EXPRESSION OF TIGHT JUNCTION PROTEINS IN DISEASES WITH COMPROMISED INTESTINAL BARRIER FUNCTION

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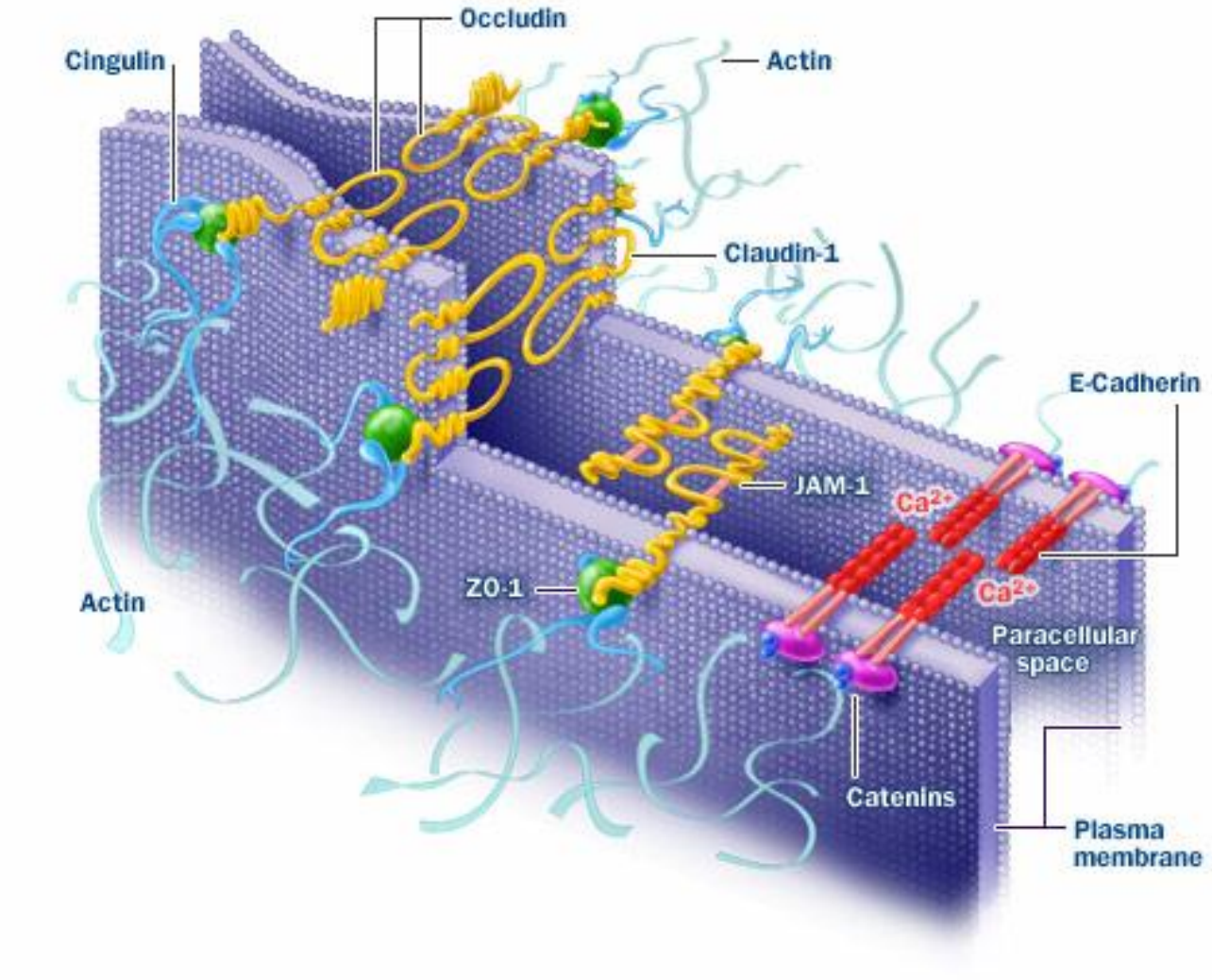
ABSTRACT

