Toxicity of gluten traces: the Italian study on gluten microchallenge

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Background

Treatment of celiac disease (CD) is based on the complete avoidance of gluten proteins in the diet, termed the gluten-free diet (GFD). Over the long term, GFD is associated with clinical, serological and histological recovery of CD. It is however almost impossible to maintain a “zero gluten level” GFD, as the dialy GFD often contains minimal amounts of gluten proteins, e.g. due to cross-contamination of gluten-free cereals during milling/storage or inclusion of wheat starch in GF food. The potential toxicity of these gluten traces is still unclear.

We previously showed that in treated celiac patients the 1-month ingestion of 100-500 mg of gliadin per day (roughly equivalent to 200-1,000 mg of gluten) is able to cause minimal changes in the architecture of the small intestinal mucosa without causing modifications in serological and clinical parameters [1]. A few studies investigated the toxicity of lower gluten doses [2-5]. This is an important issue, as the Daily ingestion of contaminating gluten is most likely to be in the range of 5-50 mg. unfortunately no firm conclusions could be drawn from these studies, as the results were primarily biased, e.g. due to retrospective study-design, lack of a control group, and no measurement of ingested gluten.

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AIM

To evaluate the consequences of the protracted ingestion of minimal daily gluten intake (either 10 or 50 mg) in a group of adult celiacs on long-term treatment with the gluten-free diet (GFD)

TYPE OF STUDY

Multicentre, prospective, randomized, placebo-controlled, double-blind

STUDY PERIOD

Years 2001-2004

SPONSOR

Italian Celiac Society (AIC)

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INCLUSION CRITERIA

- Patients with biopsy-proven CD on a GFD for at least 2 years

EXCLUSION CRITERIA

- Younger than 18 yrs
- Poor compliance to the GFD
- Abnormal results at the baseline evaluation
- Associated selective IgA deficiency

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Study-Design

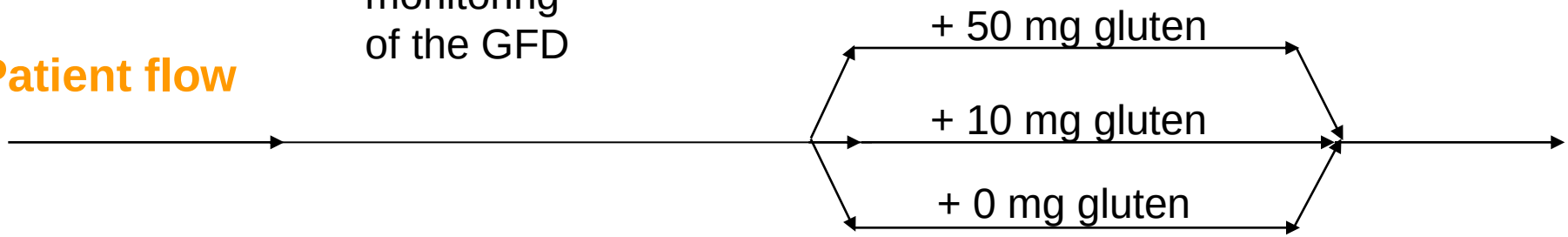
Steps

GFD ≥ 2 yrs	Run in	Baseline	Microchallenge	T_1	GFD

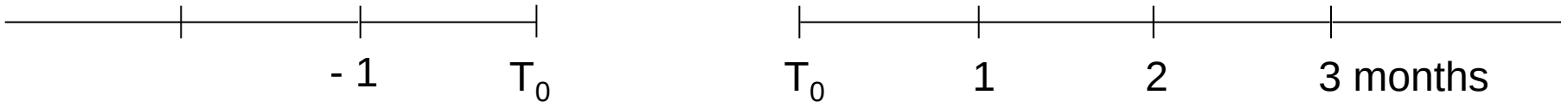
Intervention

	Informed consent Strict monitoring of the GFD	Clinical Serology SB Biopsy	Randomization Gluten exposure Monthly check	Clinical Serology SB biopsy	

