Methionine Restriction and Modification of Epithelial Tight Junction Barrier Function and Permeability

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Abstract: It is well known that caloric restriction leads to an increased longevity by forestalling age-related diseases. Dietary restriction of methionine also renders similar benefits. In this review, we report studies on the effect of methionine restriction on epithelial barrier function in a renal epithelial LLC-PK$_1$ cell, a rodent gastrointestinal model and a clinical trial using Crohn's patients. In LLC-PK$_1$ cell culture, a reduction of culture medium methionine by 80% resulted in altered tight junctional claudin composition with improved epithelial barrier function as exemplified by increased trans-epithelial electrical resistance and decreased paracellular leak to ^{14}C-*D*-mannitol. In addition to small but significant reductions in plasma and intracellular colonocyte methionine levels, dietary methionine restriction tightens intestinal epithelium and reduces mannitol permeability in rat colonic, but not ileal tissue. Crohn's patients on methionine-restricted diet reported improved symptoms as demonstrated by a reduced CDAI index, but this diet was not able to significantly reduce the plasma methionine level in patients recruited. An increased urinary lactulose/mannitol ratio was also the result of the regimen in Crohn's patients, suggesting an increased, rather than decreased ileal leakage. Overall, our results showed that reduction in dietary intake of methionine is promising in improving at least some epithelial barriers and this may be through altering tight junctional protein compositions. Methionine restriction might result in contrasting effects depending on the tissue type and possible disease conditions, thus wider range of studies are required.

Keywords: Methionine, tight junction, LLC-PK$_1$, Crohn's disease, claudin, occludin, amino acid, colon, ileum, kidney.

METHIONINE RESTRICTION IN AGING AND CANCER STUDIES

Clive McCay and colleagues reported in 1935 that the life span of rats increased by decreasing their food intake [1], making it more than 70 years since caloric restriction (CR) or dietary restriction was found to consistently increase longevity in a range of animal models [2-4]. Although its modes of action are still under investigation [2], a similar effect can be achieved in rodents through a diet low in the essential amino acid, methionine (Met) (with an elimination of related sulfur-containing amino acids, cysteine and cystine) [6-8]. In a recent study using fruit fly *Drosophila melanogaster*, the addition of Met to a CR diet reduced life span and this reduction in life span could be avoided by simply removing Met from the diet [3]. Both nutritional interventions, CR and methionine restriction (MR), do not only simply extend life span, but also forestall the onset of a variety of age-related diseases, such as cancers, obesity, diabetes, hypercholesterolemia and more [3, 4, 6, 9-11]. Since the etiology of many types of diseases involves collapse of epithelial and/or endothelial barriers [4], it is reasonable to speculate that either CR or MR, at least in part, exert their benefits through strengthening barrier functions. In this review, we are going to focus on MR and its effects on renal and intestinal barriers.

In addition to its role in protein synthesis, Met is critical in generating *S*-adenosylmethionine (SAM), the major methyl donor for a variety of biologically important reactions. After donating its methyl group, SAM is converted to *S*-adenosylhomocysteine which is in turn hydrolyzed to form homocysteine. Homocysteine can either be re-methylated to Met, or enter the trans-sulfuration pathway to form cysteine and then produce an important intracellular anti-oxidant, glutathione. Unlike normal cells, the maintenance of cancer cells is much more reliant on Met, a pivotal nutrient for tumor growth, as transformed cells are relatively incapable of transforming compensating homocysteine into Met [5]. This observation has lead to several studies focusing on the synergistic effect of MR and traditional cancer therapies through either dietary restriction of Met or by administration of methioninase (L-methionine-α-deamino-γ-lyase) [14, 15]. A number of studies on cell cultures using Met-restricted media demonstrated that MR suppresses tumor cell growth, while little harmful effect was observed on normal cells [6-8]. Similarly, a tumor inhibition effect was also observed in animal models *in vivo*. In a rat colon cancer model, for example, MR has been shown to inhibit the formation of pre-neoplastic aberrant crypt foci after the administration of the carcinogen azoxymethane [9]. Data from a phase I clinical trial conducted on 8 patients with metastatic solid tumors by Epner and colleagues indicated for the first time that diet with a reduced Met content is safe and feasible to treat patients with cancer, despite a conclusive lack of sufficient anti-tumor activity [10, 11]. A Met-free diet was used in con-

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junction with other anti-cancer agents in another two Phase I clinical trials [12, 13]. Both regimens were found to be relatively feasible and well tolerated, one of which resulted in partial response to the therapy and disease stabilization among 4 evaluated patients [13]. Despite the small patient number, these trials demonstrated for the first time in humans that MR has promise for enhancing the efficacy of chemotherapy.

