

## Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man

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(Received 13 July 1992 – Revised 8 March 1993 – Accepted 1 April 1993)

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The present study focuses on the digestion and absorption of milk and fermented milk (FM) reflected by gastro-ileal N and electrolyte movements in six healthy volunteers. The N and electrolyte content of the intestinal effluents were analysed both at the beginning of the jejunum and in the distal ileum. The gastric half-emptying time of the liquid phase was significantly ( $P < 0.05$ ) shorter for milk (35 (SE 2) min) than for FM (60 (SE 2) min). The N balance showed that 58 and 50% of ingested proteins, milk and FM respectively were absorbed between the stomach and the proximal jejunum and that 91 and 90% respectively were absorbed between the stomach and the terminal ileum in 240 min. Evaluation of mineral absorption indicated that 44 and 67% of Ca was absorbed in the duodenum after milk and FM ingestion respectively, and 41 and 11% of Ca disappeared between the jejunum and the ileum respectively. With regards to N and Ca intestinal availability, the present study confirms that FM products represent an interesting source of N as well as minerals for man. This confers on FM a beneficial effect compared with milk especially for lactase (*EC* 3. 2. 1. 108)-deficient subjects and children with persistent diarrhoea.

**Fermented milk proteins: Electrolytes: Digestion: Man**

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Fermented milk (FM) products are believed to have a high nutritional value, representing with milk an outstanding source of high-quality proteins, minerals and micronutrients for man, and are also considered to have some prophylactic properties (Hitchins & McDonough, 1989). Different sorts of FM have been developed which provide both a better shelf-life for milk by inhibiting putrefaction and an agreeable taste.

Various properties which benefit general health are attributed to FM products, many of which seem mainly related to intestinal processes. They include growth stimulation (Hargrove & Alford, 1978), prophylaxis and remedy of diarrhoea (Beau *et al.* 1990), increased lactose tolerance (Marteau *et al.* 1990; Martini *et al.* 1991), protection of gastrointestinal flora (Perdigon *et al.* 1990) and modulation of the immune system (De Simone *et al.* 1987). Like milk, FM proteins, or the products of their digestion or of their metabolism by bacteria, are believed to play a physiological role at the intestinal or even systemic level after absorption (Coste & Tomé, 1990). Moreover, it is known that microbial hydrolases partly predigest milk nutrients, and that fermented dairy products contain both a higher level of free amino acids and a more digestible form of carbohydrate than milk (Rasic *et al.* 1971; Breslaw & Kleyn, 1973; Alm, 1982). *In vivo* studies in animals suggest that this predigestion improves the bioavailability of certain key nutrients (Hargrove &

Alford, 1978; Alm, 1981) but little information is available as to whether or not this effect on the bioavailability is also observed in man.

Under these conditions the understanding of nutritional and potential prophylactic properties of FM products implies a good knowledge of their digestion in the human intestine. The present study was performed to evaluate the overall movements of N and electrolytes during the digestion and the absorption of FM in healthy human volunteers. For this purpose, six subjects were given meals composed of either milk or the same milk after fermentation. The N and electrolyte content of the intestinal effluents were analysed both at the beginning of the jejunum and in the distal ileum using the 'slow marker' perfusion technique (Modigliani *et al.* 1973).

## MATERIALS AND METHODS

### *Diets*

Bottled water (Vittel, 03270 Saint-Yorre, France) was used as the control liquid. The milk was provided by Yoplait (Sodima, 94200 Ivry, France) and contained (g/kg): crude protein (N × 6.25) 23, lipid 35, carbohydrate 45. The FM was a commercial product (Ofilus; Yoplait) containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and (g/kg): protein 36, lipid 35, carbohydrate 42. Meals were adjusted to 925 kBq with <sup>14</sup>C-labelled polyethylene glycol 4000 (<sup>14</sup>C-PEG-4000) as a non-absorbable marker of the meal's liquid phase.

### *Subjects*

Six healthy volunteers (three male and three female) ranging from 20 to 35 years of age (mean 29 years) and weighing from 60 to 70 kg (mean 64 kg) were selected according to the following criteria: (1) no history of gastrointestinal surgery, (2) absence of gastrointestinal system disorders, (3) absence of pregnancy, (4) a stable, satisfactory nutritional status and a stable body weight. The protocol was previously approved by the Ethical Committee of the Lariboisière Hospital (75010 Paris, France). All subjects gave their consent for their participation in the study.

