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A reference interval study of urinary lactulose excretion: a useful test of intestinal permeability in adults

İdrarla laktuloz atılımı için bir referans aralığı çalışması: yetişkinlerde barsak geçirgenliğini değerlendirmek için yararlı bir test

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Background/aims: *Measurement of lactulose excretion in urine following oral ingestion is used as a noninvasive method of assessing small intestinal permeability. The aim of this study was to determine the adult reference interval of urinary lactulose excretion following oral administration.* ***Methods:*** *Thirty seven volunteers (mean age 37.3 ± 15 years) with no history of gastrointestinal disease were recruited as study subjects. All subjects were fasted overnight (10 hours), emptied their bladders and then drank a 50-mL solution containing 15 mL of Duphalac (10 g lactulose) and 35 mL flavoured water. Urine was collected for six hours in a bottle. Comparisons were made for 37 samples.* ***Results:*** *In adults, the mean urinary lactulose concentration was 0.58 ± 0.39 mmol/L.* ***Conclusion:*** *This study determined the adult reference range for lactulose excreted in the urine, using a sensitive quantitative assay based on hydrolysing lactulose and enzymatically assaying fructose, one of the component monosaccharides.*

Key words: Lactulose, urine, reference interval.

Amaç: *Ağızdan alınan laktulozun idrarla atılımı, ince barsak geçirgenliğinin değerlendirilmesinde noninvaziv bir metod olarak kullanılmaktadır. Bu çalışmanın amacı, oral yolla verildikten sonra idrarla atılan laktulozun yetişkinler için referans aralığını belirlemektir.* ***Yöntem:*** *Çalışma grubu olarak, gastrointestinal hastalık öyküsü olmayan 37 gönüllü alındı (yaş ortalaması: 37.3 ± 15 yıl). Bütün denekler 10 saatlik bir açlıktan sonra sabah ilk idrarlarını attılar. Daha sonra 15 mL Duphalac (10 g laktuloz) ve 35 mL aromalı su içeren 50 mL'lik bir solüsyon içirildi. Sonraki 6 saat boyunca idrarları toplandı. 37 örnek için karşılaştırma yapıldı.* ***Bulgular:*** *Yetişkinlerde idrar laktuloz konsantrasyonu ortalama 0.58 ± 0.39 mmol/L olarak bulunmuştur.* ***Sonuç:*** *Bu çalışmada laktulozu hidrolize ederek bileşiminde bulunan monosakkaritlerden biri olan fruktozun enzimatik tayinine dayanan hassas bir kantitatif metod kullanılmış ve yetişkinlerde idrarla atılan laktuloz için referans aralığı tanımlanmıştır.*

Anahtar kelimeler: Laktuloz, idrar, referans aralığı.

INTRODUCTION

Lactulose (4-O-b-D-galactopyranosyl-D-fructose) is a synthetic disaccharide, which may be used to measure intestinal permeability (1-4). It is neither hydrolysed by human intestinal disaccharidases nor metabolised by other tissues (5), and thus transfer across the intestinal mucosa is accurately and quantitatively reflected by excretion in urine over a five-hour (6) or six-hour (7) period after oral administration.

The measurement of urinary excretion of non-metabolised sugars has been widely used as a non-invasive method of assessing mucosal integrity of the small bowel in children (8-10) and adults (12). Lactulose is an ideal compound for measuring sugar absorption because it has a negligible affinity with the monosaccharide transport system and is passively absorbed and not metabolised before urine excretion (10,11).

The permeability of the small intestine to lactulose is significantly increased in patients with small bowel pathology such as celiac disease (2,12) and Crohn's disease (13,14), whereas the absorption of monosaccharides such as xylose, mannitol (11) and L-rhamnose (2) is impaired. The urinary ratio of disaccharide and monosaccharide probe molecules can therefore be a sensitive and useful marker for detecting intestinal pathology and for monitoring response to therapeutic measures, particularly in patients with celiac disease given a gluten-free diet (8).

