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The Effect of Dietary Pectin on the Absorption of Vitamin B_{12} in Rats in Various Vitamin B_{12} Status

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Abstract

The effects of vitamin B_{12} status and pectin feeding on vitamin B_{12} absorption in rats were studied. Rats in low, medium, and high vitamin B_{12} status were fed either fiber-free or 10% pectin diet and absorption of a single oral dose of vitamin B_{12} was determined. Various vitamin B_{12} status of rats was verified by urinary methylmalonic acid and liver vitamin B_{12} measurements. Absorption of vitamin B_{12} tended to increase as the rats became deficient in vitamin B_{12}, although the difference was not significant. Pectin inhibited absorption of vitamin B_{12} regardless of the vitamin B_{12} status of the rats. The results demonstrated that inhibition of vitamin B_{12} absorption by pectin would be a possible mechanism for the impairment of vitamin B_{12} status due to chronic pectin feeding.

Key words : pectin, vitamin B_{12}, absorption

INTRODUCTION

Public interest in the importance of dietary fiber in human health has increased since Burkitt *et al.*[1] reported the correlation between lower intake of dietary fiber and higher incidence of diseases common in economically developed countries such as diabetes, obesity, heart disease, diverticulosis, and other noninfectious bowel diseases. The benificial effects of dietary fiber in human health were reported by many investigators[2~7] and increasing intake of fiber has been encouraged. However, dietary fiber was also reported to have harmful effects on health, such as its interference with micronutrient utilization[8~10]. Thus, it is important to elucidate both the beneficial and the detrimental effects of dietary fiber on human health.

Pectin, a soluble and fermentable fiber, has been reported to decrease blood cholesterol and glucose levels in normal and diabetic subjects[11~14]. It has been suggested that pectin, in the form of natural food components or supplements, could be used aggressively to prevent and treat coronary heart disease and diabetes, but for some of its complication effects on health. It has been reported that chronic feeding of pectin has negative effects on vitamin B_{12} status in rats[15~19]. Feeding pectin at the level of 5 to 15% of a vitamin B_{12} deficient semipurified diet for 10 weeks increased excretion of urinary methylmalonic acid (MMA) 9- to 21-fold and increased the rate of elimination of an injected radioactive vitamin B_{12} dose from the body of the rat[15~18]. Pectin also decreased the level of liver vitamin B_{12} in rats[19]. However, the mechanism by which pectin could have a negative influence on vitamin B_{12} status was not clear[15~19].

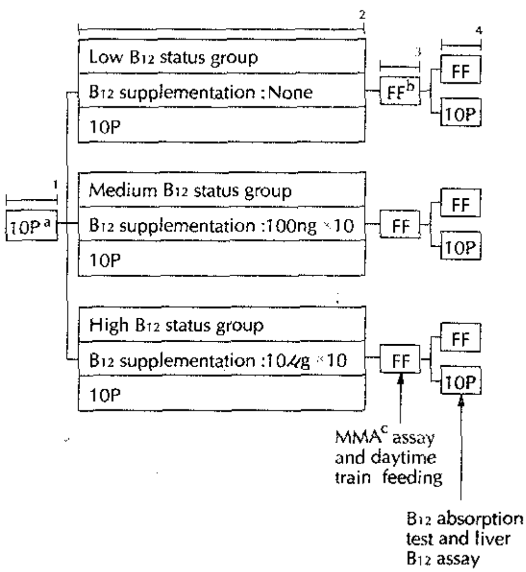
Acute pectin feeding interfered with absorption of vitamin B_{12} in rats[20]. Feeding pectin at the level of 5 to 10% of diet inhibited absorption of a single oral dose of vitamin B_{12}. Pectin intake was inversely correlated with the absorption of vitamin B_{12}. Thus, it was suggested that the possible mechanism of deteriorated vitamin B_{12} status in rats fed pectin for a long term could be the inhibition of vitamin B_{12} absorption by pectin. However, the effect of pectin on vitamin B_{12} absorption in rats fed pectin for a long term has not yet been studied. Long term pectin feeding results in lowering of vitamin B_{12} states of rats[15~19]. Since absorption of many nutrients becomes more efficient as animals become deficient[21], differences in vitamin B_{12} states would confound the interpretation of the relationship between length of feeding pectin and vitamin B_{12} absorption. The present experiment was designed to investigate the influence of vitamin

B_{12} status on vitamin B_{12} absorption. In addition, this experiment examined whether or not the negative effect of pectin on vitamin B_{12} absorption was modified by vitamin B_{12} status of rats.