### *Perfusion technique*

A gastric tube and an intestinal tube were passed from the nose to the small intestine of the volunteers as previously described (Modigliani *et al.* 1973; Mahé *et al.* 1992). The gastric tube was used for sampling postprandial gastric contents in order to determine the concentrations of the meal marker (<sup>14</sup>C-PEG-4000). The intestinal tube was used (a) to perfuse PEG-4000 into the intestine and (b) to aspirate intestinal contents, 200 mm distal to the perfusion site.

### *Experimental design*

On the night before the test meal, subjects had dinner at 20.00 hours and then fasted overnight. Starting at 08.00 hours, the intestinal and gastric tubes were positioned under radioscopic control. When the intestinal perfusion site was located at the Treitz angle (i.e. the beginning of the jejunum) with the aspiration site 200 mm distal the test lasted for 4 h. When the intestinal perfusion site was located at the terminal ileum the sampling period lasted for 8 h. Subjects were given either a 400 ml liquid test meal of milk or water or a 300 g test meal of FM, each subject serving as his own control. Starting 20 min before meal ingestion and continuing throughout the test period, a saline solution (150 mM-NaCl) containing PEG-4000 (10 g/l) was perfused into the intestine at the rate of 2 ml/min. The

20 min before meal ingestion were considered as the initial period. Every 20 min, 5 ml gastric contents were aspirated with a manual syringe. Intestinal samples were obtained by continuous suction through the distal opening of the intestinal tube. Aspirates were collected over ice and pooled at 20 min intervals. At the end of each test, gastric contents were completely aspirated and the stomach was washed with 200 ml 150 mM-NaCl solution. Subjects were not allowed to ingest food or fluids during the remainder of the collection period.

#### *Analytical methods*

The volume and pH of digesta samples were measured after homogenization. The effluents were treated with a protease inhibitor, 0.1 mM-diisopropylfluorophosphate (Sigma Chemical Co, La Verpillière, France), then frozen at  $-20^{\circ}$  and freeze-dried. PEG-4000 was measured by a turbidimetric method (Hyden, 1955) and radioactivity of  $^{14}\text{C}$ -PEG with a well-type scintillation counter (Intertechnique SL 30; Intertechnique, Plaisir, France). The total N content was determined by the pyrochemiluminescence technique (Antek 720-771 system; Soparès, Gentilly, France). Osmolality was established by measuring the freezing-points of the solutions with a micro-osmometer (Advanced Instruments, Needham Heights, Massachusetts, USA). The Na and K concentrations were determined using a flame photometer (Ciba-Corning, Gergy Pontoise, France) and the  $\text{Cl}^{-}$  concentration by the colorimetric method (Coultronics France S.A., Andilly, France). The Ca and Mg concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer, Saint-Quentin 78054, France).

#### *Calculations and statistical analysis*

The postprandial volume of gastric contents was calculated by ascending recurrence and adjusted by non-linear regression analysis (Malagelada *et al.* 1976). Gastric half-emptying time represents the time it takes 50% of the ingested liquid to be emptied from the stomach. The time of gastro-ileal transit of the liquid phase was calculated from  $^{14}\text{C}$ -PEG-4000 recovery at the ileal level. The effluent flow-rate was calculated from PEG-4000 concentration minus the perfusion volume (2 ml/min). Results were expressed as means with their standard errors. Statistical analysis was performed by using variance analysis and Tukey's studentized range test (Statistical Analysis Systems, 6.03; SAS Institute, Cary, NC, USA).

## RESULTS

### *Effluent flow-rate*

The effluent flow-rate, corrected for perfusion volume (2 ml/min), was measured every 20 min both in the jejunum and in the ileum after ingestion of either milk or FM (Fig. 1). After meal ingestion the effluent flow-rate was higher in the jejunum than in the ileum and in no case was any difference observed after milk or FM ingestion except in the ileum 20 min after FM ingestion. Following meal ingestion the jejunal flow-rate increased during the 0-40 min to approximately 6 ml/min and then returned to a basal rate of 2.5 ml/min after 160-180 min. The ileal flow-rate increased to approximately 3 ml/min during the 20-60 min period after milk ingestion and the 40-80 min period after FM ingestion. The ileal flow-rate then returned to a basal level of approximately 1 ml/min after 200 min. The calculation of the fluid balance following meal ingestion indicated an apparent gastro-jejunal net secretion and an apparent jejuno-ileal net absorption with no significant difference between milk and FM (Table 1).

