Metabolism of erythritol in humans: comparison with glucose and lactitol

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The metabolism of erythritol was assessed in six normal volunteers by measuring the amount of $^{13}CO_2$ excretion and H_2 excretion in breath, and erythritol in urine after intake of 25 g ^{13}C-labelled erythritol. The results were compared with the same variables obtained after intake of 25 g ^{13}C-labelled glucose and ^{13}C-labelled lactitol. In addition, the H_2 production by faecal flora supplemented with small amounts of erythritol, glucose and lactitol was measured in vitro, as an index of bacterial metabolism of non-absorbed substrate. In contrast to the results obtained after intake of glucose and lactitol, no increase in breath $^{13}CO_2$ and H_2 was observed after intake of erythritol, and erythritol was nearly completely recovered in urine. The in vitro experiments showed that no H_2 was formed by faecal flora from erythritol as compared with glucose and lactitol. It is concluded that erythritol is a substrate that is readily absorbed, and undergoes no metabolism by the host. If part of it escapes absorption, it is not metabolized by faecal flora.

Erythritol metabolism: Glucose: Lactitol: Humans

Recently various new sugars and sugar-alcohols have become available for nutritional and therapeutic use. They are proposed as low-energy sweeteners, less cariogenic than glucose or sucrose, but, in contrast to artificial sweeteners such as aspartame and saccharin, they provide a bulking effect to foodstuffs. Some of them, e.g. lactitol and lactulose, are also used in the treatment of constipation and portal-systemic encephalopathy. Erythritol is a C_4 polyol, naturally occurring in algae and mushrooms, with a pleasant taste and interesting physico-chemical characteristics (Oku & Noda, 1990). The aim of the present study was to determine the metabolic characteristics of erythritol in humans.

The evaluation of the energy value of a new product is cumbersome. Feeding studies can be used in animals but are difficult to perform in man (Van Es, 1987). Another technique of evaluation is based on the measurement of the fate of a dose of radiolabelled substrate (Grimble *et al.* 1988). Both techniques require a number of assumptions, and the energy value obtained should be considered as approximate. In the present study we evaluated the metabolic characteristics of erythritol by comparing it with glucose and lactitol, two substrates with a well-identified metabolism, by means of the following tests: $^{13}CO_2$ breath tests, H_2 breath tests, and measurement of urinary substrate recovery. Because colonic fermentation is an important mechanism of energy salvage for non-absorbed carbohydrates, we have also compared the amount of H_2 formed by colonic flora in a faecal incubation system to which the different substrates were added.

MATERIALS AND METHODS
Study design

Six healthy volunteers were studied, four men and two women, aged 21–25 years. After an overnight fast the subjects ingested 25 g ^{13}C-labelled glucose, [^{13}C]lactitol or [^{13}C]erythritol, in random order, dissolved in 250 ml water. There were at least 3 d between the tests. Breath samples for $^{13}CO_2$ and H_2 analysis were taken before intake of the substrate, and every 30 min during a 6 h study period. Urine was collected over 24 h in two samples: one over the first 6 h, the other over the subsequent 18 h.

To maintain the metabolic rate of the subjects as constant as possible, they remained at rest during the test. All subjects gave informed consent and the study was approved by the Ethical Committee of the Leuven University.

Substrates

All substrates originated directly or indirectly from maize or sugar cane, which are C_4 plants and contain a higher ^{13}C content than other plants. The difference in ^{13}C content between C_4 plant-derived substrates and the CO_2 in basal human breath is large enough to be used in metabolic studies (Lacroix *et al.* 1973; Schoeller *et al.* 1980; Shulman *et al.* 1983; Klein & Klein, 1985; Hiele *et al.* 1988*a*, *b*, 1989). Maize-derived glucose was obtained from Amylum, Aalst, Belgium. Erythritol was manufactured from maize glucose by Cerestar, Vilvoorde, Belgium. [^{13}C]Lactitol was prepared from ^{13}C-enriched lactose by CCA Biochem, Gorinchem, The Netherlands. [^{13}C]Lactose was obtained by putting cows on a maize diet for 5 weeks, and isolating lactose from their milk (Hiele *et al.* 1988*b*).

