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Effect of lactose hydrolysis on calcium absorption during duodenal milk perfusion

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Summary. A multi-lumen intubation system was used to study the absorption of calcium, glucose and galactose in 13 human subjects. The intubation was placed between the duodenum abdomen and proximal jejunum and the subjects were perfused with milk and lactase-supplemented milk. Lactose disappearance over a 20 cm length of intestine was used as the index of lactase activity. The subjects were assigned to one of two groups, lactase-normal and lactase-deficient.

There was linear correlation between the absorption of calcium and lactose : lactase-deficient subjects absorbed less calcium than lactase-normal subjects. Perfusion with lactase-supplemented milk enhanced calcium absorption in lactase-deficient subjects but had no effect on that of normal lactase subjects. All subjects absorbed approximately the same percentage of perfused calcium (24 %) when perfused with hydrolysed milk. These data indicate that the enhancement of calcium absorption is not a function of lactase per se, but of its hydrolytic products, glucose and galactose.

Introduction.

The role of lactose in the intestinal absorption of calcium has been studied for many years, but the results were often contradictory. Some reported an enhancing action of lactose in man (Fournier and Dupuis, 1960 ; Pansu and Chapuy, 1970) and in rats (Fournier, 1954 ; Pansu *et al.*, 1975 ; Armbrecht and Wasserman, 1979 ; Vaughan and Filer, 1960) ; others failed to demonstrate any effect in man (Debongnie *et al.*, 1979) or observed an inhibition of Ca absorption in lactose-intolerant subjects in the presence of lactose, while a positive effect was observed in lactose-tolerant subjects (Cochet *et al.*, 1983 ; Condon *et al.*, 1970 ; Kocian, Skala and Bakos, 1973). The varying observations with lactose tolerance seem to indicate that intestinal lactose lydrolysis is necessary before the enhancing effect of lactose on calcium absorption can be expressed (Debongnie *et al.*, 1979). This new hypothesis refutes earlier ones in which lactose played a direct role (Pansu *et al.*, 1975) by complexing intestinal calcium (Charley and Saltman, 1963) or by reducing intestinal pH (Kline *et al.*, 1932) after being reduced to lactate (Cochet *et al.*, 1983 ; Kocian, Skala and Bakos, 1973).

However, as yet there is little direct evidence that such hydrolysis is necessary. Experiments in which the absorption of Ca alone (Norman, Morawski and Fordtran, 1980) or in lactose-free milk and in the presence of lactose (Cochet

et al., 1983) were compared only indicated that the lactose calcium-enhancing effect varied with the subject's lactase activity. It was necessary to compare the effect of lactose and hydrolysed lactose on calcium absorption in lactase-deficient subjects to clearly show the role of lactose hydrolysis ; such a study was carried out by Debongnie *et al.* (1979), but only a small number of subjects were examined and they were of very different ages.

The present study was performed to examine the ratios of calcium absorption in subjects having different lactase activity levels in the presence of lactose and hydrolysed lactose. The duodena of human volunteers were perfused with milk or lactase-supplemented milk via a triple-lumen intubation system, and the calcium and lactose (or its hydrolytic products) levels at the two collection sites were compared. The data were analysed to determine the effect of lactose hydrolysis on calcium absorption.

Material and methods.

Subjects. — Thirteen volunteers 19 to 20 years old (6 women and 7 men) participated in the study. The procedure was clearly explained to them and each gave their total consent. Ten of the subjects were white Europeans (students at the Institute of Grignon) and three were Africans (2 blacks from Senegal and 1 Arab). All were in good health and were not taking any form of medication.

Each subject was intubated via the nose or mouth in the morning following a 12-hour fast. A mercury-weighted polyvinyl tube (Marcat) was inserted into the gastrointestinal (GI) tract until the tip was located fluoroscopically at the angle of Treitz. The perfusion site was located 30 cm above the tip. The collection sites were at the tip of the tube and 20 cm above it. The subject was perfused at a constant rate (6 ml/min) with three solutions, all maintained at 37 °C, for periods of 40-60 min each. The first perfusate was normal saline (0.85 % NaCl) (equilibrium period), the second was partially skimmed milk (45 g/l of lactose and 1.1 g/l of calcium) and the third was the same partially skimmed milk + 4 000 U/l of lactase (Maxilact LX 5 000-Gist Brocades) (22 g/l of glucose, 22 g/l of galactose, 1 g/l of lactose). The milk perfusates contained the non-absorbable volume marker 14 C-polythyleneglycol (PEG 4 000, NEN) at a concentration of 5 µCi/l. Milk pH was neutral at the first collection site and basic at the second one.