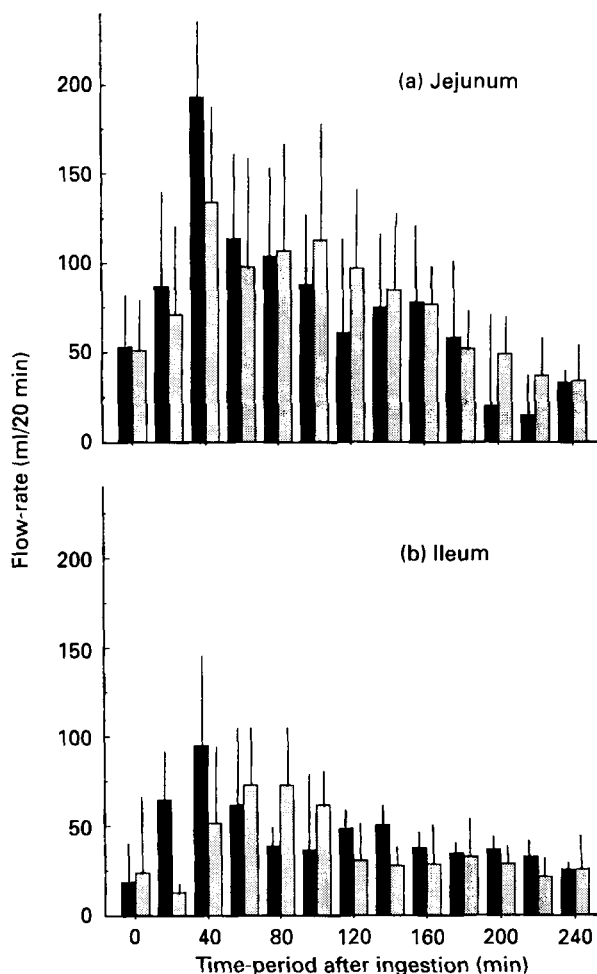


Fig. 1. Effluent flow rate (ml/20 min) profile of contents of the intestine after ingestion of 400 ml milk (■) and 300 g fermented milk (▨) in (a) the jejunum and (b) the ileum. Values are means with their standard errors represented by vertical bars for six subjects. For details of test meals and procedures, see pp. 170–171.

Table 1. Estimation of fluid movements in the 240 min following ingestion of 400 ml milk or 300 g fermented milk (FM) by healthy volunteers\*

(Mean values with their standard errors for six subjects)

Test meal	Ingested (ml)	Amount recovered (ml)				Apparent exchange†			
		Jejunum		Ileum		Gastro-jejunal		Jejuno-ileal	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Milk	400	1051	366	388	195	−651	366	+663	195
FM	280	953	365	295	115	−673	365	+658	115

\* For details of test meals and procedures, see pp. 170–171.

† A positive value indicated absorption and a negative value indicated secretion.

