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Methionine restriction fundamentally supports health by tightening epithelial barriers

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Abstract

Dietary methionine restriction (MR) has been found to affect one of the most primary tissue-level functions of an organism: the efficiency with which the epithelial linings of major organs separate the fluid compartments that they border. This process, termed epithelial barrier function, is basic for proper function of all organs, including the lung, liver, gastrointestinal tract, reproductive tract, blood–brain barrier, and kidney. Specifically, MR has been found to modify the protein composition of tight junctional (TJ) complexes surrounding individual epithelial cells in a manner that renders the complexes less leaky. This has been observed in both a renal epithelial cell culture model and in gastrointestinal tissue. In both cases, MR increased the transepithelial electrical resistance across the epithelium, while decreasing passive leak of small non-electrolytes. However, the specific target protein modifications involved were unique to each case. Overall, this provides an example of the primary level on which MR functions to modify, and improve, an organism.

Keywords

tight junction; methionine; claudin; occludin; transepithelial; barrier function

The singular importance of epithelial barriers in higher animal life

The primary importance of epithelial barrier function and fluid compartmentation to advanced animal life is seen in both phylogeny and ontogeny. Coelenterates, among the earliest forms of animal life, are essentially intestines floating in the sea; advanced animal life, on the other hand, begins developmentally as a blastocyst. Both types of life exemplify the most basic examples of compartmentation, barrier function, and cell polarity. Advanced adult animal organisms are, in essence, parallel arrays of sacs and tubes (i.e., compartments) lined by epithelia and endothelia. Homeostasis—physiological, immunological, and bioenergetic—in these organisms is intricately tied to their various barriers' ability to keep separate the different fluid compartments that they define. Although in nearly all cases the *antiluminal* compartment is the vasculature (in series with the stroma or interstitium), the various *luminal* compartments are highly unique and reflect the specialization of the

differentiated epithelial and endothelial cells that line them. Breakdown of barriers and compartmentation, as vividly seen in multiorgan failure, typically spells doom for an organism. However, barrier-function compromise need not always be so dramatic, and the subtle compromises of barrier function present in daily living can have profound impact on health and life span.

All epithelial cells that form the mucosal linings of major—and minor—organs are polar, possessing two distinct membranes, each with their own lipid composition and integral membrane proteins. For example, Na^+K^+ -ATPase is nearly always found in the antiluminal cell membrane, whereas Na^+ channels are nearly always in the luminal membrane. Such polarization of transport proteins allows for unidirectional transport for Na^+ and many other substances. This, in turn, allows for all fluid reabsorption and electrical gradients across all of the major organs of the body. Of course, if the epithelial pavement (barrier) leaks paracellularly (Fig. 1), all transport and various gradients will be short-circuited. The disease ramifications of such leaks are myriad, and have been recently reviewed.^{1,2}

There are two components to an epithelial or endothelial barrier: the cells and the gasket-like proteinaceous strands of the tight junctions (TJs) that surround every epithelial and endothelial cell. Loss of tens or thousands of cells creates an obvious barrier leak (e.g., as in gastric ulcers). Less obvious is the leak created on the molecular level when there is aberrantly altered abundance and/or phosphorylation state of one of the numerous specific proteins of the TJ. Such leaks are clinically subtle; they do not, for example, allow blood (cells) to cross a barrier, but can be responsible for a wide range of morbidities.

The TJ prevents free interchange of luminal and antiluminal fluid constituents along the paracellular route. Antigenic substances found (e.g., in bronchial/alveolar airways or the duodenal lumen) cannot therefore freely cross into the stromal/interstitial fluid compartment and engender an inflammatory cascade. Microbial infectious agents on the skin, the surface of the colon, or the cornea cannot thereby gain access to interstitial fluid and vasculature. Concerning bioenergetics, solutes transported across a barrier do not simply leak back to the fluid compartment from which they came. Each eventuality is prevented by the TJ.

