboratories) were caged for one week to allow them to acclimate to the laboratory environment and then fed a Ca^{2+}-poor basic diet (low Ca^{2+} content of about 0.03 g/kg; the feed can be regarded as calcium-free) with deionized water ad libitum for 2 weeks to deplete their Ca^{2+} storage in vivo. The animals were then randomly divided into 4 groups of 12, and according to their body weight, groups A, B, and C were injected intramuscularly with a solution of $CaCl_2$ with ^{41}Ca (54 ng) while group D was injected intramuscularly with a solution of $CaCl_2$ without ^{41}Ca to establish a control group.

The animals were kept in polyethylene cages by group and provided with deionized water ad libitum. The methods of rearing and sampling are shown in Table 1.

Groups A and B were compared to study the influence of different chemical forms of calcium supplements on the absorption rate. The rats were fed with different doses of calcium carbonate and calcium citrate (70 and 11 mg) to create equivalent daily calcium contents (per 100 g rat). Groups A and C were used to compare the influence of calcium supplements dose on the absorption rate.

After the injection of ^{41}Ca, daily stool samples were collected and the ratios of ^{41}Ca to ^{40}Ca in fecal samples were prepared for AMS measurement.

2.2 Samples preparation

Potassium-41 is the major source of interference in the AMS measurement of ^{41}Ca. Injection of CaF_3^- into the measurement system provides a 10^4-greater suppression of the ^{41}K interference than CaF^- [18, 19]. Therefore, the calcium content of the fecal samples of the rats was converted to CaF_2 and CaF_3^-, which were then extracted for AMS measurement. The procedures used for the separation of calcium from stool samples were similar to those in previous work [20, 21], with minor modifications. The ion exchange process was removed, and the recovery rate of Ca increased to over 95%. The CaF_2 was then mixed with PbF_2 powder (mass ratio 1:4) and pressed into an Al sample holder for AMS measurements.

2.3 Measurement method

Detailed AMS conditions for ^{41}Ca measurements are described elsewhere [22]. Briefly, the $^{41}CaF_3^-$ ions were selected by the injection system and injected into the accelerator. The negative molecular ions were accelerated by tandem terminal voltage (8.27 MV). Stripping foil (5 $\mu g/cm^2$) was employed to break up the molecular ions and to produce atomic ions with high charge states. The resulting positively charged ions were further accelerated at the same terminal voltage. A 90° double focusing high energy analyzing magnet was used to select $^{41}Ca^{7+}$ with an energy of 61.39 MeV. After a switching magnet, the $^{41}Ca^{7+}$ was transported to the AMS beam line, and then the $^{41}Ca^{7+}$ was selected using a 15° electrostatic detector and finally detected with an ionization chamber. The ^{41}Ca optical guidance in the accelerator was simulated with a ^{40}Ca beam using the magnetic rigidity of ^{41}Ca.

Table 1 The rearing and sampling methods for rats

Grouping	Rearing methods	Daily calcium intake	Sampling
Group A	Ca^{2+}-poor basic diet + calcium carbonate (42nd day after injection: Ca^{2+}-poor basic diet only)	70 mg/100 g	Stool sample was collected after the injection
Group B	Ca^{2+}-poor basic diet + calcium citrate (42nd day after injection: Ca^{2+}-poor basic diet only)	11 mg/100 g	As in group A
Group C	Ca^{2+}-poor basic diet + calcium carbonate (42nd day after injection: Ca^{2+}-poor basic diet only)	25 mg/100 g	As in group A
Group D	As in group A	70 mg/100 g	As in group A

