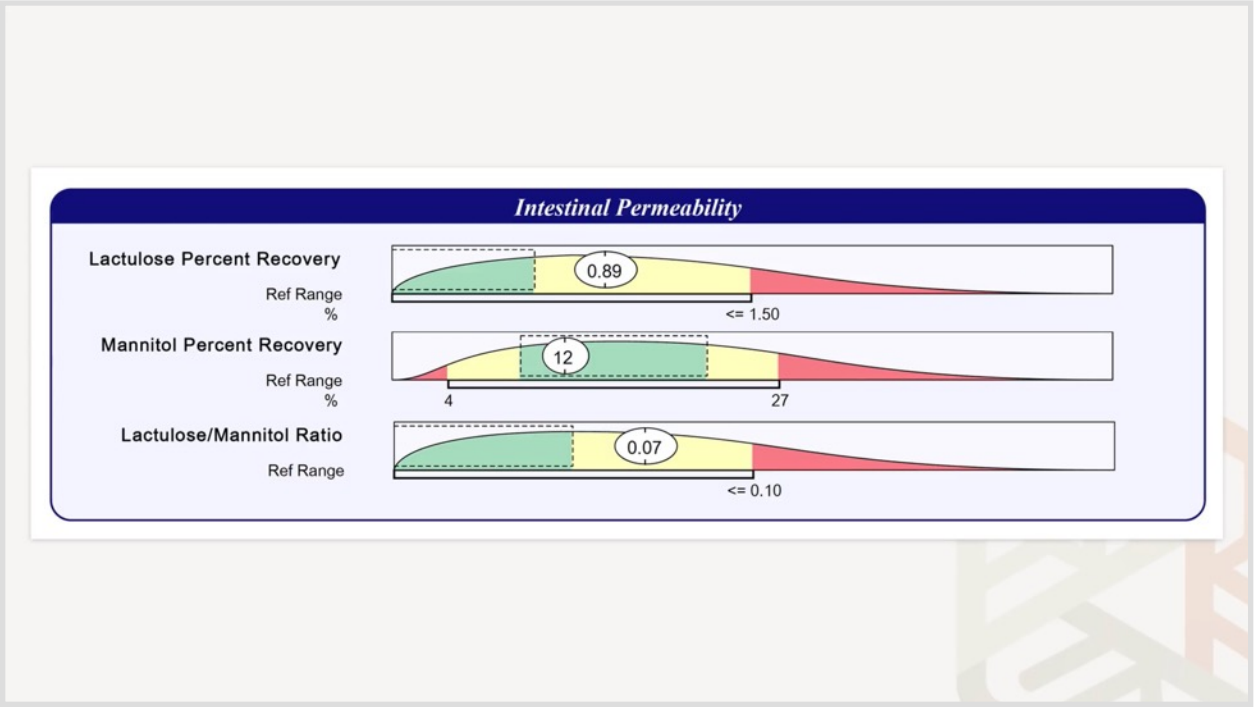
