Modulation of abdominal pain by probiotics

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Functional gastrointestinal (GI) wellbeing

Up to 70% suffer from functional GI symptoms - ¾ do not seek medical care

Chronic functional GI disturbances common – transient disturbances a rule

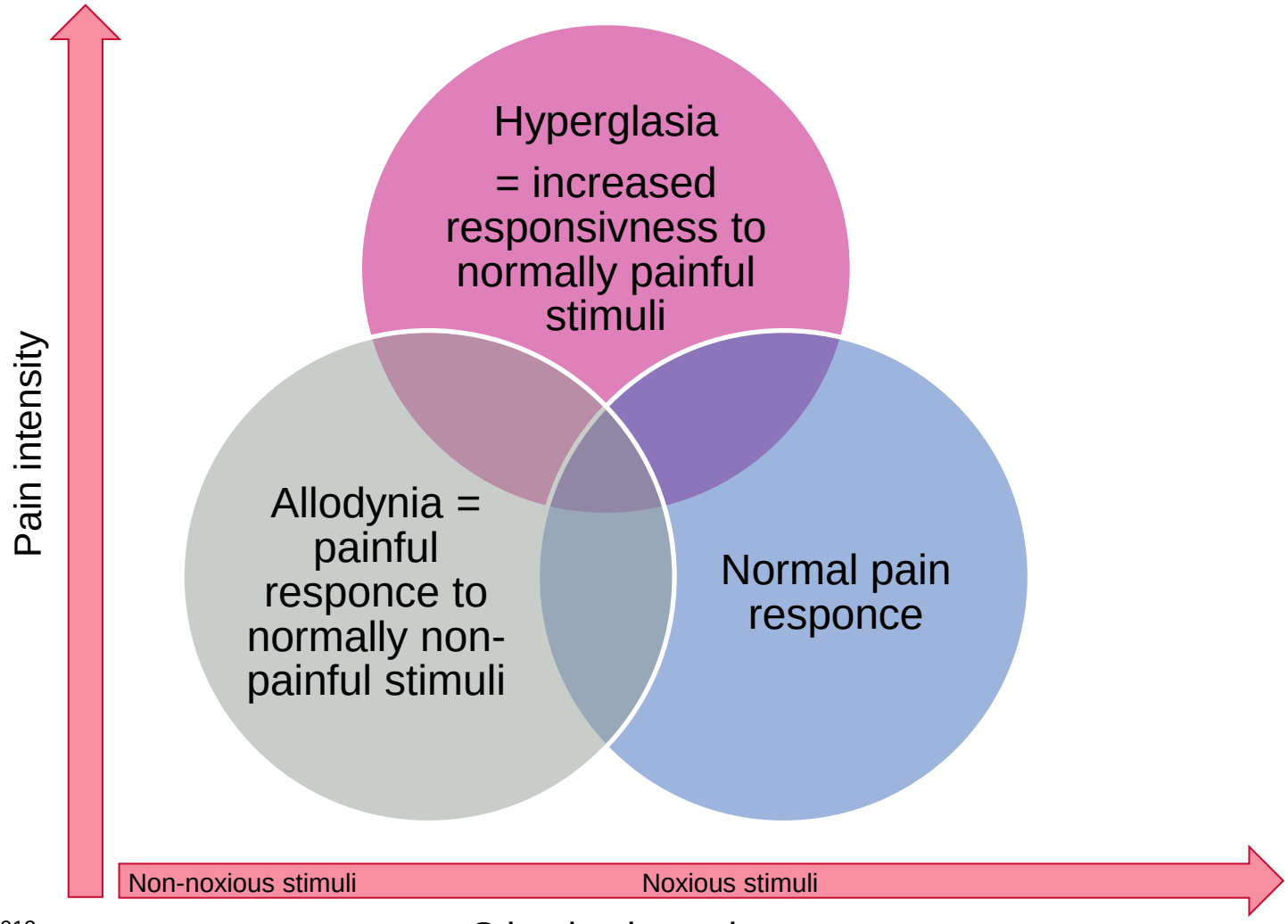
- Criteria exist for a large array of functional bowel disorders (FBDs)
  - Irritable bowel syndrome (IBS)
  - Functional bloating
  - Functional abdominal bloating
  - Functional constipation
  - Functional diarrhea
  - Functional abdominal pain syndrome

Rome III criteria for IBS
(Longstreth et al., Gastroenterol 2006)

Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following

- improvement with defecation
- onset associated with a change in frequency of stool
- onset associated with a change in form of stool

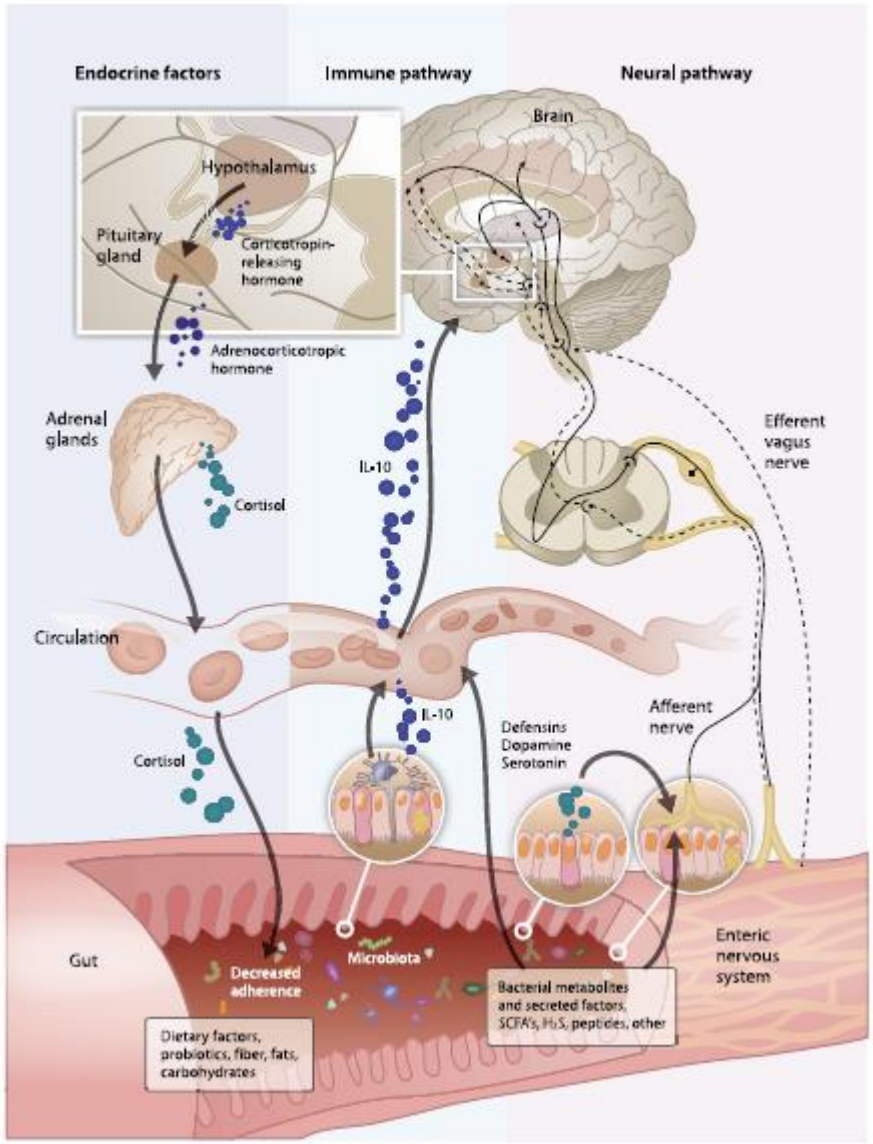
Sensitization to pain



Abdominal pain is a common symptom attributed to visceral hypersensitivity

Experimental and clinical data suggest that changes in gut flora may be a basis for the variability of abdominal symptoms observed in functional gastrointestinal disorders and may be prevented by specific probiotic administration (1-4).

