Increased lactulose/rhamnose ratio during fluid load is caused by increased urinary lactulose excretion

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NONINVASIVE ASSESSMENT of intestinal permeability in vivo is based on the measurement of urinary excretion of orally administered sugar probes. It is expressed as a ratio, usually lactulose/rhamnose or 3-O-methyl-D-glucose (3-OMG)/rhamnose. In both endotoxemic and control rats that were receiving fluid, we observed an increase in the recovery of lactulose and 3-OMG but not rhamnose in both groups, suggesting an enhancement of intestinal permeability. In the measurement of intestinal permeability, all pre- and postmucosal factors are considered equal for all sugars. We hypothesized that postmucosal factors and not changes in intestinal permeability caused the increased urinary lactulose and 3-OMG recoveries observed during fluid loading. Therefore, the effects of fluid loading on urinary excretion of the sugar probes were studied in healthy rats receiving the sugars intravenously. After intravenous injection, fluid loading increased urinary lactulose recovery threefold but not that of 3-OMG and rhamnose. In conclusion, fluid loading increases the lactulose/rhamnose ratio independent of changes in intestinal permeability. The 3-OMG/rhamnose ratio is not influenced by fluid loading.

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Initially, urinary excretion of single test substances like lactulose or polyethylene glycol was measured. However, problems with the interpretation of the data soon arose because the recovery of these substances is not only dependent on intestinal permeability itself but also on a number of other pre- and postmucosal factors (2, 3). This led to the formulation of the principle of differential urinary excretion, expressed in a ratio of two saccharides (17), such as lactulose and rhamnose (L/R) (7, 17) or lactulose and mannitol (27). It was postulated that calculation of a ratio of the recoveries of these sugars in urine is a better parameter than urinary lactulose recovery alone because incomplete dosages, reduced recovery, altered intestinal dilution, altered transit time, and altered body distribution probably have similar effects on both recoveries and thus do not influence the ratio (3, 25).

At present, determination of the ratio between the urinary excretion of the orally administered inert sugars lactulose and rhamnose or mannitol is a widely accepted clinical test for the determination of intestinal permeability. With the use of this test, it was shown that intestinal permeability is increased in situations like sepsis (20, 30, 32), burn injury (32), and villous atrophy (17).

However, the sugar test is based on a number of assumptions that may not be valid during a disease state or during fluid loading. For instance, it is assumed that urinary excretion is similar for all sugars (3). Moreover, it is known that tissue distribution is not similar for lactulose, rhamnose, and 3-OMG (3, 26). For this reason, we decided to evaluate whether the postmucosal factors apply equally to all three sugars. We focused on the effects of fluid loading because fluid is often supplemented in the diseased state to compensate for underfilling of the circulation.

METHODS

Animals. Male SPF Wistar rats (250–350 g; IFFA Credo Broekman, Someren, The Netherlands) were housed individually in metabolic cages for an adaptation period of 4 days and during the experiment. To avoid influences of differences in food intake between groups, rats were deprived of food during the experiment but water intake was ad libitum. Rats were subjected to standard 12:12-h light-dark cycle periods (7:30 A.M. to 7:30 P.M.), and room temperature was maintained at 25°C. The experiments were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals.
LACTULOSE/RHAMNOSE RATIO AND URINARY EXCRETION

Laboratory Animals (29) and were approved of by the Ethical Committee of Animal Research of Maastricht University.

Standard procedures. Anesthesia was induced by intraperitoneal injection of a mixture of ketamine (25 mg/100 g body wt, Nimatek; AUV, Cuyk, The Netherlands) and medetomidine (12.6 µg/100 g body wt; Domitor; Farmos, Espoo, Finland) in normal saline (NS, 150 mM NaCl). This caused loss of withdrawal reflexes within 5 min. When all procedures were done, the rats were injected intraperitoneally with atipazole hydrochloride (63 µg/100 g body wt; Antisedan; Farmos) to antagonize the effects of medetomidine. Ketamine does not influence intestinal motility (11). The activity of medetomidine rapidly reversed after Antisedan administration.