$^{13}CO_2$ breath tests

Breath samples were collected in aluminium-coated low-density polyethylene bags (Tesseraux container, Bürstadt, Germany). Pure CO_2 was isolated by cryogenic trapping on liquid N_2 after water removal by a methanol–CO_2–ice trap. The ratio $^{13}CO_2$:$^{12}CO_2$ was measured by isotope-ratio mass spectrometry (Finnigan MAT 250, Bremen, Germany) and expressed as $\delta^{13}C_{PDB}$ values, where $\delta^{13}C_{PDB}$ is the ^{13}C content in pro mille, expressed relative to ^{13}C content in Pee Dee Belemnite limestone:

$$\delta^{13}C_{PDB} = ((R_{sample} - R_{PDB})/R_{PDB}) \times 1000 \qquad (1)$$

and
$$R = {}^{13}C/{}^{12}C.$$

The absolute ratio ^{13}C:^{12}C of the PDB standard (R_{PDB}) is 0·0112372.

Formula 1 can be rewritten as:

$$0·001\ \delta^{13}C_{PDB} = ((R_{sample} - R_{PDB})/R_{PDB}) \qquad (2)$$

or
$$(0·001\ \delta^{13}C_{PDB} \times R_{PDB}) + R_{PDB} = R_{sample}$$
$$= ({}^{13}C/{}^{12}C)_{sample}$$
$$= (\%\,{}^{13}C/(100 - \%\,{}^{13}C))_{sample}.$$

If this equation is solved for % ^{13}C, it becomes:

$$\%\,{}^{13}C_{sample} = (0·001\ \delta^{13}C_{PDB} + 1) \times R_{PDB} \times 100/(1 + R_{PDB} \times (1 + 0·0001\ \delta^{13}C_{PDB})). \qquad (3)$$

^{13}C recovery in breath was calculated as percentage of administered dose as follows:

$$(\text{percentage recovery of administered dose/h})_t = \frac{\text{mmol excess } {}^{13}C_t \times 100}{\text{mmol excess } {}^{13}C \text{ administered}}$$

where t is sampling time, t_0 is time before substrate intake, and

$$\text{mmol } ^{13}\text{C excess}_t = \frac{(\% \, ^{13}\text{C}_t - \% \, ^{13}\text{C}_{t_0}) \times \text{mmol CO}_2 \text{ production}}{100}$$

(i.e. mmol extra ^{13}C appearing as breath CO_2 at time t, due to oxidation of the labelled substrate), and

$$\text{mmol excess } ^{13}\text{C administered} = \frac{\text{mg substrate}}{M} \times N \times \frac{(\% \, ^{13}\text{C}_S - \% \, ^{13}\text{C}_{t_0})}{100}$$

(i.e. amount of extra ^{13}C in administered substrate, relative to breath at t_0), where $\% \, ^{13}\text{C}_S$ is the percentage of ^{13}C in administered substrate, N is the number of C atoms in the compound, M is the molecular weight of compound, (mg substrate/M) $\times N$ = mmol C in substrate, $\% \, ^{13}\text{C}/100 = (^{13}\text{C}/(^{12}\text{C} + ^{13}\text{C}))$.

For our calculations, the CO_2 production was assumed to be constant and to amount to 300 mmol/m^2 body surface area per h (Shreeve *et al.* 1970). Body surface area was estimated by the weight–height formula of Haycock *et al.* (1978).

Breath $^{13}CO_2$ values after substrate intake, which differed by less than $0.5 \, \delta^{13}\text{C}_{\text{PDB}}$ units from basal levels, were considered as possibly due to changes in background, and were taken as zero. This correction factor was only important for erythritol.

Cumulative percentages of label recovery are calculated by means of the trapezoidal rule.

The ^{13}C enrichment of the substrates was measured after combustion to CO_2 (McGaw *et al.* 1988), and was found to be $-9.27 \, \delta^{13}\text{C}_{\text{PDB}}$ for glucose, $-11.73 \, \delta^{13}\text{C}_{\text{PDB}}$ for erythritol, and $-11.99 \, \delta^{13}\text{C}_{\text{PDB}}$ for lactitol.

H_2 breath tests

Breath H_2 concentrations were measured by injecting 20 ml breath samples in an H_2 monitor (GMI, Renfrew, Scotland). The result was obtained immediately and expressed as μl/l. Before each test the H_2 monitor was calibrated with a gas containing 39 μl H_2/l (Matheson, Oevel, Belgium).

Glucose and sugar-alcohols in urine

Erythritol and lactitol in urine were measured by means of a high-pressure liquid chromatography (HPLC) technique, using Waters HPLC Solvent Delivery System M45 with Waters HPLC Differential Refractive Index Detector R401 (Millipore Corporation, Milford, MA). A Shodex Ionpak column KC811 with an internal diameter of 8 mm and a length of 300 mm was used. The injected sample volume was 5 μl. The operating temperature was 75° and the flow-rate 1 ml/min. HPLC-grade water containing 0.0018 M-H_2SO_4 was used as a solvent. Glucose in urine was measured by means of an enzymic method (hexokinase, *EC* 2.7.1.1).

