Evaluation of the Lactulose/Rhamnose dual sugar test for intestinal permeability

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Aim

To evaluate a minimally invasive blood test for intestinal permeability

Introduction

A major function of the intestinal barrier, the single layer of epithelial cells interconnected by tight junction proteins, is to separate the luminal contents from the internal cellular milieu of the body. Disruption of this epithelium can lead to pathogen exposure, which in turn leads to a highly immune-reactive sub-epithelium. Once an initial insult to the epithelium has been made, an increasing number of foreign antigens may leak into the internal environment from the lumen, triggering a cycle of inflammation. Many factors can alter intestinal permeability, including: gut microbiome dysbiosis, mucus layer alterations, epithelial damage, lifestyle and dietary factors.

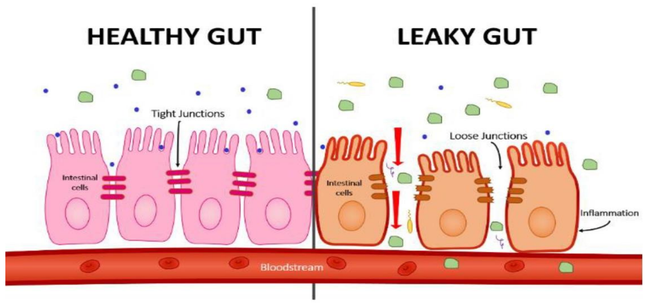
It is now recognised that increased intestinal permeability is associated with a variety of diseases such as: inflammatory bowel diseases, coeliac disease, food allergy, irritable bowel syndrome, and, more recently, obesity and metabolic diseases. In addition, all of these diseases are characterized by inflammation and subsequent debilitating symptoms that may be triggered by the translocation of luminal components into the internal host environment.

An assay of intestinal permeability can be performed based on the trafficking of a substrate via the paracellular vs intracellular epithelial pathways, and if there is a breakdown of intestinal barrier function, a substrate that cannot be transported intracellularly will appear in the blood via paracellular transport. A mixed substrate containing Rhamnose (intracellular) and Lactulose (paracellular) can be administered to test this, and uptake is measured in either urine or serum.

Although it has not been established whether altered intestinal permeability causes, or is a consequence of, inflammation, it is accepted that improved barrier function is linked to clinical remission.

We have assessed the Lactulose/Rhamnose Dual Sugar test with regard to sampling method, comparison with the Faecal Calprotectin levels and PCDAI/PUCAI clinical scores and we have defined the normal L/R range for healthy individuals.

When the intestinal barrier and tight junctions are intact, lactulose cannot enter the bloodstream



When the intestinal barrier and tight junctions are damaged, lactulose can enter the bloodstream

Rhamnose is transported intracellularly and can enter the bloodstream in either scenario.

Methods

L/R Test

Patients were instructed to fast overnight before taking a test drink containing 1g Rhamnose and 5g Lactulose. Ninety minutes after ingestion, a blood sample was collected into a lithium heparin tube, either by venepuncture or finger prick. Samples were centrifuged and the plasma separated and frozen.

250uL of sample was diluted 1:2 with ultrapure water and 300uL of 15% TCA was added to precipitate proteins and centrifuged at 1500RPM for 5 minutes. The supernatant was then treated with approx. 100ug Amberlite MB-20 resin beads (Sigma-Aldrich) to deionise and remove TCA residue. Samples were filtered through a 0.2um Nylon filter using a SINGLE StEP Filter Vial (Thomson Instrument Company, USA).

Plasma samples were batch analysed on the 930 Compact IC Flex (MetrOhm, Switzerland). A Metrosep Carb 2 – 250/4.0 column was used to separate sugars and the chromatography solvent was 250mM NaOH and 2.5mM NaAcetate in ultrapure water. An Amperometric Detector was used to quantitate the samples. Quantitation was performed by integration of the Area Under the Curve for the relevant sugars. Each run was calibrated using plasma samples spiked with 135uM, 45uM, 15uM and 5uM of Lactulose and Rhamnose.

Faecal Calprotectin Test

Stool samples were collected and frozen or refrigerated until transported to the laboratory. Samples were stored frozen at -20°C until analysis for calprotectin concentration by Quantitative Lateral Flow Assay (Bühlmann Laboratories AG, Switzerland) using the Bühlmann Quantum Blue® Reader. Calprotectin values >100ug/g are indicative of inflammation in the gastrointestinal tract.

Cohorts

Paediatric IBD group

23 children ranging in age from 6.1-17.5 years (mean 14.3 years) attending the Women's and Children's Health Network (WCHN) for investigations to diagnose or exclude IBD, or existing IBD patients, were recruited to participate in a clinical trial. L/R and faecal calprotectin tests were conducted at baseline and then 3, 6 and 12 months post recruitment. At time of publication data was not available for all time points.