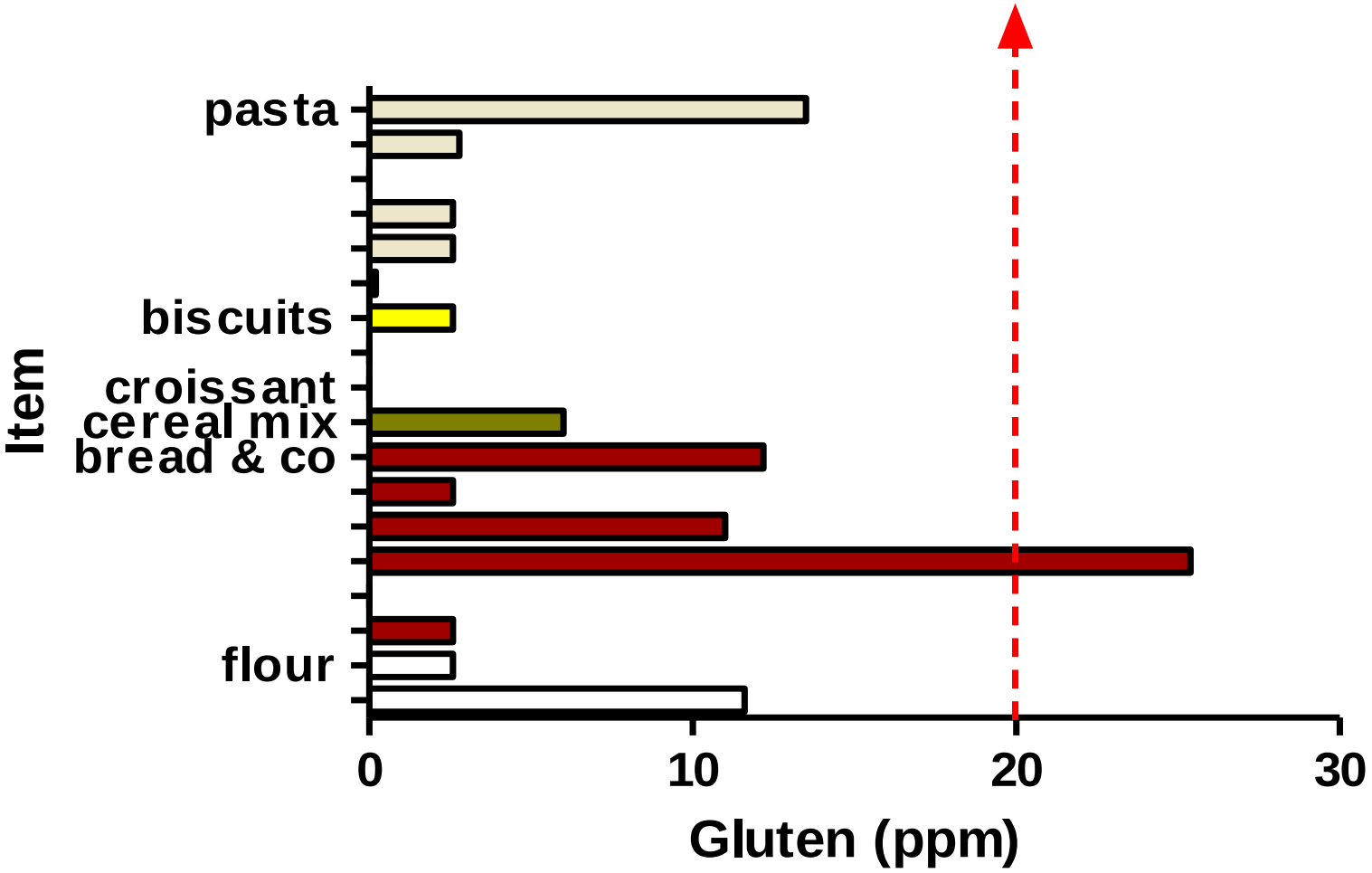
Patient flow



Timeframe



Gluten content in commercially available products in Italy



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Methods

- Purified gluten was used for the microchallenge study (Amygluten 110, Tate & Lyle, UK)
- Gluten- or lactose (placebo) containing capsules were centrally prepared
- All laboratory tests were centrally performed
- Monthly monitoring of adherence to the protocol
- Measurement of gluten contamination in commercially available GF food by ELISA (Ridascreen Gliadin, R-Biopharm AG, Germany)
- Serum AGA (ELISA) and anti-tTG (ELISA)
- Small bowel biopsy and morphometry on 10 villi, IEL count (CD3+), $\alpha\beta$ IEL count
- Control biopsies from non-celiac GE patients

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Participating centres and patients

Centre	Elegibles	Out	Patients at T_0	Out	Randomiz	Out	Patients at T_1
Ancona	3 (2F, 1M)	0	3 (2F, 1M)	1	2 (1F, 1M)	0	2 (1F, 1M)
Bari	10 (8F, 2M)	0	10 (8F, 2M)	2	8 (1F, 1M)	1	7 (6F)
Bologna	4 (3F, 1M)	0	4 (3F, 1M)	0	4 (3F, 1 M)	0	4 (3F, 1M)
Catania	6 (5F, 1M)	0	6 (5F, 1M)	2	4 (3F, 1M)	1	3 (2F, 1M)
Palermo[1]	14 (11F, 3M)	0	14 (11F, 3M)	1	13 (11F, 2M)	1	12 (11F, 1M)
Palermo[2]	2 (1F, 1M)	0	2 (1F, 1M)	0	2 (1F, 1M)	0	2 (1F, 1M)
Pavia	4 (3F, 1M)	0	4 (3F, 1M)	0	4 (3F, 1M)	0	4 (3F, 1M)
Rome[1]	4 (3F, 1M)	1	3 (2F, 1M)	0	3 (2F, 1 M)	0	3 (2F, 1M)
Rome[2]	2 (1F ,1M)	0	2 (1F, 1M)	0	2 (1F, 1M)	0	2 (1F, 1M)
	49	**1**	**48**	**6**	**42**	**3**	**39**