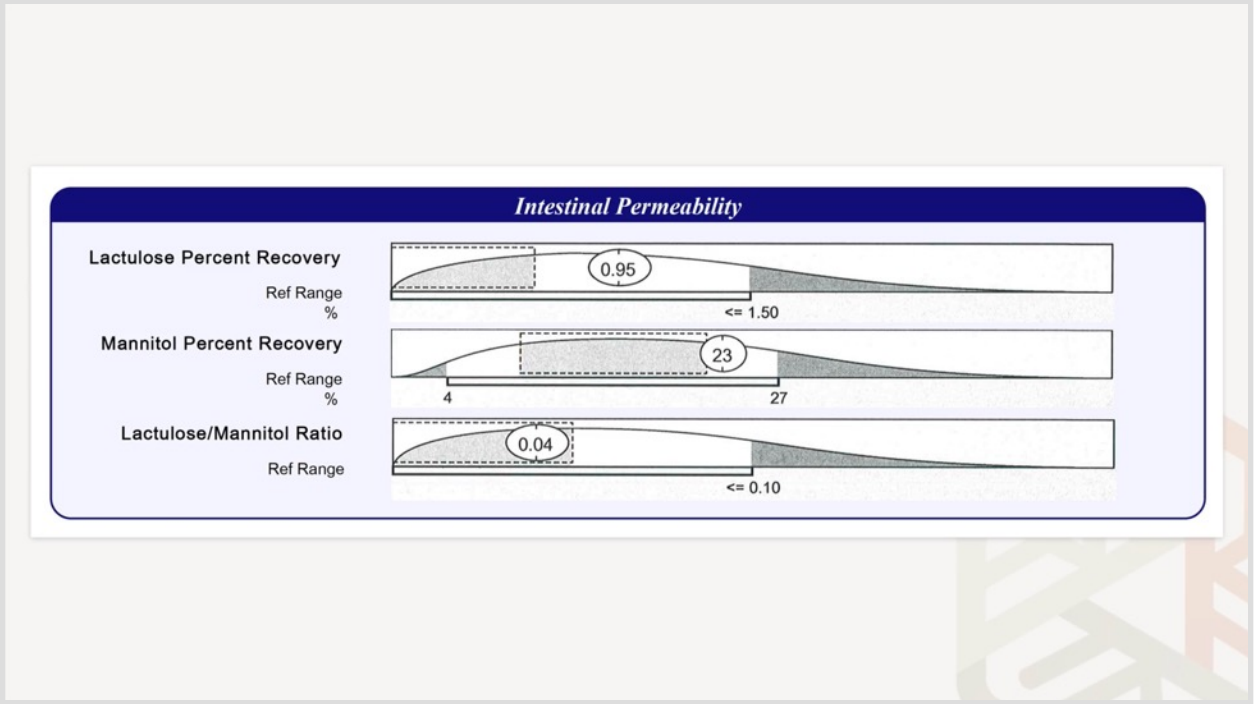
Gut: Intestinal Permeability, Part 2