The TJ has three sets of components. The first are the actual proteins that span the cell membrane into the intercellular space and literally interknit with similar proteins from neighboring cells. These are occludin and a family of different claudins.^{3,4} The specific combinations of claudins (a family of 27 unique genes and proteins in mammals) vary from one epithelial tissue type to another, providing a cell layer with its own unique TJs and barrier properties. The second set of components are junctional-associated, intracellular linker proteins (e.g., ZO-1, ZO-2, ZO-3, cingulin, and 7H6) that connect the first set of proteins to cytoskeletal proteins, the third component.

Yet, disease agents as diverse as the enterotoxins of bacteria or proteases in feces of dust mites specifically target individual TJ proteins as a means of gaining access to a more favorable environment on the other side of the barrier (and in the process can trigger significant morbidity).^{5–7} If one were to design a physiological point at which a complex organism would be vulnerable to a chronic, repetitive, downward spiraling disease scenario,

the leaky TJ would be it. Even neurological function could be subject to impairment by compromise of the paracellular barrier separating blood from cerebrospinal fluid, as the electrolyte composition of that fluid compartment is changed.⁸ Renal insufficiency, arthritis, chronic diarrhea or constipation, ocular disorders relating to the cornea or retina, auditory disorders relating to the cochlea, prostatic hyperplasia, pulmonary disease, and specific neurological disorders—all age-related pathologies—can likely be traced, at least in part, to transepithelial and transendothelial leaks.^{9–16}

Disease states associated with barrier leak

Although more examples exist, consider the following four disease states, all of which associate with leakiness—increased passive permeability—of epithelial and endothelial barriers: (1) chronic inflammation, (2) aging, (3) cancer, and (4) various infectious diseases. Inflammation is the most instructive and perhaps the most basic, being an intrinsic part of the other three.

The inflammatory state is in large measure a creation of proinflammatory cytokines, and, among the myriad effects of this large group of proteins, few are so central as their induction of transepithelial leak, both by structural modification of TJs and by induced apoptosis of the actual cells that also form the barrier.¹⁷ Lest one think that inflammation is simply wreaking nonspecific havoc on epithelial barriers, consider that the actions of specific cytokines on epithelial TJs can be quite delicate and nuanced, with almost signature-like effects on specific TJ claudins and other proteins.^{18–20} Different types of leak result from each action (DiGuilio *et al.* unpublished data), but leak, with all of its thermodynamic (and physiological) chaos, is always the net result. The immune system—an abluminal, vasculature-based entity—when activated can lead to rapid breach of barriers and luminal compartments, which can in turn lead to a downward spiral of inflammation and leak seen in diseases such as Crohn's Disease.^{21–25}

The creeping involvement of inflammation in aging, cancer, and various infectious diseases makes it unsurprising that barrier leak is an intrinsic part of each. Although it is difficult to separate indirect and direct effects here, it can still be said that aging correlates with increased epithelial barrier leak. Protein kinase C–induced increase in barrier leak across rat distal colon has been shown to increase dramatically as a function of age.²⁶ Decreased occludin immunoreactivity has been observed in rat epididymis as a function of age, a finding that correlated with increased diffusion of lanthanum across epididymal TJs;²⁷ this decrease in immunoreactivity likewise has been shown to occur in the rat blood–brain barrier as a function of age.²⁸ Finally, the mouse alveolar epithelial barrier also has been observed to become leaky in advanced age.²⁹

Electrophysiological evidence for increased barrier leak in colon cancer has existed for many years.³⁰ So too has the finding that tumor-promoting phorbol ester chemicals, as well as primary carcinogens, can induce TJ leak.^{31,32} Likewise, cancer-causing oncogene mutations have been shown to induce altered TJ composition and epithelial barrier leak.³³ In human colon mucosa, penetration of the electron-dense dye ruthenium red appeared to increase across colonocyte TJs as neoplasia progresses from hyperplastic polyps to

adenomatous polyps to villus adenomas to true adenocarcinomas.³² The phenomenon of TJ leak in cancer, and its potential role in tumor growth and metastasis, has been recently reviewed.³⁴