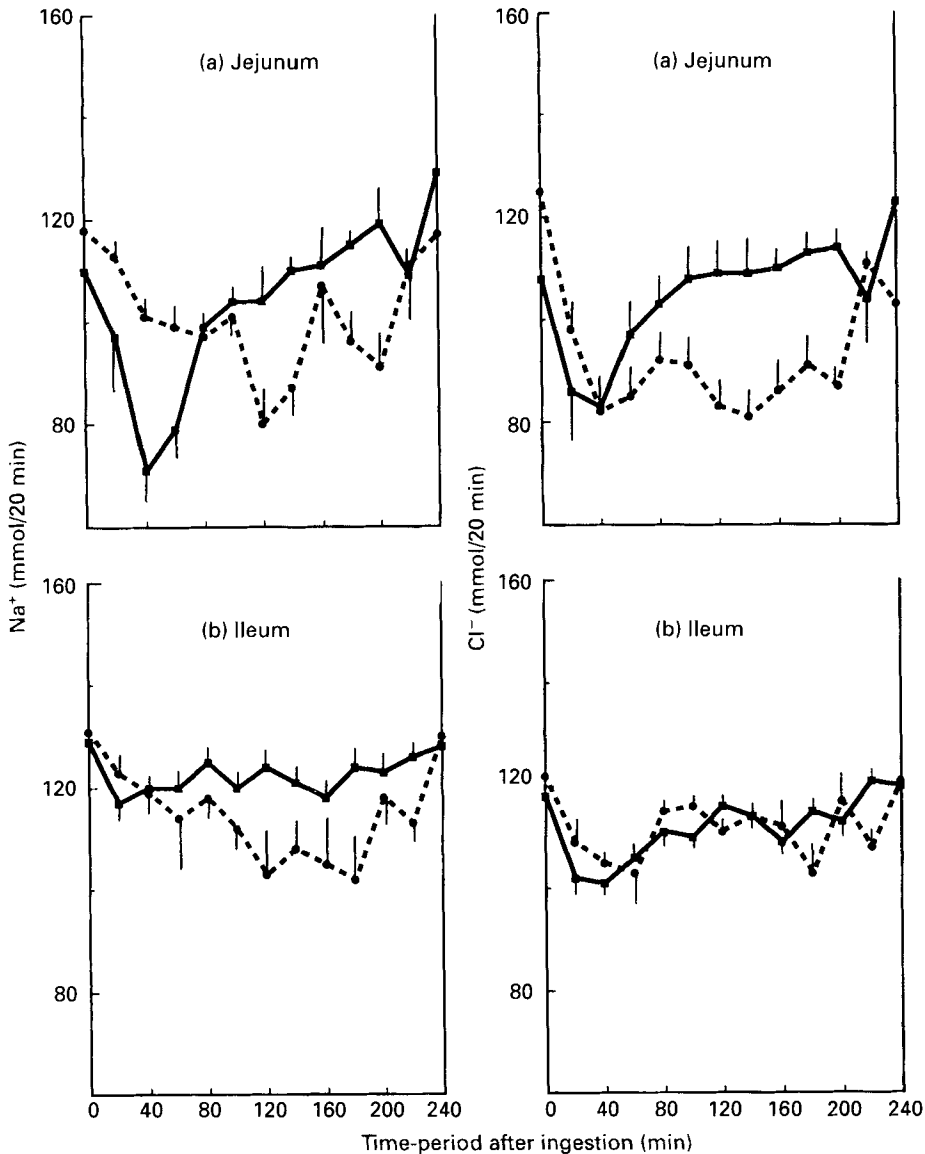


Fig. 2. Sodium ion and chloride ion profiles (mmol/20 min) in the digesta from (a) the jejunum and (b) the ileum after ingestion of 400 ml milk (■) and 300 g fermented milk (●). Points are means with their standard errors represented by vertical bars for six subjects. For details of test meals and procedures, see pp. 170–171.

#### *Osmolality, pH and ions*

In comparison with the theoretical physiological value (300 mosmol/kg), no significant variation in osmolality was observed in samples from both the jejunum and the ileum during the test period after either milk or FM ingestion (values not shown). The pH of the effluents in the jejunum decreased from 6.62 (SE 0.46) to 5.45 (SE 0.67) in 40 min and remained stable afterwards. In the ileum the pH was 7.50 (SE 0.22) in the initial phase and remained unchanged during the test period (the lowest value was 6.60 (SE 0.95) during the 80–100 min period). After meal ingestion the Na<sup>+</sup> and Cl<sup>-</sup> concentrations in the jejunum