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Subjects interrupting the protocol

Centre	Sex	Age (yrs)	Microchallenge started	Reason
Ancona	F	33	No	Abnormal histology
Bari	M	30	No	Refused randomization
Bari	F	22.	No	Thyroid carcinoma development
Catania	F	n.a.	No	Abnormal histology
Catania	F	n.a.	No	Abnormal histology
Palermo[1]	M	18	No	Abnormal histology
Rome	F	n.a.	No	Gastric polyposis
Bari	F	31	Yes (10 mg)	stopped after 8 wks (symptoms)
Palermo[1]	F	32	Yes (10 mg)	Poor adherence to the protocol
Catania	F	n.a.	Yes (50 mg)	Change of residence

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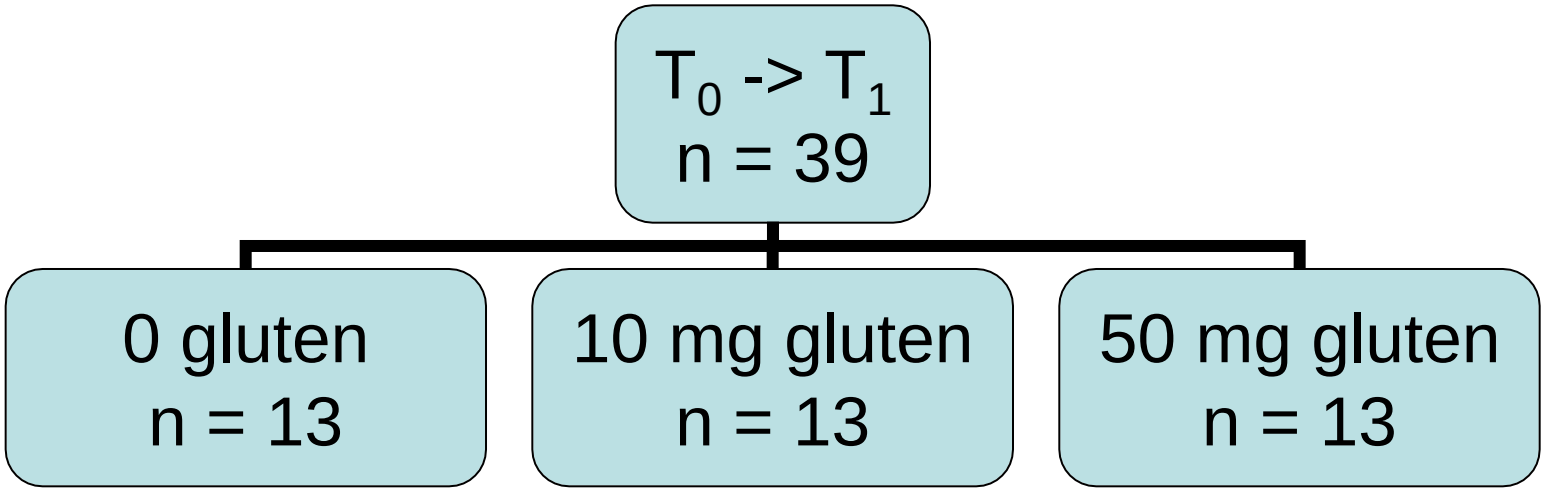
Subjects completing the study