Concerning infectious disease, it is fascinating that certain bacteria and viruses evolved the capability to induce TJ leak as part of their early etiology. Pathogenic microbial evolution seems to have focused heavily on penetrating epithelial barriers, as evidenced by two very different classes of pathogens evolving similar capabilities. This phenomenon has been excellently reviewed.³⁵ This acquired ability of a pathogen to cross barrier defenses is not surprising given the relative attractiveness to microbes of vascular compartments (substrate availability, temperature, etc.), compared to the relative inhospitableness of various luminal compartments. With regard to bacteria, a good example is *Clostridium perfringens*, whose secreted toxin, CPE, binds one or several TJ proteins as part of its mechanism of action.³⁶ On the viral side, there is hepatitis C virus, whose viral envelope proteins interact with claudin-1 (and possibly occludin and claudin-6 and -9) to facilitate viral entry into the cell and the vasculature.³⁷ It is tempting to speculate that nutrient-based modification of TJs can play a significant role in reducing pathogen invasion across epithelial junctions and barriers.³⁸

Countering barrier leak: micronutrients and nutrition

With nature and evolution favoring development of barriers and fluid compartmentation, intrinsic immunological mechanisms such as proinflammatory cytokines arose to compromise this organization. It is not surprising that adaptive responses evolved to counter such induction of leak. Certainly, the leak-reducing action of certain anti-inflammatory cytokines is an example,^{17,39} as is the (counterintuitive) reduction of barrier leak that epidermal growth factor can exert in certain epithelial layers.⁴⁰

But there is also a dawning realization in epithelial barrier research that certain foods—and specifically micronutrients—have a remarkable ability to either tighten barriers in the basal state and/or oppose the induction of leak in the pathophysiological state. With the exception of certain fundamental nutrients such as glutamine,⁴¹ the nutritional approaches to enhancement of TJ barrier function have focused largely on the area of micronutrients and nutraceuticals.^{42–44} Already, increasing numbers of publications have reported that trace minerals such as zinc, bioflavonoids such as quercetin, and certain unusual alkaloids such as berberine can have dramatic beneficial effects on epithelial-barrier function with very significant physiological dividends to the organism.^{45–47} In contrast, it is highly unusual to find the subtraction of a dietary component causing a major salutary effect on epithelial barrier function. Reduction of saturated fat may be one such example,²⁶ but the reduction of sulfur-containing amino acids in the diet may prove to be far more powerful, as this appears to engineer a fundamental—and positive—remodeling of TJ complexes in very different epithelial cell layers.

Reduction of methionine has a general effect of tightening epithelial barriers

Aging is both a cellular phenomenon and a tissue and organ phenomenon. The reality of cellular senescence aside, what people are affected by as they move beyond the age of 50 is a tissue- and organ-based eventuality: whether it is reduced air exchange in the lungs, vascular changes in the eyes, impaired fluid balance across the colon, or the many inflammatory-based tissue changes found in gerontological medicine, a conclusion seems to be that organs and tissues decline with advance in years.

Our research regarding effects of methionine restriction (MR) on epithelial barrier function was undertaken on the basis of an overriding hypothesis: because epithelial and endothelial barrier function is so intrinsic to healthy animal physiology, the regimens of caloric restriction, and in particular MR, would achieve life span extension, and a forestall age-related morbidity in part, because of a salutary effect on epithelial barrier function. It is on this premise that we have researched the effects of MR on epithelial barrier function in renal and gastrointestinal models. As will be discussed below, MR not only modifies TJ complexes but also achieves improved barrier function in renal and gastrointestinal models epithelial models, which highlights the medical importance of MR and the biological centrality of barrier function.

Using the well characterized renal proximal tubule-like *in vitro* model of the LLC-PK₁ epithelial cell layer, we found⁴⁹ that general restriction of sulfur-containing amino acids for 7 days successfully lowered cystine, cysteine, methionine, and taurine intracellular pools (Fig. 2), while not dramatically affecting cell viability, replication, or differentiation. The percentages shown for sulfur amino acids are relative to those present in α -modified Minimum Essential Medium.⁴⁹ The experiments used dialyzed serum so that the relative amounts of sulfur amino acids are in fact as shown; these reduced values are based on the values used in prior rodent feed studies,⁴⁸ and the amounts needed to maintain cell proliferation and differentiation (as described in Ref. 49). Associated with these reduced intracellular levels of sulfur-containing amino acids was a subtly altered renal epithelial cell layer that demonstrated increased transepithelial electrical resistance and decreased transepithelial (paracellular) leak of the non-electrolyte D-mannitol (Fig. 3).⁴⁹ These twin findings suggest that the TJ complexes between cells had been structurally modified to allow for their decreased permeability. Given that the basal state of any functional epithelial layer is already tight, it is surprising that MR could result in an epithelium that was significantly tighter. This permeability/physiological effect of MR was reflected in a compositional change to the renal epithelial TJs, with significantly decreased TJ proteins, claudin-3 and -7, while claudin-4 and -5 were elevated (Fig. 4).⁴⁹ These changes are underscored by the lack of effect on the ubiquitous TJ proteins occludin and claudin-1.⁴⁹ Thus, MR resulted in compositional/structural changes in a cell layer's TJ complexes that included tighter junctional seals and less barrier leak. These results are described in Ref. 49.