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MATERIALS AND METHODS

Subjects

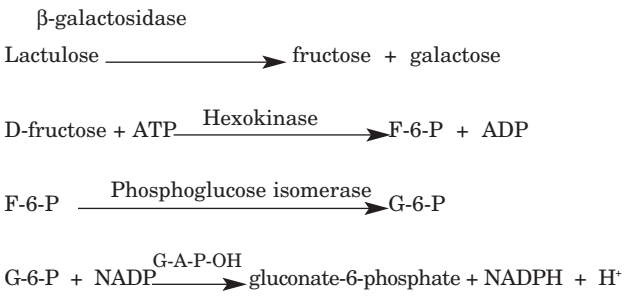
Thirty-seven volunteers (mean age 37.3 ± 15, range 18-68 years) with no history of gastrointestinal disease were recruited as study subjects. No drug use, including diuretics was permitted during the test period. Evaluation of smoking history, height and weight was made at the time of examination. Subjects reporting any regular cigarette use within the year before the examination were classified as smokers. Body mass index was calculated as weight in kilograms divided by the square of height in meters.

Collecting Samples

Urine samples were collected as follows: all subjects were fasted overnight (10 hours), empied their bladder and then drank a 50-mL solution containing 15 mL of Duphalac (10 g lactulose) and 35 mL flavoured water. Breakfast was offered one hour later and urine was then collected for six hours in a bottle. No preservative was used because the urine was stored in a refrigerator and the test performed immediately after the collection period.

Principle of Assay

The basic principle of the assay was first described as a manual method by Behrens et al. (7). The enzymatic hydrolyses of lactulose is via the following pathway:



The NADPH formed in the reaction is estimated by measuring the change in absorbance at 340 nm.

Chemicals

Lactulose, triethanolamine hydrochloride, magnesium sulphate, adenosine-5'-triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), hexokinase/glucose-6-phosphate dehydrogenase (G-6-P-DH), phosphoglucoisomerase, and β-Galactosidase were obtained from Sigma Chemical Co.

Preparation

Triethanolamine buffer is prepared by dissolving 5.6 g triethanolamine HCl and 740 mg MgSO4 in 50 mL distilled water. The pH is adjusted to 7.5 with 1 mol/L NaOH and is diluted to 100 mL.

The following enzymes are diluted with ammonium sulphate, 3.2 mol/L, to obtain the enzyme activities indicated. Hexokinase/glucose-6-phosphate dehydrogenase (HK/G-6-P-DH): 190U/ 95 U/mL; 1 unit of hexokinase will catalyse the phosphorylation of 1.0 µmol of glucose per minute at pH 8.5, 1 unit of G-6-P-DH oxidizes 1.0 mmol of glucose-6-PO_4 per minute to 6-phosphogluconate at pH 7.4, 25oC in the presence of NADP. Stored frozen. b-Galactosidase 500 U/mL; 1 unit hydrolyses 1.0 mmol of ortho-nitrophenyl-b-D-galactopyranoside per minute to ortho-nitrophenol and galactose at pH 7.3 and 37°C. Stored frozen. Phosphoglucoisomerase 350 U/mL; 1 unit converts 1.0 mmol of D-fructose-6-PO4 to D-glucose-6-PO4 per minute at pH 7.4 at 25°C. Stored at 0-5°C.

Aqueous solution of lactulose is prepared to 1.45 mmol/L concentration by dilutions of a stock solution (2.9 mmol/L).

Buffer/enzyme cocktail

Sufficient cocktail, freshly prepared for the number of samples to be analyzed æ 2.73 mL of cocktail containing 1 mL triethanolamine /MgSO4 , 2 mg ATP, 2 mg NADP, 20 mL hexokinase/glucose-6-phosphate dehydrogenase and 1.73 mL distilled water æ is added to each cuvette containing the hydrolysed sample of lactulose.