MATERIALS AND METHODS

Experimental design and vitamin B_{12} absorption test

Thirty six male Sprague-Dawley rats (mean body weight of 72g) received a vitamin B_{12} deficient fiber-free (FF) diet for 4 days and then a vitamin B_{12} deficient 10% pectin (10P) diet for 7 weeks (a 5 week depletion period and a 2 week repletion period) as is shown in Fig. 1. The 10P diet was prepared by adding pectin (Sigma Chemical Co., St. Louis, MO, USA) to the basal fiber-free diet. The composition of the basal diet is shown in Table 1. The approximate polygalacturonic acid concentration and methoxy content of pectin were 86% and 9.9%, respectively. Rats were housed individually in stainless steel wire-bottomed cages and located in a room where temperature (20 ~24° C), humidity (55~65%), and lighting cycle (0700-1900h light and 1900-0700h dark) were controlled. Diet and water were allowed *ad libitum*. The rats were trained to drink 0.5ml of 10% sucrose solution every other day in order to avoid traumatic method of dose administration later.



[1] Depletion period (5 weeks)
[2] Repletion period (2 weeks)
[3] Transition period (1 week)
[4] Dosing period (2 days)
[a] Vitamin B_{12} deficient 10% pectin diet
[b] Vitamin B_{12} deficient fiber-free diet
[c] Methylmalonic acid

Fig. 1. Experimental design.

To develop different vitmain B_{12} states of rats, animals received different amounts of vitamin B_{12} supplement during the repletion period. Rats were supplemented with 10 doses of 0, 100, or 10,000 ng of vitamin B_{12} in 0.5ml of 10% sucrose solution to yield three groups whose corresponding vitamin B_{12} states were low, medium, and high. Following the repletion period all rats were fed a vitamin B_{12} deficient FF diet for one week (transition period). During the last three days of the transition period, daytime train-feeding was developed. Rats had their food cups removed overnight and returned in the morning. At the end of the transition period, rats were individually transferred into metabolic cages and 24-hour urine samples were collected. Urinary MMA was measured by the colorimetric analysis developed by Giorgio and Plaut[22].

On the first day of the dosing period, rats in each vitamin B_{12} status were randomly divided into two groups and were offered either a vitamin B_{12} deficient FF or a 10P diet during the daytime. On the follo-

Table 1. Composition of the fiber-free diet

Ingredient	Amount (g/100g diet)
Soy protein[1]	20.0
Corn starch[2]	70.0
Corn oil[3]	5.0
Salt mix[4]	3.5
Vitamin mix[5]	1.0
D,L-methionine[1]	0.5

[1] Teklad Test Diets, Madison, WI, USA
[2] Miwon Food Co., Korea
[3] Baeksul Food Co., Korea
[4] Rat mineral mix, provided : (in mg/100g of diet) $CaCO_3$, 725 ; $CaHPO_4$, 1130 ; Na_2HPO_4, 651 ; KCl, 730 ; $MgSO_4$, 230 ; $MnSO_4 \cdot H_2O$, 15.4 ; $CuSO_4$, 1.3 ; ferric citrate (16.7% H_2O), 15.1 ; $ZnCO_3$, 2.1 ; KIO_3, 0.1
[5] Vitamin B_{12}-free mix prepared in glucose, provided : (in mg/100g diet) D-biotin, 0.20 ; choline bitartrate, 100 ; folic acid, 1.00 ; nicotinic acid, 5.00 ; D-calcium pantothenate, 5.00 ; pyridoxine · HCl, 1.50 ; (in IU/100g of diet) retinyl acetate, 1,000 ; ergocalciferol, 125 ; D,L-α-tocopherol acetate, 5.00

wing day a vitamin B_{12} absorption test was carried out. In the morning rats received 2g of their assigned diet and then 0.5ml of 10% sucrose solution containing 0.1μCi of 100ng ^{57}Co-vitamin B_{12} dose (Amersham Co., Arlington Heights, IL, USA) in a petri dish. The rats voluntarily consumed the food and ^{57}Co-vitamin B_{12} dose without intubation or any other trauma. After consumption of the test dose each petri dish was filled with Radiacwash solution (Radiac with EDTA, Atomic Products Co., NY, USA) for 24 hours. Any ^{57}Co recovered in the Radiacwash solution was measured by γ-counter (1282 CompuGamma CS Gamma Counter, LKB-Wallac Instrument, Turrku, Finland). The administered dose adjusted for any dose left in the petri dish was considered consumed dose.