The lactulose-mannitol test involves measuring levels of these two sugars in the urine after the patient consumes them orally. Mannitol is an oligosaccharide with a molecular weight of 182 daltons and a molecular radius of up to 0.4 nanomicrons. Lactulose is an oligosaccharide with a molecular weight of 342 daltons and a radius of 0.42 nanomicrons. The different molecular weights of these molecules help to determine whether permeability is transcellular or paracellular. Mannitol is small enough to pass directly through the cells, which would be transcellular, whereas lactulose is a larger size, means it would pass between the cells, paracellular. Because of the smaller size, in healthy people, mannitol is expected to be present in urine in a larger amount, maybe 10 to 30 percent of the orally ingested dose, whereas lactulose would expected to be recovered at less than 1 percent of the oral dose. The integrity of the gut barrier depends on proper functioning of the paracellular route, so if the ratio of lactulose to mannitol is higher than expected, that would be indicative of small intestine permeability. The change in the lactulose-mannitol ratio can indicate an increase in lactulose absorption via the paracellular route because of decreased phyllis height or impaired function of tight junctions, or decreased mannitol absorption due to decreased surface area of the gut villi.

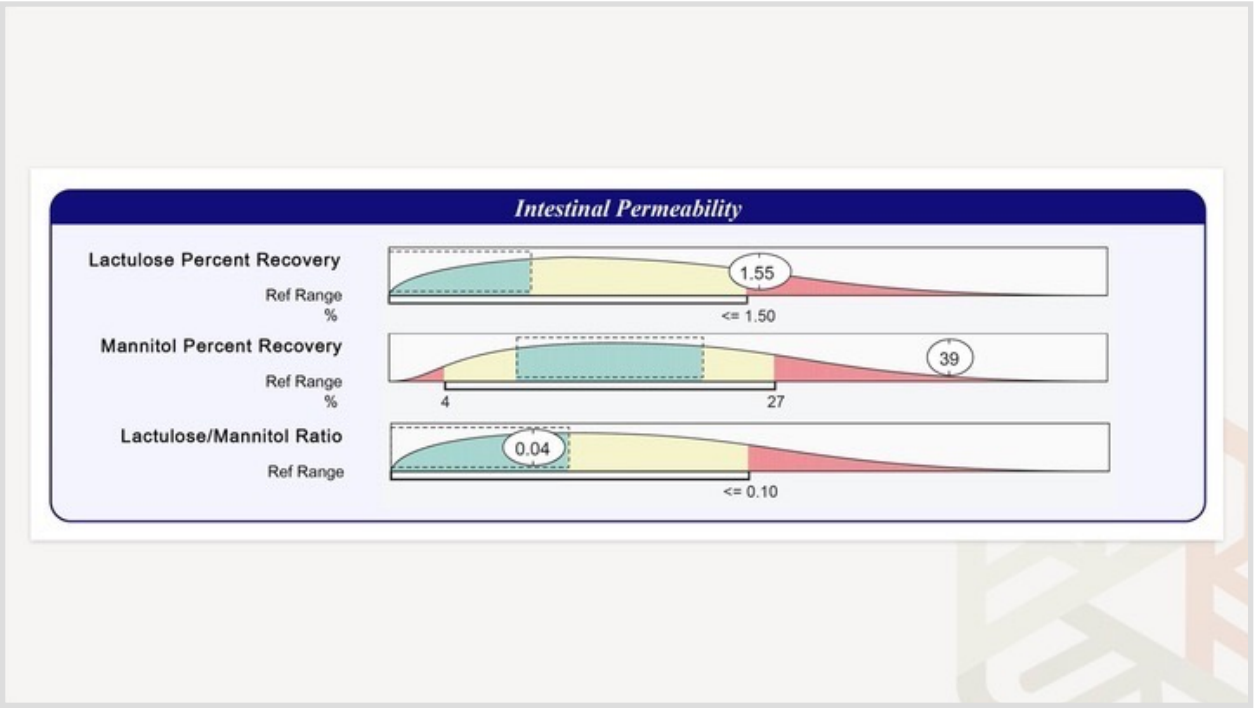


So when we do order this test, which, as I mentioned before, isn't all that often, we use Genova's Intestinal Permeability Assay, and here's a result on the slide. You can see that lactulose is borderline high and mannitol is normal. This creates a borderline lactulose-to-mannitol ratio. This patient is a 30-year-old female with interstitial cystitis and vulvodynia, and she had this test prior to starting her work with us. We identified cryptosporidium and SIBO; we treated these and then retested her intestinal permeability markers, and her results normalized. Some patients may need

additional support for restoring their gut barrier integrity after addressing the trigger markers, and we'll cover this in more detail in the treatment section, if they still have markers for intestinal permeability after you address these things.



In this result on the slide, the ratio is normal. Both lactulose and mannitol are high-normal, so I wouldn't diagnose intestinal permeability based on these results alone, but typically we'd run this together with the antigenic permeability screen, Cyrex Array 2, which we'll talk about shortly, and if that's positive, I might take additional steps to treat, and if it's normal, I wouldn't.

