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Effect of Casein-Derived Peptides on D-Xylose Absorption Assessed by H_2 Breath Test in Normal Volunteers

CARLOS DEFILIPPI, MD, ANA MARIA MADRID, MD, KARIME SALAS, LUIS MICHEA, MD, and NESTOR LAGOS, PhD

Studies have shown a promoting effect of food on small intestinal absorption. Casein hydrolysate seems be more effective in increasing of D-xylose absorption in dogs than the whole protein and lactulose. The purpose this study was to analyze the effect of groups of peptides derived from casein hydrolysate on the absorption of D-Xylose and intestinal transit time in normal subjects. Seven normal volunteers participated in the study. Three peptide fractions were isolated from casein enzymatic hydrolysate by means of a preparative HPLC silica column. On separate days subjets drank test solutions containing lactulose, D-xylose, and D-xylose with one of three peptide groups. The hydrogen breath test was used to indirectly estimate D-xylose absorption and orocecal transit time. Two peptide fractions when added to D-xylose were followed by an increased absorption characterized by decreased H_2 production. A nonstatistically significant increase of orocecal transit time was observed with these peptides.

KEY WORDS: casein; peptides; D-xylose; absorption

Studies have shown that the introduction of food into the small intestine increases the absorption of water and electrolytes from an isolated loop (1). Meal-induced jejunal absorption was observed in response to different nutrients and also after administration of nonnutritive substances (2). Previous studies have suggested that different factors might be involved in the effect of promoting absorption: cholinergic stimulation and transmembrane calcium fluxes (1, 3), stimulation of opioid receptors (4), and hormonal secretions (5). Therefore, it is possible that this promoting effect on absorption might be enhanced with some nutrients compared to others. According to this point of view, studies in our laboratory (6) have shown that replacement of soy protein by casein in a mixture of proteins, fat, and carbohydrates continuously infused in the duodenum, markedly increased absorption in dogs with duodenal and ileal cannulas. In additional studies we found that casein hydrolysate infusion was followed by an increase of D-xylose absorption compared to the whole protein (7), suggesting that this effect is related to the presence of peptides derived from casein into the small intestine.

Recently we have separated, by means of a silica column, three groups of peptides according to their hydrophobicity. Infusion of one group of peptides was followed by a significantly increased D-xylose absorption in dogs compared to the other two peptides (8). Other aspects that must be considered are the mechanisms involved in the increased absorption observed

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From the Department of Physiology and Biophysics and Department of Medicine, Faculty of Medicine, University of Chile, Santiago, Chile.

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Address for reprint requests: Carlos Defilippi, MD, Department of Physiology and Biophysics, Faculty of Medicine, University of Chile, Casilla 70005, Santiago, Chile.

after nutrient administration. Are they acting directly to enhance transport of solute or indirectly because of changes in others factors, such as motility and transit time?

The possibility that motor changes might be involved was analyzed in the previously mentioned studies: the casein and casein hydrolyzate effect on D-xylose absorption (7) was related to small intestinal motor changes, characterized by a decreased frequency as well as the percentage of propulsive waves. By contrast, in studies with elementary diets (6), no changes in the small intestinal motor pattern were observed in the presence of casein and casein hydrolysate, compared to soy protein and soy protein hydrolysate. Cyclic motor activity also remained unchanged and no differences in frequency, amplitude, and propulsive activity of small intestinal contractions were observed after infusion of groups of peptides derived from casein hydrolysate, independent of the effect on D-xylose absorption (8).

These apparently contradictory results might depend on the characteristics of nutrients analyzed and also on marked differences of the effect of nutrients on absorption and motor activity between species has been observed (9).

The aims of this study were to confirm the effect of groups of peptides derived from casein hydrolysate on absorption of D-xylose and orocceal transit time in normal volunteers by means of a noninvasive method such as the H_2 breath test.

MATERIALS AND METHODS

Seven volunteers (four men, three women; mean age 23.4 years, range 18–28 years) participated in the study. They were all healthy, with no gastrointestinal complaints and no history of operations. They had not received antibiotics or prokinetic drugs for at least four weeks before the studies.

Subjects were not entered in the study if they exhaled H_2 at a concentration less than 15 ppm after the administration of 25 g of D-xylose or had suspected bacterial overgrowth defined as basal values greater than 15 ppm of alveolar H_2.

This study was approved by the Ethical Committee of the University Hospital of the University of Chile, and all subjects gave informed written consent.