Seeing the same results in different epithelial models, but with distinct molecular actions, underscores the biological significance of MR inducing on an organism to perform at an intrinsically higher level of efficiency. When a sulfur-containing, amino acid restricted diet

was fed to Sprague Dawley rats for a period of 28 days, as described by Ramalingam *et al.*,⁵⁰ reduction of sulfur-containing amino acid pools in colonocytes was statistically significant, but much less than that observed for the cell culture studies described above. Methionine decreased by approximately 10%, taurine by approximately 30%, and cystine was unchanged. However, a similar set of changes occurred physiologically in colon epithelial barrier function compared to the renal epithelial changes described above. Specifically, transepithelial ¹⁴C-D-mannitol leak decreased across the colon mucosa (Fig. 5);⁵⁰ transepithelial electrical resistance likewise increased. It is noteworthy that, whereas in both the LLC-PK₁ renal epithelial cell layer and the rat colon model, the net effect of sulfur-containing amino acid restriction was improved barrier function, this was achieved in the colon model by modulation of different TJ proteins than those that were modified in the renal model. In LLC-PK₁ cell layers, MR produced no change in occludin and a significant decrease in claudin-3, whereas in rat colon epithelium MR⁵⁰ led to, on average, an approximately 300% increase in claudin-3 and a dramatic posttranslational—presumably phosphorylation-based—change in occludin (Fig. 6).⁵⁰ These rat colon studies are summarized in Ref. 50.

Recent research has suggested that specific claudins have specific functional roles. Claudins 1, 3, 4, 5, and 18 are often classed as sealing claudins, whereas claudins 2, 10, 16, and perhaps 7 are pore-forming claudins.^{51,52} However, it is also well understood, and discussed in the recent reviews,^{51,52} that claudin extracellular loops interdigitate in homotypic and heterotypic interactions. This means that changes in individual claudins may not necessarily be as significant as overall changes in the relative amounts of claudins to each other. One must therefore be very careful to not over-interpret the meaning of induced changes in individual claudins. The data that we show describing MR-induced changes in claudin proteins broadly shows that MR influences the composition of tight junctional complexes. The permeability data we show, both electrophysiological and radiochemical, point to claudin compositional changes as having resulted in decreased leak across the epithelial cell layer; which specific claudin changes account for this decreased leak remain to be determined. We report only changes in claudin amount and then only in detergent-soluble cell lysates. One will need to also consider the effects of MR on claudin compartmentalization within the cell, as well as posttranslational effects on the claudins, such as phosphorylation state changes, both of which are known to affect permeability.

Conclusions

The conclusion of the above discussion is that restriction of sulfur-containing amino acids leads to distinct, tissue-specific effects on epithelial cell TJ complexes, with decreased junctional leak being the common end point, despite securing such decreased permeability through different target protein changes. The surprise here is not that sulfur-containing amino acid restriction modifies TJ seals; a host of pathogens and inflammatory mediators have also achieved this. The surprise is that, unlike the action of microbial pathogens or proinflammatory proteins, the net result of the changes to the TJ complex is a tighter complex, not one with greater leak.