In addition to its synergistic effect in chemotherapy of original tumors, MR has also demonstrated its ability in decreasing the motility and invasiveness of existing cancer cells [6, 14, 15]. Studies using murine or human melanoma cells showed that MR suppresses attachment and motility of melanoma cells by the inhibition of specific integrin expression, in turn through integrin-mediated focal adhesion kinase (FAK) phosphorylation [6]. In prostate cancer cells, MR not only induced apoptosis, but also reduced the invasiveness of two different prostate cell lines [16]. This effect seemed to be via a FAK/extracellular-regulated kinase (ERK)-dependent pathway [16] and Raf/Akt survival pathway [17]. MR also modulates g-protein activity and the relative abundance and phosphorylation of the motility proteins cofilin and profilin [14]. Since the effect of Akt on cell survival requires glucose metabolism, the latest research from the same group revealed that MR-induced cell death depends on alterations of glucose metabolism [18].

The underlying mechanism by which MR exerts its effects on either aging or cancers still needs to be resolved. However some specific metabolic sensors may be involved in response to the concentration changes of circulating and/or tissue levels of Met and its various metabolites. Many of those sensors are extremely well conserved in evolution and often act autonomously within the cell. They possibly include the target of rapamycin (TOR) [19], AMP-activated protein kinase (AMPK) [20], and the "anti-aging" sirtuin proteins (SIT2 or SIRT1 in mammals specifically) [21, 22]. They detect specific metabolic changes in amino acids, AMP and NAD^+, respectively. These sensors are sometimes tightly intertwined [23], making pathway identification of MR or CR mechanisms more intricate. Instead of regulating a large number of downstream sensory and signaling genes individually to achieve longevity, research has shown that SAM could modulate those genes in general by binding to riboswitches and/or affecting DNA methylation/polyamine synthesis [24].

THE EFFECT OF METHIONINE RESTRICTION ON EPITHELIAL TIGHT JUNCTIONS

Despite the lack of exact mechanism, the positive effects of MR in mammalian biology and physiology are apparent. We propose that such benefits may in part involve the improvement of the overall epithelial barrier effectiveness, by making the barriers less affected by environmental and microbial stress and challenges. To this end, MR effects on the epithelial tight junction (TJ) apparatus are a pivotal component that deserves attention.

Depending on the specific organ, epithelial tissue of any kind either separates the external environment from the bloodstream (such as skin, cornea and nasal mucosae), or separates luminal internal fluids from the bloodstream, such

as in the gastrointestinal (GI) and urinary tracts. These barriers are not impermeable however. For example, xenobiotics including bacterial toxins can gain entry into the inner (vascular) compartment through either transcellular or paracellular routes. Tight junctions (or zonula occludens) are positioned at the apical end of the epithelial lateral intercellular space, serving as a selectively permeable barrier governing the degree of paracellular leakiness [25, 26]. Disease often results in leaky epithelial barriers, allowing abnormally increased paracellular permeability of substances [4]. This makes discovering methods that can improve barrier function very clinically desirable. The following sections summarize and discuss our studies on MR in modulating TJs and epithelial barriers in cell culture, animal models and a small clinical trial.

MR EFFECTS ON THE LLC-PK₁ RENAL EPITHELIUM

Attempting to investigate the effect of MR diet on epithelial TJ barriers, we began by mimicking the MR diet used in longevity studies *in vivo* for use with the LLC-PK₁ renal epithelial cell line *in vitro*. A Met-free, cysteine-free and cystine-free alpha-modified "base" MEM medium was used with 10% dialyzed fetal bovine serum (to eliminate any sulfur-containing amino acids contributed from the serum) [27]. A final formulation of 0.285 mM cysteine (50%), 0 mM cystine, and 0.01 mM Met (10% of normal alpha-MEM levels) was able to allow normal cell division, polarization and differentiation. This was exemplified by the presence of lumen negative, 1 mV potential differences, apical microvilli and Na^+-dependent sugar transport, hallmark features of LLC-PK₁ epithelium [28]. Vectorial salt and fluid transport was evident in post-confluent LLC-PK₁ epithelium cultured with the modified MR medium (see the domes in Fig. **1**), with minor differences in morphology compared to cells in control medium.

