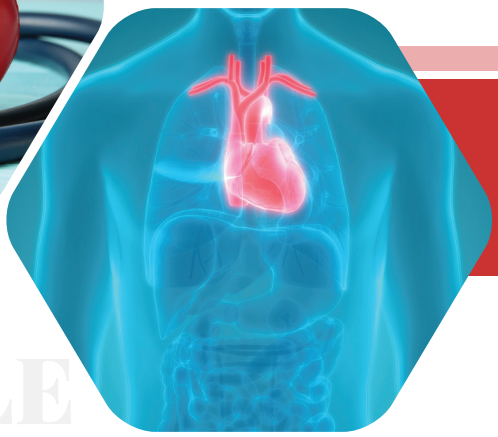
Kaneka® PROBIOTICS



White Paper

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# CARDIO

## Promoting Overall Heart Health with Floradapt® Cardio.*

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# Introduction

Heart health month brings to the front of our minds that most important organ. More than ever, people of all ages are concerned with maintaining their heart health, whether by diet, exercise or other lifestyle factors, including supplements. The wide range of supplements bearing the "supports a healthy heart" promise includes an array of ingredients focusing on several mechanisms. One of the newer and very intriguing offerings is probiotics. Newly introduced strains offer clinically shown benefits for healthy cholesterol metabolism.

# Why Cholesterol Matters

First, a basic primer on cholesterol to remind us why it is important for heart health: Despite the bad rap cholesterol gets, it has a number of important roles in the body. It is a fatty compound produced by the liver which is used to make hormones (think male and female sex hormones), vitamin D and bile. It is also a critical part of the membranes that surround all cells in the body, keeping them supple but not too permeable. The body can make its own cholesterol and foods like meat and cheese contribute to the body's overall pool.

Dietary cholesterol can't enter the bloodstream from the intestine without the aid of bile and lipoprotein carriers. The names of these carriers will be familiar to you: low density lipoprotein (LDL) and high density lipoprotein (HDL). When they have attached to a cholesterol molecule, they are known as LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C). LDL-cholesterol in the blood is susceptible to being oxidized, particularly if there is a low level of antioxidants in the blood. This is where cholesterol's functioning in the body starts to turn bad. Oxidized LDL-cholesterol (ox-LDL) has different characteristics than un-oxidized: it literally becomes sticky. This stickiness causes it to stick to itself and also to the vessel walls. These deposits, called plaques, are an integral part of hardening and narrowing of the arteries in atherosclerosis. During this process, an inflammatory response ensues, which is even more damaging to the vessel. If plaque formation continues for a long time, the cholesterol plaques become thick and can block arteries, causing a reduction in blood flow. This leads to reduction in the oxygen supply, which causes additional tissue damage. If a plaque ruptures, the resulting blood clot can cause a sudden blockage. This is the reason for many strokes and heart attacks (Keaney 2000).

Current research supports the idea that probiotics, particularly of the Lactobacillus genus, can positively affect cholesterol metabolism and promote cardiovascular health.

# Floradapt® Cardio and Lactobacillus plantarum Strains

Floradapt® Cardio is a patented, clinically tested, once-a-day combination of three Lactobacillus plantarum probiotic strains that have been shown to enhance overall heart health through beneficial effects on cholesterol metabolism in healthy individuals.* The Lactobacilli are a group of lactic acid-producing bacteria with documented cholesterol-lowering properties, with Lactobacillus plantarum being the predominant species found in the human intestine.

The strains used in the Floradapt® Cardio formula were isolated via a screening process specifically designed to identify strains that beneficially effect cholesterol metabolism while promoting health benefits along the gut-brain-axis (Bosch et al. 2014).

Out of 550 strains screened, the L. plantarum CECT 7527, 7528 and 7529 strains showed a highly superior ability to survive the acidic environment of the stomach and intestines. They also showed an ability to adhere to intestinal cells that was four times higher than the other common strains used as positive controls (L. plantarum 299v, L. plantarum VSL#3) (Bosch et al. 2014). Adherence to intestinal cells is required for successful colonization of the intestine.

Using genetic sequencing, CECT 7527, CECT 7528 and CECT 7529 were identified as being part of the Lactobacillus plantarum species. The strains were shown not to have genes conveying antibacterial resistance; this is important so as not to spread the trait of resistance to other undesirable bacteria and thereby reduce the overall effectiveness of antibiotics.