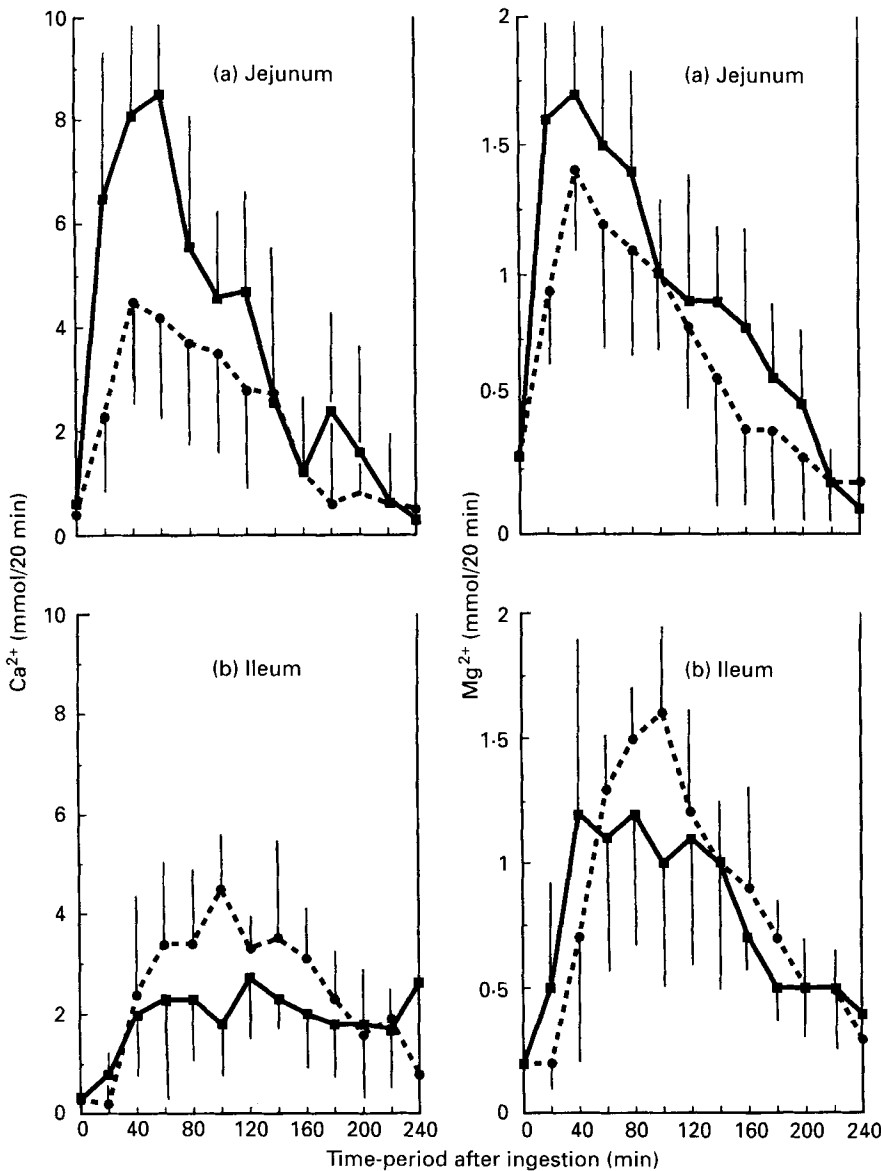


Fig. 3. Magnesium ion and calcium ion profiles (mmol/20 min) in the digesta from (a) the jejunum and (b) the ileum after ingestion of 400 ml milk (■) and 300 g fermented milk (●). Points are means with their standard errors represented by vertical bars for six subjects. For details of test meals and procedures, see pp. 170–171.

decreased significantly to approximately 90 mmol ( $P < 0.05$ ) and returned to a basal level of approximately 120 mmol after 120 min, whereas in the ileum no difference was observed after milk and FM ingestion (Fig. 2). After milk and FM ingestion the recoveries of both Ca and Mg were increased significantly ( $P < 0.05$ ) between 20 and 120 min in the jejunum and between 40 and 150 min in the ileum and then returned to the initial levels (Fig. 3).

The calculation of the ion balance (Table 2) indicated a net apparent secretion of NaCl, a net apparent absorption of  $\text{Ca}^{2+}$  and no significant movement of  $\text{K}^+$  and  $\text{Mg}^{2+}$  at the gastro-jejunal level. All ions showed an apparent net absorption at the jejuno-ileal level.

Table 2. Estimation of ion movements in the 240 min following ingestion of 400 ml milk or 300 g fermented milk (FM) by healthy volunteers\*

(Mean values with their standard errors for six subjects)

		Amount ingested (mmol)	Amount recovered† (mmol)				Apparent ion exchange‡			
			Jejunum		Ileum		Gastro-jejunal		Jejuno-ileal	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cl <sup>-</sup>	Milk	11.2	84.0	43.4	23.3	22.9	-72.8	43.4	+60.7	22.9
	FM	7.9	52.8	25.9	13.2	9.5	-44.9	25.9	+39.6	9.5
Na <sup>+</sup>	Milk	6.4	79.6	38.1	32.7	22.3	-73.2	38.1	+46.9	22.3
	FM	4.8	68.9	39.1	14.2	9.4	-64.1	39.1	+54.7	9.4
K <sup>+</sup>	Milk	16	17.9	3.8	4.5	1.4	-1.9	3.8	+13.4	1.4
	FM	11	14.0	4.4	4.1	1.5	-2.9	4.4	+9.9	1.4
Ca <sup>2+</sup>	Milk	11.8	6.6	1.2	1.7	0.8	+5.3	1.2	+4.9	0.8
	FM	11.3	3.6	0.8	2.4	1.0	+7.7	0.8	+1.2	1.0
Mg <sup>2+</sup>	Milk	1.7	1.5	0.3	0.8	0.2	+0.2	0.3	+0.7	0.2
	FM	1.0	1.2	0.2	0.8	0.1	-0.2	0.2	+0.4	0.1

\* For details of test meals and procedures, see pp. 170-171.

† Calculated as: total recovered - perfused (72 mmol NaCl/240 min).

‡ A positive value indicated absorption and a negative value indicated secretion.