Intragastric administration of sugar probes. Under anesthesia, endotoxemia was induced in rats (n = 17) by subcutaneous injection of 2 mg/kg Escherichia coli 055:B5 lipopolysaccharide (LPS) (Sigma, St. Louis, MO) in sterile NS (4 ml/kg body wt) at 0 and 8 h (18, 22). Control animals (n = 15) received a sham injection of sterile NS. Because rats that are given LPS tend to stop drinking and thus become hypovolemic, rats were fluid loaded at 0 and 8 h (10 ml sterile NS/100 g body wt sc). Control animals (n = 5) did not obtain the fluid but were anesthetized and given a sham injection (Fig. 1B). At 8 h, rats were given an intravenous injection of 0.5 ml of a sterile isosmolar mixture of lactulose (1.5 mM, Sigma), rhamnose (2.3 mM, Fluka) and 3-OMG (3.0 mM) in NS. This dosage is different from the dosage that was given in the intragastric experiment because it was based on the urinary sugar recoveries that are found under normal conditions after intragastric administration. Because the urinary recovery of the paracellularly transversing lactulose is much lower than the recovery of the transcellularly transversing sugars rhamnose and 3-OMG after intragastric administration, the amount of lactulose given via the intravenous route is much lower than the amount of lactulose given via the enteral route. Urine was collected from 8–14 h and from 14–32 h, the volumes were measured, and 1 ml was frozen in liquid nitrogen and stored at −80°C.

Biochemical determination and calculations. Rats were housed in individual metabolic cages in which all urine produced by the rat was collected in a clean tube situated under the cage. We tested the stability of sugars in urine collected this way and found that they were very stable (data not shown). Therefore, in this setup there is no need for preservatives.

Biochemical determination of urinary lactulose, rhamnose, and 3-OMG concentrations was performed as described previously (23). The urinary recovery of lactulose, rhamnose, and 3-OMG were calculated as (7)

\[
Recovery(%) = \frac{C_{\text{urine}} \cdot V_{\text{urine}}}{C_{\text{intake}} \cdot V_{\text{intake}}} \times 100
\]

where \( C \) = concentration and \( V \) = volume.

In the second experiment, the cumulative recoveries were calculated by adding the 14–32 h recovery to the 8–14 h recovery (Fig. 1B).

The ratio between lactulose and rhamnose, which indicates intestinal permeability, was calculated as (7)

\[
L/R = \frac{\text{Recovery}_{\text{lactulose}}}{\text{Recovery}_{\text{rhamnose}}}
\]

The ratio between 3-OMG and rhamnose, which indicates active glucose transport, was calculated as (26)

\[
3-\text{OMG}/R = \frac{\text{Recovery}_{\text{3-OMG}}}{\text{Recovery}_{\text{rhamnose}}}
\]

Statistical analysis. Results are presented as means ± SE. Levels of significance were set at \( P < 0.05 \). Time effects within groups were tested using the Wilcoxon’s signed-rank test (19). Differences between groups were tested using the Mann-Whitney’s test (19).
RESULTS

Intragastric administration of sugar probes. The urine production during the 10–16 h period was not different between the two groups (fluid, 7.0 ± 0.3 ml/100 g body wt; fluid + LPS, 8.3 ± 0.5 ml/100 g body wt), which indicates that 70–80% of the fluid given is excreted within the 8-h period. At 16 h, arterial hematocrit was 44% in both groups.

Recovery of lactulose (Table 1) was 0.19% in the control measurement and increased in both experimental groups at 10–16 h to 0.73% (fluid) and 1.11% (fluid + LPS). Urinary rhamnose recovery was 9.3% and did not change in time or between groups. The recovery of 3-OMG was increased from 60% to 91% in both groups. As a result, both L/R and 3-OMG/R were increased in both groups (Table 2). No effects of LPS treatment on L/R and 3-OMG/R were observed.

Intravenous administration of sugar probes. When sugars were administered intravenously, the recovery of lactulose (Fig. 2A) was 26% in the control group when urine was collected for 6 h and increased to 80% after fluid loading. After 24 h urine collection, lactulose recovery reached 49% in the control group and 65% in the fluid group. The 6 h recovery of rhamnose was 11.1% and was independent of fluid support or urine collection period (Fig. 2B). The recovery of 3-OMG was 64% after 6 h urine collection and was not changed by fluid administration. When urine was collected for a longer period, 3-OMG recovery increased marginally to 74% after 24 h collection.

Due to increased lactulose recovery after fluid administration, urinary L/R, calculated with 6 h collection data, was increased from 0.39 ± 0.03 in the control group vs. 1.32 ± 0.22 in the fluid group (Table 3). At 24 h urine collection, L/R increased to 0.74 ± 0.09 in the control group and to 1.81 ± 0.19 in the fluid group.

After intravenous sugar injection, urinary 3-OMG/R amounted to 1.0 and did not change in time or due to fluid support until 24 h, when 3-OMG/R was 1.14 ± 0.02 in the control group and 1.03 ± 0.04 in the fluid group (Table 3).