The L. plantarum strains, CECT 7527, CECT 7528 and CECT 7529 are patented under European patent EP 2 485 743 B1 and U.S. patent 8,668,906 B2 for use in modulating serum cholesterol in humans or animals.

These L. plantarum strains are known commercially by their brand names as:

- KABP™-011 (CECT 7527)
- KABP™-012 (CECT 7528)
- KABP™-013 (CECT 7529)

# Clinical Evidence on Cholesterol Metabolism

Two randomized, double-blind, placebo-controlled trials have tested the efficacy of L. plantarum KABP™-011, KABP™-012 and KABP™-013 strains in Floradapt® Cardio.

In one study, 60 people with high cholesterol who were not taking any cholesterol- lowering drugs took Floradapt® Cardio (containing 1.2 billion CFUs) or placebo once per day for 12 weeks (Fuentes et al. 2013). After 12 weeks, subjects in the Floradapt® Cardio group had a reduced total cholesterol (TC) by 13.6%. This result was significantly better than placebo ($p<0.05$) and lasted for 4 weeks after cessation of Floradapt® Cardio administration. A sub-group analysis showed that subjects who started the study with the highest levels of TC (251-300 mg/dl) saw the greatest benefit, with significant reductions in TC (-17.4%), LDL-C (-17.6%), and oxidized LDL-C (-15.6%); all $p<0.5$ compared to placebo. This shows that the cholesterol modulating effects were more pronounced in those with the greatest need.

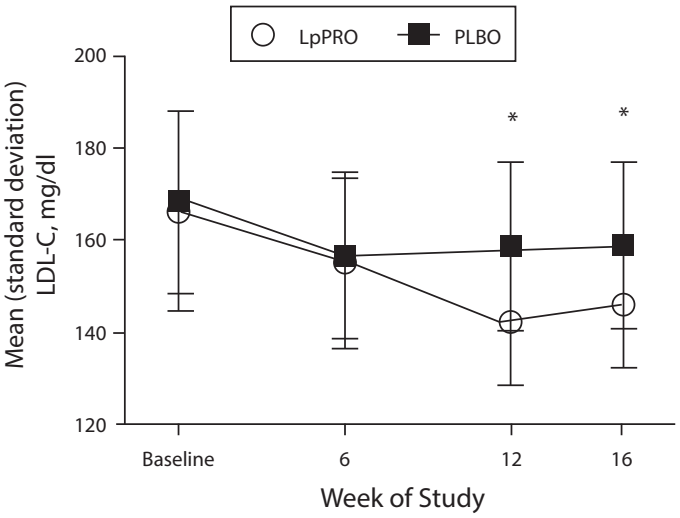


Figure 1. Mean (standard deviation) LDL-C concentrations (mg/dL) at each timepoint for subjects receiving *Lactobacillus plantarum*-containing probiotic (LpPRO) or placebo (PLBO) capsules (Fuentes et al. 2016). *$p<0.001$ between treatments.

Fuentes et al. conducted another study using the same design (n=60) and found similar results (Fuentes et al. 2016). After taking Floradapt™ Cardio for 12 weeks, subjects with high cholesterol had significant reductions in TC, LDL-C (Figure 1), LDL-C/HDL-C ratio, oxidized LDL-C (all $p<0.001$), and triglycerides (TG, $p<0.05$) compared to placebo. As in the first study, these improvements lasted for 4 weeks after discontinuing Floradapt™ Cardio. The study investigators noted, "The magnitude of the LDL-C reduction is similar to that shown for recommended dietary interventions such as plant sterols and viscous fibers."

In a meta-analysis of 15 trials (n=976) using Lactobacillus to lower cholesterol, the Floradapt™ Cardio strains had the greatest ability to lower cholesterol from among all the strains tested, including L. acidophilus L-1, L. plantarum 299v, L. plantarum TENSIA, L. fermentum, L. reuteri NCIMB, L. sporogenes, L. salivarius UCC118, and L. rhamnosus CGMCC1.3724 (Wu et al. 2017). The Fuentes (2013) trial described above was included in this meta-analysis. See Figures 2 and 3 below on page 6.

