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Importance of Lactose in Yogurt for Mineral Utilization

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Mineral utilization was assessed in mature female rats fed yogurt-based diets or casein-based diets that contained quantities of sucrose, lactose, or lactose's component sugars (glucose, galactose) equivalent to the amount of lactose found in yogurt and one of two levels of calcium (4 or 8 mg of Ca/g of diet). Ingestion of the high level of calcium depressed the efficiency of absorption of calcium, magnesium, and zinc. Although ingestion of purified lactose, but not yogurt, improved apparent absorption of magnesium and zinc, the effect of lactose on calcium absorption is questionable. An effect of lactose on calcium absorption could only be observed among rats fed the high level of calcium if less rigorous statistical tests (LSDs) were applied. Diet digestibility was strongly correlated with apparent absorption of calcium, magnesium, and zinc. However, changes in the colon induced by the dietary treatments (i.e., pH and β-glucurondiase activity of colonic contents) were not correlated to apparent absorption of minerals.

Lengemann et al. (1957) suggested that the greater bioavailability of calcium from milk than from plant sources was probably due to the lactose content of milk. A number of investigators have reported that lactose improved calcium absorption in intestinal preparations (Vaughan and Filer, 1960; Favus and Angeid-Backman, 1984; Armbrecht and Wasserman, 1976). However, recent studies have shown few statistically significant differences in the utilization of calcium from dairy products containing lactose and from calcium supplements not containing lactose (Greger et al., 1987; Sheikh et al., 1987; Recker et al., 1988; Behling and Greger, 1988). Scrimshaw and Murray (1985) summarized the apparently inconsistent data on lactose–calcium interactions in this manner: "The bulk of the evidence indicates a favorable or neutral effect of lactose on Ca absorption."

Some of the controversy over the importance of lactose may reflect differences in the physiological state of experimental animals or human subjects. Lactose is not believed to affect the saturable transcellular process of calcium absorption that is regulated by vitamin D but does appear to affect the nonsaturable paracellular pathway of calcium absorption in the gut (Bronner, 1987). As calcium intakes increase, the relative importance of the nonsaturable paracellular transport system increases (Bronner, 1987); presumably sensitivity to lactose also increases.

The maturity of animals may also affect responses to lactose. Armbrecht et al. (1979) noted that active transport of calcium and the level of vitamin D dependent calcium-binding protein decreased in the intestine of rats as they matured. This would presumably increase the relative importance of the nonsaturable process in mature animals. However, as rats mature, intestinal lactase levels tend to decline (Leichter, 1973). Several investigators have observed that dietary lactose did not improve calcium absorption among lactase-deficient humans (Kocian et al., 1973; Cochet et al., 1983). However, other researchers have observed that carbohydrates that escape absorption in the proximal gut enhance calcium absorption distally (Kelly et al., 1984; Amman et al., 1988).

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Table I. Composition of Diets (%)

	4 yogurt	8 yogurt	4 lactose or 4 sucrose	8 lactose or 8 sucrose	4 glucose/ galactose	8 glucose/ galactose
yogurt, freeze-dried	33.3	66.7				
casein[a]	6.9		19.8	19.8	19.8	19.8
DL-methionine[a]	0.3	0.3	0.3	0.3	0.3	0.3
sucrose/lactose			9.5[e]	19.0[e]		
glucose					5.0	10.0
galactose[a]			3.3	6.6	8.3	16.6
corn oil[b]	1.0	1.0	1.0	1.0	1.0	1.0
butter fat, anhydrous[a]	12.0	12.0	12.0	12.0	12.0	12.0
cellulose[a]	5.0	5.0	5.0	5.0	5.0	5.0
mineral mixtures, modified AIN-76[c,d]	3.5	3.5	3.5	3.5	3.5	3.5
vitamin mixture, AIN-76[a,d]	1.0	1.0	1.0	1.0	1.0	1.0
choline bitartrate[a]	0.2	0.2	0.2	0.2	0.2	0.2
calcium carbonate preparation			1.16	2.33	1.16	2.33
cornstarch	36.8	10.3	43.24	29.27	42.74	27.27