Purification of Peptide Fractions. The peptide fractions were isolated from casein enzymatic hydrolysate (Sigma, cat. # C 0626). Briefly, 3 g of casein enzymatic hydrolysate were dissolved in 12 ml isopropanol–H_2O–acetic acid (4:2:1, v/v). After 30 sec of sonication, the solution was centrifuged for 5 min at 10,000 *g* at room temperature. The supernatant was applied to a preparative HPLC column (2× 25 cm, done by us) with Silica Gel (870-230 mesh, ASTM, Merck). The fractions were eluted at a flow rate of 1.4 ml/min with isopropranol–H_2O–acetic acid (4:2:1 v/v) as the mobile phase. Fractions of 3.9 ml by tube were collected. The fractions were concentrated in the Speed Vac Plus SC 210A (Savat) and then analyzed by one-dimensional high-efficiency thin-layer chromatography (HE-TLC). HE-TLC plates were obtained from Merck (Silica Gel 60, 20 × 20 cm). Chromatographic separation were developed in the solvent systems: isobutanol–H_2O–pyridine–acetic acid–acetonitrile (40:20:10:1:10, v/v). The HE-TLC plates were dried under a stream of N_2 (g) and the spots on the plates were visualized with 0.1% ninhydrin–ethanol–collidine–acetic acid at 100°C by 5 mins. Three major fractions were isolated, tested, and named fraction I (tubes 2–19 were pooled together), fraction II (tubes 20–26) and, fraction III (tubes 27–50) (Figure 1).

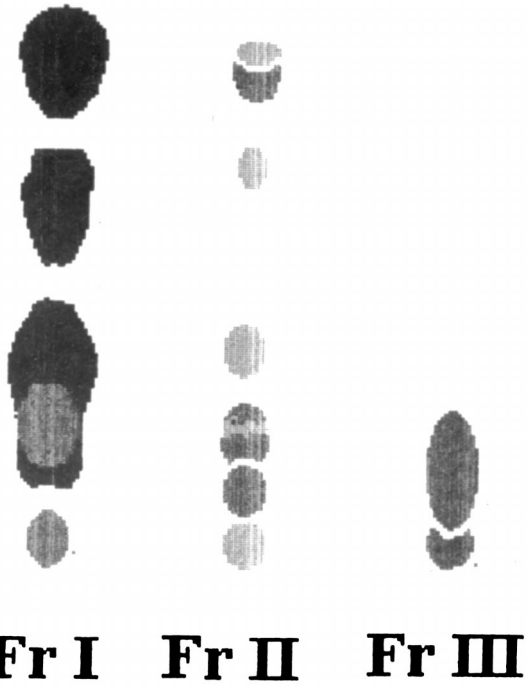

Fig 1. Thin-layer chromatography of casein derived peptides showing three different groups of peptides: fractions I, II, and III.

Design. The day before the tests, patients were asked to eat at 8 PM a standarized meal consisting of chicken, rice and gelatin. Then subjects remained fasting until the next morning. After a basal recording of the H_2 concentration, the subjects received in the following order, on separate days: (1) 25 g D-xylose (Sigma Chemical Co., St. Louis, Missouri). and (2) 12.5 g of lactulose (Duphalac, Reid-Rowell, Marietta, Georgia); then at random 25 g of D-xylose and each one of the peptide groups (fractions I, II, and III). All these substances were dissolved in 300 ml of distilled H_2O. Subjects drank test solutions within a period of 5 min.

Five test studies were performed on each subject, separated by at least five days between each test.

End expiratory H_2 concentrations were measured, using an H_2-sensitive electrode (H_2 Lactoscreen, Hoek Loos Schiedan Holland Apparatus) and expessed as part per millon. Breath samples were collected every 10 min for a period of 200 min.

TABLE 1. CHARACTERISTICS OF H_2 BREATH TEST AFTER LACTULOSE, D-XYLOSE, AND D-XYLOSE WITH THREE FRACTIONS OF CASEIN PEPTIDES

	[H_2] basal (ppm)	*OCTT (min)*	*[H_2] peak (ppm)*	*× [H_2] (ppm)*	*× Area (ppm × min)*
Lactulose	3.9 ± 1	57 ± 7	67.7 ± 8	41.4 ± 6	6532 ± 1120
D-Xylose	5.7 ± 1	67 ± 12	25.2 ± 1.4	14.1 ± 1.4	1841 ± 241
D-Xylose + Fr. I	5.5 ± 1	60 ± 12	24.4 ± 7	12.9 ± 4.5	1747 ± 631
D-Xylose + Fr. II	5.0 ± 1	105 ± 9	11.4 ± 1.8	4.5 ± 1.5	347 ± 121
D-Xylose + Fr. III	4.7 ± 1	108 ± 16	13.8 ± 2.7	6.0 ± 0.9	414 ± 56

Data Analysis. The following parameters were measured: (1) H_2 basal concentration was defined as the mean concentrations before D-xylose administration. (2) Orocecal transit time (OCTT) was defined as the time between D-xylose ingestion and the first of at least three consecutive increases of H_2 concentration. (3) Area under the curve was defined as the area observed between mean basal concentration and H_2 levels at each point. (4) Mean H_2 concentration was considered as the mean of each hydrogen concentration measurement over basal. (5) Peak H_2 concentration was defined as the maximal value obtained in each experiment.