1. Verdu EF et al. Gut 55, 182-90 (2006)
2. Kamiya T et al. Gut 55, 191-6 (2006)
3. Kajander K et al. Aliment. Pharmacol. Ther. 22, 387-94 (2005)
4. O'Mahony L et al. Gastroenterology 128, 541-551 (2005).



Microbiome – gut – brain

-Bi-directional

-Early life development essential for balanced function

-Endocrine, immune, neural and intestinal factors

-Stressors can disturb (psychological, infectious, etc.)

(Chichlowski and Rudolph, JNM, 2015)

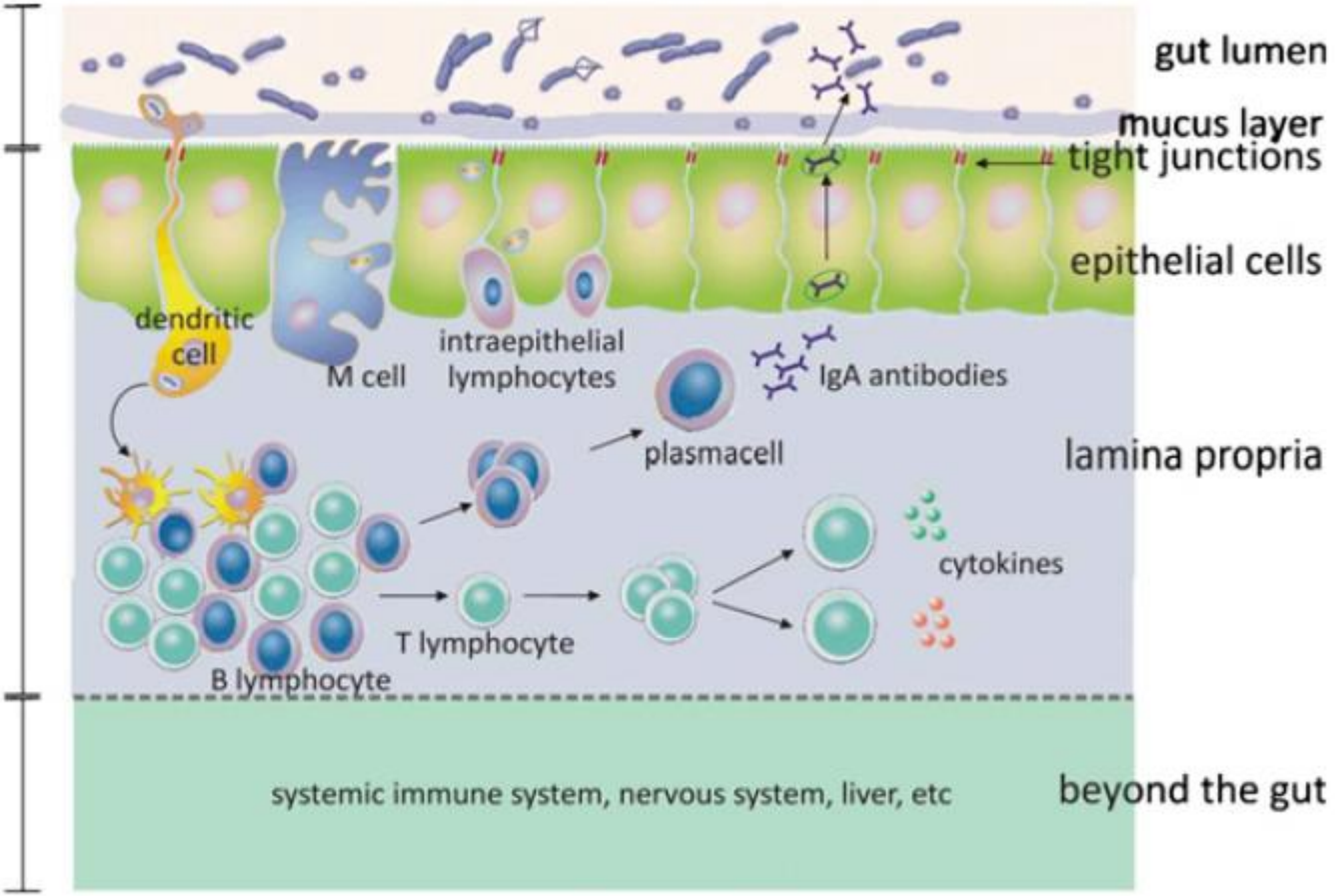
Levels of action of probiotics

”Live microbes that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO 2002; Hill et al., 2014)

Recilience
Composition
Metabolites

Tight junction integrity
Epithelial cell proliferation
Immune effects
Mucosal gene expression

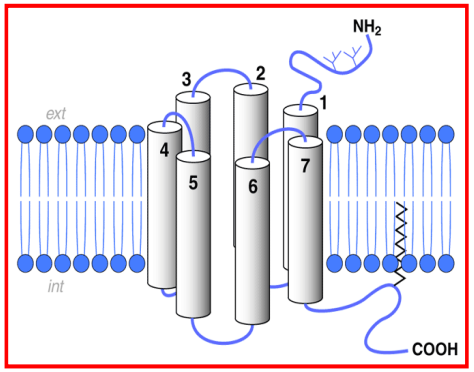
Systemic immunity
Host health



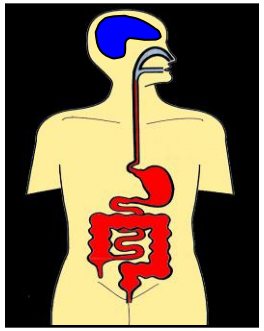
Rijkers et al., 2010

Key regulators of pain

- 3 receptors (GPCR) are mainly involved in the regulation of pain *:
  - Mu opioid receptor (MOR)
  - Cannabinoid receptor (CB)-1
  - Cannabinoid receptor (CB)-2



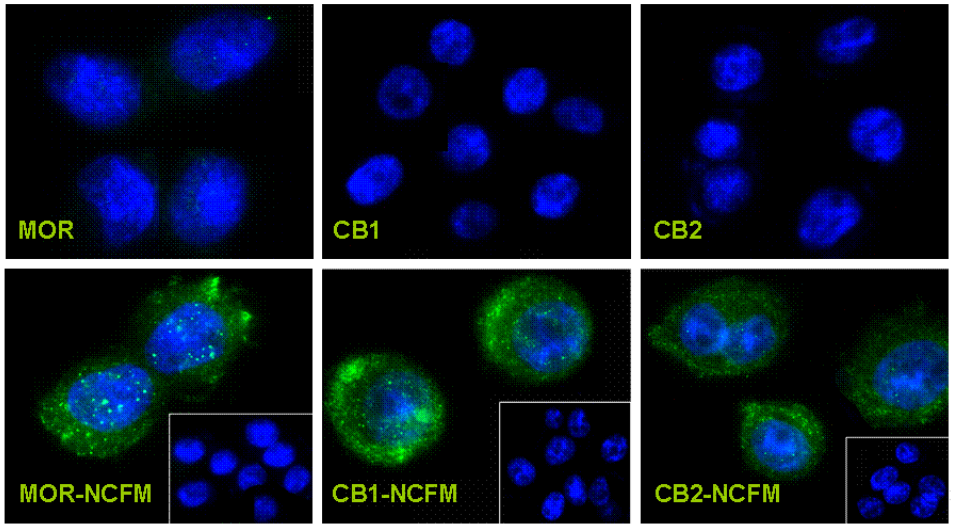
- All receptors are widely expressed in the central nervous system and in peripheric tissues, like gut epithelium* :
  - Enteric nervous system
  - Lymphocytes, macrophages, DC
  - Epithelial cells



*Philippe D et al. Gut (Epub ahead of print), Massa F et al. J. Clin. Invest. 113, 1202-1209 (2004), Stein C et al. Nat Med 8, 1003-1009 (2003), Philippe D et al. J. Clin. Invest. 111, 1329-1338 (2003), D'Argenio G et al. FASEB. J. 20, 568-570 (2006).