[a] Teklad test diets (Madison, WI). [b] Mazola corn oil (Best Foods, Englewood Cliffs, NJ). [c] Supplies (mg/kg of diet): NaCl, 2590; $K_3C_6H_5O_7{\cdot}H_2O$, 7700; K_2SO_4, 1820; MgO, 840; manganous carbonate, 123; ferric citrate, 140; zinc carbonate, 28; cupric sulfate, 10.5; KIO_3, 0.35; $Na_2SeO_3{\cdot}5H_2O$, 0.35; $CrK(SO_4)_2{\cdot}2H_2O$, 19.2. 18 270 mg of Na_2HPO_4/kg of diet was added to the mineral mixture to replace the 17 500 mg of $CaHPO_4$ (calcium source) omitted from the standard formulation. [d] AIN-76 formulation described by American Institute of Nutrition (1977). [e] Lactose used in diets 4 lactose and 8 lactose; sucrose used in diets 4 sucrose and 8 sucrose.

The primary purpose of this study was to assess the relative effect of yogurt-based diets and casein-based diets supplemented with quantities of lactose or its component sugars (glucose, galactose) equivalent to that found in yogurt on the utilization of calcium, magnesium, and zinc by mature rats. A secondary purpose was to determine whether the levels of calcium fed would alter the sensitivity of mature animals to lactose.

PROCEDURE

Diets and Animals. Forty-eight female, Sprague-Dawley, retired breeder rats (Harlan Sprague–Dawley, Indianapolis, IN) were fed one of eight dietary treatments (Table I). The diet providing 4 mg of Ca/g of diet (diet 4 yogurt) was determined to contain 8.5% lactose and 2.6% galactose; diet 8 yogurt was determined to contain 18.4% lactose and 5.4% galactose. Diets formulated to contain similar amounts of lactose as the yogurt-containing diets were called diets 4 lactose and 8 lactose, respectively. Diets formulated to provide the same amounts of disaccharide, but as sucrose, as the lactose-containing diets were called diets 4 sucrose and 8 sucrose. Finally diets formulated to contain the same counts of glucose and galactose as would be present in the yogurt-based diets, if all the lactose were hydrolyzed, were called diets 4 glucose/galactose and 8 glucose/lactose.

Plain low-fat yogurt (Dannon Co., White Plains, NY) was freeze-dried in a pilot-size lyophilizer (Virtis Co., Gardiner, NY) for use in the diets. The diets were formulated to contain either 4 or 8 mg of Ca/g of diet from the freeze-dried yogurt or an oyster shell preparation (Marion Laboratories, Inc., Kansas City, MO). In those diets in which yogurt was not the protein source, casein was used. All diets contained 18% protein from either yogurt and/or casein, except the diet providing 8 mg of Ca/g from yogurt (diet 8 yogurt), which unavoidably contained 23% protein. Fat provided about 28% of the energy in these diets; this is closer to the typical fat intake of Americans than is the standard AIN diet formulation (Science and Education Administration, 1980). The analyzed mineral contents of the diets are listed in Table II.

Rats were housed individually in stainless steel wire-bottomed cages. The facilities met the standards of the American Association for Accreditation of Laboratory Animal Care.

Deionized water and food were offered ad libitum. Food consumption was recorded daily. Rats were weighed twice a week.

Sample Collection and Analyses. On day 30 of the study, each rat was fed one 2-g meal of its respective diet in which 9 μCi of ^{45}Ca as $CaCl_2$ (Du Pont–New England Nuclear, Wilmington, DE) had been blended. Rats were fasted overnight prior to the meal to ensure their immediate consumption of the meal. No other food was given to the rats for 8 h after the meal.

Table II. Analyzed Mineral Content of Diets

treatment	Ca, mg/g	Mg, mg/g	Zn, μg/g
4 yogurt	3.76	0.84	32
8 yogurt	7.51	1.25	41
4 lactose	3.98	0.52	25
8 lactose	7.85	0.57	26
4 glucose/galactose	4.09	0.54	25
5 glucose/galactose	7.68	0.56	23
4 sucrose	4.23	0.57	27
8 sucrose	7.26	0.53	24
pooled SD[a]	0.39	0.01	1

[a] Three portions of each diet analyzed.