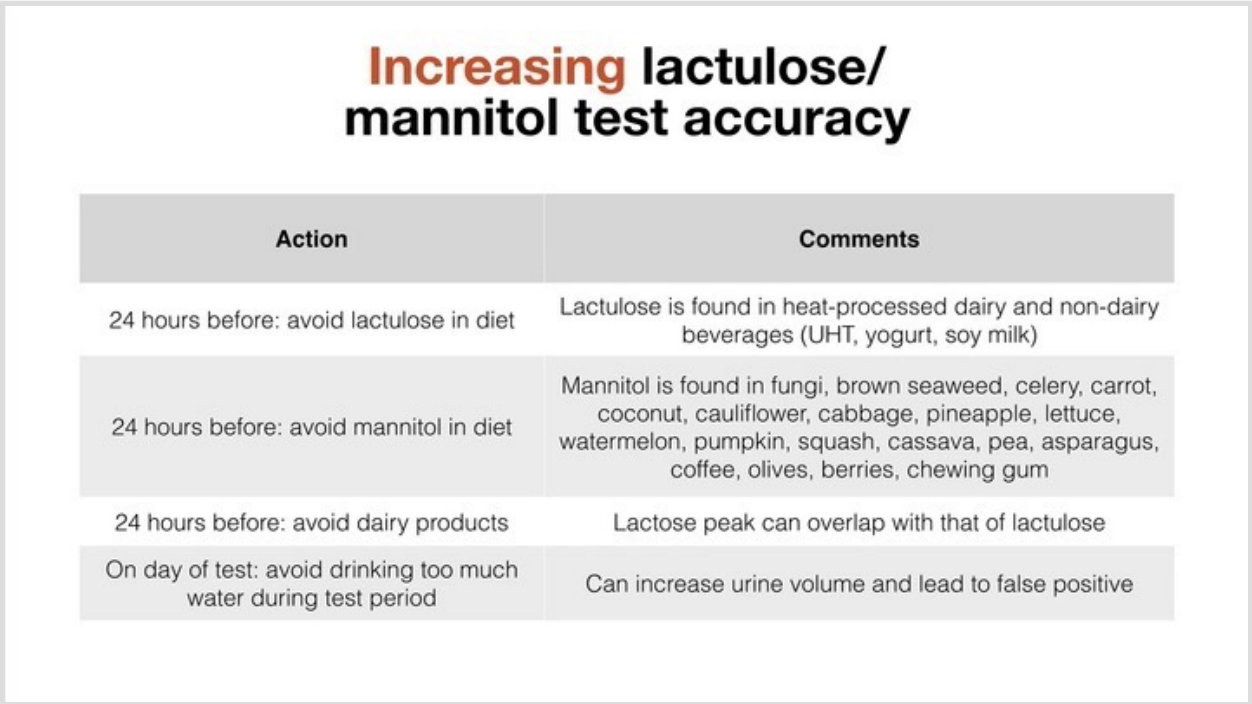


Here's a similar result on this slide; in this case, both lactulose and mannitol are high, above the reference range, so one possibility for this is metabolic dysfunction. Studies have shown that obese women have had increased lactulose and mannitol expression but a normal lactulose-to-mannitol ratio. Animal models have shown that obesity is associated with increased intestinal growth, which augments the absorption of both lactulose and mannitol, and another study showed that higher insulin levels led to increased paracellular and transcellular permeability. So in this case, we're seeing an increase in intestinal permeability, but the cause is likely to be metabolic dysfunction rather than gut pathology. So again, we always want to be thinking about underlying causes, and just because you see gut permeability doesn't necessarily mean that the problem is originating in the gut.

Another possibility, and this is one of the criticisms of lactulose-mannitol testing, is that transport of lactulose and mannitol through the barrier is not necessarily an indicator of tight junction malfunction. Many factors can influence the uptake of these sugars, including GI motility, use of medications like NSAIDS, smoking, alcohol intake, variations in gastric emptying, surface area of the intestine, mucosal blood flow, and renal clearance. Only molecules larger than 5,000 daltons can change permeability of the intestinal epithelial cells and result in T-cell response and production of cytokines and antibodies. Lactulose and mannitol are below 500 daltons, so they may not be appropriate as challenge molecules to determine pathological intestinal permeability. Studies have shown the lack of correlation between the permeation of inert sugars and macromolecules, which means just because lactulose and mannitol are getting through the gut barrier, that doesn't mean that larger macromolecules, like proteins, which would be expected to trigger immune dysfunction, are getting through. It would be better to use larger sugars that are 12,000 to 15,000 daltons that are similar in size to food proteins and endotoxins because those are the antigens of concern when it comes to intestinal permeability. These larger molecules would be

more suitable markers for measuring permeability to the antigens that we're concerned about. But that's being done right now, and lactulose and mannitol, as I said, are below 500 daltons, so that's one of the biggest issues with this kind of testing.

Another challenge with intestinal barrier testing is that barrier dysfunction may not be expressed all the time in particular conditions. It can range from mild to severe dysfunction, manifested continuously, or intermittent dysfunction, manifests only when the intestine is challenged. Susceptibility to barrier dysfunction can be detected using a challenge test with aspirin. You give 1,300 milligrams of aspirin the night before the test and again on the morning of the test, and studies using this challenge have shown that patients with non-alcoholic fatty liver disease, for example, don't have intestinal permeability all the time, but can easily develop it when they're exposed to barrier stressors like aspirin. I wouldn't recommend doing this in patients with kidney or liver disease, or anyone else who has contraindications for that dose of aspirin.