L. acidophilus NCFM® can reduce gut pain – pre-clinical data

- Mechanism: modulation of pain-reducing receptor expression in the intestine
- Shows direct interaction between a probiotic and host nervous system receptors



nature medicine

Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors

Christel Rousseaux[1–3], Xavier Thuru[1–3,10], Agathe Gelot[4–6,10], Nicolas Barnich[7], Christel Neut[1–3], Laurent Dubuquoy[1–3], Caroline Dubuquoy[1–3], Emilie Merour[1–3], Karen Geboes[8], Mathias Chamaillard[1–3], Arthur Ouwehand[9], Greg Leyer[9], Didier Carcano[9], Jean-Frédéric Colombel[1–3], Denis Ardid[4–6] & Pierre Desreumaux[1–3]

Rousseaux et al., 2007:
L. acidophilus NCFM induces expression of analgesic ('anti-pain') receptors in tissue culture cells

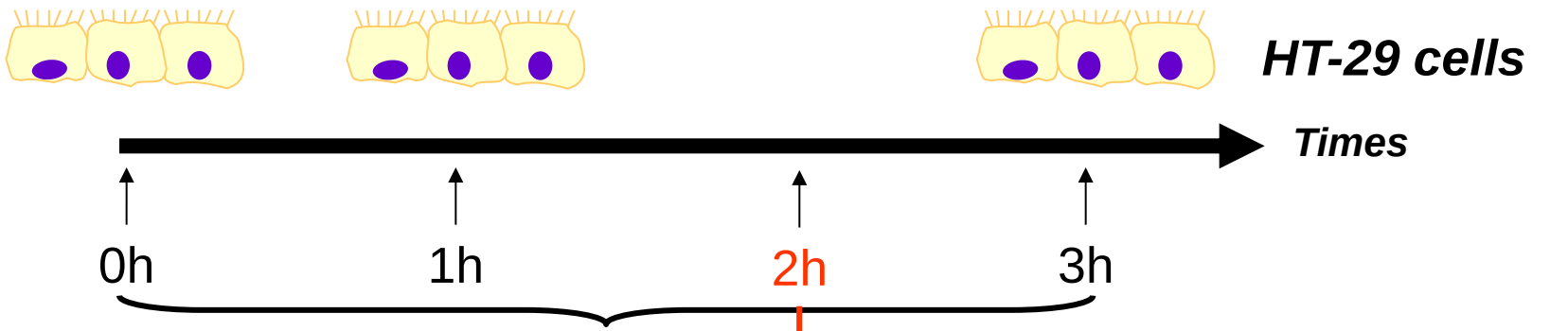
Aims of the study : In vitro experiments

To determine whether particular probiotic strains:

may induce expression of mu opioid (MOR) and cannabinoid 1 and 2 (CB1 and CB2) receptors on epithelial cells

and contribute to the modulation and restoration of normal visceral pain perception

Stimulation of human epithelial cells with probiotics and intestinal bacteria

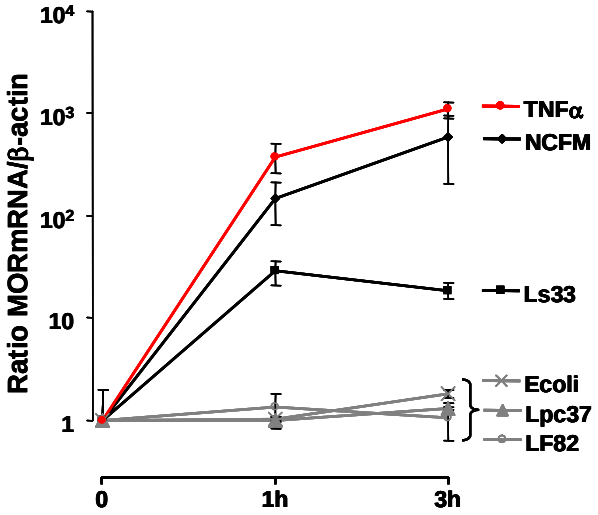


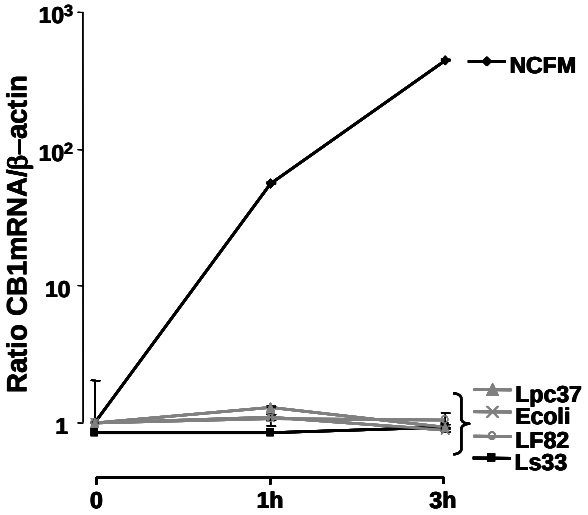
- ***Lactobacillus*** **(100 cfu/cell)**
  - *L. acidophilus* NCFM
  - *L. salivarius* Ls33
  - *L. paracasei* Lpc37
- ***Bifidobacterium*** **(100 cfu/cell)**
  - *B. lactis* Bi07
  - *B. lactis* Bl04
- **Escherichia coli** ***(100 cfu/cell)***
  - Commensal (cEc)
  - Adherent-invasive (LF82)

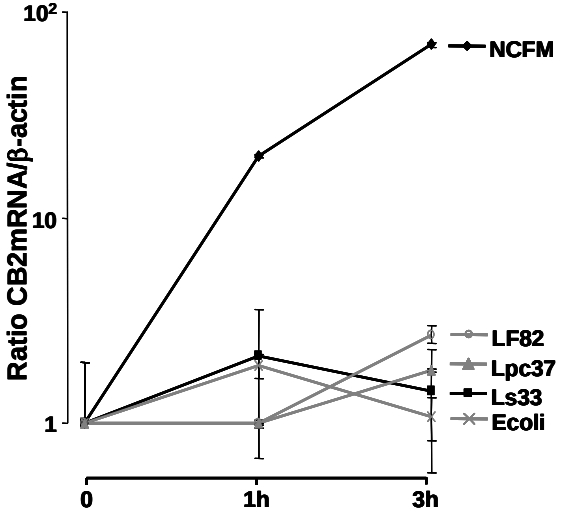
TNFα (10 ng/ml)*

*: Philippe D *et* al. *Gut* (Epub ahead of print)

Only *L. acidophilus* NCFM strains induced significant expression of MOR, CB1 and CB2 mRNA by epithelial cells