Measurement of sugar and calcium absorption. — No samples were collected during initial saline perfusion. The second solution was perfused for 40 min (equilibrium period) before intestinal collections began simultaneously at the two aspiration sites. Two-ml fractions were collected manually with a syringe at a constant rate (sampling time : 10 min) for a period of 50 min, providing 5 samples per subject per type of milk. Each sample was homogenized and analysed for its sugar and calcium contents. Lactose, glucose and galactose were analysed by gas-chromatography (Carlo-Erba) using ribitol as an internal standard. Calcium was measured in a calcimeter (Corning) and PEG concentration was determined by liquid scintillation (Kontron). The ratio of calcium and sugar concentrations to

total radioactivity was calculated for each sample (expressed as a percentage of the perfusate). The average concentrations of the five fractions were determined and analysed statistically using t-tests or correlation tests.

Results.

1. *Intestinal lactose disappearance as a measure of lactase activity.*

The subjects were assigned to one of the two following groups according to intestinal lactose content :

Group 1. Subjects in which lactose concentrations at the lower collection site (tip) were at least 20 % lower than those at the upper collection site. Hydrolytic products were not considered detectable as they would be absorbed very quickly after hydrolysis. The lactase activity of this group was considered to be normal : mean lactose disappearance over 21 cm of intestine = 25.8 % $\pm$ 5.5 ; n = 5 of lactose perfused.

Group 2. Subjects whose lactose concentrations between the two collection sites differed by less than 20 % ; these were considered to be lactase-deficient : mean lactose disappearance = 8.0 % $\pm$ 2.8 ; n = 8 of lactose perfused. The three African subjects in this group showed a total absence of lactase, as no difference in lactose concentration was observed between the two collection sites. All variations in fluid volume resulting from the unabsorbed lactose were corrected by the concentration of 14 C-PEG.

2. *Intestinal calcium absorption.*

Figure 1 shows the percentage of calcium lost between the two collection sites. This was taken to be the percentage of perfused calcium absorbed in the 20 cm length of intestine. Percentage of calcium absorbed :

$$100 - \frac{\text{Ca (2)}}{\text{Ca (1)}} \times \frac{\text{PEG (1)}}{\text{PEG (2)}} \times 100$$

(1) at the upper collection site ; (2) at the lower collection site ; Ca = sample calcium concentration ; PEG = sample total ^{14}C radioactivity.

a) *Perfusion with raw milk.* — Alactasic subjects were characterized by a high (25 to 50 %) dilution of intestinal PEG C14 in the jejunum as compared with the duodenum. There was no change in the PEG concentration between the two collection sites in lactase-normal subjects. A significant difference in calcium absorption was seen between the normal and the lactase-deficient groups ($P < 0.005$). The normal group absorbed 24.4 % of the calcium perfused, three times more than the lactase-deficient group (8.1 % of calcium absorption). In this last group, the three completely alactasic subjects absorbed a non-measurable amount of calcium in the experimental 20 cm of duodenum-jejunum. Calcium absorption versus lactose absorption curves were plotted for each subject and analysed for correlation. The regression line (fig. 2) obtained had a correlation coefficient of 0.778.

b) *Perfusion with hydrolysed milk.* — There was a significant increase ($P < 0.005$) in calcium absorption in the lactase-deficient group when hydro-

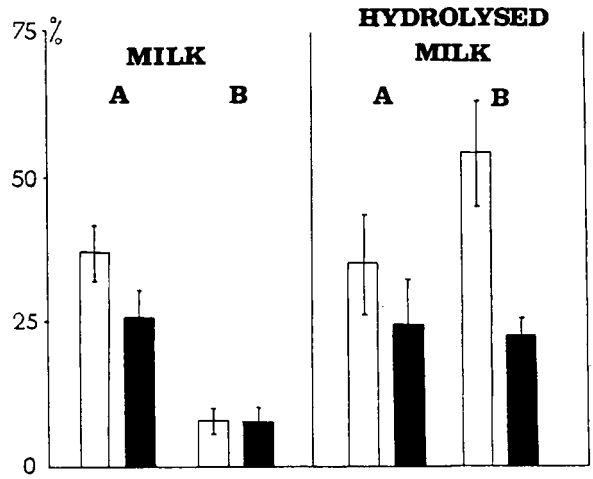

FIG. 1. — *Differences in percentage of perfused amounts of calcium* (black histograms) *and monosaccharides* (white histograms) *absorbed between duodenum and jejunum from milk and hydrolysed milk.* The mean + SEM is shown for the two groups with normal (A) and deficient (B) lactase activity.

lysed milk was perfused. No such difference was observed in subjects with normal lactase activity. The two groups absorbed the same amount of calcium (24 %) when perfused with hydrolysed milk.