There are several other ways to increase the accuracy of lactulose-mannitol testing. Patients should avoid lactulose and mannitol in the diet for at least 24 hours before the test. The table here on the slide indicates where these things are found in the diet. Lactulose is found in heat-processed dairy and non-dairy beverages like soy milk; mannitol is found in fungi, brown seaweed, celery, carrot, coconut, cauliflower, cabbage, pineapple, lettuce, watermelon, pumpkin, squash, cassava, pea, asparagus, coffee, olives, berries, and chewing gum, so quite a few foods and things that are commonly consumed. Avoid drinking too much fluid during the test period. This can influence the lactulose-mannitol ratio and lead to a false negative result. You want to instruct patients to avoid dairy products for 24 hours prior to the test because the lactose peak can overlap with that of lactulose. And note that some sources advise patients to avoid significant alcohol consumption; use of NSAIDs like aspirin, ibuprofen, etc.; and acute stress for a

few days before the test because all of these can increase intestinal permeability. But given what we just talked about with the aspirin challenge, it might be better not to avoid those things; they could be challenges to see how the gut barrier is responding to intake of those substances, especially if the patient consumes them normally.

Lactulose mannitol test interpretation matrix

Analyte	Result	Analyte	Result	Analyte	Result	Indication
Lactulose	High	**Mannitol**	Normal	**L/M Ratio**	High	**Increased IP**
Lactulose	High	**Mannitol**	Low	**L/M Ratio**	High	**Increased IP + malabsorption**
Lactulose	High	**Mannitol**	High	**L/M Ratio**	Normal	**Increased IP d/t insulin resist.**
Lactulose	Normal	**Mannitol**	Normal	**L/M Ratio**	Normal	**Normal IP**
Lactulose	Normal	**Mannitol**	Low	**L/M Ratio**	High	**Malabsorption**
Lactulose	Normal	**Mannitol**	High	**L/M Ratio**	Low	**↑ transcellular permeability**

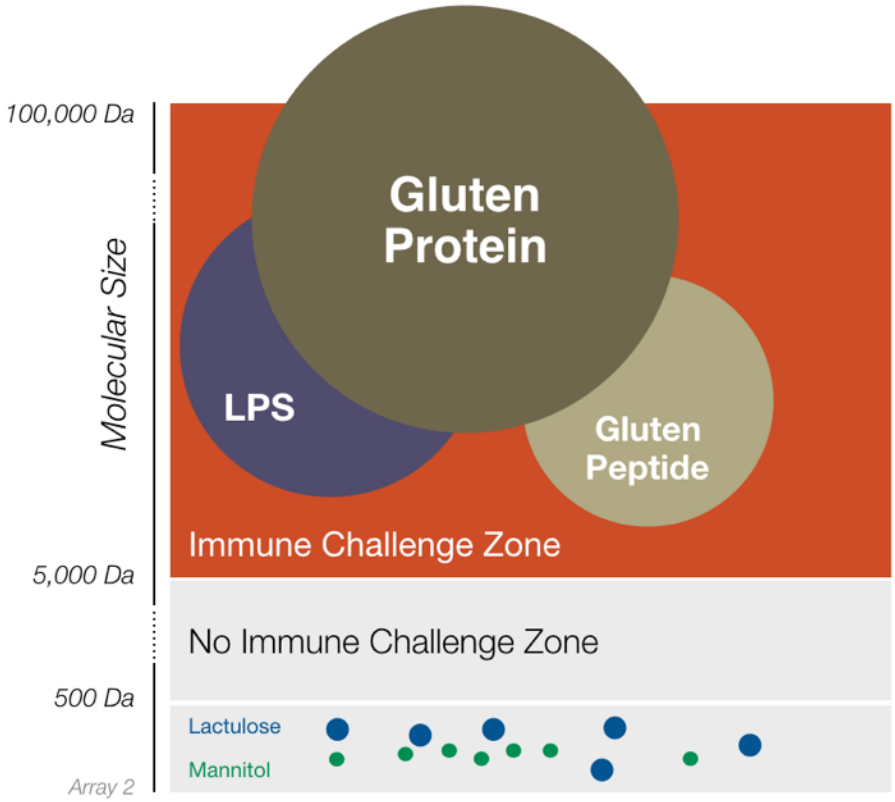
With all of this in mind, here is an interpretation matrix that I created for lactulose and mannitol testing, and it's on this slide and the next slide and we're going to make it into a handout so you can print it out and use it as a quick reference in your clinic. So, when lactulose is high and mannitol is normal, that would lead to a high lactulose-mannitol ratio and that would indicate increased intestinal permeability because more of that lactulose, which is larger, is getting through the barrier. When lactulose is high and mannitol is low, that would also lead to a high lactulose-mannitol ratio, and it would indicate both increased intestinal permeability and malabsorption because the mannitol is not getting through as it normally should. When lactulose is high and mannitol is also high, that would lead to a normal mannitol lactulose ratio, and as we discussed a few slides back, that would be increased intestinal permeability due to metabolic dysfunction or insulin resistance. When mannitol is normal and lactulose is normal, of course that's a normal lactulose-mannitol ratio and normal intestinal permeability. When lactulose is normal and mannitol is low, that would lead to a high lactulose-mannitol ratio, and it would be an indicator of malabsorption. When lactulose is normal and mannitol is high, that would lead to a low lactulose-mannitol ratio, and it would suggest an increase in transcellular permeability, because that is how mannitol, if you recall, gets through the barrier, is right directly through the cell.

