Kaneka PROBIOTICS

White Paper

Floradapt®

Intensive G.I.

The Effects of Floradapt® Intensive GI on Gut Discomfort

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

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Introduction

So many people experience gut discomfort that unfortunately, for many it becomes a way of life. Symptoms can include abdominal pain, bloating and distension, gas, nausea, constipation and/or diarrhea, all of which lead to a lower quality of life.

Gut dysbiosis, which refers to an imbalance of the microflora in the intestine, can also play a role in gut discomfort. While the causes of gut dysbiosis are not well understood, altered immune function and visceral hypersensitivity are thought to be involved.

Current pharmaceutical treatments for various types of gut discomfort and dysbiosis are not always effective and can have problematic side effects, particularly with long term use (Zhang et al. 2016).

Floradapt® Intensive GI and Gut Discomfort

Floradapt® Intensive GI is a combination of two *Lactobacillus plantarum* strains plus *Pediococcus acidilactici* that can be taken once-a-day and that has been clinically shown to improve gut health and comfort.* The *Lactobacilli* are a group of lactic acid-producing bacteria known for their benefits for digestive balance and health, with *Lactobacillus plantarum* being the predominant species found in the human intestine.

The strains used in the Floradapt® Intensive GI formula were isolated via a screening process specifically designed to highlight strains that have a beneficial effect on intestinal health while also showing the ability to withstand gastrointestinal conditions (Espadaler *et al.* 2013).*

Floradapt® Intensive GI
Three Distinct Mechanisms of Action

Chosen from among 100 strains, the KABP™-021, KABP™-022, KABP™-023 strains showed a good ability to survive the acidic environment of the stomach and intestines as well as highly oxidizing conditions that can occur concurrent with gut discomfort (Espadaler *et al*. 2013; Lorenzo-Zúñiga et al. 2014). They also showed an ability to adhere to intestinal cells that was comparable to *L. plantarum* 299v, with P. acidilactici being better than a strain considered to be superior in its adhesion capacity (*L. rhamnosus* GG) (Espadaler *et al*. 2013). Adherence to intestinal cells is required for successful colonization of the intestine.

In addition to these essential basic properties, the chosen strains showed three important mechanisms of action that make them especially beneficial for gut health.

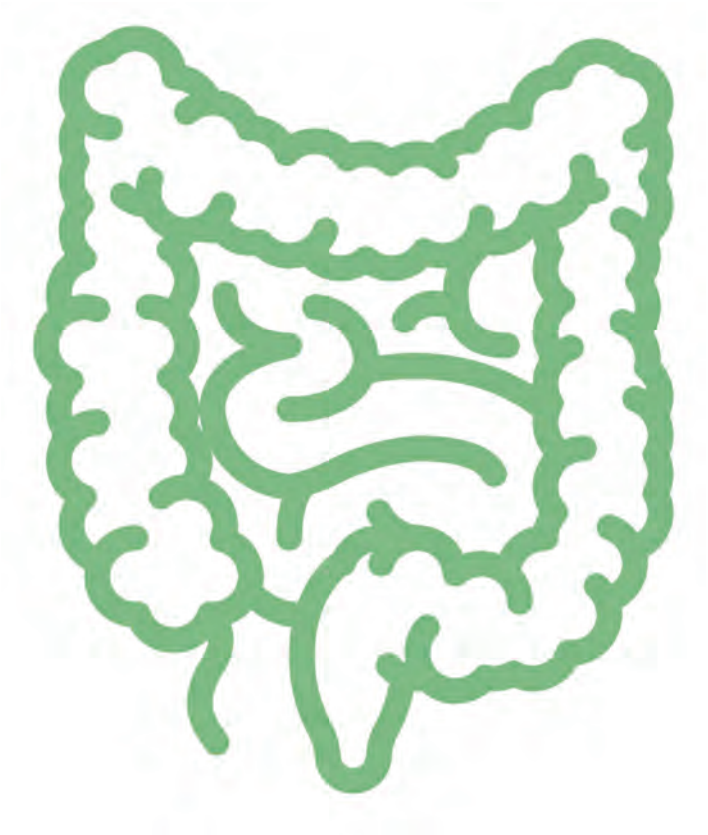
- KABP™-021
- KABP™-022
- KABP™-023

First, the two *Lactobacillus* strains (KABP™-021 and KABP™-022) were shown to produce acetylcholine whereas other Lactobacillus strains (L. reuteri, L. rhamnosus GG) produced none, and Bacillus infantis produced one quarter of the amount (AB-Biotics 2018). Acetylcholine has been shown to inhibit the release of pro-inflammatory cytokines in macrophage cultures (Borovikova et al. 2000). The strains also produced the short chain fatty acids acetate, propionate and butyrate (Espadaler *et al.* 2013); acetate is known to be a possible inhibitor of IL-2 and IL-8 (Ishiguro *et al*. 2014). It is also a nutrient source for the cells of the intestine.