Continuous TJs were confirmed by immunoflourescent staining of ZO-1, an important TJ-associated protein, in both media (Fig. **2**). The cell number per unit was also counted using this method and equal cell number per unit area was calculated under both conditions. This suggests an equivalent liner junctional density in both Met-restricted and control media. Based on this, the significantly higher transepithelial electrical resistance (R_t) that was observed in confluent LLC-PK₁ epithelium in MR medium (Fig. **3A**) is not due to a junctional density difference. Furthermore, the considerably tighter epithelium grown in MR medium was accompanied by an unchanged short-circuit current (I_{scc}). This indicates that the transepithelial Na^+ transport activity at both apical and basolateral side of the cell sheet was not altered by a reduced Met level in the cell culture medium (Fig. **3B**). Apical-to-basolateral flux of ^{14}C-*D*-mannitol, a paracellular transport marker, was also measured to generate further support for an MR-induced change in TJ permeability (Fig. **3C**). A nearly 75% reduction was observed in the transepithelial mannitol diffusion rate in the MR condition implying an appreciable decrease in the leakiness of the LLC-PK₁ TJs to non-electrolytes.

To further confirm that the increased R_t and decreased mannitol permeability seen in Met restricted LLC-PK₁ cells is due to modified TJs, a few TJ protein abundance assays
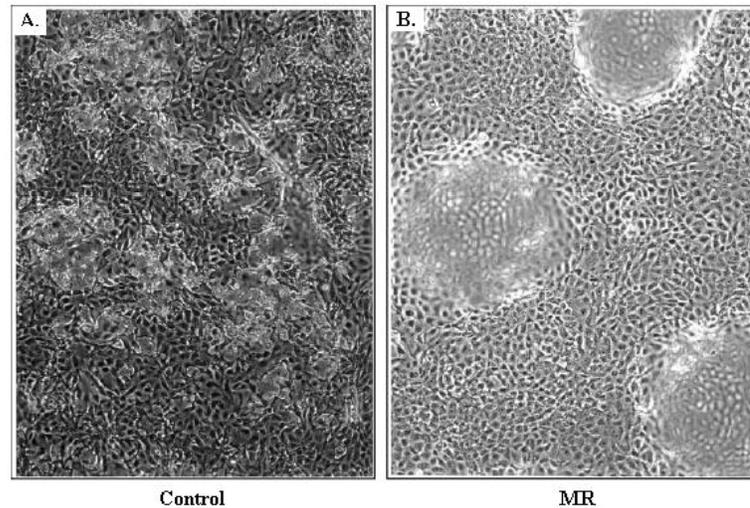
Fig. (1). Phase contrast micrograph of the one week postconfluent cultures of control vs. MR conditions. Cells were seeded into 75 cm^2 tissue culture flasks and allowed to reach confluence (one week). Flasks were than re-fed in either control (100% cycsteine/ 100% cystine/100% Met) or Met-restricted (50% cycsteine/0% cystine/10% Met) media on the day of confluence, again 4 days later and again at one week postconfluence. Results are typical and show the three-dimensional, fluid-filled domes characteristic of confluent LLC-PK$_1$ cultures, with the domes being larger in MR medium **(B)**. The control condition **(A)** resembles a typical one week postconfluent LLC-PK$_1$ cell sheet with numerous but very small domes; the MR condition appears more like a typical freshly confluent LLC-PK$_1$ cell sheet. Since in LLC-PK$_1$, doming is indicative of a healthy, polarized cell sheet that is actively and transepithelially transporting ions. This photograph suggests that MR medium did not have negative impact on the basic functions of LLC-PK$_1$ cells as transporting epithelia.
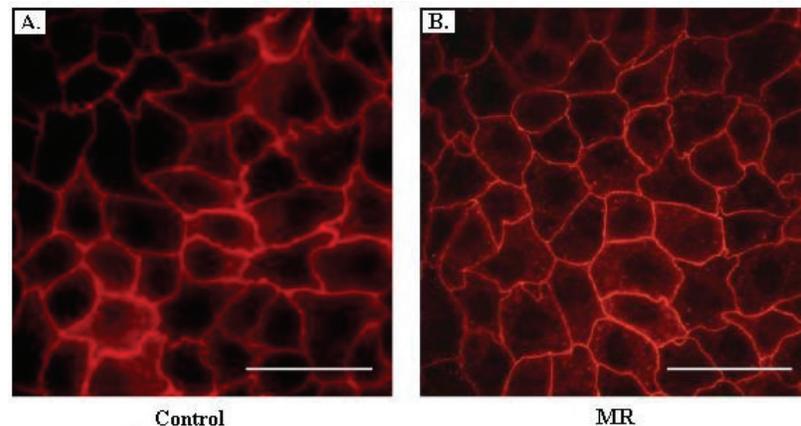