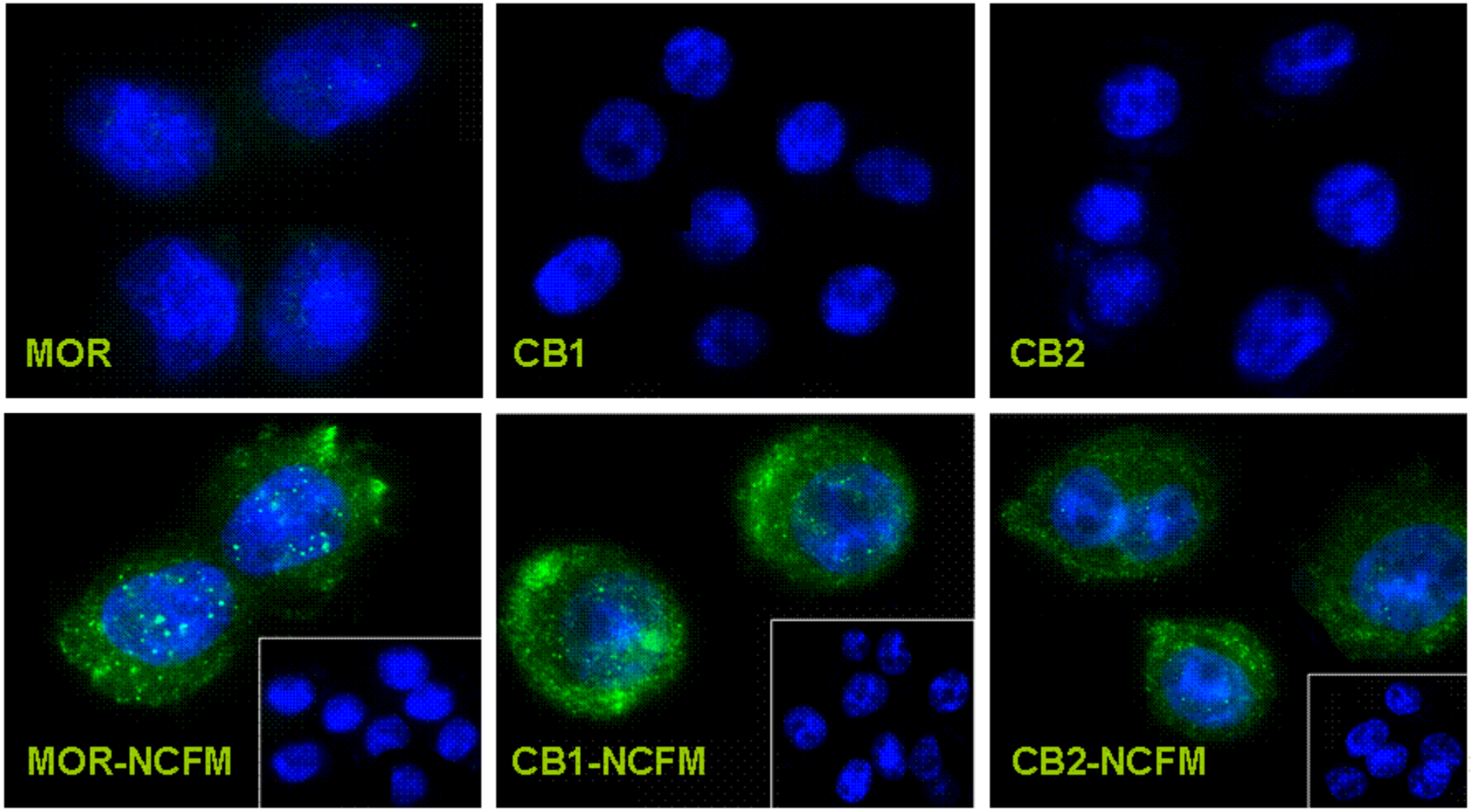




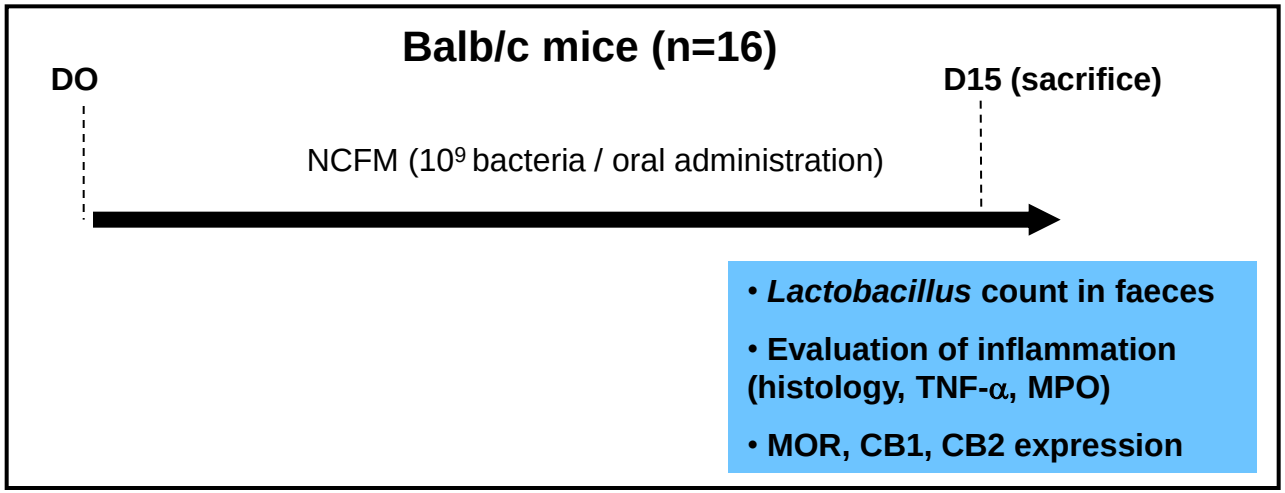


- *L. salivarius* (Ls33) induced MOR mRNA expression
- *L. paracasei (Lpc37), B. lactis* Bi07 and BL04 strains, and the two controls *E. coli* were ineffective

L. acidophilus NCFM strains induced expression of MOR, CB1 and CB2 protein by HT-29 epithelial cells

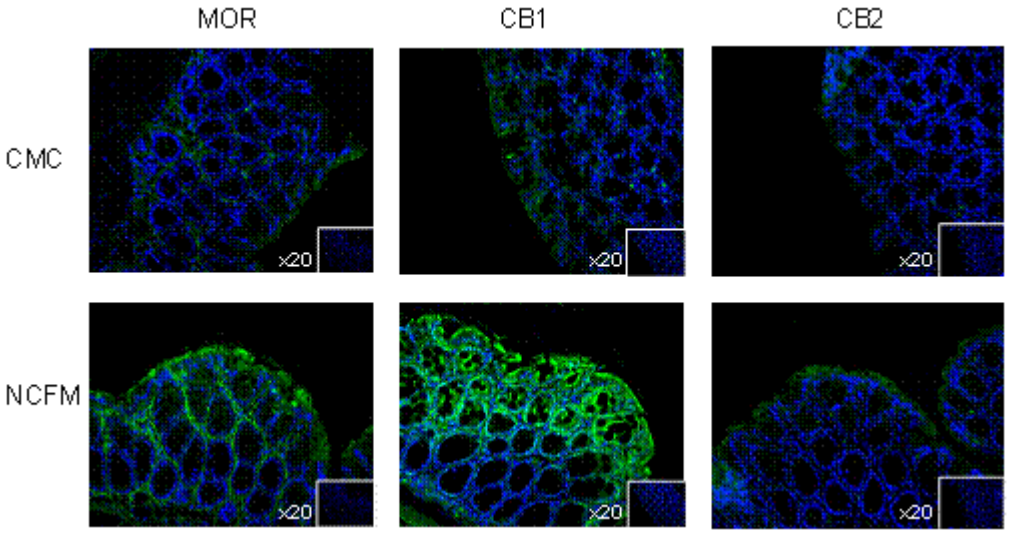


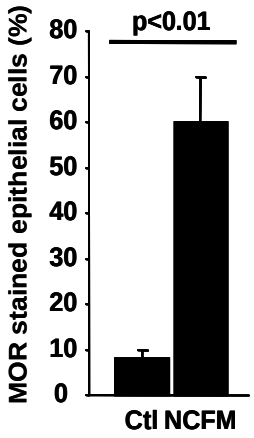
NCFM induced MOR , CB1 and CB2 mRNA expression in vivo in mice

