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 \( ^{13} \text{C} \)-labelled glucose, \([^{13} \text{C}] \text{lactitol} \) or \([^{13} \text{C}] \text{erythritol} \), in random order, dissolved in 250 ml water. There were at least 3 d between the tests. Breath samples for \(^{13} \text{C} \text{O}_2 \) and \( \text{H}_2 \) 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 \(^{13} \text{C} \) content than other plants. The difference in \(^{13} \text{C} \) content between \( C_4 \) plant-derived substrates and the \( \text{CO}_2 \) 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. 1988a, b, 1989). Maize-derived glucose was obtained from Amylum, Aalst, Belgium. Erythritol was manufactured from maize glucose by Cerestar, Vilvoorde, Belgium. \([^{13} \text{C}] \text{lactitol} \) was prepared from \(^{13} \text{C} \)-enriched lactose by CCA Biochem, Gorinchem, The Netherlands. \([^{13} \text{C}] \text{Lactose} \) was obtained by putting cows on a maize diet for 5 weeks, and isolating lactose from their milk (Hiele et al. 1988b).

\(^{13} \text{C} \text{O}_2 \) breath tests

Breath samples were collected in aluminium-coated low-density polyethylene bags (Tesseraux container, Bürstadt, Germany). Pure \( \text{CO}_2 \) was isolated by cryogenic trapping on liquid \( \text{N}_2 \) after water removal by a methanol–\( \text{CO}_2 \)–ice trap. The ratio \(^{13} \text{C} \text{O}_2 :^{12} \text{C} \text{O}_2 \) was measured by isotope-ratio mass spectrometry (Finnigan MAT 250, Bremen, Germany) and expressed as \( \delta^{13} \text{C}_{PDB} \) values, where \( \delta^{13} \text{C}_{PDB} \) is the \(^{13} \text{C} \) content in pro mille, expressed relative to \(^{13} \text{C} \) content in Pee Dee Belemnite limestone:

\[
\delta^{13} \text{C}_{PDB} = \frac{(R_{\text{sample}} - R_{\text{PDB}})}{R_{\text{PDB}}} \times 1000
\]

and

\[
R = \frac{^{13} \text{C} / ^{12} \text{C}}.
\]

The absolute ratio \(^{13} \text{C} :^{12} \text{C} \) of the PDB standard \( (R_{\text{PDB}}) \) is 0.0112372.

Formula 1 can be rewritten as:

\[
0.001 \delta^{13} \text{C}_{PDB} = \frac{(R_{\text{sample}} - R_{\text{PDB}})}{R_{\text{PDB}}}
\]

or

\[
(0.001 \delta^{13} \text{C}_{PDB} \times R_{\text{PDB}}) + R_{\text{PDB}} = R_{\text{sample}} = \frac{(^{13} \text{C} / ^{12} \text{C})_{\text{sample}}}{(\%^{13} \text{C} / (100 - \%^{13} \text{C})}_{\text{sample}}.}
\]

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

\[
\%^{13} \text{C}_{\text{sample}} = (0.001 \delta^{13} \text{C}_{PDB} + 1) \times R_{\text{PDB}} \times 100 / (1 + R_{\text{PDB}} \times (1 + 0.0001 \delta^{13} \text{C}_{PDB})).
\]

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

\[
(\text{percentage recovery of administered dose/h})_t = \frac{\text{mmol excess }^{13} \text{C}_t \times 100}{\text{mmol excess }^{13} \text{C} \text{ administered}}
\]
where \( t \) is sampling time, \( t_0 \) is time before substrate intake, and

\[
\text{mmol } ^{13}\text{C excess}_t = \frac{\left( ^{13}\text{C}_t - ^{13}\text{C}_0 \right)}{100} \times \text{mmol CO}_2 \text{ production}
\]

(i.e. mmol extra \(^{13}\text{C}\) appearing as breath \( \text{CO}_2 \) at time \( t \), due to oxidation of the labelled substrate), and

\[
\text{mmol excess } ^{13}\text{C administered} = \frac{\text{mg substrate}}{M} \times N \times \frac{\left( ^{13}\text{C}_s - ^{13}\text{C}_0 \right)}{100}
\]

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

For our calculations, the \( \text{CO}_2 \) 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 \(^{13}\text{C} \) 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 \(^{13}\text{C}\) enrichment of the substrates was measured after combustion to \( \text{CO}_2 \) (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 \( \text{H}_2 \) concentrations were measured by injecting 20 ml breath samples in an \( \text{H}_2 \) monitor (GMI, Renfrew, Scotland). The result was obtained immediately and expressed as \( \mu \text{l/l} \). Before each test the \( \text{H}_2 \) monitor was calibrated with a gas containing 39 \( \mu \text{l} \) \( \text{H}_2/\text{l} \) (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 \( \mu \text{l} \). The operating temperature was 75\(^\circ\) and the flow-rate 1 ml/min. HPLC-grade water containing 0.0018 M-\( \text{H}_2\text{SO}_4 \) was used as a solvent. Glucose in urine was measured by means of an enzymic method (hexokinase, \text{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\(^\circ\) 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 \( \text{Na}_2\text{HPO}_4 \), 12 H\( \text{O}_2 \) and 6.34 g \( \text{NaH}_2\text{PO}_4 \). 2 H\( \text{O}_2/\text{l} \). 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. The vials were tightly closed with a rubber cap and aluminium crimp seal, and incubated at 37°C. 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. \textit{Individual values of }$^{13}$CO$_2$\textit{ excretion 1.5 and 6 h after intake of glucose and lactitol, and }$^{13}$CO$_2$\textit{ excretion after 6 h:}$^{13}$CO$_2$\textit{ excretion after 1.5 h for healthy volunteers*}

<table>
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<th>Subject no.</th>
<th>$^{13}$CO$_2$ excretion 1.5 h</th>
<th>$^{13}$CO$_2$ excretion 6 h</th>
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</tr>
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<td>7.54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0.49</td>
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<tr>
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<td>0.91</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.89 4.54</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Lactitol</td>
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<td>1.02</td>
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<td>6</td>
<td>1.20 6.35</td>
<td>5.29</td>
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</tr>
</tbody>
</table>

*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.

\textit{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 $P \leq 0.05$ 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.

\textbf{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.
As is shown in Fig. 3, the amount of H₂ 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 ($P \leq 0.001$) 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 $^{13}$CO₂ 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 $^{13}$CO₂ excretion curve and the amount of breath H₂ 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 Grimbé 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 $^{14}$CO₂ 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 $^{13}$CO₂ excretion, a low value for $^{13}$CO₂ excretion after 6 h: $^{13}$CO₂ excretion after 1.5 h, and absence of H₂ production. The values found for cumulative amount of $^{13}$CO₂ excretion, which is a measure for exogenous glucose oxidation, are in good agreement with data reported by other investigators (Ebner et al. 1979). After lactitol intake the cumulative $^{13}$CO₂ excretion was also high, but the time-course was different and the $^{13}$CO₂ excretion after 6 h: $^{13}$CO₂ excretion after 1.5 h ratio was high. This value and the high H₂ production seem typical for a substrate that is not absorbed but which undergoes colonic fermentation.

After intake of erythritol neither the $^{13}$CO₂ nor the H₂ 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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