Fermentation of substrates by faecal flora

Faecal samples were collected from six healthy volunteers eating a regular Western diet. The subjects did not suffer from gastrointestinal complaints and had not used antibiotics for at least 6 months. Faecal samples were placed in the refrigerator at 4° immediately after voiding until further processing. After a maximum of 12 h they were diluted with nine times their weight of cooled phosphate buffer, containing 28.8 g Na_2HPO_4 . 12 H_2O and 6.34 g NaH_2PO_4 . 2 H_2O/l. After homogenization, 20 ml faecal suspension was transferred into
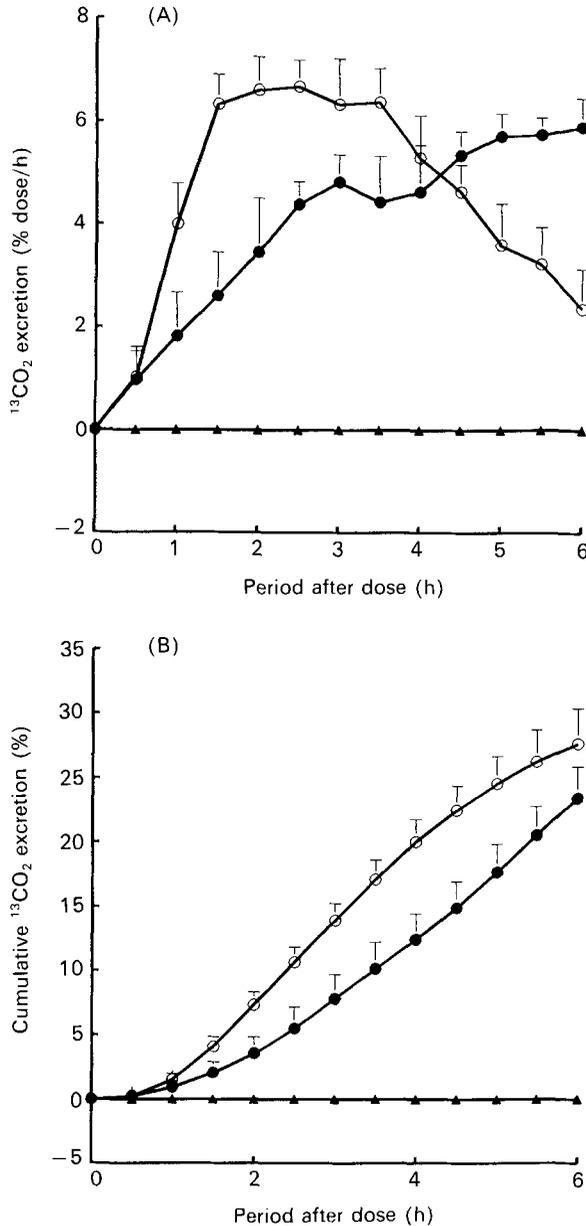
Fig. 1. (A) Rate of $^{13}CO_2$ excretion (% dose/h) after ingestion of 25 g ^{13}C-labelled glucose (○), lactitol (●) or erythritol (▲). (B) Cumulative amount of $^{13}CO_2$ excretion (% administered dose) after ingestion of 25 g ^{13}C-labelled glucose (○), lactitol (●) or erythritol (▲) in human volunteers. For details of procedures, see pp. 170–171.

each of four 100 ml vials. Glucose (50 mg) was added to the first vial, 50 mg erythritol to the second vial and 50 mg lactitol to the 3rd vial; no substrates were added to the 4th vial. The vials were tightly closed with a rubber cap and aluminium crimp seal, and incubated at 37°. To maintain anaerobic conditions, all manipulations were done under an atmosphere of N_2, and the buffer solution was flushed with N_2. After 6 h the H_2

Table 1. *Individual values of* $^{13}CO_2$ *excretion 1·5 and 6 h after intake of glucose and lactitol, and* $^{13}CO_2$ *excretion after 6 h:* $^{13}CO_2$ *excretion after 1·5 h for healthy volunteers**

| | Subject no. | $^{13}CO_2$ excretion | | $^{13}CO_2$ excretion 6 h:1·5 h |
		1·5 h	6 h	
Glucose	1	5·53	0	0
	2	7·54	0	0
	3	5·15	2·52	0·49
	4	5·68	2·32	0·41
	5	5·24	4·76	0·91
	6	8·89	4·54	0·51
Lactitol	1	3·68	3·75	1·02
	2	2·82	7·28	2·58
	3	1·00	4·36	4·36
	4	5·95	6·33	1·06
	5	3·17	7·33	2·31
	6	1·20	6·35	5·29

* For details of procedures, see pp. 170–171.

concentration in the head-space of the incubation vials was measured by means of the GMI H_2-monitor.