The net result of this particular change is an organism that functions at greater thermodynamic efficiency. Whether an epithelial layer vectorially absorbs or secretes a particular solute, there will simply be less backleak involved as a result of MR. Such backleak would give rise to a physiological futile cycle that simply burns ATP with no useful work performed. In addition, there would be a decreased background level of inflammation in various tissues in the organism, because there would be decreased leak of immunogenically active molecules from tissue luminal compartments into the interstitial fluid and vasculature. The role that either outcome would play in extending life span is a matter for future research. But the positive role that each would play in an organism's systemic physiology and background level of inflammation seems obvious.

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References

1. Mullin JM, et al. 2005; Keynote review: epithelial and endothelial barriers in human disease. *Drug Discov Today*. 10:395–408. [PubMed: 15808819]
2. Sawada N, et al. 2003; Tight junctions and human diseases. *Med Electron Microsc.* 36:147–156. [PubMed: 14505058]
3. Tsukita S, Furuse M. 2000; The structure and function of claudins, cell adhesion molecules at tight junctions. *Ann N Y Acad Sci*. 915:129–135. [PubMed: 11193568]
4. Gonzalez-Mariscal L, et al. 2003; Tight junction proteins. *Prog Biophys Mol Biol*. 81:1–44. [PubMed: 12475568]
5. Wan H, et al. 2001; The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy*. 31:279–294. [PubMed: 11251630]
6. Balkovetz DF, Katz J. 2003; Bacterial invasion by a paracellular route: divide and conquer. *Microbes Infect*. 5:613–619. [PubMed: 12787737]
7. Berkes J, et al. 2003; Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut*. 52:439–451. [PubMed: 12584232]
8. Plumb J, et al. 2002; Abnormal endothelial tight junctions in active lesions and normal-appearing white matter in multiple sclerosis. *Brain Pathol*. 12:154–169. [PubMed: 11958369]
9. Simon DB, et al. 1999; Paracellin-1, a renal tight junction protein required for paracellular Mg²⁺ resorption. *Science*. 285:103–106. [PubMed: 10390358]
10. Kitajiri SI, et al. 2004; Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. *Hear Res*. 187:25–34. [PubMed: 14698084]
11. Gow A, et al. 2004; Deafness in Claudin 11-null mice reveals the critical contribution of basal cell tight junctions to stria vascularis function. *J Neurosci*. 24:7051–7062. [PubMed: 15306639]
12. Huber JD, Egleton RD, Davis TP. 2001; Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci*. 24:719–725. [PubMed: 11718877]
13. Clayburgh DR, Shen L, Turner JR. 2004; A porous defense: the leaky epithelial barrier in intestinal disease. *Lab Invest*. 84:282–291. [PubMed: 14767487]
14. Giebel SJ, et al. 2005; Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. *Lab Invest*. 85:597–607. [PubMed: 15711567]
15. Han X, et al. 2004; Increased iNOS activity is essential for pulmonary epithelial tight junction dysfunction in endotoxemic mice. *Am J Physiol Lung Cell Mol Physiol*. 286:L259–267. [PubMed: 12896879]