Fig. (2). Immunoflourescence micrographs of LLC-PK$_1$ cell sheets fed with control (A) vs. MR (B) medium. Cells were seeded at confluent density on permeable (0.4 μm pore size) Millipore PCF filter ring assemblies and after 24 hours kept on control or MR media for one week. Filters were then excised from the rings and stained with Cy3-labeled 2° antibody against the 1° antibody for the junction-specific protein, ZO-1. Scale bar = 20 μm.

were conducted. Western immunoblots on transmembrane TJ proteins occludin and claudins were chosen because they are key components of the actual physiological barrier function of the TJ [29, 30]. As shown in Fig. (**4**), MR did not significantly change the abundance of total occludin or claudin-1 or -2, but it did induce noticeable decreases in the abundance of claudin-3 and -7 in LLC-PK$_1$ epithelium, and significant increases in the abundance of claudin-4 and -5. Although 1) the changes in remaining claudin proteins and any phosphorylation status of these proteins are still to be examined, and 2) so far there is no definitive link between particular TJ protein changes and permeability changes, a clear pattern could still be drawn that MR is changing the structural components of the TJ barrier and the permeability of the barrier. These changes are one of extremely few interventions that are known to improve, rather than compromise, TJ barrier function [31].

MR EFFECTS ON RAT SMALL INTESTINE AND COLON

In order to extend our previous renal epithelial cell culture studies on restricted Met to a more medically relevant and a less reductionist system, we chose to follow the LLC-PK$_1$ study with a rat model to investigate whether MR also enhances epithelial barrier properties in gastrointestinal tissue.

Similar to the diet used by Richie *et al.,* (2004) in their longevity studies, a diet with protein (casein) content
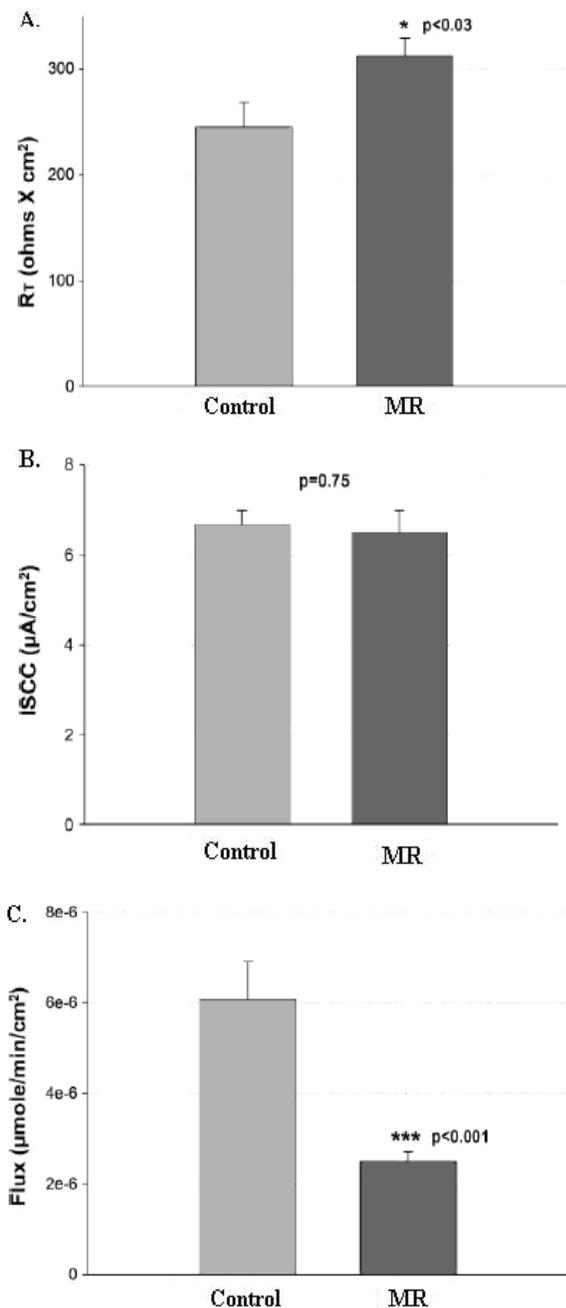
Fig. (3). MR medium-treated LLC-PK₁ cell sheets exhibit a significant increase in R_t (A), a comparable I_{scc} (B), and a significant decrease in transepithelial ^{14}C-D-mannitol flux (C), as compared to controls. Cells were seeded at confluent density onto permeable (0.4 μm pore size) Millipore PCF filter ring assemblies and after 24 hours kept on control or MR media for one week before potential difference (PD) and I_{scc} readings were taken using Ag/AgCl electrodes in series with NaCl agar bridges and a modified Ussing chamber set-up, respectively. Ohm's law was used to calculate R_t. The radiotracer probe, ^{14}C-mannitol, was put in the dish media basolaterally and allowed to incubate for 3 hours, during which time samples were taken from the ring media apically. Flux was calculated using linear regression of cpm vs. time. Data shown represent the mean ± standard error of the mean (SEM), n=9 cell sheets. * and *** denote p<0.05 and p<0.001 significances, respectively, as tested by Student's *t*-test, assuming equal variances.

