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Clinical impact of low amount of gluten

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Disclosure

Scientific consultant for Dr. Schär Food

Thanks, Dr Dicke: gluten is toxic for CeD patients... but what about a minimal amount of it?

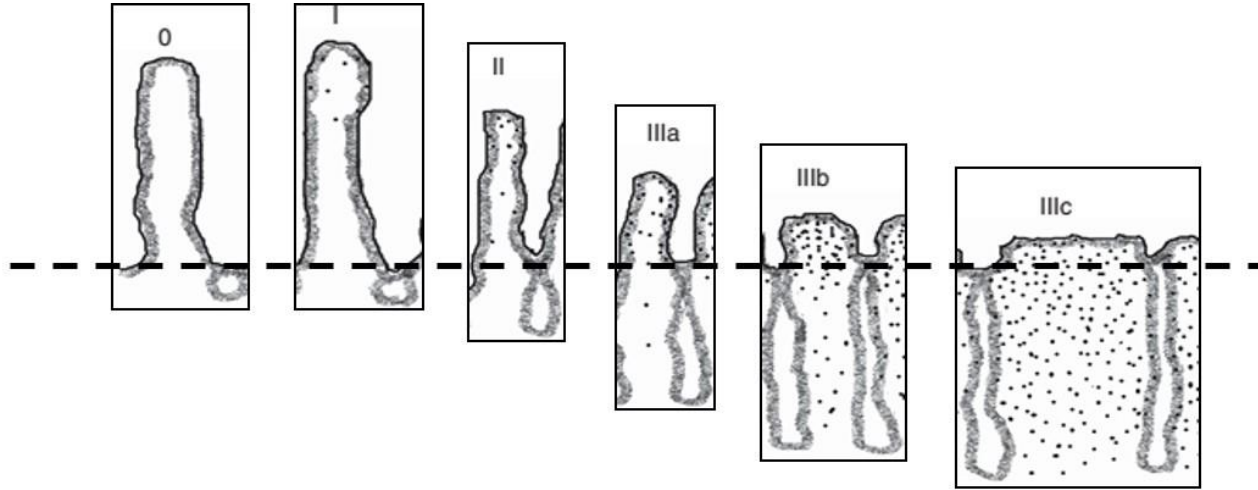


*WK Dicke,
1905 - 1962*

Gluten dose-effect relationship in CeD

- The intake of gluten of European people on a regular diet is **10-15 g/day**
- The toxic effect of gluten in CeD patients is typically related to the **cumulative daily dose**, however a single gluten intake may cause transient symptoms
- The effects of the gluten dose and its clinical consequences may be investigated in terms of: (a) symptoms, (b) laboratory tests (e.g. TGA antibodies), (c) **intestinal biopsy changes** (hystology, biopsy proteome score)
- A prolonged **gluten microchallenge** is required (4-12 weeks) to establish the **NOAEL**

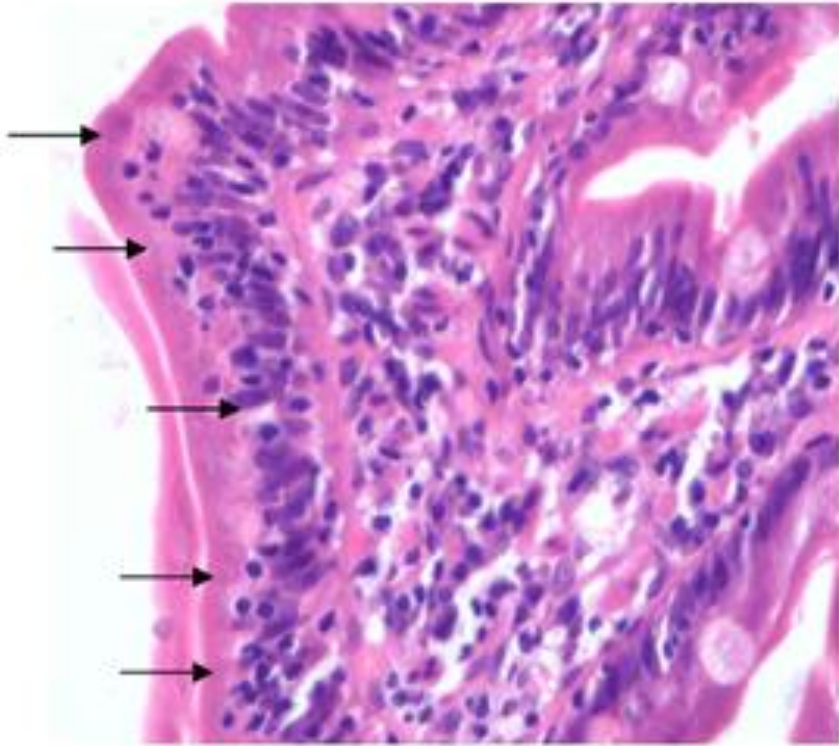
Intestinal morphology is the best biomarker of celiac health