Background. The relationship between the increased intestinal permeability observed in several acute and chronic diseases and the expression of tight junctions (TJ) proteins is not well established. Aim aim was to investigate the expression at transcriptional level of 3 transmembrane TJ proteins, Occludin, Claudin-1, Claudin-2 and of 2 scaffold TJ proteins, zonula occludens-1 (ZO-1) and Myosin 9B in intestinal biopsies from patients affected by celiac disease (CD), Crohn's disease (CrD), peptic ulcer disease (PUD) and type 1 diabetes (IDDM). Methods. Samples of small intestinal mucosa were taken from the second/third portion of the duodenum from all the subjects studied. After total RNA extraction and cDNA synthesis, quantitative real time polymerase chain reaction assays with SYBR Green was performed. Data were normalized by using 18S rRNA as standard.
Results. are shown in the Table. A significant down-regulation of all 5 genes studied was observed in patients affected by celiac disease before gluten-free diet, while no significant change was observed in IDDM patients. Conclusions. The down-regulation of TJ transmembrane or/and intracellular components observed in all but IDDM might explain the increased intestinal permeability reported in these pathological conditions. The meaning of TJs disruption in the pathogenesis of each of this pathological condition warrants further investigation. The normal transcriptional level observed in celiacs on GFD suggests that the down-regulation of TJs in CD is reversible and secondary to gliadin exposure rather than to a genetic predisposition.

	Cldn-1	Cldn-2	Occl	ZO-1	Myo9B
Healthy Controls (N=5)	1	1	1	1	1
CD (N=5)	↓125 fold **	↓ 2000 fold *	↓9 fold ***	↓ 100 fold**	↓ 1000 fold **
CD on GFD (N=2)	↓1.3 fold	↓3.3 fold	↓2.1 fold	↓1.6 fold	↓1.1 fold
IDDM (N=5)	↑2.1 fold	↓1.6 fold	↑1.1 fold	↑1.1 fold	↑2.1 fold
Crohn's disease (N=2)	↓25 fold	↓143 fold	↓25 fold	↓2.5 fold	↓ 400 fold
Peptic ulcer (N=2)	↓ 1000 fold	↓ 9000 fold	↓4 fold	↓ 9000 fold	↓20 fold

BACKGROUND



Intestinal epithelial tight junctions (TJ) play a crucial role in regulating the paracellular pathway.

As the relationship between the increased intestinal permeability observed in several acute and chronic diseases and the expression of TJ-associated proteins is not well established,

AIM of THE STUDY

was to investigate the expression at transcriptional level of:
3 transmembrane TJ proteins:
Occludin, Claudin-1, Claudin-2
2 scaffold TJ proteins:
zonula occludens-1 (ZO-1), Myosin 9B

in intestinal biopsies from patients affected by peptic ulcer disease, celiac disease (CD), Crohn's disease, insulin dependent diabetes mellitus (IDDM)

METHODS

HUMAN INTESTINAL TISSUE:

Samples of small-intestine mucosa were taken from the second/third portion of the duodenum from subjects undergoing upper gastrointestinal (GI) endoscopy.

Patients included:

1. 5 subjects with active CD at diagnosis
2. 2 subjects with CD on gluten-free diet from at least two years
3. 2 subjects with Crohn's disease
4. 5 subjects with IDDM
5. 2 subjects with peptic ulcer disease
6. 5 healthy controls

METHODS: Quantitative polymerase chain reaction

Total RNA was extracted using TRizol RNA purification protocol. RNA concentration was read at 260 nm by spectrophotometer (Beckman coulter DU530, UV/vis). The ratio 260/280 was determined for each sample.

cDNA synthesis. Two micrograms of total RNA were reverse transcribed with the High-Capacity cDNA Archive Kit (Applied Biosystem, Foster city, CA, USA).

Quantitative polymerase chain reaction with the TaqMan procedure. This reaction was performed with the SYBR Green PCR Master Mix (Applied Biosystems, manufactured by Roche, NJ, USA) and was run on an Applied Biosystems 7500 Fast Real-Time PCR System. All reactions were performed in duplicate; the ROX reference dye was applied in every reaction. Data were normalized by using 18S rRNA as standard. mRNA-specific primers were designed for the following human genes:

1. occludin (*OCC*)
2. claudin-1 (*CLDN-1*)
3. claudin-2 (*CLDN-2*)
4. zonula occludens 1 (*ZO-1*)
5. myosin 9-beta (*MYO IXB*)