Statistics

$^{13}CO_2$ and H_2 breath values were compared by means of a two-way analysis of variance, with the administered substrate and the subject taken as classification variables. The Ryan-Einot-Gabriel-Welsch multiple-range test was used to compare the effect of substrate on individual means (SAS Institute Inc., 1988). For this purpose $P \leqslant 0.05$ was considered as statistically significant. Values are given as means with their standard errors.

The amounts of H_2 in faecal incubation experiments were normalized by logarithmic transformation, and thereafter analysed by the same method.

RESULTS
$^{13}CO_2$ breath tests

The rate of $^{13}CO_2$ excretion after intake of the different substrates is shown in Fig. 1(A). After intake of glucose there was a rapid rise in breath $^{13}CO_2$ which reached a maximum after 2–3·5 h. After lactitol intake ^{13}C excretion in breath increased more slowly than after glucose intake, but continued to rise until the end of the 6 h study period. No rise in $^{13}CO_2$ excretion was detectable after intake of erythritol. The oxidation rate after lactitol intake was significantly lower than that after glucose intake from 1·5 h to 2·5 h, and significantly higher from 5 to 6 h. This difference in label excretion profiles between glucose and lactitol was clearly illustrated by the ratio $^{13}CO_2$ excretion rate after 6 h: $^{13}CO_2$ excretion after 1·5 h. This value was always lower than 1 for glucose, and always higher than 1 for lactitol (Table 1). The cumulative amount of $^{13}CO_2$ excretion is shown in Fig. 1(B).

H_2 breath tests

The pattern of breath H_2 excretion after intake of the different substrates is shown in Fig. 2. From 2 to 6 h after intake of lactitol a rise in breath H_2 was observed which was significantly higher than that after intake of the other substrates.
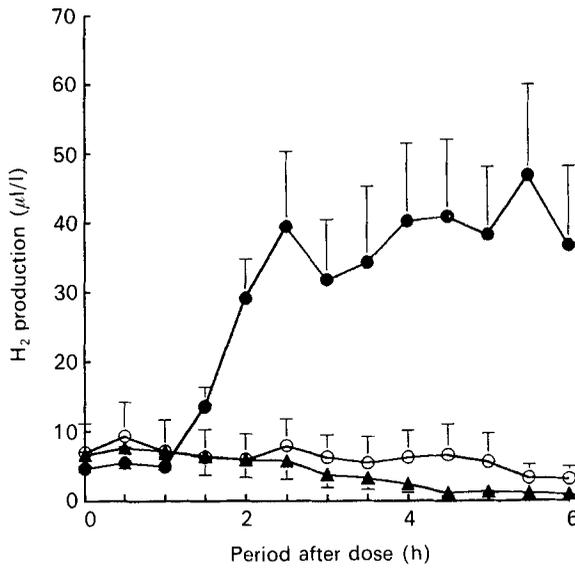
Fig. 2. H_2 excretion after ingestion of 25 g ^{13}C-labelled glucose (○), lactitol (●), or erythritol (▲) in healthy volunteers. For details of procedures, see pp. 170–171.
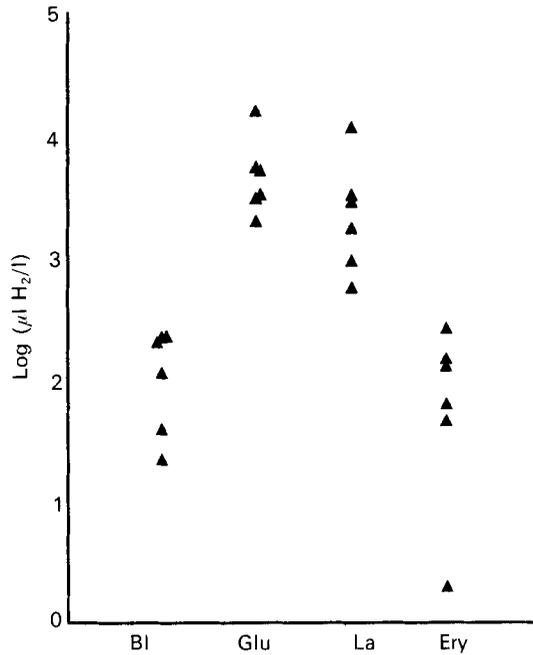
Fig. 3. Amount of H_2 produced by faecal samples from healthy volunteers after incubation with different substrates. Bl, blank; Glu, glucose; La, lactitol, Ery, erythritol. For details of procedures, see pp. 171–173.