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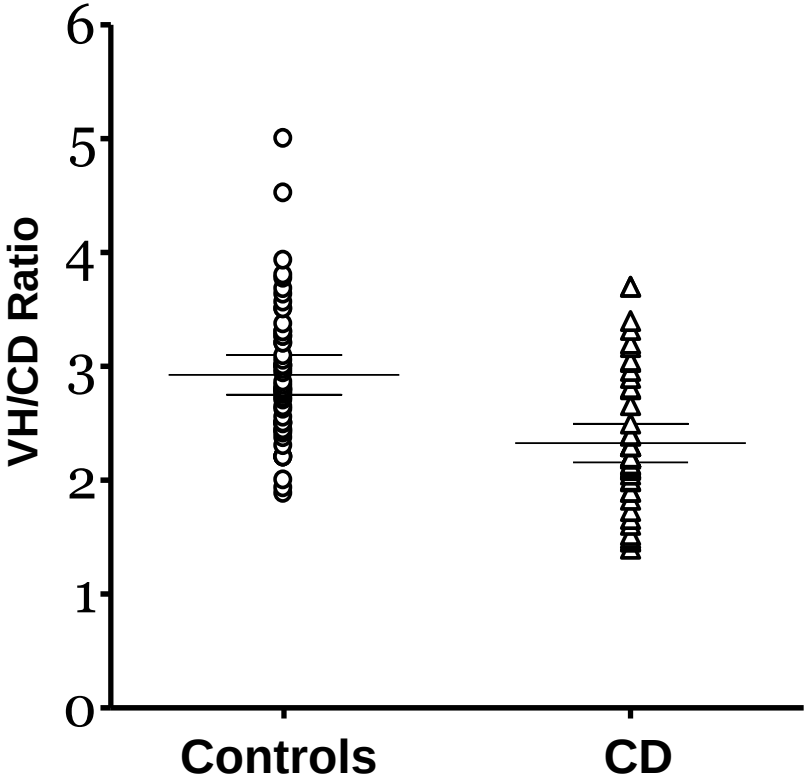
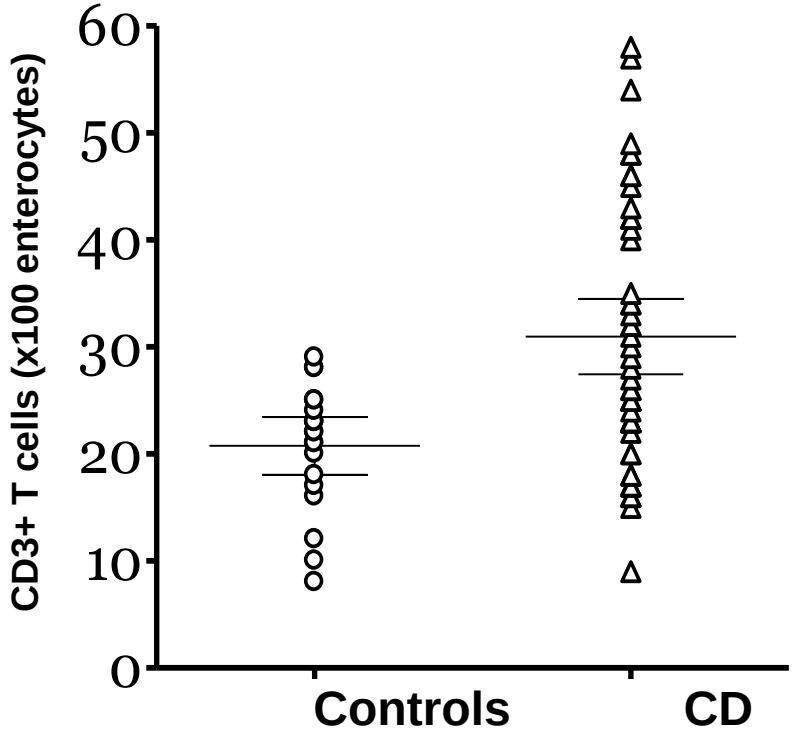
Biopsy findings at baseline[1]





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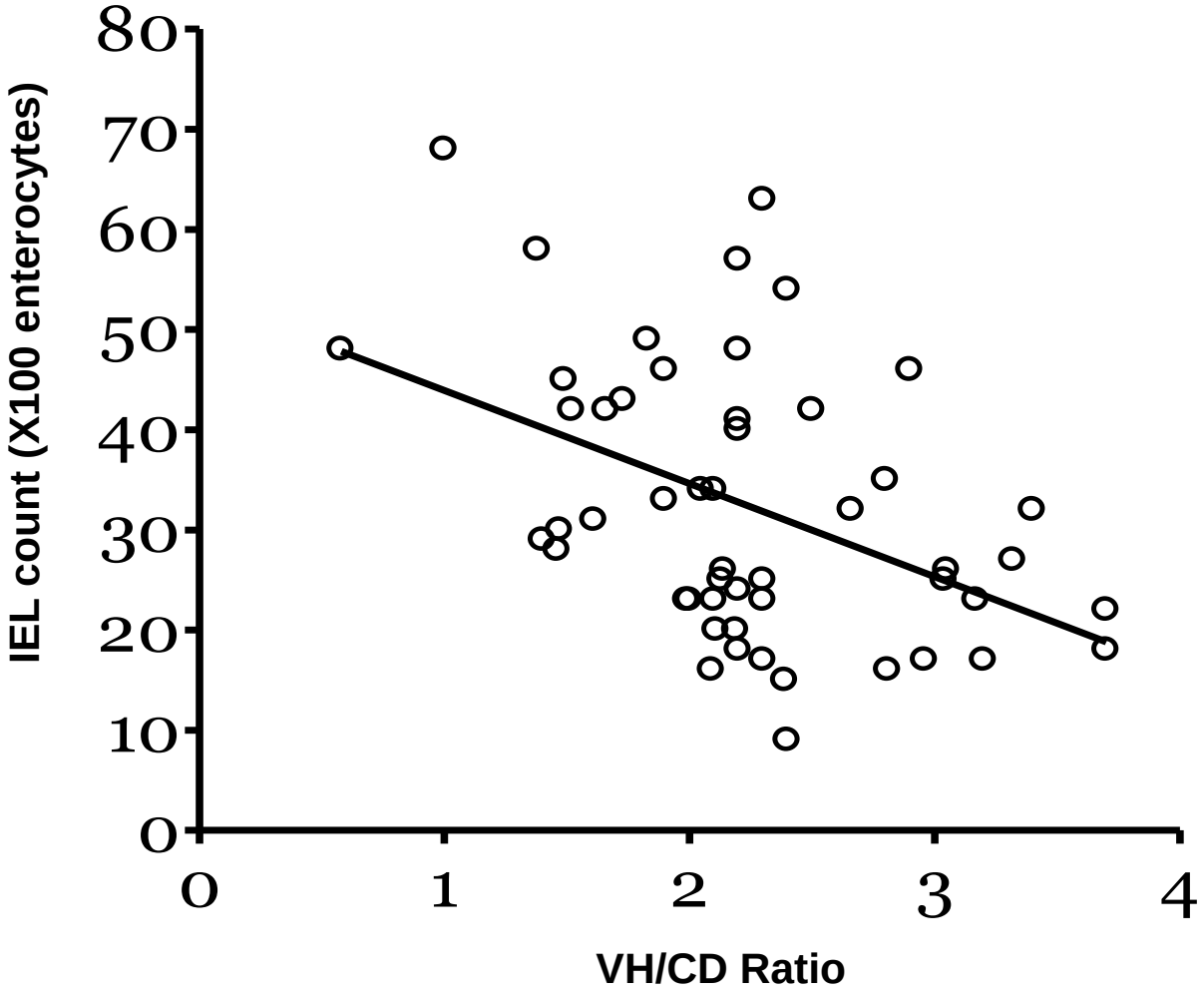
Biopsy findings at baseline[2]