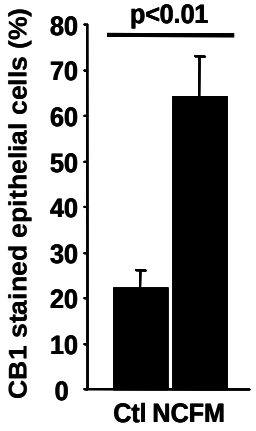


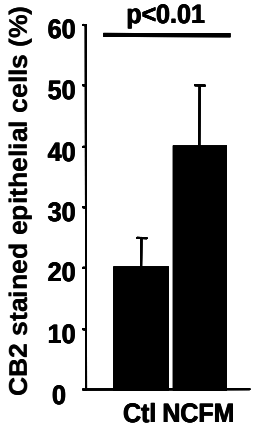
NCFM induced MOR , CB1 and CB2 mRNA expression in vivo in mice

- No macroscopic, histologic inflammation in mice treated with NCFM
- No modification of MPO and TNF-α colonic concentrations in NCFM treated mice compared to untreated animals
- Induction of MOR, CB1 and CB2 expression in NCFM treated mice

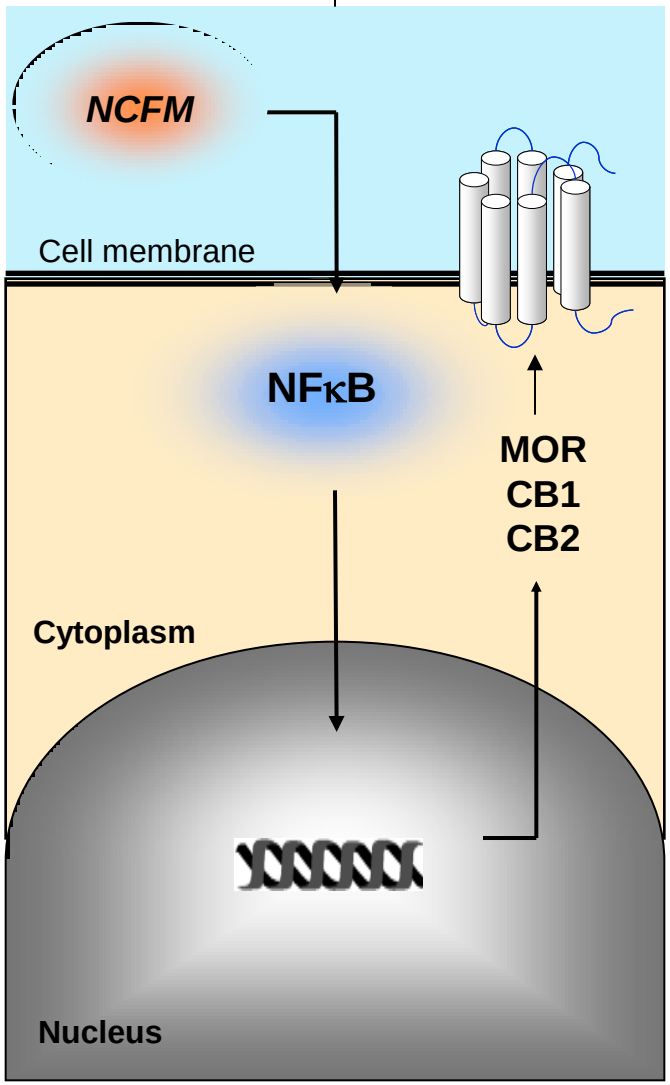


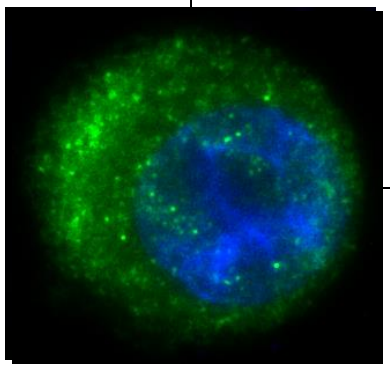






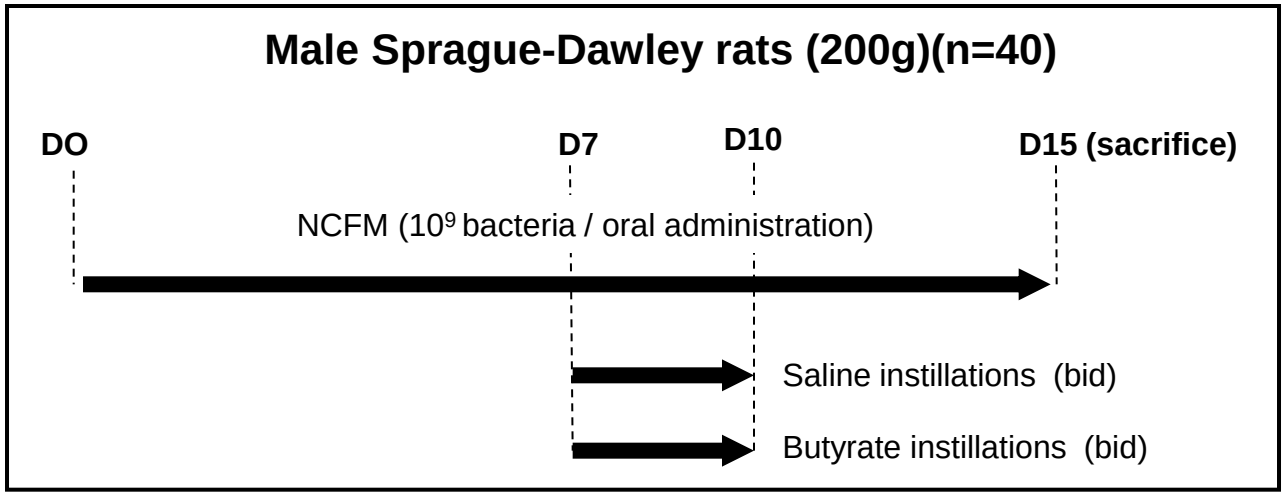
NCFM strains induced MOR, CB1 and CB2 expression in epithelial cells through the NFkB pathway

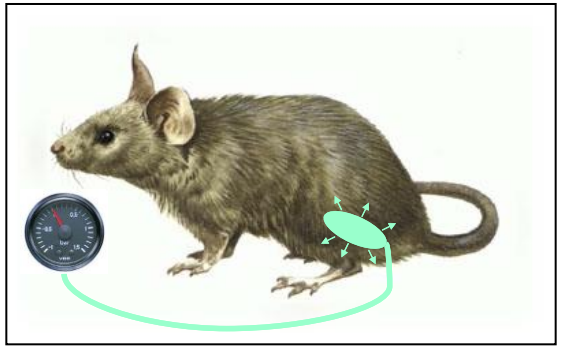




What is the functional role of NCFM-induced analgesic receptors ?