Second, Floradapt® Intensive GI strains were shown in in vitro studies to be good inhibitors of pathogenic (gram-negative) bacteria such as E. coli and P. aeruginosa and the yeast C. albicans, which are known to be high in certain conditions of gut discomfort and dysbiosis, but the strains did not inhibit good bacteria such as those of the Bacteroides genus (Espadaler *et al.* 2013).

Third, KABP™-023 (*Pediococcus acidilactici*) was shown in a pilot study to be a high producer of polyphosphate granules, compared to *Lactobacillus* strains, especially LGG (*Lactobacillus rhamnosus)* which produced none. In a pilot study, polyphosphate granules were shown to reduce intestinal permeability, which detrimental to good intestinal health (Loré*et al.* 2016).

The *L. plantarum* KABP™-021, KABP™-022, KABP™-023 strains are patented under US patent number US10,155,015 for use in conditions of the gastrointestinal tract and promotion of intestinal health.



Clinical Evidence

Human studies have assessed the effects of the Floradapt® Intensive Gut on gut health and comfort (Espadaler et al. 2013; Lorenzo-Zúñiga V et al. 2014). Lorenzo-Zúñiga V *et al.* (2014) conducted a multicenter, randomized, double-blind, placebo-controlled trial in subjects (n=83, aged 20-70 years) with irritable bowel syndrome (IBS). Subjects received high dose ($1\text{-}3 \times 10^{10}$ cfus/d) or low dose ($3\text{-}6 \times 10^{9}$ cfus/d) probiotic administration or placebo once daily for 6 weeks. The ratio of the strains in the formula was 1:1:1. The primary outcome measure was quality of life as assessed by standardized questionnaire with a secondary outcome measure of probiotic effects on gut-related anxiety and global symptom relief, also measured by questionnaire (the Visceral Sensitivity Index, or VSI).

There were no differences in baseline characteristics between the groups. There was a statistically significantly greater improvement in quality of life at both three and six weeks for both the low-dose ($p=0.071$ and $p=0.023$, respectively) and high-dose groups ($p=0.041$ and $p=0.017$, respectively) compared to placebo (on the order of 18 vs 12 points of improvement in treatment vs placebo). There was no statistical difference between the low- and high-dose groups ($p=0.024$).

Clinical Evidence (continued)

A post-hoc analysis showed that the number of subjects in both probiotic groups together having a good response (≥ a 15-point improvement from baseline) was statistically significantly higher than in the placebo group (p=0.009). Similarly, there was a statistically significantly greater improvement in gut-related anxiety at six weeks for both the low-dose and high-dose groups (p=0.015 and p=0.033, respectively) compared to placebo (on the order of 10 vs 7 points of improvement in treatment vs placebo).

A recent meta-analysis assessed the effects of probiotics on IBS (Zhang *et al.* 2016). Probiotics tested included single species or combinations of *Lactobacillus*, *Bifidobacterium* and *Bacillus coagulans.* Twenty-one randomized, controlled trials including 1275 subjects with IBS were included, all using overall symptom response as the primary endpoint. Probiotic treatment was associated with more improvement than placebo for both overall symptom response (RR: 1.82, 95 % CI 1.27 to 2.60) and quality of life (QoL)(SMD: 0.29, 95 % CI 0.08 to 0.50), but not for individual IBS symptoms.

In a randomized, double-blind, placebo-controlled pilot study in subjects (n=25, aged 45.4 ±14.8 years; 10 males) with lactose intolerance received 1×10^9 cfus/d of each strain in Floradapt® Intensive Gut (n=18) or placebo (n=7) once daily for 8 weeks. The probiotic group had a lower mean symptom score compared to both baseline and placebo ($p<0.001$ and $p<0.018$, respectively), while there was no difference in placebo versus baseline. In particular, there was a significant decrease in symptoms of abdominal plain, flatulence and abdominal noises (all $p<0.05$), with no adverse events noted (Cano-Contreras et al. 2019).