Excretion of substrates in urine

During the first 6 h after substrate intake there was a cumulative urinary recovery of 52·2 (SE 2·5)% of the administered dose of erythritol, which amounted to 84·1 (SE 3·3)% after 24 h. No significant amounts of lactitol and glucose were found in urine.

Fermentation by faecal flora

As is shown in Fig. 3, the amount of H_2 formed by the faecal flora after 6 h was not higher after incubation with erythritol than that for the blank incubation. However, these amounts were significantly lower ($P \leqslant 0.001$) than those after incubation with glucose and lactitol.

DISCUSSION

In the present study the metabolic characteristics of erythritol were evaluated by comparing them with the metabolic characteristics of glucose and lactitol. The total $^{13}CO_2$ excretion in breath after intake of the ^{13}C-labelled substrates is determined by both the subject's own metabolism, and bacterial metabolism. The shape of the $^{13}CO_2$ excretion curve and the amount of breath H_2 excretion were used as indices of colonic bacterial metabolism. Urinary excretion was used as a measure for absorption without subsequent metabolism.

Several techniques have been proposed for the assessment of the energetic value of sugars and sugar-alcohols. Energy balance studies, in which all energy intakes and losses are measured after intake of a basal diet and after substitution of part of this diet by the substrate of interest, are probably the most accurate (Van Es *et al.* 1986; Van Es, 1987). However, as discussed by Van Es (1987), the extrapolations and the possible errors in the measurement of energy intake and loss require cautious interpretation of the results. Moreover, the technique is difficult to perform and requires good co-operation from the volunteers. Another possible approach was described by Grimble *et al.* (1988) who estimated the energy value of lactitol by measuring ^{14}C excretion in breath, urine and faeces after intake of $[^{14}C]$lactitol. This method assumes that all $^{14}CO_2$ in breath originates from bacterial metabolism and subsequent endogenous metabolism of volatile fatty acids formed in the colon. Therefore, it can only be applied to substrates already known to be inert for endogenous metabolism and, therefore, it cannot be applied to substrates such as xylose, xylitol, or sorbitol. Moreover, the conclusions depend largely on the validity of the formula of Miller & Wolin (1979) describing the stoichiometry of colonic fermentation.

Although the method used in the present study does not allow determination of the energy value, it has the advantage of being applicable to study important metabolic characteristics of a large range of well-known and newly developed substrates. Glucose, which stands model for a completely absorbed and metabolized substrate, was characterized by a high cumulative $^{13}CO_2$ excretion, a low value for $^{13}CO_2$ excretion after 6 h : $^{13}CO_2$ excretion after 1.5 h, and absence of H_2 production. The values found for cumulative amount of $^{13}CO_2$ excretion, which is a measure for exogenous glucose oxidation, are in good agreement with data reported by other investigators (Ebiner *et al.* 1979). After lactitol intake the cumulative $^{13}CO_2$ excretion was also high, but the time-course was different and the $^{13}CO_2$ excretion after 6 h : $^{13}CO_2$ excretion after 1.5 h ratio was high. This value and the high H_2 production seem typical for a substrate that is not absorbed but which undergoes colonic fermentation.

After intake of erythritol neither the $^{13}CO_2$ nor the H_2 excretion in breath increased. This means that after oral intake of 25 g erythritol it is not metabolized endogenously nor by the bacterial flora. A high proportion of erythritol was excreted in urine after 24 h. A small percentage was, however, not recovered in urine. Therefore, it is possible that a small amount of erythritol reached the colon where it was not metabolized by the colonic flora. Indeed, the faecal incubation experiments showed that erythritol is not metabolized by the faecal flora, in comparison with glucose and lactitol. Faecal excretion of erythritol may have passed unnoticed because its excretion in faeces was not analysed. It should be noted that both the *in vivo* and *in vitro* experiments were performed with non-adapted subjects or their faeces. Therefore, we cannot preclude that some bacterial metabolism occurs after an adaptation period.

From the present study it can be concluded that erythritol is a substrate that seems to be readily absorbed but undergoes no further metabolism by the host. If part of it escapes absorption, it is not substantially metabolized by faecal flora.

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