Glucose and galactose were absorbed at similar rates by each subject, but these rates differed slightly between the two groups. In group 1, glucose and galactose absorption during hydrolysed milk perfusion was similar to lactose absorption during milk perfusion (36.0 % of glucose, 34.0 % of galactose and 35 % of lactose perfused). In group 2, perfusion with hydrolysed milk resulted in a 6-fold increase in milk sugar absorption (53.8 % of galactose and 52.8 % of glucose *vs* 8.2 % of lactose perfused). These subjects seemed to absorb free sugars at a higher rate than the lactase-normal subjects, but the difference was not significant. The two black Africans, who were totally alactasic, absorbed surprisingly high amounts of galactose and glucose (about 80 % of the perfused sugar).

Discussion.

The ability of human subjects to absorb calcium and glucose-galactose from milk and hydrolysed milk has been examined. An intubation system was used with duodenal milk perfusion and sample collection at two distal sites, one duodenal and the other jejunal. Although possessing certain unphysiological characteristics, this technique allowed quantification of lactase activity by measuring the disappearance of lactose between the duodenum and jejunum and has been proposed as one of the most suitable methods of performing this type of study (Bond and Levitt, 1976).

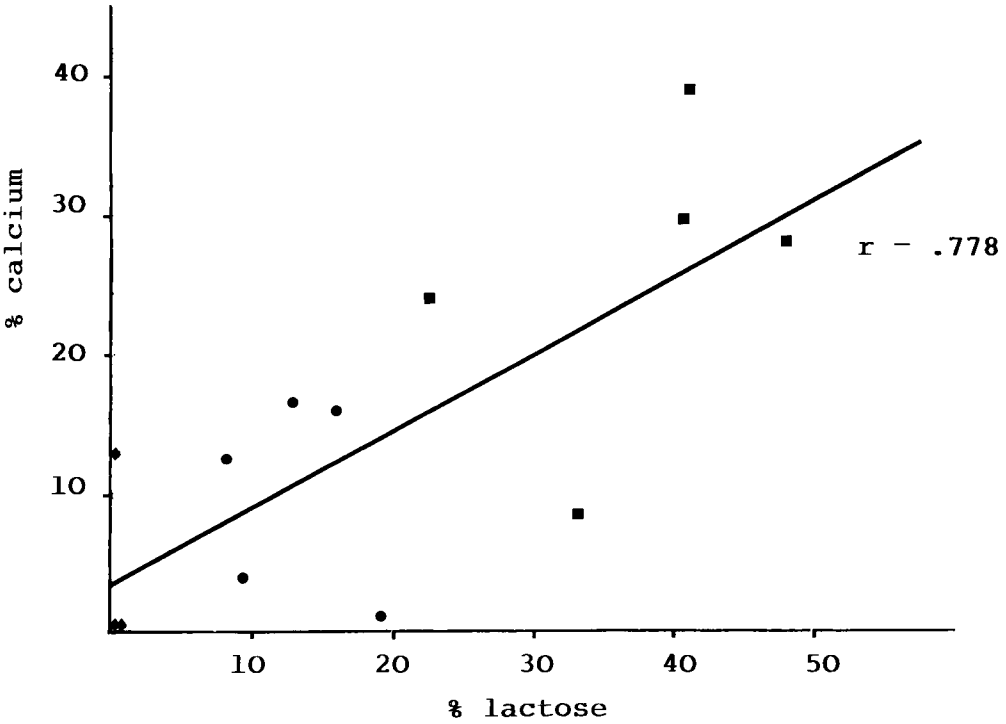

FIG. 2. — *Relationship between the percentage of calcium absorbed and of lactose lost between duodenum and jejunum during milk perfusion.*
■ : normal-lactase group ; ● : low-lactase group ; ◆ : zero-lactase group.

The results show a positive correlation between calcium absorption and lactase activity in all the subjects. This finding supports previous studies reporting a variation in the effect of lactose on calcium absorption according to lactase activity (Cochet *et al.*, 1983 ; Condon *et al.*, 1970) : lactose had a positive effect in lactose-tolerant subjects but no effect or a negative one in intolerant subjects. In the present study, the absorption of milk calcium was significantly greater in subjects with normal lactase activity than in lactase-deficient subjects (26 % of Ca absorbed *vs* 8 %). These data are consistent with those reported by Debongnie *et al.* (1979) who used a similar intubation sampling system, but the milk was ingested orally and the samples collected at the terminal ileum. Control subjects absorbed 65 % of milk calcium and lactase-deficient subjects 28 % ; this is 2 or 3 times greater than the absorption levels seen in the present study. However, the length of intestine over which absorption was tested was much shorter in the present work and the ileum is known to be the major site of calcium absorption in rats (Marcus and Lengemann, 1962). The differences observed between the two experiments are thus understandable, and it can be assumed that direct duodenal perfusion does not affect milk calcium absorption. Nevertheless, the Ca

absorption values obtained in the present study are difficult to compare with those of other reports because of the differences in such parameters as calcium source, intestinal segment examined and method used. Perfusion with hydrolysed milk induced a very significant increase in Ca absorption by lactase-deficient subjects, but had no effect on calcium absorption by lactase-normal subjects. In contrast to our finding with milk perfusion, calcium absorption levels were equal in all subjects during hydrolysed milk perfusion and were independent of the subject's lactase activity levels (24 %).