replaced by an essential amino acid mixture was used [32]. The MR diet contained 20% of the Met that is present in the control diet. For the same reason as in the cell culture study, other (non-essential) sulfur-containing amino acids (cysteine and cystine) were removed from MR diet. Rats (starting weight of ~400g and 7-8 weeks old) in both groups had free access to food and water. The reduction of the overall nitrogen content of the diet as result of MR was compensated by a minor increase in glutamate level [33].
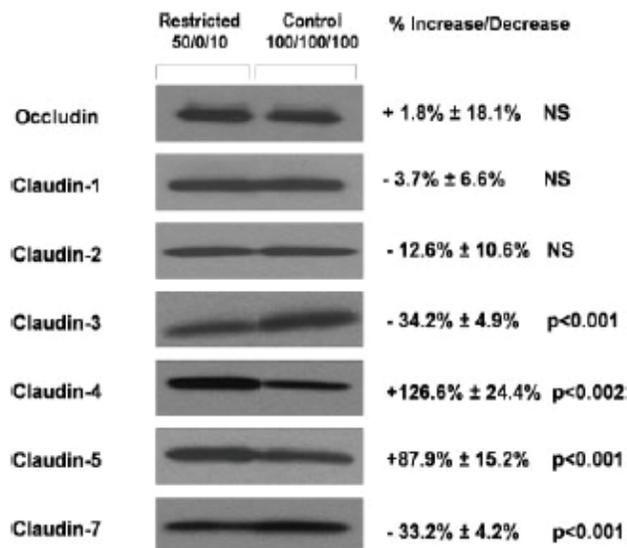

Fig. (4). Western immunoblots of integral TJ proteins in control and MR-treated LLC-PK₁ cell sheets. Cell sheets in the same condition as described in Fig.1 were scraped and processed for Western blots. Results shown are from the membrane fraction (Triton-soluble) and representative of n=4 runs using four different cell passages. The percentage changes represent the mean ± SEM and were calculated by densitometry of the appropriate bands of the four separate PAGE and Western immunoblots. Significance was determined using Student's *t*-test, assuming equal variances.

Based on the observed appearance and activity level, rats in both groups appeared healthy throughout the experimental period (from 3-4 days to up to 9 weeks) [34]. While rats on control chow gained weight consistently, those on MR diet lost about 10% of their initial weight for the first 2-3 weeks before subsequently beginning to gain weight. However the weight was maintained at approximately 93% of the starting weight by the end of the 9-week study (Fig. **5**). Although the initial weight dip has never been reported by others, previous studies did indicate that chronic dietary MR reduces the deposition of adipose tissue [35, 36]. A recent study by Hasek *et al.,* (2010) also showed a significant decrease in body weight and adiposity of MR rats relative to the control group after the initial 2 weeks [37]. The authors speculated that this is possibly due to a metabolic inefficiency with higher maintenance requirements induced by MR diet. In the same report, researchers also documented that when MR diet was applied to rats after physical maturity (6 month of age), they did not increase body weight during the 26-week long study, maintaining 96% of their initial weight.

With the exception of glucose and BUN (blood urea nitrogen) levels, all plasma electrolytes and non-electrolytes
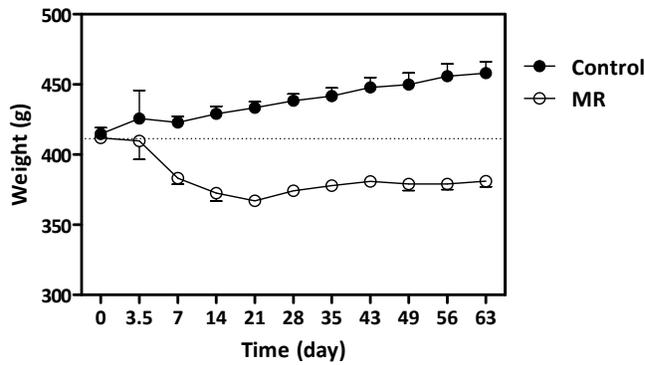
Fig. (5). Body weight of animals on control and MR diets. Data spans 9 weeks of the study and is reported as mean ± SEM.

tested in MR rats including serum albumin, total protein content, serum creatinine and prealbumin (a sign of a significant deviation from normal food intake), remained at comparable levels to those in the control group. Arguably these changes in blood analyses and initial weight loss are not considered to be unhealthy.