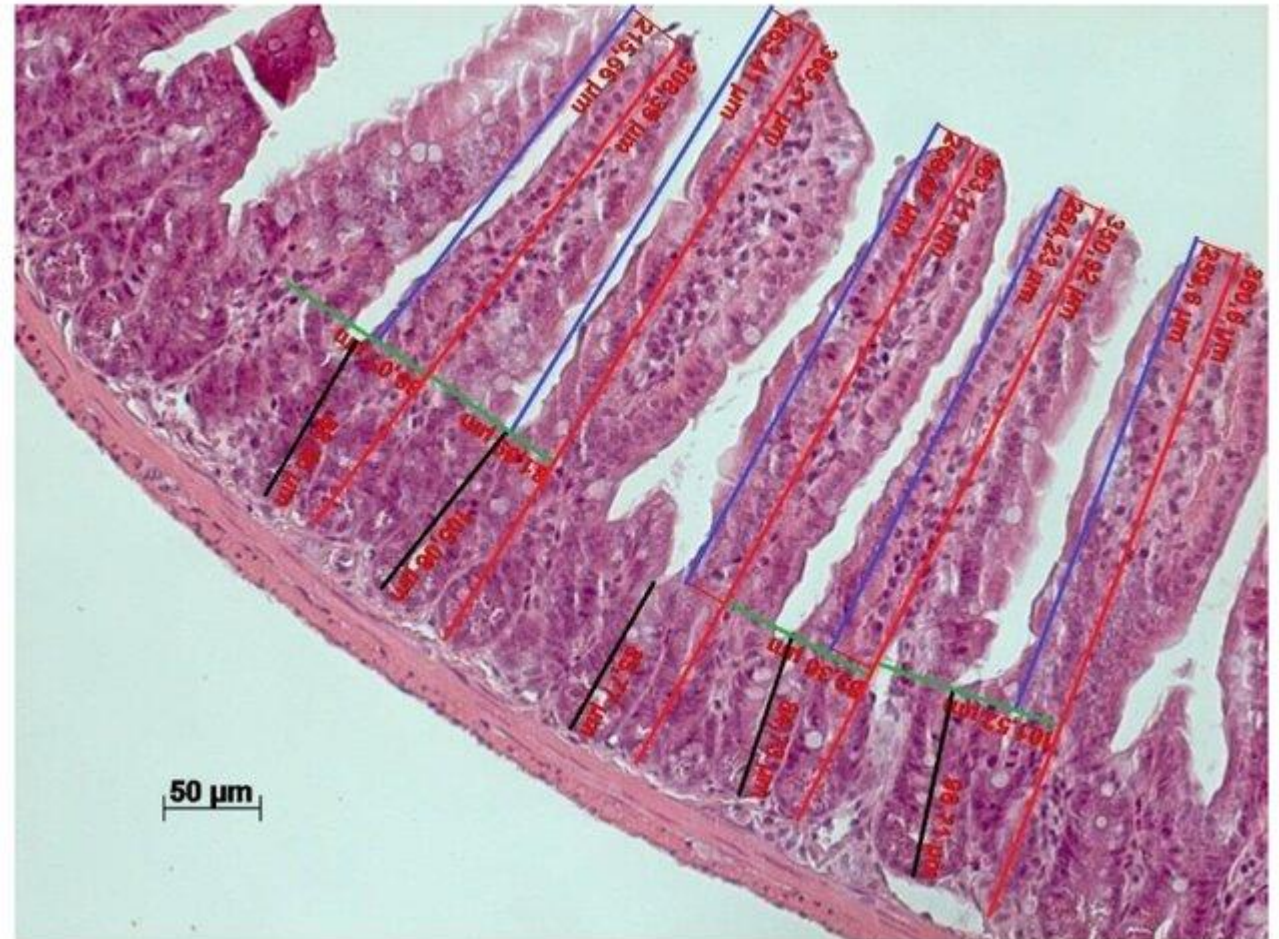
	Marsh-Oberhuber Grade					
	0	1	2	3a	3b	3c
Descriptor		Infiltrative	Hyperplastic	Partial villous atrophy	Subtotal villous atrophy	Total villous atrophy
Villous architecture	Normal	Normal	Normal	Shortened, blunt	Clearly atrophic	Complete loss
Crypt architecture	Normal	Normal	Enlarged	Enlarged	enlarged	Severe hyperplasia
IEL	Normal (<25 x 100 enterocytes)	Increased	Increased	Increased	Increased	Increased
Vh:Cd ratio	>3	>3	>2 - <3	>1 - <2	<0.5 - <1	<0.5

Intestinal morphometry parameters: the Vh/Cd ratio and the IEL count

Intraepithelial lymphocyte (IEL) count



Villous height/Crypt depth (Vh/Cd) ratio



Dose-dependent effects of the protracted ingestion of small amount of gliadin in CeD patients