Evaluation of the functional role of NCFM-induced analgesic receptors in rats measured by colorectal distension

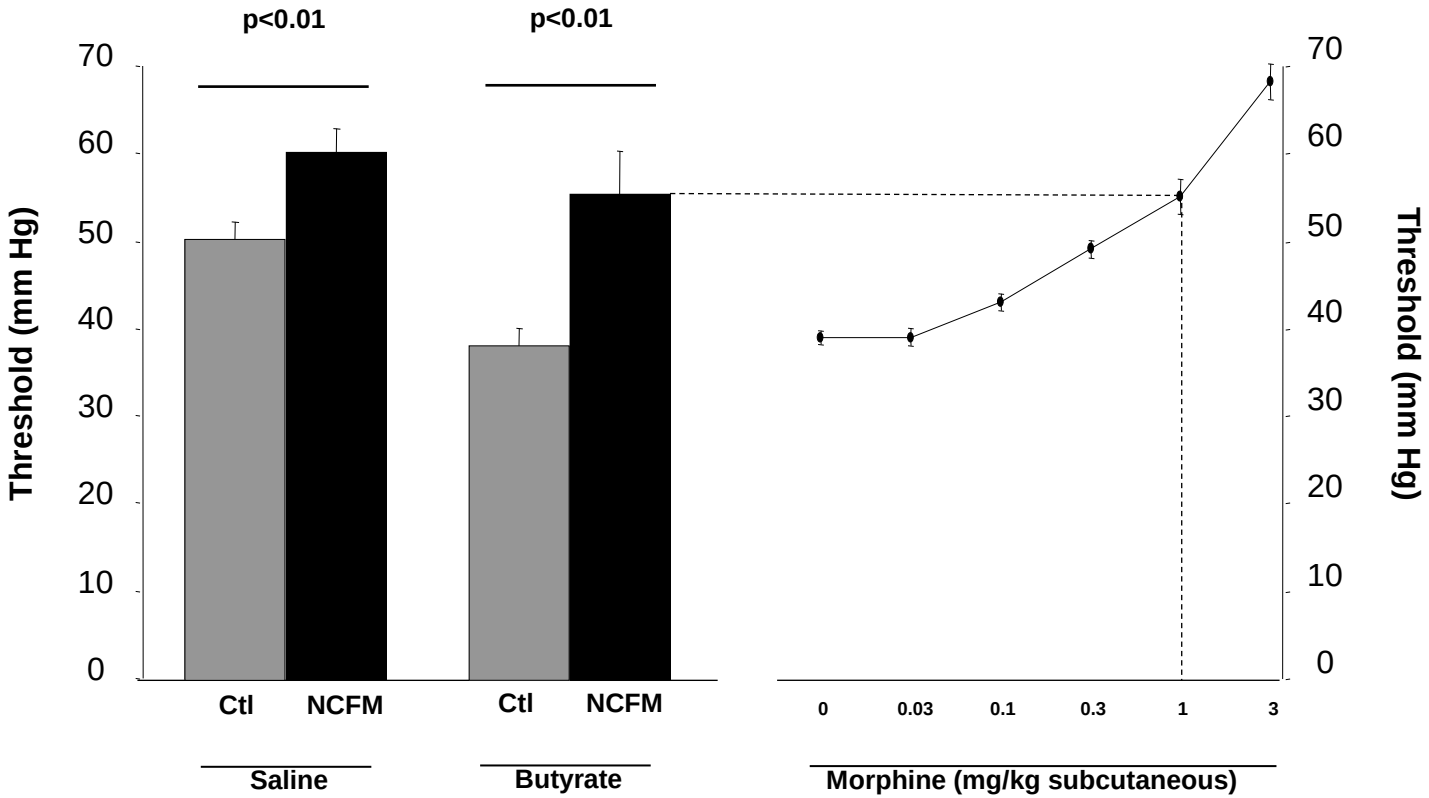




*Colorectal distension after inflation of a balloon inserted intrarectally and connected to a barostat system**

*Verdu EF et al. Gut 55, 182-190 (2006), Kamiya T et al. Gut 55, 191-6 (2006). Bourdu S et al. Gastroenterology 128, 1996-2008 (2005)

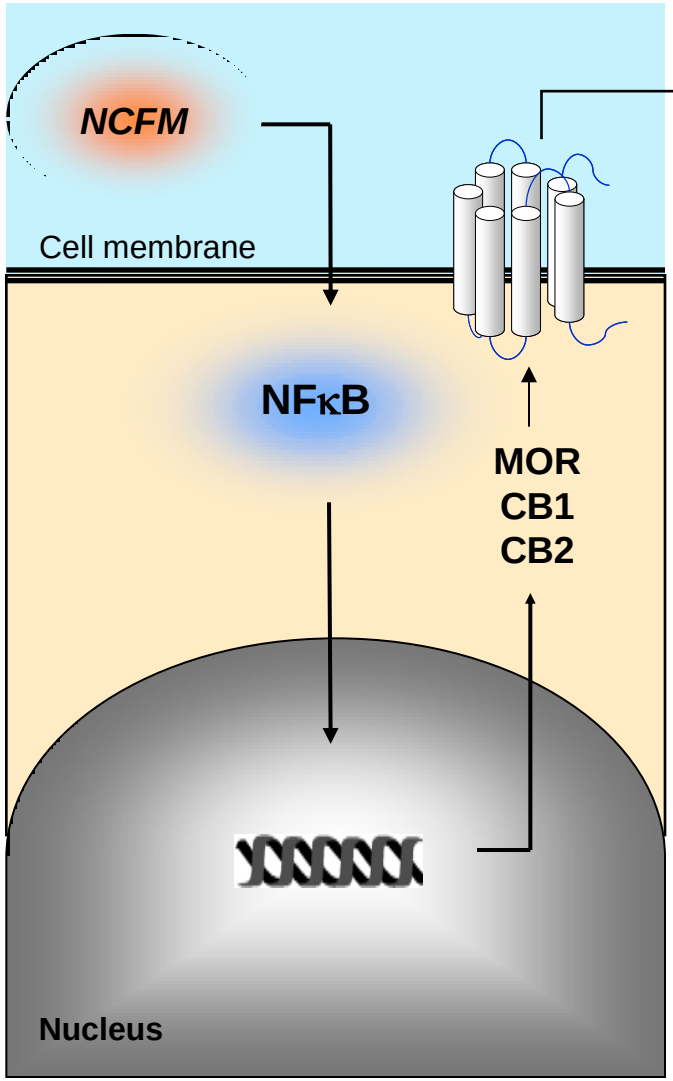
NCFM administration induced modulation and restoration of visceral pain perception



- NCFM decreased visceral perception allowing a 20% increase of pain threshold
- and a 44% increase of pain threshold in rat with colonic hypersensitivity
- NCFM mediated a similar effect than 1mg/kg of morphine s/c

*Bourdu S et al. Gastroenterology 128, 1996-2008 (2005)

NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut



NCFM increases pain threshold in the gut

CONCLUSION

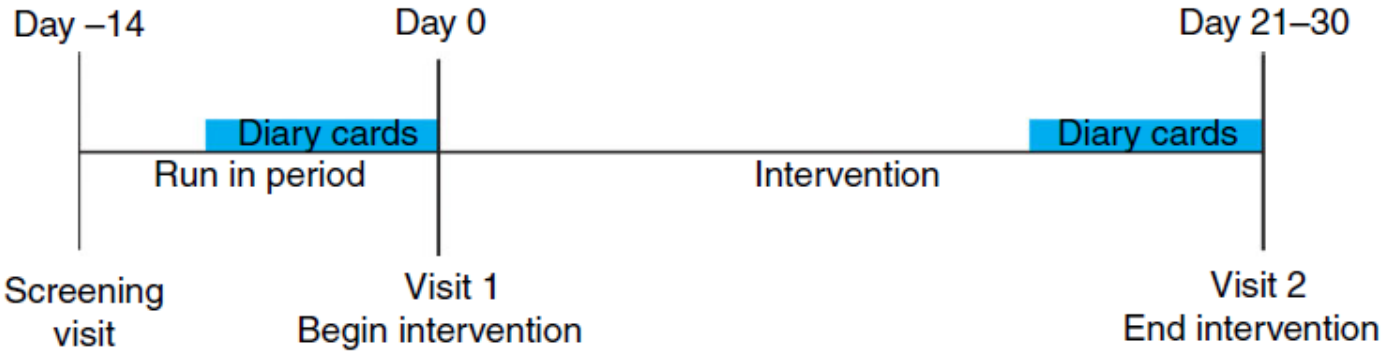
L. acidophilus NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut

