Effect of feeding cereal-legume diets to rats on in vitro absorption of ^{45}Ca

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Received 18 August 1989; accepted 3 April 1990

Summary. The in vitro absorption of calcium from the duodenum was significantly less in a group of rats fed on a wheat diet than in a group fed a wheat and Bengal gram (70:30) diet.
Key words. In vitro absorption; calcium; wheat; Bengal gram.

Cereal-legume combinations are extensively consumed as staple food in developing countries, in contrast to the industrially advanced countries where animal protein consumption is very high[1]. The prevalence of kidney stone disease[2] and osteoporosis[3] has increased progressively in affluent societies during the last two or three decades, and this has been ascribed to the high intake of animal proteins. The effects of different proteins on calcium metabolism, however, vary with the amount and type of amino acids furnished by each protein. The effect of different cereal-legume mixed diets on the absorption of calcium is not known to any considerable extent. Dietary protein may influence calcium absorption by affecting the intestinal muco proteins. Therefore, the present study was planned to determine the effect of feeding cereal-legume diets on the absorption of ^{45}Ca in the rat intestine, using an in vitro method.

Materials and methods

Wheat chapatis (unleavened bread) and two kinds of wheat and Bengal gram chapatis (80:20 and 70:30) were prepared in the laboratory. The chapatis were dried at 60 ± 1 °C and ground to a fine powder for chemical analysis and for the preparation of diets for feeding to rats. Wheat chapatis and wheat and Bengal gram (80:20 and 70:30) chapatis were analysed for protein, ether extract, crude fibre[4], neutral detergent fibre (NDF)[5], phytin phosphorus[6] and calcium[7].

To study the effect of dietary protein on the in vitro absorption of calcium (^{45}Ca) in rats, the method of Akedo et al.[8] was used. Thirty-two weanling rats, about 28 days old, were divided into four groups and fed the experimental diets ad libitum for ten weeks. The various diets, a wheat chapati diet (D_1), a wheat and Bengal gram (80:20) chapati diet (D_2), a wheat and Bengal gram (70:30) chapati diet (D_3), and a casein diet (D_4) were formulated at 10% protein level. The level of fibre in all the diets was maintained constant at 5% and the level of fat at 10%. The diets were mixed with adequate amounts of vitamin mixture[9] and salt mixture[10]. The composition of the various diets is given in table 2.

At the end of the experimental period, the rats were fasted for 30 h and anaesthetized with solvent ether. A piece of duodenum about 5 cm in length was removed, washed with Krebs' Ringer phosphate buffer (pH 7.4) and used as a sac with the mucosa inside. The sac was filled with 0.4 ml of Krebs' Ringer phosphate buffer containing 0.2% glucose and 0.4 µCi $^{45}Ca - CaCl_2$. On the serosal side, the sac was bathed in 30 ml of Krebs' Ringer phosphate buffer containing 0.2% glucose. The tubes were incubated for 30 min in a water bath maintained at 37 °C. During the incubation, oxygen was bubbled through the contents. At the end of incubation (30 min), 100 µl of sample was taken from the serosal side and added to a vial containing 10 ml of scintillation fluid[11]. Radio activity was measured on liquid scintillation counter (Beckman LS-100). The intestinal segment was taken out of the buffer, blotted on filter paper and weighed. The results of the relative absorption of ^{45}Ca from the duodenum were expressed[12] as ^{45}Ca counts per min/100 µl serosal fluid/100 mg of fresh tissue/30 min

Table 1. Chemical composition of chapatis (on dry matter basis)

Chapatis	Crude protein (%)	Ether extract (%)	Crude fibre (%)	Neutral detergent fibre (%)	Phytin P (mg/100 g)	Calcium (mg/100 g)
Wheat chapati	12.47	1.06	3.11	8.51	197.0	50.0
Wheat/Bengal gram chapati (80:20)	15.97	1.79	3.46	17.30	189.0	70.0
Wheat/Bengal gram chapati (70:30)	16.67	1.87	3.92	20.12	185.0	85.0

Table 2. Composition of diets fed to the rats (g/100 g of diet)