# Three Distinct Mechanisms

Other research is elucidating a number of mechanisms that may contribute to the cholesterol modulating effects of probiotics in the intestine. Several of these mechanisms have been demonstrated in vitro for the Floradapt® Cardio strains.

One of these mechanisms involves bile acids. Floradapt® Cardio strains were shown to produce an enzyme called bile salt hydrolase (BSH). BSH deconjugates bile acids, hindering their function. Lactobacilli with BSH activity survive and colonize the lower small intestine where the bile circulation (enterohepatic cycle) takes place (Kumar 2012). In vitro tests showed Floradapt® Cardio strains produced more BSH enzyme than other high-producing BSH strains of L. plantarum (229v and VSL#3) (Table 1) (Bosch et al. 2014).

When BSH is deconjugated, it cannot emulsify cholesterol for intestinal absorption into the blood. Without bile emulsification, the undigested cholesterol passes through the digestive tract in the feces, effectively removing it from the body. Floradapt® Cardio strains have also been shown to directly bind cholesterol onto their cell surface in vitro, which can keep it from being absorbed into the bloodstream (Bosch et al. 2014). This action of preventing absorption of cholesterol and removing it from the intestine through the feces is the first mechanism of action.

Normally, bile is transported back to the liver and reused after it is absorbed through the intestine, in a process called enterohepatic recirculation (Begley et al. 2006). When probiotic bacteria break down bile with BSH, there is a deficit of bile and the liver is forced to make more bile. Since cholesterol is used to make bile acids, this draws down the body's cholesterol pool, thereby reducing total cholesterol. This action of causing more cholesterol to be used up to produce needed bile is the second mechanism of action which may be associated with the beneficial effects on cholesterol metabolism observed in clinical studies.

Table 1. Bile salt hydrolase (BSH) and media cholesterol lowering activity (%) of the three Floradapt® Cardio strains (KABP™-011, KABP™-012 and KABP™-013) compared to two common commercial Lactobacillus plantarum controls (299v and VSL#3) (Bosch et al. 2014).

| STRAIN | BSH | % Cholesterol Reduction |
|---|---|---|
| Lp KABP™-011 | 2.10 | 10.10 |
| Lp KABP™-012 | 2.53 | 9.60 |
| Lp KABP™-013 | 3.17 | 9.30 |
| Lp 299v | 1.70 | 4.58 |
| Lp VSL#3 | 2.00 | 7.40 |

# Floradapt® Cardio and Cholesterol Metabolism

Like many probiotic bacteria, Lactobacillus plantarum strains produce short-chain fatty acids (SCFA) from dietary fiber (Table 2). SCFAs inhibit the body's own production of cholesterol by decreasing the expression of enzymes required for cholesterol synthesis in the liver (den Besten et al. 2013). They can also decrease the redistribution of cholesterol from the blood to the liver (Dora et al. 2002). This action of SCFAs inhibiting the body's production of cholesterol is the third mechanism of action.

Table 2. SCFA (propionic and butyric acid) production of the three Floradapt® Cardio strains and a common commercial Lactobacillus plantarum control (Lp 299v) (Bosch et al. 2013).

| STRAIN | Propionic acid (mg/L) | Butyric acid (mg/L) |
|---|---|---|
| Lp KABP™-011 | 15.5 | 14.2 |
| Lp KABP™-012 | 12.2 | 12.3 |
| Lp KABP™-013 | 44.9 | 21.6 |
| Lp 299v | 12.9 | 9.2 |

# Summary of Capabilities

Floradapt® Cardio is a synergistic combination of unique, high-performing probiotics select-ed from wild strains of Lactobacillus plantarum. Clinical trials have shown a once-a-day dose of Floradapt® Cardio taken for 12 weeks can be of benefit to cholesterol metabolism (Fuen-tes et al. 2013; Fuentes et al. 2016). There are three mechanisms of action documented in vitro associated with healthy cholesterol levels:

Production of BSH that can break down bile, thus preventing the emulsification and absorption of dietary cholesterol; Direct binding of dietary cholesterol, interfering with its absorption into the blood; Production of SCFAs from dietary fiber that may inhibit the body's own production of cholesterol in the liver