The apparent gastro-jejunal absorption of Ca<sup>2+</sup> was significantly higher ( $P < 0.05$ ) for FM than for milk but the overall gastro-ileal absorption yield was not significantly different for milk and FM.

#### *Transit of the liquid phase of the meals*

After milk and FM ingestion, gastric emptying and both gastro-jejunal and gastro-ileal transit time of the liquid phase of the meal were calculated from either <sup>14</sup>C-PEG-4000 disappearance in the stomach or appearance at the collection site (jejunum or ileum; Fig. 4).

The gastric emptying of the liquid phase of FM appeared delayed compared with that of milk (Fig. 4(a)). The gastric emptying curve of the liquid phase was fitted with the power exponential model:  $VR = \exp(-at^b)$ , where VR was the meal fraction remaining in the stomach at a given time (Elashoff *et al.* 1982). The power parameter ( $b$ ) did not differ from 1 for milk but significantly ( $P < 0.05$ ) differed from 1 for FM (1.02 (SE 0.45) and 1.91 (SE 0.60) respectively). This indicated that milk, but not FM, exhibited a simple exponential gastric emptying pattern. The gastric half-emptying time of milk was significantly ( $P < 0.05$ ) shorter than that of FM (35 (SE 2) and 60 (SE 2) min respectively). At the jejunum level (Fig. 4(b)) the same delay was observed for the liquid phase of FM in comparison to milk, as indicated by <sup>14</sup>C-PEG-4000 appearance. The time for 50% jejunal delivery of <sup>14</sup>C-PEG-4000 was significantly shorter for milk than for FM (40 and 60 min respectively). At the ileal level the difference was still observed between milk and FM (Fig. 4(c)) considering that the <sup>14</sup>C-PEG-4000 was not detectable in the 20 min period after FM ingestion. Nevertheless, the time for 50% ileal delivery of the liquid phase did not vary for milk and FM (85.1 (SE 29.8) and 87.2 (SE 16.2) min respectively). These results indicated a duodeno-ileal liquid phase transit time of 45 min for milk and of 27 min for FM.

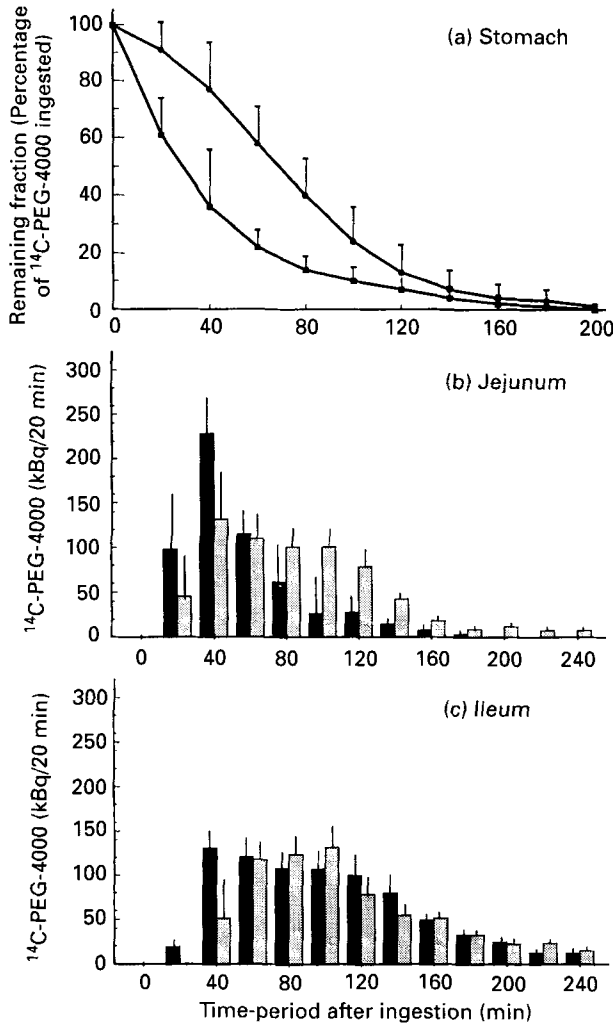


Fig. 4. (a) Gastric emptying pattern (%) and  $^{14}\text{C}$ -labelled polyethylene glycol (molecular weight 4000;  $^{14}\text{C}$ -PEG-4000; kBq/20 min) profile in (b) the jejunum and (c) the ileum of the liquid phase after ingestion of 400 ml milk (■, ■) and 300 g fermented milk (●, □). Values are means with their standard errors represented by vertical bars for six subjects. For details of test meals and procedures, see pp. 170–171.