The primary result of the study is that restricted dietary Met intake improved rat colonic epithelial barrier function with an onset as early as 2 weeks on MR diet and maintained at least for up to 9 weeks. We observed significant increases in the R_t of distal colon tissue sheets from rats fed on MR diet compared to tissue from rats on control diet (Fig. **6A**). This was accompanied by a significant reduction in the transepithelial flux of ^{14}C-D-mannitol across distal colon in MR rats (Fig. **6B**). Both parameters imply a decrease in colonic paracellular leak and an improvement in the TJ barrier function. Similar to what was observed in the LLC-PK$_1$ study, no significant changes in colon I_{scc} were noted. This suggests a comparable Na^+ reabsorption or Cl^- secretion from the colonocytes and that the difference seen in transepithelial R_t was in fact the result of a difference in the TJ permeability, rather than altered transcellular permeability.

In addition to changes in TJ permeability, there are several other factors that may in part be responsible for the enhanced barrier function observed in rats on MR diet. The morphological architecture and cell proliferation of rat colon tissues were therefore evaluated by hematoxylin & eosin staining and a semiquantitative Ki-67 staining, respectively. The gross histology, as well as the architecture of the crypts (i.e. length and frequency), and the general thickness of the colonic mucosa was similar in both control and MR groups. In both groups, the Ki-67 labeled cells were located in the appropriate compartments within the epithelium in a similar fashion, most densely right above the base of the crypts, indicating similar cell proliferation between the groups. There was no difference in the rate of apoptosis, or the observed mitotic frequency across the groups either. Overall, MR diet did not seem to alter the morphology or cell kinetics of the rat colonic mucosa.

We did see a moderate but significant decrease in the plasma and intracellular colonocyte Met levels in MR, which could possibly lead to a decreased degree of methylation of macromolecules including DNA. Since DNA methylation is one of the important epigenetic mechanisms known to alter
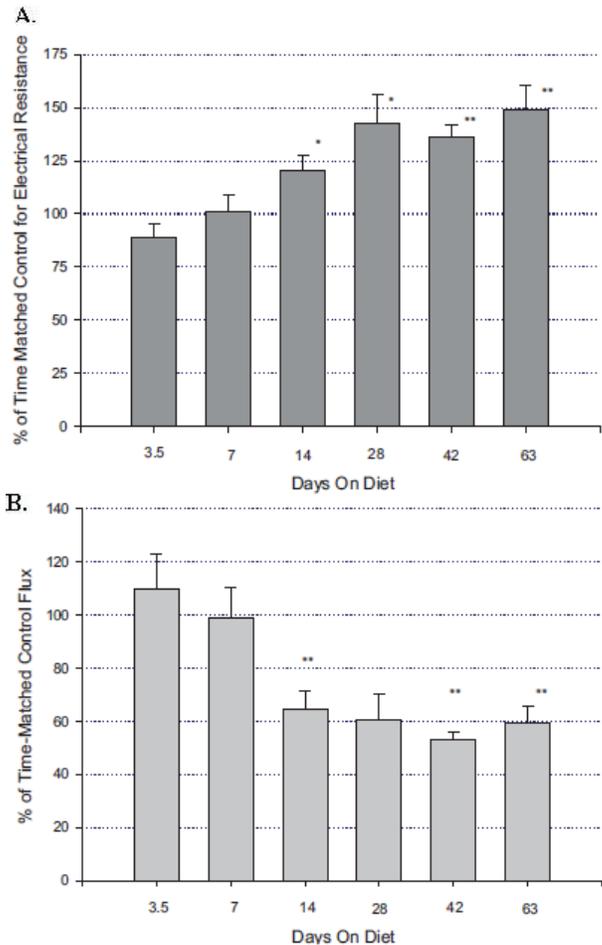

Fig. (6). Time course of MR-induced changes in rat colon epithelial barrier functions: (A) the relative change of R_t compared to time-matched controls, and (B) transepithelial mannitol flux across the same tissues. Error bars indicate mean ± SEM (n=6 to 12 separate time-matched tissues). * and *** denote p<0.05 and p<0.001 significances, respectively, as tested by Student's *t*-test, assuming equal variances.

gene expression [38, 39], future work by our group will focus on the linkage between MR-related DNA methylation and TJ modifications.