Protocol

100 µL urine is added to 100 mL triethanolamine /MgSO4 buffer and 50 mL b-galactosidase in a 4-mL disposable cuvette. For each urine sample, a separate cuvette is prepared without b-galactosidase in order to estimate free fructose. The cuvettes are gently agitated at 20-25 °C for two hour following which 2.73 µL of the buffer/enzyme cocktail is added. The absorbance at 340 nm is recorded against a blank cuvette, containing cocktail and water instead of urine. 20 mL of phosphoglucoisomerase is then added to the cuvettes giving a final volume of 3.0 mL. The change in absorbance is repeatedly recorded until the reaction is complete (4-6 min).

Statistical analysis

Statistical analysis was carried out with the SPSS statistical program. Ranking the observations from smallest to largest, the range of values from the 2.5th to the 97.5th percentile (Mid-95%) covered the central 95% of values precisely.

Table 1. Descriptive statistics for study group

	n	`X ± SD
Age (years)	37	37.3 ± 15
BMI (kg/m^2)	37	24.6 ± 4.2
Urine volume (mL/6 h)	37	406 ± 298
Urinary Lactulose Concentration (mmol/L)	37	0.58 ± 0.39

Table 2. Reference interval in mmol/L for lactulose in urine of adults.[a]

	$f_{0.025}$	$f_{0.975}$	Median
Lactulose (mmol/L)	0.047	1.377	0.48

a Fractiles 0.025 and 0.975 ($f_{0.025}$, $f_{0.975}$) and median are shown.

RESULTS

For total group, the mean urinary lactulose concentration was 0.58 ± 0.39 mmol/L. The means of age, BMI and urine volume were 37.3± 15 years, 24.6± 4.2 kg/m^2 and 406±298 mL/6 hours respectively. Table 1 shows descriptive statistics for the whole group.

Reference Interval

Reference values were obtained with samples from 37 fasting healthy subjects (17 females and 20 males) free of intestinal disorders. The reference interval for adults, of urinary lactulose in mmol/L shown in Table 2, where 0.025 and 0.975 fractiles and the median are shown. Sugar excretion during the six-hours period was determined as 0.047-1.377 (median 0.48).

DISCUSSION

Measurement of the urinary excretion of two or more orally administered nonmetabolized sugar probes of different sizes (mono- and disaccharides) has been established as an excellent noninvasive approach for the assessment of intestinal permeability in humans (10).

Contrast radiology, endoscopy, and histology are usually used to establish a definitive diagnosis of absorptive pathology of the intestine. There is a natural reluctance to subject patients to time consuming, expensive and unpleasant procedures in pursuit of a comparatively rare disease, especially if the presenting symptoms are vague (14).

Small bowel diseases are difficult to investigate, with jejunal and ileal anomalies often requiring invasive examinations because noninvasive tests are almost always nonspecific for the diagnosis of diseases involving damage of the intestinal mucosa (10).

In addition, these techniques sometimes fail to show the disease, particularly if it is confined to the small bowel, where radiological studies may be inconclusive and biopsy difficult (14).

In various diseases affecting the small bowel, the damaged mucosa is abnormally permeable, and the absorption of molecules may be increased through a "leak", or decreased because of diminished surface area. This may lead to increased permeability of larger molecules and/or to impaired transcellular passage of smaller molecules (10).

Although histologic examination of the small intestinal mucosa from a peroral biopsy sample remains the gold standard for evaluation of mucosal integrity, this diagnostic technique is not always available. Even if biopsy and histologic examination are available, many physicians prefer to use a noninvasive test to verify the need for a biopsy first. Such a test must be sensitive, precise and accurate. The test substance should be easily measured in biological fluids, results should be obtained rapidly and the test should be relatively inexpensive (9).

Intestinal permeability indicates the capacity of the intestinal absorptive surface to permit passive penetration by watersoluble molecules. It may be studied using orally administered probe molecules which are passively absorbed, are resistant to enzymatic digestion, are not metabolised and are subject to quantitative renal excretion. Measurement of the urinary excretion of such probes then provides a measure of intestinal absorption (14).

Measurement of lactulose in urine after oral administration is well established in the non-invasive investigation of small intestinal absorption pathways in adults (7,15,16).