Unfortunately, we could not obtain carrier-free ^{45}Ca. The specific activity of the ^{45}Ca was 23.84 mCi/mg of $CaCl_2$. That means only 0.14 μg of calcium as $CaCl_2$ was added to meals that contained approximately 8 or 15 mg of calcium. This would be negligible. However, work by Schwartz et al. (1982) suggests that during the processes of digestion the extrinsic label does not completely exchange with intrinsic calcium. Similarly, Weaver et al. (1987) observed different percent absorptions of ^{45}Ca among rats fed intrinsically and extrinsically labeled spinach but observed similar absorption of ^{45}Ca from intrinsically and extrinsically labeled kale. Together the data suggest that the utilization of extrinsic ^{45}Ca will reflect thegeneral effects of the dietary treatments on dietary calcium utilization but the absolute values may vary.

Fecal samples were collected on days 30–34 of the study. The feces were dried to a constant weight, cleaned of foreign adhering matter, and ground to a fine powder.

Rats were anesthetized and killed on day 35 by exsanguination after being fasted overnight. Tibias were removed and frozen in acid-washed plastic containers. Two pellets were expressed from each rat's colon into separate test tubes. The pellets were weighed and diluted 10-fold with distilled water. The pH of one pellet and the β-glucuronidase activity (Shiau and Chang, 1983) and protein (Lowry et al., 1951) content of the other pellet were determined.

Diets, tibias, and fecal samples were analyzed for calcium, magnesium, and zinc by atomic absorption spectroscopy as described previously (Greger and Snedeker, 1980). The lactose and galactose contents of the yogurt and diets were determined enzymatically (Boehringer Mannheim GMBH, 1980).

Bovine liver standard (SRM 1577a) or milk standard (SRM 1549) obtained from the National Bureau of Standards (NBS) were analyzed with each batch of experimental samples. Samples of the liver standard were determined to contain 116 ± 2 (SEM) μg of Zn/g (n = 18) and 610 ± 5 μg of Mg/g (n = 8); the standard was certified to contain 123 μg of Zn/g and 600 μg of Mg/g. Samples of the milk standards were determined to con-

Table III. Calcium Utilization by Mature Rats Fed Two Levels of Calcium and Various Sugars[a]

treatment	app absorption, %		tibia concn	
	Ca	$^{45}CaCl_2$	Ca, mg/g	^{45}Ca, % of dose
4 yogurt	18 ± 4	80 ± 2	188 ± 3	0.44 ± 0.07
8 yogurt	8 ± 5	73 ± 2	180 ± 4	0.24 ± 0.03
4 lactose	25 ± 4	78 ± 2	182 ± 5	0.60 ± 0.08
8 lactose	19 ± 3	77 ± 2	188 ± 3	0.43 ± 0.09
4 glucose/galactose	27 ± 4	81 ± 1	185 ± 6	0.83 ± 0.19
8 glucose/galactose	7 ± 3	72 ± 2	185 ± 1	0.39 ± 0.04
4 sucrose	24 ± 3	77 ± 3	197 ± 3	0.45 ± 0.08
8 sucrose	5 ± 3	69 ± 1	198 ± 4	0.39 ± 0.04
statistical effect of[b]				
Ca level	0.001	0.001	NS	0.001
sugar source	NS	NS	0.001	0.01
interaction	NS	NS	NS	NS

[a] Means ± SEM (n = 6). [b] Comparisons of means assessed with analysis of variance and differences indicated as significantly ($p < 0.001$ or $p < 0.01$) or not significantly different (NS).

tain 46.7 ± 0.5 μg of Zn/g (n = 14), 1.30 ± 0.02% Ca (n = 28), and 0.117% Mg (n = 24); the standard was certified to contain 46.1 μg of Zn/g, 1.30% Ca, and 0.120% Mg.

Portions of the test meals, ground fecal samples, and ashed tibia samples were suspended in counting fluids (Biosafe II, Research Products International, Mount Prospect, IL), and radioactivity was measured in a β-scintillation counter (Beckman LS 3801; Beckman Instruments, Irvine, CA) set at the photopeak for ^{45}Ca. The counts were corrected for background and decay and expressed as a percentage of the dose given.