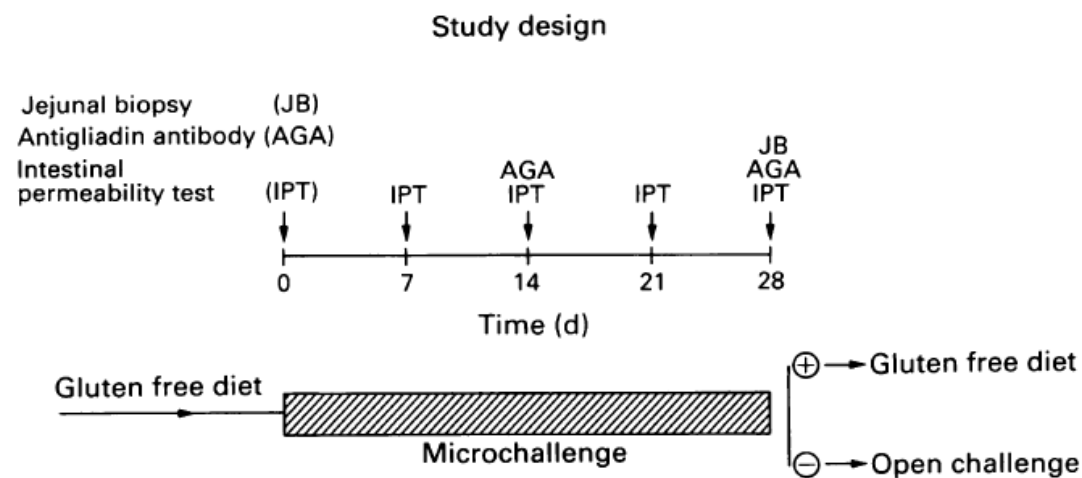
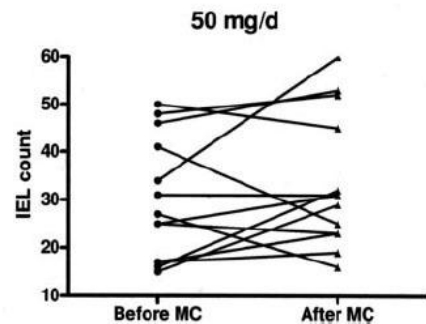
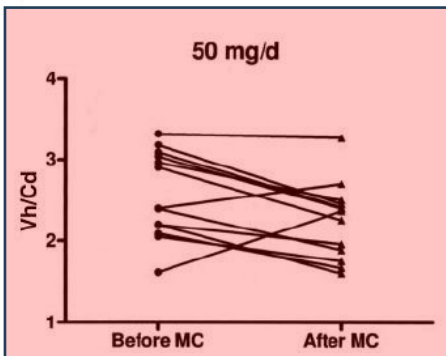
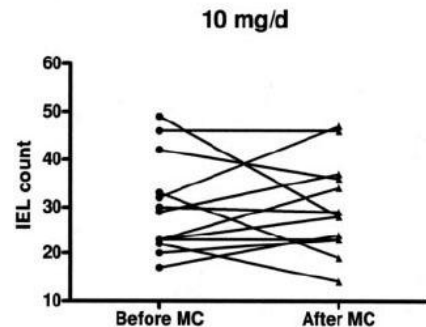
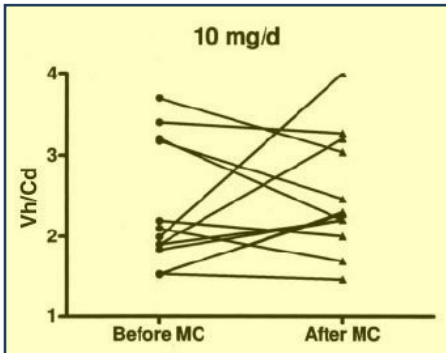
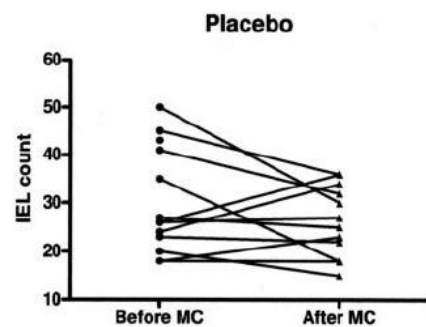
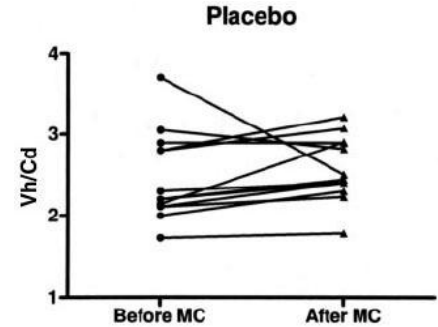


TABLE II *Results of the jejunal morphometric study and of the sugar intestinal permeability test (mean (SD)) in the study groups*

	Gliadin 100 mg/day		Gliadin 500 mg/day	
	T ₀	T ₁	T ₀	T ₁
Jejunal morphometry:				
Villous height (μm)	269 (47)	263 (34)	251 (26)	* 210 (27)†
Crypt depth (μm)	178 (34)	200 (29)	158 (16)	* 185 (24)
Villous height/crypt depth	1.5 (0.1)	* 1.3 (0.2)	1.6 (0.1)	* 1.1 (0.1)†
Intraepithelial lymphocytes (× 100)	11 (3)	* 19 (6)	10 (1)	* 25 (5)
Intestinal permeability test:				
Cellobiose/mannitol % (× 100)	2.8 (1.7)	3.3 (2.3)	2.8 (2.0)	5.8 (2.8)

*T₁ v T₀: p<0.01; †T₁-500 mg v T₁-100 mg: p<0.01.

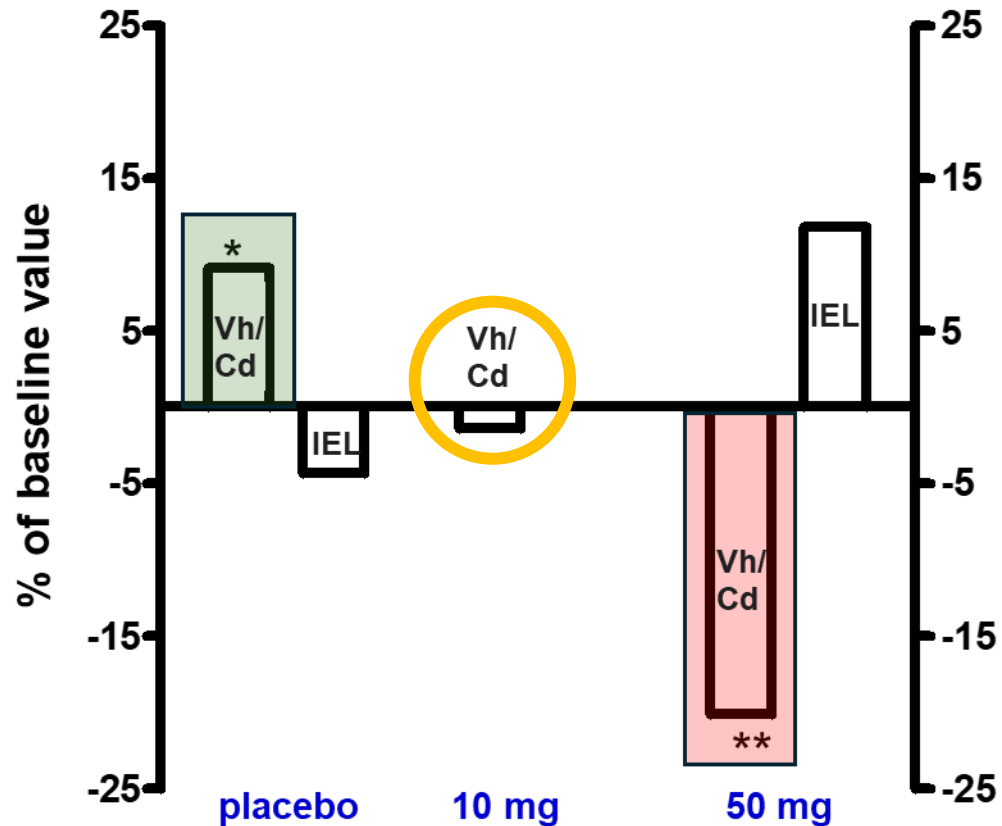
Insights from the first prospective, double-blind, placebo-controlled, low-gluten challenge trial