Lactulose mannitol test interpretation matrix
(Cont.)

Analyte	Result	Analyte	Result	Analyte	Result	Indication
Lactulose	Low	**Mannitol**	High	**L/M Ratio**	Low	**↑ trans ↓ para permeability**
Lactulose	Low	**Mannitol**	Normal	**L/M Ratio**	Low	**↓ paracellular permeability**
Lactulose	Low	**Mannitol**	Low	**L/M Ratio**	Normal	**Malabsorption?**

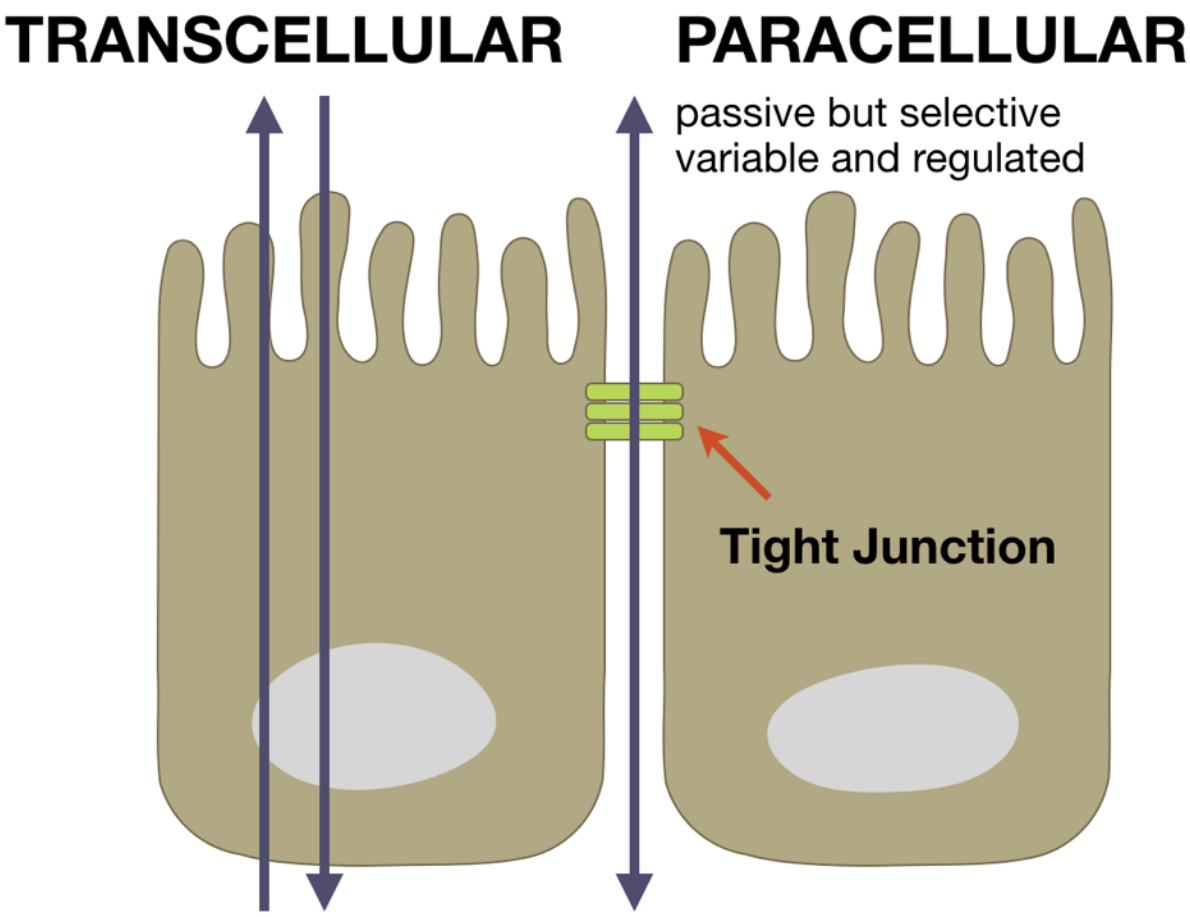
When lactulose is low and mannitol is high, that would also be a low lactulose-to-mannitol ratio and an increased transcellular permeability and a decreased paracellular permeability. When lactulose is low and mannitol is normal, it's again a low lactulose-to-mannitol ratio and it signals a decrease in paracellular permeability. And finally, when lactulose is low and mannitol is low, that would be a normal lactulose-mannitol ratio and an indicator of malabsorption.

Antigenic permeability screen



Adapted from: Cyrex Array 2 Clinical Applications Guide. http://cyrexlabs.com

Okay, the second test we'll talk about is the antigenic permeability screen. This was developed by Dr. Aristo Vajdani in Cyrex Labs, in large part because of the deficiencies of the lactulose-mannitol test that we just discussed. Since lactulose and mannitol are small molecules, they don't necessarily initiate an immune response, and Dr. Vajdani wanted to create a test that would better reflect pathological barrier permeability. So instead of using larger sugars, the 15,000-to-20,000 dalton sugars, he decided to screen for antibodies to proteins and bacterial endotoxin, since these are the major concern in terms of immune reactivity. The uptake of immunogenic molecules, aka antigens, from