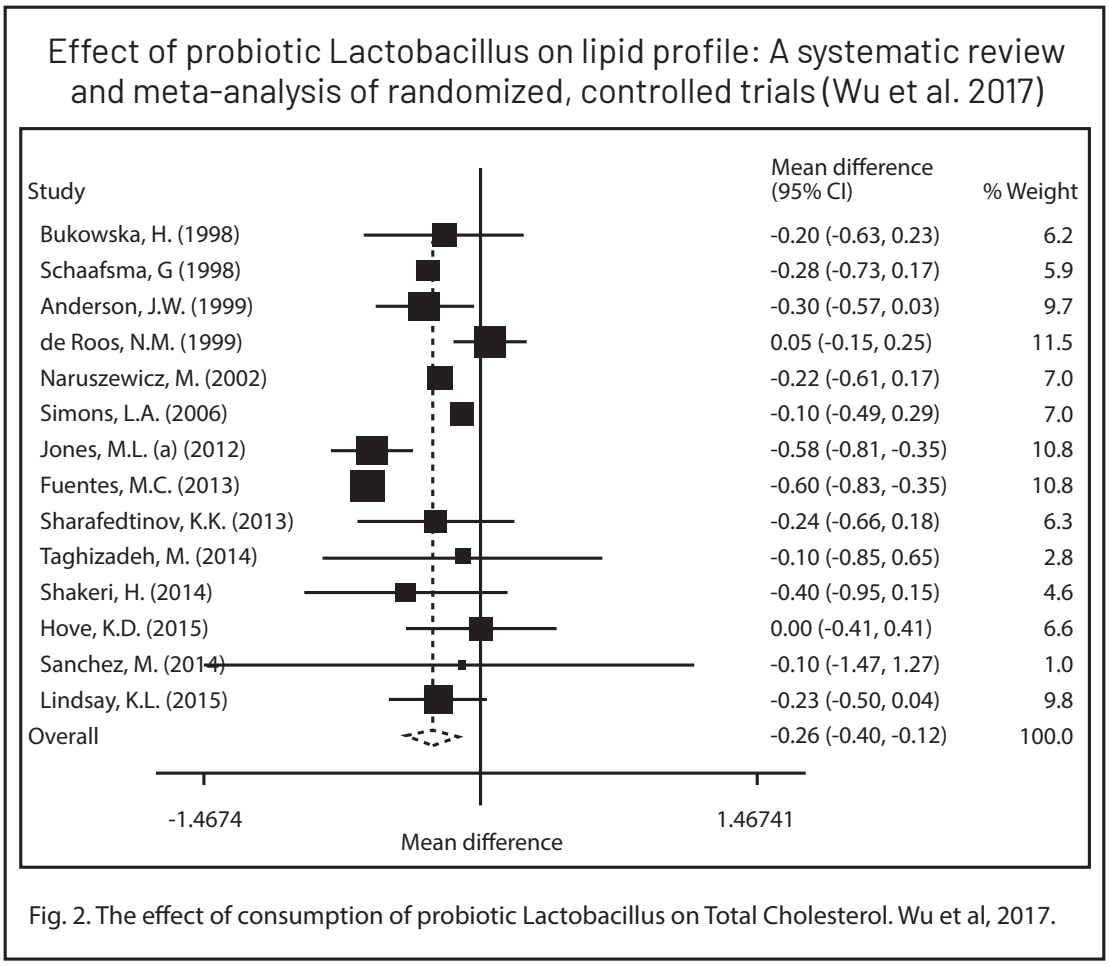


Fig. 2. The effect of consumption of probiotic Lactobacillus on Total Cholesterol. Wu et al, 2017.