Additional food was allowed after dosing. Rats were sacrificed by open heart puncture at 6 hours after dosing. Stomach, small intestine, cecum, colon, and liver samples were collected and ^{57}Co recovered in the stomach, small intestine, cecum, and colon was measured by γ-counter. The counts recovered in each sample were expressed as a percentage of the consumed dose. Since pectin appears to affect the gastrointestinal transit rate[23-25], vitamin B_{12} absorption was expressed as relative absorption. The consumed dose minus ^{57}Co recovered in stomach and small intestine was defined as available dose[20], since vitamin B_{12} is absorbed in the small intestine[26,27]. Available dose represented the vitamin B_{12} dose which had a chance to contact absorption sites in the small intestine. The consumed dose minus ^{57}Co recovered in gastrointestinal tract (stomach, small intestine, cecum, and colon) was considered absorbed dose. Relative absorption was defined as absorbed dose as a percentage of available dose.

Liver samples were first counted by γ-counter to measure ^{57}Co recovery and then the vitamin B_{12} content of liver was measured by competitive binding radioassay[28-30]. The amount of ^{57}Co in the liver derived from the absorption test was too small (less than 1% error) to interfere with the radioassay using ^{57}Co-vitamin B_{12}. The liver samples were treated with papain-cyanide solution (Sigma Chemical Co., St. Louis, MO, USA) to extract vitamin B_{12} from protein binders and vitamin B_{12} was measured using vitamin B_{12}/folate dual RIA kit (Amersham Co., Arlington Heights, IL, USA).

Statistical analysis

One-way analysis of variance was performed on the data of urinary MMA and liver vitamin B_{12} and two-way analysis of variance was performed on the data of relative absorption to detect significant differences between groups. Logarithmic or arcsine transformation of the data was performed if it was necessary to more nearly satisfy the assumptions about the variances and distribution of the observations[31,32]. Tukey's multiple range test with a procedure-wise error rate of 0.05 was used as a follow-up procedure.

RESULTS AND DISCUSSION

To develop various vitamin B_{12} states in rats, a depletion and repletion regimen was used. All rats became poor in terms of vitamin B_{12} status during the depletion period and then were divided into low, medium, and high status groups according to the amounts of vitamin B_{12} supplement offered during the repletion period. Long term feeding of pectin has been reported to change morphology and function of intestinal mucosa[33,34], so generalized malabsorption could be introduced by long term pectin feeding. In this experiment a FF diet was offered to the rats during the transition period to allow the intestinal mucosa to recover from the possible changes induced by chronic pectin feeding. Following the transition period the rats were fed either a FF or a 10P diet and absorption of a single oral dose of vitamin B_{12} was studied.

To verify different vitamin B_{12} status in the rats, urinary MMA excretion and levels of vitamin B_{12} in the liver were measured. The average amounts of urinay MMA were 103, 18, and 8.1μmol/day in the low, medium, and high vitamin B_{12} status groups, respectively (Table 2). Urinary MMA serves as an indicator of vitamin B_{12} deficiency since vitamin B_{12} is a cofactor for the enzyme methylmalonyl CoA mutase[35]. MMA excretion in the low status group increased significantly compared with the medium and the high status groups. Although MMA excretion of the medium group was significantly higher than that of the high status group, the average excretion of both the medium and the high status groups did not exceed 18μ mol/day.

The levels of liver vitamin B12 are shown in Table 3. The average liver vitamin B12 concentration of the medium status group (18.9ng/g liver) was significantly higher than that of the low status group (8.4ng/g liver). Liver vitamin B12 concentration of the high status group (75.7ng/g liver) was significantly elevated compared with that of the medium status group. Thus, the low status group was vitamin B12 deficient and the high status group was in a good vitamin B12 state. The liver vitamin B12 content of the medium status group was between the high and the low groups, but its vitamin B12 status was good enough to prevent elevated excretion of MMA. The mean body weights of the low, medium, and high status groups were 324, 328, and 330g, respectively.

Relative absoption is shown in Table 4. When a FF diet was fed, relative absorptions of the low, medium, and high status groups were 62.0%, 56.0%, and 53.2%, respectively, and there was no statistically significant difference among groups. Although the differences were not significant, there was a trend in the relationship between relative absorption and vitamin B12 status. Relative absorption tended to increase as the vitamin B12 status of rats became poor. However, within the range of vitamin B12 status assigned in this experiment, significant differences in relative absorption depending on vitamin B12 status were not observed. When a 10P diet was fed, relative absorptions of the low, medium, and high status group were 40.7%, 38.8%, and 33.2%, respectively. There was no statistically significant difference among groups. Relative absorption of rats fed the 10P diet (37.6%) was significantly lower than that of rats fed the FF diet (57.1%) regardless of their vitamin B12 status.