16. Zhu JX, et al. 2004; Improvement of barrier function and stimulation of colonic epithelial anion secretion by Menoease Pills. *World J Gastroenterol.* 10:2514–2518. [PubMed: 15300895]
17. Al-Sadi R, Boivin M, Ma T. 2009; Mechanism of cytokine modulation of epithelial tight junction barrier. *Front Biosci.* 14:2765–2778.
18. Watson CJ, et al. 2005; Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J Cell Sci.* 118:5221–5230. [PubMed: 16249235]
19. Adams RB, Planchon SM, Roche JK. 1993; IFN-gamma modulation of epithelial barrier function. Time course, reversibility, and site of cytokine binding. *J Immunol.* 150:2356–2363. [PubMed: 8450217]
20. Mullin JM, et al. 1997; Different size limitations for increased transepithelial paracellular solute flux across phorbol ester and tumor necrosis factor-treated epithelial cell sheets. *J Cell Physiol.* 171:226–233. [PubMed: 9130471]
21. Mankertz J, Schulzke JD. 2007; Altered permeability in inflammatory bowel disease: pathophysiology and clinical implications. *Curr Opin Gastroenterol.* 23:379–383. [PubMed: 17545772]
22. Zeissig S, et al. 2007; Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut.* 56:61–72. [PubMed: 16822808]
23. Bruewer M, Samarin S, Nusrat A. 2006; Inflammatory bowel disease and the apical junctional complex. *Ann N Y Acad Sci.* :242–252.
24. Fakhoury M, et al. 2014; Inflammatory bowel disease: clinical aspects and treatments. *J Inflamm Res.* 7:113–120. [PubMed: 25075198]
25. Hollander D, et al. 1986; Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann Intern Med.* 105:883–885. [PubMed: 3777713]
26. Mullin JM, et al. 2002; Age- and diet-related increase in transepithelial colon permeability of Fischer 344 rats. *Dig Dis Sci.* 47:2262–2270. [PubMed: 12395899]
27. Levy S, Robaire B. 1999; Segment-specific changes with age in the expression of junctional proteins and the permeability of the blood-epididymis barrier in rats. *Biol Reprod.* 60:1392–1401. [PubMed: 10330098]
28. Mooradian AD, Haas MJ, Chehade JM. 2003; Age-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1). *Mech Ageing Dev.* 124:143–146. [PubMed: 12633933]
29. Tankersley CG, et al. 2003; Changes in lung permeability and lung mechanics accompany homeostatic instability in senescent mice. *J Appl Physiol* (1985). 95:1681–1687. [PubMed: 12794030]
30. Davies RJ, et al. 1989; Detection of the cancer-prone colon, using transepithelial impedance analysis. *Arch Surg.* 124:480–484. [PubMed: 2930357]
31. Mullin JM, O'Brien TG. 1986; Effects of tumor promoters on LLC-PK1 renal epithelial tight junctions and transepithelial fluxes. *Am J Physiol.* 251:C597–602. [PubMed: 3464217]
32. Soler AP, et al. 1999; Increased tight junctional permeability is associated with the development of colon cancer. *Carcinogenesis.* 20:1425–1431. [PubMed: 10426787]
33. Mullin JM, et al. 2005; Ras mutation impairs epithelial barrier function to a wide range of nonelectrolytes. *Mol Biol Cell.* 16:5538–5550. [PubMed: 16176977]
34. Martin TA, Jiang WG. 2009; Loss of tight junction barrier function and its role in cancer metastasis. *Biochim Biophys Acta.* 1788:872–891. [PubMed: 19059202]
35. Guttman JA, Finlay BB. 2009; Tight junctions as targets of infectious agents. *Biochim Biophys Acta.* 1788:832–841. [PubMed: 19059200]
36. Fujita K, et al. 2000; Clostridium perfringens enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein. *FEBS Lett.* 476:258–261. [PubMed: 10913624]
37. Krieger SE, et al. Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by neutralization of E2-CD81-claudin-1 associations. *Hepatology.* 51:1144–1157.

38. Mullin JM, Raines J, Livornese LL Jr. Retrofitting the battlements: tight junction remodeling as a novel antimicrobial approach. *Future Med Chem.* 7:9–13. [PubMed: 25582329]
39. Madsen KL, et al. 1997; Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion. *Gastroenterology.* 113:151–159. [PubMed: 9207273]
40. Soler AP, Laughlin KV, Mullin JM. 1993; Effects of epidermal growth factor versus phorbol ester on kidney epithelial (LLC-PK1) tight junction permeability and cell division. *Exp Cell Res.* 207:398–406. [PubMed: 7688317]
41. Marc Rhoads J, Wu G. 2009; Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids.* 37:111–122. [PubMed: 19130170]
42. Amasheh M, et al. 2009; Barrier effects of nutritional factors. *Ann N Y Acad Sci.*
43. Shimizu M. 2010; Interaction between food substances and the intestinal epithelium. *Biosci Biotechnol Biochem.* 74:232–241. [PubMed: 20139625]
44. Suzuki T, Hara H. 2011; Role of flavonoids in intestinal tight junction regulation. *J Nutr Biochem.* 22:401–408. [PubMed: 21167699]
45. Amasheh M, et al. 2008; Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. *J Nutr.* 138:1067–1073. [PubMed: 18492835]
46. Mercado J, et al. 2013; Enhancement of tight junctional barrier function by micronutrients: compound-specific effects on permeability and claudin composition. *PLoS One.* 8:2013.
47. Valenzano MM, DiGuilio KM, Mullin JM. 2015; Comparative Effects of the Micronutrients Zinc, Quercetin, Berberine, Indole and Butyrate on CACO-2 Barrier Function and Tight Junction Composition. *PlosOne.* 10:e0133926.
48. Orentreich N, et al. 1993; Low methionine ingestion by rats extends life span. *J Nutr.* 123:269–274. [PubMed: 8429371]
49. Skrovanek S, Valenzano MC, Mullin JM. 2007; Restriction of sulfur-containing amino acids alters claudin composition and improves tight junction barrier function. *Am J Physiol Regul Integr Comp Physiol.* 293:R1046–1055. [PubMed: 17567720]
50. Ramalingam A, et al. Dietary methionine restriction improves colon tight junction barrier function and alters claudin expression pattern. *Am J Physiol Cell Physiol.* 299:C1028–1035.
51. Tamura A, Tsukita S. Paracellular barrier and channel functions of TJ claudins in organizing biological systems: advances in the field of barriology revealed in knockout mice. *Semin Cell Dev Biol.* 36:177–185.
52. Gunzel D, Yu AS. Claudins and the modulation of tight junction permeability. *Physiol Rev.* 93:525–569.