- **3 study groups:**
 - 50 mg gluten/day ($n = 13$)
 - 10 mg gluten/day ($n = 13$)
 - placebo ($n = 13$)
- The gluten microchallenge disclosed large **inter-individual variability** in the sensitivity to gluten traces at any given gluten dose.
- Some CeD patients showed worsening after ingesting only 10 mg gluten/d, whereas a few had an apparent improvement after the 3-mo challenge with 50 mg gluten/day.
- Despite this wide individual variability, it was shown that **50 mg gluten/day**, if introduced for 3 months, were sufficient to cause a **significant decrease in the Vh/Cd index** in treated CeD patients.

Catassi C, Fabiani E, et al. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr.* 2007 Jan;85(1):160-6.

Insights from the first prospective, double-blind, placebo-controlled, low-gluten challenge trial



* Significantly improved

** Significantly different from placebo

- The **50-mg group** showed significant **worsening** of the **Vh/Cd** index.
- A significant **improvement** in the **Vh/Cd** index was observed in the **placebo** group, likely reflecting a “Hawthorne effect”, but not in the 10-mg and 50-mg groups.
- Because of the limited number of patients, we were not able to reach firm conclusions about the safety/toxicity of 10 mg gluten/day
- A **NOAEL (No Observed Adverse Effect Level)** was not definitely determined

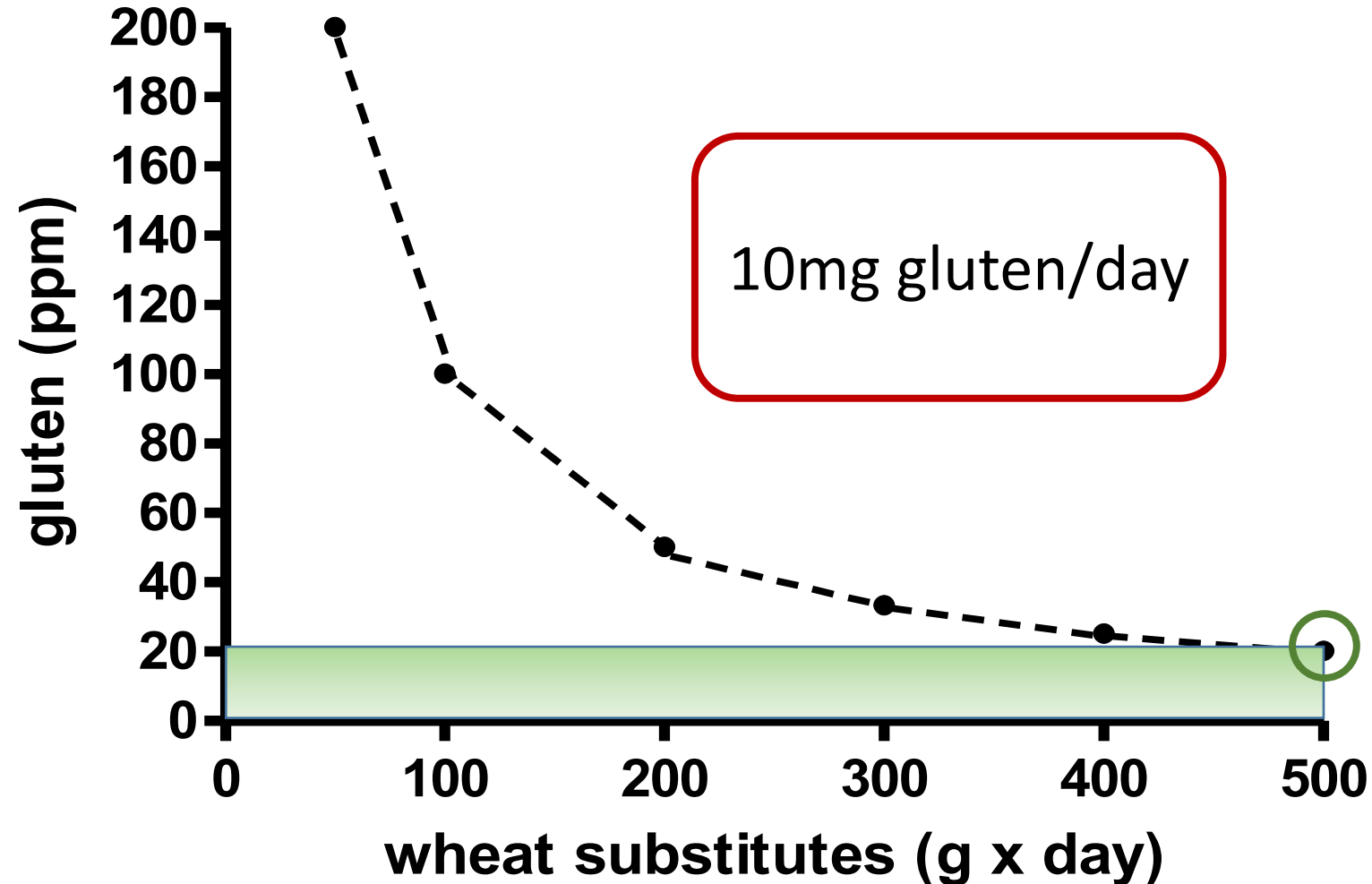
Inter-Individual Variability in Gluten Tolerance