#### Nitrogen movements

The quantity of N present in the intestinal effluent was measured in the jejunum and the ileum after either water, milk or FM ingestion. Before ingestion of the test meal the initial N flow-rates in the jejunum and the ileum were 7.8 (SE 3.6;  $n$  9) and 4.3 (SE 3.6;  $n$  12) mmol/h respectively. The ingestion of water was accompanied by the recovery of 27.1 (SE 4.3) mmol endogenous N in 240 min at the jejunum and 17.1 (SE 8.6) mmol endogenous N at the ileum. After either milk or FM ingestion (Fig. 5) the N content similarly peaked in the 20–40 min period, remained at an intermediate level in the 60–100 min period and progressively returned to the initial level in the 100–180 min period. The N content was significantly higher ( $P < 0.05$ ) than initial values in the 20–120 min period after either milk or FM ingestion. In the ileum the N profile peaked in the 40–60 min period and decreased progressively.

The calculation of the N balance (Table 3) implies an evaluation of the part of



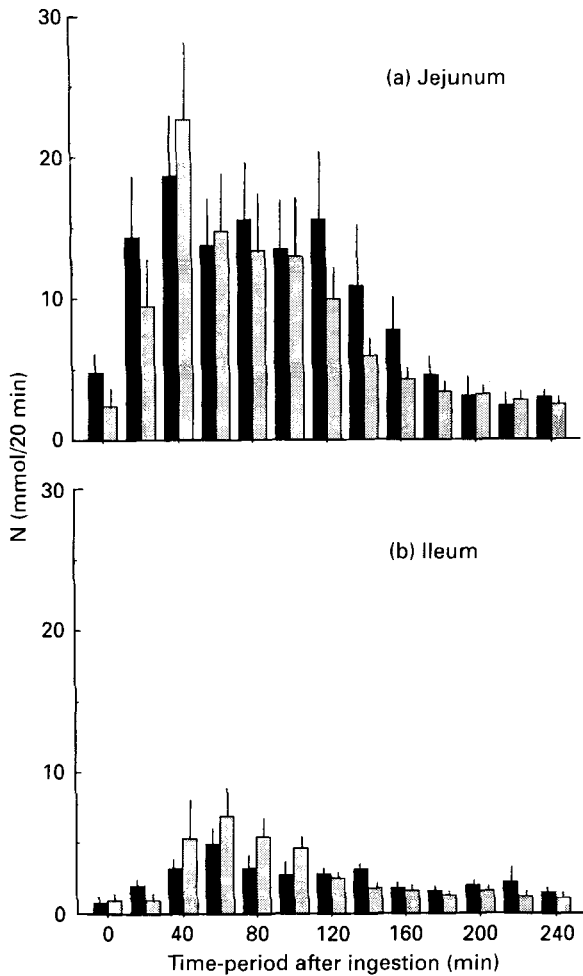


Fig. 5. Nitrogen profile (mmol/20 min) of the digesta in (a) the jejunum and (b) the ileum after the ingestion of 400 ml milk (■) and 300 g fermented milk (▨). Values are means with their standard errors represented by vertical bars for six subjects. For details of test meals and procedures, see pp. 170–171.

Table 3. Estimation of nitrogen yield in jejunal and ileal digesta 240 min after water, milk and fermented milk (FM) ingestion by healthy volunteers\*

(Mean values with their standard errors for six subjects)

N ingested (mmol)		N recovered (mmol)											
		Total				Exogenous†				Net disappearance (%)			
		Jejunum		Ileum		Jejunum		Ileum		Jejunum		Ileum	
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Water	0	27.1	4.3	17.1	8.6	—	—	—	—	—	—	—	
Milk	105.1	71.5	25.1	26.4	6.6	44.4	25.1	9.3	6.6	57.7	20.5	33.4	8.4
FM	123.4	89.1	24.5	29.6	8.8	61.9	24.5	12.5	8.8	49.8	19.8	40.0	7.1

\* For details of test meals and procedures, see pp. 170–171.

† Endogenous N was evaluated after water ingestion.

endogenous N secreted in the intestine. Taking the values obtained after water ingestion for the endogenous N, the present results show that 58% of the milk and 50% of the FM were absorbed between the stomach and the proximal jejunum and 33 and 40% between the proximal jejunum and the terminal ileum respectively. The overall N absorption was 91.2 (SE 6.3)% for milk and 89.9 (SE 7.2)% for FM, and there was no significant difference between the two meals.

