

Response to reviewer's comments

We thank the reviewer for their comments and have altered the manuscript accordingly so as to make it more readable and accurate.

Major

1. "Tight Junctions" should be the first section in the review, followed by "Epithelial Barrier Function in IBD"

We have changed the order of these sections accordingly

2. The sentence "Water, small ions and low molecular weight molecule typically use paracellular routes..." is not an accurate statement and should be modified. Ion channels and transporters play a very important role in ion and nutrient transport. Also there should be a mention of the difference between tight and leaky epithelia.

We agree that the use of the word "typically" here is unclear and we have altered this section to enhance clarity on this issue:

Ions and solutes pass transcellularly utilising channels, carriers and transporting ATPases<sup>[1]</sup>. The paracellular pathway also regulates permeability to water, ions and low molecular weight molecules (<600kDa). The differences in paracellular permeability between different epithelia such as the proximal or distal nephron, has led to the concept of "leaky" or "tight" epithelia. In disease states, epithelia may become more "tight" or more "leaky" such as is seen in inflammatory bowel diseases <sup>[1]</sup>.

3. The sentence: "The majority of tight junctions are cation selective" is another inaccurate generalization. .(Check: Tight junction, selective permeability, and related diseases, Susanne M. Krug, Jörg D. Schulzke, Michael Fromm and a review by J R Turner: The role of Molecular Remodeling in Differential Regulation of Tight junction Permeability). Please modify

We have modified this statement accordingly.

TJs may be size and/ or charge selective and prevent contact between the proteins of the two cell poles: the basolateral and apical cell membranes<sup>[10]</sup>. While many tight junction proteins have properties of increased barrier formation, others form size and/or charge selective channels or pores<sup>[1]</sup>.

4. “Chicken or Egg?” section should have subheadings. It is very difficult to read and follow. The sections: “Tight Junctions” and “Therapeutic Opportunities” could also greatly benefit from subheadings.

We have added subheadings to enhance readability

5. Therapeutic Opportunities: The paragraph: “Short chain fatty acids...” , it is not clear to the reader how this is therapeutic. Please add a sentence about the expected source of butyrate or SCFA in the diet and a note about the effect of claudin 2 downregulation.

We have clarified this and added a sentence regarding the expected source of butyrate

In the colon, anaerobic bacterial fermentation of undigested polysaccharides leads to the production of short chain fatty acids, particularly acetic, proprionic and butyric acids..... Butyrate might be postulated to have a role in maintaining barrier function via tight junction regulation.

Minor

1. Novel “therapies” that alter .... The word “therapies” should be replaced by “compounds”

We have changed the word “therapies” to “compounds”