Statistics. In order to estimate differences between each experimental condition, the sign rank test was used; $P < 0.05$ was considered statistically significant. Results are presented as the mean ± SE.

RESULTS

Basal H_2 concentration was 5 ± 0.3 ppm for the entire group of experiments, and no statistically significant differences were observed between each experiment (Table 1). Peak H_2 concentrations values are shown in Table 1. As expected, maximal values were observed with lactulose. Peak H_2 concentration with D-xylose was statistically significantly lower (68.7%) than that observed with lactulose. A statistically significant decrease of peak H_2 concentration was also observed when fractions II and III were added to D-xylose. A decrease of H_2 concentration of 55.8% and 53.6% compared with D-xylose alone was observed with group II and III peptides, respectively, suggesting an improvement of D-xylose absorption. No statistically significant differences between these two fractions were observed. In contrast, variations of peak H_2 concentration of D-xylose were not statistically significant when peptide fraction I was added to the test solution. Similar findings were observed when the mean hydrogen concentration of D-xylose was analyzed (Table 1).

The shorter OCTT was observed with lactulose and D-xylose; no statistically significant differences between lactulose and D-xylose were observed. An increase in the duration of the intestinal transit time was observed with the addition of peptide fractions II and III to D-xylose solution. However, in three studies with fraction II, OCTT was not determined because no increase of H_2 concentration over basal values was observed. The increase of OCTT did not achieve statistical significance compared to that observed with lactulose and D-xylose. OCTT in experiments whith fraction I peptides was similar to that observed with lactulose and D-xylose alone (Table 1).

An attempt to more accurately estimate D-xylose absorption was performed by measuring the area under the curve. Statistically significant differences were seen between lactulose with an estimated mean area of 6532 ± 1120 ppm × min, compared to 1841 ± 241 ppm × min observed with D-xylose, representing a decrease of 71.8% (Table 1). A statistically significant additional decrease of H_2 production was observed when fraction II and III peptides were added to the D-xylose, with a 81.1% reduction of the area under the curve of exhaled H_2 with group II peptides and 77.5% with group III pepides. No statistically significant differences between both groups of peptides was observed. The area under the curve observed after administration of fraction I peptides was not statistically significantly different from D-xylose.

Individual variations of the area under the curve are shown in Figures 2 and 3. A decrease of the area under the curve was observed comparing lactulose and D-xylose. A decrease of the area was also seen in all the experiments with fractions II and III. By contrast marked individual variations were observed with fraction I.

DISCUSSION

This study shows that groups of peptides separated from casein hydrolysate markedly increased D-xylose absorption as estimated by the H_2 breath test. D-xylose has several advantages for absorption studies. In dogs during duodenal infusion, D-xylose blood levels are very sensitive, paralleling those of glucose and showing variations following changes of motility (10).

Casellas et al (11) showed that the 5-hr hydrogen breath test was sensitive enough to establish malab-

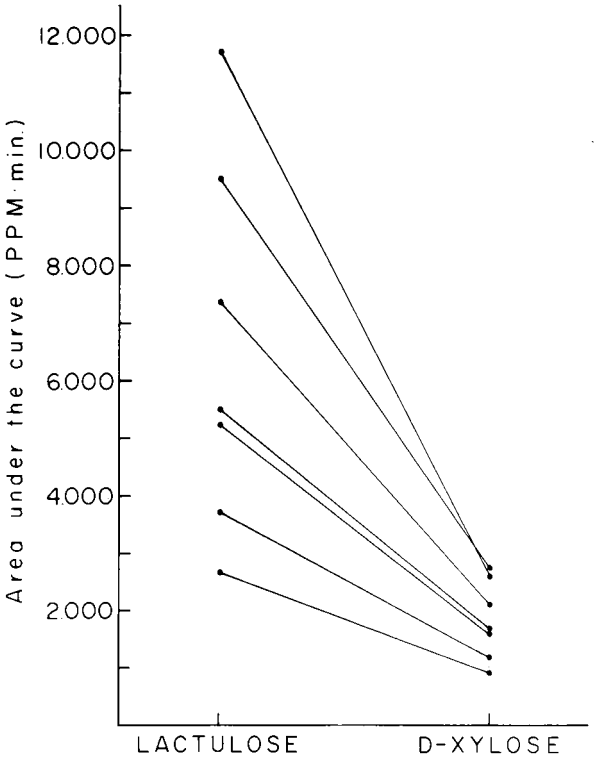

Fig 2. Individual variations of the area under the curve observed after administration of lactulose and D-xylose.