Fig. 1. Dose-response relationship between increasing gluten doses and the percentage of patients with celiac disease experiencing adverse effects, based on a Gaussian distribution of gluten intolerance (from Wikipedia, modified); CeD: Celiac disease.

Catassi C, Monachesi C, et al. Lessons from low-gluten challenge studies in celiac disease: Is it time to reconsider the gluten threshold in gluten-free food? Dig Liver Dis. 2025 May;57(5):655-656.

Gluten threshold: from the gluten threshold to the safe concentration in gluten-free products



The Codex Definition of Gluten-Free and worldwide standards



Under the guidelines established by the **Codex Alimentarius Commission**, a joint **FAO-WHO** body, food labeled and sold as “gluten-free” may contain up to **20 mg/kg** (parts per million, or ppm) of gluten. This threshold aims to limit **daily gluten intake to less than 10 mg**, which would require consuming as much as 500 g of gluten-free products containing 20 ppm of gluten.

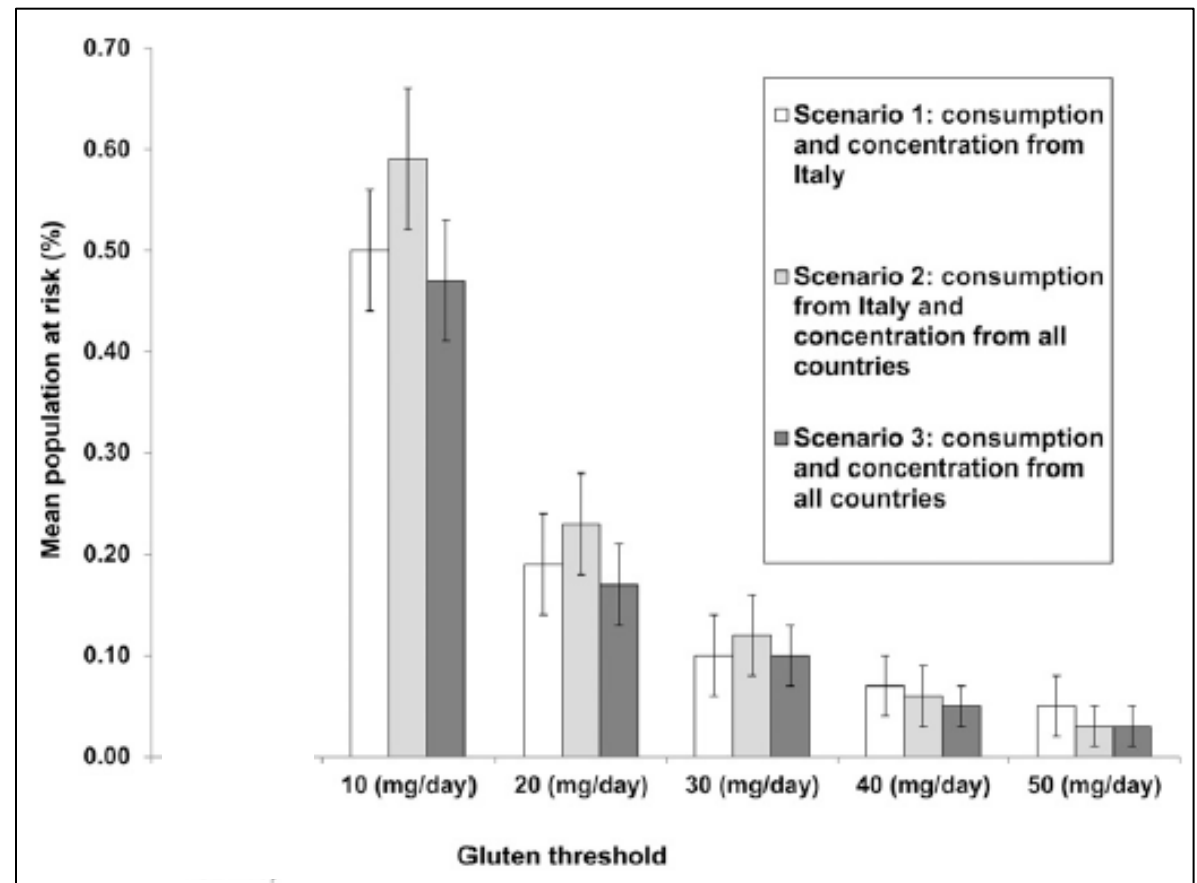
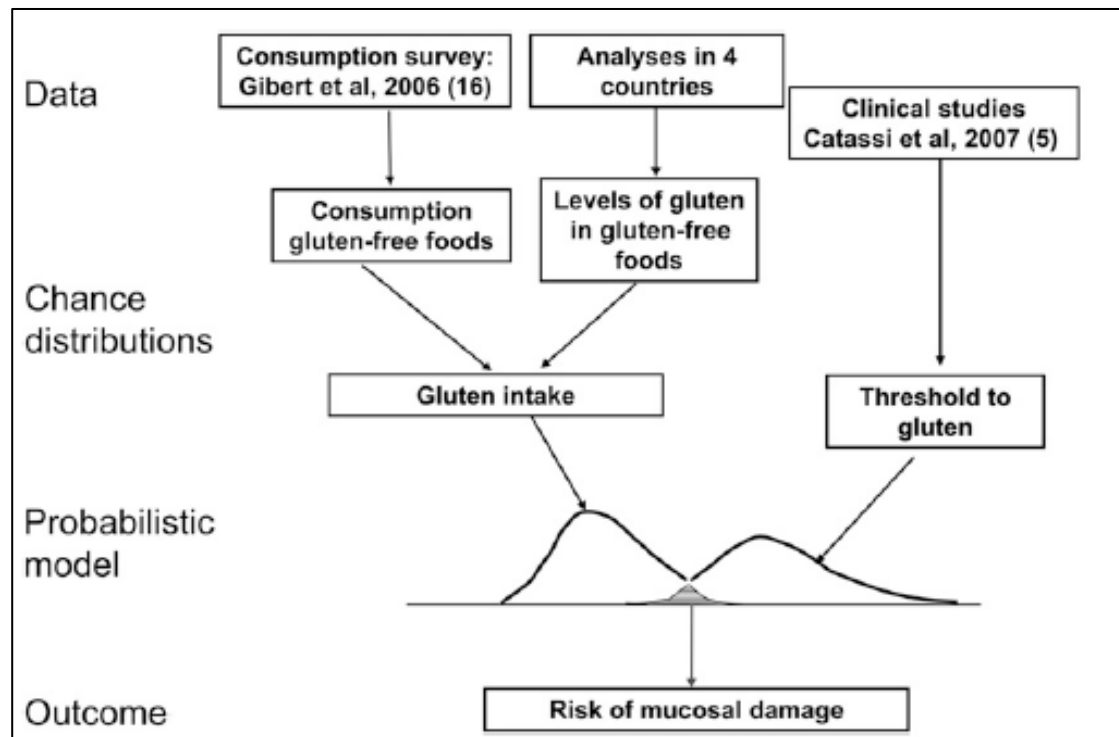
2008