DISCUSSION

In this study, effects of fluid load on urinary recoveries of lactulose, rhamnose, and 3-OMG after an intragastric or intravenous dose of a sugar solution were measured. The fluid loading was performed in LPS-

### Table 1. Urinary recovery of lactulose, rhamnose, and 3-O-methyl-D-glucose after intragastric administration in control and endotoxin-treated fluid-loaded rats

<table>
<thead>
<tr>
<th></th>
<th>Fluid</th>
<th>Fluid + LPS</th>
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</thead>
<tbody>
<tr>
<td>Lactulose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.19 ± 0.04</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>10–16 h</td>
<td>0.73 ± 0.08*</td>
<td>1.31 ± 0.51†</td>
</tr>
<tr>
<td>Rhamnose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.3 ± 1.2</td>
<td>11.1 ± 1.3</td>
</tr>
<tr>
<td>10–16 h</td>
<td>8.7 ± 1.3</td>
<td>9.6 ± 1.0</td>
</tr>
<tr>
<td>3-OMG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>60 ± 2.2</td>
<td>71 ± 5.2</td>
</tr>
<tr>
<td>10–16 h</td>
<td>91 ± 3.4†</td>
<td>91 ± 2.5†</td>
</tr>
</tbody>
</table>

Values are percentages of intragastric dose. Time indicated (10–16 h) is time period of urine collection (see Fig. 1A). 3-OMG, 3-O-methyl-D-glucose; LPS, lipopolysaccharide. *P < 0.05 vs. control; †P < 0.01 vs. control.

### Table 2. Urinary lactulose/rhamnose ratio and 3-OMG/rhamnose ratio after intragastric administration of lactulose, rhamnose, and 3-OMG in control and endotoxin-treated fluid-loaded rats

<table>
<thead>
<tr>
<th></th>
<th>Fluid</th>
<th>Fluid + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.024 ± 0.006</td>
<td>0.019 ± 0.003</td>
</tr>
<tr>
<td>10–16 h</td>
<td>0.102 ± 0.021†</td>
<td>0.114 ± 0.021†</td>
</tr>
<tr>
<td>3-OMG/R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.26 ± 0.85</td>
<td>6.91 ± 0.49</td>
</tr>
<tr>
<td>10–16 h</td>
<td>13.44 ± 2.25†</td>
<td>10.57 ± 0.97†</td>
</tr>
</tbody>
</table>

Values are ratios. L/R, lactulose/rhamnose ratio; 3-OMG/R, 3-O-methyl-D-glucose/rhamnose ratio. Time indicated (10–16 h) is time period of urine collection (see Fig. 1A). *P < 0.05 vs. control; †P < 0.01 vs. control.
treated rats because these rats tend to stop drinking (12, 14). The amount of fluid given equals twice their daily oral intake (31) and was injected subcutaneously to act like a slow infusion. It was found in the present study that fluid loading increases the L/R, usually considered to indicate an increase in intestinal permeability. It was shown, however, that this increase is caused by increased urinary lactulose excretion, independent of changes in intestinal permeability. Active intestinal glucose transport, as assessed with 3-OMG/R, seems a more reliable marker of intestinal function.

After intragastric administration of the sugars, control lactulose recovery was similar to results in healthy rats (7) but somewhat lower than values measured in humans (4, 9, 10, 16, 27). Increased lactulose recovery was expected in the LPS-treated group only (20), but was found in both groups, suggesting an effect of fluid loading. Control rhamnose recovery was similar to other data in rats (7) and within the range for human rhamnose recovery (8, 16). No changes in rhamnose recovery were seen due to LPS challenge or fluid administration. These findings show that the small intestinal absorptive surface remained unchanged after LPS challenge or fluid load (17).

After intravenous injection of the sugars we measured a relatively low urinary lactulose recovery, in contrast to values obtained in dogs (13), cats (21), and humans (4, 9), and these differences in lactulose recovery probably reflect species differences (13). Also, 3-OMG and rhamnose recovery were not complete after intravenous injection. The incomplete recovery of 3-OMG and rhamnose suggests that these saccharides may be metabolized by rats, although tracer studies would be necessary to determine the site and metabolic pathway involved. Variations in metabolism of the sugars, caused by physiological or pathophysiological events, could account for the changed two-sugar ratios.

Because the lactulose recovery increased and the rhamnose recovery was unchanged after intragastric administration of the sugar probes, the L/R increased in fluid-loaded endotoxin-treated and control animals. We also obtained this result in fluid-loaded pigs (5).

In our second experiment, we administered the sugars intravenously, so that only postmucosal factors can account for the changes observed in this experiment. After intravenous injection of the sugars, a time effect both in fluid-loaded and control animals was observed in the recovery of lactulose but not in the recovery of rhamnose. Thus a difference between lactulose and rhamnose regarding the rate of urinary excretion was observed. As a result, the ratio of lactulose and rhamnose was increased in time. The length of urine collection is thus of great importance if one wants to compare sugar recovery data and two-sugar ratios with data reported in the literature. Because the doses of lactulose, rhamnose, and 3-OMG that were given in the intravenous experiment were based on the recoveries of these sugars after intragastric administration, we expect that time effects in urinary recoveries of sugars also exist if one administers the sugars intragastrically.