Clinical Evidence (continued)

In a pilot animal study using a mouse colitis model (knock-out IL-10C57B6J mice), young mice treated with 10^9 cfus/d of Floradapt® Intensive GI probiotic strains, VSL#3, a medical food, or placebo for 10 weeks (n=12 in each arm) showed delayed onset of colitis symptoms compared to placebo and VSL#3 (both $p<0.01$) with a statistically significantly larger portion of animals showing no colitis symptoms (no histological or anatomical markers of intestinal inflammation present), compared to placebo after 10 weeks ($p<0.01$) (Lorén *et al.* 2016).

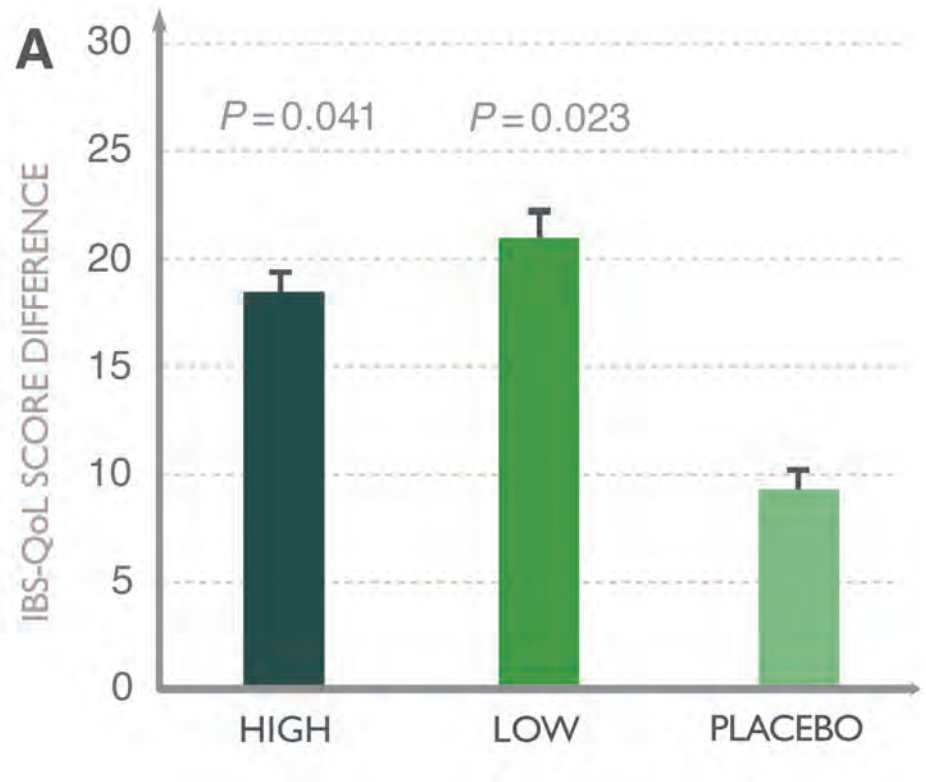


Figure 1 Irritable bowel syndrome related quality of life score improvement compared to baseline after 42 d of treatment. A: Global scores improved significantly more in both treatment groups than placebo (Kruskall-Wallis test); B: Among the different domains, the mental status showed a significant improvement when compared to placebo.

Three Distinct Mechanisms (continued)