Ingredients	Wheat diet (D_1)	Wheat & Bg* diet (80:20) (D_2)	Wheat & Bg* diet (70:30) (D_3)	Casein diet (D_4)
Wheat chapati	80.3	–	–	–
Wheat & Bengal gram (80:20) chapati	–	62.6	–	–
Wheat & Bengal gram (70:30) chapati	–	–	60.0	–
Casein	–	–	–	12.2
Salt mixture	2.7	2.7	2.7	2.7
Vitamin mixture	1.0	1.0	1.0	1.0
Cellulose	1.9	1.6	1.1	5.0
Groundnut oil	8.9	8.2	8.1	10.0
Corn starch	5.2	23.9	27.1	69.1

*Bg = Bengal gram.

Table 3. Effect of different diets on in vitro absorption ^{45}Ca in rats

Diet	^{45}Ca absorbed (CPM)*	Relative percentage of absorption of ^{45}Ca
D_1-Wheat chapati	34.17 ± 6.15	32.4 (36.7)**
D_2-Wheat & Bengal gram (80:20) chapati	81.77 ± 10.58	77.5
D_3-Wheat & Bengal gram (70:30) chapati	92.99 ± 8.81	88.2
D_4-Casein	105.51 ± 7.94	100.0
C.D. at 5%	31.24	

* These are the values expressed as ^{45}Ca counts per min (CPM)/100 µl of serosal fluid/100 mg fresh tissue/30 min incubation at 37 °C.
** The value given in parentheses is the ^{45}Ca absorption as a percentage of the value for the D_3 diet fed group.

incubation at 37 °C. Results were statistically analysed for analysis of variance.

Results and discussion

The results indicate (table 3) that the rats fed on wheat and Bengal gram (80:20 and 70:30) chapati diets (D_2 and D_3) absorbed more than twice the amount of ^{45}Ca (CPM/100 µl of serosal fluid/100 mg tissue) as compared to the group fed on a wheat chapati diet (D_1). The difference in ^{45}Ca absorption between groups with wheat and Bengal gram diets (D_2 and D_3) and those with casein diet (D_4) was nonsignificant. The absorption of ^{45}Ca in the wheat diet group (D_1) was only 36.7% of that for the wheat and Bengal gram (70:30) diet (D_3). The data clearly reveal that the supplementation of cereal diets with Bengal gram significantly improved calcium absorption, and this may be due to the improved amino acid composition of these diets. It has been reported that the deficiency of essential amino acids in the diet may cause a drop in the calcium-binding activity of muco-proteins [13] and in calcium absorption in vitro and in vivo.

The protein content was higher and there was less phytin phosphorus in wheat and Bengal gram (80:20 and 70:30) chapatis as compared to wheat chapati (table 1). As all the diets fed to rats were prepared at 10% protein level, the total content of wheat in the wheat chapati diet was higher (see table 2). The diets which contained Bengal gram therefore had a lower phytic acid content. This may also have contributed to the better calcium absorption observed in vitro. As the consumption of animal proteins over long periods is known to produce some deleterious effects, cereal-legume mixed diets may be used in place of animal proteins without much effect on the calcium absorption.

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0014-4754/90/101016-02$1.50 + 0.20/0

Irradiation of the head by ^{60}Co opens the blood-brain barrier for drugs in rats

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Received 5 February 1990; accepted 1 June 1990

Summary. The passage of 6 model drugs; acetylsalicylic acid, chloramphenicol, ethimizol, carbisocaine, heptacaine, and diazepam, through the blood-brain barrier, was determined in unirradiated control rats and in animals 1, 3, and 7 days after irradiation of the head only with a dose of 25 Gy from a ^{60}Co source. The brain uptake index (BUI), which compares the uptake of the test substance with that of ^3H_2O 5 s after their injection into the common carotid artery, was significantly increased in comparison with unirradiated controls 7 days after irradiation, for all substances tested except for ethimizol. For acetylsalicylic acid and chloramphenicol it was also significantly increased in the other time intervals. The less lipophilic substances showed a greater relative increase of BUI than the more lipophilic ones.
Key words. Blood-brain barrier; irradiation; drugs.