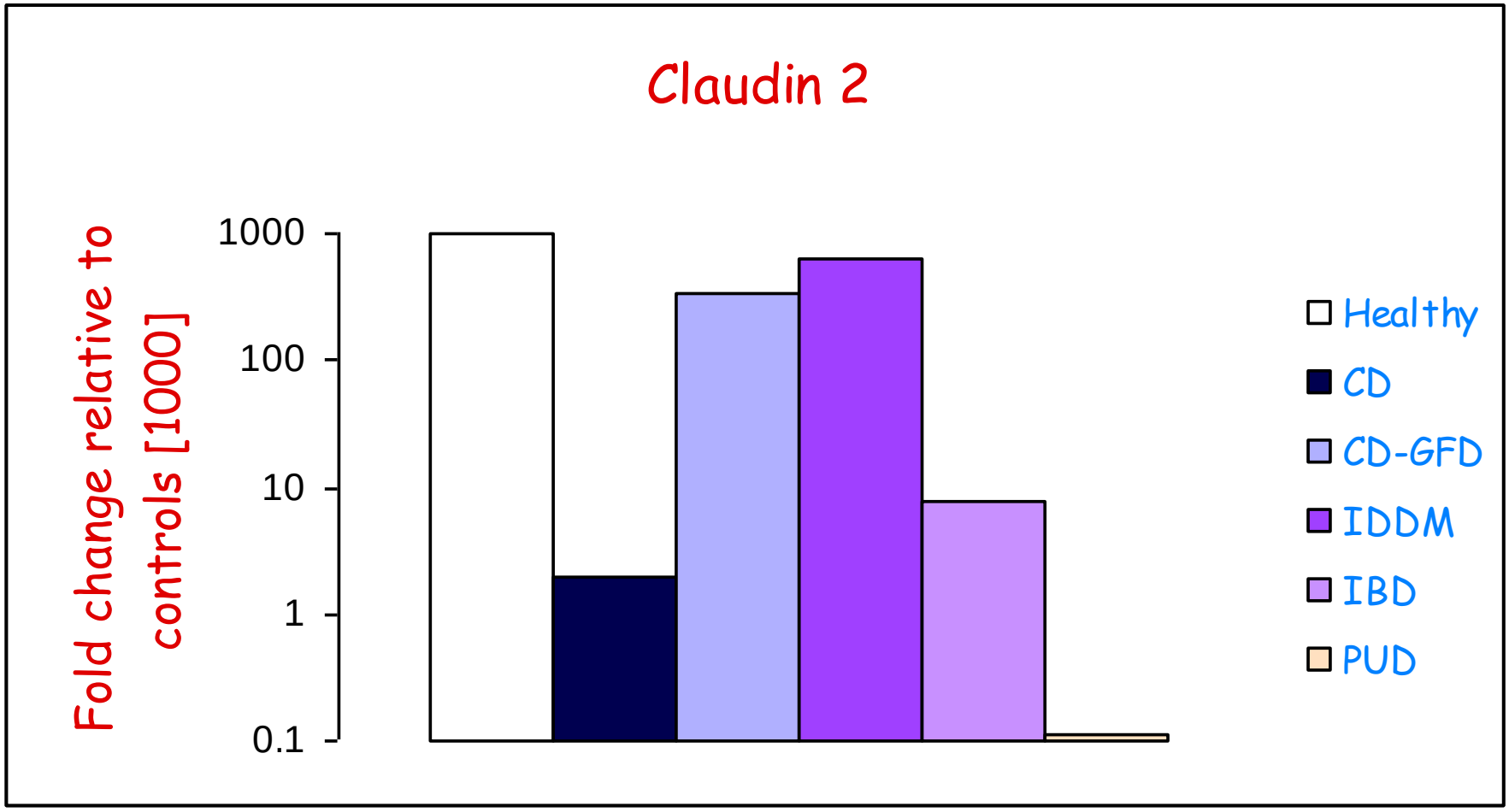
RESULTS

A significant down-regulation of all 5 genes studied was observed in patients affected by celiac disease before gluten-free diet, while no significant change was observed in IDDM patients