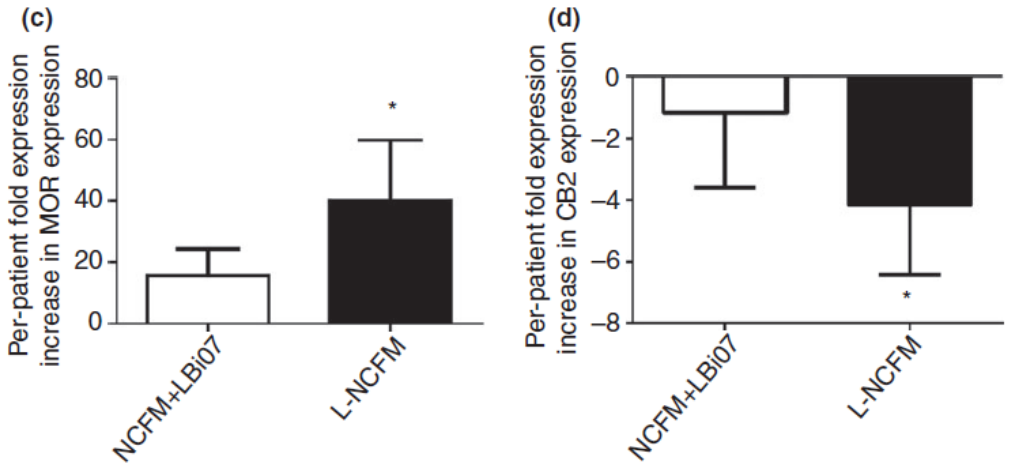
L. acidophilus NCFM® can reduce gut pain – human intervention

AP&T Alimentary Pharmacology and Therapeutics

Lactobacillus acidophilus NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study

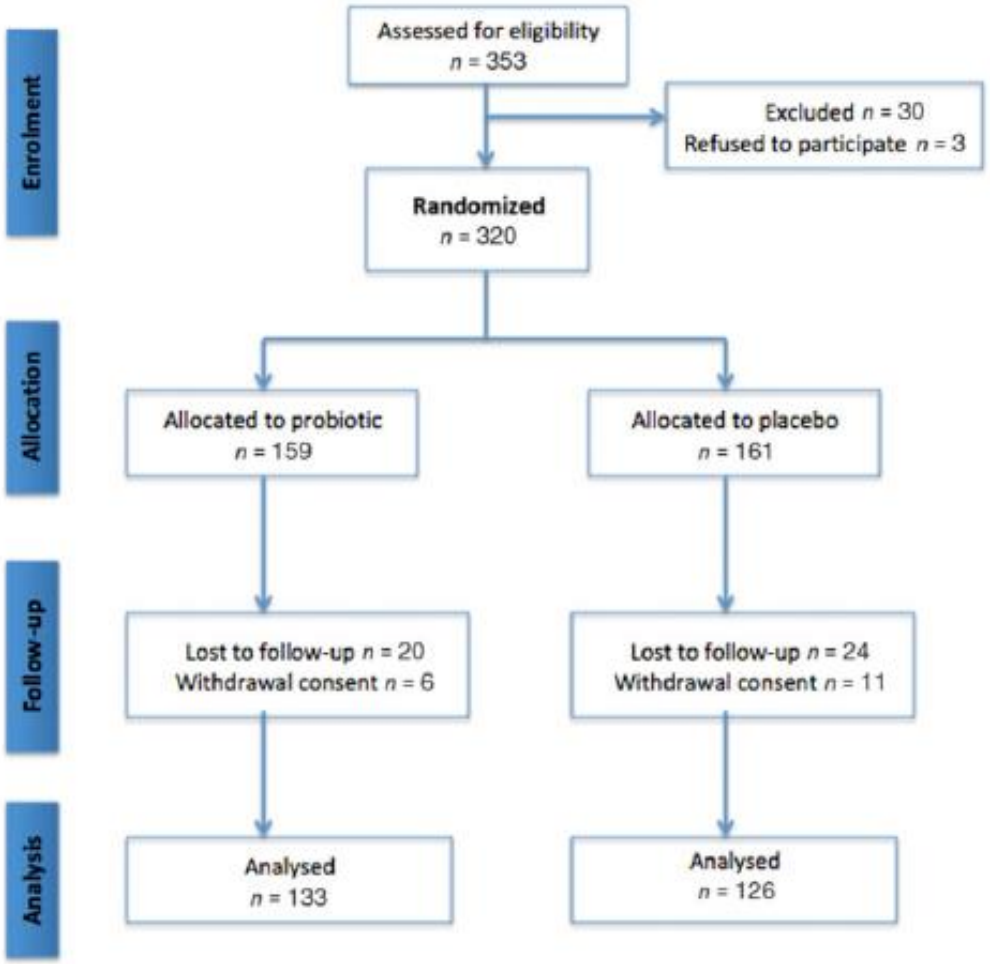
T. Ringel-Kulka*[,1], J. R. Goldsmith†[,1], I. M. Carroll†, S. P. Barros‡, O. Palsson†, C. Jobin†[,§] & Y. Ringel†





Ringel-Kulka et al. 2014

Reduction of post-colonoscopy pain (D'Souza et al., 2015)



Probiotic:
NCFM and Bi-07 1.25 × 10^{10} CFU each

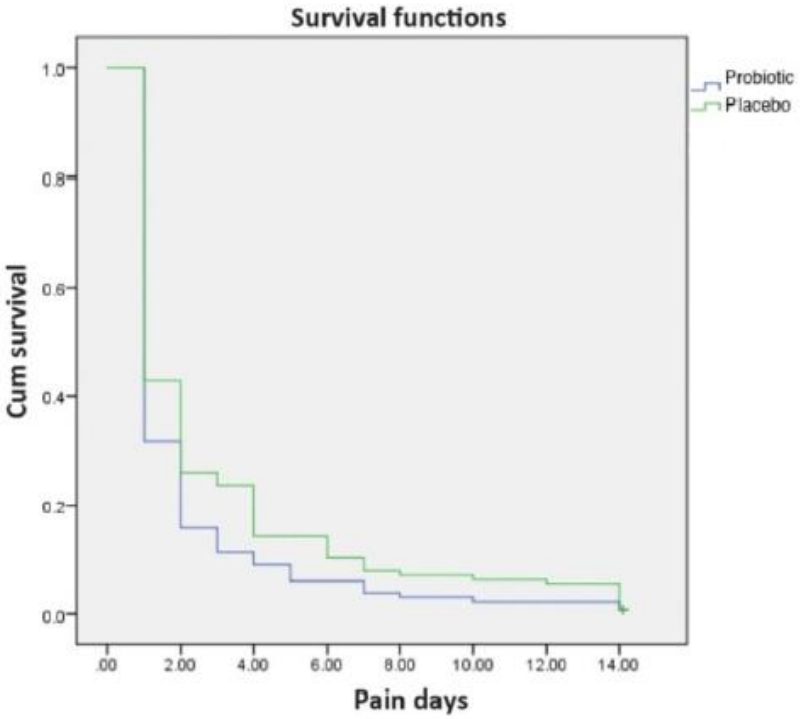


Fig. 2. Kaplan–Meier survival curve for differences in pain resolution between probiotic and placebo (P = 0.028).