The time necessary for half-maximal excretion was ~3 h in fluid-loaded animals and 24 h in controls. Moreover, the initial excretion rate was threefold higher in fluid-loaded than in control animals. After 6 h urine collection, the increase in lactulose recovery after intragastric administration was fourfold, similar to the threefold increase after intravenous administration. Therefore, the increase in lactulose recovery and in L/R caused by fluid support observed after intragastric administration of the sugar probes can be fully explained by increased urinary lactulose excretion and thus does not give information regarding intestinal permeability or barrier function. Postmucosal factors, possibly renal handling or changes in tissue distribution, cause the increase in urinary lactulose recovery in fluid-loaded animals. The tissue distribution of the three sugars is different in that lactulose cannot enter cells and therefore has an extracellular distribution (25), whereas rhamnose and 3-OMG do enter cells and therefore may be present both intra- and extracellularly (3, 26). If fluid loading changes the urinary recovery of extracellular lactulose, one would expect that some effect would also be present for rhamnose and 3-OMG, since these saccharides also have some extracellular distribution. The effect on urinary lactulose recovery has taken place between entering the blood and the appearance in urine. The kidney is the main organ influencing this pathway. Therefore, in our opinion, changes in renal handling cause the increased urinary lactulose recovery during fluid loading. In the renal proximal tubule, water reabsorption occurs via transcellular and paracellular pathways. In a variety of circumstances, in which there is a reduction of net proximal Na⁺ reabsorption, enhanced movement of raffinose or sucrose, like lactulose markers of extracellular space, from the peritubular capillary into the lumen has been demonstrated (24). Isotonic saline infusion reduces oncotic pressure in humans (1). If capillary uptake is impeded by a reduction in peritubular capillary oncotic pressure, the interstitial hydrostatic pressure will rise as active Na⁺ transport continues, favoring backflow across the tight junctions (24). This may be the mechanism by which saline loading increases urinary lactulose recovery.

However, we cannot exclude another, unknown, postmucosal factor. The isotonic saline loading that was performed in this study may have caused changes in

### Table 3. Urinary L/R and 3-OMG/R after intravenous administration of lactulose, rhamnose, and 3-OMG in control and fluid-loaded rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fluid</th>
</tr>
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<tbody>
<tr>
<td>L/R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>0.39 ± 0.03</td>
<td>1.32 ± 0.22†</td>
</tr>
<tr>
<td>24 h</td>
<td>0.74 ± 0.09</td>
<td>1.81 ± 0.19†</td>
</tr>
<tr>
<td>3-OMG/R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>0.99 ± 0.02</td>
<td>0.97 ± 0.03</td>
</tr>
<tr>
<td>24 h</td>
<td>1.14 ± 0.02</td>
<td>1.03 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are ratios. Times indicated are time period of urine collection (see Fig. 1B). *P < 0.05 vs. control; †P < 0.01 vs. control.
systemic parameters as plasma renin activity, plasma aldosterone (15), and the corticotropin and cortisol response to endotoxin stimulation (6). It is not known whether an influence on postmucosal factors is also present in humans and/or patient populations. Systemic and renal handling may be different in rats and humans. Information concerning these issues is lacking at present. Also, it is not known at present whether smaller amounts of fluid loading decrease the rise in lactulose recovery. However, changes in urinary excretion may also apply to critically ill patients with fluid shifts or patients with renal insufficiency, obscuring the interpretation of obtained L/R. It could be necessary to include tests with intravenously injected lactulose in these patient studies.

During fluid support, the recovery of 3-OMG is also increased after intragastric administration, resulting in an increased 3-OMG/R. However, since neither rhamnose nor 3-OMG recovery is changed by fluid administration after intravenous administration, the increase in 3-OMG/R cannot be explained by changes in renal excretion. Thus intestinal active glucose transport indeed seems to be increased after fluid administration.

In conclusion, fluid loading increases the urinary recovery of lactulose, but not rhamnose, after intravenous or intragastric administration of the sugar probes. As a result, the L/R is increased independently of changes in intestinal permeability. Therefore, the assumption that postmucosal factors influence the urinary recoveries of all inert sugars equally is not valid, and thus care must be taken when interpreting urinary recoveries of lactulose, but not rhamnose, after intravenously administered simple sugars. As an extended view, it may also apply to critically ill patients with fluid challenge is due to fluid load (Abstract). Eur. J. Gastroenterol. Hepatol. 10: A22–A23, 1998.

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