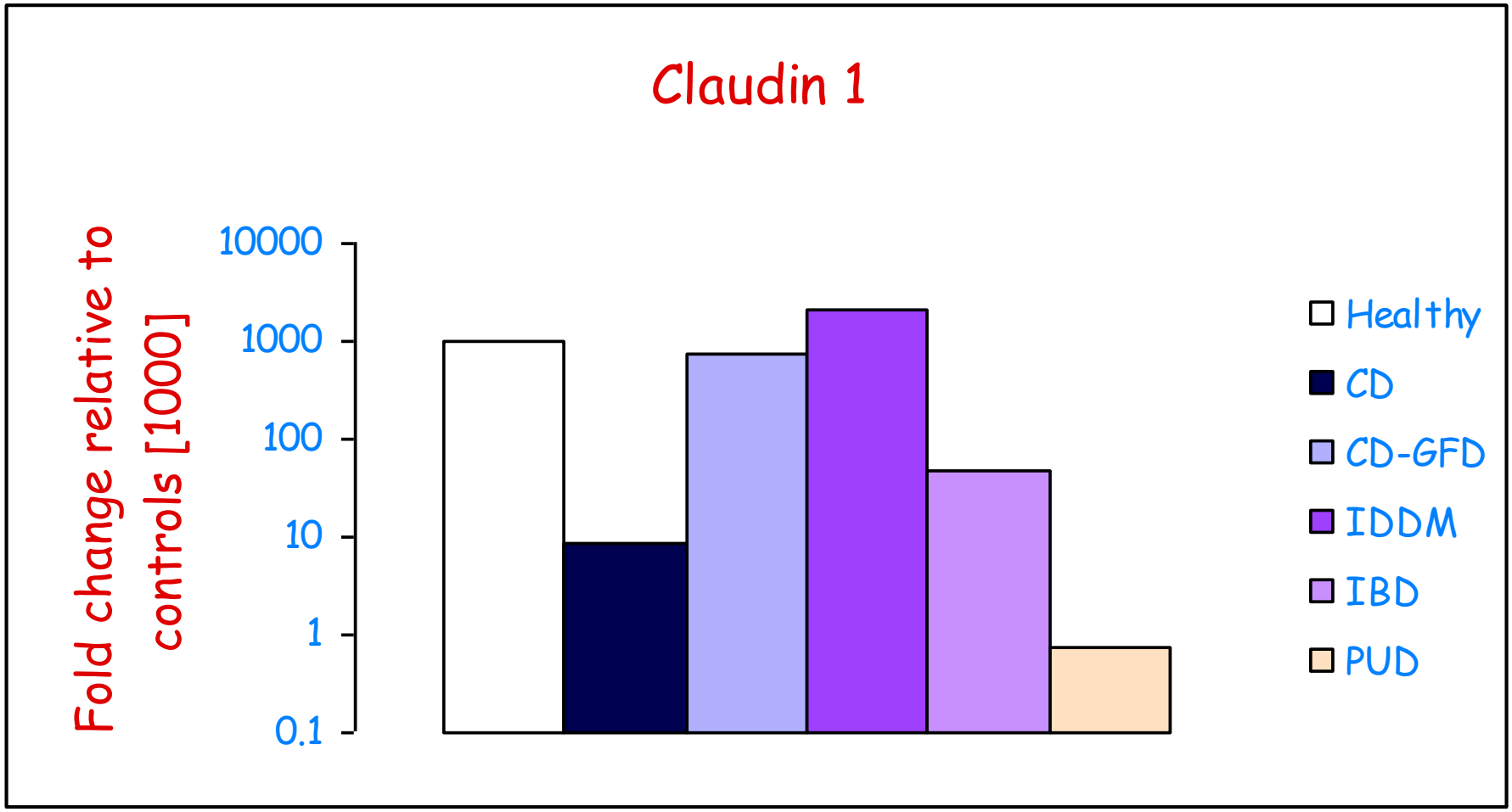
	Cldn-1	Cldn-2	Occl	ZO-1	Myo9B
Healthy Controls (N=5)	1	1	1	1	1
CD (N=5)	↓125 fold **	↓ 2000 fold *	↓9 fold ***	↓ 100 fold**	↓ 1000 fold **
CD on GFD (N=2)	↓1.3 fold	↓3.3 fold	↓2.1 fold	↓1.6 fold	↓1.1 fold
IDDM (N=5)	–2.1 fold	↓1.6 fold	–1.1 fold	–1.1 fold	–2.1 fold
Crohn's disease (N=2)	↓25 fold	↓143 fold	↓25 fold	↓2.5 fold	↓ 400 fold
Peptic ulcer (N=2)	↓ 1000 fold	↓ 9000 fold	↓4 fold	↓ 9000 fold	↓20 fold