sorption of different etiologies. In spite that, this test has not been validated in normal subjects; in the previously mentioned study, the H_2 breath test was able to detect normalization of mucosal hystology in treated celiac disease patients. In the present study we have observed that measurement of exhaled H_2, in spite of marked individual variations, can easily differentiate between a nonabsorbable and an absorbable carbohydrate in the same subject. After addition of two casein-derived fractions of peptides, an additional decrease of H_2 production was observed, suggesting an increase of D-xylose absorption. In three experiments H_2 concentration remained at basal levels that might be interpreted as no D-xylose reaching colonic H_2-producing flora and that D-xylose was completely absorbed. The other possibility is that a delayed increase in H_2 production might not be noticed during the observation period. Another study (12) showed that in 20 normal subjects, a shortened 3-hr observation period failed to detects the peak H_2 concentration in only two occasions compared to the 5-hr test. Since, in the present experiments, H_2 remained at basal levels for 200 min, the probability of missing an increase of H_2 as a consequence of prolonged OCTT seems to be low. In contrast, a third group of peptides had no effect on D-xylose absorption, showing that this specific effect depends on the characteristics of the peptide fraction.

The active peptides represent a fraction of nonpolar peptides retained for a longer time in the column. Neutral and nonpolar amino acids must be predominant in fractions II and III. This finding of an increase of D-xylose absorption stimulated by some

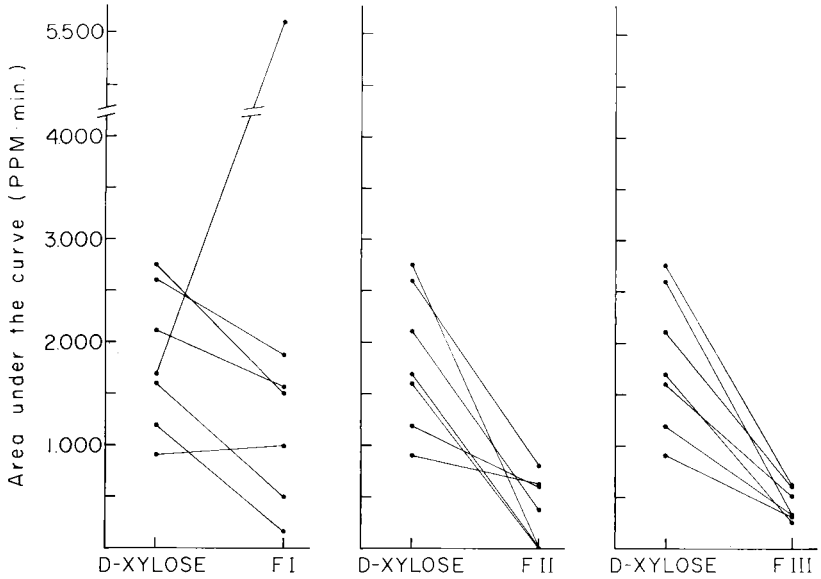

Fig 3. Individual variations of the area under the curve observed after administration D-xylose and D-xylose with fractions I, II, and III peptides.

casein-derived peptides is in accord with a similar observation in man using the whole protein (13), and in dogs using casein hydrolysate and measuring plasma levels of D-xylose (7). Since this marked effect on absorption was observed with small amounts of peptides, we can postulate that very active products are present in peptides obtained from passage through the silica column.

Several studies have shown that both natural peptides, derived from digestion of casein and wheat gluten in the alimentary tract, as well as synthetic analogs can modify several gastrointestinal functions. The amino acid sequence of these small peptides has been determined and they are called β-casomorphins (14, 15). Although in the present experiments the structure of peptides was not determined, we can speculate that β-casomorphins might be present in the isolated fractions.

Two mechanisms might be involved in the effect of casein-derived peptides: changes in motor activity that in turn could improve absorption and a direct effect on absorption. Both effects have been described in studies with β-casomorphins. The delayed transit time observed in the present study is in accordance with previous observations in normal volunteers and in experimental animals (16–18). The lack of statistically significant differences might be explained in part because data on OCTT are not available in experiments in which D-xylose was completely absorbed.

More recent evidence suggests that D-xylose absorption in the human small intestine is entirely diffusional (19) and therefore it must be driven by actively transported solutes. β-Casomorphins have been shown to stimulate absorption of sodium and chloride in the rabbit ileum (20).

The present study, based on indirect estimation of D-xylose absorption, cannot provide any additional evidence on the mechanims involved. We also cannot speculate on whether a similar effect might be expected for other substances, such as nutrients requiring transport mechanism. In summary, an improved D-xylose absorption was observed by adding to D-xylose two fractions of peptides obtained after passage of casein hydrolysate through a silica column.

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