This technique may also have a useful role in the assessment of the extent of disease activity. To evaluate this further, studies to examine the association between intestinal permeability and the findings on endoscopic and radiologic investigation may be appropriate.

Normal P-P plot of the urinary lactulose, BMI, age and urine volume is shown in Figure 1. Urine lactulose concentration lies in the 0.047 – 2.931

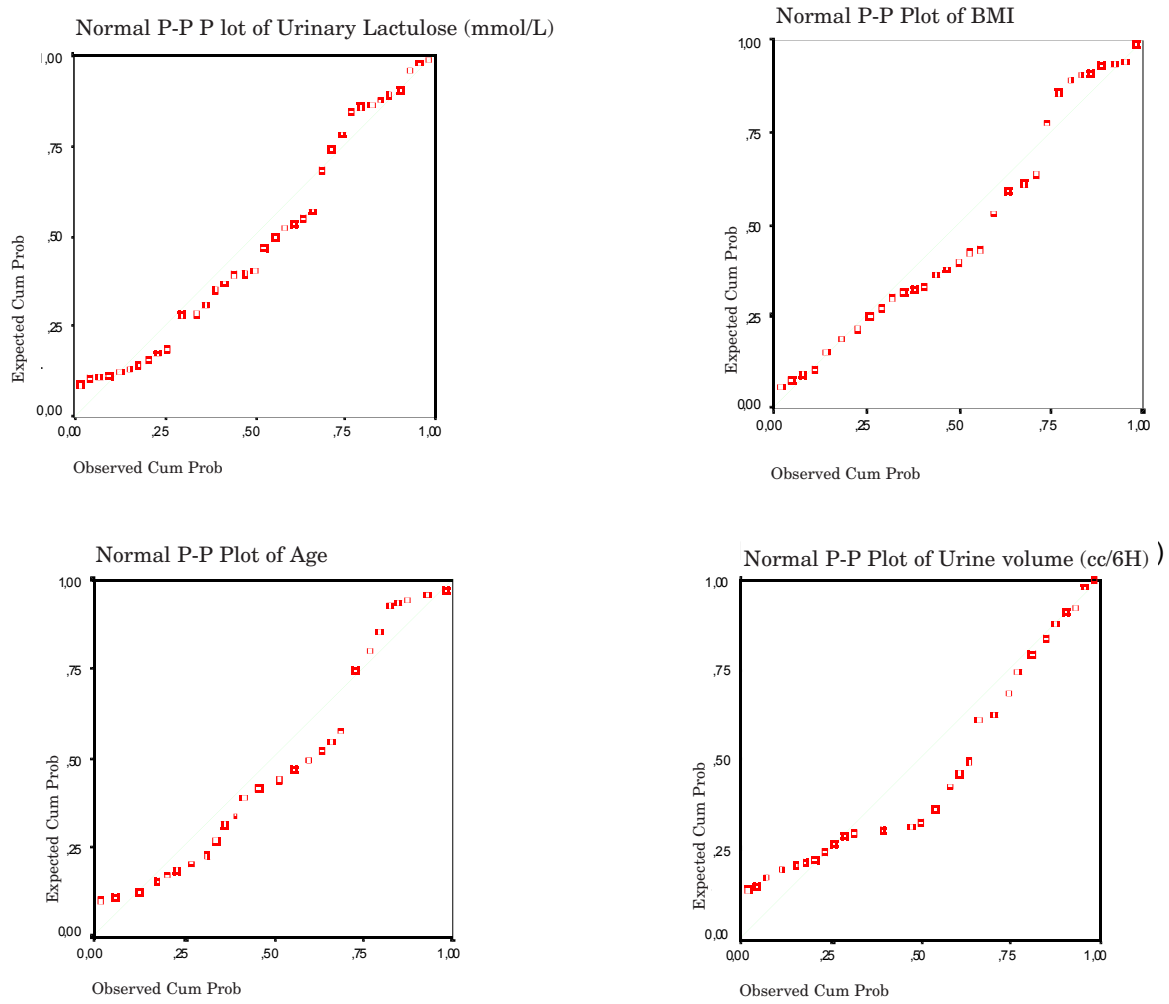


Figure 1. Normal P-P plot for urinary lactulose, BMI, age and urine volume

mmol/L range in this study. Northrop et al. (17) have reported that following a standard permeability test dose of 2 g lactulose/10 kg body weight (up to a maximum of 10 g), the concentration of this sugar in urine generally lies in the 0.15 - 2.9 mmol/L range. These results are compatible with those of the present study.