Variable	Statistic	Probiotic (n = 133)	Placebo (n = 126)	P value
Bloating	Mean	2.000	2.517	0.111
	Standard deviation	1.996	3.054	
Pain	Mean	1.993	2.779	0.032
	Standard deviation	2.398	3.361	
Return of normal bowel habit	Mean	3.054	3.422	0.280
	Standard deviation	2.198	3.156	

Lactose intolerance symptoms

Reference	Subjects	Delivery format	Dose	Conclusion
Montes RG, et al. 1995. Effect of milks inoculated with Lactobacillus acidophilus or a yogurt starter culture in lactose-maldigesting children. J Dairy Sci. 78: 1657-1664.	20; 5-16 y	Milk containing probiotic	10^{10} cfu/d	H_2 excretion not reduced but symptoms alleviated; different meachanism than in the case of regular yogurt starter cultures

20 lactose mal-digesting children (5-16 yr)

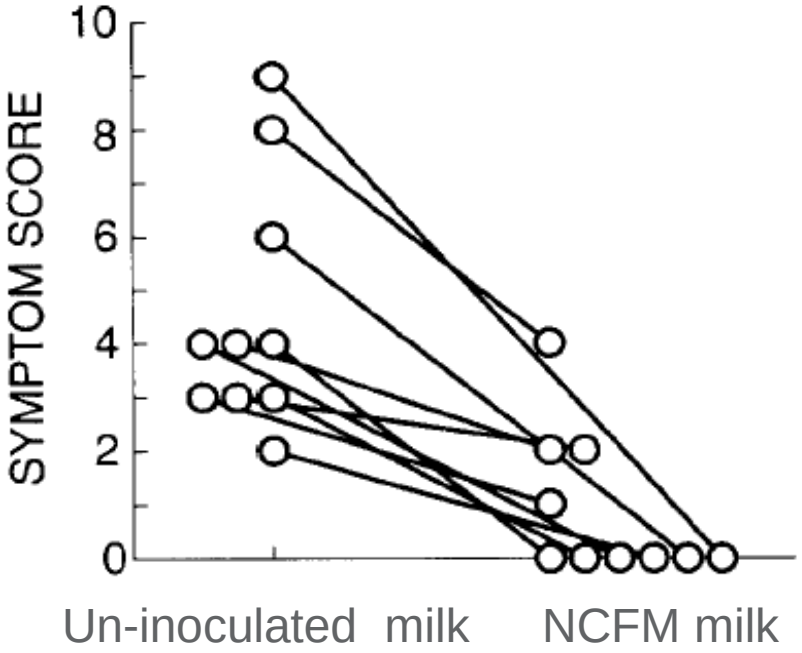
Single blinded study

Symptoms and breath H_2 excretion evaluated

10^{10} cfu NCFM® in milk (11.6g lactose)

Compared to:

- **10^{10} *S. thermophilus***
- **Plain milk**



Combined symptom score of abdominal pain, bloating, gas, cramps, flatus, abdominal rumbling

Montes, et al. 1995

Unpublished clinical trial with NCFM - Study design

- **391 subjects included**

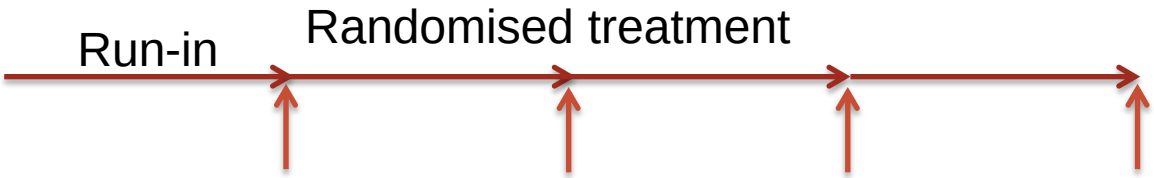
- Divided over three treatments:
  - placebo (MCC)
  - 1 billion NCFM/day
  - 10 billion NCFM/day

- Study design:
  - 8 week run-in
  - 12 week treatment
  - 4 week washout

- Faecal samples and quesionaires:
  - 0, 4 12 and 16 weeks

Table 14.1.1 Number of randomized patients per center

Study Site	Placebo	L.acidophilus (low)	L.acidophilus (high)
Helsinki	92	92	92
Turku	39	37	39
Total	131	129	131



Lyra et al., unpublished

Study outcomes

Primary Objective:

Examine the effect of probiotic capsules on alleviating irritable bowel syndrome (IBS) symptoms

Secondary Objectives:

Examine the effect of probiotic capsules on adequate relief of IBS symptoms

Examine the effect of probiotic capsules on elevating the IBS-related quality of life

Examine the effect of probiotic capsules on alleviating anxiety and depression

Examine the effect of probiotic capsules on stool consistency and bowel movement frequency

Assess the response effect of probiotic capsules on fecal microbiota

Assess characteristics of the fecal microbiota in relation to health status, demographic data and responsiveness to treatment

Assess the response effect of probiotic capsules on product safety

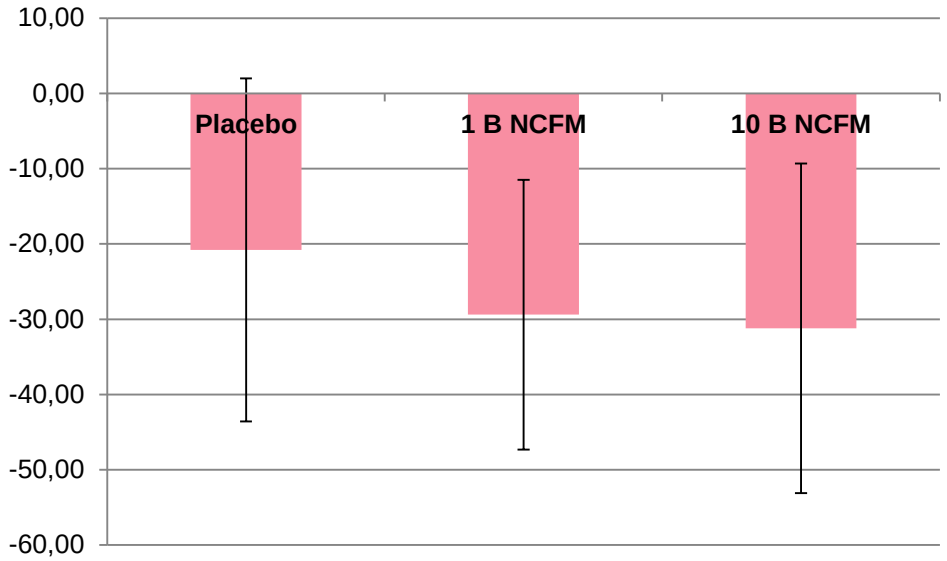
For all IBS scores the within group differences were significant.

In predefined analyses placebo effect too high to allow significant difference between treatments.

Lyra et al., unpublished

Post-hoc analyses

Reduction in abdominal pain among participants with moderate to severe pain at baseline



-baseline vs week 12

-baseline pain VAS score >35

-for combined active groups visceral pain reduced significantly compared to placebo

T-test for the change from BL in IBS-SSS Abdominal pain for patients with baseline > 35

ITT population

	Mean difference	95% CI Lower	95% CI Upper	Standard error	t-value	P-value
Week 12: L.acidophilus (high+low) vs. placebo	-9.5184	-18.8658	-0.1709	4.7036	-2.02	0.0460

Lyra et al., unpublished

To conclude

Functional bowel disorders common – Visceral pain a common symptom

- Visceral allodynia and hyperglasia may sensitize to pain

Increased gut permeability, low-level inflamation, microbial inbalance can induce sensitization to intestinal pain

- Probiotics may counteract these

L. acidophilus NCFM can directly influence the expression of pain-relieving receptors in the gut

- Pre-clinical study demonstrating mechanism-of-action and clinical data confirming the effect

The miracles of science™