National Standards		
US, Canada, Europe, UK <20ppm	Argentina <10ppm	Australia, New Zealand, Chile No detectable gluten (3ppm)

What happened next?

Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation¹⁻³

Anna Gibert, Astrid G Kruizinga, Susanna Neuhold, Geert F Houben, Miguel A Canela, Alessio Fasano, and Carlo Catassi





Article

Gluten Contamination in Naturally or Labeled Gluten-Free Products Marketed in Italy

Anil K. Verma ^{1,*}, Simona Gatti ^{1,2}, Tiziana Galeazzi ¹, Chiara Monachesi ¹, Lucia Padella ¹, Giada Del Baldo ², Roberta Annibali ², Elena Lionetti ^{1,2} and Carlo Catassi ^{1,2}

Table 1. Level of gluten contamination in the 200 examined products.

Gluten Content (ppm)	Number of Products	Median (Range) (ppm)	Mean $\pm$ SD (ppm)
<10	173	<5 (<0.5–9.3)	n.a.
10–20	9	13.9 (10.4–17.1)	14.1 $\pm$ 2.2
>20	18	31.7 (20.4–126.2)	49.2 $\pm$ 35.9

Products exceeding 20ppm (18 out of 200)

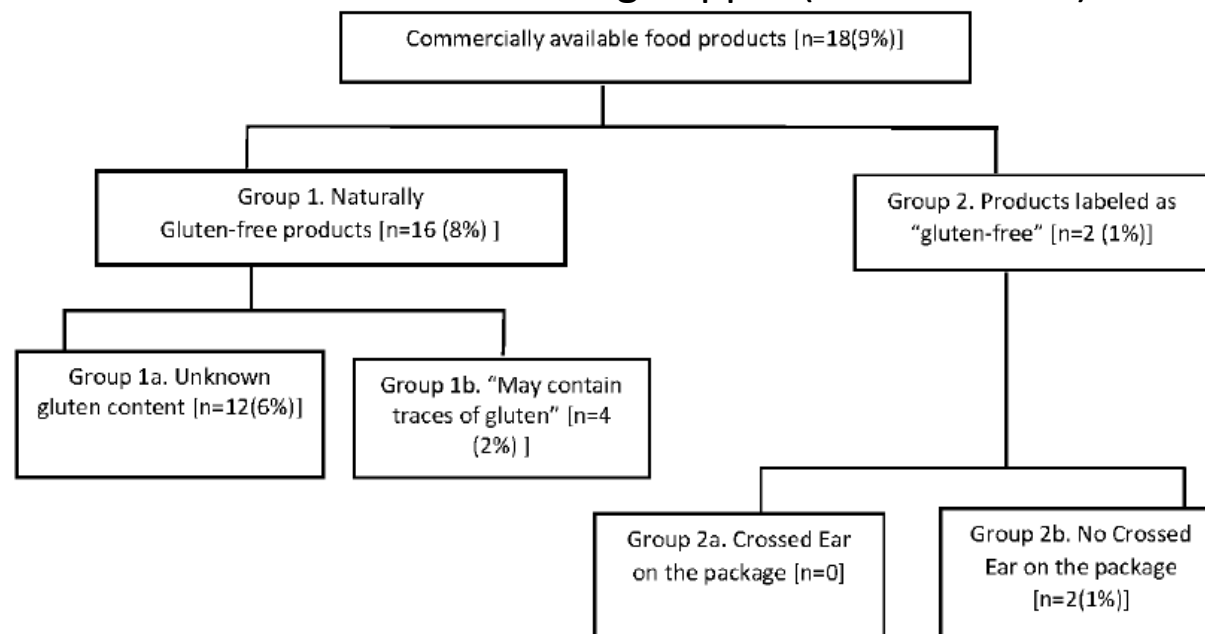


Table 1. Studies on the rate of gluten contamination in natural and certified gluten-free foods, as well as food service products.