Percent apparent absorption of minerals, $^{45}CaCl_2$, and dry matter (percent digestibility) were calculated by

$$[(\text{intake} - \text{fecal loss})/\text{intake}] \times 100$$

The effects of dietary treatments were evaluated by analysis of variance with the general linear model (GLM) (SAS Institute Inc., 1985). When the treatments caused significant differences, the means were compared by two different tests, i.e., factorially for effect of level of calcium, type of carbohydrate, and their interactions and by tests for least significant differences (LSDs). Pearson correlation coefficients were calculated.

RESULTS AND DISCUSSION

These mature rats weighed an average of 274 g and consumed an average of 11.1 g of feed daily. The dietary treatments did not affect either variable.

Calcium Utilization. Rats absorbed calcium and $^{45}CaCl_2$ less efficiently and retained less of the dose of ^{45}Ca in tibias when fed diets that contained 8 mg of Ca/g of diet rather than those that contained 4 mg of Ca/g of diet (Table III). The levels of calcium fed were 160% and 80% of the level of calcium recommended as optimal by the American Institute of Nutrition (1977). The average woman (23–50 years) in the United Statesis believed to consume 70% of the recommended dietary allowance (RDA) for calcium (Science and Education Administration, 1980). But some groups, i.e., Consensus Development Panel on Osteoporosis (1984), suggest that women should consume as much 188% of the RDA for calcium daily. Thus, the levels of calcium fed were not extreme. Moreover, absorption of calcium by the nonsaturable, paracellular system, which is sensitive to lactose in in situ gut loops, should predominate when high levels of calcium are fed (Bronner, 1987).

We believe that one reason for the apparent discrepancies in conclusions on lactose–calcium interactions among studies is the sensitivity of the statistics used to evaluate the data. For example, the interpretation of our data on the effect of dietary carbohydrate on calcium and $^{45}CaCl_2$ absorption depend on the manner in which statistical analyses are performed. When the data were analyzed with a factorial model of analyses of variance, the source of dietary carbohydrate was not found to significantly affect apparent absorption of calcium (Table III). However, when less reliable tests of least significant differences were applied, there were significant ($p < 0.05$) differences. Then, the ingestion of lactose appeared to improve calcium absorption when the high level of calcium was fed, i.e. 19% absorption vs 8%, 7%, or 5% when yogurt, glucose/galactose, or sucrose, respectively, was fed. There were no differences in calcium absorption among rats fed the various carbohydrate sources when the low level of calcium was fed. Either statistical test is valid to apply to these data.

The apparent absorption of the ^{45}Ca from $CaCl_2$ was much higher than the apparent absorption of the total dietary calcium. This may reflect several factors. Extrinsic calcium does not necessarily equilibrate with intrinsic calcium (Schwartz et al., 1982; Weaver et al., 1987). Fecal losses of total calcium may reflect endogenous secretions more than fecal losses of ^{45}Ca in the 4 days after a test meal. Thus, absorption of ^{45}Ca would be greater than apparent absorption of total calcium. Even so, the effects of the dietary treatments were similar. Factorial analysis demonstrated that ingestion of 8 vs 4 mg of Ca/g of diet depressed apparent absorption of ^{45}Ca but that the source of dietary carbohydrate had no effect. Alternatively, use of the less reliable LSD tests demonstrated that the apparent absorption of ^{45}Ca was greater among rats fed lactose rather than sucrose with 8 mg of Ca/g of diet.

Interestingly the variable indicating calcium utilization that appeared to be most sensitive to the form of carbohydrate in the diet was retention of ^{45}Ca in bone. Those rats fed glucose/galactose or lactose retained significantly more ^{45}Ca in bone than those fed yogurt as determined by factorial analysis. The suggests other factors in yogurt may have counteracted the effect of lactose and its component sugars on calcium utilization.