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

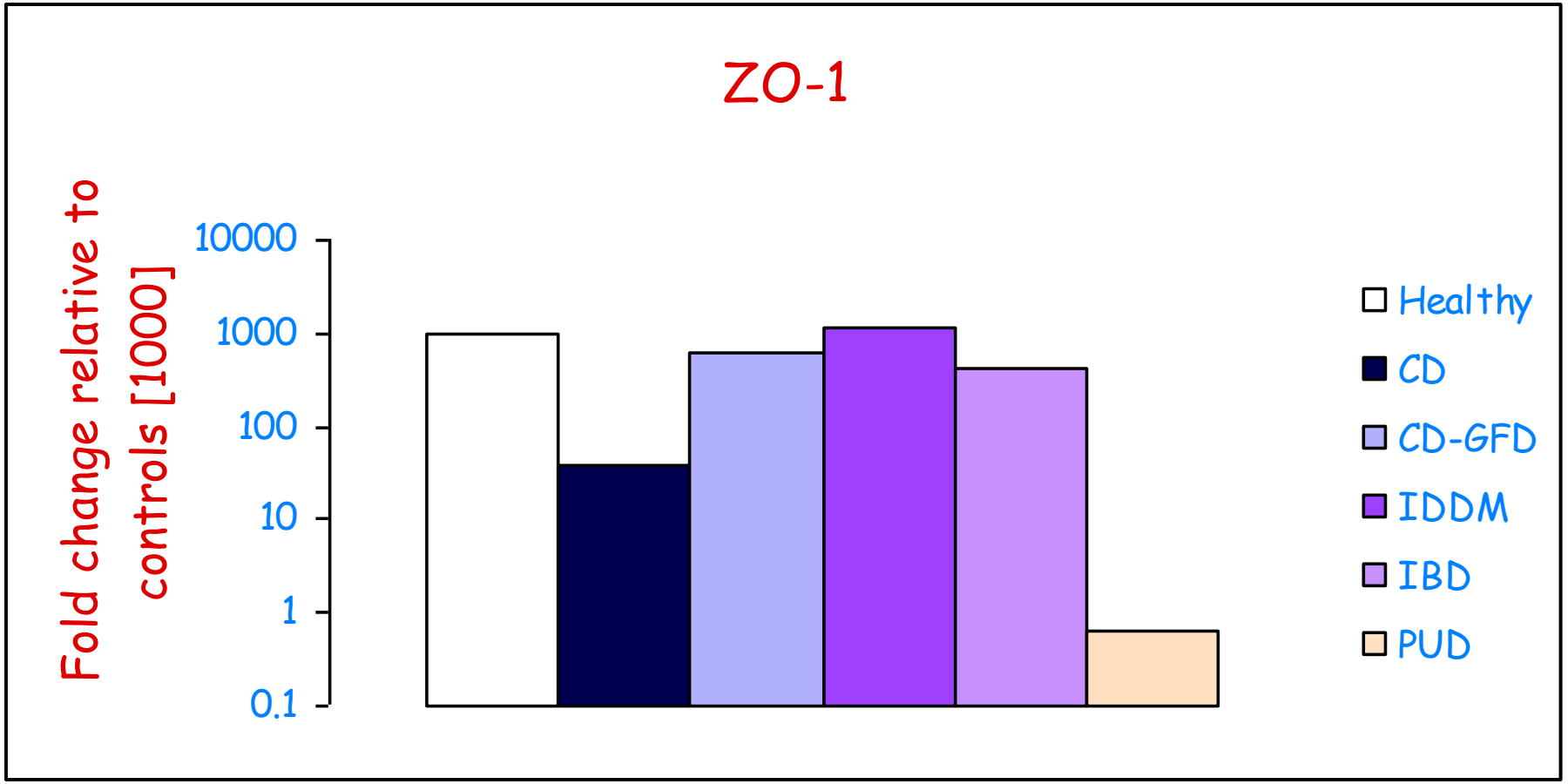
RESULTS


Claudin 2
Fold change relative to controls [1000]
1000
100
10
1
0.1
Healthy
CD
CD-GFD
IDDM
IBD
PUD