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Clinical findings

Symptoms	*Placebo*	*10 mg*	*50 mg*
None	6	8	7
Abdominal pain and distension	2	1	2
Anemia and/or iron deficiency	1	0	0
Loss of appetite	0	0	1
Bloating, mood changes	2	1	0
Apthous stomatitis	0	0	1
Constipation	2	0	0
Headache, abdominal distention	1	0	0
Weight loss	0	0	1

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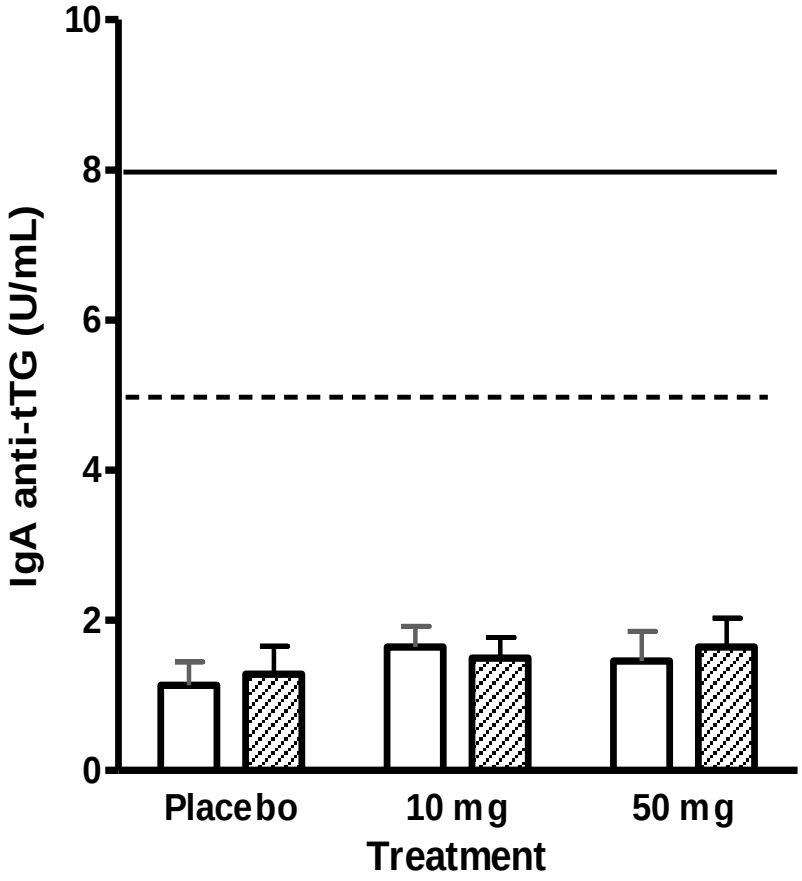
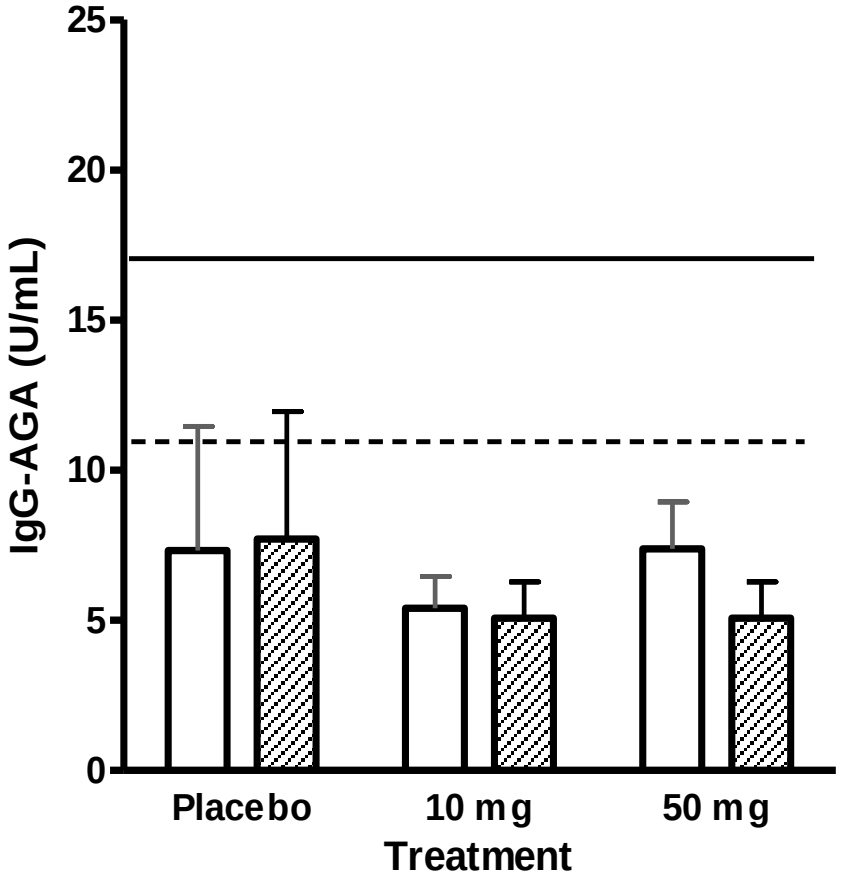
Serological findings