Using RT-PCR technique, dietary MR was confirmed to affect the transcription of certain TJ genes. An 80% increase in claudin-3 mRNA was seen in rat colonic mucosal scrapes from rats on MR diet, while no significant change in occludin expression was noted (Fig. **7A**). Western blot analysis also revealed an increase in claudin-3 protein abundance in MR compared to the control diet group (Fig. **7B**). There was no significant change in claudin-1 or -7. Unlike LLC-PK$_1$ cells, claudin-2, -4, -5 and -6 were however not detectable in rat distal colon scrapes. Despite no change in occludin abundance, there is an additional occludin protein band, 3-5 kDa above the original occludin band, which suggests a possible post-translational modification of occludin protein by MR diet.

In summary, for the first time in an animal model, we demonstrated that restricted intake of Met improved colonic
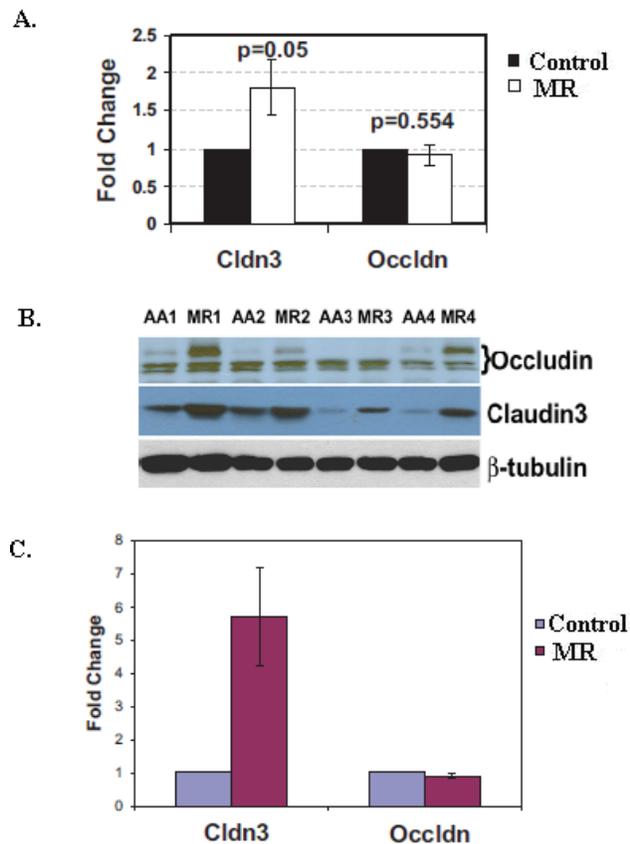
A.

B.

C.

Fig. (7). Methionine restriction alters mRNA expression and protein abundance of certain TJ proteins in the distal colon of rats on control vs. MR diet. (A). RT-PCR of CLDN3 and OCLN expression as normalized with GAPDH value. Error bars indicate mean ± SEM (n=3 tissues); **(B).** Western blots analysis showing the steady-state level of TJ proteins (labels indicate the appropriate matched animal pairs) and **(C).** quantitation of claudin-3 and occludin levels as normalized with β-tubulin levels in each lane (mean ± SEM, n=4 tissues).

epithelial barrier function. It is worth pointing out that restricted Met intake may have different impact on other tissues and organs, depending on their specific TJs and TJ regulation pathways. For example, in parallel to the rat colon study, separate rat studies under the same control and MR conditions were carried out for 3 weeks to investigate the effect of MR on rat ileal tissue. In contrast to what was observed in rat colon, there was no significant change in R_t, I_{scc}, or transepithelial flux values of tracers of two differences molecular weights (^{14}C-*D*-mannitol of 182 Da and ^{3}H-*D*-lactulose of 342 Da) across distal ileal mucosal sheets. This suggests that dietary MR-induced barrier improvement might be tissue specific, thus each organ should be treated and studied differently.

MR EFFECTS ON CLINICAL PATIENTS WITH CROHN'S DISEASE

The intestinal barrier in patients with Crohn's disease is characterized by radiolabeled tracer leakage and lower electrical resistance and/or impedance, which in part can apparently be traced back to alterations in claudin proteins [40,

41]. In light of the ability of dietary MR to enhance epithelial barriers in both cell culture and rat models, a clinical study aiming to improve compromised GI barrier in patients with Crohn's disease was proposed and carried out. The 2-year study recruited 24 adult Crohn's disease patients, 17 of whom completed the study. Subjects in the trial were required to be on a 4-week meat- and fish-free diet with very sparing dairy, designed to lower dietary intake of Met by over 60%. Low nitrogen intake as a result of the overall low protein consumption was corrected by supplementation with twice daily Hominex®-2, a Met-free, amino acid-based nutrient formula produced by Abbott Labs. A small cohort of 5 patients was also randomized to take a sham diet with white meat and unrestricted dairy intake.