Type of Products	Study Country	<i>n</i>	Percentage of Food Containing >20 mg/kg of Gluten	References
Gluten-free-labeled products	Ireland	260	10%	McIntosh et al., 2011 [20]
	Italy, Spain, Germany, and Norway	205	0.5%	Gibert et al., 2013 [21]
	United States	78	21%	Lee et al., 2014 [22]
	Brazil	20	16%	Oliveira et al., 2014 [23]
	United States	275	10%	Sharma et al., 2015 [24]
	Spain	3141	12%	Bustamante et al., 2017 [25]
	Turkey	200	17.5%	Atasoy et al., 2017 [26]
	Italy	56	0%	Bianchi et al., 2018 [27]
	Brazil	180	3%	Farage et al., 2019 [28]
	United States	5624	32%	Lerner et al., 2019 [29]
	Indian	160	36%	Raju et al., 2020 [30]
Naturally gluten-free products	United States	22	32%	Thomson et al., 2010 [31]
	Canada	640	9.5%	Koerner et al., 2013b [32]
	United States	186	19%	Sharma et al., 2015 [24]
	Indian	160	10%	Raju et al., 2020 [30]
Naturally or certified gluten-free products	Lebanon	173	6%	Hassan et al., 2017 [33]
	Italy	200	9%	Verma et al., 2017 [34]
	Brazil	130	22%	Farage et al., 2017 [35]

High Clinical Trial Effect (Hawthorne Effect) in Celiac Disease

Larazotide Acetate for Persistent Symptoms of Celiac Disease Despite a Gluten-Free Diet: A Randomized Controlled Trial

Daniel A. Leffler,¹ Ciaran P. Kelly,¹ Peter H. R. Green,² Richard N. Fedorak,³ Anthony DiMarino,⁴ Wendy Perrow,⁵ Henrik Rasmussen,⁵ Chao Wang,⁵ Premysl Bercik,⁶ Natalie M. Bachir,⁷ and Joseph A. Murray⁸

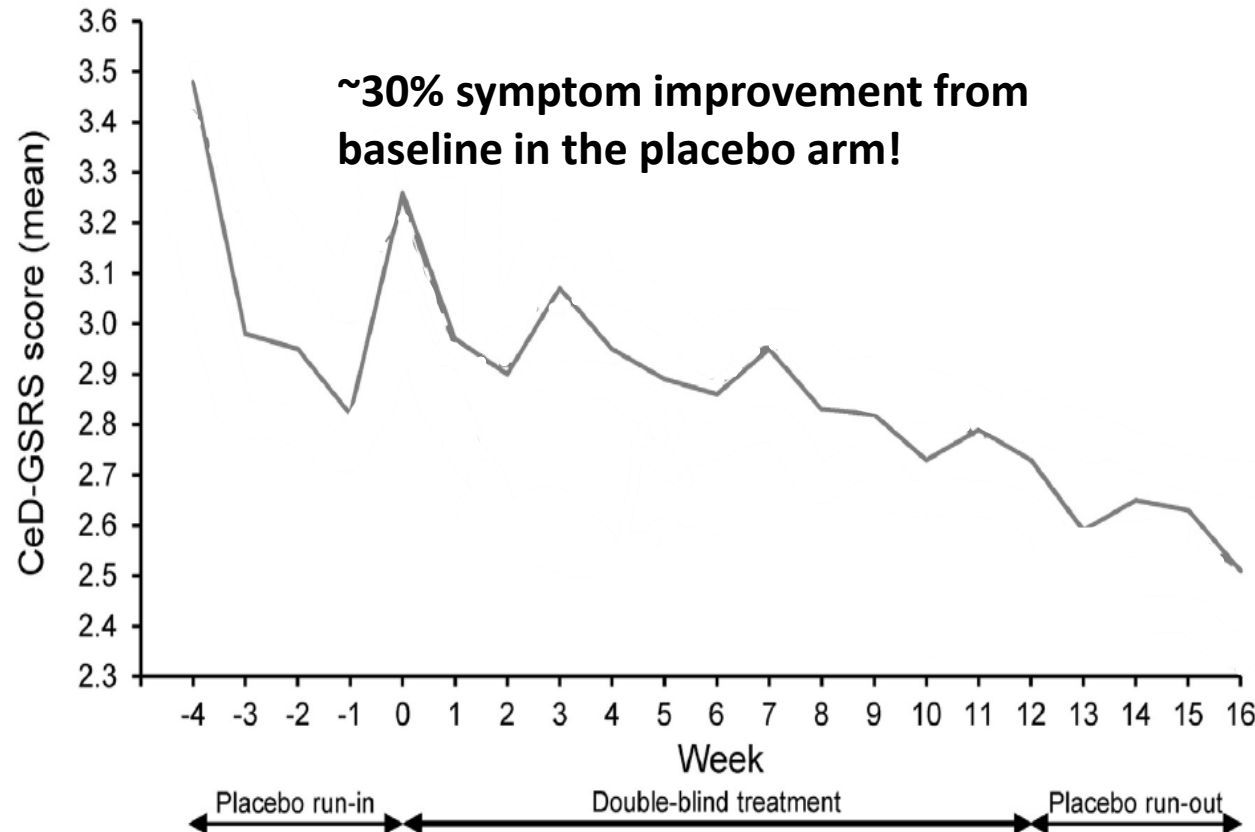


No Difference Between Latiglutenase and Placebo in Reducing Villous Atrophy or Improving Symptoms in Patients With Symptomatic Celiac Disease

Joseph A. Murray,¹ Ciarán P. Kelly,² Peter H. R. Green,³ Annette Marcantonio,⁴ Tsung-Teh Wu,⁵ Markku Mäki,⁶ and Daniel C. Adelman,⁴ on behalf of the CeliAction Study Group of Investigators



Mean weekly average on-treatment CeD-GSRS score



2

1,95

1,9

1,85

1,8

1,75

1,7

1,65

1,6

1,55

1,5

~25% histologic improvement from baseline in the placebo arm!