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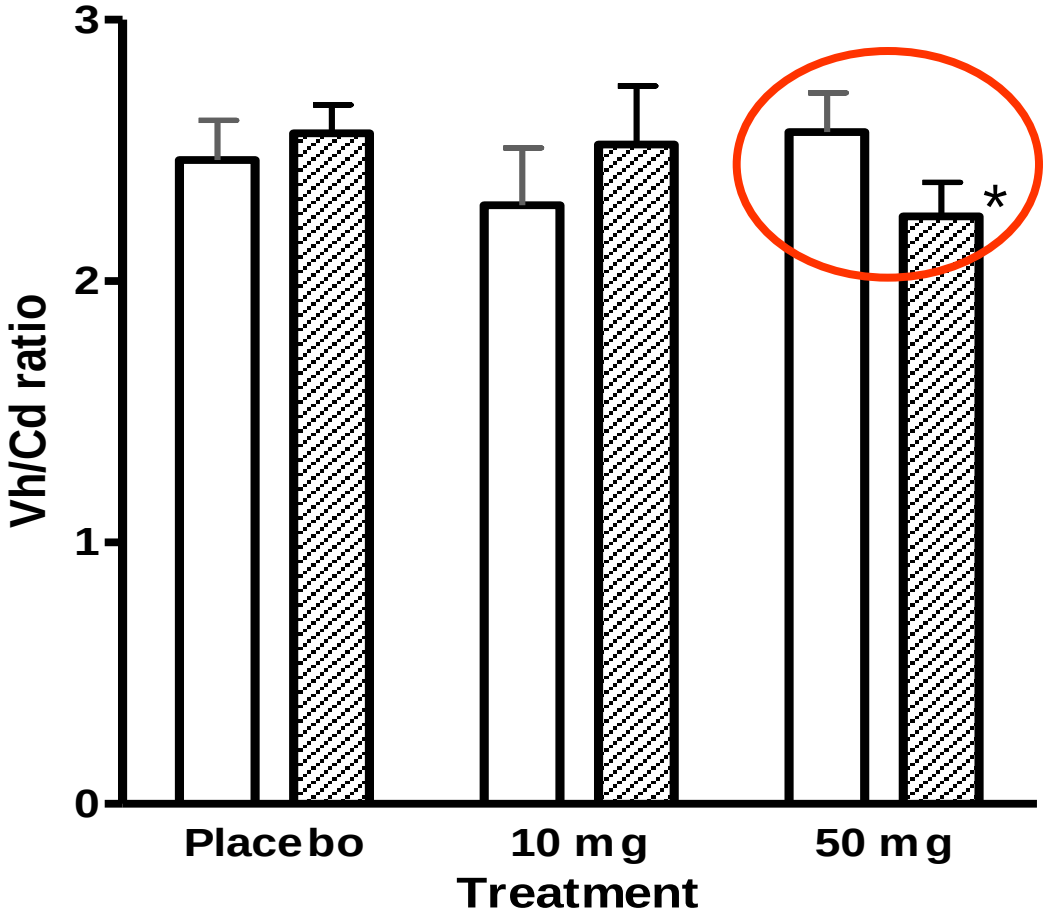
Morphometry findings[1]