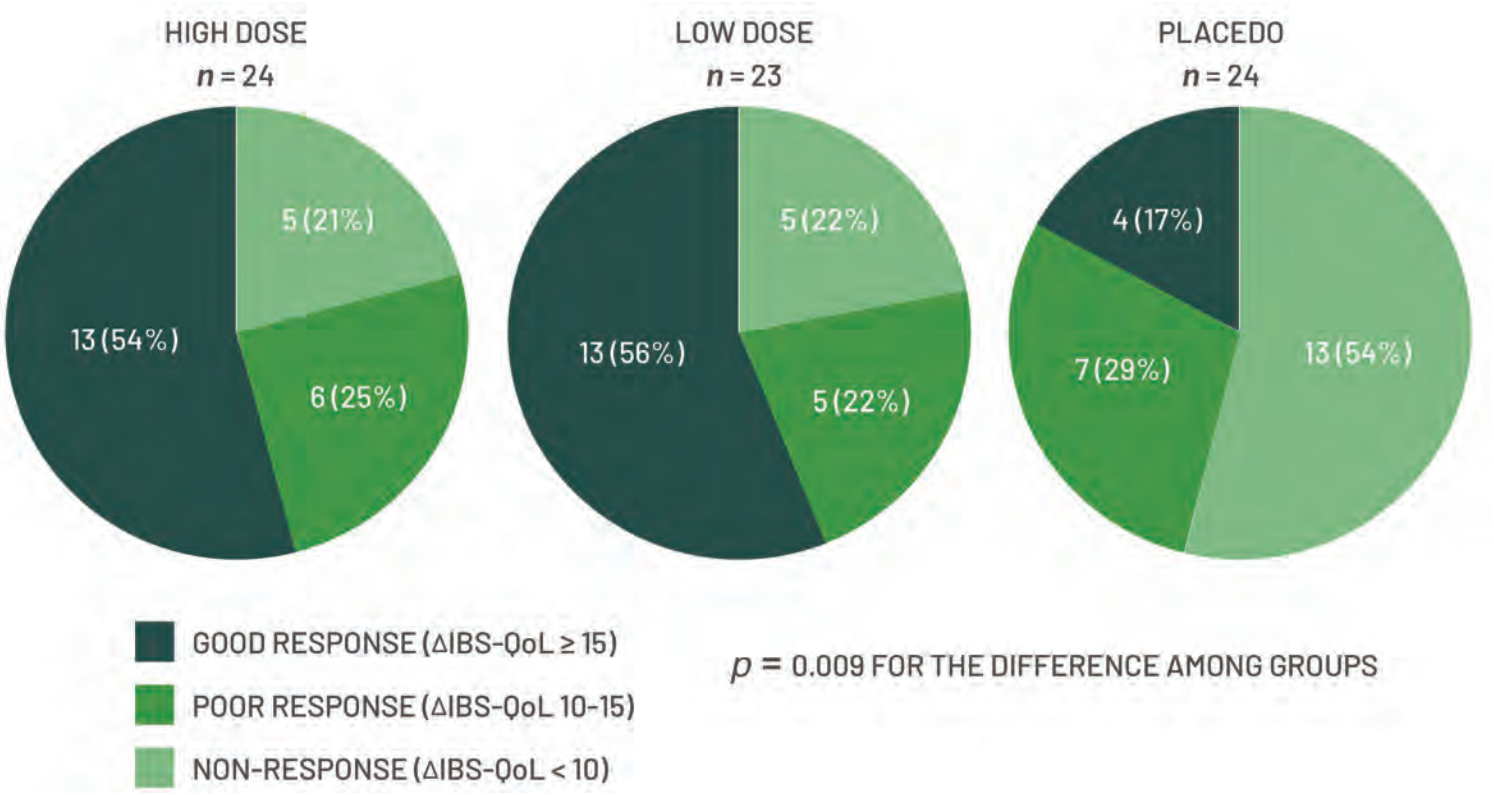


Figure 2 **Irritable bowel syndrome-related quality of life score response to probiotic and placebo therapy.** Good response was defined as score improvement ≥ 15 points; poor response as score improvement 10-15 points; and non-response as score improvement < 10 points. The number of responders (score increment > 10) was significantly larger in both groups of patients treated with probiotics than in those treated with placebo (x^2 test). IBSQoL: Irritable bowel syndrome-related quality of life.

Summary of Capabilities

Floradapt® Intensive GI is a synergistic combination of unique, high-performing probiotic strains selected from wild strains of *Lactobacillus plantarum* and *Pediococcus acidilactici.* Clinical trials have shown a once-a-day dose of Floradapt® Intensive GI can be of benefit for gut discomfort and overall gut health and microbial balance.*

There are three mechanisms of action documented in vitro associated with gut benefits:

- Production of acetylcholine and short chain fatty acids, which have been shown to inhibit the release of pro-inflammatory cytokines.
- Inhibition of pathogenic (gram-negative) bacteria such as E. coli and P. aeruginosa and the yeast C. albicans, which are known to be high in certain conditions of gut dysbiosis.
- Production of polyphosphate granules, which are known to impact intestinal permeability

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