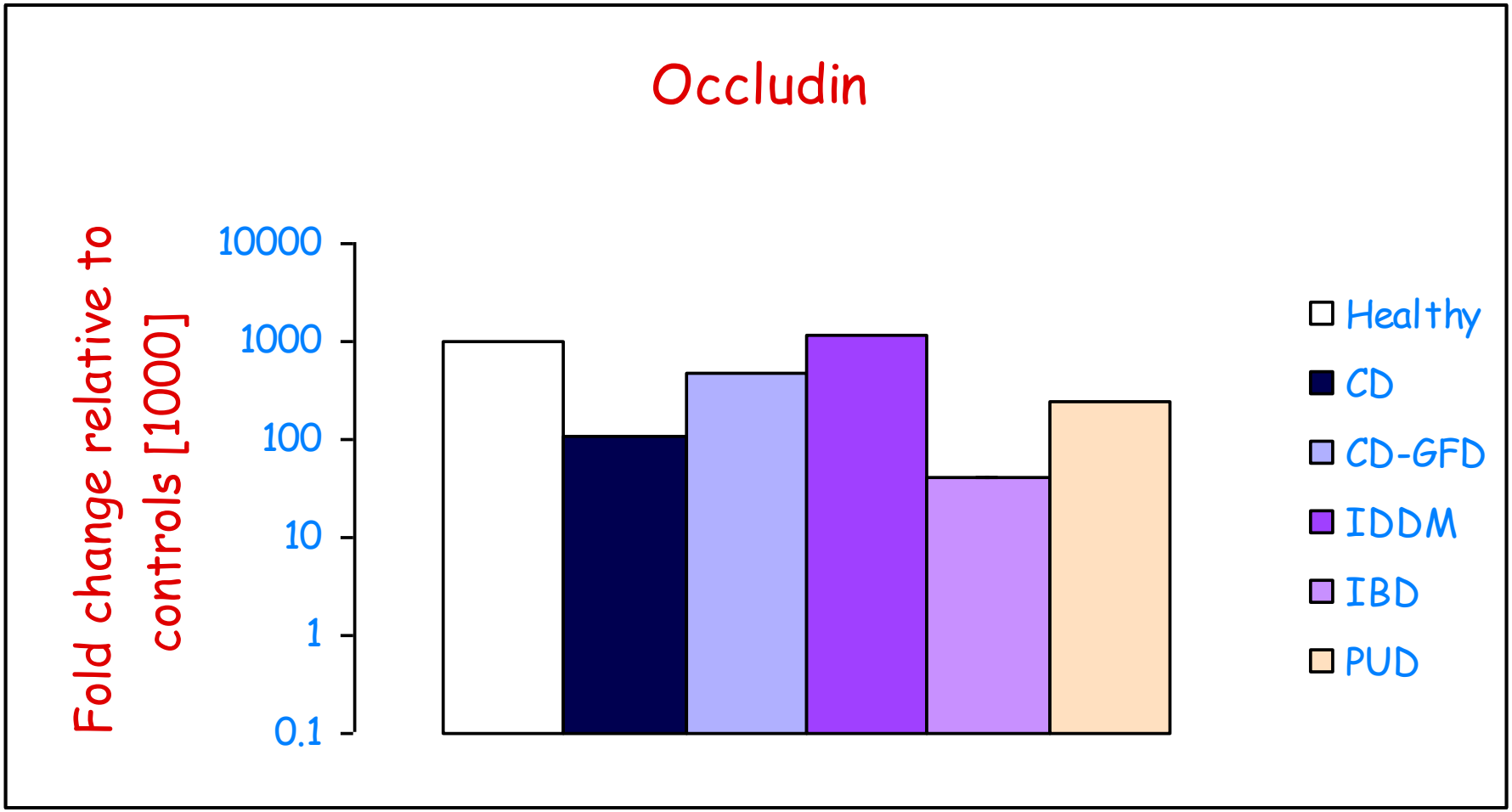
RESULTS


Claudin 1
Fold change relative to controls [1000]
10000
1000
100
10
1
0.1
Healthy
CD
CD-GFD
IDDM
IBD
PUD

RESULTS


ZO-1
Fold change relative to controls [1000]
10000
1000
100
10
1
0.1
Healthy
CD
CD-GFD
IDDM
IBD
PUD