- ***50 mg significantly different from placebo (Kruskal-Wallis test)***

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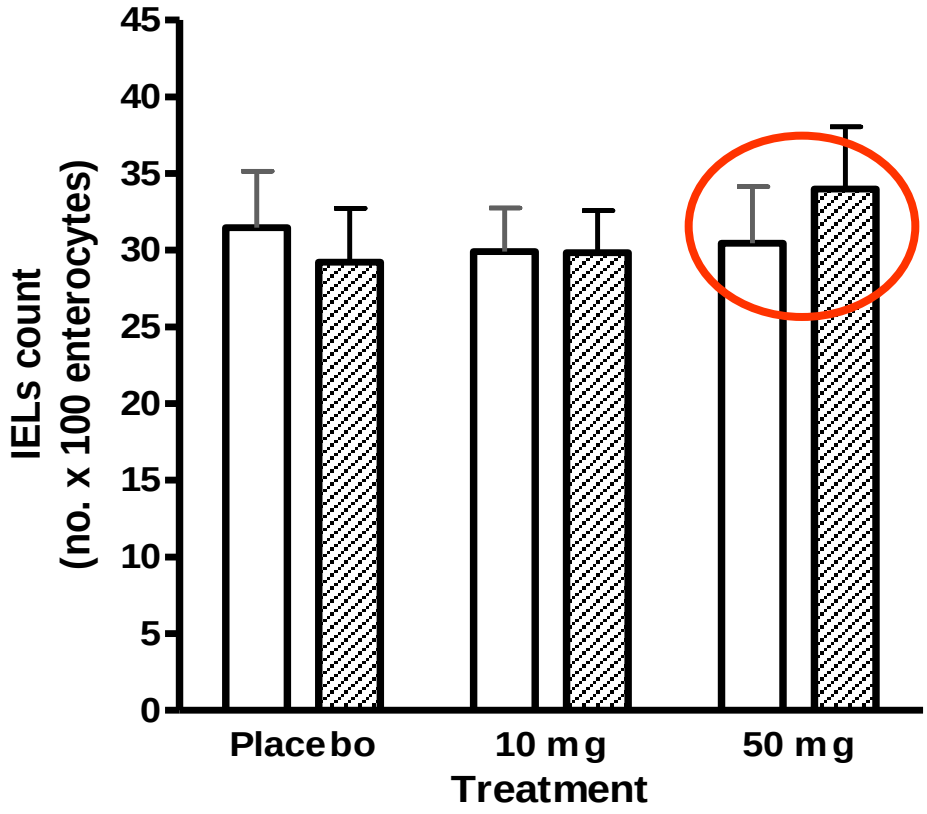
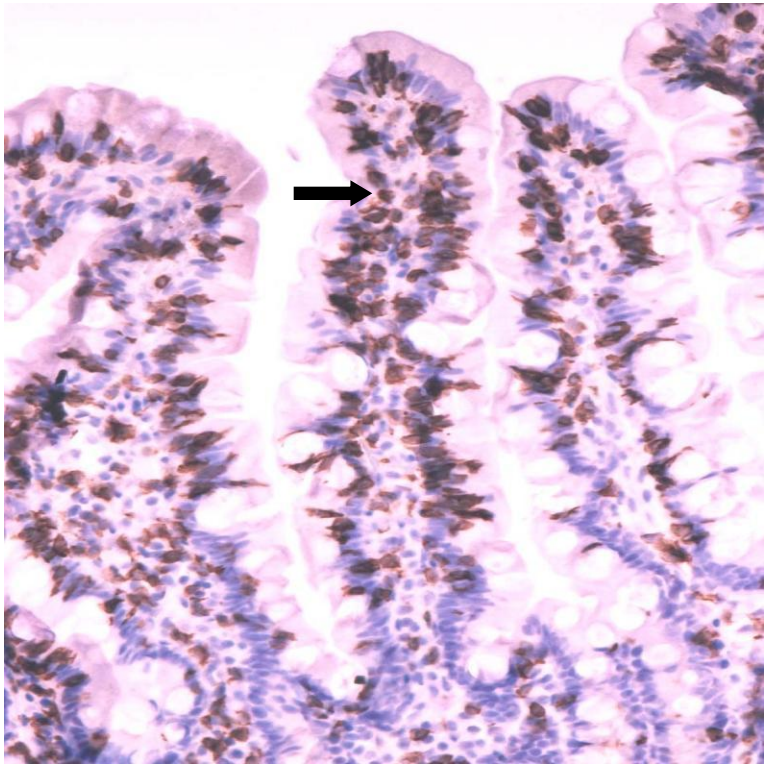
Morphometry findings[2]