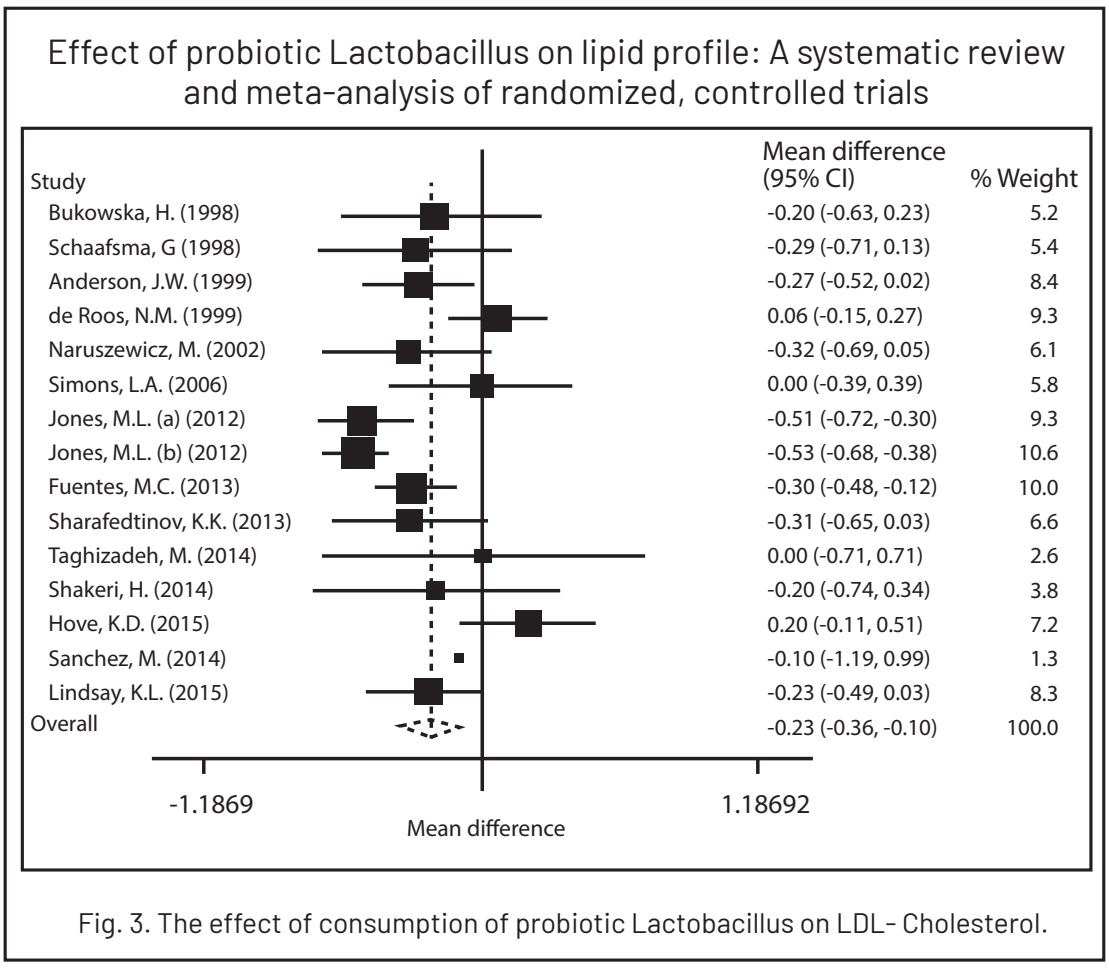


Fig. 3. The effect of consumption of probiotic Lactobacillus on LDL- Cholesterol.

# The Genesis of Floradapt®

Kaneka has been at the forefront of developing novel probiotic solutions for a variety of health applications. From inception to finished product, Floradapt® stands alone because of our unique process of developing clinically validated probiotic formulations.

Floradapt® products are based on a mechanistic approach to identify optimal strains for specific health applications. The strain library is a collection of over 1000 different, wild-type strains strategically sourced from around the world and populations with good health yet little exposure to modern lifestyle and amenities. As a result, our strains are derived from healthy individuals with "conserved microbiomes" that have not lost their health functionality,
therefore providing a diverse microbiome which is more adept at achieving better homeostasis. Probiotic researchers frequently see non-conserved microbiomes in western societies, where the diversity of the gut microbiota is reduced because of exposure to antibiotics and antifungals, among other factors. Hosts with conserved microbiomes have abundant, acid-resistant smart strains that can offer superior efficacy for a variety of health applications, including gut, digestion, immunity, infant colic, women's, and cardiovascular health.

Floradapt® contains science-based strains for a targeted clinical application, which may be formulated into proprietary products. Our published, randomized, double-blind,
placebo-controlled clinical trials utilized actual dosages appropriate for commercial dietary supplement products. What this means for the brand is that the finished dosages are designed to contain the exact amounts, potency, strains, and delivery modality used in the clinical trials.

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