RESULTS


Occludin
Fold change relative to controls [1000]
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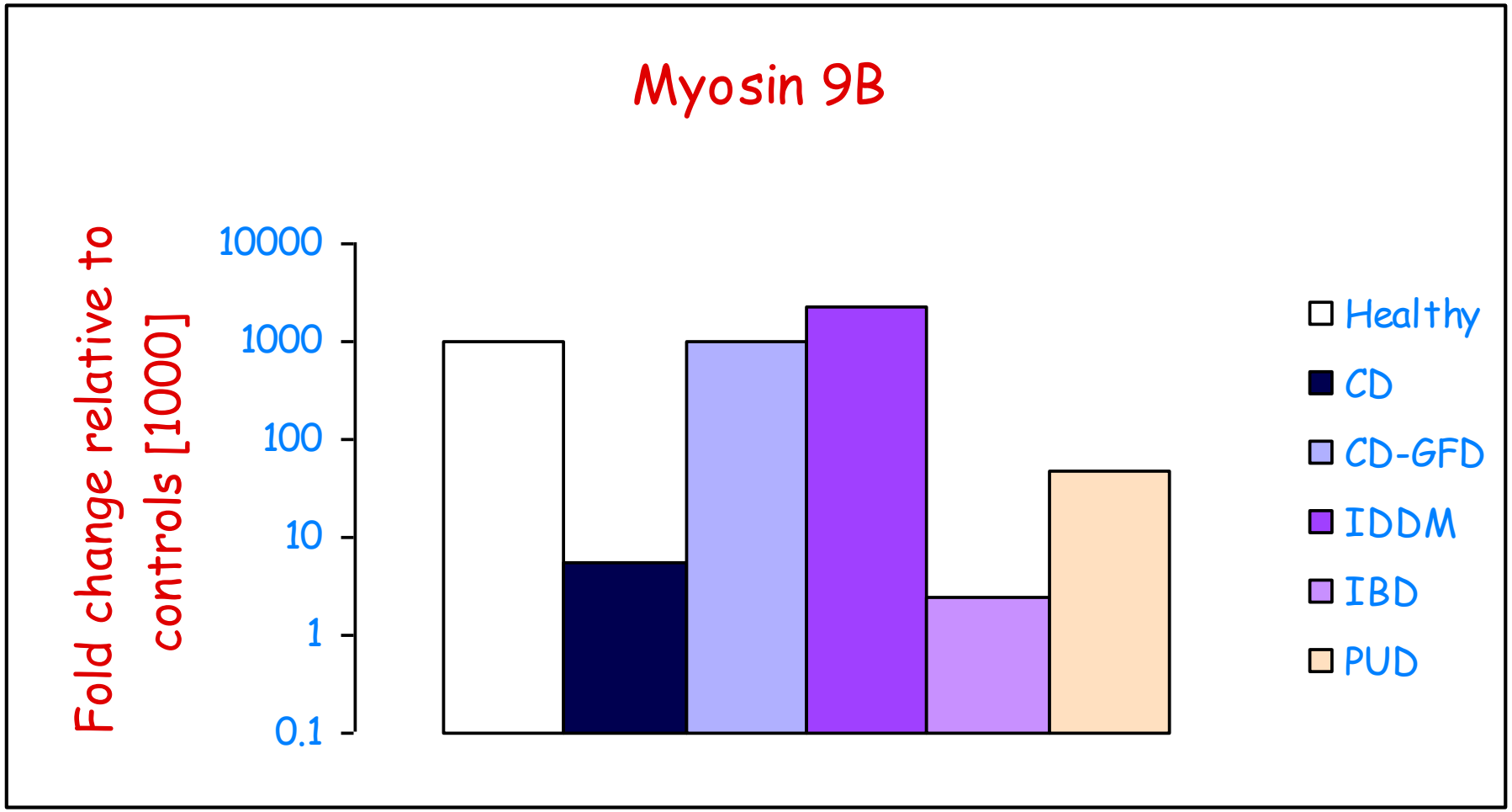
1000
100
10
1
0.1
Healthy
CD
CD-GFD
IDDM
IBD
PUD

RESULTS


Myosin 9B
Fold change relative to controls [1000]
10000
1000
100
10
1
0.1
Healthy
CD
CD-GFD
IDDM
IBD
PUD

CONCLUSIONS

1. The down-regulation of TJ transmembrane or/and intracellular components observed in all but IDDM might explain the increased intestinal permeability reported in these pathological conditions.

2. The meaning of TJs disruption in the pathogenesis of each of this pathological condition warrants further investigation.

3. The normal transcriptional level observed in celiacs on GFD suggests that the down-regulation of TJs in CD is reversible and secondary to gliadin exposure rather than to a genetic predisposition.