Crohn's disease activity indices (CDAIs) were computed for all patients at 3 different times: before the diet, at the end of the diet, and 4 weeks after resuming a normal diet. This score is a research tool utilized to quantify the symptoms of patients with Crohn's disease, and is considered to be the gold standard clinically for assessing Crohn's disease activity [42]. Results revealed a statistical decrease of the score (from 128 ± 13 to 106 ± 11) (P < 0.02), showing a symptomatic improvement while on the diet. However, this could have been due to the elemental nature of a diet supplemented with Hominex®-2, rather than a decreased Met level. It should also be noted that the near vegan nature of the diet involved a voluntary increase in plant protein intake, and hence roughage, which may not have been salutary, given these patients' condition with a chronic inflamed gut. Patients on the sham diet (without red meat, but allowing chicken, fish and dairy) showed no significant change in CDAI scores before vs. end of the diet.

In contrast to what was seen in cell culture and rat study, amino acid analysis of plasma Met revealed a merely 10% non-significant reduction at the end of the 4-week diet as compared to their pre-diet level. The lack of an effect of the MR diet on plasma Met concentration suggests that either the dietary MR was not stringent enough, and /or the cellular concentrative Met transport systems can overcome this level of decreased intake, and/or the human body can hydrolyze labile protein stores, possibly in the liver and kidney, to make up Met intake deficit. With this being said, we did observe a small (12%) but significant decrease in plasma Met seen in a previous trial with 5 healthy control subjects on the same dietary MR plan. In neither group did we observe a significant change in body weight while on the diet. In comparison, an approximately 40% decrease of plasma Met was previously reported on the first day of a Met-free regimen, which was a more stringent, formula-based Met-free diet [12].

A significant (17%) decrease in serum homocysteine was however observed among Crohn's patients in our trial. A recent report studying sulfur amino acids in MR rats using a similar diet to the rat chow that was used in our studies revealed a near 150% increase in serum homocysteine level [43]. It was suspected that there occurred a possible MR-induced inhibition of homocysteine trans-sulfuration due to the MR diet. This discrepancy could be due to a species difference, and/or the fact that laboratory rats are more compliant to the MR diet, and/or an improved vitamin B

intake with the Hominex®-2 supplement to the Crohn's patients who may normally have impaired ileal vitamin B absorption.

The unchanged plasma Met concentration was accompanied by an insignificant, yet 20% increase of urinary lactulose/mannitol (L/M) ratio, which was due to an increased lactulose leak in over 60% of patients with mannitol leak staying constant. This may relate to Crohn's patients, having a chronically inflamed GI tract in need of supra-normal rates of tissue regeneration and repair. Despite an unaltered plasma Met level, it is still possible that the intracellular enterocyte Met level was affected. If this was the case the MR diet could pose a contraindication for their condition by decreasing rates of protein synthesis and cell restitution.

Most of our patients enrolled in the study had only mild-moderate disease, with roughly half receiving TNF immunomodulatory medications. Overall, Crohn's patients and healthy controls, on either MR or sham diet, tolerated the regimen and managed their disease well throughout the trial period.

SUMMARY

With evidence more than ever revealing the actions of disease pathogens and mediators on TJs, sculpturing a more resilient barrier by minimizing junctional leakiness may prove to be an exceedingly beneficial way to prevent and/or treat certain diseases. Dietary modifications, such as MR appear to be one of the modalities that show promise in offsetting disease-introduced barrier leakiness. In our studies using cell culture and animal models, we demonstrated that dietary MR can indeed remodel TJs and improve barrier function. Implementing these findings clinically will clearly require many further studies focusing on dietary implementation, target-tissues, and targeted disease states. In addition, a thorough insight into the molecular mechanism of MR is also needed. Such understanding may then lead to more practical dietary approaches to barrier enhancement than MR itself.

CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

CR   =   Caloric restriction

GI   =   Gastrointestinal

I_{scc} =   Short-circuit current

Met   =   Methionine

MR   =   Methionine restriction

R_t =   Transepithelial electrical resistance

SAM   =   *S*-adenosylmethionine

TJ   =   Tight junction

ACKNOWLEDGEMENT

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