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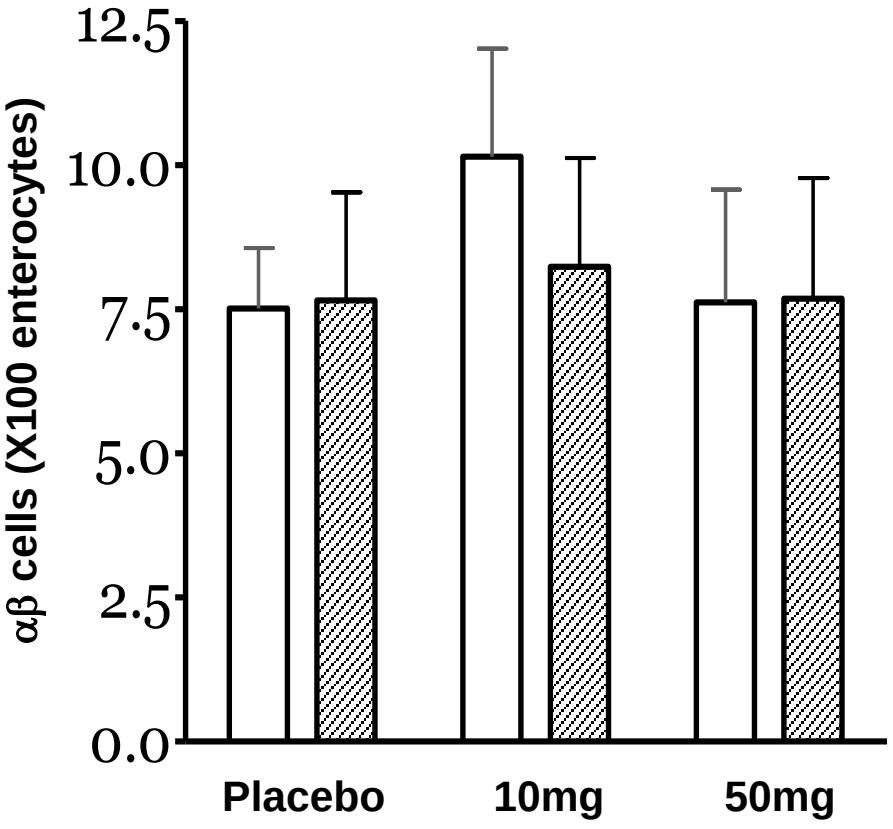
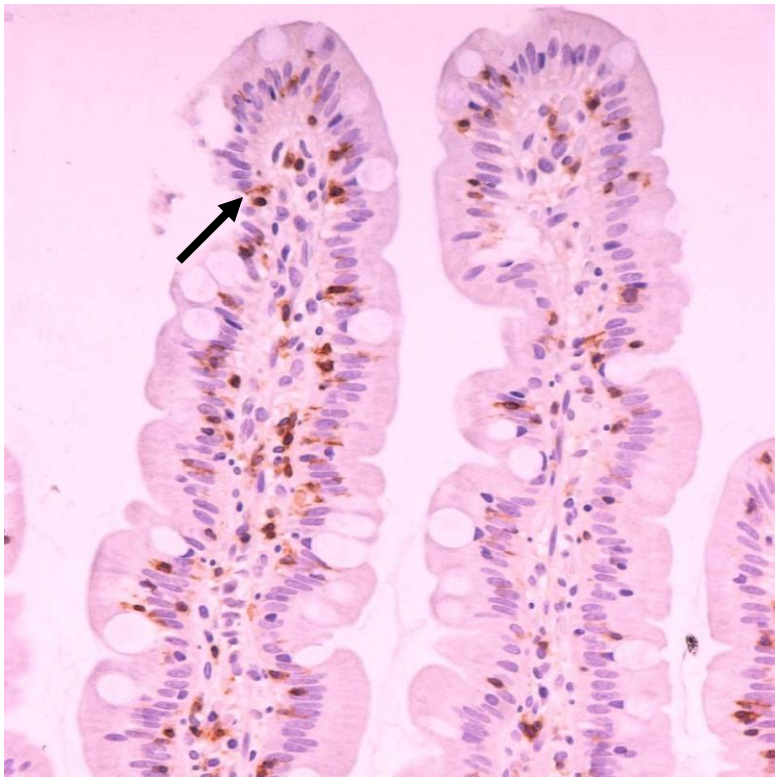
Morphometry findings[3]





Tolerable daily intake of gluten and ppm of gluten in food for celiacs

	50 g	*100 g*	*200 g*	*300 g*
200 ppm	10 mg	20 mg	40 mg	60 mg
100 ppm	5 mg	10 mg	20 mg	30 mg
50 ppm	2.5 mg	5 mg	10 mg	15 mg
20 ppm	1 mg	2 mg	4 mg	6 mg

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MAJOR FINDINGS

- The histological picture of the small intestinal mucosa did not revert to normal in celiacs on a strict GFD
- No clinical or serological change with either 10 or 50 mg of daily gluten
- Patients challenged with 50 mg/day of gluten for 3 months showed minimal histological changes in comparison with the placebo and the 10 mg groups
- Based on average intake of GF food (up to 300 g/day) a 20 ppm limit appears a safe threshold for gluten contamination

References

1. Catassi C, Rossini M, Rätsch IM, Bearzi I, Santinelli A, Castagnani R, et al. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. Gut 1993; 34: 1515-9.
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