Baseline

Week 12

Courtesy of Dan Leffler, presented at the Celiac Disease On the Horizon symposium. NYC May 1–2, 20

Cytokine release after gluten ingestion differentiates coeliac disease from self-reported gluten sensitivity

Jason A Tye-Din^{1,2,3} , Gry I Skodje^{4,5,6}, Vikas K Sarna^{4,7,8}, John L Dzuris⁹, Amy K Russell¹, Gautam Goel⁹, Suyue Wang⁹, Kaela E Goldstein⁹, Leslie J Williams⁹, Ludvig M Sollid^{4,7,8}, Knut EA Lundin^{4,7,8} and Robert P Anderson⁹

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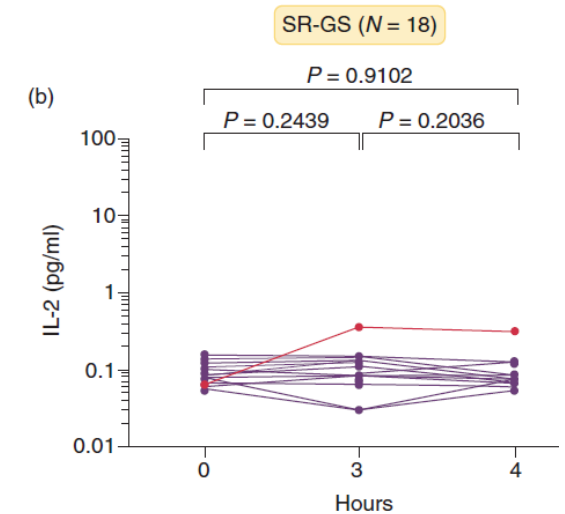
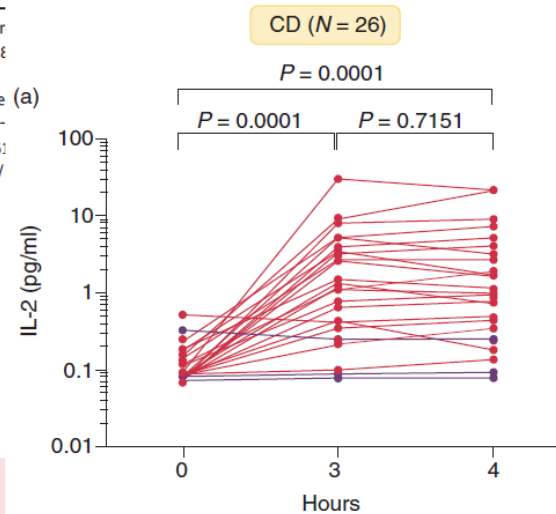

Key summary

Current knowledge

- Gluten challenge for weeks or months is necessary to diagnose coeliac disease in people following a gluten-free diet.
- One-off gluten ingestion increases circulating cytokines, particularly interleukin (IL)-2, 8 and 10, in coeliac disease.

What is new?

- Cytokine release after bolus gluten challenge differentiates coeliac disease from self-reported gluten sensitivity.
- Absence of cytokine release after gluten predicted coeliac disease patients who could resume an unrestricted diet without relapse after 1 y.
- Bolus gluten challenge with serum IL-2 at 3–4 h could replace extended gluten challenges for diagnosis of coeliac disease in patients already on a gluten-free diet.
- Early gluten-mediated immune activation present in coeliac disease is absent in people who self-report gluten sensitivity.



- IL-2 is an early marker of T-cell activation and indicates that the immune system is reacting to gluten
- IL-2 release begins at just 3 mg of gluten, while no response is seen at 2 mg (unpublished data).
- The correlation between the IL-2 test and the histological damage of the SI mucosa is still unknown

Do we need further studies on the gluten threshold

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YES

- A NOAEL of gluten in CeD has not definitely been established
- Many CeD patients show incomplete healing of the small intestinal mucosa after long-term GFD (most likely unrelated to the consumption of 20 ppm products)
- the IL-2 test could represent a simple tool for further investigating the gluten threshold provided that correlation with chronic damage of the small intestinal mucosa will be shown

Do we need further studies on the gluten threshold

YES

- A NOAEL of gluten in CeD has not definitely been established so far
- Many CeD patients show incomplete healing of the small intestinal mucosa after long-term GFD (most likely unrelated to the consumption of 20 ppm products)
- the IL-2 test could represent a simple tool for further investigating the gluten threshold if correlation with chronic damage of the small intestinal mucosa will be shown

HOWEVER

- Adverse events clearly related to 20-ppm GF products have never been reported
- Some labeled GF products are already close to the «no detectable gluten» level
- Not a single study investigated whether the healing of the SI mucosa of CeD patients is better in countries adopting the «no detectable gluten» threshold than in countries adopting the 20ppm threshold
- Breaks in the the GFD are most likely related to voluntary or unintentional trasgressions or cross-contamination of processed gluten-free ingredients
- Further lowering of the gluten threshold may increase psycho-social burden on patients

The Ancona Research Group on Celiac Disease, year 2023



THANKS FOR
YOUR
ATTENTION!