Overall our data suggest that a primary reason for the controversy in the literature on the effect of dietary lactose on calcium absorption is that the interaction of lactose with calcium is a fairly weak one. Differences in the total dietary regime, such as the level of calcium fed or the source of lactose (i.e., as an isolated chemical or in yogurt), and differences in the sensitivity of the statistical methods affect whether data from a study appeared to provide support for this interaction. Thus, the importance of lactose in improving calcium absorption in adult animals under practical situations is probably limited. Work of others suggest that this interaction may have

Table IV. Magnesium and Zinc Utilization by Mature Rats Fed Two Levels of Calcium and Various Sugars[a]

treatment	app absorption, % Mg	app absorption, % Zn	tibia concn Mg, mg/g	tibia concn Zn, μg/g
4 yogurt	53 ± 3	6 ± 4	3.19 ± 0.04	162 ± 4
8 yogurt	46 ± 3	-1 ± 4	3.08 ± 0.07	159 ± 8
4 lactose	64 ± 3	17 ± 3	3.08 ± 0.05	163 ± 3
8 lactose	66 ± 2	15 ± 3	3.14 ± 0.11	163 ± 5
4 glucose/galactose	55 ± 4	9 ± 5	2.97 ± 0.07	167 ± 4
8 glucose/galactose	47 ± 1	-3 ± 3	3.05 ± 0.06	162 ± 3
4 sucrose	56 ± 2	15 ± 3	3.07 ± 0.05	160 ± 6
8 sucrose	44 ± 4	1 ± 2	3.08 ± 0.09	158 ± 5
statistical effect of[b]				
Ca level	0.005	0.005	NS	NS
sugar source	0.001	0.005	NS	NS
Interaction	NS	NS	NS	NS

[a] Means ± SEM ($n = 6$). [b] Comparison of means assessed with analysis of variance and differences indicated as significantly ($p < 0.001$ or $p < 0.005$) or not significantly different (NS).

greater practical implications among young animals (Kobayashi et al., 1975; Ghishan et al., 1982).

Magnesium and Zinc Utilization. Rats fed diets with added lactose apparently absorbed magnesium and zinc more efficiently as assessed by factorial analysis than rats fed the other diets (Table IV). Other investigators have observed the addition of lactose to a diet enhanced apparent absorption or retention of magnesium and zinc (Lengemann, 1959; Forbes, 1961; Ghishan et al., 1982), but generally nutritionists have emphasized the seemingly less consistent effect of lactose on calcium absorption. Ingestion of additional calcium depressed apparent absorption of both magnesium and zinc in these mature rats as has been observed previously (Seelig, 1964; Greger, 1981).

Bone mineral levels of young rats, but not usually of mature rats, are sensitive to dietary changes (Greger et al., 1987; Behling and Greger, 1988). Accordingly, the variation in calcium intakes had no effect on bone calcium, magnesium, or zinc concentration during this 1-month study (Tables III and IV). Bone calcium concentrations were elevated among rats fed sucrose rather than yogurt, lactose, or glucose/galactose. This was not related to the absorption data. However, Kang et al. (1979) observed that rats accumulated more calcium in their kidneys when fed sucrose rather than starch.

Although the yogurt-containing diets provided as much lactose to rats as diets 4 lactose and 8 lactose, the former did not promote mineral absorption. This cannot be related to hydrolysis of lactose by microorganisms in the yogurt during storage or in the gut (Kolars et al., 1984). The lactose content of the diets as determined by repeated analyses remained constant during storage. The yogurt had been freeze-dried. Tamine and Deeth (1980) found no viable microorganism in frozen yogurt.

The yogurt contributed additional magnesium to the diet. Previously Greger et al. (1987) noted that supplemental inorganic magnesium depressed absorption of calcium. However, the additional magnesium in milk and yogurt did not appear to depress calcium absorption in mature and young rats in previous studies (Greger et al., 1987; Behling and Greger, 1988). Perhaps other factors in the yogurt counteracted any beneficial effects of the lactose in yogurt. The yogurt-containing diets were slightly less digestible than diets 4 lactose and 8 lactose (Table V).

It does not appear that any effects of lactose on mineral absorption can be ascribed to its component sugars per se as suggested by Kobayashi et al. (1975). The glucose/galactose-containing diets were formulated to provide similar amounts of glucose and galactose as the lactose-containing diets would upon hydrolysis. However, ingestion of glucose and galactose did not have beneficial effects on mineral absorption.