the lumen plays a significant role in the pathogenesis of gastrointestinal and autoimmune disease. Less than 10 percent of subjects with a genetic susceptibility to autoimmunity progressed to clinical disease in their lifetime, so this suggests that environmental triggers like toxic chemicals, infections, and dietary proteins are involved and play a significant role in the development of autoimmune disease. Cyrex Array 2 was designed to measure whether such triggers are provoking pathological intestinal permeability and leading to inflammation and disease.



The intercellular tight junctions of the intestinal epithelial barrier control the equilibrium between tolerance and immunity to non-self antigens. When the zonulin-occludin pathway is dysregulated in genetically susceptible individuals, intestinal and extraintestinal inflammatory and autoimmune disorders will occur. The intestinal tight junctions allow the passage of macromolecules from the intestine into the submucosa, and the regional lymph nodes stimulate the immune system to mount cellular and humoral immune responses against various tissues or organs, and this mechanism's been confirmed in several different autoimmune diseases, from celiac disease to type 1 diabetes to rheumatoid arthritis to multiple sclerosis. Studies have also found elevated serum zonulin levels in 70 percent of patients within an average of three-and-a-half years of onset of autoimmune disease, so to summarize, we have a lot of research showing that the zonulin-occludin pathway and the disruption in gut barrier permeability is directly related and precedes in many cases the development of autoimmune disease.