#### DISCUSSION

Milk products are currently assumed to represent a good source of nutrients and minerals for man. In the present study the double-lumen-tube technique was used to collect digesta at the proximal jejunum and terminal ileum and to quantify the lumen movements of water, electrolytes and N after milk and FM ingestion in human subjects. When comparing milk and FM ingestion the results showed: (1) reduced gastric emptying rate, gastro-jejunal transit of the liquid phase and gastro-jejunal absorption of N for FM, (2) increased gastro-jejunal absorption of Ca for FM, (3) increased jejuno-ileal transit time of the liquid phase and N absorption from FM, (4) no difference in water, electrolyte and N exchanges and in the overall gastro-ileal transit and absorption.

It appeared that fermentation mostly modifies the digestion of milk at the gastro-jejunal level without affecting the overall digestive balance of water, electrolytes and N. Gastric processes are the first steps following food ingestion. Their main functions are the homogenization of the chyme and the gradual delivery of nutrients for digestion and absorption in the small intestine. Results showed a delayed gastric emptying of the liquid phase for FM in comparison with milk, mainly due to a reduced amount of liquid emptied during the first 20 min following meal ingestion. This reduction could be due to either a hormonal effect (Ruskone *et al.* 1980; Hunt *et al.* 1985; Houghton *et al.* 1990), or to food consistency and viscosity (Houghton *et al.* 1987; Siegel *et al.* 1988).

Interestingly, the N flow-rate in the duodenum presented a biphasic profile after both milk and FM ingestion, but the gastric emptying of the liquid phase was delayed after FM ingestion in comparison with milk. Milk proteins are composed of two major components: soluble whey protein and micellar casein. It was previously observed in short-bowel patients that after milk ingestion whey proteins remain soluble in the stomach and rapidly empty with the liquid phase partly intact, whereas caseins clot in the stomach and present a delayed gastric emptying mainly in the form of degraded products (Mahé *et al.* 1991). The present results suggest that, in the case of FM, N is partitioned into two liquid phases with differential viscosities and transit. These results suggest that the gastric clotting of proteins is less effective with FM than with milk. It was noted at the ileal level that these differences were no longer present, due to the absorption of the major proportion of N in the small intestine. Although N transit appeared slightly modified after fermentation, the overall gastro-ileal absorption was unchanged in comparison with milk and remained very high (about 90%).

Differential apparent absorption of K, Ca and Mg was observed since the Ca was absorbed mainly in the duodenum, whereas the K and the Mg were apparently absorbed in the jejuno-ileum. Moreover, the overall apparent disappearance of minerals from the lumen indicated an apparent absorption from exogenous milk minerals of 85.4 (SE 7.1)% for Ca and 53.9 (SE 13.3)% for Mg. In the same way, the overall apparent disappearance of minerals from the lumen indicated an apparent absorption from exogenous FM minerals of 78.2 (SE 8.7)% for Ca and 18.0 (SE 12.4)% for Mg. These results show that FM is an excellent alternative to milk as a source of Ca particularly for lactase (EC 3.2.1.108)-deficient subjects. The differences in the absorption of minerals and the low apparent absorption of Mg could be more due to secretion of K and Mg in the proximal part of the

intestine than to differences in the site of absorption of the ions. Schaafsma *et al.* (1988) have demonstrated that the intestinal absorption of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in rats is lower with FM than with milk whereas other results in humans have shown that Ca absorption from milk and FM does not significantly differ (Smith *et al.* 1985; Recker *et al.* 1988; Lewis *et al.* 1989). Our results confirm that, due to its nature, mineral bioavailability from milk products is slightly superior to that from plant foods and probably does not significantly differ from milk when given in quantities representative of normal dietary serving sizes.

Protein absorption begins in the duodenum but the jejunum and the ileum are also important for protein digestion (Nixon & Mawer, 1970; Adibi & Mercer, 1973; Chung *et al.* 1979). In conclusion, our results suggest that the denaturation and partial hydrolysis of milk proteins and other physical-chemical changes that occur during fermentation are not significant with regard to N and mineral bioavailability in humans. Thus, FM as a source of nutrients and minerals could be beneficial in the treatment of lactase-deficient humans (Marteau *et al.* 1990) and children with persistent diarrhoea (Beau *et al.* 1990). In all cases, FM is apparently a good product for human subjects with regard to N and Ca intestinal availability.

This work was supported in part by grants No. 663/38 EEC/ONILAIT (No. 9317 B INRA/SODIMA). The authors wish to thank Miss Suzanne Salter for her English assistance.

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