Healthy Paediatric Control group

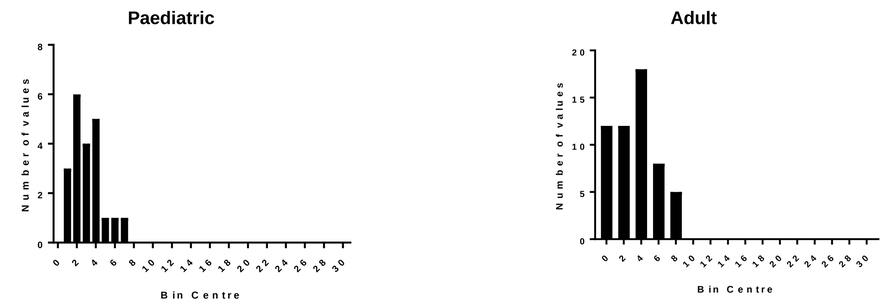
33 children, ranging in age from 6 to 20 years (mean 11.7 years), from the Women's and Children's Health Network (WCHN) Paediatric Outpatients were recruited for intestinal permeability testing between 2014 and 2017. They were symptom free at the time of testing and not from a Gastroenterology clinic.

Healthy Adult Control group

50 individuals were recruited between 2010-2015, ranging in age from 18 to 66 years (mean 26 years). Participants completed a questionnaire to rule out underlying gastrointestinal disorders prior to performing the L/R test. Plasma samples were collected by venepuncture for all participants, and 19 of these also had samples collected at the same time by finger prick.

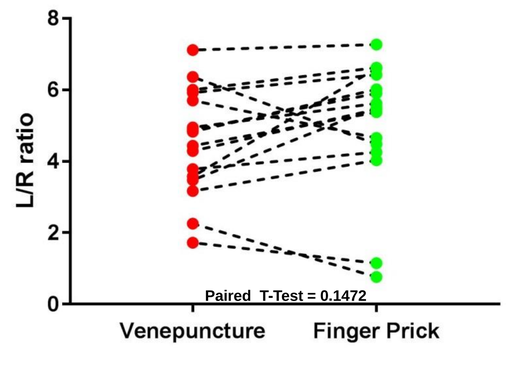
Results

Fig 1: Frequency distribution of Lactulose/Rhamnose ratio in healthy individuals



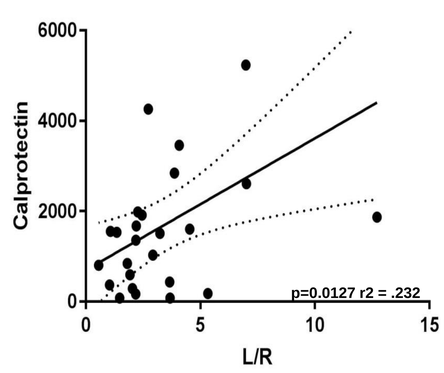
The distribution of L/R ratios when divided into 30 groups reveals a left skewed distribution with an average value in bin 4 for both children and adults.

Fig 2: Fingerprick vs. Venepuncture



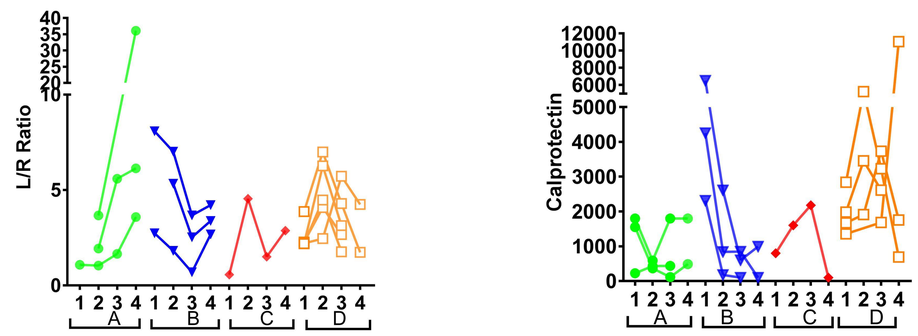
The measured value for L/R ratios from standard venepuncture is not statistically different to the L/R ratio value when the sample is collected by finger prick from the same individual.

Fig 3: Faecal Calprotectin vs. L/R



There is a positive correlation between intestinal permeability values and paired faecal calprotectin determined at the same time in the same individuals.

Fig 4: Comparison of L/R and faecal calprotectin in paired paediatric IBD samples



When paediatric IBD cohort participants are separated into 4 curated groups (A-D) based on their intestinal permeability measures over 4 time points (1-4) approximately 3 months apart, patterns of permeability can be observed (left). When the same participants are tested for faecal calprotectin at the same time points, there are similarities in the behaviour of the measures in each curated group.

Conclusions

- Using healthy paediatric and adult cohorts we have established a statistically robust cut off for the normal range of intestinal permeability.
- There is no statistical difference in the Lactulose/Rhamnose ratio observed between serum samples obtained by venepuncture vs fingerpick, suggesting that the fingerpick is a reliable method which can be used in place of the more invasive venepuncture. This may be particularly useful when performing the test in paediatric cohorts.
- There is a positive correlation between faecal calprotectin levels and intestinal permeability, measured by Lactulose/Rhamnose ratio, suggesting that the L/R test is a useful surrogate for measuring intestinal damage. A key feature of the L/R test is that it is a direct functional measure of intestinal barrier function, rather than a surrogate for permeability.

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