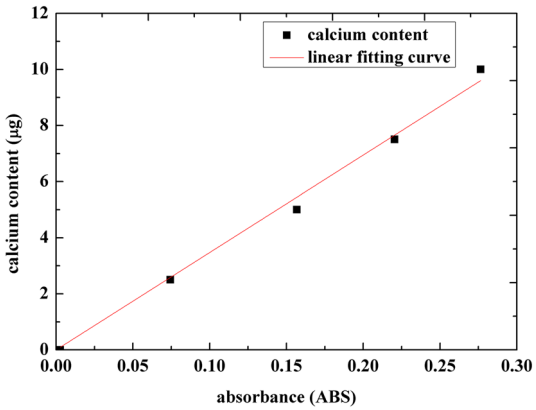

Fig. 1 Linear fitting curve of standard samples

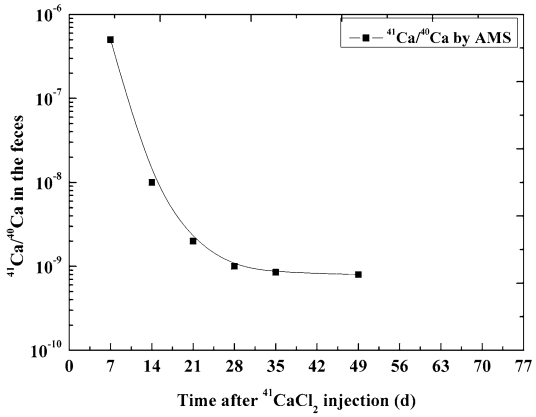

Fig. 2 The ratio of ^{41}Ca/^{40}Ca in the rat vs feces after injecting ^{41}Ca

The samples were disposed of by ashing in a muffle (the sample was prepared using an identical chemical procedure to that of the ^{41}Ca sample) and then processed in a Type AA700CRT atom absorption spectrophotometer (Perkin ELMER, Singapore) to determine the Ca^{2+} contents in the feces. The results of the measurement and a linear fitting curve of standard samples are shown in Fig. 1.

A linear fit is obtained in Fig. 1:

$$y = 34.726x, \quad (1)$$

where y is the calcium content, x is the absorbance, and the linear regression coefficient was $R^2 = 0.9973$; this equation was used to calculate the calcium content in the actual sample.

3 Results and discussion

Figure 2 shows the ratio of ^{41}Ca to ^{40}Ca in the rat vs feces after injecting ^{41}Ca. The ratio of ^{41}Ca to ^{40}Ca in feces tended to reach equilibrium on the 28th day after injection. As a result, the average value of ^{41}Ca/^{40}Ca in feces can be used to represent the actual value from the 35th to 49th day (including the 42nd day) after injection. On the 42nd day after injection, the rats were exclusively fed a Ca^{2+}-poor basic diet and deionized water to obtain the ratio of ^{41}Ca to ^{40}Ca in feces without calcium intake. This value can be used as the ^{41}Ca/^{40}Ca ratio of endogenous origin.

The calcium absorption rate of each rat was replaced by the absorption rate of calcium per 100 g of rat to account for the differences in weight between rats. The results of these measurements are shown in Table 2.

Using these data, we obtained the following results in Table 3.

4 Conclusion

Generally, the actual calcium absorptivity is significantly higher than the apparent calcium absorptivity in rats ($p < 0.05$). Based on the actual calcium absorptivity data,

Table 2 The results of measurements

Grouping	Calcium intake (mg/100 g rat)	Total fecal calcium (mg/100 g rat)	^{41}Ca/^{40}Ca in feces	^{41}Ca/^{40}Ca of endogenous origin
Group A	28 ± 0.1	9.08 ± 0.78	(8.43 ± 1.22) × 10^{-10}	(4.87 ± 0.14) × 10^{-9}
Group B	28 ± 0.1	9.74 ± 0.83	(7.17 ± 1.15) × 10^{-10}	(3.98 ± 0.16) × 10^{-9}
Group C	10 ± 0.1	1.25 ± 0.11	(9.07 ± 1.21) × 10^{-10}	(2.16 ± 0.15) × 10^{-9}
Group D	28 ± 0.1	8.88 ± 0.69	5 × 10^{-13}	5 × 10^{-13}

Table 3 Calcium absorptivity

Grouping	Apparent calcium absorptivity (%)	True calcium absorptivity (%)
Group A	67.57 ± 6.79	73.19 ± 3.60
Group B	65.21 ± 2.96	71.47 ± 4.01
Group C	87.50 ± 1.10	92.74 ± 1.81

the absorption rates of calcium carbonate and calcium citrate are approximately equal at the same dose, indicating that the calcium absorption rate in SD rats is not influenced by the chemical formula of calcium supplements. On the other hand, the amount of absorbed calcium in the intestine depended on the calcium intake. When intake is low, active transcellular calcium transport in the duodenum is upregulated, and a larger proportion of calcium is absorbed by the active process than by the passive paracellular process that prevails in the jejunum and ileum. Bioavailability of the calcium source—digestibility and solubilization—plays a role under low calcium intake conditions but is relatively unimportant when calcium intake is high. Our experimental results are consistent with this theory within a certain range: the greater the calcium intake, the lower the calcium absorption rate, and small doses of calcium can be nearly completely absorbed. This match between theory and experiment validates the method of measuring calcium absorptivity by ^{41}Ca labeling endogenous calcium.

In conclusion, a monitoring method for endogenous calcium absorptivity using rats and ^{41}Ca tracing was developed based on CIAE-AMS, and encouraging results were obtained. The method is currently restricted by factors such as limitations in biological diversity and sample size. A more detailed experimental program is being developed, and further exploration will be implemented in the near future.

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