Figure 1. Diagrammatic cross section of a simple epithelial barrier. Reproduced with permission from Ref. 49.

Figure 2.

Intracellular sulfur-containing amino acid levels decline sharply in sulfur-containing, amino acid–restricted culture medium. LLC-PK₁ cell layers were placed under either control (100% cysteine/100% cystine/100% methionine) (red bar) or sulfur-containing amino acid–restricted (50% cysteine/0% cystine/10% methionine) (blue bar) conditions for 1 week before being harvested and amino acid pools analyzed. Reproduced with permission from Ref. 49.

Effect of Restricting Sulfur-Containing Amino Acids
on Transepithelial Mannitol Flux

Figure 3.

Sulfur-containing, amino acid–restricted cell sheets exhibit a significant increase in transepithelial electrical resistance and a significant decrease in transepithelial ^{14}C -mannitol flux. LLC-PK $_1$ cell layers were kept on control (100% cysteine/100% cystine/100% methionine) (red bar) or sulfur-containing, amino acid–restricted (50% cysteine/0% cystine/10% methionine) (blue bar) conditions for 1 week before measuring transepithelial electrical resistance and transepithelial mannitol leak. Data shown represent the mean $\pm$ standard error of $n = 9$ cell sheets. Significance was determined by Student's t -test, assuming equal variances. Reproduced with permission from Ref. 49.

Figure 4.

Western immunoblots of integral tight junction proteins in sulfur-containing, amino acid-restricted (left) versus control (right) conditions. LLC-PK₁ cell layers were maintained at confluence in either control (100% cysteine/100% cystine/100% methionine) or sulfur-containing amino acid-restricted conditions (50% cysteine/0% cystine/10% methionine) for one week, then harvested as described.⁴⁹ Immunoblots shown and the percentage increase/decrease represents the mean ± standard error, and was calculated by densitometry of the appropriate bands of 4 separate polyacrylamide gel electrophoresis and western immunoblots of the membrane fraction of cells. Significance was determined using Student's *t*-test, assuming equal variances. Reproduced with permission from Ref. 49.

Figure 5.

MR diet affects TJ barrier function. ^{14}C -mannitol flux (pmole/min/cm²) across distal colon tissues from rats on control and MR diets for 28 days ($n = 5$ tissues per group). Values shown represent mean $\pm$ standard error. $P < 0.05$, Student's t -test, two-tailed. Reproduced with permission from Ref. 50.

Figure 6.

MR of the diet of Sprague-Dawley rats alters mRNA expression and protein abundance of certain tight junction components. (A) RT-PCR of specific tight junction protein mRNA in the distal colon of animals on control and methionine-restricted diet ($n = 3$). (B). Western blot analysis showing the steady state level of tight junction proteins (top panel: occludin; middle panel: claudin-3) in the distal colon of animals on control and MR diet (numbers indicate animal pairs). Sample loading was normalized to β -tubulin levels (bottom panel). Reproduced with permission from Ref. 50.