It has been reported that chronic pectin feeding deteriorates vitamin B12 status in rats[15~19] and acute pectin feeding impairs vitamin B12 absorption in rats with good vitamin B12 status[20]. In this experiment the inhibitory effect of pectin on vitamin B12 absorption was not modified by vitamin B12 status of the rats. It is concluded that inhibition of vitamin B12 absorption by pectin would be a possible mechanism for the deterioration of vitamin B12 status by chronic pectin feeding.

Table 2. Urinary methylmalonic acid excretion[1,2]

Group	(n)[3]	Urinary MMA (μmol/day)
Vitamin B12 status		
Low	(12)	103 ±27[c]
Medium	(12)	18 ±6.0[b]
High	(12)	8.1±2.6[a]

[1] Urinary methylmalonic acid excretion was measured durning the transition period.
[2] Values are means±SD. Variables were transformed using logarithmic function for the analysis and then analyzed by one-way ANOVA. Tukey's multiple range test was used as a follow-up test. Means differ significantly ($p<0.05$) if they do not share a common alphabetic superscript.
[3] Number of rats per group.

Table 3. Liver vitamin B12[1,2]

Group	(n)[3]	Liver vitamin B12 (ng/g)
Vitamin B12 status		
Low	(12)	8.4±0.9[a]
Medium	(12)	18.9±3.8[b]
High	(12)	75.7±8.6[c]

[1] Liver vitamin B12 was measured after vitamin B12 absorption test.
[2] Values are means±SD. Variables were analyzed by one-way ANOVA. Tukey's multiple range test was used as a follow-up test. Means differ significantly ($p<0.05$) if they do not share a common alphabetic superscript.
[3] Number of rats per group.

Table 4. Relative absorption of ^{57}Co-vitamin B12 dose[1]

Group	(n)[2]	Relative absorption (%)
Individual groups		
FF-Low	(6)	62.0±11.0
FF-Medium	(6)	56.0±12.8
FF-High	(6)	53.2± 9.1
10P-Low	(6)	40.7± 6.3
10P-Medium	(6)	38.8± 4.9
10P-High	(6)	33.2± 7.3
Pooled groups		
Dietary treatments		
FF	(18)	57.1±11.1[b]
10P	(18)	37.6± 6.7[a]
Vitamin B-12 status		
Low	(12)	51.4±14.1
Medium	(12)	47.4±12.9
High	(12)	43.2±13.1

[1] Values are means±SD. Variables were transformed using arcsine function for the analysis and then analyzed by two-way ANOVA. Tukey's multiple range test was used as a follow-up test. Means differ significantly ($p<0.05$) if they do not share a common alphabetic superscript.
[2] Number of rats per group.

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상이한 비타민 B_{12} 영양상태에 있는 흰쥐에 있어서 식이중의 펙틴이 비타민 B_{12}의 흡수에 미치는 영향

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요 약

흰쥐의 비타민 B_{12} 영양상태가 비타민 B_{12}의 흡수에 미치는 영향을 조사하고, 펙틴의 비타민 B_{12} 흡수 저해 효과가 동물의 비타민 B_{12} 영양상태에 따라 어떻게 변화하는지 조사하였다. 흰쥐에게 비타민 B_{12}가 결여된 펙틴식이를 장기간 공급하여 비타민 B_{12} 결핍상태로 만든 후, 상이한 양의 비타민 B_{12}를 투여하여 비타민 B_{12} 영양상태가 불량, 중간, 그리고 양호한 군으로 나누었다. 각 군의 비타민 B_{12} 영양상태는 소변으로 배설되는 methylmalonic acid의 양과 간의 비타민 B_{12} 농도로 조사하였다. 각 군에게 무섬유식이 또는 펙틴식이를 제공하면서, 구강으로 투여한 비타민 B_{12}의 흡수정도를 측정하였다. 비타민 B_{12} 영양상태가 불량한 동물은 양호한 상태의 동물보다 비타민 B_{12}의 흡수율이 높은 경향을 보였으나, 유의적인 차이는 없었다. 펙틴섭취는 동물의 비타민 B_{12} 영양상태에 무관하게 비타민 B_{12}의 흡수를 저해시켰다. 따라서 장기간 펙틴을 섭취한 동물에서 볼 수 있는 비타민 B_{12} 결핍상태는 단기간 섭취한 펙틴의 효과인 비타민 B_{12}의 흡수 저해 효과가 누적되어 발생된다고 결론 지을 수 있다.