Measurement of the passive transfer of disaccharides across the intestinal mucosa and then their excretion in urine has become a useful tool in clinical practice and research (2,3,11). Their adoption will clearly allow this useful test of small bowel integrity and function to become a routine clinical tool (18).

REFERENCES

1. Muller W, Walker-Smith J, Schmerling DH, et al. Lactulose: a gas liquid chromatography method of determination and evaluation of its use to assess intestinal mucosal damage. Clin Chim Acta 1969; 24: 45-9.
2. Menzies IS, Laker MF, Pounder R, et al. Abnormal intestinal permeability to sugar in villous atrophy. Lancet 1979; 24: 1107-9.
3. Pearson AD, Eastham J, Laker MF, et al. Intestinal permeability in children with Crohn's disease and coeliac disease. Br Med J 1982; 285: 20-1.
4. Beach RC, Menzies IS, Clayden GS, Scepes Jan W. Gastrointestinal permeability changes in the preterm neonate. Arch Dis Child 1982; 57: 141-5.
5. Dahlquist A, Gryboski JD. Inability of the human small intestinal lactase to hydrolyse lactulose. Biochim Biophys Acta 1965; 110: 635-6.
6. Menzies IS. Absorption of intact oligosaccharides in health and disease. Biochem Soc Trans 1973;2:1042-7.
7. Behrens RH, Docherty H, Marinos E, Neale G. A simple enzymatic method for the assay of urinary lactulose. Clin Chim Acta 1984; 137: 361-7.
8. Hamilton I, Hill A, Bose B, et al. Small intestinal permeability in pediatric clinical practice. J Pediatr Gastroenterol Nutr 1987; 6: 697-701.

9. Lifschitz CH, Shulman RJ. Intestinal permeability test: are they clinically useful? J Pediatr Gastroenterol Nutr 1990; 10: 283-7.
10. Marsilio R, D'Antiga L, Zancan L, et al. Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics. Clin Chem 1998; 44: 1685-91.
11. Cobden I, Dickinson RJ, Rothwell J, Axon ATR. Intestinal permeability assessed by the excretion of two molecular probes in coeliac disease. Br Med J 1978; 2: 1060.
12. Johnston SD, Smye M, Watson RGP, et al. Lactulose-mannitol intestinal permeability test: a useful screening test for adult coeliac disease. Ann Clin Biochem 2000; 37: 512-9.
13. Pearson ADJ, Eastham EJ, Laker MF, et al. Intestinal permeability in children with Crohn's disease and coeliac disease. Br Med J 1982; 285: 20-1.
14. Murphy MS, Eastham EJ, Nelson R, et al. Intestinal permeability in Crohn's disease. Arch Dis Child 1989; 64: 321-5.
15. Travis S, Menzies I. Intestinal permeability: functional assessment and significance. Clin Sci 1992; 82: 471-88.
16. Fleming SC, Duncan A, Russell RI, Laker MF. Measurement of sugar probes in serum: an alternative to urine measurement in intestinal permeability testing. Clin Chem 1996; 42: 445-8.
17. Northrop CA, Lunn PG, Behrens RH. Automated enzymatic assays for the determination of intestinal permeability probes in urine. 1. Lactulose and lactose. Clin Chim Acta 1990; 187: 79-88.
18. Lunn PG, Northrop CA, Northrop AJ. Automated enzymatic assays for the determination of intestinal permeability probes in urine. 2. Mannitol. Clin Chim Acta 1989; 183: 163-70.