These results are somewhat different from those reported by Pansu and Chapuy (1970) who found a higher lactose enhancement of calcium absorption in alactasic subjects than in normal lactasic ones. However, in the latter study, lactose and calcium were ingested as a bolus containing very high quantities (500 mg of Ca, 100 g of lactose). The present study is closer to those of Debongnie *et al.* (1979) and Kobayashi *et al.* (1975), both of which showed that hydrolysed lactose resulted in an increase in calcium absorption. However, the first one reported that hydrolysed lactose had a similar positive action in both control and lactase-deficient subjects, while we observed this effect only in the lactase-deficient ones. These discrepencies may result from the method of measuring lactase deficiency (expired H_2, direct intestinal absorption or plasma glucose measurement). The enhanced calcium absorption by lactase-deficient subjects during hydrolysed milk perfusion could be related to the restoration of their capacity to absorb glucose and galactose. These two sugars were surprisingly well absorbed by totally alactasic subjects (double the efficiency of the other groups). However, it should be remembered that both subjects were Africans who might differ genetically from the controls.

In contrast to the findings of Gray and Santiago (1966), the present study indicates that these two sugars are transported at the same rate and that the presence of these hexoses in a disaccharide form (lactose) does not change the rate of absorption, as shown by the identical absorption levels between lactose and glucose-galactose in lactase-normal subjects. The results of Vaughan and Filer (1960), showing that galactose has a greater effect on ileal Ca absorption than does glucose, are difficult to understand if sugar transport is involved in Ca absorption. The enhanced Ca absorption observed with a glucose polymer (Kelly *et al.*, 1984) might be interpreted as being due to glucose accumulation and its absorption following polymer hydrolysis.

These results can be well explained by the hypothesis of Norman, Morawski and Fordtran (1980) which proposes that the Ca-absorption-enhancing action of glucose is a consequence of water movement across the mucosa induced by glucose transport. The resulting increase in Ca concentration should activate its absorption by active transport. A Ca-glucose-galactose-cotransport system might also be envisaged. Whatever the details of the mechanism, the present study suggests that the origin of increased Ca absorption is not the lactose itself, but its hydrolytic products, glucose and galactose, and that it probably involves their transport mechanism.

This study, together with those of Debongnie and Kobayashi, clearly indicates that lactose pre-hydrolysis can increase the absorption of calcium in

alactasic subjects. While further studies are required on a larger group of subjects to clarify certain differences between these studies, the evidence seems to indicate that the ingestion of hydrolysed milk may be a good method of enhancing the calcium status in young alactasic subjects and in osteoporotic subjects who are often lactase-deficient (Newcomer *et al.*, 1978 ; Norman, Morawski and Fordtran, 1980).

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Résumé. *Hydrolyse du lactose et absorption intestinale du calcium du lait.*

Cette étude a pour objectif de déterminer le rôle de l'hydrolyse préalable du lactose du lait sur l'absorption intestinale du calcium, en fonction de l'activité lactasique du sujet. Treize volontaires, 7 hommes et 6 femmes, ont été intubés avec une sonde à triple canal, dont l'extrémité est localisée au niveau de l'angle de Treitz. Successivement, du sérum physiologique (pour équilibration), du lait et du lait à lactose hydrolysé sont perfusés à vitesse constante. Les reflux intestinaux correspondant au duodénum et au jéjunum proximal (distance de 20 cm entre ces 2 points) sont recueillis et analysés. La quantité de lactose absorbée entre ces 2 points a été choisie comme indice de l'activité lactasique du sujet. Les résultats mettent en évidence une bonne corrélation entre cette valeur et la quantité de calcium absorbée sur le même segment au cours de la perfusion du lait, celle-ci étant nulle pour les sujets totalement alactasiques. Les sujets ont été regroupés en tolérants (au- delà de 20 % de lactose absorbé sur le segment) et faiblement tolérants (au-dessous de 20 % d'absorption) au lactose. Le taux d'absorption du calcium du lait est significativement plus faible chez les sujets à basse activité lactasique, mais augmente fortement ($p < 0,05$) après hydrolyse préalable du lactose. Ce taux est alors équivalent à celui mesuré chez le groupe à forte activité lactasique, qui reste identique, que le lait soit hydrolysé ou non. L'effet activateur du lactose sur l'absorption intestinale du calcium apparaît donc bien lié, non au lactose per se, mais à ses produits d'hydrolyse, le mélange glucose-galactose.

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