Table V. Fecal pH and β-Glucuronidase Activity and Dry Matter Digestibility of Mature Rats Fed Various Sugar Sources and Calcium Levels

treatment	colonic content, pH	β-glucuronidase act. of colonic content,[a] units/mg protein	diet digestibility,[b] %
4 yogurt	7.30 ± 0.21[c]	113 ± 14	91.9 ± 0.2
8 yogurt	7.67 ± 0.17	123 ± 26	89.6 ± 0.5
4 lactose	7.65 ± 0.17	54 ± 9	92.2 ± 0.4
8 lactose	7.58 ± 0.13	87 ± 13	90.6 ± 0.3
4 glucose/galactose	7.50 ± 0.15	107 ± 22	92.8 ± 0.4
8 glucose/galactose	7.84 ± 0.13	126 ± 17	90.7 ± 0.4
4 sucrose	7.35 ± 0.20	112 ± 33	92.6 ± 0.3
8 sucrose	7.74 ± 0.14	143 ± 10	91.0 ± 0.2
statistical effect of[d]			
Ca level	0.05	NS	0.0001
sugar source	NS	0.05	0.05
interaction	NS	NS	NS

[a] Unit = micrograms of phenolphthalein produced/hour per milligram of protein. [b] Digestibility = [(intake of dry matter − fecal loss of dry matter) × 100]/intake of dry matter. [c] Means ± SEM ($n = 6$). [d] Comparisons of means assessed with analysis of variance and differences indicated as significantly ($p < 0.0001$ or $p < 0.05$) or not significantly different (NS).

Gut Function and Flora. Kelly et al. (1984) and Amman et al. (1988) have noted that carbohydrates that escape absorption in the proximal intestine enhance calcium absorption in the remainder of the gut. The carbohydrates that escape absorption in the proximal gut also become substrate for gut flora. We thought the metabolic activity of this flora might be assessed through measurement of β-glucuronidase activity and pH of the colonic contents.

Factorial analysis demonstrated that rats fed yogurt absorbed dry matter in their diets less efficiently than rats fed sucrose or glucose/galactose; average digestibilities were 90.8% vs 91.8% and 91.7%, respectively. The digestibility of the lactose-containing diets was 91.4%.

More dry matter was available as substrate for gut flora in rats fed yogurt and to lesser extent isolated lactose. However, factorial analysis indicated that the form of dietary carbohydrate had no effect on the pH of colonic contents (Table V). Furthermore, rats ingesting isolated lactose had decreased β-glucuronidase activity as compared to rats fed yogurt, glucose/galactose, or sucrose.

Dietary calcium levels had a more consistent effect on these variables. Rats fed 8 mg of Ca/g of diet rather than 4 mg of Ca/g absorbed total dry matter less efficiently. The increased amount of material that entered the lower gut of animals fed 8 mg of Ca/g of diet rather than 4 mg of Ca/g of diet was not associated with a significant change in β-glucuronidase activity, but there was a tendency for enzyme activity to be increased. Furthermore, the antacid capacity of calcium was evident as the pH of the colonic contents of rats fed the higher level of calcium was elevated.

Diet digestibility was highly correlated to apparent absorption of calcium ($r = 0.802$, $p < 0.001$), magnesium ($r = 0.451$, $p < 0.005$), and zinc ($r = 0.708$, $p < 0.001$). This suggests the importance of generalized gut function on mineral absorption. However, changes in the colon induced by the dietary treatments (i.e., pH and β-glucuronidase activity of colonic contents) were not related to mineral absorption.

In general, the addition of lactose to diets fed to mature rats had less effect on calcium utilization and diet digestibility than doubling the amount of calcium in the diet. However, the impact of dietary lactose on magnesium and zinc utilization deserves further study. The relative importance of lactose–mineral interactions appears to be modified by several factors, including the level of calcium fed and the source of dietary lactose (i.e., in isolated form or in yogurt).

Registry No. Ca, 7440-70-2; Mg, 7439-95-4; Zn, 7440-66-6; lactose, 63-42-3; sucrose, 57-50-1; glucose, 50-99-7; galactose, 59-23-4; β-glucuronidase, 9001-45-0.

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